STRATIFIED MEDICINE: METHODS FOR EVALUATION OF PREDICTIVE BIOMARKERS

by

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Abstract

Background: Stratified medicine was defined as the use of biomarkers to select patients more likely to respond to a treatment or experience an adverse event.

Aims: To investigate the hypothesis that there is a mismatch between the theoretical proposals and practice of predictive biomarker research, focusing on the clinical utility stage.

Methods: Methodological research was identified in a systematic review of frameworks for staged evaluation of predictive biomarkers. Actual research supporting 50 real cases identified in European Medicines Agency licensing was analysed. A case study of recent research into ERCC1 in non-small cell lung cancer was undertaken. Existing discrepancies between the theory and practice were identified and possible reasons and consequences of these were discussed.

Findings: A mismatch between theory and practice was identified. It appeared to be a result of both the practice not following some theoretical requirements, and the underdevelopment of methodology for certain situations. Areas of clinical research with insufficient relevant methodology were identified.

Conclusions: The major research priorities identified in this thesis were development of a clear hierarchy of biomarker research designs and development of methodology related to the biomarker threshold.

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CHAPTER 1. INTRODUCTION

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In 1992 Barbara Bradfield was told her breast cancer has come back. It was aggressive, spread to her neck and lungs. Her disease was declared terminal. She agreed to participate in a phase I trial of a new drug, developed especially to treat her type of cancer. A year later, at the end of the trial, her scans showed no evidence of disease. Five years later there was still no evidence of recurrence[2] and according to most recent sources, she remains cancer-free.[3] The drug, trastuzumab, was amongst the first targeted cancer treatments developed and later became a standard treatment for patients with human epidermal growth factor receptor 2 (HER2) overexpressing breast cancer.[4]

Stories such as this often captivate the public and give hope for future developments of treatments well suited to every individual patient, but initial enthusiasm is often not supported by further research.

What can be done to hear more of the success stories in the future and minimise the failures in the area which has become known as stratified medicine? With the aim of leading to better care for patients, this thesis will attempt to identify biomarker research that has been carried out to a good standard. It will also suggest improvements to the conduct of such research.

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In order to set the scene for this thesis, an understanding of some concepts around stratified medicine is required. These are briefly discussed below.

1.1 FACTORS INFLUENCING DRUG EFFECTS

It is sufficient to read a clinical trial report, even for a targeted drug such as trastuzumab, to realise that drugs do not always have the same effect in all patients: only some will benefit and there will usually be a group experiencing a serious adverse event.

A wide spectrum of factors that can influence an individual's response to treatment has been reported in the literature. These include:

- Genetic factors, such as the presence and characteristics of therapeutic targets, drug-metabolising enzymes, drug transporters, or targets of adverse drug reactions,[5-7]
- The setting in which the treatment is administered,[5]
- Patient's compliance with treatment,[5]
- Environmental factors, such as diet, or concomitant medication[5,6]
- Other factors – for example age, or circadian rhythm.[5,6]

The scale of the variability of response to treatments may be appreciable. According to some authors, drugs may not have the desired effect in 30-40% of patients and treatments such as chemotherapy in some cases are only beneficial for 30% of patients.[8,9] In addition, adverse effects associated with drug treatment can be a serious problem. A review of European studies that were published from 2000 onwards found that the percentage of all hospital admissions that was associated with adverse drug reactions ranged from 0.5% to 12.8% (median 3.5%).[10]

This clearly demonstrates the need for ways to predict which patients are most likely to benefit from a given treatment and in whom the treatment is likely to cause unacceptable harm.

1.2 STRATIFIED MEDICINE AND BIOMARKERS

A number of different terms have been used to describe the changes brought about by the use of new tools that help to provide patients with more individualised treatments. These include personalised, tailored, individualised, precision and stratified medicine. There are numerous definitions of these terms in the literature and often these are used

interchangeably. Some authors, however suggest more subtle distinctions and an example of these will be used to better explain the scope of this thesis.

Figure 1.1 demonstrates a simple model with empirical and personalised medicine at opposite ends of a spectrum and stratified medicine in the middle. Empirical medicine may be defined as prescribing the same treatment to all patients with a particular condition without taking into account their individual characteristics (although in practice this extreme is uncommon). An example of such treatments may be provided by non-steroidal anti-inflammatory drugs.[9] At the other end of the spectrum, personalised medicine proposes patient treatment customised for each individual.[11] This concept would describe treatments such as therapeutic cancer vaccines prepared using the patient's own tumour tissue.[12]

Somewhere between these two extremes lies stratified medicine where "a patient can be found to be similar to a cohort that has historically exhibited a differential therapeutic response using a biomarker that has been correlated to that differential response."[9]

Figure 1.1 Position of stratified medicine on the spectrum between empirical and personalised medicine and the focus of each term[11]

A broad classification of such patient cohorts based on different levels of response has also been suggested,[13] where patients are grouped into:

1) Responders – those with a positive response to a drug,
2) Super responders – a subgroup of responders with exceedingly good response to a treatment that is statistically distinguishable from the remaining responders; this group may not exist for all treatments,
3) Nonresponders – for whom the drug does not have any effect,
4) Negative responders – patients who suffer from unacceptable adverse effects.

The use of a biomarker (or biological marker) is central to the concept of stratified medicine. One of the most widely cited definitions of a biomarker is that proposed by a US

National Institute of Health workshop: "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.*"[14]

Although the term "biomarker" is often used in the context of stratified medicine, some authors suggest it would be more appropriate to talk about a "classifier", which could be described as a mathematical function which translates single or multiple biomarker values into categories (for example likely or unlikely to respond to treatment) that may be used for decision-making.[15,16] However, in practice the term "biomarker" appears to be used in preference to "classifier" and has arguably a broader scope. In addition, the term "classifier" is often used to indicate signatures based on, for example, multiple genes. Thus this thesis will follow the common nomenclature and use the term "biomarker".

Not every biomarker that can be measured will be directly relevant to stratified medicine. The most crucial here are predictive biomarkers, which have been variously defined. Some of the examples of these definitions are:

- "*a marker that predicts the differential efficacy (benefit) of a particular therapy based on marker status*"[17]
- "*measured at baseline to identify patients who are likely or unlikely to benefit from a specific treatment*"[16]
- used to select patients for treatment based on the "*estimation of probability of response to a particular agent*"[18]
- separating "*a population with respect to the outcome of interest in response to a particular (targeted) treatment*"[19]

What all these definitions have in common is that the predictive biomarkers are used:

1) for prediction of patient outcome (either in terms of efficacy or safety), and
2) in the context of a particular treatment.

These two points will be used throughout the thesis to identify relevant biomarker cases and methodologies. Further, predictive biomarkers are classed within the thesis based on their purpose as either predicting treatment efficacy or safety.

The use of a predictive biomarker in the context of a particular treatment differentiates it from a prognostic one, which is "*associated with a differential outcome regardless of the therapy given, even if choice of therapy is available*".[17] Prognostic biomarkers, such as cancer stage may distinguish populations where different treatments are appropriate.

However these would not guide the choice of a particular treatment based on differential response to that treatment.[17]

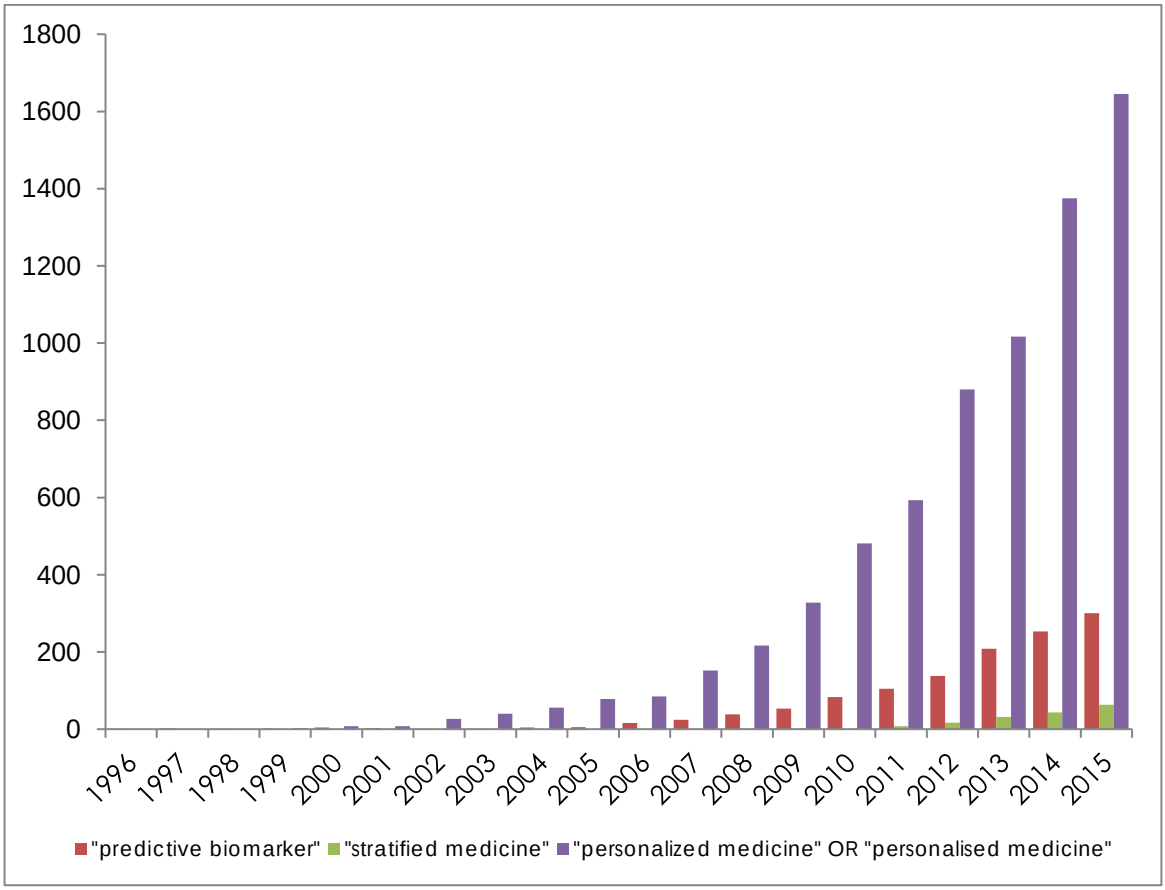
This distinction may prove more complex in certain cases. For example, HER2 expression in breast cancer (BC) was identified in the late 1980s as a prognostic biomarker: women whose tumours overexpressed HER2 have a more aggressive cancer resulting in shorter survival time.[20] However, for treatment with trastuzumab this biomarker also has a predictive role. Therefore, since the introduction of this targeted treatment it is no longer possible to argue that HER2 expression *"is associated with a differential outcome regardless of the therapy given."*[17]

Another type of biomarker that needs to be mentioned here are diagnostic biomarkers. These are *"used in people with signs or symptoms to aid assessing whether they have a condition."*[21] An example of a diagnostic biomarker is the presence of a BCR/ABL fusion gene which needs to be confirmed to diagnose chronic myeloid leukaemia (CML).[22] Again, the distinction is not simple as over time with better understanding of biology, biomarkers once considered predictive may become part of a redefinition of the disease or its subtype, and thus a diagnostic biomarker.[23] For example, a recent paper suggested dividing acute myeloid leukaemia into 11 genomic subgroups,[24] one of which is based on a biomarker previously used as predictive of response to arsenic trioxide (t(15;17) translocation).[25]

A final type of biomarker that requires mention are biomarkers used for dose selection. For example, levels of CYP2C9 enzyme could potentially be used for improved selection of a warfarin dose in anticoagulation therapy. These biomarkers can have a huge impact on treatment benefit, as inappropriate dose could limit the efficacy or result in serious adverse effects.[7] However, these are considered outside of the scope of this thesis.

There are other types of biomarkers, such as monitoring, screening, staging or predisposition biomarkers.[26] These however will not be discussed here, as they are of little relevance to the scope of this thesis.

To provide an indication of the growing use of some of the discussed concepts, a quick search of PubMed was carried out in March 2016, the results of which are shown in Figure 1.2. It produced only 189 hits for "stratified medicine". However "predictive biomarker" produced over 1.3 thousand hits and "personalised (or personalized) medicine" over 7.5 thousand. The great popularity of the term "personalised medicine" most likely results from its use in a wide number of contexts, including what this thesis will call "stratified medicine".



Carried out 17.03.2016. Prior to 1996 only hits for "personalised (or personalized) medicine" were obtained – one in 1990 and one in 1971. Data for 2016 not shown, as does not account for an entire year.

Figure 1.2 Number of hits in PubMed for some terms related to stratified medicine

1.3 BIOMARKER MEASUREMENT IN BIOLOGICAL SAMPLES

Before discussing methodological issues around predictive biomarkers, a brief outline of biomarker measurement is necessary to provide the reader with an appreciation of the practical issues that need to be addressed when incorporating a biomarker into a clinical study.

In terms of the biological characteristic that is measured, the most important types of biomarkers that have been used as predictive are generally molecular in nature and include:

1) Chromosome-level – referring to the characteristics of entire chromosomes. There are a number of biomarkers of this type, one of the most relevant being reciprocal translocations. This term describes the exchange of molecular fragments between two different (non-homologous) chromosomes.[27] One of the most recognised

examples is the Philadelphia chromosome – a shortened chromosome 22, resulting from an exchange of defined fragments between chromosome 9 and 22.[28]

2) Gene level biomarkers – referring to the presence of certain genetic traits. Two types are important here:

    - Presence of a variant of a gene (allele) - such as that of the human leukocyte antigen, class I B (HLA-B*5701 allele) which predicts that patients are likely to show a hypersensitivity reaction to abacavir,[29]
    - Mutation within a gene - the permanent change in the nucleotide sequence, for example KRAS mutation which has been used to predict lack of response to epidermal growth factor receptor (EGFR) targeted drugs.[18]

    These are often considered constant. However, for tumours and viral genomes the mutation rate is high resulting in these biomarkers changing over time, often resulting in development of resistance to a previously effective treatment.[30]

3) Gene expression level biomarkers – referring to the presence and amount of a given protein,[18] such as HER2 or excision repair cross-complementation group 1 (ERCC1) expression. One of the major challenges is that these biomarkers are by nature continuous. Yet, in order to be used for predicting patients' response these often need to be dichotomised.[31] Another important challenge is that gene expression can be measured directly at protein level or at messenger RNA level and these two approaches do not necessarily provide comparable results.[32] Some of the issues around the different methods of measuring protein expression biomarkers will be investigated in more detail in Chapter 7, using ERCC1 as an example.

Other biomarker types have also been investigated, although more rarely, such as functional magnetic resonance imaging as a potential predictor of response to antidepressant treatment in major depressive disorder.[33]

The above biomarker types can be measured in a variety of biological specimens, which include:

- Tumour tissue – in which the majority of biomarkers predictive of treatment efficacy in cancer, such as HER2 expression are measured,
- Patients' healthy tissue – this is particularly relevant to biomarkers predicting treatment safety and biomarkers identifying a subset of patients with an inherited

condition. An example of the first situation is the presence of HLA-B*5701 allele, which is used to predict adverse events associated with abacavir treatment.[34-36] An example of the second kind is the presence of the G551D mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in cystic fibrosis patients.[37]

- Viral genome – generally biomarkers predicting efficacy of treatments for viral diseases, such as viral resistance biomarkers in human immunodeficiency virus (HIV) infection treatment.

There is a variety of laboratory methods that have been developed to measure biomarkers, often with multiple assays available for the same purpose. To be used in a clinical setting, such laboratory methods need to be "fit-for-purpose", which often requires demonstration of acceptable analytical parameters:

- Precision (or reproducibility[1]) – where repeated measurements on the same sample made under the same laboratory conditions result in the same biomarker values,[18]
- Accuracy – reflecting how close the biomarker measurement is to the true value.[18]

These may often depend on a wide variety of factors other than the type of the laboratory assay used, such as the concentration of reagents or timing of laboratory procedures.[1] In any case, establishment of the precision of a biomarker test may be relatively easy compared to accuracy, for which there may be no reference standard against which to compare the biomarker assay.[18]

Apart from the analytical parameters, the biomarker measurement can be influenced by both pre- and post-analytical factors. Pre-analytical factors include for example the biological sample collection method, processing and storage.[1] Post-analytical factors encompass issues around the reporting and provision of the laboratory data to the clinic.[38]

Although a lot of attention has been given to the analytical factors, there are suggestions in the literature that the majority of errors in hospital laboratories may be due to pre- or post-analytical factors.[39,40] Some of the pre-analytical issues will be investigated in Chapter 7, focusing on biological specimen collection and processing.

Finally, factors such as biological rhythms and diet have also been cited in the literature as potentially influencing laboratory measurements.[38] Although these may appear irrelevant to predictive biomarker measurement, there is at least one case (HER2 expression in BC)

where evidence suggests the biomarker values may fluctuate in women during the menstrual cycle.[41]

1.4 PREDICTIVE BIOMARKER DEVELOPMENT

A number of strategies have been suggested for taking a biomarker from discovery to the clinic. These aim to ensure control of the factors influencing biomarker measurement and use of only "fit-for-purpose" predictive biomarkers in clinical practice. A systematic review of these will be reported in Chapter 2 where different models proposed in the literature will be discussed. Figure 1.3 provides a simple overview of the biomarker development process and some of the major concepts are discussed below.

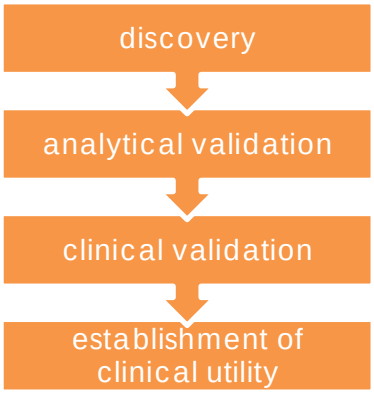


Figure 1.3 Overview of predictive biomarker development process

The initial discovery stage aims to identify biomarkers which may be used as predictive of treatment effect.[18,42] There are two general approaches to this:

- Knowledge-driven – based on known disease pathogenesis and/or pharmacological mechanism of action of a drug. This approach is limited by the extent of knowledge of disease biology and the validity of the assumptions made.[43-45]
- Data-driven – using high-throughput techniques to identify one or more biomarkers that differ between cohorts (for example responders and non-responders). This approach may require large sample sizes and involves a high risk of producing false positive associations.[43-45]

These two approaches may be combined and it has been suggested this may be the optimal strategy, particularly in the development of multi-marker classifiers.[43-45]

However, a very common situation is an opposite sequence of events, where discovery of treatments follows the identification of a particular molecular target. The presence of the drug target later becomes a predictive biomarker.[46] An example of such a process is the development of trastuzumab to target HER2 on tumour cell surface,[20] which will be described in Chapter 2.

In either case of discovery sequence, it has been postulated the next step should be analytical validation of the biomarker(s). This stage investigates the analytical parameters of the laboratory assay such as precision and accuracy discussed above.[18,47-49] It also aims to define the laboratory procedures for measurement of the biomarker, such as biological specimen processing times.[44] The importance of this stage will be one of the problems addressed by Chapter 7.

If the biomarker assay has demonstrated satisfactory analytical validity, the next stage involves clinical validation. This stage focuses on establishing a correlation between the biomarker values and the clinical outcome.[18,43,49]

The final stage, evaluating clinical utility, provides information on whether the biomarker improves patient care. It usually requires availability of a laboratory assay with sufficiently short turn-around time to inform clinical decisions.[50] Usually on completion of this stage a decision is made about the implementation of a predictive biomarker in clinical practice.[47-49,51,52] This stage will be the focus of Chapters 3-6 and will also be important for Chapter 7.

1.5 Regulation in Europe

The majority of predictive biomarkers used in clinical practice fall under the class of *in vitro* diagnostics. These are regulated by the EU Directive, 98/79/EC on *in vitro* diagnostic medical devices, which is currently under revision.[53]

According to the Directive to access the European market the manufacturer mainly requires to ensure the performance of the assays is relatively safe and that the assay performs (in terms of for example accuracy) as described in the technical documentation. In most cases this is assessed based on the documents provided by the manufacturer to a notified body. This includes a range of information, such as a description of the technology, description of the quality system, results of evaluation of assay performance and results of stability tests. Certain cases are listed in the Directive (for example HLA-B allele testing), where additional measures are required. These may involve either the notified body testing the assay performance or auditing the quality assurance

system.[54] These measures correspond to establishing the analytical validity of a predictive biomarker described above in section 1.4.

There is no formal process for further evaluation of predictive biomarkers to be used in clinical practice. However often drugs are licensed in populations that have been identified by a predictive biomarker. In a large number of cases this licensing is done by the European Medicines Agency (EMA), as in Europe a centralised drug evaluation by the EMA is required for drugs for treatment of a number of conditions, drugs obtained from biotechnology processes and all drugs used for rare conditions (orphan medicines). Companies can also apply for a centralised marketing authorisation of other drugs.[55] Although the EMA does not license biomarkers, it evaluates drugs in groups of patients which can be defined by predictive biomarkers (for example trastuzumab is licensed for use in HER2 overexpressing BC patients).[56] Therefore reviewing EMA licensing decisions is likely to give a broad overview of the impact of predictive biomarkers on treatment selection since 1995 (when EMA was established[57]).

The European system appears to be ensuring limited evidence standards for predictive biomarkers prior to their implementation in clinical practice, and this will be further investigated in Chapter 5 and 6. An alternative approach has been put into place in the USA, where the Food and Drug Administration (FDA) evaluates both the biomarker assay and the new drug. New biomarkers intended as predictive are evaluated in this context prior to the FDA considering the drug.[58]

1.6 STUDY DESIGNS

The main focus of this thesis is on studies which are undertaken in the context of the clinical utility stage. These provide information to enable a decision on the routine implementation of a predictive biomarker in clinical practice. Some of the designs more typically utilised for the prior stages of biomarker development, have also been used for such decision-making, as will be discussed in Chapter 5 and 6. These designs are briefly described below and illustrated using patient flow diagrams. For simplicity, in the diagrams, the treatment expected to be more beneficial in biomarker positive patients is labelled as "experimental treatment" and the treatment that comprises standard care or placebo as "control treatment". For controlled studies a two-arm design is shown, although in practice multiple arms may be included in a such study.

1.6.1 RANDOMISED TRIALS WITH BIOMARKER INTEGRAL TO DESIGN

This group of study designs is often considered in the theoretical literature as the most appropriate for evaluation of the clinical utility of a predictive biomarker.[18] The different randomised designs proposed in the literature are outlined below.

1.6.1.1 ENRICHMENT DESIGN

As shown in Figure 1.4, in the enrichment (also known as targeted) design the biomarker is used to restrict entry into the trial. The experimental drug, expected to be beneficial only in biomarker positive patients, is compared to a control intervention.[15,18,48,49,59-61]

Such a trial provides information on the best treatment for biomarker positive patients.[60,61] If the biomarker is truly predictive, implementation of such a design offers advantages in terms of a reduced sample size compared to a trial without entry restriction.[15,60,61] However, although only biomarker positive patients are treated, recruitment and biomarker evaluation needs to be undertaken in the entire population with the condition.[60,61]

One of the major limitations of this design is that it does not demonstrate the utility of the biomarker. It may therefore result in denying beneficial treatment to some of the patients identified as biomarker negative.[61]

As the treatment is not evaluated in the biomarker negative patients, a strong biological rationale is required for excluding this group.[15,48,49,59] It should only be undertaken using an assay which reliably identifies the biomarker positive patients.[60,62]

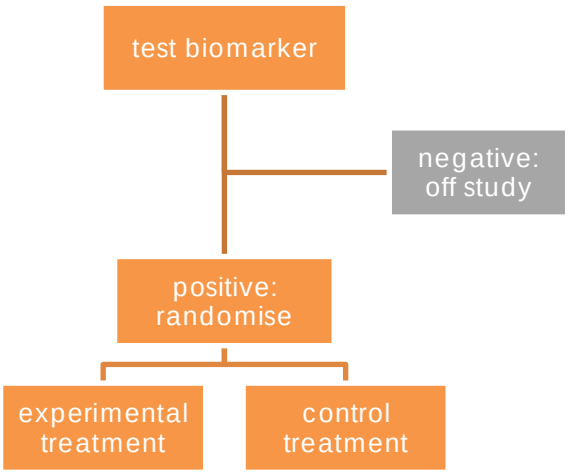


Figure 1.4 Patient flow in an enrichment design

1.6.1.2 STRATIFIED DESIGN

In a stratified design the biomarker status is used as a stratification factor and patients are randomised to treatment within each biomarker defined stratum,[59,62] as shown in Figure 1.5. Usually patients are stratified into biomarker positive and negative groups, however in some cases a stratum defined by an unknown biomarker status may also be included.[63] The stratified design may be most appropriate when a biomarker has been developed, but uncertainty remains about the effect of the treatment in biomarker negative patients.[49]

This study design can provide information on the benefit of the treatment in all patients and within each biomarker-defined subgroup.[61] It can also allow an indirect evaluation of the biomarker-based treatment strategy.[17,61]

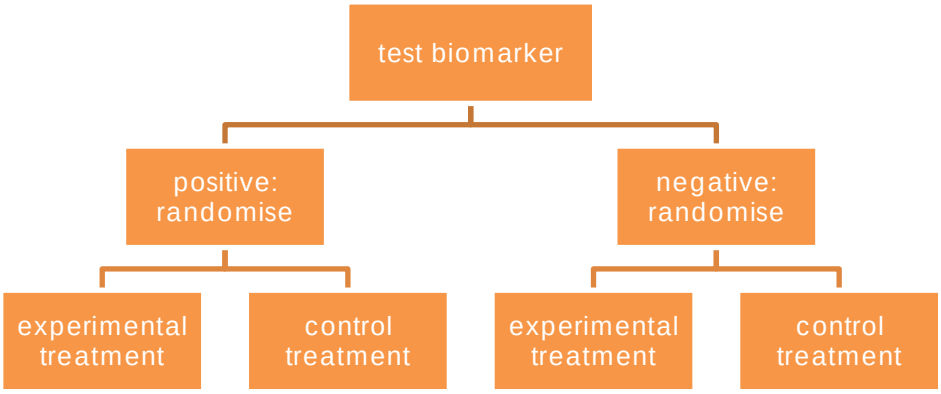


Figure 1.5 Patient flow in a stratified design

Some authors suggest that stratification is only important in smaller trials, where uneven patient distribution between subgroups may impact on the interpretation of the results.[48,61] A statistical test of interaction between the biomarker status and treatment effects is possibly the most suitable method of data analysis in this design, however recruitment of a large enough sample to ensure sufficient power may be problematic.[17,48,49]

1.6.1.3 BIOMARKER STRATEGY DESIGN

In the biomarker strategy design shown in Figure 1.6 patients are randomised to either treatment guided by the biomarker status or irrespective of it.[15,17,59,61,62] Outcomes in both arms are compared and thus the predictive value of the biomarker is assessed.[17] Importantly, this design allows a direct evaluation of the consequences of implementing the predictive biomarker in clinical practice.[1,61,62] One of the important problems is

selection of the treatment strategy for patients with an unknown biomarker status, due to, for example, assay failure. [61]

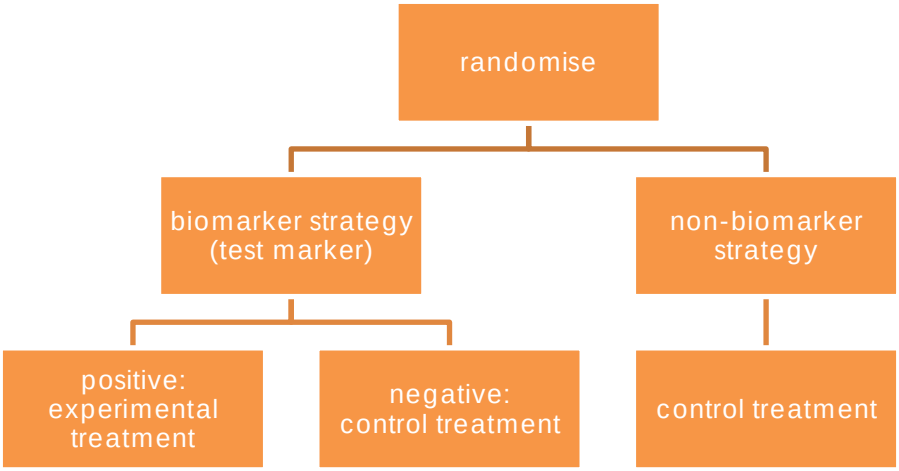


Figure 1.6 Patient flow in a biomarker strategy design

A major drawback of this design is that the biomarker strategy arm may be superior even if the biomarker is not predictive, as long as the experimental treatment provides more benefit irrespective of the biomarker status.[61] This has been, to some extent, addressed by the proposition of a modified biomarker-strategy design (shown in Figure 1.7). In such a study patients in the non-biomarker strategy arm are randomised to one of the two treatments, rather than receiving the control intervention only. If the biomarker status is measured in both arms, this allows the assessment of the clinical utility of the biomarker.[17]

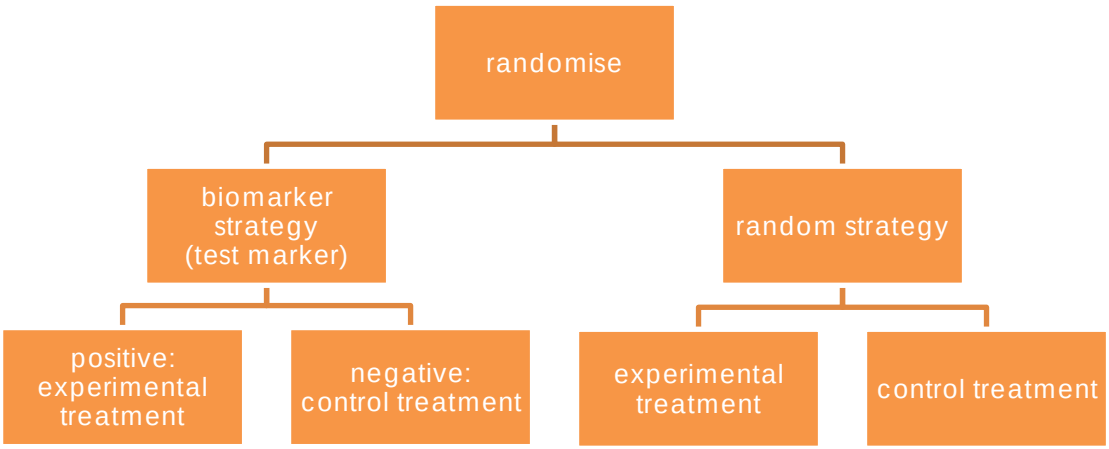


Figure 1.7 Patient flow in a biomarker strategy design with randomisation in control arm

An important advantage of the biomarker-strategy design is that it allows testing of complex strategies with multiple biomarkers and treatments.[61] However, a major concern in particular for the modified version is that this design may require a huge sample size.[15] This is largely due to a proportion of patients in both study arms receiving the same treatment, thus diluting any effects.[1] It has been suggested that a stratified design might be a more efficient option in most cases.[61]

1.6.1.4 ADAPTIVE DESIGNS

A number of adaptive designs have been proposed in the literature and a recent review of these is available.[64] Some examples of such trials are discussed below.

One of these designs has been referred to as adaptive patient design. It is proposed to be utilised in a situation when there is suspicion that a biomarker is predictive, however it is likely that the treatment may offer benefit to biomarker negative patients as well. One variant of this design is shown in Figure 1.8.

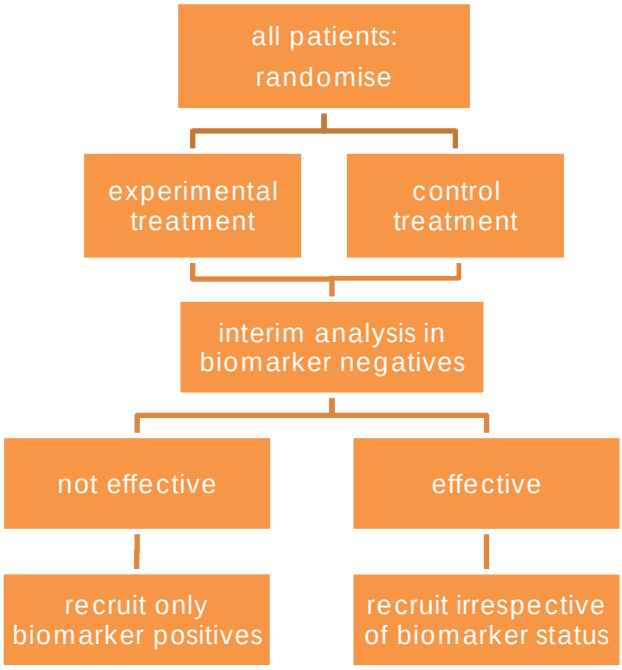


Figure 1.8 Patient flow in an adaptive patient design with interim analysis in biomarker negative patients

In the beginning patients are included irrespective of their biomarker status. An interim analysis is then undertaken in biomarker negative patients who are assumed less likely to benefit from the experimental treatment. Depending on the results of the analysis either patients irrespective of the biomarker status, or only biomarker positive are recruited for

the remaining part of the trial.[48,64] Such a trial allows evaluation of the biomarker and maximisation of recruitment of patients who are more likely to benefit.[62] However, this design requires evaluation of outcomes relatively soon after treatment initiation to enable the interim analysis. In the case when the biomarker is predictive, it also requires more patients to be recruited than an enrichment trial.[48]

Another example of an adaptive design that has been suggested for evaluation of predictive biomarkers is an adaptive signature design.[64] This design is proposed for situations when no predictive biomarker is available at the start of the trial. As shown in Figure 1.9, patients are included irrespective of the biomarker status in two stages and randomised to either experimental or control treatment. The data from the first stage are used to identify a biomarker (usually a multi-marker classifier). In stage II the biomarker status is prospectively evaluated in newly recruited patients and randomisation continues as in stage I.

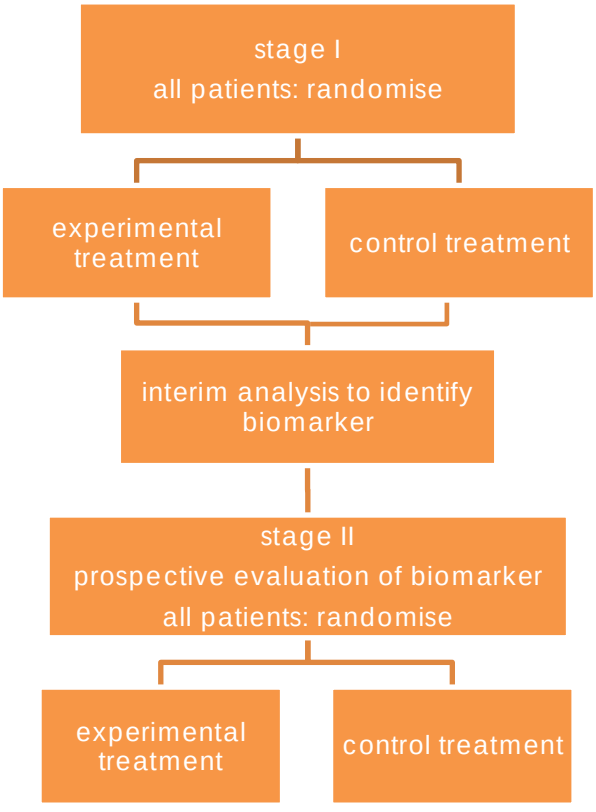


Figure 1.9 Patient flow in an adaptive signature design

At the end of the trial, two analyses are undertaken:

1) comparison of the experimental and control treatment irrespective of the biomarker status in patients from both stages,

2) comparison of the experimental and control treatment in the biomarker positive patients who were recruited in stage II.

The overall significance level is split between the two analyses.[65]

1.6.2 Randomised trials with biomarker not integral to the design

1.6.2.1 Prospective-retrospective design

Although a prospective trial designed to evaluate the clinical utility of a predictive biomarker would be ideal, some authors argue that it may not always be feasible or ethical.[1,15] A prospective-retrospective study can be undertaken if:

- information emerges on a potential predictive biomarker for which there is an assay of acceptable analytical validity, and
- archived biological specimens are available for the vast majority of patients from a completed randomised trial independent of the data which generated the biomarker hypothesis.

A schematic representation of such a study is shown in Figure 1.10. In this case a protocol should be developed to test the biomarker hypothesis prior to any analysis of archived specimens. This should be then used to undertake a study utilising the available biological specimens and collected patient data.[1,62]

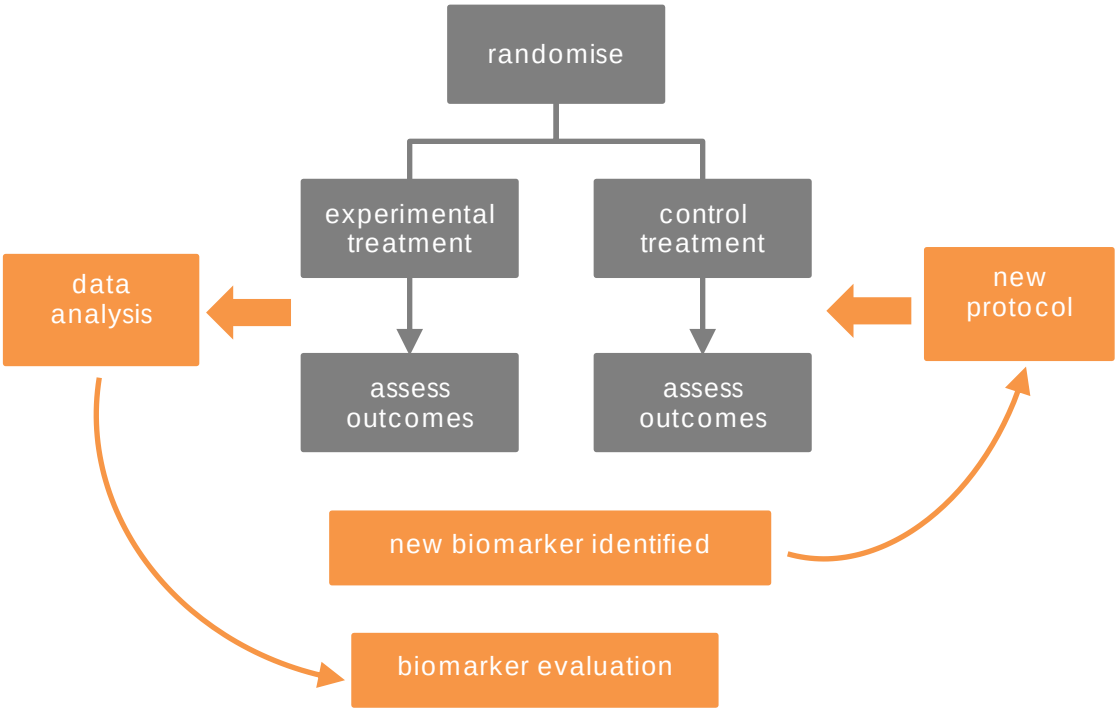


Figure 1.10 Patient flow in a prospective-retrospective study

This study design can potentially address similar questions to a stratified design and, if well conducted, the results can be treated as coming from a prospective randomised trial.[62] However, problems exist regarding the possible bias which may be introduced by the missing or not analysable biological specimens. Another concern may be the difference in the tissue processing and storage procedures between the archived biological specimens and the biological samples that would be used to measure the biomarker status in the future patients in clinical practice. These discrepancies may potentially lead to results not being applicable in clinical practice.[62]

1.6.2.2 SUBGROUP ANALYSES OF RANDOMISED TRIALS

Subgroup analyses of data from randomised trials are not in any way specific to the area of stratified medicine. However they have a very important role, as by definition this field attempts to identify subsets of patients with differential treatment effects. In trials with such analyses patients are enrolled irrespective of their biomarker status. Three types of subgroup analysis may be undertaken that are encountered in this thesis: prospective, retrospective and cross-sectional.

In the prospective type, a subgroup analysis based on the biomarker status is planned in the study protocol. The biomarker may be measured in the beginning of the trial or later on, using baseline biological specimens.[66,67] Data may be analysed in a similar way to that coming from a stratified design and identical information can be obtained. However, such analyses may often be underpowered and subject to limitations associated with multiple testing.[68-71]

In the retrospective (or *post hoc*) type a biomarker subgroup of interest is identified only after the conclusion of the trial. The biomarker status is then measured (using, for example, archival tumour samples) and a subgroup analysis is performed.[67] Again, this analysis can provide similar information to a stratified design, however the probability of false positive findings is much higher.[69,71] It has been suggested this analysis may be more suitable for earlier stages of biomarker development, rather than evaluation of clinical utility.[62]

The concept of cross-sectional analysis emerged from examples of trials looking at viral resistance biomarkers in HIV infection.[72,73] In such an analysis, on completion of a randomised trial, a subgroup of patients from one or from multiple treatment arms is identified based on their outcome - usually lack or loss of response to treatment. The biomarker (or a panel of biomarkers) is evaluated in this group of patients only. Such an analysis is comparable to a cross-sectional study and can only provide information on the prevalence of the biomarker in the patients with a particular outcome. In spite of their

obvious limitations, such analyses are undertaken due to the possibility of development of treatment resistance during a trial.[74,75] Such resistance biomarkers are later used to choose treatments for new patients.

1.6.3 Non-randomised studies

Non-randomised studies evaluating biomarkers are usually considered relevant to development stages prior to evaluation of clinical utility. This may be generally true, however for rare diseases and drugs demonstrating extraordinary benefit, such study designs may be used to make decisions on biomarker and drug use in clinical practice. These designs have not been described extensively in theoretical literature, however they will be encountered in Chapters 5-7.

1.6.3.1 Single arm study including only biomarker positive patients

Figure 1.11 shows the patient flow in a single arm study including only biomarker positive patients. All patients are given the experimental treatment and only activity of the drug in biomarker positive patients can be demonstrated.[76]

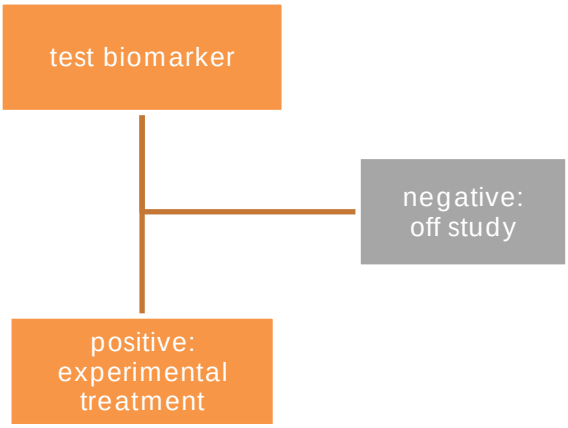


Figure 1.11 Patient flow in a single arm study including only biomarker positive patients

1.6.3.2 Single arm biomarker-strategy study

This study design was identified from ongoing studies, for example where ERCC1 expression was used to select treatments for patients.[77] As shown in Figure 1.12, the design includes patients irrespective of their biomarker status and allocates them to a biomarker-based treatment, corresponding to one arm of the biomarker strategy randomised controlled trial (RCT). Under the assumption that the biomarker is not prognostic, the

biomarker negative subgroup may provide some comparison for the biomarker positive patients.

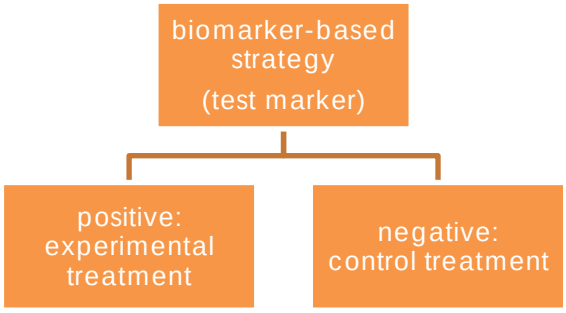


Figure 1.12 Patient flow in a single arm biomarker-based strategy study

1.6.3.3 SINGLE ARM STUDY INCLUDING PATIENTS IRRESPECTIVE OF BIOMARKER STATUS

In this design all patients are included irrespective of their biomarker status and all receive the same experimental treatment, as shown in Figure 1.13. The biomarker is measured in all of the included patients to identify a subgroup responding to the treatment.[78]

Figure 1.13 Patient flow in a single arm study including patients irrespective of biomarker status

1.6.4 UMBRELLA AND BASKET TRIALS

Umbrella and basket trials can offer minimisation of costs and increase in efficiency of conducting research into treatments with associated predictive biomarkers.

1.6.4.1 UMBRELLA TRIAL

Umbrella trials investigate multiple drugs associated with multiple predictive biomarkers. They may recruit patients with one or a variety of diseases and different molecular

characteristics.[79] As shown in Figure 1.14, patients are assigned to the appropriate treatment based on their biomarker status.[80,81] As multiple biomarkers can often be evaluated using the same platform, this approach can offer substantial efficiency gains. In addition, in clinical areas where targeted treatments may only be appropriate for a small proportion of patients, these trials offer large improvements in terms of recruitment. Umbrella trials may be both randomised, including a control group as in the case of FOCUS4[82] and non-randomised as in case of National Lung Matrix trial.[83]

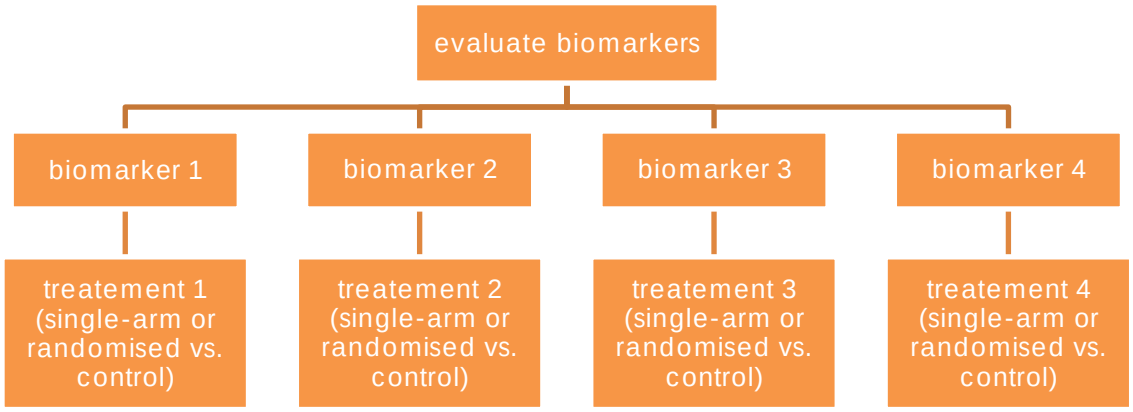


Figure 1.14 Patient flow in an umbrella trial

1.6.4.2 BASKET TRIAL

Basket trials investigate a single drug in a range of diseases (usually cancers).[79,80] At least three variants of this design can be identified based on the inclusion criteria. In these studies patients are:

1) included based on similar molecular characteristics,[79,80]
2) positive for a range of biomarkers which are likely drug targets,[81,84]
3) included irrespective of their biomarker status and a range of potential biomarkers is evaluated to discover predictive biomarkers.[84]

Basket trials are often utilised as discovery tools to rapidly screen for response in different disease settings.[85] Based on the results of such trials, cohorts which show the most promising results can be expanded.[81] An example of such a trial is investigating vemurafenib in patients with a range of V600 mutation positive tumours.[86]

1.7 AIMS OF THE THESIS

Poor study design and inadequate reporting of studies have been identified as a major obstacle to progress in the field of stratified medicine.[18] There are a number of methodological papers postulating the most appropriate ways to develop predictive biomarkers and the best study designs to evaluate clinical utility. Anecdotal evidence suggested there might be a large discrepancy between the theory and practice of stratified medicine. This thesis investigates the hypothesis that there is a mismatch between the theoretical proposals and practice of predictive biomarker research, focusing on the final stage of biomarker evaluation prior to implementation in clinical practice. It will investigate the theory and actual research supporting 50 real cases. Existing discrepancies between the theory and practice will be identified. The possible reasons for such discrepancies and the resulting limitations of predictive biomarkers available in clinical practice will be explored. In addition, areas of clinical research with insufficient relevant methodology will be identified.

Figure 1.15 provides an overview of the position of parts of this thesis (Chapters 2-7) relative to the theory and practice of stratified medicine. This structure was chosen to first introduce the biomarker development process in Chapter 2 and thus provide context for the identification of clinical research practice in Chapters 3-5. Chapter 6 will then compare the identified research practice to theoretical literature. Chapter 7 will return to the practical aspects and focus on issues related to the biomarker assay.

Chapter 2 will expand the basic framework described in section 1.4 and introduced in Figure 1.15. It will try to answer the question of what frameworks have been proposed in the literature and whether there appears to be consensus - at least on some elements of such frameworks. Based on the findings of a systematic review the most appropriate strategies for development of "fit-for-purpose" predictive biomarkers will be identified. The aims, relevant research designs and criteria for entry into and completion of each stage of biomarker development will be discussed. As the clinical utility stage is the major focus of this thesis, Chapter 2 will identify not only what this stage should comprise, but also the necessary prerequisites.

Chapter 3 will address the question on what predictive biomarkers have been included in EMA licensing. It will report on the identification of the dataset of real cases of predictive biomarkers which have been considered for marketing in the European Union. These predictive biomarkers come from both indications and contraindications of drugs considered by the EMA for licensing. Positive and negative recommendations are included and these will form the starting point for Chapters 4-6. The identified biomarker

cases will be characterised in terms of biomarker type, disease area and drug type. Licensing over time will also be considered to establish whether an expansion of stratified medicine is noticeable.

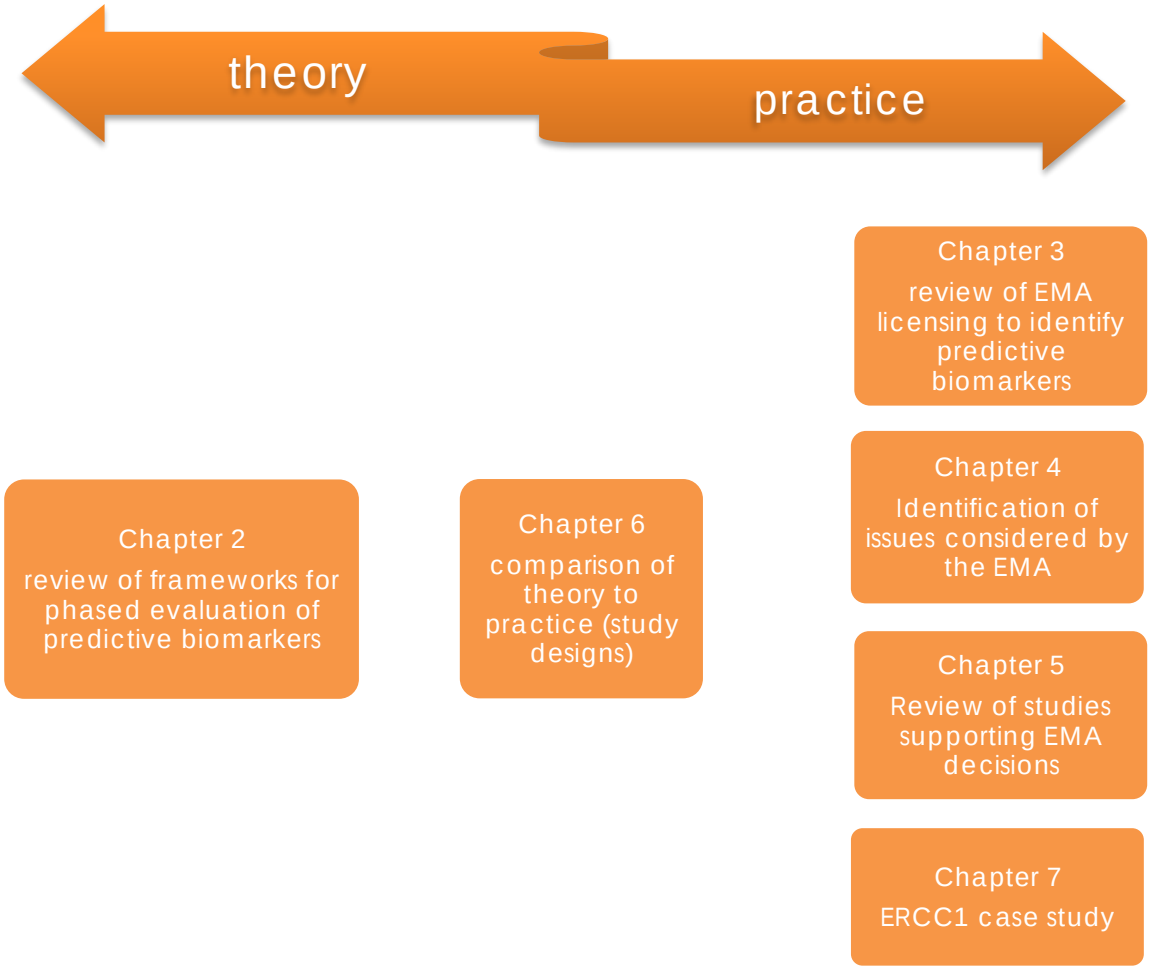


Figure 1.15 Structure of the thesis

EMA decisions will then be investigated further in Chapter 4, where an attempt will be made to find out what issues were important in decisions on licensing of a drug with a predictive biomarker in the indication. A text analysis of available documentation will explore these issues and the themes that emerge from the analysed documents will be described. Attention will also be given to the critical issues which resulted in the licensing refusal for a small number of drugs with an associated predictive biomarker.

The themes identified in this Chapter 4, together with general methodological concerns will form the basis of criteria used in Chapter 5. This chapter will aim to investigate what level of evidence was sufficient to include a predictive biomarker in an indication by analysing the clinical trials that supported the EMA decisions. The study designs and

strength of evidence behind each recommendation will be considered. The evidence supporting drugs with a positive and negative licensing recommendation will be compared. Consideration will be given to the evidence standards emerging from the analysis that appear sufficient in practice to use a predictive biomarker in the clinic.

After examining the studies supporting EMA decisions in Chapter 5, these will be compared in Chapter 6 to the methodology suggested in the literature. A methodological framework based on a systematic review describing study designs relevant to development and evaluation of clinical utility of predictive biomarkers has been published.[78] The evidence collected in Chapter 5 will be used to assess the validity and limitations of the framework. Study designs used in practice, but not included in the methodology will be identified. The strength of the evidence supporting EMA decisions will further be evaluated.

Chapter 7 will return to issues earlier in the predictive biomarker development process. It will try to answer what are the reasons and consequences of lack of standardisation in laboratory methods used for biomarker evaluation. It will investigate the impact of problems with analytical validity of a biomarker on trials undertaken for evaluation of clinical validity and utility. A case study of ERCC1 expression in non-small cell lung cancer (NSCLC) to predict response to cisplatin will be undertaken to address these issues. A survey of trials that were either ongoing or completed since 2007 will be reported. This will explore the variability in analytical and pre-analytical factors between trials and the motivation for the choice of particular biomarker assays. Consideration will be given to the impact of the identified variability on the usability of the trial results to inform clinical practice.

Chapter 8 will provide a discussion of the main issues identified throughout this thesis and draw conclusions on the strengths and limitations of the research practice in the area of stratified medicine and the necessary future developments in trials methodology.

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CHAPTER 2. FRAMEWORKS FOR STAGED EVALUATION OF PREDICTIVE BIOMARKERS – A SYSTEMATIC REVIEW

This chapter was presented as an oral presentation:

Malottki K, Smith H, Deeks J, Billingham L. Systematic review of frameworks for staged evaluation of predictive biomarkers, Methods for Evaluating Medical Tests and Biomarkers Symposium, 20 July 2016, Birmingham, UK

Author Contributions

Kinga Malottki designed the review, designed and carried out the searches, screened all titles and abstracts, assessed full texts for inclusion, extracted data and summarised the frameworks and wrote the chapter
Holly Smith checked 15% of titles and abstracts, assessed full texts for inclusion
Jon Deeks commented on the design of the review, summary of the frameworks and the chapter
Lucinda Billingham commented on the design of the review, summary of the frameworks and the chapter

Abstract

Background: A number of predictive biomarkers have changed clinical practice. However, there are also examples where potential predictive biomarkers failed at a late stage of development. Such cases of failure, together with the need to optimise the use of resources, suggest a structured approach to biomarker development is necessary. In contrast to drugs, no model for phased evaluation is in place for predictive biomarkers.

Aims: To identify existing frameworks for staged evaluation of predictive biomarkers and the stages these propose. For each identified stage, explore the outcomes, relevant study designs and requirements for entry into and completion. To compare the frameworks.

Methods: A systematic review of papers suggesting a framework for staged evaluation of predictive biomarkers was undertaken. These were identified through broad searches of MEDLINE, EMBASE and additional internet searches. Data were extracted on the characteristics of the frameworks and the stages they contained. Identified frameworks were compared and grouped based on the context in which the predictive biomarker was to be developed and the stages proposed. Information on each identified stage within each model was summarised and compared across models.

Findings: 23 papers were identified that described a framework for staged evaluation of predictive biomarkers. These were grouped into four models: (I) general predictive biomarker development, (II) integrated into phased drug development, (III) development of a multi-marker classifier and (IV) development of marker predicting treatment safety. The most complete was model I (general), which comprised stages of: pre-discovery, discovery, analytical validation, clinical validation, clinical utility and implementation. The remaining models contained most of the same stages, however models II and III did not contain analytical validation and model IV clinical validation. The stages in models II-IV corresponding to those in model I were occasionally merged or subdivided. Different terminology was also used to describe similar concepts. Relevant study designs were described for all stages, however there seemed to be consensus mainly for the clinical utility stage, where RCTs designed to evaluate the biomarker were advocated (including enrichment, stratified and biomarker-strategy designs). The appropriate time to finalise the biomarker assay and select the threshold for continuous biomarkers was rarely mentioned. Where discussed, there was little agreement on these issues.

Conclusions: The identified models suggest the need to consider the context in which the biomarker is developed. There was a large overlap between the four models, suggesting consensus on at least some of the research steps that may be necessary prior to predictive biomarker implementation into clinical practice.

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2.1 BACKGROUND

In drug development there is a widely accepted model, which identifies phases usually necessary for the authorisation of a drug therapy for use in clinical practice: drug discovery, pre-clinical research and phase I-III studies.[87,88] There are exceptions where this sequence is not exactly followed. For example drugs for rare diseases may be implemented in clinical practice based on early phase trials or observational studies.[89] However, this model forms the basis of drug development programs which, for a particular reason, may introduce necessary modifications.[89,90]

No such generally accepted model appears to be in place for staged evaluation of predictive biomarkers. Different organisations have proposed their own frameworks, such as the Food and Drug Administration (FDA) Drug-Diagnostic Co-Development Concept Paper.[47] In addition, there have been numerous publications proposing models for staged evaluation. However, to date no consensus has been reached on the best way to develop predictive biomarkers. This may be a result of some of the complexities in the field of stratified medicine. One of the major issues is the possibility of discovery of predictive biomarkers at different times in relation to the drug with which they link. For example, some biomarkers may be discovered prior to the drug (in targeted treatments), while others will only emerge after the drug has been given market access.[91]

A range of predictive biomarkers have been successfully implemented in clinical practice and these will be reviewed in Chapter 3. However, examples of failure at late stages of development, such as that of ERCC1 expression in NSCLC (investigated in Chapter 7), suggest the need for more structured approaches. As in the case of drug development, opportunity costs of research into potential predictive biomarkers need to be considered. It is therefore crucial, at each stage of the biomarker development process, to collect information allowing rational decision-making regarding whether and how to proceed further.[92] Criteria are necessary that can be used for such decisions about entry into and completion of stages. It is particularly important to have a standardised approach to guide whether a potential predictive biomarker should enter a late phase trial.

2.2 AIMS

This systematic review aimed to investigate the path that should be followed in the development of a predictive biomarker to ensure that only biomarkers fit-for-purpose are used for treatment selection.

In particular, this encompassed identification of existing frameworks for staged evaluation of predictive biomarkers. For all identified frameworks the number and characteristics of the proposed stages were investigated. Within each stage information was sought on the aim of the stage, information to be collected, relevant study designs and requirements for entry into and completion. The process which led to the development of these frameworks was also investigated, as this was considered a potential indicator of the strength of these proposals.

It was also of interest whether any criteria could be identified that would indicate that the biomarker development should be terminated. This would potentially take place when the biomarker is unlikely to complete a particular stage or result in a clinically meaningful tool.

In addition, it was hypothesised that the models of staged evaluation may depend on the clinical context (for example development of a predictive biomarker alongside a treatment or at a later stage).

To provide real-life context for the systematic review of frameworks proposed in the literature, a case study of the discovery and development of HER2 expression to predict response to trastuzumab in BC was undertaken.

2.3 METHODS

This section will first describe the systematic review, the main focus of this chapter. An outline of the methods relevant to the case study will be included at the end of this section.

2.3.1 SYSTEMATIC REVIEW OF FRAMEWORKS FOR PHASED EVALUATION OF PREDICTIVE BIOMARKERS

2.3.1.1 SEARCHES IN BIBLIOGRAPHIC DATABASES AND THE INTERNET

Broad searches were undertaken in MEDLINE and EMBASE. These were supplemented by internet searches, which included websites of drug licensing agencies (EMA, FDA). The complete search strategies are reported in Appendix 3. These employed a combination of terms for:

- the area of stratified medicine (such as "predictive biomarker" or "pharmacogenomics"),
- staged evaluation (for example "hierarchical model"), and

- framework (for example "framework" or "guideline").

Both index and text terms were used. All identified references were imported into an EndNote X7 (Thomson Reuters) database.

2.3.1.2 IDENTIFICATION OF INCLUDED FRAMEWORKS

The titles and abstracts of all papers identified in the searches were screened for inclusion by one reviewer using the criteria reported in Box 2.1.

Box 2.1 Inclusion criteria for the systematic review of frameworks for staged evaluation of predictive biomarkers

Inclusion: papers or resources proposing a complete framework or part of a framework (more extensive than a single stage) for staged evaluation of predictive biomarkers from discovery to clinical implementation. Exclusion: papers or resources not meeting the inclusion criteria (for example clinical trials), not published in English and conference abstracts.

Papers not published in English, although potentially relevant, were excluded due to financial and time constraints. It was anticipated that conference abstracts would contain insufficient detail to provide information useful for this chapter and were also excluded.

Full texts of all papers potentially meeting inclusion criteria were obtained.

A second reviewer independently checked a random sample of 15% titles and abstracts. In cases of disagreement, a full text was obtained for papers identified as potentially relevant by at least one reviewer.

Two independent reviewers assessed the full texts for inclusion in the review using the same criteria as in the screening stage. All disagreements were resolved by discussion.

Reference lists of all papers assessed in full text were screened to identify additional potentially relevant papers. If identified, these were added to the EndNote database and assessed for inclusion, first based on the title and abstract and then, if potentially relevant, a full text was obtained.

2.3.1.3 DATA EXTRACTION

Data were extracted into an Excel 2010 (Microsoft) spreadsheet from each included framework. The data extraction items were focused on the stages and their content and are shown in Box 2.2.

Box 2.2 Data extraction items for review of frameworks for staged evaluation of predictive biomarkers

- suggested stages of biomarker development,
- description of these stages,
- the aim of each stage,
- the outcome of each stage – referring to the information or product (for example finalised assay) available as a result of a stage,
- requirements for a biomarker to enter each stage,
- requirements for biomarker to complete each stage,
- criteria for biomarker development to be stopped at a given stage,
- study designs relevant to each stage,
- information regarding clinical context,
- basis of the framework – defined as the information source or process on which the proposed framework was based,
- country from which the framework originated.

A data extraction table is provided in Appendix 4.

2.3.1.4 DATA ANALYSIS

Frameworks were compared to identify any shared approaches to staged evaluation. This was undertaken based on the situation to which they were applicable and the stages proposed. These items were used to construct models which best reflected the approaches proposed in multiple frameworks.

For each constructed model, the identified stages were summarised. Descriptions of reported study designs relevant to each stage, outcomes of each stage, entry and completion criteria were included. Particular attention was also given to identification of the stage within the framework when a procedure for biomarker evaluation should be finalised, resulting in a standardisation of biomarker evaluation from that point forwards.

The optimal time when a threshold for continuous biomarkers should be established was also investigated.

The stages proposed in these models were compared and contrasted. This was undertaken to find whether there is consensus in the literature with regard to the most appropriate strategies for predictive biomarker development.

2.3.2 CASE STUDY

The case study on discovery and development of HER2 expression to predict response to trastuzumab was intended mainly to provide context and illustrate the process of predictive biomarker development. Internet searches were undertaken for review articles describing the development of HER2 expression to predict response to trastuzumab. The citations in these review papers were utilised to identify relevant primary studies. Websites of the FDA and EMA were also searched to identify relevant documentation. The research undertaken in the process of HER2 development was summarised in chronological order to best reflect the events that led to the introduction into clinical practice of both the biomarker and the drug.

2.4 CASE STUDY OF HER2 EXPRESSION TO PREDICT RESPONSE TO TRASTUZUMAB IN BREAST CANCER

In this case study attention will be given to the research into the biomarker and drug, as this is an example of drug-biomarker co-development. The majority of the research was conducted by or with the participation of scientists working for Genetech, the company which developed trastuzumab and marketed it in the USA.[20,93] A more detailed description of this case study is available in Appendix 5.

2.4.1 HER2 DISCOVERY AND CLINICAL SIGNIFICANCE

In the mid-1980s a new protein involved in cancer was discovered. Due to its structural similarity to EGFR, it was thought to also be a growth factor receptor, although its ligand was unknown. This protein was therefore called "human EGF receptor 2", or HER2 for short.[94,95]

The first large-scale study exploring the clinical significance of HER2 was published in 1987. It analysed tumour tissue from 189 women with BC. The most important part of the study focused on 86 tumours from patients with node-positive disease. A strong correlation was

found between HER2 amplification (evaluated using Southern blotting) and the number of nodes involved. It also appeared to be a good prognostic factor of survival and time to relapse – second only to the number of nodes involved. Importantly, the vast majority of HER2 genes evaluated for amplification were not mutated.[96] Subsequent attempts at replication of these findings in small studies led to mixed results.[97]

A large retrospective study, including 526 patients with BC was undertaken by scientists involved in the original study of HER2 clinical significance. It found that in 345 node-positive patients HER2 amplification (evaluated using Southern blotting) was an independent predictor of both relapse and overall survival (again, second only to the number of nodes involved). However, a clear association was not seen in patients with node-negative disease.[98] This study also explored the relationship between HER2 gene amplification and expression at the level of RNA (evaluated using Northern blotting) and protein (evaluated using immunohistochemistry (IHC) and Western blotting). There was a strong correlation between the results of all four laboratory techniques. However, results obtained using Northern and Western blotting showed the weakest association with clinical outcomes.[98]

The suggestion that increased expression of the HER2 proto-oncogene (non-mutated gene) may be sufficient to drive cancer was then confirmed in *in vitro* experiments.[99]

2.4.2 Drug discovery and development

In 1989 a number of mouse antibodies against HER2 were investigated *in vitro*. One of these, muMAb 4D5, led to the most extensive inhibition of tumour proliferation and showed high specificity for HER2. This antibody was selected by Genetech for further development.[20]

In vivo proof of concept studies that followed showed satisfactory effects of the antibody.[20] However, as muMAb 4D5 was a molecule of mouse origin, it was likely that using it in human patients would result in an immune reaction – production of human anti-mouse antibodies. This was confirmed in a phase I trial including 12 patients with HER2 overexpressing breast and ovarian tumours. It also showed that muMAb 4D5 was well tolerated and localised to tumour tissue.[20]

To address the immune reaction, a series of humanised antibodies were prepared and investigated *in vitro*. Of these, huMAb 4D5-8 showed the highest affinity for HER2 and one of the best inhibitions of cell proliferation. It also appeared to have little effect on a cell

line from normal tissue, thus promising limited adverse events.[100] HuMAb 4D5-8 later became known as trastuzumab, or Herceptin.[97]

Animal research suggested trastuzumab may have synergistic interactions with certain chemotherapeutic drugs such as cisplatin, docetaxel and cyclophosphamide. It was also demonstrated to have an acceptable safety profile when administered over a long period to a range of animals including primates.[97]

2.4.3 PHASE I TRIALS

Three phase I trials were undertaken between June 1992 and March 1993. All of these trials included patients with refractory metastatic BC overexpressing HER2 (assay NR). One trial (Ho407g, n=16) investigated a single dose of trastuzumab and two trials a weekly dose schedule - either as monotherapy (Ho452g, n=17),[101] or in combination with cisplatin (Ho453g, n=15).[102]

Administration of trastuzumab was shown to be safe. A dose limiting toxicity was not reached.[103] There was also no evidence of development of an anti-trastuzumab immune response. Encouragingly, four patients in the combination trial demonstrated objective response to treatment.[20]

Based on these results, a dose schedule for phase II trials was established involving a 250 mg loading dose followed by a 100 mg weekly dose.[104]

2.4.4 PHASE II TRIALS

Phase II trials recruited patients between March 1993 and September 1996 and evaluated trastuzumab as monotherapy(Baselga 1996,[104] n=39 and Cobleigh 1999,[105] n=222), or in combinations with cisplatin (Pegram 1998,[106] n=39). Cobleigh 1999 introduced a new dose schedule based on body mass.[105]

All trials were single-arm and evaluated the drug only in HER2 IHC positive patients. The assay utilised muMAb 4D5 across all three trials. However, in Cobleigh 1999 CB11* was also used.[105] The threshold for considering patients as HER2 positive was also changed from ≥25% of cells staining positive in the first trial[104] to >10% cells staining either lightly (2+) or strongly (3+) in the subsequent two trials.[105,106]

* The reason why and how these antibodies were combined was NR

The percentage of patients who responded to monotherapy (either CR or PR) was 11%[104] and 15%.[105] Nineteen percent of the patients responded to the combination of trastuzumab with cisplatin.[106] The median time to loss of response ranged from 5.1 to 5.4 months. In the combination trial the median overall survival was 13 months (range 0, >30 months). The drug was also shown to be relatively safe.[104-106]

2.4.5 Development of commercial assay (HercepTest)

In December 1996 Genetech started a partnership with a diagnostics company (DAKO) to develop a commercial HER2 expression assay - HercepTest.[93,107]

In one study HercepTest was compared to the assay used in clinical trials (IHC, antibody NR) using 548 tumour specimens. Concordance between the two assays ≥75% was assumed acceptable. The results demonstrated a 79% (95% CI: 76, 82%) concordance. The HercepTest sensitivity was 0.79 (95%CI: 0.73, 0.83) and specificity 0.78 (95% CI: 0.73, 0.83).[108]

Another study used 168 breast tumours, which had previously been characterised using five different methods of HER2 evaluation. HercepTest was shown to have 85% concordance (95% CI: 78, 89%), a sensitivity of 0.6 (95% CI: 0.5, 0.7) and a specificity of 1 (95% CI: 0.95, 1).[108]

A number of reproducibility studies were also undertaken (for example lot-to-lot).[108]

In 1998 HercepTest was approved by the FDA to aid assessment of eligibility for trastuzumab treatment.[93,107]

2.4.6 Phase III trial

A randomised phase III trial (H0648g, also included in datasets in Chapter 5 and 6) recruited 469 HER2 positive patients with progressive metastatic BC between June 1995 and March 1997. Their biomarker status was determined using IHC (antibody NR), where 2+ or 3+ staining in >10% of cells was classed as positive. The trial compared trastuzumab with chemotherapy (anthracycline, cyclophosphamide, or paclitaxel) to chemotherapy alone.[109]

It demonstrated that the median time to progression for trastuzumab added to chemotherapy was 7.4 months and for chemotherapy alone 4.6 months ($p<0.001$). The combination of trastuzumab and paclitaxel appeared the most beneficial. Cardiac

dysfunction was observed in 51 patients treated with trastuzumab, although the mechanism was unclear.[109]

2.4.7 Marketing authorisation

The FDA considered trastuzumab in a fast-track process and it was approved in 1998 in combination with paclitaxel for first-line treatment of HER2 positive metastatic BC patients and as monotherapy for second and third line therapy.[93]

An application was submitted to the EMA in the beginning of 1999 and was approved in the middle of 2000 in a similar indication.[110]

2.5 Findings of the systematic review of frameworks for phased evaluation of predictive biomarkers

Having described the case of the development of HER2 expression for prediction of response to trastuzumab, this chapter will now focus on how such a process was described in the methodological literature. This case study will then be used in the Discussion to explore how well the theoretical proposals match this example of research practice.

2.5.1 Identification of included papers

The review process is shown in Figure 2.1. MEDLINE and EMBASE searches resulted in a total of 26,624 records. An additional 63 records were identified in internet searches. All of these were imported into an EndNote database. After removal of duplicates, 16,268 references remained. Screening of reference lists of papers assessed in full text resulted in addition of four references to the database.

All titles and abstracts were assessed for inclusion by one reviewer and 2700 were checked by a second reviewer. The second reviewer identified two potentially relevant references[111,112] missed by the first reviewer. These two papers were obtained in full text and subsequently both were excluded.

Due to the complexity in applying the inclusion criteria to the titles and abstracts, a full text was sought for 340 papers. The full text could not be obtained for 13 references, which are reported in Appendix 6. 296 papers were excluded based on the assessment of the full text. 277 of these did not describe a framework and 19 did not include predictive biomarkers in their scope. A list of excluded full texts together with reasons for exclusion is reported in Appendix 7. Data were extracted on the 23 complete frameworks.

Inclusion criteria were met by 31 papers. Of these, 23 described a complete framework and eight[113-120] focused only on a fragment of such a framework. These, for example, described only the stages of analytical and clinical validation. Given the extent of information available from the papers describing a full framework and potential difficulty in matching the papers describing a fragment of a framework to any model, the eight papers reporting a fragment of a framework were not analysed.

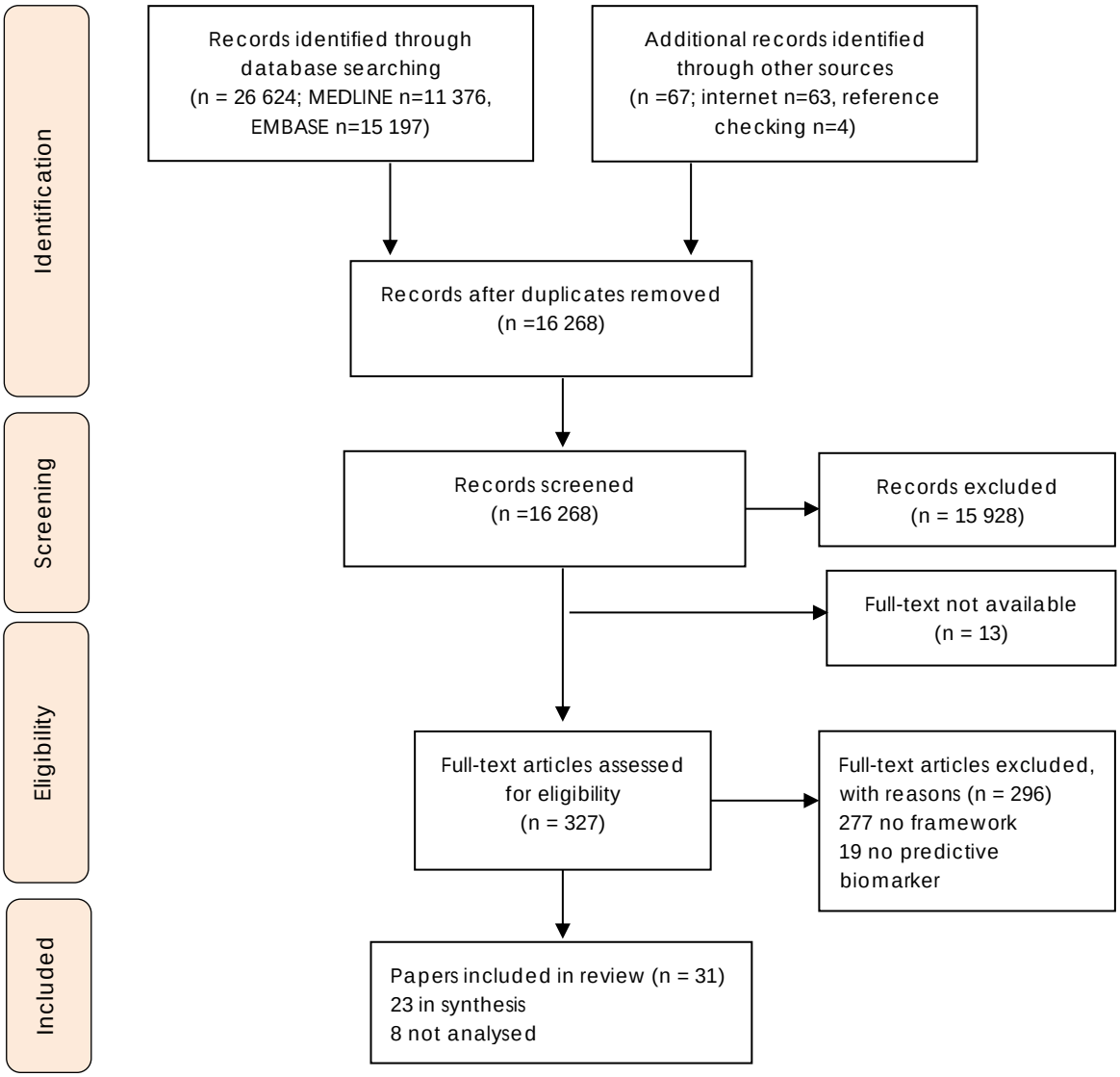


Figure 2.1 Flow chart for review of frameworks for staged evaluation of predictive biomarkers

2.5.2 IDENTIFIED MODELS FOR STAGED DEVELOPMENT OF PREDICTIVE BIOMARKERS

Identified frameworks were grouped into four models (shown in Box 2.3).

These models are discussed in more detail in sections 2.5.3 - 2.5.6 below. Model I is described in detail. Models II-IV are considered special cases of this model and are therefore only briefly summarised and a full description is available in Appendix 8.

Box 2.3 Models identified from the review of frameworks for staged evaluation of predictive biomarkers

Model I (general) described stages for development of predictive biomarkers that appeared to be applicable to any context,

Model II (alongside phased drug development) tied the biomarker development to the phases of drug development,

Model III (multi-marker classifier) described development of a multi-marker classifier and focused mainly on construction of a statistical model for the classifier,

Model IV (safety biomarker) focused on biomarkers predicting safety of treatments already available in the clinic.

2.5.3 Model I (general)

2.5.3.1 Identified frameworks

Eleven papers described frameworks matched to model I (general).[18,43,44,47,121-127] The details of these papers are reported in Table 2.1. All of these were published in 2010 or later. They included four to seven stages and in some cases stages were also sub-divided into multiple sub-stages. Four of the frameworks focused on predictive biomarkers or companion diagnostics,[18,47,121,125] while the remaining seven had a wider scope and described, for example, development of any biomarker. One of the papers focused on proteomic biomarkers.[127] Where specified, the disease areas for which the frameworks were proposed were cancer,[18,43,44,122] autism (suggesting this was generalisable to complex diseases in general)[121] and neurological and neuropsychiatric disorders.[124]

The authors came mainly from the USA and European countries. The grounds on which the framework was proposed were only provided in four papers: an FDA concept paper,[47] a process used by the Early Detection Research Network[43] and considerations emerging from real-life examples.[125,127]

The order of stages in the identified frameworks was generally in agreement with that proposed for model I, as shown in Table 2.2. All frameworks included the discovery and clinical utility stage. Four frameworks included a pre-discovery stage.[47,121,125,127] Analytical

validation was not mentioned in two,[121,124] clinical validation in four,[121,122,124,127] and implementation in four.[44,47,121,125] Ecker 2013[121] and Kaur 2013[124] differed most from the proposed model. On completion of the discovery stage these two frameworks proposed proceeding immediately into the utility stage.

Table 2.1 Frameworks matched to model I

Framework	Stages (number)	Scope	Country/ region	Basis
Alymani 2010[18]	4 (+6 sub-stages)	predictive biomarkers for solid tumours	UK	NR
Ecker 2013[121]	6 (+ 2 sub-stages)	development of targeted treatment for autism (complex diseases)	UK, Switzerland	NR
FDA 2015[47]	4 (+ 4 sub-stages)	predictive in vitro biomarkers; dug-diagnostic co-development	USA	USA drug regulator concept paper; for discussion, not for implementation
Goosens 2015[122]	4	any cancer biomarker	USA, Switzerland	NR
Heckman-Stoddard 2012[43]	5	any cancer biomarker	USA	based on Early Detection Research Network process to guide biomarker development for early detection
Henry 2012[44]	5 (+ 2 sub-stages)	any cancer biomarker	USA	NR
Horvath 2010[123]	6	any biomarker	Hungary, Australia	NR
Kaur 2013[124]	4	pharmacogenomics in neurological and neuropsychiatric diseases	India	NR
Love 2012[125]	7	companion diagnostics; written from the perspective of drug/ diagnostic developing company	USA	consideration of biomarker cases
Majkic 2011[126]	5	any biomarker	Serbia	NR
Mischak 2012[127]	6 (+4 sub-stages)	any proteomic biomarker	International	considerations in a real-life example

NR – not reported

Table 2.2 Labels and stage numbers in identified frameworks matched to model I

Stage	Alymani 2010[18]	Ecker 2013[121]	FDA 2005[47]	Goossens 2015[122]	Heckman-Stoddard 2012[43]	Henry 2012[44]	Horvath 2010[123]	Kaur 2013[124]	Love 2012[125]	Majkic 2011[126]	Mischak 2012[127]
pre-discovery		(1-5) (1) Human phenotypes (2) Human genotype(s) (3) Animal models (4) Cellular assays (5) Drug dev. and screening	(1) Basic research						(1) Defining unmet need		(1) Initial disc. and validation
discovery	(1) Disc.	(5) BM dev.	(2) Prototype design or disc.	(1) BM disc.	(1) Preclinical exploratory studies	(1-2) (1) Id. of a potential BM (2) Dev. of a candidate BM	(1-2) (1) Association of disease with a new BM (2) Potential use of new BM in practice	(1-2) (1) Id. of markers (2) Validation and interpretation of data (pre-clinical mechanistic)	(2) BM discovery	(1) Preclinical investigation	(1-2) (1) Initial disc. and validation (2) panel advice
analytical validation	(2) BM validation (2.1) method dev. (2.2) pre-study validation (2.3) in-study validation		(3) Preclinical dev. (3.1) Analytical validity (3.2) Preclinical pilot feasibility studies	(2) BM assay dev. and analytical validation	(2) Clinical assay dev.	(3) Analytic validity (3.1) analytic validity (3.2) pre-analytic validity	(3) Analytic validity		(3) Technical assay validation	(2) BM validity assessment	(3.1-3.2) (3.1) BM evaluation in appropriate samples (3.2) panel advice

Stage	Alymani 2010[18]	Ecker 2013[121]	FDA 2005[47]	Goossens 2015[122]	Heckman-Stoddard 2012[43]	Henry 2012[44]	Horvath 2010[123]	Kaur 2013[124]	Love 2012[125]	Majkic 2011[126]	Mischak 2012[127]
clinical validation	(3) BM qualification (3.1) retrosp. study (3.2) prosp. study (BM evaluation as secondary objective)		(4) Clinical dev. (4.1)Clinical validity		(3) Retrosp. longitudinal repository studies	(4) Clinical validity	(4) Clinical validity (efficacy)		(4) Clinical validation	(3-4) (3) retrosp. epidemiological studies (4) prosp. clinical studies	
clinical utility	(3.3) Prosp. study (BM evaluation as primary objective)	(6) Clinical trials	(4.2) Clinical utility	(3) Validation of clinical utility	(4) Prosp. study	(5) Clinical utility	(5) Clinical utility (effectiveness)	(3) Functional interpretation	(5) Clinical utility	(5) Randomised clinical studies	(4) (4.1) intervention trial (4.2) panel advice
implementation	(4) Clinical implementation			(4) Clinical implementation	(5) Cancer control		(6) Clinical impact (efficiency)	(4) Clinical implementation	(6-7) (6) medical utility (7) commercial adoption		(5-6) (5) implementation in clinical practice (6) feedback mechanism

BM – biomarker; dev. – development; disc. – discovery; id. – identification; prosp. – prospective; retrosp. – retrospective;

2.5.3.2 STUDY DESIGNS AND OUTCOMES OF EACH STAGE

This section will summarise the identified study designs that were considered relevant to each stage by the frameworks matched to this model. It will also provide an overview of what were perceived as the desirable information and products to be obtained as a result of each stage (the outcomes).

Pre-discovery

As shown in Table 2.3 there were no study designs discussed in the identified frameworks for the pre-discovery stage.

Where reported, the outcome of this stage was focused around identification of the clinical need and context for biomarker development.[121,125,127] One paper defined this further as development of a Target Product Profile and criteria for success.[125] One paper also required development of animal models and cellular assays as well as the drug.[121]

Table 2.3 Model I: pre-discovery stage

Framework	Study design	Outcome
Alymani 2010[18]	N/A	N/A
Ecker 2013[121]	NR	• Define a clinical need • Develop cellular assays and animal models of a pathologic feature • Drug development
FDA 2015[47]	NR	NR
Goosens 2015[122]	N/A	N/A
Heckman-Stoddard 2012[43]	N/A	N/A
Henry 2012[44]	N/A	N/A
Horvath 2010[123]	N/A	N/A
Kaur 2013[124]	N/A	N/A
Love 2012[125]	NR	• prepare Target Product Profile • define criteria for success - in therapeutic terms and fulfilling Target Product Profile
Majkic 2011[126]	N/A	N/A
Mischak 2012[127]	NR	Define clinical need and context

N/A – not applicable; NR – not reported

Discovery

The identified frameworks suggested a wide range of strategies for biomarker discovery, as shown in Table 2.4.

Table 2.4 Model I: discovery stage

Framework	Study design	Outcome
Alymani 2010[18]	can be optimised by combining: • clinical correlation studies: analysis of bio-specimens from patients • investigation in multiple preclinical model systems (*in vitro* and *in vivo*).	• ideally understanding of biology • correlation between biomarker and outcome may be sufficient
Ecker 2013[121]	NR	biomarker discovery
FDA 2015[47]	discovery NR meeting with regulator to discuss development	NR
Goosens 2015[122]	• *"most optimal setting is prospective sample collection and follow-up based on a fully predefined protocol"* (costly and lengthy) • *"retrospective analysis of samples archived as part of previously completed prospective trials (prospective-retrospective design)"* - shorter time • *biobank in which biospecimens and complete clinical annotations are prospectively accumulated"* (based on protocols for cohort or case-control studies) • *In practice, biomarker discovery is often based on* ***"samples of convenience"*** • Initial validation in a separate independent patient sample, but cross-validation possible	• biomarker discovery • initial validation
Heckman-Stoddard 2012[43]	• two possible (complementary) approaches: 1) *"knowledge-based"* - only selected markers 2) *"unbiased"* - large number of biomarkers • preclinical studies: "could include animal models, cell lines, or clinical samples"	biomarker discovery
Henry 2012[44]	multiple approaches; discussed two: 1) candidates based on biology understanding 2) "discovery" approach - using techniques such as high-throughput sequencing and mass spectroscopy to identify one or more biomarkers that differ between cohorts; need careful design to minimise false positives	biomarker discovery
Horvath 2010[123]	• "Case-control studies are often sufficient" • "'Decision analytic modelling could be a cost-saving approach for assessing the potential clinical utility of the new biomarker in various practical scenarios.	• biomarker discovery • assessment of potential clinical utility
Kaur 2013[124]	*"human population based lab research"* (Candidate gene and genome wide approach) Cell-culture or animal model based approach	• biomarker discovery • understanding of biology
Love 2012[125]	NR	• characterise information provided by the biomarker(s) • identify appropriate platform • identify body site for imaging or sample type
Majkic 2011[126]	correlative laboratory studies	• correlation of biomarker with biological phenomenon • improve assay: reliability and sensitivity; standardise
Mischak 2012[127]	NR discovery studies advice from multidisciplinary panel	• biomarker discovery and initial validation • panel guidance on sample collection and study design

NR – not reported

Three papers postulated two general and not mutually exclusive approaches: knowledge-based and data-driven.[43,44,124] Where reported, the appropriate study design involved at least one of the two types of studies:

- pre-clinical: cell line and animal model research,[18,43,124]
- clinical correlative studies: mainly using case-control and cohort methodology.[18,44,122,123]

Two papers also discussed the need for initial validation of discovery,[122,127] either using an independent sample or cross-validation.[122] Another paper proposed conducting decision analytical modelling to evaluate the potential clinical utility.[123] Two papers suggested obtaining advice on further research either from the regulator,[47] or a multidisciplinary panel.[127]

Apart from identification of a candidate biomarker it was also suggested this stage should ideally provide understanding of the biology linking the biomarker to the outcome.[18,124] Definition of the parameters of the biomarker assay was also discussed as an outcome of this stage.[125,126]

Analytical validation

The design of studies to evaluate analytical validity was rarely reported, as shown in Table 2.5. One paper claimed there was no generally accepted standard.[18] The designs reported in three papers generally involved comparing the results of the assay to a known reference standard using biological specimens from patients.[18,47,122] Two papers also proposed feasibility or pilot studies to be conducted at this stage.[18,47]

The outcomes of analytical validation included test accuracy (for example sensitivity, specificity),[18,43,44,47,122,123] precision[18,47,123] and reproducibility.[18,44,125] Establishing the biomarker cut-off was suggested in two papers.[43,47] One of these identified the possible necessity for a grey zone between biomarker positive and negative patients. Such a grey zone is a set of biomarker values for which a treatment decision would not be biomarker-dependent due to uncertainties in biomarker evaluation and clinical outcome assessment.

At this stage frameworks also proposed developing a platform for use in clinical practice,[47,122] and assay standard operating procedures.[18,43] It was also indicated that biological variability of the biomarker should be investigated,[44,126] as well as factors related to biological sample handling.[44] The need for identification of the target population was also discussed.[47]

Table 2.5 Model I: analytical validation

Framework	Study design	Outcome
Alymani 2010[18]	no accepted standard - guided by Good Clinical Laboratory Practice and other standards, may not always be applicable feasibility studies to assess reagent availability; *uses "controls (from patient samples or suitable surrogates) that contain known concentrations of the biomarker "* *"test the assay on real patient samples (…) to confirm consistent performance"*	method development and preliminary validation • parameters including: *"selectivity, sensitivity, different choice of controls, different analyte recovery methods, precision, accuracy and reproducibility"* • *" stability of the analyte in controls and patient samples*" • produce an analytical report • *"construct a standard operating procedure"* • generate valid patient data • *"identify any issues that may occur when analysing real patient samples"*
Ecker 2013[121]	NR	NR
FDA 2015[47]	analytical studies: • *"using an independent prospective clinical dataset, or by testing retrospectively banked specimens from the original studies"* • *"pilot studies to determine relevant populations to be studied to establish clinical test performance and target cut- off points in biological specimens"*	• assay performance including sensitivity, specificity, precision • validate the test platform • establish test cut-offs taking into account clinical and analytical factors (may include a grey zone) • identify the populations to be studied
Goosens 2015[122]	Usually by analytic validity studies *"assaying the same set of samples by both the assay used in the initial discovery and the clinical deployment platform to determine robustness and reproducibility of the measurements"*	• adapt biomarkers to a clinical platform • accuracy and reliability of the platform
Heckman-Stoddard 2012[43]	NR	• for binary assay - sensitivity and specificity • if cut-off not known - use ROC curve • biomarker status association with patient and disease characteristics
Henry 2012[44]	NR	• standard operating procedures • sensitivity, specificity and robustness of assay; • reproducibility within and between laboratories
Horvath 2010[123]	analytic validity studies (details NR)	• "e.g. technical sensitivity, specificity, imprecision and trueness" • "quality control procedures" • analytical characteristics improved if needed
Kaur 2013[124]	N/A	N/A
Love 2012[125]	NR	assay reproducibility in relevant sample types
Majkic 2011[126]	NR	• ability to distinguish between different phenotypes/ outcomes; • reference values and individual variation
Mischak 2012[127]	• recommended by panel • present results to panel	NR

N/A – not applicable; NR – not reported; ROC – receiver operator characteristic

Clinical validation

There was a variety of clinical validation study designs discussed, as shown in Table 2.6. Two papers suggested this stage should begin with a retrospective study (further details NR), followed by a prospective one.[18,126] One of these papers further describes this prospective study as a clinical trial investigating the drug and including the biomarker hypothesis as a secondary objective.[18] One paper suggested conducting only a retrospective study using stored samples (further details NR),[43] another proposed a case-control design[44] and one a diagnostic accuracy study.[123] One paper suggested this stage should be carried out in parallel with phase I and II drug trials.[47] One framework postulated that, if positive, the results of the clinical validation study should be reproduced in an independent dataset.[44]

The proposed outcomes of this stage included sensitivity and specificity for clinical end-point prediction,[18,47,123,125] positive predictive value (PPV), negative predictive value (NPV) and diagnostic likelihood ratios.[47] One paper suggested that the threshold for a continuous biomarker or multi-marker classifier should be developed at this stage.[43] Other outcomes included linearity of the biomarker,[125] biological variability[43] and correlation between biomarker and outcome.[126]

Clinical utility

Most of the papers suggest evaluation of clinical utility in an RCT.[18,43,47,121-124,126] Different study designs were suggested depending on the situation, as detailed in Table 2.8. These are described in more detail in Chapter 1 and briefly these were:

- biomarker-strategy with or without randomisation in the control arm,[18]
- prospective subgroup analysis of an RCT,[47]
- biomarker stratified,[18,47]
- enrichment,[47]
- prospective-retrospective.[47,122]

It was also noted that a systematic review or meta-analysis of multiple trials may be used.[123,124] One paper suggested that depending on circumstances case-control or cohort methodology can be used rather than an RCT.[124]

Table 2.6 Model I: clinical validation

Framework	Study design	Outcome
Alymani 2010[18]	clinical studies; no formal guidelines • " *start by retrospectively analysing material from prior well-controlled studies from which high quality sample material as well as clinical outcome (…) is available (…) collection of tissues should be prospectively determined"* • as alternative materials from bio-banks – useful for discovery (less robust) • After retrospective studies, a *"prospective clinical study for the biomarker (…) as an adjunct to a clinical trial of which the primary objective is to test the efficacy of a drug"* sample collection should be prospective and follow *"standard operating procedures and other clinical trial regulatory guidance"*	" sensitivity and specificity for clinical end-point determination"
Ecker 2013[121]	N/A	N/A
FDA 2015[47]	In parallel with phase I and II studies: • *"studying the test in relation to the intended clinical outcome in patient subgroups with and without the analyte of interest"* • *"based on information known from analytical studies and based on pilot studies or careful analysis to determine relevant populations (…) clinical test performance"* and target cut-offs • *should use the same "endpoints used to indicate the clinical utility"* of biomarker and provide information on the clinical impact of a test result	for dichotomous/ dichotomised outcomes: • clinical sensitivity and specificity • PPV and NPV in patient population similar to intended indication • additionally positive and negative diagnostic likelihood ratios may be investigated
Goosens 2015[122]	N/A	N/A
Heckman-Stoddard 2012[43]	*"retrospective analysis using stored samples"*	• determine if biomarker predicts outcome • continuous biomarker: find threshold • multiple markers: compare, develop combinations, assess if sequential testing adds information • assess within-individual variability
Henry 2012[44]	• often use of convenience samples; • patients representative of the clinical question; • cases and controls should be similar; • sample handling and processing should be similar and biomarker assessment blind to group • observation of apparent clinical validity *"needs to be reproduced in a completely independent set of samples in order to confirm validity"* • consider independent validation by completely independent researchers	show that *"biomarker reliably divides the overall population of interest into two distinct groups"* (such as more or less likely to have an event)
Horvath 2010[123]	*"usually investigated in diagnostic accuracy studies in a representative spectrum of patients, in order to obtain the clinical sensitivity and specificity of the test"*	clinical sensitivity and specificity of the test
Kaur 2013[124]	N/A	N/A
Love 2012[125]	NR	• appropriate reproducibility (precision and accuracy) • appropriate sensitivity and specificity • linearity over the range of possible test results for intended use
Majkic 2011[126]	retrospective epidemiological studies followed by prospective clinical studies	correlation between biomarker levels and clinical outcomes
Mischak 2012[127]	N/A	N/A

N/A – not applicable; NR – not reported; PPV – positive predictive value; NPV – negative predictive value

In the majority of the papers the outcome of this stage was assessing clinical utility of the biomarker.[18,43,47,122,123,125-127] Some also mentioned confirmation of biomarker performance[43,122] and evaluation of biomarker variability in response to administered drugs.[126] According to one paper, the outcome of this stage should be new indications and drugs on the market.[124]

Implementation

The implementation stage is shown in Table 2.7. It referred to activities that would normally follow marketing of the biomarker. At the point of implementation only three frameworks report further collection of data: through cohort studies or case reviews,[43] health technology assessment and clinical guideline development,[123] or an unspecified feedback mechanism.[127]

The outcomes of this stage were focused on implementation of the biomarker in clinical practice which involved incorporation of regulation into clinical guidelines[122] and reimbursement.[122,125] According to some frameworks impact on spending[123,127] and population health should also be evaluated.[43]

Table 2.7 Model I: implementation

Framework	Study design	Outcome
Alymani 2010[18]	NR	NR
Ecker 2013[121]	N/A	N/A
FDA 2015[47]	N/A	N/A
Goosens 2015[122]	NR	Implementation in clinical practice: • regulatory approval, • commercialisation, • coverage by health insurance companies, • incorporation in clinical practice guidelines
Heckman-Stoddard 2012[43]	*"may include cohort designs or clinical case reviews"*	real-life impact of the biomarker on reducing the burden of disease in the population
Henry 2012[44]	N/A	N/A
Horvath 2010[123]	• health technology assessment • guideline recommendations	investigate *"ethical, legal, financial or social implications of testing"*
Kaur 2013[124]	NR	improved therapeutic response
Love 2012[125]	NR	• establish if the test is used to make treatment decisions that improve patient outcome • establish if the test (and treatment) are an appropriately reimbursed standard of care
Majkic 2011[126]	N/A	N/A
Mischak 2012[127]	*"feedback mechanisms to evaluate cost-effectiveness, clinical adoption, problems in routine application, unanticipated collateral problems"*	• implement in practice (may be on a limited scale if data on hard endpoints not robust) • collect data on *"cost-effectiveness, clinical adoption, problems in routine application, unanticipated collateral problems"*

N/A – not applicable; NR – not reported

Table 2.8 Model I: clinical utility

Framework	Study design	Outcome
Alymani 2010[18]	prospective study - primary objective: "*evaluation of the predictive power of the biomarker*" Three designs suggested: • biomarker-strategy (no randomisation in control arm) • biomarker-strategy (randomisation in control arm) *"for example, if one treatment is better than the other in both biomarker-positive and biomarker-negative patient subgroups. It may also allow a retrospective assessment of an alternative classification"*; relatively inefficient • stratified (more efficient, allows testing for interaction and of biomarker prognostic impact; no direct assessment of predictive impact of biomarker; cannot be used if more than one biomarker and more than two treatments or outcomes other than efficacy assessed)	clinical utility
Ecker 2013[121]	Clinical trial	NR
FDA 2015[47]	As a phase III trial; possible designs: • ***"simple two**-arm randomization comparing a treatment and a control (...) with the results from the diagnostic test (...) used as a prespecified stratification factor in the post-hoc statistical analysis"* – would " *allow for identification of a treatment by diagnostic test result interaction [if] results of the testing will not be readily available at the clinical sites"* • *randomization within differing strata by diagnostic test result* • enriched – "*careful explanation and justification of the enrichment technique used (diagnostic test, demographic information, other)"* needed • "*in cases where the analyte is stable and where collection bias (including spectrum bias, verification bias, and sampling bias) can be carefully characterized and addressed, prospectively designed retrospective clinical utility studies*" possible	clinical utility
Goosens 2015[122]	*Ideally, (...) statistically well- powered prospective trials*" if infeasible - prospective- retrospective design and/or biobank/biorepository samples could be used	• clinical utility; • confirm performance
Heckman-Stoddard 2012[43]	Prospective trial	• establish if biomarker predicts outcome; • PPV of the biomarker
Henry 2012[44]	NR	• assay useful in clinical practice • includes benefit-to-harm ratio
Horvath 2010[123]	RCT, a systematic review or meta-analysis of multiple RCTs most appropriate	clinical utility
Kaur 2013[124]	• correlation of markers with multiple outcomes in large independent populations; depending on resources, ethical issues and outcomes assessed this could be case-control, cohort or RCT; • meta-analysis of such studies	new indications and drugs on market
Love 2012[125]	NR	• biomarker provides actionable information in relevant context • improved treatment decisions
Majkic 2011[126]	RCT	• improved treatment • "*influence of drugs on biomarker values*"
Mischak 2012[127]	• intervention trial as recommended by panel • present to panel	benefit of biomarker

N/A – not applicable; NR – not reported; RCT – randomised controlled trial; PPV – positive predictive value;

2.5.3.3 CRITERIA FOR ENTRY AND COMPLETION OF STAGES

Criteria for a potential predictive biomarker to enter into and complete a particular development stage were provided in six papers.[18,43,47,123,124,127] These are shown in Table 2.9.

None were discussed for pre-discovery.

For discovery, one paper claimed there are no accepted standards for entry or completion.[18]

To commence analytical validation, papers suggested a validation plan needs to be in place,[18] modelling needs to confirm there is potential for the biomarker to show desired clinical utility,[123] or a multidisciplinary panel decides the biomarker can enter this stage based on discovery data.[127] One paper suggested there are no standards for completion of this stage[18] and another proposed a panel recommendation as the criterion.[127]

Entering into clinical validation appeared to be dependent on the existence of a suitable biomarker test based on the analytical validation.[18,43,47] One paper suggested the cut-off needs to be selected.[47] With regard to completing this stage, it was stated in one paper that there are no standards.[18]

Progression to the clinical utility stage, where reported, appeared to require satisfactory analytical validity[47,127] and in one case having completed biomarker discovery (however this paper suggested proceeding to clinical utility immediately after discovery).[124] For completion one paper suggested that considerations are similar to any other clinical hypothesis and usually require data from two or more adequate trials, although this may depend on the situation.[47] Another paper claimed this was "*traditionally settled by debate, consensus and time*".[18]

Implementation generally required demonstration of clinical utility[18,124,127] No criteria for completion were discussed.

Table 2.9 Model I: entry (in) and completion (out) criteria for stages

Stage	Alymani 2010[18]	Ecker 2013[121]	FDA 2005[47]	Goossens 2015[122]	Heckman Stoddard 2012[43]	Henry 2012[44]	Horvath 2010[123]	Kaur 2013[124]	Love 2012[125]	Majkic 2011[126]	Mischak 2012[127]
pre-discovery	N/A	NR	NR	N/A	N/A	N/A	N/A	N/A	NR	N/A	NR
discovery	No standard	NR	NR	NR	NR	NR	NR	NR	NR	NR	In: NR Out: satisfactory discovery and initial validation
analytical validation	In: validation plan Out: no standard	N/A	NR	NR	NR	NR	In: *"modelling confirms potential clinical utility"* Out: NR	N/A	NR	NR	In and out: panel recommendation
clinical validation	In: validation must be fit-for-purpose validation (full not necessary) Out: no standard	N/A	In: test analytically characterised; cut-off selected Out: NR	N/A	Good clinical assay	NR	NR	N/A	NR	NR	N/A
clinical utility	In: NR *Out: "settled by debate, consensus and time"*	NR	In: test analytically characterised; cut-off selected Out: usually data from two or more adequate trials	NR	NR	NR	NR	In: Completion of discovery Out: NR	NR	NR	In and out: panel recommendation
implementation	In: qualified biomarker (clinical validity and utility) Out: NR	N/A	N/A	NR	NR	N/A	NR	In: Completion of clinical utility Out: NR	NR	N/A	In: panel recommendation Out: NR

N/A – not applicable; NR – not reported;

2.5.4 Model II (alongside phased drug development)

2.5.4.1 Identified frameworks

Three papers were identified that were matched to model II (alongside phased drug development). Two of these focused on predictive[128,129] and one on any -omics biomarkers.[130] As shown in Table 2.10, all of these frameworks matched well to the stages in model II. The process which led to construction of these frameworks was NR.

Table 2.10 Labels and stage numbers in identified frameworks matched to model II

Stage	Garcia 2011[128]	Hodgson 2009[129]	Lin 2009[130]
Pre-clinical	(1)	(1)	(1-3)
	Preclinical discovery and analytical assay validation	Pre-clinical	(1) Discovery (2) Internal validation (3) External validation
phase I trial	(2)	(2)	(4)
	Phase I trial clinical qualification	Phase 1 (biomarker validation)	clinical trial (phase I, II)
phase II trial	(3)	(3)	(4)
	Phase II trial clinical qualification	Phase 2 trial against comparator in biomarker +ve and -ve patients	Clinical trial (phase I, II)
phase III trial	(4)	(4)	(5)
	Phase III trial clinical qualification	Phase 3 Preparation for commercial launch	Large clinical trial (phase III)
implementation			(6)
			Continued surveillance

2.5.4.2 Study designs and outcomes of each stage

Little detail was available in the identified papers on study designs appropriate for each stage and the outcomes of these stages. The main relevant points are summarised below.

<u>Pre-clinical</u>

The relevant designs for this stage were: literature reviews,[129,130] pre-clinical models,[129] case-control studies and data-mining.[130] One framework suggested that an initial discovery study should be replicated for external validation.[130]

This stage should aim to identify the candidate biomarker,[129,130] provide understanding of the biomarker biology and assess performance characteristics of the assay.[130]

Phase I trials

No relevant study designs were described in the papers.

The main outcomes were "clinical usefulness"[128] and evaluability and prevalence of the biomarker.[129]

Phase II trials

The proposed designs for this stage were either stratified or, for prolonged stable disease, randomised discontinuation. However, if sufficient evidence is available from a phase I expansion cohort, a study may not be needed.[128] According to one paper this should provide evidence on the usefulness of the biomarker in a clinical setting and reproducibility, validity and variability of the assay.[128] Another paper suggested conducting a benefit/risk ratio in all patients and biomarker-defined subgroups would be the aim[129]

Phase III trials

This stage should involve randomised trials:

- with biomarker-based inclusion criteria for biomarkers with high predictive value (most likely referring to an enrichment design), or
- stratified for biomarkers with low predictive value.[128]

As a result of such a trial, information on biomarker utility should be obtained.[128,129] One paper also suggested the biomarker assay should be finalised at the end of this stage.[129]

Implementation

None of the papers provided details of the implementation stage.

2.5.4.3 CRITERIA FOR ENTRY AND COMPLETION OF STAGES

Criteria for entry into three stages (pre-clinical, phase II and phase III trial) were available from two papers.[128,129] Completion was only discussed in one paper for the phase II trial stage.[128]

To enter the pre-clinical stage some understanding of the drug mechanism of action was required.[128]

For entry into a phase II trial, *"data on biomarker evaluability and prevalence and estimate of effect size"* was needed.[129] To complete phase II, clinical validity of the biomarker should be demonstrated.[128]

For entry into phase a III biomarker trial, clinical validity of the biomarker needed to be shown.[128]

2.5.5 Model III (multi-marker classifier)

2.5.5.1 Identified frameworks

There were seven papers[13,45,50,52,131-133] that described frameworks matched to model III (multi-marker classifier). None of the frameworks was focused exclusively on predictive biomarkers.

As shown in Table 2.11, the order of stages in the identified frameworks was generally in agreement with that proposed for model III with one exception (Ginsburg 2006[132]). Three papers reported the basis of the proposed framework and this was: literature searches,[133] a committee with members from a variety of backgrounds,[50] and experience from an observational study.[45]

2.5.5.2 Study designs and outcomes of each stage

<u>Pre-discovery</u>

Study designs in this stage reflected the different sources of data to be used later for discovery. These were: cell-line experiments,[131] genetic association studies[132] and a pilot of a multicentre study.[45]

The outcomes of this phase generally included formulation of the question to be addressed,[45] data collection,[50,131-133] a validated biomarker discovery platform[132,133] and a protocol for a multicentre study.[45]

<u>Identification of candidate biomarkers</u>

The study designs suggested for this stage were mainly focused around different statistical techniques, such as two-sample t-tests,[131] or a variety of pattern recognition techniques[132]. Two approaches were suggested that can be used on their own or in combination: non-hypothesis driven and hypothesis driven discovery.[45] It was also proposed that the findings should be replicated in a new study and biological plausibility studies carried out.[132]

The only outcome of this stage discussed was obtaining a set of candidate biomarkers.

Table 2.11 Labels and stage numbers in identified frameworks matched to model III (multi-marker classifier)

Stage	Cho 2012[131]	Ginsburg 2006[132]	Ioannidis 2011[133]	IOM 2012[50]	Matsui 2013[52]	Shahzad 2012[45]	Simon 2005[13]
pre-discovery	(1-2) (1) Data collection (2) Quality control/ pre-processing	(1-2) (1) Biomarker discovery (2) Clinical and biological data collection	(1) (1) Analytical tools	(1.1.1) (1) Discovery and Test Validation Stage (1.1)Discovery Phase (1.1.1) Step 1: Data Quality Control		(1.1-1.2) (1) phased approach (1.1) Clinical phenotype consensus definition (1.2) Establishment of study logistics (1.2.1) initial protocol (1.2.2) feasibility studies (1.2.3) pilot studies (1.2.4) problem identification (1.2.5) trouble shooting (1.2.6) protocol modification (1.2.7) individual training (1.2.8) new protocol	
identification of candidate biomarkers	(3) (3) Identification of candidate biomarkers	(1, 2.1) (1) Biomarker discovery (2.1) Biomarker validation	(2) (2) Clinically oriented discovery	(1.1.2) (1.1.2) Step 2: Computational Model Development and Cross-Validation	(1.1) (1) Developing genomic signatures (1.1) gene screening	(1.3) (1.3) Candidate gene discovery	(1) (1) developing a genomic classifier
prediction model development	(4) (4) Construction of prediction model	(3) (3) Predictive model development	(2) (2) Clinically oriented discovery	(1.1.2) (1.1.2) Step 2: Computational Model Development and Cross-Validation	(1.2 - 2.1) (1.2) ranking and selection (2) prediction analysis (2.1) development of predictor	(1.4-1.5.1) (1.4) Differential Gene List Validation/ Verification (1.5) Molecular Classifier Algorithm Development (1.5.1) identification of classifier genes and cutoff	(1) (1) developing a genomic classifier

Stage	Cho 2012[131]	Ginsburg 2006[132]	Ioannidis 2011[133]	IOM 2012[50]	Matsui 2013[52]	Shahzad 2012[45]	Simon 2005[13]
internal validation	(4) Construction of prediction model		(3) Validation	(1.1.2) Step 2: Computational Model Development and Cross-Validation	(2.2) Clinical validation of predictors	(1.5.2) Independent Testing of Selected Classifier Genes	(2-3) (2) Internal validation of a classifier in developmental studies (3) Evaluating if classifier is superior to existing prognostic factors
external validation	(4) Construction of prediction model	(5) Implementation (5.1) Development of diagnostic test	(3) Validation	(1.1.3 - 1.2) (1.1.3) Step 3: Confirmation on an Independent Dataset (1.1.4) Step 4: Release of Data, Code, and the Fully Specified Computational Procedures to the Scientific Community (1.2) Test Validation Phase (1.2.1) Analytical Validatiton (1.2.3) Clinical/ Biological Validation (1.2.4) implementation of the new test in the workflow and quality management system of the CLIA-certified laboratory	(2.2) Clinical validation of predictors	(1.6) External Classifier Validation/ Testing	(4) Translation of platforms and demonstrating assay reproducibility

Stage	Cho 2012[131]	Ginsburg 2006[132]	Ioannidis 2011[133]	IOM 2012[50]	Matsui 2013[52]	Shahzad 2012[45]	Simon 2005[13]
clinical utility	(5) Independent validation of prediction	(4) Decision support tool development	(4) Clinical application	(2) Evaluation for Clinical Utility and Use Stage	(3) Biomarker-Based Clinical Trials for Assessing Clinical Utility	(2) Comparison against standard of care & personalized use	(5) Independent validation of genomic classifier
implementation		(5) Implementation (5.2) Health professional and public education (5.3) Development of clinical guidelines (5.4) Regulatory oversight in laboratories (5.5) Cost-effectiveness (5.6) Privacy	(4-5) (4) Clinical application (5) Post-clinical appraisal				

Prediction model development

Again, a variety of statistical models were suggested including linear discriminant analysis and support vector machines.[131]

Two major tasks within this stage were identified:

1) selection of biomarkers to be included in the classifier (feature selection) and
2) construction of the prediction model.[13,50,52]

Internal validation

Internal validation was often described as involving two main approaches:

- Split-sample – where the available sample of patients is divided into a training set used for model development and a separate test set used for testing the performance of the classifier,[13,45,50,52]
- Cross-validation – where statistical techniques using a single set of patients for both development and validation of the classifier are implemented.[13,45,50,52,131,133]

The outcome of internal validation was assessment of the performance of the classifier in terms of measures of accuracy such as classification error rate,[13,131] AUC ROC,[131] sensitivity, specificity, PPV and NPV.[13]

External validation

This stage was generally synonymous with conducting a new study using an independent sample of patients. Some of the frameworks suggest this should be a large-scale[133] and/or multicentre study,[50,131] involving a diverse population,[133] relevant to the intended use of the test.[50,52]

The outcomes of this stage generally involved showing the adequate performance of the classifier. In some papers this was defined in terms of test accuracy,[45,52,132] precision[45,132] and reproducibility.[13,45,132] Other outcomes included demonstrating the generalisability of the classifier,[50,133] specifying the type of the analyte to be used,[132] investigating the influence of specimen handling on classifier results,[45] developing a prototype biomarker platform[132] and standardisation of the classifier.[13,50] Cost-effectiveness of the test was mentioned by one paper in this stage.[132]

It was also suggested that data and computer code used for the classifier should be made available to the scientific community, or at least the regulators to provide independent verification of the results obtained.[50]

Clinical utility

The clinical utility stage generally required carrying out an RCT. The proposed designs, depending on the situation, were:

- enrichment,
- biomarker-strategy,
- prospective subgroup analysis of an RCT, and
- prospective-retrospective.[13,50,52]

The possibility of undertaking an observational study was also suggested.[133] It was also mentioned that if the event rate is very low, a single-arm trial including only biomarker positive patients may be appropriate.[13]

This stage should lead to establishing the clinical utility[13,50,52,131,133] and a fully developed, standardised classifier.[131,133] It should also provide information on the feasibility of using the classifier in clinical practice.[52]

Implementation

This stage was to investigate cost-effectiveness of the biomarker.[132,133] Issues such as education, policy and regulation of the test should be considered.[132] Conducting an audit of the actual use in practice and cost-utility of the biomarker was also discussed.[133]

2.5.5.3 Criteria for entry and completion of stages

Criteria for entry into at least some of the first six stages were reported in five of the six papers.[13,45,50,52,131,133] One paper reported criteria for completion of one stage (external validation).[50]

Prior to pre-discovery, the phenotype of interest should be defined (for example the outcome to be predicted)[45]. It was also suggested that consideration should be given to whether it is possible to develop a classifier to address a given problem.[50]

For entry into the discovery stage it was proposed that data of adequate quality [50] or adequately normalised[131] should be available. Study infrastructure and logistics needed to be established.[45]

For entry into prediction model construction the requirements provided were a manageable number of candidate genes[131] and data of sufficient quality.[50]

To initiate internal validation, a predictive model needed to be completed.[50,52]

For external validation, a complete model[50,52] and satisfactory results from internal validation were required.[13,50] Completion of internal validation needed full definition of the multi-marker classifier.[50]

Before entry into the clinical utility stage, completion of discovery and validation of the model was required.[50,133] According to one paper, at this point the classifier needs to be *"locked-down"*.[50]

2.5.6 Model IV (Safety)

2.5.6.1 Identified frameworks

Two papers were identified that described a framework that matched model IV (safety). Both of these focused on a situation when drugs are already on the market when new biomarkers predictive of safety are identified. They were based on the experience and practice of organisations that aim to discover and develop safety biomarkers.[51,134]

As shown in Table 2.12, the frameworks agreed relatively well with the proposed model, however neither of the two papers matched exactly the stages proposed.

Table 2.12 Labels and stage numbers in identified frameworks matched to model IV

	Loo 2010[51]	Matheis 2011[134]
surveillance	(1) Active surveillance, patient recruitment and collection of data and biomaterial	(NR as stage, but setting up a dedicated biobank discussed)
discovery	(2) Identification of gene variants and replication of findings	(1) Candidate biomarker identification
pre-clinical	(3) Pharmacokinetic and functional validation	(NR as stage, but referred to as pre-requisite to stage 2)
analytical validation		(2) Exploratory phase
clinical utility	(4) Prospective clinical studies to evaluate diagnostic utility	(3) Confirmatory phase
implementation	(5) Determination of the cost-effectiveness of diagnostic testing	(4) Submit for regulatory approval

2.5.6.2 STUDY DESIGNS AND OUTCOMES OF EACH STAGE

The study designs and outcomes relevant to each stage were described in little detail in the identified frameworks.

Surveillance

For this stage recruitment of patients with adverse events and matched controls[51] or establishment of a dedicated biobank[134] was to be undertaken.

None of the papers discussed the outcomes relevant to surveillance.

Discovery

One paper suggested the discovery stage should be based on a case-control study utilising a candidate gene approach supplemented by genome wide association studies (GWAS). The findings should then be replicated in a new study in a different population.[51] The other paper advocated the use of a literature review, databases and a biobank.[134]

None of the papers discussed the outcomes of this stage.

Pre-clinical

Pre-clinical investigation was to be based on *in vitro* and animal model studies.[51]

None of the papers discussed the outcomes of this stage.

Analytical validation

This stage was to involve a small study in healthy subjects and patients comparing the biomarker test under investigation to the reference standard.[134]

The suggested outcomes to be assessed were the performance of the assay (including sensitivity, specificity, PPV, NPV, or ROC AUC), biological variability of the biomarker and stability of the analyte after sampling.[134]

Clinical utility

Studies addressing clinical utility of the biomarker differed between the two frameworks. These were:

- a prospective clinical trial aiming to establish the utility of the predictive biomarker in preventing adverse events,[51]

- a study in a large patient population ("proof of performance") aiming to establish biomarker performance and threshold. [134]

Implementation

There was no information on the study designs or outcomes of the implementation stage.

2.5.6.3 CRITERIA FOR ENTRY AND COMPLETION OF STAGES

These criteria were described only in Matheis 2011 for two stages.[134]

Entry into the analytical validation stage was to be based on pre-clinical and clinical evidence supporting the biomarker. The validation protocol should also be discussed with the regulatory agencies. Completion of this stage was to be based on "assay acceptance criteria" defined prior to undertaking the validation.

For biomarker utility, only entry criteria were described. These were the presentation of the results of analytical validation to regulatory agencies.

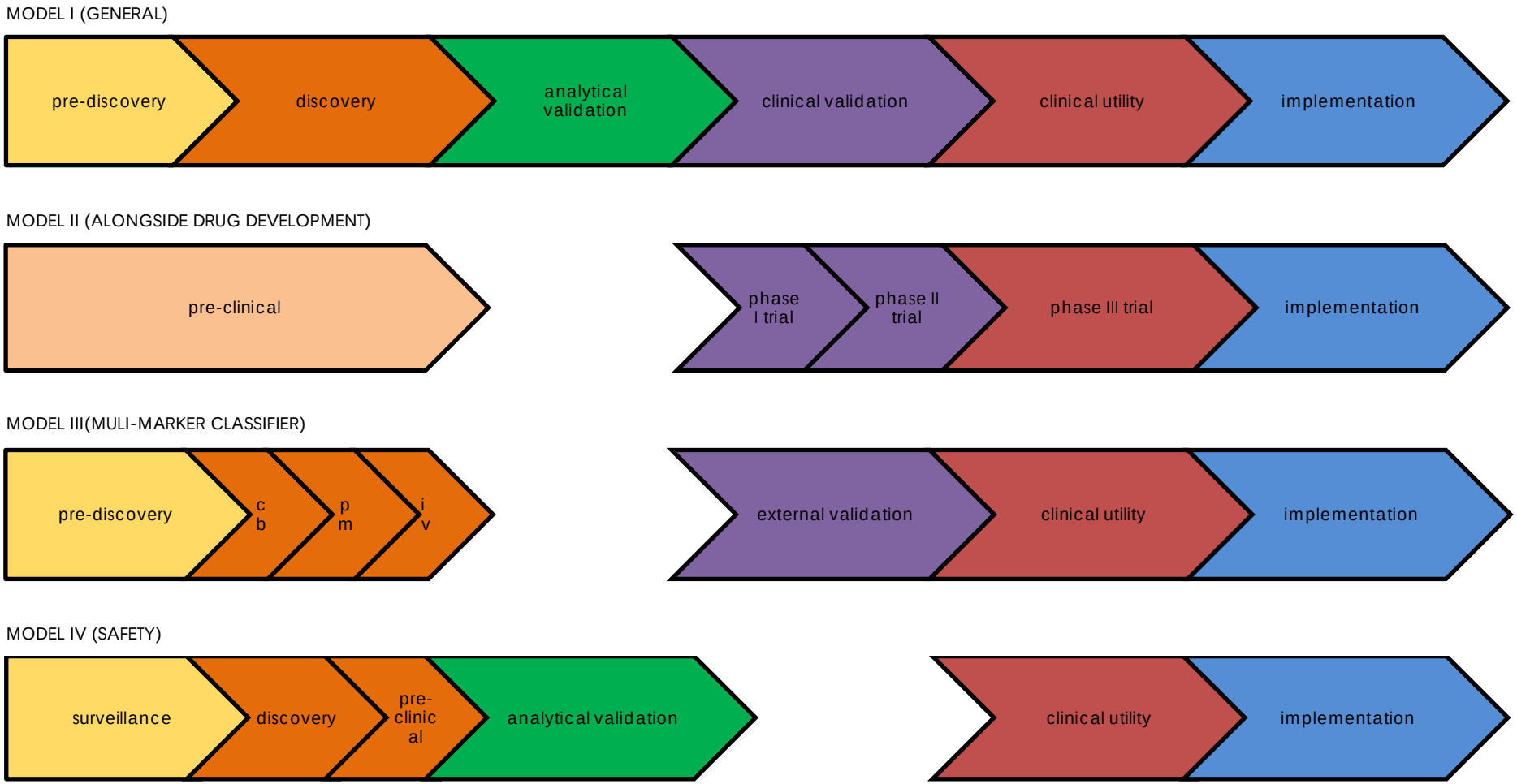
2.6 COMPARISON OF MODELS

2.6.1 IDENTIFIED STAGES IN CONTEXT OF MODEL I

A general comparison of the stages included in different models based on their aims is provided in Figure 2.2. This was based on a comparison with model I, as it appeared to describe the most complete pathway. The similarities and differences between models, relative to stages of model I, are discussed below.

Pre-discovery

A pre-discovery stage was identified in model I, III and IV (where it was called "surveillance"). In model II it appeared to be merged with discovery as pre-clinical research. This generally included activities such as collection of data,[132] ensuring the data are of sufficient quality,[50,131,133] and formulation of the question to be addressed within the discovery stage.[45,121,125]



cb – candidate biomarkers; pm – prediction model; iv – internal validation; colours used to indicate stages with similar aims

Figure 2.2 Stages of biomarker development addressing similar objectives in models I-IV

Discovery

This stage was present in all models. As mentioned above, in model II it was merged with pre-discovery activities. In model III three stages were mapped to the discovery stage in model I: identification of candidate biomarkers, development of the predictive classifier and internal validation of the predictive classifier.

A wide variety of discovery designs was identified and these included utilisation of one or more of:

- pre-clinical models (*in vitro* and *in vivo*),[18,43,124,128]
- correlative and/or case-control studies using biological specimens and data from patients,[18,43,51,122-124,126,130,132,134]
- literature reviews.[45,128,130,134]

Model III was somewhat different in terms of research designs, as it focused on the utilisation of statistical techniques for discovery, without providing much detail on the study methodology.[52,131-133]

Some papers proposed two complementary approaches:

1) Knowledge-driven – where identification of a new biomarkers is based on knowledge of disease biology and drug mechanism of action,[43-45,51,124]
2) Data-driven – where biomarkers are identified based on the association between the biomarker value and outcome, for example in GWAS.[43-45,51,124]

It was suggested that at this stage the understanding of the biological function of the biomarker would be ideal,[18,124,126,130] however as this is not always possible correlation to a clinical outcome could be sufficient.[18]

Two papers suggested that prior to discovery, consideration should be given to the feasibility of developing a biomarker to address a given question.[50,130] The proposed criteria were based on cost-effectiveness[130] and required assay parameters such as sensitivity or specificity.[50]

Analytical validation

Analytical validation was only included in model I and IV. However, some frameworks within the other two models included some forms of analytical validation at a different stage - for example during pre-discovery[133] and external validation[50] in model III.

The details of relevant study designs were rarely reported. These generally appeared to be comparing the biomarker assay to a reference standard and using either specimens from patients, or positive and negative controls,[122] in a manner similar to concordance studies for HercepTest described in section 2.4.5.

It was usually considered that this stage is when the analytical parameters of an assay should be established.[18,43,44,47,121-123] Finalising standard operating procedures was also discussed.[18,44]

Clinical validation

Stages corresponding to clinical validation in model I have been included in model II (phase I trial and phase II trial) and III (external validation). The aim was often to show the biomarker predicts a clinical outcome in a new dataset, independent of that used for discovery.[18,43-45,47,52,125,126,132] The relevant study designs varied depending on the model and the individual frameworks within the model. These included:

- retrospective correlative studies using archived specimens,[18,43,126]
- prospective studies where the biomarker does not form part of the primary hypothesis,[18,126]
- RCT with biomarker-based primary hypothesis,[128]
- analysis of samples from bio-banks.[18]

Model III papers generally referred to studies using an independent set of samples for external validation, however the design was not described in detail.[13,45,50,52,131-133]

Clinical utility

All models contained a clinical utility stage. The majority of the frameworks agreed that the best study design for assessment of clinical utility of a predictive biomarker would be a large prospective study, preferably an RCT. The exact methodology would depend on the situation, for example the feasibility of conducting such a trial. The strength of the evidence supporting the use of the biomarker should also be taken into account when designing such a trial. The most commonly discussed RCT designs correspond to those outlined in Chapter 1:

- biomarker-strategy (with or without randomisation in the control arm),[13,18,50,52]
- stratified,[18,47,50,128]
- enrichment,[13,47,50,52,128]

- prospective subgroup analysis of an RCT,[13,47,50,52]
- prospective-retrospective analysis of an RCT.[13,47,50,122]

It was also noted that a systematic review or meta-analysis of multiple trials may be used.[123,124] It was proposed that in some circumstances, for example when ethical considerations preclude an RCT, case-control or cohort methodology may be acceptable.[124,133] One paper suggested that a single-arm trial including only biomarker positive patients may be acceptable in cases where there is a low event rate.[13]

Implementation

This stage was included in all four models and focused on the steps necessary for the biomarker to become part of standard clinical practice. These included health technology assessment and implementation of biomarkers in clinical guidelines.[122,123,132,133] Some frameworks also mentioned audit of the use of the biomarker in actual clinical practice.[127,133]

2.6.2 BIOMARKER THRESHOLD AND FINAL ASSAY

The point at which the biomarker threshold and the final assay should be specified was rarely provided. Where discussed, it was proposed that the biomarker threshold should be defined as a result of:

- analytical validation (model I),[47]
- clinical validation (model I),[43] or
- clinical utility studies(model IV).[134]

Nine papers provided recommendations on when the final form of the assay or classifier should be specified:

- after analytical validation (model I),[47]
- after a phase III trial (model II),[129]
- after external validation (model III),[13,50,131-133]
- on completion of the clinical utility stage (model III).[131,133]

Finalising the assay at the end of the analytical validation was also indirectly suggested in papers proposing that by then, the analytical parameters of an assay should be established[18,43,44,47,121-123] and standard operating procedures finalised.[18,44]

2.7 DISCUSSION

This chapter reports the first systematic review of frameworks proposed in the literature for phased evaluation of predictive biomarkers. A similar approach has previously been utilised in a review of frameworks for phased evaluation of diagnostic tests.[135]

Broad literature searches were undertaken and inclusion decisions were made by two independent reviewers. This ensured that the possibility of missing relevant papers was minimised. Some papers which may potentially be considered relevant, such as the Cancer Research UK roadmap, were excluded, as insufficient detail was available to identify whether these in fact proposed a staged biomarker development process.[136]

Data extraction and synthesis was carried out by one reviewer only. This involved a number of subjective judgements, as for example on the aim and classification of biomarker development stages. It is possible that if undertaken by another reviewer, the classification would differ to some extent.

Twenty three papers were included in the synthesis. Of these, only nine reported being based on real-life experience or work of a multi-disciplinary group.[43,45,47,50,51,125,127,133,134] The remaining twelve did not report any basis for the proposed process.

All identified frameworks were mapped to four models which emerged from the literature review. The identified models differed with respect to their purpose and context. Model I provided the most general framework for phased evaluation of predictive biomarkers, without specifying the type of the biomarker or the stage of the drug life-cycle. Model II focuses on development of a predictive biomarker integrated into the standard drug development programme. Model IV focused on the development of biomarkers predictive of treatment safety, identified after the drug has been made available in clinical practice. Model III was distinguished by the fact that it aimed to describe development of a multi-marker classifier. Also its main focus was on the statistical techniques involved. For this reason, it also appeared to differ most from the remaining three models – both in terms of the stages proposed and in the language used.

The clinical area for which the frameworks were proposed was identified in 10 cases. In most cases it was cancer.[13,18,43,44,122,128,129] The other diseases were: autism,[121] cardiovascular,[130] and neurological and neuropsychological.[124] This aspect did not seem to influence the type of the proposed framework.

As shown in Figure 2.2, it was possible to identify general similarities in sequence and aims of stages across the different models. These comparisons were using model I as a starting

point, as it arguably assumed the broadest perspective. Model I included the stages summarised in Table 2.13.

Table 2.13 Summary of stages in model I

Stage	Description
Pre-discovery	focusing mainly on question formulation and data collection
Discovery	aiming at the identification of biomarkers for further development; ideally two approaches should be combined here: knowledge-driven and data-driven
Analytical validation	aiming to establish the accuracy of the biomarker assay
Clinical validation	Investigating the correlation between the biomarker and the outcome
Clinical utility	evaluating the benefit of the use of the biomarker in clinical practice
Implementation	focusing on measures which may ensure uptake of the biomarker in clinical practice

The study designs proposed for each stage varied between different models and papers. In some cases, such as analytical validation, there was little information on the most appropriate research methodology. The most detail was reported for the clinical utility stage, where different RCTs with the biomarker integral to their design were generally considered ideal. However it was acknowledged in a number of frameworks that this may not always be feasible.

There also seemed to be little mention or agreement on the time when the assay should be finalised and - for continuous biomarkers –the threshold established. This ranged from completion of the analytical validation stage[47] to completion of clinical utility.[131,133,134]

An interesting point was raised with regard to the biological plausibility of the biomarker being predictive. A number of frameworks argued that a good understanding of the role of the biomarker in the disease and drug mechanism should be achieved.[18,124,126,130] However, one of the papers acknowledged this may not always be possible and correlation of the biomarker value with a clinical outcome could be sufficient.[18]

It was also of interest for this review to identify criteria for entry into and completion of stages, also referred to in the literature as "decision gates".[92] This was very rarely discussed beyond claiming that satisfactory results of the current or previous stage should be obtained (for completion and entry criteria respectively).

One paper stressed the importance of involving a multidisciplinary panel at each stage when a decision on further research is to be made.[127] Other papers postulated

involvement of regulatory bodies in such decisions.[47,50,134] This appears to be a rational approach, as addressing biomarker development from one perspective may lead to substantial omissions. This was, for example observed in the frameworks matched to model III, which appeared to be focusing on the statistical analysis of classifiers and neglected issues associated with the laboratory procedures.

Criteria for termination of the biomarker development programme were generally not discussed. However, two papers noted that prior to undertaking any research, the feasibility of development of a biomarker useful in addressing a given clinical problem should be considered.[50,130]

Section 2.4 described the development of HER2 expression and trastuzumab. This was, in fact, an example of a drug developed to target a particular biomarker. Based on the available literature, the sequence of stages here involved:

- biomarker discovery,
- drug discovery (based on biomarker),
- drug pre-clinical development,
- drug early clinical development (phase I and II),
- parallel biomarker analytical validation and phase III trial (using a different assay to that later implemented in clinical practice),
- implementation.

This scenario appears closest to model II, however there are some important differences. In particular, the phase III trial utilised a different assay to evaluate patents' HER2 status to that which was later implemented in clinical practice. It appears that the use of a different assay in the phase III trial might be a serious limitation, particularly that it demonstrated a concordance with the assay later used in practice of only 79%. Although this discrepancy could be a result of using a relatively old case study, there are still a number of situations when multiple assays are available to evaluate the same biomarker. It is not always clear whether their results are comparable, as in the case of multiple PD-L1 assays in lung cancer.[137] These issues will be addressed in more detail in Chapter 7.

Finally, the proposed models are all based on the assumption that a phased system is advantageous. However, there is some disagreement in the literature and more flexible approaches have been suggested. For example, the Pharmaceutical Research and Manufacturers of America proposal suggests investigating whether biomarkers meet evidence standards for a given application, rather than adhering to particular stages.[138]

These flexible approaches can offer an alternative to a staged system. Even if such a point of view is taken, the existing frameworks may still be useful as a source of evidence standards to address particular issues.

2.8 CONCLUSIONS

To summarise the findings of the systematic review four models for predictive biomarker development were constructed based on the identified frameworks. The most complete, in terms of the aims, was model I (general), which included the following stages:

1) Pre-discovery
2) Discovery
3) Analytical validation
4) Clinical validation
5) Clinical Utility
6) Implementation

There were some broad similarities between the different models, however they did not always include the same stages. There was a large variety of study designs suggested for each stage, especially outside of evaluation of clinical utility. Very little information was available on the entry and completion criteria for each stage. The time for "locking" the final assay was rarely commented on. Where reported, it was completion of analytical validation, prior to or after the clinical utility stage. Similarly, defining the biomarker threshold was rarely discussed and where included, there was little agreement on when it should take place. Some papers suggested it should be established on completion of the analytical validation or the clinical utility stage. The clinical area which the frameworks were addressing did not seem to influence their content.

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CHAPTER 3. PREDICTIVE BIOMARKERS IN EUROPEAN MEDICINES AGENCY LICENSING - A SYSTEMATIC REVIEW

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Author Contributions

Kinga Malottki, led the design of the review, created all databases, applied inclusion criteria, extracted data, analysed the results and wrote the chapter
Mousumi Biswas, applied inclusion criteria and commented on the interpretation of the results
Jon Deeks advised on the design, interpretation of the results and the chapter
Richard Riley advised on the design, interpretation of the results and the chapter
Lucinda Billingham advised on the design, interpretation of the results and the chapter
Charles Craddock provided clinical advice where needed and advice on the interpretation of the results
Philip Johnson provided clinical advice where needed and advice on the interpretation of the results

Abstract

Aims: To investigate how many and what predictive biomarkers are currently included in European Medicines Agency licensing of drugs.

Background: Stratified medicine is often heralded as the future of clinical practice. A key part of stratified medicine is the use of predictive biomarkers, which identify patient subgroups most likely to benefit or least likely to experience harm from an intervention. However the impact of stratified medicine on treatment practice is unknown.

Methods: Indications and contraindications of all drugs considered by the EMA and published on their website were screened to identify predictive biomarkers. For all included Biomarker-Indication-Drug (B-I-D) combinations data were collected and summarised on the type of the biomarker, whether it selected a subgroup of patients based on efficacy or safety, therapeutic area, marketing status, date of licensing decision, date of inclusion of the biomarker in the indication or contraindication, and on orphan designation.

Findings: 49 B-I-D combinations were identified over 18 years. These included 37 biomarkers and 41 different drugs. All identified biomarkers were molecular. Six drugs (relating to 10 B-I-D combinations) had an orphan designation at the time of licensing. The identified B-I-D combinations were mainly used in cancer and HIV treatment, but also in hepatitis C and three other indications (cystic fibrosis, hyperlipoproteinemia type I, and methemoglobinemia). In 45 B-I-D combinations biomarkers were used as predictive of drug efficacy and in four of drug safety. It appeared that there was an increase in the number of B-I-D combinations introduced each year, however the numbers were too small to identify any definite trends.

Conclusions: Given the large body of literature documenting research into potential predictive biomarkers and extensive investment into stratified medicine, relatively few predictive biomarkers were included in licensing. These were also limited to a small number of clinical areas (mainly cancer and HIV). This might suggest a need for improvement in methods of translation from laboratory findings to clinical practice.

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3.1 BACKGROUND

3.1.1 ROLE OF STRATIFIED MEDICINE

Drugs are rarely effective in all patients and may be associated with serious adverse events.[8] The challenge of stratified medicine is to identify predictive biomarkers that identify patient subgroups (or strata) with a differential therapeutic response to a linked intervention, allowing more appropriate and effective use of interventions to maximise patient benefit and minimise the occurrence of serious adverse events.[9,139] Predictive biomarkers are defined particular to a treatment for a condition, where biomarker values are associated with differential efficacy or safety profile of that treatment.[16-19] The use of predictive biomarkers promises a more appropriate choice of treatment and it can also help to rationalise funding decisions, avoiding costs of futile treatment and of adverse events. However, the additional cost of measuring the marker has to be taken into account. Examples of successful use of predictive biomarkers include BC treatment with drugs such as tamoxifen, where it is prescribed to women who are oestrogen receptor positive,[140] or trastuzumab, which is only given to patients with HER2 overexpression in their tumour.[20]

Chapter 2 reviewed the potentially long and complex process of developing predictive biomarkers and there is a large body of literature documenting research into these.[141,142] Millions of pounds have been invested into stratified medicine, both in industry and through programs from funding bodies such as the Medical Research Council[143] and Cancer Research UK.[144] However, it has been hypothesised that stratified medicine has not been implemented in practice as much as expected. This chapter provides evidence of the impact of stratified medicine research to date and, if less than expected, this will highlight the need to review the underlying reasons and address the problems.

3.1.2 CHOICE OF DATA SOURCE

As mentioned in Chapter 1, in Europe a centralised drug evaluation by the EMA is required for a large number of drug types, including those with an orphan designation, granted to drugs intended for the treatment of a life-threatening or chronically debilitating condition which is either affecting no more than 5 in 10,000 people in the EU or when the revenue is unlikely to cover the investment in drug development.[145] Companies can also apply for a centralised marketing authorisation of other drugs.[55] Although the EMA does not license biomarkers, it evaluates drugs in groups of patients which can be defined by predictive biomarkers (for example trastuzumab is licensed for use in HER2 overexpressing BC patients, as described in the case study in Chapter 2).[56]

Therefore reviewing EMA licensing decisions is likely to give a broad overview of the impact of predictive biomarkers on treatment selection since 1995, when the EMA was established[57].

3.2 AIMS

This systematic review aimed to find out how many and what predictive biomarkers were included in the indications and contraindications considered by the EMA. The disease areas where predictive biomarkers have been used and whether there was any trend over time suggesting an increase in the use of predictive biomarkers were also investigated.

3.3 METHODS

3.3.1 UNIT OF ANALYSIS

A Biomarker-Indication-Drug (B-I-D) combination was defined as the unit of analysis, relating to the use of a predictive biomarker with a particular drug (defined here as the active substance rather than the trade name) for a particular condition or disease.[16-19] This was done to separate cases such as for example

- HER2 expression used to predict response to trastuzumab in BC, and
- HER2 expression used to predict response to trastuzumab in stomach cancer.

Safety biomarkers can be used in more than one disease area to predict the same adverse event. Therefore these indications were grouped into one B-I-D combination, as long as the adverse events predicted were the same.

3.3.2 DATABASE OF DRUGS IN EMA LICENSING

A database of all drugs identified in EMA licensing was created in Microsoft Excel 2010, which included the drug name, licensing status, indication and contraindication. The indications and contraindications of all drugs listed on the EMA website in either European Public Assessment Reports or Pending Decisions[146,147] (accessed on the 17th of January 2013) were included in the database.

3.3.3 SELECTION OF B-I-D COMBINATIONS

Selection of relevant B-I-D combinations was carried out in two stages, using the criteria shown in Box 3.1.

In the first stage all database entries were screened by two independent reviewers to identify those potentially including a predictive biomarker in the indication or contraindication. If an entry was identified by at least one of the reviewers as potentially relevant, it was included in the second stage of screening.

Box 3.1 Inclusion and exclusion criteria for identification of B-I-D combinations containing predictive biomarkers

To be included the biomarker had to:

1) be used in the indication and/or contraindication of the drug,
2) be associated with a particular treatment,
3) identify a subgroup of patients with a particular disease eligible for treatment with the drug.

Excluded biomarkers were:

1) associated with a non-therapeutic substance (for example vaccines),
2) not used as predictive, including:
    - used for diagnosis, screening or forming part of the disease definition (already established for defining a disease) or established disease subtype,
    - prognostic only (associated with outcome regardless of treatment and not predictive of treatment response[1])
    - associated with another treatment (for example the biomarker was not associated with the differential efficacy or toxicity of the drug of interest, but another drug given in combination with the drug of interest).

In the second stage, a list of potential B-I-D combinations was created in MS Excel based on the entries identified in the first stage. The list of potential B-I-D combinations was assessed by two independent reviewers using the inclusion/exclusion criteria shown in Box 3.1. This stage utilised, as necessary:

- information in the Summary of Product Characteristics (which sets out the information on the drug obtained in the assessment process and summarises its properties and clinical use together with the clinical trial evidence that was considered by the EMA[148]),

- the Scientific Discussion (which discusses the properties and clinical evidence in more detail),
- ICD10 classification,[149]
- targeted internet searches and
- expert advice.

Any disagreements between reviewers applying inclusion criteria were resolved by discussion.

3.3.4 DATA EXTRACTION

For the included B-I-D combinations data were collected using the information available on the EMA website into an Excel spreadsheet on items shown in Box 3.2.

Box 3.2 Data extraction items for the review of predictive biomarkers in EMA licensing

- type of the biomarker used as predictive,
- whether it selected a subgroup of patients based on efficacy or safety,
- therapeutic area,
- marketing status,
- date of licensing decision,
- date of inclusion of the biomarker in the indication or contraindication,
- presence of an orphan designation

To provide a context for the review, data were also collected on the total number of drugs licensed each year with and without an orphan designation.

3.3.5 DATA ANALYSIS

The majority of the data were summarised narratively. The numbers of B-I-D combinations considered each year were summarised using bar graphs. The time from the initial licensing of the drug to inclusion of the predictive biomarker was plotted. When drugs were discussed, this referred to the active substance, unless explicitly stated that the brand names were considered (as in Figure 3.3).

3.4 FINDINGS

As shown in Figure 3.1, 883 entries in the database of EMA licensing were screened, corresponding to an 18-year period (1995-2012). After the first stage of screening 203 potentially relevant B-I-D combinations were identified from 100 entries, as some indications and contraindications contained multiple potentially relevant biomarkers.

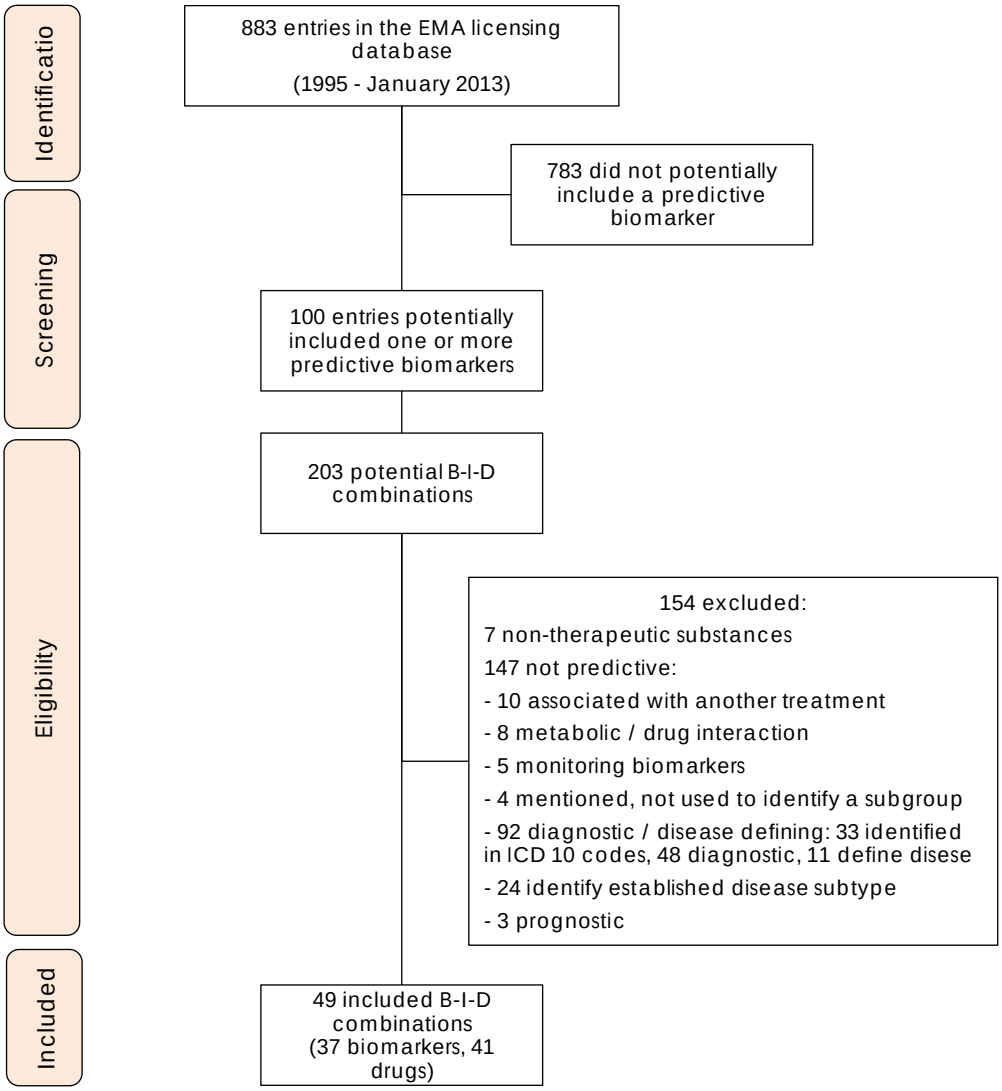


Figure 3.1 Flow diagram for the systematic review of predictive biomarkers in EMA licensing

In the second stage of screening 154 of these were excluded because the potential B-I-D combinations included a non-therapeutic substance (such as contrasting agent) in seven cases, or the biomarker was not predictive in 147 cases. Details of excluded potential B-I-D combinations are reported in Appendix 9. Forty nine B-I-D combinations were identified, including 37 biomarkers and 41 different drugs.

Most of the identified drugs were authorised, with the exception of:

- gemtuzumab ozogamicin (Mylotarg) – refused;
- zoledronic acid (Zometa) – pending (with negative recommendation);
- imatinib (Glivec) in one of the five identified indications (aggressive systemic mastocytosis) - withdrawn prior to refusal;
- amprnavir (Agenerase) – withdrawn;
- nelfinavir (Viracept) – withdrawn;

3.4.1 B-I-D COMBINATIONS OVER TIME

The number of new B-I-D combinations considered by the EMA each year is shown in Figure 3.2. It increased overall from zero or one per year in the late 1990s, to a maximum of 7 in 2011 and 2012. This was however not a steady increase, as this number showed fluctuation between 2000 and 2006, a decrease between 2006 and 2010, followed by an increase in the number in 2011 and 2012.

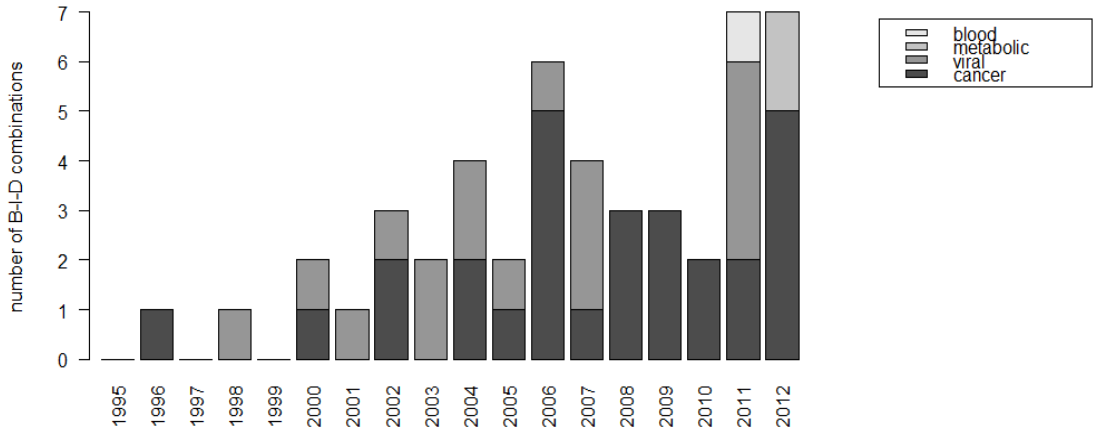
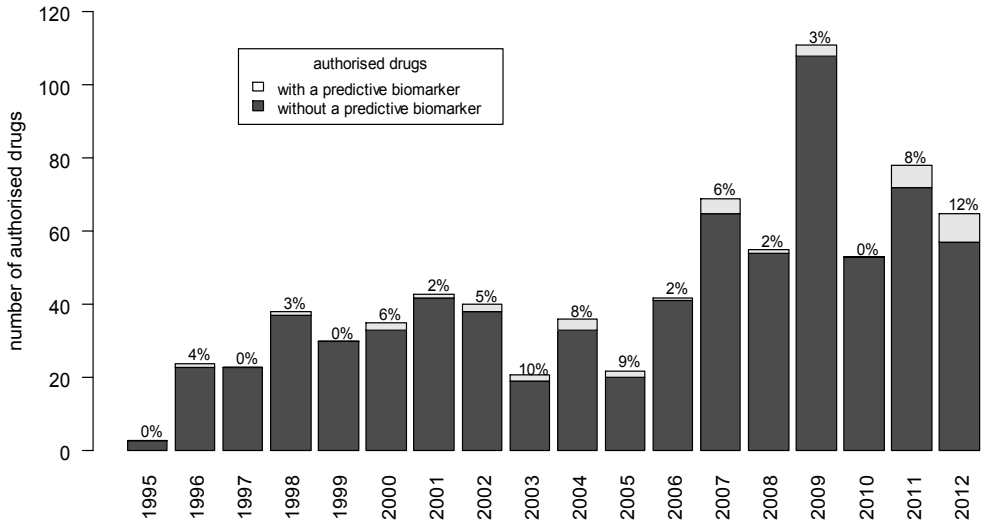


Figure 3.2 Number of new B-I-D combinations considered each year by disease area (includes biomarkers added after the drug was initially licensed)

When drug brands (including generic drugs) are considered, a predictive biomarker was included in the indication or contraindication when the drug was first licensed in 35 cases. For one drug (Xeloda, with capecitabine as the active substance) the date of inclusion of the biomarker was unclear from the documentation. For the remaining drugs the time from the initial licensing decisions to the inclusion of a predictive biomarker ranged from zero (initial decision included predictive biomarker) to ten years, as shown in Figure 3.3.

Drug brand names were used, as these correspond to individual licensing decisions; filled circles indicate a generic drug; Cap – capecitabine; MC – metylthionium chloride

Figure 3.3 Time from initial licensing of a drug to inclusion of a predictive biomarker in the indication or contraindication



Data for 2012 include 4 generic drugs (all with capecitabine as the active substance)

Figure 3.4 New drugs authorised each year with and without a predictive biomarker in the indication or contraindication (excludes biomarkers added after the drug was initially authorised)

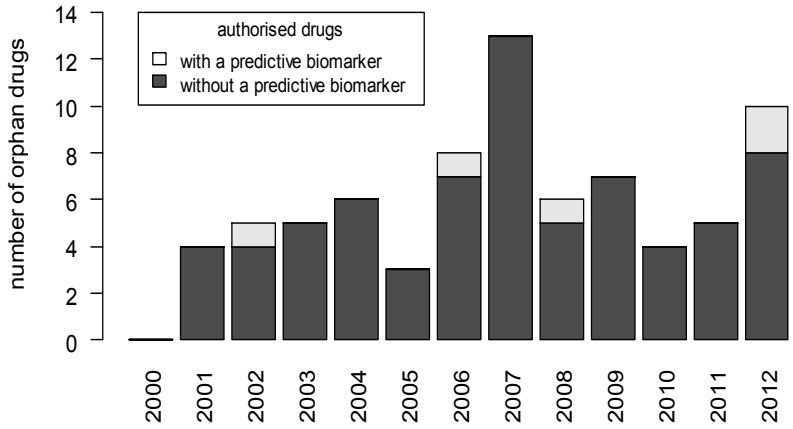
The proportion of new drug brands (including generic drugs) that already contain a predictive biomarker in the indication of contraindication was considered at the time of the first licensing decision. This increased over time and was close to 10% in 2003, 2004, 2005, 2011 and 2012 (shown in Figure 3.4).

3.4.2 DRUGS WITH AN ORPHAN DESIGNATION

As shown in Figure 3.5, six drugs associated with a predictive biomarker had an orphan designation at the time of licensing, however for two it was removed at the end of the marketing exclusivity period.[150] One of the six drugs (imatinib) was associated with five different predictive biomarkers in five different indications. Therefore, in total there were 10 B-I-D combinations including a drug with an orphan designation and this constituted 20% of all the identified B-I-D combinations.

3.4.3 IDENTIFIED B-I-D COMBINATIONS

The identified B-I-D combinations all contained molecular predictive biomarkers. Only four biomarkers were used to predict adverse events (reported in Table 3.1), while 33 were used to predict treatment efficacy (reported in Table 3.2).



Includes drugs where orphan designation was later removed

Figure 3.5 New orphan drugs authorised each year with and without a predictive biomarker in the indication or contraindication (excludes biomarkers added after the drug was initially authorised)

Most of the indications were for treatment of cancer (29 B-I-D combinations) and viral diseases, mainly HIV (17 B-I-D combinations). The remaining biomarkers were used to stratify metabolic and blood disorders (cystic fibrosis, hyperlipoproteinemia type I, and methemoglobinemia).

Table 3.1 Biomarkers predictive of adverse events identified in the review of EMA licensing

Biomarker	Indication	Drug
DPD deficiency	Colorectal Neoplasms Colonic Neoplasms Stomach Neoplasms Breast Neoplasms	Capecitabine (Xeloda and generic drugs: Capecitabine Accord; Capecitabine Krka; Capecitabine Medac; Capecitabine Teva)
DPD deficiency	Stomach Neoplasms	tegafur / gimeracil / oteracil (Teysuno)
HLA-B*5701 allele	HIV Infections	Abacavir (Kivexa; Trizivir; Ziagen)*
NADPH reductase deficiency	Methemoglobinemia	Methylthioninium chloride (Methylthioninium chloride Proveblue)

* HLA-B*5701 allele is predictive of hypersensitivity to abacavir, which is present in three three drugs: Kivexa (abacavir / lamivudine); Trizivir (abacavir / lamivudine / zidovudine); Ziagen (abacavir)

3.5 Discussion

Stratified medicine is promoted as key to the future of medicine, and is currently one of the most active areas of clinical research. This review likely provided the first indication of the number and nature of predictive biomarkers included in licensing in Europe based on the drug indications and contraindications on the EMA website. Forty nine B-I-D combinations were identified. All identified biomarkers were molecular. The identified B-I-D combinations were mainly used in cancer and HIV treatment, with only five used in other disease areas.

Table 3.2 Biomarkers predictive of efficacy identified in the review of EMA licensing

Biomarker	Indication	Drug
ALK gene rearrangement	Carcinoma, Non-Small-Cell Lung	Crizotinib (Xalkori)
BRAF V600 mutation	Melanoma	Vemurafenib (Zelboraf)
CCR5 tropism	HIV Infections	Maraviroc (Celsentri)
CD-33 expression*	Leukemia, Myeloid, Acute	gemtuzumab ozogamicin (Mylotarg)‡
EGFR expression	Colorectal Neoplasms	Cetuximab (Erbitux)
EGFR expression	Carcinoma, Non-Small-Cell Lung	Erlotinib (Tarceva)
EGFR mutation	Carcinoma, Non-Small-Cell Lung	Erlotinib (Tarceva)
EGFR mutation	Carcinoma, Non-Small-Cell Lung	Gefitinib (Iressa)
EpCAM expression	CancerAscites	Catumaxomab (Removab)
FIP1L1-PDGFR rearrangement	Hypereosinophilic Syndrome	Imatinib (Glivec)#
G551D mutation in CFTR gene	Cystic Fibrosis	Ivacaftor (Kalydeco)‡
genotype 1 HCV	Hepatitis C, Chronic	Boceprevir (Victrelis)
genotype 1 HCV	Hepatitis C	Telaprevir (Incivo)
HER2 expression	Breast Neoplasms	Lapatinib (Tyverb)
HER2 expression	Breast Neoplasms	Trastuzumab (Herceptin)
HER2 expression	Stomach Neoplasms	Trastuzumab (Herceptin)
HER2 expression	Breast Neoplasms	Everolimus (Afinitor)
HER2 expression **	Breast Neoplasms	pertuzumab (Perjeta)
Hormone dependency	Prostatic Neoplasms	Degarelix (Firmagon)
Hormone receptor expression**	Breast Neoplasms	zoledronic acid (Zometa)
Hormone receptor expression	Breast Neoplasms	Everolimus (Afinitor)
Kit (CD 117) expression	Gastrointestinal Stromal Tumors	Imatinib (Glivec)#
Kit (D816V) mutation***	Aggressive Systemic Mastocytosis	Imatinib (Glivec)#
KRAS mutation	Colorectal Neoplasms	Cetuximab (Erbitux)
KRAS mutation	Colorectal Neoplasms	Panitumumab (Vectibix)
LPL protein detectable	Hyperlipoproteinemia Type I	alipogene tiparvovec (Glybera)‡
oestrogen receptor expression	Breast Neoplasms	Fulvestrant (Faslodex)
oestrogen receptor expression	Breast Neoplasms	Toremifene (Fareston)
PDGFR gene rearrangements	Myelodysplastic-Myeloproliferative Diseases	Imatinib (Glivec)#
Philadelphia chromosome	Precursor Cell Lymphoblastic Leukemia-Lymphoma	Dasatinib (Sprycel)‡
Philadelphia chromosome	Precursor Cell Lymphoblastic Leukemia-Lymphoma	Imatinib (Glivec)#
t(15;17) translocation	Leukemia, Promyelocytic, Acute	arsenic trioxide (Trisenox)#
viral resistance mutations***	HIV Infections	Amprenavir (Agenerase)
viral resistance mutations	HIV Infections	atazanavir sulphate (Reyataz)
viral resistance mutations	HIV Infections	Darunavir (Prezista)
viral resistance mutations	HIV Infections	efavirenz / emtricitabine / tenofovir disoproxil (Atripla)
viral resistance mutations	HIV Infections	Emtricitabine (Emtriva)
viral resistance mutations	HIV Infections	emtricitabine /rilpivirine /tenofovir disoproxil (Eviplera)
viral resistance mutations	HIV Infections	Enfuvirtide (Fuzeon)
viral resistance mutations	HIV Infections	fosamprenavir calcium (Telzir)
viral resistance mutations	HIV Infections	lopinavir / ritonavir (Kaletra)
viral resistance mutations***	HIV Infections	Nelfinavir (Viracept)
viral resistance mutations	HIV Infections	rilpivirine hydrochloride (Edurant)
viral resistance mutations	HIV Infections	tenofovir disoproxil fumarate (Viread)
viral resistance mutations	HIV Infections	Tipranavir (Aptivus)

* refused, **pending, ***withdrawn,‡ drug designated an orphan medicine, # orphan designation has been removed at the end of exclusivity period

It is likely that the 49 identified B-I-D combinations from the EMA database do not represent a complete list of the predictive biomarkers used in practice as some B-I-D combinations could have been considered by national regulatory agencies, particularly for drugs considered before EMA was established in 1995. In addition, EMA licensing is not compulsory for some disease areas, such as mental health. However, a number of drugs with indications in depression of schizophrenia have been considered by the EMA. Therefore although this approach may not provide a complete list of all predictive biomarkers used in Europe, relatively few are likely to have been omitted, particularly from recent years.[57]

The fact that some of the identified B-I-D combinations included biomarkers introduced to an indication of an already licensed drug suggests that at least to some extent stratification occurring after the initial licensing of a drug was captured. However, the actual extent to which this takes place in clinical practice is difficult to evaluate. Furthermore, as mentioned in Chapter 1, in Europe there is no formal process for licensing predictive biomarkers for clinical use beyond the requirements of the *in vitro* diagnostic Directive.[54] Therefore a number of such biomarkers may be used in practice without inclusion in drug licensing.

Several types of biomarkers were excluded. These included biomarkers used for dose adjustments as they do not directly predict efficacy or adverse events (although inappropriate dose selection could limit the treatment efficacy or cause adverse events).[7] This review also excluded prognostic biomarkers. In practice these can be used for treatment selection (as for example Oncotype DX[151]), however this is based on the assessment of need for treatment rather than probability of patients responding to a particular therapy.[1] Only biomarkers associated with drug treatments were investigated. Other biomarkers may be used in practice with non-drug treatments (for example radiotherapy[152]).

The definition of a predictive biomarker can be difficult to apply, as over time predictive biomarkers may become part of a new definition of the disease or its subtype[23] and therefore be classed as diagnostic. In this evaluation diagnostic biomarkers (for example these included factor IX deficiency, or genetic testing for familial lipoprotein lipase deficiency) were excluded, as well as biomarkers used to identify an established subtype of a disease (mainly ST segment elevation and non-ST segment elevation myocardial infarction).

The spectrum of diseases where predictive biomarkers have been successfully developed is relatively narrow. This suggests a possible need for more research in other clinical areas.

The vast majority of the B-I-D combinations were associated with treatment efficacy and only four with safety. As adverse events associated with some treatments could be serious, the possibility to screen out patients at high risk prior to commencing treatment would be beneficial.

A relatively large proportion of the drugs with an associated predictive biomarker identified in this review had an orphan designation. This seems surprising, as convincing evidence to support the use of a drug in a subgroup of patients with a rare condition might be difficult to obtain, due to the small numbers of patients available to test the hypotheses.

It is difficult to provide accurate estimates of the extent of research into potential predictive biomarkers, however it has been suggested in 2011 that the number of publications on different biomarkers (not only predictive) was in the area of 15,000.[141] Another paper published in 2009, which reviewed genetic markers evaluated as potential predictors of response to treatment, found that 541 different genes were investigated as potential predictive biomarkers in 1,668 papers.[142] It can be reasonably expected that this number largely increased since these papers were published. This review shows that few predictive biomarkers have been included in licensing relative to this large body of literature documenting numerous potential predictive biomarkers. Therefore, in spite of the substantial investment in research, the promise of stratified medicine is probably not yet being realised to a large extent. The reasons for this might include poor translation of findings of laboratory studies into clinical context (the frameworks for which were discussed in Chapter 2), or the failure to identify effective predictive biomarkers and treatments. Even though it is becoming easier and cheaper to gather huge sets of genomic data, its interpretation is challenging, which can potentially hinder translational research. Recognising this, initiatives have been undertaken both in the USA (National Institutes of Health and the Food and Drug Administration) and UK (Medical Research Council) to promote the translation of basic research into clinical practice.[143] Also the availability of datasets such as the Cancer Cell Line Encyclopaedia and a similar UK initiative might contribute to the faster progress of stratified medicine.[153,154] The relatively small number of predictive biomarkers identified in licensing might also indicate the need for more sound methodological standards for biomarker discovery and development.[155] The evidence supporting these 49 B-I-D combinations will be considered in Chapter 5 and Chapter 6 to find out what evidence standards seem to be sufficient in practice. However first, Chapter 4 will explore the issues considered by the EMA when evaluating the evidence supporting the identified B-I-D combinations.

3.6 CONCLUSIONS

This chapter likely provides the first indication of the number and nature of predictive biomarkers included in licensing in Europe using systematic review methodology. Given the large body of literature documenting research into potential predictive biomarkers and extensive investment into stratified medicine, relatively few predictive biomarkers were included in licensing. These were also limited to a small number of clinical areas.

Forty nine B-I-D combinations were identified over 16 years, which included 37 biomarkers and 41 different drugs. There appeared to be an increase in the number of B-I-D combinations introduced each year, however the numbers were too small to attempt the identification of any trend. All identified biomarkers were molecular. The clinical areas were mostly limited to cancer and HIV treatment. The other indications were hepatitis C, cystic fibrosis, hyperlipoproteinemia type I, and methemoglobinemia. Of the identified 49 B-I-D combinations, ten included a drug with an orphan designation.

The relatively low number of identified predictive biomarkers could potentially indicate the need for improvement in methods of translation from laboratory findings to clinical practice.

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CHAPTER 4. ISSUES IN INCLUSION OF PREDICTIVE BIOMARKERS IN EMA LICENSING – A TEXT ANALYSIS

Author contributions:
Kinga Malottki led the design of the study and carried the text analysis; drafted the chapter
Jon Deeks advised on the design, text analysis and the chapter
Lucinda Billingham advised on the design, text analysis and the chapter

Acknowledgements
Dr Jonathan Mathers provided advice on possible approaches to this analysis

Abstract

Background: The review of drugs considered by the EMA with a predictive biomarker in the indication or contraindication described in Chapter 3 identified 49 B-I-D combinations. This chapter focuses on the rationale provided by the EMA for their recommendations and the issues that seemed to be considered important when considering indications and contraindications containing predictive biomarkers.

Aim: To explore the rationale provided by the EMA to support the inclusion of predictive biomarkers in drug indications and contraindications; to identify criteria used and compare these to an initial *a priori* framework.

Methods: A framework analysis of Scientific Discussions and Assessment Reports obtained from the EMA website was undertaken. Seven initial criteria were used as a starting point and as new criteria emerged from the data, they were added to the framework. For each recommendation it was noted which criteria were mentioned and whether they were met.

Findings: The analysis identified 41 different criteria and these were grouped into 13 themes. The themes that were most frequently commented on were: population, study design, clinical efficacy evidence, toxicity, context, pre-efficacy evidence supporting the drug. An analysis of critical issues in negative recommendations highlighted the importance of clinical efficacy, but also identified a number of other crucial themes such as population and study design. A case study highlighted the importance of the context in interpretation of study results.

Discussion: A comprehensive text analysis was undertaken that identified the issues discussed in EMA documentation relevant to B-I-D combinations. Although it was possible to identify certain criteria that were used more frequently or that appeared critical in negative recommendations, it was difficult to identify any clear patterns indicating the necessary or sufficient criteria. Also how the same criteria were applied to different B-I-D combinations seemed to be very dependent on the context in which the B-I-D combination was considered. The findings of this text analysis need to be considered as exploratory due to some limitations. The text analysis was undertaken by one reviewer only and therefore it is possible that some issues were missed. It is also likely that these results are influenced by selective reporting in EMA documents of the issues discussed.

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4.1 BACKGROUND

The review of drugs considered by the EMA with a predictive biomarker in the indication or contraindication described in Chapter 3 identified 49 B-I-D combinations. The next stage of research was planned to investigate the evidence supporting EMA recommendations for these B-I-D combinations. For this purpose a set of seven criteria was proposed (as described below in section 4.3.3).

Prior to applying these criteria an exploratory text analysis reported in this chapter was carried out to identify the issues considered important by the EMA while issuing recommendations for these drugs. This was undertaken to possibly modify the list of criteria considered relevant, should important new issues be identified. The criteria used by the EMA were also of interest, as these would imply at least some of the issues important for implementation of predictive biomarkers in clinical practice. In particular of interest were criteria for inclusion of predictive biomarkers in indications and contraindications which could be considered:

- necessary conditions - if the condition was not met, the indication containing the predictive biomarker would be rejected.[156]
- sufficient conditions - if the entire set of such conditions was met, the indication containing the predictive biomarker would be accepted.[156]

The Scientific Discussions and Assessment Reports were analysed as these documents provided a record of the discussions that lead to issuing recommendations. Within these documents the focus was on the discussion sections, as these provided judgments, rather than only summarising the evidence available. This was undertaken using a framework analysis using the proposed seven criteria as a starting point.

4.2 AIMS

This framework analysis aimed to explore the rationale underpinning the EMA recommendations on inclusion of predictive biomarkers in drug indications and contraindications. It also undertook to compare these criteria with seven proposed in an initial framework. In particular, the questions shown in Box 4.1were addressed.

Box 4.1 Questions addressed by text analysis of EMA documents

1) What criteria have been used by the EMA to support their decisions?
2) What is the relationship between the criteria used by the EMA and the criteria proposed in the initial framework, as described in section 4.3.3 below?
3) Are there any criteria which can be considered necessary conditions for a predictive biomarker to be included in an indication?
4) Is there a set (or sets) of criteria which can be considered sufficient conditions for a predictive biomarker to be included in an indication?
5) Is there any variation in the criteria used depending on the clinical area, biomarker type (efficacy or safety) or presence or absence of an orphan designation?

4.3 METHODS

4.3.1 DATA SOURCE

Scientific Discussions and Assessment Reports were obtained from the EMA website for all B-I-D combinations identified in the review described in Chapter 3. These documents were chosen, as they aim to report on the evidence and discussions underpinning EMA recommendations on drug licensing.

After a drug is granted marketing authorisation, the EMA publishes the Committee for Human Medicinal Products (CHMP) report as the European Public Assessment Report (EPAR), which includes "the reasons for its opinion in favour of granting authorisation, after deletion of any information of a commercially confidential nature". For negative decisions the EMA also publishes such information together with reasons for the refusal.[157]

The EPARs do not have clearly identified authors, although the rapporteurs responsible for an evaluation of a particular drug can be identified. These documents follow a standard layout, defined by a template.[158] An EPAR contains either a Scientific Discussion or an Assessment Report and these documents outline the evidence and reasoning behind the recommendations issued by the EMA.

The length of the Scientific Discussions or Assessment Reports varies substantially between different EPARs and for the included B-I-D combinations it was between five[159] and 147 pages.[160] The structure of these documents is generally similar, as it follows a template, as shown in Box 4.2.

Box 4.2 Standard sections included in EMA documents

1. Introduction – providing background information about the disease, available treatment options, the new drug and its possible position in the treatment pathway.
2. Chemical, pharmaceutical and biological aspects – including information on issues such as drug manufacturing, chemical composition and stability.
3. Toxico-pharmacological aspects - including information on pharmacokinetics, pharmacodynamics and toxicology, usually based on preclinical studies.
4. Clinical aspects – providing information on the investigation of the drug in humans, including information from a range of studies, with the level of evidence depending on the particular drug in question; usually includes a discussion of clinical efficacy.
5. Clinical safety – providing details of the investigation of safety in all patients that have been exposed to the drug; usually includes a discussion of clinical safety.
6. Overall conclusion and benefit-risk assessment.

In some of the documents certain sections were not present. For example, when the document discussed an extension of a therapeutic indication of an already marketed drug, the introduction or the chemical, pharmaceutical and biological aspects were often omitted.

For the purpose of this analysis, the introduction and the last three sections were of primary interest, as the focus was the identification of criteria applied to clinical-level data. Therefore, information was sought mainly in these sections.

4.3.2 Framework analysis

Framework analysis of EMA documents was conducted by one reviewer. This approach to text analysis was taken, as it offered a structured process to data collection and analysis.[161] It also permitted the use of an initial framework as a starting point and comparison of data obtained in the review to that framework.[161,162] This approach not only allowed the identification of the criteria used by the EMA, but also exploration of how these related to the criteria considered important prior to undertaking this analysis.

The initial *a priori* framework was constructed based on background information and team discussion.[161,162] Data collection from identified documents was undertaken

following an iterative approach. In each iteration a sample of five B-I-D combinations (four for the last iteration, due to the total number of B-I-D combinations identified) was compared with the proposed framework and the information on whether the proposed criteria were discussed and met was recorded. If new criteria were identified from a document, the framework was expanded to incorporate these.

4.3.3 INITIAL FRAMEWORK

The initial framework was constructed from criteria considered important in assessing the methodology and strength of evidence supporting inclusion of a biomarker in a drug indication. An initial overview of the evidence underlying B-I-D combinations was utilised to support the selection of the criteria, before the reports were read in full detail. The initial framework comprised the criteria shown in Box 4.3.

Box 4.3 Initial criteria

1) The population in studies was in accordance with the population identified by the drug indication.
2) The design of the studies supporting the inclusion of the biomarker in the indication was appropriate.
3) The primary outcome assessed in the supporting studies was appropriate.
4) The sample size in studies was sufficiently large.
5) The biomarker status was available for a sufficiently large proportion of patients in the studies.
6) The patients with available biomarker status were representative of the whole trial population.
7) There was sufficient evidence supporting the biomarker based on the results for the primary outcome.

4.3.4 IDENTIFICATION OF SAMPLES FOR ITERATIONS

Each iteration comprised five B-I-D combinations selected to provide a maximum variation sample[163] - to provide a range of B-I-D combinations in terms of disease areas considered, type of the biomarker (efficacy or safety), orphan status and the final EMA decision. The aim of this structure of the analysis was to increase the chance of identifying new criteria as early as possible in the process. This ensured that as new criteria emerged, these were included in the current and following iterations and thus the possibility of missing criteria was minimised. This was particularly important as the analysis was

performed by one reviewer only. The sample for the first iteration was selected so that the five documents described drugs belonging to all of the below categories:

- a cancer treatment,
- an HIV treatment, and
- a treatment for a disease other than cancer or HIV,
- an efficacy biomarker,
- a safety biomarker,
- a drug with an orphan designation,
- a drug without an orphan designation,
- an authorised drug,
- a rejected drug.

The following samples were identified using the same criteria as the first iteration, unless no more B-I-D combinations in a given category were left. In that case they were substituted by B-I-D combinations meeting other criteria from the list above.

4.3.5 DATA COLLECTION

All texts were compared with the initial framework. For each criterion it was recorded whether it was mentioned and considered by the EMA to be met. A criterion was considered as mentioned if it was present in any discussion section of the document. Statements made elsewhere in the document were only included if they were expressed as a normative statement (providing a judgement of facts). Factual (or positive) statements outside of a discussion section were not considered relevant. The difference is demonstrated in the example of two possible statements on the same issue:

> Factual statement: *The number of patients included in all studies was 455.* (excluded)
>
> Normative statement: *The number of patients included in all studies was 455 and was considered sufficient.* (included)

A quote from the original document to support this was also recorded. *De novo* criteria were added to the initial framework and from that point it was recorded whether any were mentioned and/or met in the subsequent B-I-D combinations. All information was recorded in an Excel spreadsheet.

4.3.6 DATA SUMMARY

The identified criteria were grouped into themes based on their subject. The criteria and themes were discussed in the context of the initial framework (outlined in section 4.3.3). The frequency of the use of each criterion and theme in positive and negative recommendations was summarised. Variations in criteria used were explored based on factors outlined in aim 5:

- clinical area,
- biomarker type (efficacy or safety),
- presence or absence of an orphan designation.

An attempt was made to identify criteria that may be considered necessary and sufficient for marketing authorisation of a drug with a predictive biomarker in the indication or contraindication. This was undertaken taking into account the constraints of a relatively small sample of available EPARs. To identify the necessary conditions for marketing authorisation criteria and themes were considered that were:

- not met by at least one negative recommendation and
- at least partially met, unclear or not mentioned for all positive recommendations.

For the set of sufficient conditions for marketing authorisation, it was attempted to identify the minimum set of criteria and themes met in positive recommendations.

4.3.7 PILOTING

The first iteration of the framework analysis was used to pilot the data collection process. In case of difficulties in carrying out the planned analysis, modification would be made. However, as there were no issues in undertaking the pilot iteration, the text analysis was carried out without modifications.

4.4 FINDINGS

4.4.1 EMA RECOMMENDATIONS IDENTIFIED

Details of the selection of cases for text analysis are presented in the flow diagram in Figure 4.1. A Scientific Discussion or an Assessment Report was obtained for 48 B-I-D combinations. For two B-I-D combinations the relevant Scientific Discussion or Assessment Report was not available (HLA-B*5701 allele in treatment of HIV infection with abacavir and D816V mutation in c-Kit in treatment of systemic mastocytosis with imatinib). An additional B-I-D combination was also identified from the analysis of the documents

available on the EMA website[§] (EGFR expression in treatment of NSCLC with cetuximab). In addition a single B-I-D combination provided two recommendation cases, as the EMA first issued a negative recommendation and later revised it to a positive one (LPL protein expression in treatment of hyperlipoproteinemia type I with alipogene tiparvovec). This meant there were 49 cases for the analysis.

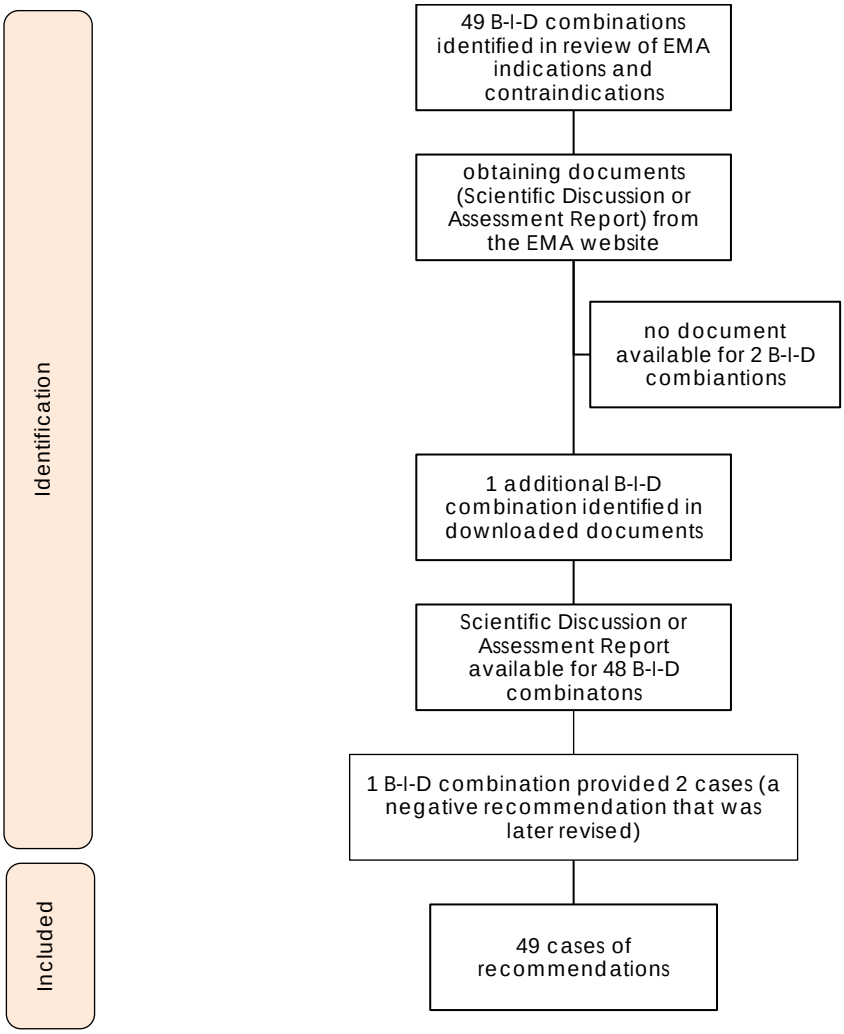


Figure 4.1 Flow diagram for identification of cases for framework analysis of EMA recommendations

4.4.2 IDENTIFIED CRITERIA

In total, 41 different criteria were identified, including the seven originally proposed and these are listed in Box 4.4 and Box 4.5. Where possible, the criteria identified were grouped

[§] This B-I-D combination (cetuximab for treatment of EGFR expressing NSCLC) was only identified on downloading all Scientific Discussion documents from the EMA website. The relevant indication was not mentioned on the EMA website, but one of the downloaded documents contained a discussion of the negative recommendation

into common themes. These criteria were identified during different iterations from 45 positive and four negative recommendations.

4.4.3 Themes based on the original criteria

As well as collecting information on the use of the original seven criteria, new criteria that appeared to fall within the same theme were added to the list (as shown in Box 4.4). The themes were generally based on the original criteria with the exception of the theme relating to results for the primary outcome. The themes are described below, however detailed descriptions of the criteria falling within each theme are not included, but are provided in Box 4.4 and their use reported in section 4.4.5.

4.4.3.1 Theme 1: Population

This theme included one criterion – whether the population in the studies supporting the B-I-D indication was in accordance with the indication. The population characteristics were usually mentioned, however normative statements were not always provided. An example of where this criterion was not met is:

> *"the pivotal trials with gemtuzumab included an ill-defined population, which in many cases could have been exposed to intensive re-induction chemotherapy. This population does not correspond to the claimed indication. Because the studies and claimed populations are different, it is impossible to extrapolate the results observed to the claimed indication."*[164]
>
> (CD-33 expression – AML - gemtuzumab ozogamicin)

4.4.3.2 Theme 2: Study design

This theme focused on the appropriateness of the study design. It comprised four criteria, as shown in Box 4.4, encompassing different features of study design. In cases when the design was judged as appropriate, a brief statement was usually provided, such as:

> *"The design and duration of studies is in line with regulatory requirements"*[165]
>
> (G551D mutation - cystic fibrosis – ivacaftor)

When the design was considered inadequate, more detail was usually given:

> *"according to CHMP guidelines, this full application should have been based on data generated by randomised controlled clinical trials rather than by open-label, non-comparative studies"*[164]
>
> (CD-33 expression – AML - gemtuzumab ozogamicin)

Box 4.4 Themes based on the original criteria including newly identified items falling within the same theme

Theme 1: Population – population in studies was in accordance with the population identified by the drug indication*

Theme 2: Study design

2.1. design of the studies supporting the inclusion of the biomarker in the indication was appropriate*

2.2. amendments to trials were acceptable

2.3. imbalances between groups in trials were acceptable or no imbalances were present

2.4. level of possible bias was acceptable

2.5. statistical approach to data analysis was adequate

Theme 3: Primary outcome - primary outcome assessed in the studies was appropriate*

Theme 4: Sample size - sample size in studies was sufficiently large*

Theme 5: Proportion of patients with biomarker status available - biomarker status was available for a large enough proportion of patients in the studies*

Theme 6: Subgroup with available biomarker representative - patients with available biomarker status were representative of the whole trial population*

Theme 7: Clinical efficacy evidence

7.1. sufficient evidence supporting conclusions based on the results for the primary outcome

- 7.1.a. irrespective of biomarker
- 7.1.b. supporting the biomarker (including enrichment design)*

7.2. sufficient evidence supporting conclusions based on the results for the secondary outcomes

- 7.2.a. irrespective of biomarker
- 7.2.b. supporting the biomarker (including enrichment design)

7.3. sufficient evidence supporting conclusions based on the results for any outcomes

- 7.3.a. irrespective of biomarker
- 7.3.b. supporting the biomarker (including enrichment design)

7.4. sufficient evidence to conclude clinical benefit

- 7.4.a. in all patients, irrespective of the biomarker status
- 7.4.b. in the biomarker-defined group of patients (including enrichment design)

7.5. consistency of results across subgroups (other than defined by the biomarker)

7.6. sufficiently long-term data provided

7.7. results reproduced in more than one study

* original a priori criteria

4.4.3.3 THEME 3: PRIMARY OUTCOME

This theme comprised one criterion, which reflected whether the primary outcome used in studies supporting the B-I-D combination was appropriate.

Again positive judgements were usually in the form of a brief statement, while negative opinions on the primary outcome provided more detail, as for example:

> *"due to the short survival expectancy in this group of patients, OS [overall survival] would have been a more adequate primary endpoint"*[166]
>
> (HER2 expression - Breast Neoplasms – lapatinib)

4.4.3.4 THEME 4: SAMPLE SIZE

This theme comprised one criterion – whether the sample size in included studies was adequate. It appeared that the information on the sample size was mainly provided as part of a descriptive summary of studies, rather than a criterion for judgement on the strength of supporting evidence, as in the following example:

> *"A total of 356 patients with EGFR-expressing metastatic colorectal cancer (…) received the combination treatment of cetuximab with irinotecan."*[167]
>
> (EGFR expression - colorectal neoplasms – cetuximab)

4.4.3.5 THEME 5: PROPORTION OF PATIENTS WITH BIOMARKER STATUS AVAILABLE

This theme comprised one criterion – whether the proportion of patients form studies supporting the B-I-D combinations with an available biomarker status was sufficiently large. Such information was discussed in few cases, for example:

> *"The SAG [Scientific Advisory Group] expressed concerns about the results submitted, in particular about the large amount of missing data with respect to EGFR mutation status which should have been controlled by design and conduct of the clinical studies. In this respect, the clinical studies presented were considered to be inadequate..."* [168]
>
> (EGFR mutation – NSCLC – gefitinib)

4.4.3.6 THEME 6: SUBGROUP WITH AVAILABLE BIOMARKER REPRESENTATIVE

This theme again comprised only one criterion – whether patients in the studies supporting the B-I-D combinations with an available biomarker status were representative of all the patients in these studies.

This criterion was mentioned in only one of the 49 analysed documents, where it was considered not met:

> *"[Referring to last line setting] In the KRAS evaluable population, there is an imbalance with respect to ECOG PS favouring the experimental arm. (…) [Referring to second line setting] There is an imbalance with respect to age favouring the experimental arm in the KRAS evaluable population. Comparing FAS [full analysis set] with KRAS, it is noticed that ECOG PS tended to be poorer in the KRAS population"*[169]
>
> (KRAS mutation - colorectal neoplasms – cetuximab)

4.4.3.7 THEME 7: CLINICAL EFFICACY EVIDENCE

Initially this theme included the strength of evidence supporting the biomarker (or drug – for enrichment designs) based on results for the primary outcome. However, analysis of EMA documents showed that the decisions were based on a wider range of outcomes and also took into account efficacy evidence irrespective of the biomarker status. This theme was expanded to include criteria evaluating whether there was sufficient evidence to conclude that the drug provided clinical benefit, which involved a judgement of the importance and relevance of the efficacy findings from studies. Other criteria considered within this theme are reported in Box 4.4.

4.4.4 NEW THEMES IDENTIFIED

Twenty criteria were identified that did not fall within the themes constructed based on the initial seven criteria. These were grouped into six themes, as shown in Box 4.5.

4.4.4.1 THEME 8: TOXICITY

This theme comprised one criterion – whether the drug toxicity was considered acceptable. This was usually commented on in the context of treatment efficacy, as for example:

> *"In conclusion, in the particular context of the proposed indication, Trisenox presented an acceptable safety profile, even if some particular concerns remained in the monitoring some adverse events..."*[170]
>
> (t(15;17) translocation and/or PML/RAR-α gene – APL- arsenic trioxide)

Box 4.5 New criteria and themes identified in the text analysis of EMA documents

Theme 8: Toxicity – drug toxicity was acceptable

Theme 9: Context

9.1. drug offers more acceptable route/ mode of delivery
9.2. no cross resistance with other available treatments observed
9.3. sufficient evidence available to support the B-I-D combination compared to other available treatment options
9.4. drug addressed an unmet clinical need
9.5. biomarker prognostic of poor outcome for standard care
9.6. novel mechanism of drug action/ new active substance

Theme 10: Biomarker test

10.1. availability of biomarker test on the market or clear guidelines for test
10.2. biomarker test is accurate
10.3. information on how the biomarker was assessed in studies was sufficient
10.4. correlation between continuous biomarker level and treatment effect

Theme 11: External evidence

11.1. supporting evidence from drugs with similar mechanism of action
11.2. evidence from previous studies (for example when indication is being broadened)
11.3. conclusions supported by published meta-analysis or other literature sources

Theme 12: Pre-efficacy evidence supporting the biomarker

12.1. evidence from pre-clinical studies to support biomarker
12.2. evidence from mechanism of action to support biomarker

Theme 13: Pre-efficacy evidence supporting the drug

13.1. quality of the product acceptable
13.2. preclinical studies provide sufficient information
13.3. sufficient evidence available to support selected dose and/or duration of treatment
13.4. ADME/ pharmacokinetics characterised sufficiently

4.4.4.2 THEME 9: CONTEXT

This theme focused on the wider context in which the drug was considered and included criteria such as availability of the treatments, how novel the drug was, whether the

biomarker used to identify patients for treatment with the drug was also prognostic of a poor outcome for standard care (details provided in Box 4.5).

The wording was usually relatively brief, as for example:

> *"crizotinib has successfully addressed a high unmet medical need for a relatively rare NSCLC subtype"*[171]
>
> (ALK gene rearrangement – NSCLC – crizotinib)

4.4.4.3 THEME 10: BIOMARKER TEST

This theme comprised four criteria focusing on the performance of the biomarker test used in studies supporting the B-I-D combinations and the availability of a biomarker test for use in clinical practice. For example, the use of such a criterion was identified in a case where the EMA document appeared critical of the available biomarker tests:

> *"It is also acknowledged that EGFR status may be subject to measurement error (false-positive and false-negative results, nonvalidated methods, arbitrary cut-off values for defining positive patients, etc.)"*[172]
>
> (EGFR expression – NSCLC – erlotinib)

4.4.4.4 THEME 11: EXTERNAL EVIDENCE

This theme focused on evidence outside of the B-I-D combination that could potentially provide some additional information to support the decision. This could involve evidence from drugs with a similar mechanism of action, studies of the same drug used in a different indication or published literature.

An example of evidence from drugs with similar mechanism is shown below:

> *"Even though publications are generated in a different indication, the general scientific knowledge of AAV [adeno-associated virus] vectors has increased in particular with regard to long term expression of protein and knowledge related to long term safety of AAV vector therapies. These data should also be taken into consideration for Glybera using a broader approach."*[160]
>
> (LPL protein expression - hyperlipoproteinemia type I - alipogene tiparvovec)

4.4.4.5 THEME 12: PRE-EFFICACY EVIDENCE SUPPORTING THE BIOMARKER

This theme included two criteria, which judged whether the predictive biomarker was supported by pre-clinical studies and the proposed mechanism of drug action. For

example, *in vitro* studies were considered to support the association of particular virus mutations with resistance to a drug:

> "*HIV-1 resistance, as observed in vitro and in HIV-1 infected patients to emtricitabine develops as the result of changes at codon 184 causing the methionine to be changed to a valine of the HIV reverse transcriptase.*"[173]
>
> (viral resistance – HIV – emtricitabine)

4.4.4.6 THEME 13: PRE-EFFICACY EVIDENCE SUPPORTING THE DRUG

This theme comprised four criteria which judged whether evidence from preclinical, pharmacodynamics and pharmacokinetic studies was sufficient. The criteria were often discussed in detail, however brief normative statements were also provided, as for example:

> "*The pharmacokinetics profile of emtricitabine was well defined*"[173]
>
> (viral resistance – HIV – emtricitabine)

It also included acceptable quality of the drug, which was usually commented on in a brief statement, such as:

> "*The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC*"[174]
>
> (DPD deficiency - colorectal, colonic, stomach and breast neoplasms – capecitabine)

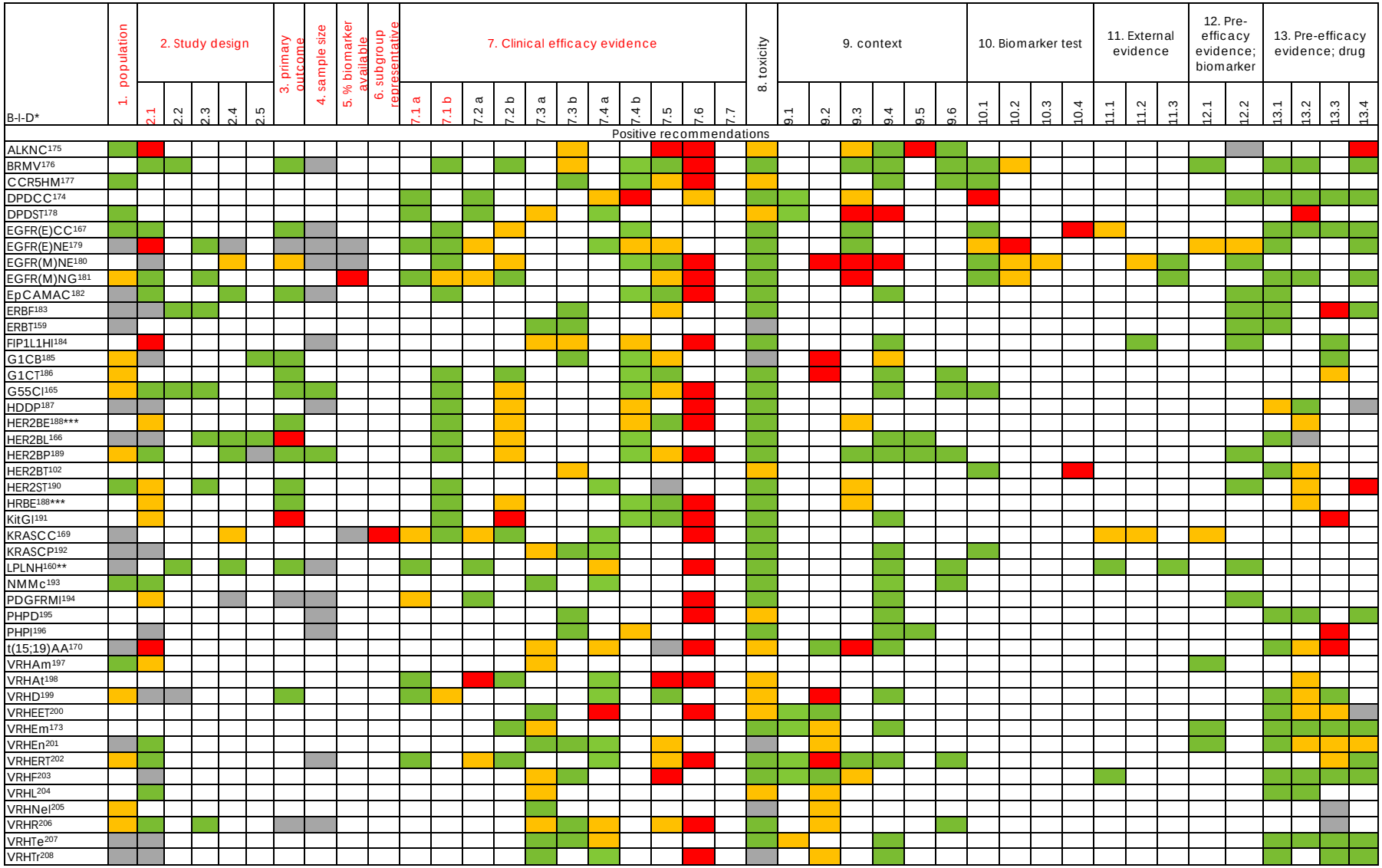
4.4.5 USE OF CRITERIA

The use of different criteria within each B-I-D combination is shown in Table 4.1, while the frequency of use of different criteria is shown in Figure 4.2 for B-I-D combinations with positive recommendations and Figure 4.3 for those given negative recommendations.

4.4.5.1 THEME 1: POPULATION

At least some of the population characteristics were usually reported (in 31 of 49 recommendations). These were usually either judged as at least partly representative of the population in the drug indication, or only reported without provision of a normative statement. In two negative recommendations the study population was judged as not in accordance with the drug indication.

Table 4.1 Criteria identified in the framework analysis grouped by theme and the extent to which these were met in EMA documents



B-I-D*	1. population	2. Study design					3. primary outcome	4. sample size	5. % biomarker available	6. subgroup representative	7. Clinical efficacy evidence										8. toxicity	9. context						10. Biomarker test				11. External evidence			12. Pre-efficacy evidence; biomarker		13. Pre-efficacy evidence; drug			
		2.1	2.2	2.3	2.4	2.5					7.1 a	7.1 b	7.2 a	7.2 b	7.3 a	7.3 b	7.4 a	7.4 b	7.5	7.6	7.7		9.1	9.2	9.3	9.4	9.5	9.6	10.1	10.2	10.3	10.4	11.1	11.2	11.3	12.1	12.2	13.1	13.2	13.3	13.4
Negative recommendations																																						
CD33AG[164]																																						
EGFR(E)NC[209]																																						
HRBZ[210]																																						
LPLNH[160]**																																						

Red text – *a priori* criteria; black text – *de novo* criteria; *the abbreviated B-I-D combinations are explained in Appendix 10; **initially rejected, authorised after revision; *** based on one scientific discussion document

Details of each criterion are reported in Box 4.4 and Box 4.5

Criterion met
Criterion partly met
Criterion not met
Criterion mentioned, but unclear whether met
Criterion not discussed

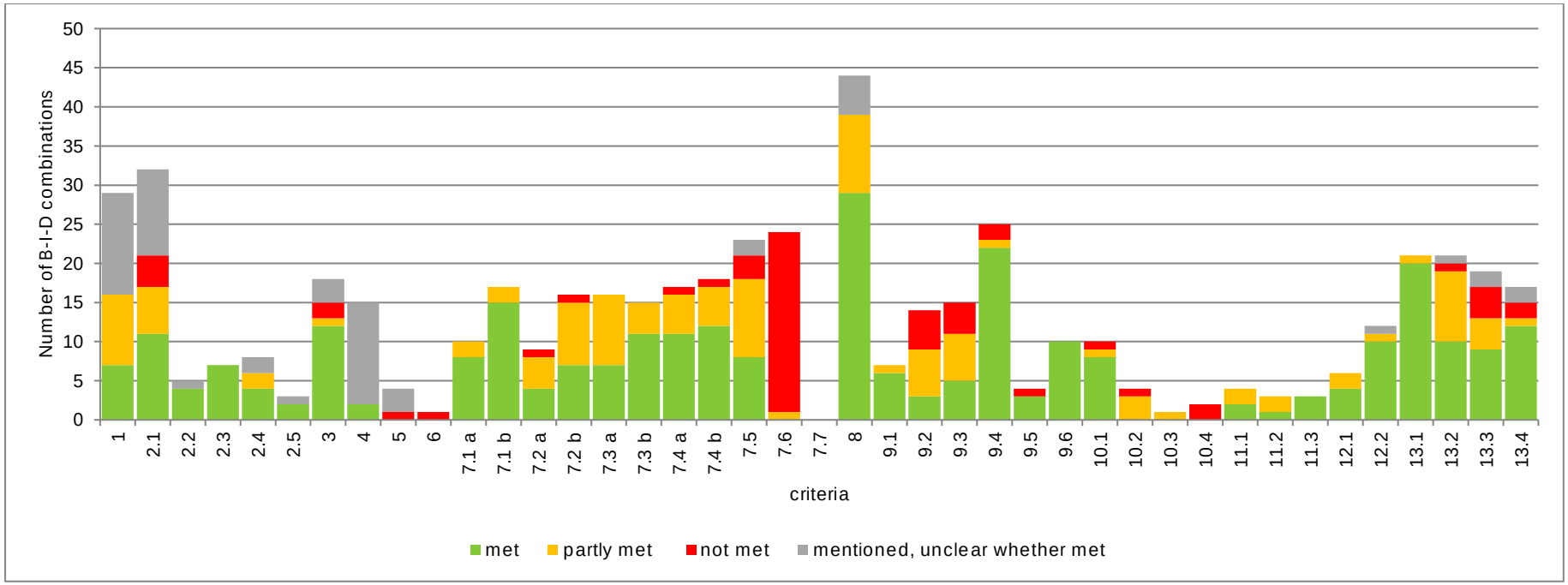


Figure 4.2 Number of criteria mentioned in EMA documents supporting positive recommendations

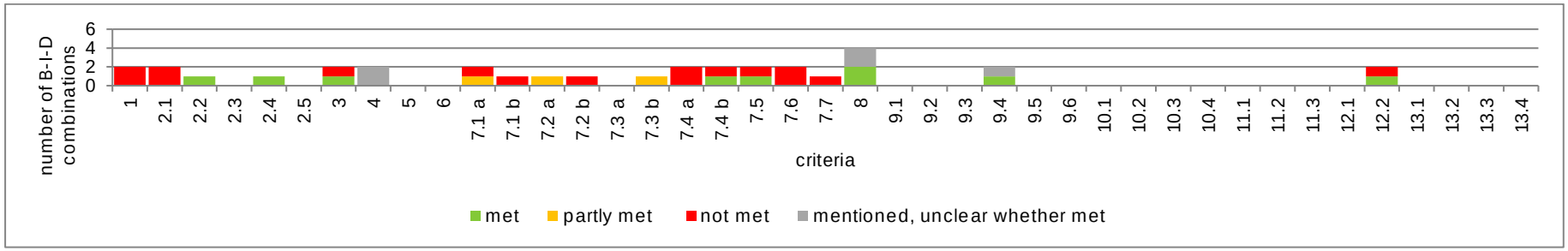


Figure 4.3 Number of criteria mentioned in EMA documents supporting negative recommendations

4.4.5.2 THEME 2: STUDY DESIGN

The appropriateness of the study design (criterion 2.1) was commented on in 34 of 49 documents. It was judged as appropriate in 11 recommendations and partly adequate in six. When the design was judged as partly appropriate, various issues were discussed (such as randomisation, blinding, choice of comparator, or stratification factors) and only some of these were considered appropriate. The study design was considered inadequate in six recommendations, in four of which it did not preclude the drug from being authorised. In ten cases it was not clear whether the design was judged as appropriate.

The remaining criteria within this theme were commented on infrequently (in three to nine recommendations) and they were mostly met. Clearly some of these were not applicable to all contexts – for example not all trials are amended to an extent that requires commenting on.

4.4.5.3 THEME 3: PRIMARY OUTCOME

The primary outcome was mentioned in 20 of the 49 cases. It was judged as appropriate in 11, partly appropriate in one, inappropriate in three and it was not clear in three cases.

When it was partly met, this was due to either only some of the studies supporting the B-I-D combination being judged as having an appropriate primary outcome, or there were some reservations regarding the appropriateness of the outcome.

The primary outcome was judged inappropriate either because an outcome was of limited clinical relevance, or because a more appropriate outcome (overall survival) could have been measured.

4.4.5.4 THEME 4: SAMPLE SIZE

The sample size was mentioned in 17of 49 recommendations, however a judgement on whether it was sufficient was only provided in two (in both judged as adequate).

4.4.5.5 THEME 5: PROPORTION OF PATIENTS WITH BIOMARKER STATUS AVAILABLE

This criterion was mentioned in four of 49 recommendations and it was not met in one and unclear in three. This infrequent use of this criterion is partly due to nineteen of the recommendations in the framework analysis being based on studies including only biomarker positive patients. Therefore the biomarker status was available by definition for all of the included patients. In the remaining cases the proportion of patients with an

available biomarker status did not seem to be an important concern. In the case when it was judged as too small, it did not preclude licensing of the drug in an indication defined by the biomarker.

4.4.5.6 THEME 6: SUBGROUP WITH AVAILABLE BIOMARKER REPRESENTATIVE

This criterion was mentioned in only one of the 49 analysed documents, where it was considered not met, however this did not preclude drug licensing. Again, one of the reasons for this criterion not being often mentioned may be frequent utilisation of studies including only biomarker positive patients.

4.4.5.7 THEME 7: CLINICAL EFFICACY EVIDENCE

In most recommendations (45 of 49) the results were discussed for

- both primary (criteria 7.1 a and b) and secondary (criteria 7.2 a and b)outcome, and/or
- any outcome - without consideration whether it was primary or secondary (criteria 7.3 a and b).

In three cases the evidence discussed was based on the primary outcome only. In one case the results were discussed for the secondary outcome (to support the biomarker) and for any outcome (to support the drug in all patients, irrespective of biomarker status). In 13 cases the discussion of efficacy results did not include any data relevant to the biomarker in question.

Generally, the evidence in terms of efficacy results seemed to be mainly considered either sufficient or partly sufficient. There were some exceptions, where either all discussed results were considered unsatisfactory (two cases of negative recommendations), or only the evidence reported for secondary outcomes was insufficient, with satisfactory evidence for primary outcomes (two cases, both authorised).

Apart from the judgment on the strength of evidence, there was usually (in 37 of 49 recommendations) some comment on the clinical relevance of the benefit provided by the drug (in all patients and/or those identified by the biomarker, criteria 7.4 a and b)). In most cases the criterion of clinically relevant benefit was considered met or partially met. In five cases it was not met (three of these were rejections).

In 25 of the 49 recommendations it was also considered whether the results varied for different subgroups of the population other than defined by the biomarker status, for

example age (criterion 7.5). Consistency of results was met in nine cases (one of which was rejected) and partially met in 10. The results were considered inconsistent across subgroups in four cases (including one negative recommendation). In two cases the results for different subgroups were discussed, but no conclusions on their consistency were drawn.

In 26 of the 49 recommendations it was also discussed whether sufficiently long term follow-up data were available (criterion 7.6), or whether further follow-up would be required. In one case this criterion was partly met, as sufficiently long-term follow-up data were available only for a subgroup of the population identified by the indication. In the remaining cases the follow-up was not considered sufficiently long-term. However in most of these the follow-up data were requested as a post-marketing measure.

For one negative recommendation the positive results of one study were not replicated in another study (criterion 7.7) and this was commented on as a substantial limitation to the credibility of these results.

4.4.5.8 THEME 8: TOXICITY

Toxicity was considered in 48 of the 49 recommendations. In 31 recommendations it was considered acceptable (including two rejections), in ten it was considered partially acceptable and in seven it was unclear whether it was acceptable (including two rejections).

4.4.5.9 THEME 9: CONTEXT

At least one criterion included in this theme was mentioned in 40 of the 49 included recommendations.

Within this theme the most frequently mentioned criteria were:

9.2 no cross-resistance with other available treatments observed (mentioned in 14 of 49 recommendations, at least partially met in nine and not met in five),

9.3 sufficient evidence available to support the B-I-D combination compared to other relevant treatment options (mentioned in 15 of the 49 recommendations, at least partially met in 11 and not met in four),

9.4 drug addressed an unmet clinical need (mentioned in 27 of 49 recommendations, at least partially met in 24, not met in two and unclear in one).

The remaining criteria were mentioned less often and were usually met, apparently providing further support to a positive recommendation.

4.4.5.10 Theme 10: Biomarker test

Interestingly, criteria relevant to the biomarker test theme were not mentioned very often – only in ten of the 49 recommendations. The one most frequently mentioned (ten of the 49 identified recommendations) was the availability in Europe of the biomarker test or clear guidelines on how to perform it (criterion 10.1). In four recommendations for authorised drugs the accuracy of the biomarker test was discussed (criterion 10.2). The remaining two criteria within the biomarker test theme (10.3 and 10.4) were mentioned only in one and two documents respectively.

4.4.5.11 Theme 11: External evidence

External evidence was mentioned in seven of the 49 recommendations and for all of these the criteria were at least partially met. In four cases evidence from drugs with a similar mechanism of action was discussed (criterion 11.1). Evidence from previous studies for example in a different indication (criterion 11.2) and from published literature (criterion 11.3) were mentioned in three cases each.

4.4.5.12 Theme 12: Pre-efficacy evidence supporting the biomarker

In 17 of the 49 recommendations evidence from studies undertaken prior to efficacy trials was used to support the biomarker. The most frequently cited criterion within this theme (14 recommendations, 12 at least partially met, 1 not met, 1 unclear) was the evidence from the mechanism of drug action (criterion 12.2). Also evidence from pre-clinical studies (criterion 12.1) was taken into consideration in six recommendations where it was at least partially met.

4.4.5.13 Theme 13: Pre-efficacy evidence supporting the drug

Evidence from lower level studies undertaken prior to efficacy trials was mentioned frequently (33 of 49 recommendations) and whether it was considered appeared to be mainly an artefact of the report structure, rather than any particular concerns.

Acceptable quality of the drug (criterion 13.1) was mentioned in 21 recommendations and was at least partly met in all of these. Sufficiency of information from preclinical

studies (criterion 13.2) was commented on in 21 recommendations (at least partly met in 19, not met in one and unclear in one). Evidence to support selected dose and/or duration of treatment (criterion 13.3) was commented on in 19 recommendations (at least partly met in 13, not met in four and unclear in two). Criterion of sufficiently characterised drug pharmacokinetics (criterion 13.4) was commented on in 17 recommendations (at least partly met in 13, not met in two and unclear in two).

4.4.6 Necessary conditions

The potential necessary criteria for authorisation of a drug with a biomarker in the indication or contraindication were identified as those not met by at least one of the negative recommendations, but at least partially met, unclear or not discussed for all positive recommendations. As there were only four negative recommendations with a Scientific Discussion or Assessment Report document available, these conclusions are limited. The identified necessary criteria were:

- Theme 1: Population – population in studies was in accordance with the population identified by the drug indication - this was not met in two refusal recommendations.[164,210]
- Theme 7: Clinical efficacy evidence
    - 7.1.a. sufficient evidence supporting conclusions based on the results for the primary outcome irrespective of biomarker - this was not met in one refusal recommendation.[164]
    - 7.1.b. sufficient evidence supporting conclusions based on the results for the primary outcome supporting the biomarker (including enrichment design) - this was not met in one refusal recommendation.[164]
    - 7.7. Results reproduced in more than one study - this was not met in one refusal recommendation.[160]
- Theme 12: Pre-efficacy evidence supporting biomarker
    - 12.2. evidence from mechanism of action to support biomarker - this was not met in one refusal recommendation.[209]

For all of these criteria there were no positive recommendations where these were not met.

4.4.7 SUFFICIENT CONDITIONS

Based on the data collected it is difficult to identify sufficient criteria for authorisation. It appears that the majority of the criteria identified from the analysed documents were largely a result of selective reporting of issues important within the context of a given B-I-D. Therefore it did not seem possible to identify a set of sufficient criteria.

Some of the criteria appeared to be important in the majority of the analysed documents. In the majority of the positive recommendations at least one criterion from these themes was completely or partly met:

- Theme 7: Clinical efficacy evidence,
- Theme 8: Toxicity – toxicity acceptable,
- An at least one criterion from these themes:
    - Theme 9: Context,
    - Theme 10: Biomarker test,
    - Theme 11: External evidence,
    - Theme 12: Pre-efficacy evidence supporting the biomarker,
    - Theme 13: Pre-efficacy evidence supporting the drug,

There was one EMA document where there was no information on any of these criteria.[210] There was also one refusal case where all of the above conditions were met.[211]

This set of criteria is therefore not complete and it does not allow to completely distinguish between the positive and negative recommendations.

4.4.8 VARIATION DEPENDING ON CLINICAL AREA

Twenty nine of the 49 identified cases discussed cancer treatments – the most frequent in this dataset were breast, NSCLC and colorectal cancers. Sixteen recommendations discussed treatments for viral diseases: 14 HIV-1 (including 13 "viral resistance" biomarkers and one other) and two genotype 1 HCV treatments. Four recommendations were classed as blood/metabolic disease areas and included only orphan drugs.

Cancer was the only clinical area where each individual criterion was mentioned at least once and, as it was the disease area with the largest number of recommendations included in this analysis; it substantially shaped the dataset as a whole. For the majority of

the identified criteria there were few differences between clinical areas. The criteria where the main differences occurred were:

- Theme 3: the primary outcome was discussed less frequently for viral diseases (19%) than for the other clinical areas (55%).
- Criteria 7.1.-7.3: study results were more commonly discussed with separation into primary and secondary outcomes for cancer and blood/metabolic diseases (66% and 75% respectively) than for viral diseases (31%), where they were usually discussed together for all outcomes.
- Criterion 7.4: clinical benefit in the biomarker-defined subgroup was mentioned more frequently for blood/metabolic diseases (75%) than the remaining disease areas (56%).
- Criterion 9.1: acceptable mode of delivery was referred to most frequently in viral diseases (31%), only mentioned in two of 29 cancer recommendations (7%) and not mentioned for blood/metabolic diseases.
- Criterion 9.2: cross-resistance with other available treatments was more commonly discussed in viral diseases (75%), in only two cancer recommendations (7%) and none of the blood/metabolic diseases. This is possibly due to inclusion in the dataset of only chronic viral diseases, often requiring a change in the treatment regimen due to arising resistance. Similar reasoning could however be applied to cancer, where multiple lines of therapy are also common.
- Criterion 12.2: evidence from mechanism of action was used most frequently to support the biomarker in cancer (45%), one blood/metabolic (25%) and no viral disease recommendations.

4.4.9 Variation depending on the type of the biomarker

There were only three recommendations where the biomarker was predictive of treatment safety and in the remaining 46 recommendations biomarkers were predictive of treatment efficacy. A comparison of criteria used in recommendations for efficacy biomarkers with the three toxicity cases did not show any clear differences.

4.4.10 Variation depending on presence of an orphan designation

In ten of the 49 recommendations the drug had an orphan designation. This included eight positive and two negative recommendations.

For most of the identified criteria there were no obvious differences in how frequently different criteria were mentioned or met. The criteria where the main differences occurred were:

- Theme 2: in drugs with orphan designation there was no discussion of the statistical approach to data analysis, however it was only mentioned in three non-orphan cases (8%).
- Theme 3: the primary outcome was discussed more frequently for orphan (60%) than for non-orphan drug recommendations (36%).
- Theme 4: the sample size was discussed more frequently for orphan (80%) than for non-orphan(26%) drug recommendations. However for both of these drug types it was usually only mentioned, without providing any normative statements.
- Criterion 7.6: sufficiently long term follow-up was mentioned more frequently for orphan drugs (80%) than non-orphan drugs (41%) and for both groups it was rarely met.
- Criterion 9.2: cross-resistance was discussed less frequently for orphan drugs (20%) than for non-orphan (31%).
- Criterion 9.3: comparison with other available treatments was discussed less frequently for orphan drugs (10%) than for non-orphan (36%).
- Criterion 9.4: addressing an unmet clinical need was mentioned in most of the orphan drug recommendations (80%) and less frequently for non-orphan drugs (41%).
- Theme 10: with one exception[165] (criterion 10.1), issues related to the biomarker test were not discussed for orphan drugs. These were however relatively rarely discussed for the non-orphan drugs as well (23%).

4.4.11 Critical issues in B-I-Ds with negative recommendations

There were four negative recommendations. Based on the available documents it was attempted to identify issues which appeared to be critical for the negative recommendations. The main issues that were identified are reported below for each of the four recommendations together with the relevant themes.

4.4.11.1 GEMTUZUMAB OZOGAMICIN FOR TREATMENT OF CD33-POSITIVE AML

Critical issues:
Theme 1: population in the studies not in accordance with indication
Theme 2: inappropriate study design
Theme 7: limited information on efficacy

The indication considered for this drug was treatment of adult patients with first relapse AML who are not candidates for other intensive re-induction chemotherapy. The main points raised in the Assessment Report are described below.[164]

It was stated in the EMA document that at least some of the patients included in the studies were actually eligible for other cytotoxic regimens. This was confirmed by supportive studies where some of the patients were treated with high-dose chemotherapy and allogenic hematopoietic stem cell transplantation after treatment with gemtuzumab.

It was also raised that a randomised trial would have been both possible and more appropriate, however this did not seem to be the most important concern.

As it was considered that the population in the studies was not representative of the "theoretical situation where no other treatment option is available", informal comparisons were undertaken with other available treatments for patients in first relapse. Based on these it was concluded that gemtuzumab results in only a modest rate of complete remission: 13% of patients in the studies compared to 20-70% for other available treatments based on published literature. It was also noted that there is little information on the duration of the response and other clinically relevant outcomes such as overall survival.

4.4.11.2 CETUXIMAB FOR TREATMENT OF EGFR EXPRESSING NSCLC

Critical issues:
Theme 2: concerns about post-hoc data analysis
Theme 7: limited benefit inconsistent across different outcomes
Criterion 12.2: trial data inconsistent with drug mechanism of action

The indication under consideration was first line treatment of EGFR-expressing NSCLC in combination with platinum-based chemotherapy.[209]

The drug was initially rejected as the overall survival benefit it provided was considered "modest" (HR=0.88, 95%CI: 0.795-0.969, p=0.01 based on a pooled analysis of trials, about

one month improvement in median OS). These results were also not supported by convincing effects on PFS. Such benefit was considered insufficient to outweigh the safety concerns. This was however not a unanimous decision, and some members of the CHMP had a divergent position, for example:

> *"The lack of convincing supportive data in terms of PFS was considered not to be critical because the overall pattern was generally consistent with OS in terms of a favourable treatment effect.*
>
> *There is an unmet medical need particularly in patients with tumors of non-adenocarcinoma histology."*[209]

It was also discussed that there appeared to be no effect of KRAS mutation status on treatment efficacy, and therefore EGFR signalling was possibly not related to treatment activity (two out of four studies included only patients with EGFR expressing tumours). This issue however appeared to be of lesser importance.

The applicant requested the opinion to be re-examined for a population of patients under 65 years of age. It was observed that in the older patients there were more deaths early after initiation of treatment, which had a large impact on the efficacy and safety profile. The selection of patients based on age was justified by claiming that older patients were more frail and suffered from more comorbidities than younger ones.

In this group of patients the point estimate of OS was slightly better (HR= 0.85; 95% CI: 0.72-0.99; p=0.043, median OS benefit of about 1.5 months). However the conclusions of the CHMP did not change. The OS effect was still considered "modest" and not supported by PFS data.

Additionally, concerns were expressed about the *post-hoc* analysis of data. It was pointed out that this was prone to bias and multiplicity problems. Also the safety profile for patients <65 years was similar to that for all patients in terms of percentage of patients experiencing serious adverse events. Therefore, a negative opinion was confirmed, although a minority of the CHMP again disagreed.

4.4.11.3 ZOLEDRONIC ACID FOR TREATMENT OF HORMONE RECEPTOR POSITIVE BREAST CANCER

Critical issues:
Theme 1: background therapy not representative of current practice
Criterion 7.7: results not replicated (data from one trial only)

Zoledronic acid (belonging to a class of drugs known as bisphosphonates) was considered as a treatment for hormone receptor positive early BC in premenopausal women. Zoledronic acid was already used to prevent bone resorption (break-down) in cancers involving the bone and thus to avoid skeletal events. This new application was withdrawn by the applicant after a negative opinion issued by the CHMP.[210]

One of the major concerns was that the background therapy was considered unrepresentative of current European practice, as it did not include adjuvant chemotherapy. Another important concern seemed to be that the anti-tumour activity of zoledronic acid was to some extent surprising:

> *"The direct anti-tumor activity of zoledronic acid appears very promising, but it also represents, at least conceptually, a new property of this potent bisphophonate."*[210]

Therefore, in spite of very "promising results" of the trial that was submitted in support of the indication (disease free survival compared to endocrine therapy alone HR=0.66; 95% CI: 0.48, 0.90), the CHMP considered the fact that the results were not replicated to be of utmost importance (theme 7):

> *"So far, the adjuvant benefit of zoledronic acid has only been demonstrated in one, single trial which remains one of the major concerns."*[210]

4.4.11.4 ALIPOGENE TIPARVOVEC FOR LPL PROTEIN POSITIVE LIPOPROTEIN LIPASE DEFICIENCY

Critical issues:
Criterion 7.4: data on clinically relevant outcome not considered robust
Criterion 7.7: insufficient evidence of long term benefit

This drug is discussed in more detail in the case study in section 4.4.12 and therefore the critical issues for this decision are only briefly outlined here.

The CHMP rejected this application due to two main reasons:

- Data on pancreatitis attacks (clinical outcome) were not considered robust and it was not unlikely that the observed improvement in this outcome was due to factors other than the treatment investigated.
- Insufficient evidence of long term benefit of treatment in terms of surrogate outcomes was available.[160]

4.4.12 Case study: detectable levels of LPL protein in treatment of LPL deficiency with alipogene tiparvovec

This B-I-D combination was selected for the case study, as it was given a positive recommendation after an initial negative one. Therefore this case study could potentially provide some insight into what criteria were important for the EMA when considering drugs with predictive biomarkers in the indication or contraindication. This B-I-D combination is considered in more detail to allow a better understanding of the rationale underpinning EMA recommendations.

4.4.12.1 Background information on LPL deficiency and alipogene tiparvovec

LPL deficiency is a rare recessive disease with prevalence of the disease judged as one to two people per million in the EU.[160] It is diagnosed in adults, but symptoms can appear in children and include severe abdominal pain, colicky pains and pancreatitis. The complications of the disease include acute pancreatitis which may be life threatening and also lead to a chronic pancreatic insufficiency.[212]

People with this disease do not produce a functional LPL enzyme.[213] LPL is mainly found in skeletal and heart muscle, as well as fat tissue.[212] It is involved in clearance from the body of triglyceride-rich chylomicrons, which are normally produced after a meal and deliver triglycerides to different parts of the body. The lack of functional LPL results in high levels of fasting chylomicrons and high plasma triglyceride concentration.[213] Chylomicrons are in turn thought to be responsible for the clinical symptoms. When the drug was considered by the EMA there was no treatment available and management of LPL deficiency was mainly through extremely low-fat diet.[212]

Alipogene tiparvovec (trade name Glybera) is a gene therapy drug comprising a non-integrating adeno-associated viral vector which is used to deliver functional copies of the LPL gene to cells.[160] The treatment is given on a single occasion as a number of intramuscular injections at different sites. It requires 12 weeks of immunosuppression following the injection.[160] The treatment cannot be repeated, as all patients treated with

alipogene tiparvovec developed a high and persistent immune response to the drug, precluding retreatment.[160,213,214]

4.4.12.2 NEGATIVE RECOMMENDATIONS

Alipogene tiparvovec has been granted an orphan designation in 2004 and the application for marketing authorisation was first submitted in December 2009 in an indication that did not contain the biomarker:

> *"Glybera is indicated for the long term correction of lipoprotein lipase deficiency, to control or abolish symptoms and prevent complications in adult patients clinically diagnosed with lipoprotein lipase deficiency (LPLD)."*[160]

This application was given a negative recommendation by the EMA in June 2011 due to insufficient evidence supporting clinical benefit, however as this indication did not contain a predictive biomarker, it will not be considered in detail here.

In July 2011 the EMA was asked by the applicant to re-examine their decision. Additional information and effectiveness and safety analyses were also provided.

The drug was first re-examined by Committee for Advanced Therapies (CAT), which concluded that the benefit of the drug

> *"is sufficiently demonstrated in selected patients as defined by the restricted indication in patients diagnosed with LPL deficiency and suffering from at least one pancreatitis episode despite dietary fat restrictions. The indication is restricted to patients with detectable levels of LPL protein"*[160]

Although this is not explicitly stated in the documentation provided, it appears that it was CAT that limited the indication to patients with a history of pancreatitis episodes and introduced the biomarker. The CHMP disagreed with this opinion with one of the main reasons being insufficient evidence of long term benefit. It was also stated that the data on pancreatitis attacks (clinical outcome) cannot be considered robust and the observed change in pancreatitis rates may be due to factors other than the investigated treatment. Again, the drug was given a negative recommendation. There were however divergent positions (information on their content was not available). Details of the criteria considered in this recommendation are reported in Table 4.2.

4.4.12.3 POSITIVE RECOMMENDATION

Following the refusal, the European Commission asked for a re-examination of the CHMP recommendation. The Applicant was asked whether they supported an indication *"restricted to patients with detectable levels of LPL protein".*[160]

As the applicant agreed, they were asked to provide an overview summarising data for this patient group. Detailed, individual patient data on 12 patients relevant to this indication were provided. Based on these it was concluded by both CAT and CHMP that, considering the "totality of evidence" the previously identified issues were resolved. In particular the issues below were discussed.

The CHMP noted that the efficacy data based on pancreatitis events alone were insufficient to support the conclusions due to its limitations. However they noted that when equal-length of pre- and post- treatment periods are considered for each patient, less events occurred post-treatment. This data were also considered to be supported by weight gain in three patients that was not associated with pancreatitis or abdominal pain. This was considered consistent with diet violation not causing disease symptoms. It was also observed that there was a reduction in hospital admissions and intensive care unit stays post-treatment. Further evidence supporting the positive opinion was based on laboratory measures: observations in seven patients of LPL mass, LPL activity and LPL enzyme function, as well as vector DNA expression (unclear in how many patients this was measured).

In making its recommendation,

> *"The CHMP (…) took into consideration the extreme rarity of the condition and the high degree of unmet medical need, particularly in patients with severe or recurrent pancreatitis events"*[160]

and considered the "totality of evidence". It was concluded that each individual component of the data on its own was subject to limitations and should not be considered in isolation. As a result it was concluded that based on:

> *"- the persistence of LPL activity in patients who had had biopsies (8 biopsies performed in 7 patients; one patient had two biopsies, the first at 18 weeks and the second at 52 weeks),*
>
> *- the evidence of an effect on lipids, in particular the post prandial CM, (in 5/5 patients at 14 weeks and 3/3 patients at 52 weeks),*
>
> *- the evidence presented on the reduction in the rate of pancreatitis"*[160]

the positive effect of alipogene tiparvovec was confirmed in this group of patients. The concerns raised in the rejection recommendation were considered resolved (by majority of CHMP members). There was also a minority divergent opinion noted, which mainly concluded that:

> "*the grounds for refusal initially voted have not been satisfactorily resolved and there are still uncertainties on the robustness and the relevance of the clinical results submitted*"[160]

Details of the criteria considered in this recommendation are reported in Table 4.2.

4.4.12.4 Difference between the positive and negative recommendation

It does not appear that there was a large change in the quantity or quality of data available between the negative and positive recommendations. It seems however that there was a change in the interpretation of the data, particularly for the results of included studies. In the positive recommendation the "totality of evidence" approach was emphasised, possibly implying that a positive trend across different outcomes to some extent compensates for the study limitations. It was also highlighted that this treatment is the first drug available for this patient population.

4.5 Discussion

The framework analysis of 49 EMA recommendations resulted in identification of 41 criteria, which were grouped into 13 themes. These criteria were discussed in the analysed documents with varying frequency. The most frequently mentioned themes were:

- Theme 1: Population
- Theme 2: Study design
- Theme 7: Clinical efficacy evidence
- Theme 8: Toxicity
- Theme 9: Context
- Theme 13: Pre-efficacy evidence supporting the drug

This analysis found that although there are some criteria which were considered more frequently than others in the context of EMA licensing recommendations, it is very difficult to identify any that can be considered sufficient or necessary.

Table 4.2 Criteria considered in recommendations for LPL protein in treatment of LPL deficiency with alipogene tiparvovec (only themes and criteria where information was reported are included)

Theme and/or criterion	Negative recommendation	Positive recommendation
1. Population		Reported, but no normative statement
2. Study design 2.2. amendments to trials acceptable	The primary efficacy outcome was changed from plasma triglyceride levels to postprandial chylomicrons (pp-CM) as a surrogate efficacy marker – the CHMP agreed this was acceptable	
2.4. Level of possible bias acceptable		*"Considering the combination of the rarity of the indication as well as the fact that this is an autosomal recessive disorder with different levels of genetic penetration, a high consistency in the results is challenging to achieve. A lack of full consistency is acknowledged as a limitation of the data, but this does not rule out a favourable effect of Glybera."*
3. Primary outcome	pp-CM was considered acceptable	
4. Sample size		*"Acknowledging the limited dataset in the sub group of 12 patients with severe or multiple pancreatitis attacks, the CHMP discussed the pancreatitis results on the basis of individual patient data"*
7.1.a. Sufficient evidence supporting conclusions based on the results for the primary outcome irrespective of biomarker	*"there is currently insufficient data on pp-CMs to demonstrate the efficacy of Glybera based on only 3 patients at 52 weeks (of the 27 patients enrolled in the clinical trial programme), even taking into account the extreme rarity of the disease"*	*"Looking at the totality of the available evidence for efficacy, the CHMP considered (...) the evidence of an effect on lipids, in particular the post prandial CM, (in 5/5 patients at 14 weeks and 3/3 patients at 52 weeks), (...) and concluded by majority, that there was sufficient evidence to confirm a positive effect on Glybera in this sub group of severe patients with a high degree of unmet medical need"*
7.2.a. Sufficient evidence supporting conclusions based on the results for the secondary outcomes irrespective of biomarker		*"Looking at the totality of the available evidence for efficacy, the CHMP considered* *- the persistence of LPL activity in patients who had had biopsies (8 biopsies performed in 7 patients; one patient had two biopsies, the first at 18 weeks and the second at 52 weeks),(...)* *- the evidence presented on the reduction in the rate of pancreatitis and concluded by majority, that there was sufficient evidence to confirm a positive effect on Glybera in this sub group of severe patients with a high degree of unmet medical need."*
7.4.a. Sufficient evidence to conclude clinical benefit irrespective of biomarker	The main issues were: • lack of robust data on pp-CM • retrospective analysis of pancreatitis data did not provide evidence of efficacy in terms of a clinically meaningful reduction in pancreatitis • follow-up data on pancreatitis were insufficient (relatively short duration of post-treatment follow-up and large variability in historical annual pancreatitis rates)	It was considered that: • effect of the treatment on pp-CM is biologically significant, even though it was tested on a small number of patients; pp-CM *"data at 52 weeks (n=3 pts) suggest the presence of a metabolically relevant amount of LPL activity"* • *"The evidence generated for the reduction of pancreatitis events and severity of attacks, although hampered by statistical*

	• it could not be excluded that the observed decrease of pancreatitis risk is due to other factors (lifestyle and diet changes) • there was no evidence of a relationship between pancreatitis events and reduction in hospital admissions and ICU stays	*limitations, suggested that Glybera leads to a clinically relevant reduction of pancreatitis risk."* • *"This is also supported by the reduction in hospital admissions and ICU stay"* • *"The evidence generated by the overall efficacy data, acknowledging the limitations, is considered to be sufficiently robust"*
7.6. Sufficiently long term data provided	Insufficient follow-up to conclude persistence of LPL activity and impact on annual rates of pancreatitis (which have very high year to year variability)	Acknowledged that the follow-up was relatively short
8. Toxicity acceptable	The main risk was considered to be that the treatment requires 12 weeks of immunosuppression which was considered acceptable	
9.4. Drug addressed an unmet clinical need		The group of patients for whom the treatment is intended was described as *"sub group of severe patients with a high degree of unmet medical need"*
9.6. Novel mechanism of action/ new active substance		alipogene tiparvovec was qualified a new active substance
11.1. Supporting evidence from drugs with similar mechanism		Data on other gene therapies using the same virus as the vector (mode of gene delivery) were used to support long term safety.
11.3. Conclusions supported by published meta-analysis or other literature sources		*"Residual baseline level of LPL is also important due to potential immunogenicity/tolerance and there should be further follow-up in this respect since it has been published that antibodies can in some instances neutralize LPL"*
12.1. Evidence from mechanism of action to support biomarker		*"In order to prevent an immune response against the transgene protein, treatment is restricted to patients with detectable levels of LPL protein"*

Interestingly, as these were all recommendations that included a predictive biomarker, there was relatively little attention given to any issues associated with the biomarker test (theme 10). Criteria within this theme were commented on only in ten of the 49 analysed recommendations.

An analysis of critical issues in negative recommendations identified a number of criteria which seemed to be important in these cases. These were often criteria within theme 7: clinical efficacy evidence. In these cases, for various reasons, the evidence supporting the drug efficacy was considered insufficient. Other critical issues included, for example, criteria from theme 1: population and theme 2: study design.

In one case a B-I-D combination was first given a negative recommendation and after EMA reconsidered the evidence, drug authorisation was recommended. This was analysed in more detail in a case study. This suggested that to a large extent it was not new evidence that influenced the change in the recommendation, but a different perspective adopted by the EMA, with more weight given to the context (lack of any alternative treatment and rarity of the condition) and a "totality of evidence" approach.

The context (theme 9) was considered very frequently (40 of 49 recommendations) and it seemed to influence interpretation of evidence on efficacy (theme 7) and toxicity (theme 8).

The initial seven criteria formed the basis for construction of the first seven themes. Of these, some appeared important both due to being frequently referred to in EMA documents and forming the basis of negative recommendations. However, some of these initial criteria did not seem to be of high importance (particularly theme 5: proportion of patients with biomarker status available and theme 6: subgroup with available biomarker status representative).

There are a number of limitations to these findings, as the framework analysis was carried out by one person. There is therefore a possibility that some criteria were missed. The classification of criteria proposed here is to a very large extent subjective and a different person conducting the framework analysis may identify different criteria and themes.

It is also likely that the identified criteria are to a large extent a result of selective reporting by the EMA. It has been suggested that documents should be considered in the context of authorship, their purpose and intended readers.[215,216] These documents were written only to a very small degree as a record of discussions and may not reflect all the issues that were considered important. However these were the only available documents on these issues. A further identification of important issues could have been undertaken by

interviews with members of CHMP, however this was considered outside of the scope of this exploratory analysis.

4.6 CONCLUSIONS

Forty one criteria grouped into 13 themes have been identified in the framework analysis of 49 EMA recommendations for B-I-D combinations containing predictive biomarkers. It was not possible to identify sufficient or necessary criteria with any degree of certainty.

It also appears that the way criteria were applied (as highlighted by a case study of alipogene tiparvovec) was largely context-dependent.

CHAPTER 5. EVIDENCE SUPPORTING EMA RECOMMENDATIONS

Author contributions
Kinga Malottki lead the design of the study, carried out the analysis and drafted the chapter
Jon Deeks contributed to the design of the study, commented on data analysis and chapter
Lucinda Billingham contributed to the design of the study, commented on data analysis and chapter

Acknowledgements:
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Abstract

Background: Chapter 4 investigated the rationale for EMA recommendations for drugs with a predictive biomarker in the indication or contraindication that were identified in Chapter 3. All the 41 identified criteria used by the EMA were considered for use in this chapter. The focus of the current chapter is on the evidence base supporting the inclusion of a particular predictive biomarker in drug indications and contraindications.

Aim: To analyse the evidence supporting EMA recommendations including a predictive biomarker reported in the EMA documentation and thus to identify evidence standards in positive licensing recommendations and contrast them with the evidence supporting the B-I-D combinations where licensing was refused.

Methods: The criteria used to evaluate the evidence were: (1) population in accordance with the population identified by the drug indication, (2) design of the biomarker evaluation, (3) the type of primary outcome, (4) sample size (of patients with an available biomarker status) in studies; (5) findings - evidence supporting the biomarker based on the results for the primary outcome. These criteria were used to construct radial plots. In addition, the replication of findings of a single study and the consistency of the biomarker assay within a B-I-D combination were investigated.

Findings: Based on this analysis it seems that for biomarkers predicting treatment safety the evidence requirements were minimal, as in three of the four B-I-D combinations the biomarker predicting adverse events was based on the understanding of the drug action and/or metabolism. Evidence standards were not clear for biomarkers predicting treatment efficacy. In two cases of negative recommendations for non-orphan drugs, it appears that the promising results of a single study have not been replicated in another study. However, this was also the case for some of the B-I-D combinations which received a positive recommendation. For drugs with an orphan designation there was also little, if any, clear difference between the evidence base supporting the positive and negative recommendations.

The analysis also identified poor reporting as an important issue limiting the evaluation of the studies and implementation of their findings.

Conclusions: No clear evidence standards were identified in this analysis. These findings possibly highlight the importance of the context in which the B-I-D combinations were assessed. However, more consistent evidence standards may be needed to ensure optimal patient treatment.

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5.1 Background

Chapter 4 based on EMA documentation analysed the rationale for EMA recommendations for drugs with a predictive biomarker in the indication or contraindication. It attempted to identify criteria used by the EMA to support these recommendations. This resulted in the identification of 41 different criteria grouped into 13 themes. Some of these criteria were important from the perspective of drug licensing decisions in general (for example satisfactory quality of the product), but not specific to predictive biomarker evaluation.

It was considered that licensing decisions result in the use of a particular predictive biomarker in clinical practice, as mentioned in Chapter 1 and Chapter 3. It was therefore expected that the analysis of this evidence base would provide an indication of the evidence standard sufficient to establish clinical practice that includes the use of a predictive biomarker to select patients for treatment with a given drug. This would correspond to the clinical utility stage discussed in detail in Chapter 1 and Chapter 2. The studies of interest were often grouped into main and supportive in the EMA documentation, with the main studies providing evidence most relevant to a particular indication.

5.2 Aims

The aim of this chapter was to review the evidence that supported recommendations including a predictive biomarker reported in the EMA documentation. This was undertaken to identify evidence standards supporting positive licensing recommendations and contrast these with the evidence supporting the B-I-D combinations where licensing was refused. This was considered within different groups of B-I-D combinations:

- where the biomarker predicted treatment efficacy and the drug did not have an orphan designation,
- where the biomarker predicted treatment efficacy and the drug had an orphan designation,
- where the biomarker predicted treatment safety.

5.3 METHODS

5.3.1 DATA SOURCES

Data were collected for studies for which it was reported in the EMA documentation that these evaluated the biomarker forming part of the B-I-D combination for use in clinical practice.

Scientific Discussions and Assessment Reports were the main source of information on studies supporting B-I-D combinations. These were described in more detail in Chapter 4. For the majority of B-I-D combinations containing biomarkers predicting efficacy the relevant data were contained in the Clinical Efficacy section of the Scientific Discussion or Assessment Report. In some cases, for example where the biomarker was added at a later stage, other relevant document sections were also used as a data source. For example for LPL protein expression use in hyperlipoproteinemia type I to predict response to alipogene tiparvovec the original assessment (and therefore the Clinical Efficacy section) did not contain the biomarker. The relevant information was therefore only found in the re-assessment section of the document.

For biomarkers predicting adverse events, information on the biomarker was not reported (NR) within one section. Therefore data were collected from any section of the report where these were mentioned. In one case the study was only reported for each drug containing the active substance in a separate document (Procedural steps taken and scientific information after the authorisation).[217-219]

For each relevant study identified within the EMA documentation additional searches were undertaken for any published papers and online information (such as trial summaries available on manufacturer's website). These additional searches were used to supplement information on trial design, biomarker assay used and biological sample collection. However, as the EMA often used data from ongoing trials, study results were only collected from the EMA documentation.

5.3.2 SELECTION OF CRITERIA FOR EVALUATION OF THE EVIDENCE BASE

5.3.2.1 THEMES CONSIDERED

In Chapter 4, which explored the rationale for EMA recommendations, the identified criteria were grouped into 13 themes. These were all considered for use in this analysis.

5.3.2.2 SELECTION OF CRITERIA FOR ANALYSIS OF EVIDENCE SUPPORTING EMA RECOMMENDATIONS

Criteria within each theme were considered, taking into account the following issues:

- frequency of use of these criteria in EMA documents,
- identification as a critical issue in negative recommendations,
- methodological rationale,
- their relevance to the final stage of predictive biomarker evaluation prior to implementation in clinical practice (corresponding to clinical utility stage),
- feasibility of evaluation of these criteria.

Where possible, multiple criteria and themes were merged into one. The selection of criteria for use in this chapter is discussed below. The final criteria are shown in Box 5.1.

THEME 1: POPULATION

This theme was relatively frequently mentioned within the EMA discussions. It was also one of the critical issues in negative recommendations. The similarity between the population within studies and the target population (identified by the drug indication) appears to be of high importance for both the general licensing decisions and the predictive biomarker question. It also appeared important from the methodological perspective, as exemplified by its common use in methodological quality tools and publications[220-224] Therefore this criterion was selected to be used in this chapter. However, as only evidence supporting the predictive biomarker was of interest, consideration was narrowed down to study participants with an available biomarker status.

THEME 2: STUDY DESIGN

Study design was also frequently mentioned by the EMA, although it was not always considered in the context of biomarker evaluation. Within this theme there were five different criteria, however some of these seemed to be applicable only to certain cases (such as acceptability of protocol amendments). Other criteria, even though they might be of high importance (for example whether the level of bias in studies was acceptable), might be difficult to measure and to a certain extent overlap with the study design criterion. Therefore the initial criterion of whether the study design was appropriate to evaluate the clinical utility of a predictive biomarker was used. This was again supported by methodological tools and literature.[17,220-223,225]

THEME 3: PRIMARY OUTCOME

Appropriate choice of the primary outcome was mentioned relatively frequently in the EMA discussions and even though it is not only biomarker specific, it is an important criterion when considering the strength of evidence supporting a change in clinical practice.[220-223,226] It was therefore used in this chapter. It was however acknowledged that it might be difficult to assess how appropriate an outcome may be without detailed knowledge of each of the identified disease areas. As obtaining expert advice for all B-I-D combinations was not feasible, this criterion was focused on the type of the primary outcome. This was determined based on the level of objectivity in outcome measurement and its clinical relevance.

THEME 4: SAMPLE SIZE

The sample size was mentioned relatively frequently in the EMA discussions, although often there was no judgement on whether it was sufficient. This is an important criterion, as a larger sample size will increase the certainty in the findings of a study.[223,227] Initially this criterion was to consider all patients included in a study and be complemented by the proportion of patients with an available biomarker status (Theme 5, discussed below). However, it appeared these two criteria could be merged into one – the total number of patients with available biomarker status. The possibility that the patients with an available biomarker status may not be representative of the target patients would be captured by the population criterion.

Therefore, the sample size criterion has been modified to only include patients with available biomarker status.

THEME 5: PROPORTION OF PATIENTS WITH BIOMARKER STATUS AVAILABLE

This criterion was rarely mentioned in EMA discussions and did not appear as one of the critical issues. The relevance of this criterion to the review was discussed above and it was partly incorporated into sample size.

THEME 6: SUBGROUP WITH AVAILABLE BIOMARKER REPRESENTATIVE

This criterion was mentioned only in one of the documents analysed in Chapter 4. This criterion was however considered important and was therefore incorporated into the population criterion, as described above.

THEME 7: CLINICAL EFFICACY EVIDENCE

Some form of evidence supporting the clinical efficacy has been mentioned in all of the analysed documents. It was also one of the main critical issues in negative recommendations. The results of the study with respect to the biomarker were selected as these were considered crucial for establishing whether it is predictive. It would be ideal to consider all the outcomes measured within each study, however, within a realistic time frame, such approach would not be feasible for all the identified B-I-D combinations. Therefore the results considered will be limited to the primary outcome, as this is the outcome for which the most important evidence could be expected.[223]

This however raised the issue of how to address the B-I-D combinations where the biomarker is predictive of drug toxicity. Arguably, from the point of view of evaluating the biomarker, the best case would have been evaluation in trials with drug safety as the primary outcome. However it was likely that this was not the case, as there may have been ethical and logistic limitations to such research. Therefore, for safety biomarkers the approach was more flexible.

In such cases the initial criterion was therefore modified to include the relevant safety results and therefore has been renamed to "Findings".

There were two additional criteria identified within this theme that were potentially relevant. The first one – whether sufficiently long-term data were available was not feasible to measure objectively and therefore was not included as a criterion here. The second one – whether the results were reproduced in more than one study will not be addressed directly, however it will be illustrated by the number of studies supporting each B-I-D combination and the results of these studies.

THEME 8: TOXICITY

Although the criterion of acceptable drug toxicity was of high importance, it was not considered relevant to the strength of evidence supporting biomarkers predictive of treatment efficacy. For biomarkers predictive of treatment toxicity, the safety results have been incorporated into the criterion of findings. Therefore this theme will not be explicitly considered in this analysis.

THEME 9: CONTEXT

This theme was mentioned by the EMA in most cases. It was also considered the reason for recommendation change in the case study described in Chapter 4. Clearly different

evidence may be considered sufficient in a case where the new drug is the only treatment, as compared to disease areas where multiple options are available. However in practice it might be extremely difficult to measure the context objectively, especially for all the B-I-D combinations identified. Therefore, even though it is an important issue in appraisal of the evidence, the context will not be considered within this analysis.

THEME 10: BIOMARKER ASSAY

Issues related to the biomarker assay were not discussed frequently. The choice of the biomarker assay is important, as different laboratory procedures may not give comparable results (as will be discussed in the example of ERCC1 measurement in Chapter 7).Analytical validity of the biomarker evaluation procedures was generally not commented on. It would also be difficult to use for this analysis, as identifying relevant research could be problematic.

However, the consistency of laboratory procedures used across different studies is also important and it will be identified in the summary.

THEME 11: EXTERNAL EVIDENCE

The external evidence (such as from drugs with similar mechanism) has only been rarely mentioned and it generally did not contribute to the main data supporting the recommendations. Its impact on the strength of the evidence base may also be marginal and questionable. It will therefore not be used in this chapter.

THEME 12: PRE-EFFICACY EVIDENCE SUPPORTING THE BIOMARKER

Lower level evidence (such as from the drug development programme) supporting the biomarker was only rarely mentioned and is not directly relevant to this chapter, which focuses on the evaluation of a biomarker for use in clinical practice (clinical utility). Therefore this theme will not be used in this chapter.

THEME 13: PRE-EFFICACY EVIDENCE SUPPORTING THE DRUG

Similarly to theme 12, as this is lower level evidence not directly relevant to clinical utility, it will not be used in this chapter.

5.3.3 FINAL CRITERIA

The final criteria that were used for the evaluation of the evidence base reported in EMA documents are summarised below in Box 5.1.

Box 5.1 Criteria that were used for evaluation of evidence base reported in EMA recommendations

1. Population – population with the biomarker status available in studies was in accordance with the population identified by the drug indication
2. Study design - design of the studies supporting the inclusion of the biomarker in the indication was appropriate
3. Primary outcome – the type of primary outcome assessed in the studies
4. Sample size - sample size (of patients with an available biomarker status) in studies was sufficiently large
5. Findings - evidence supporting the biomarker based on the results for the primary outcome (including enrichment design); for safety biomarkers secondary outcomes were considered if primary outcomes were irrelevant

- ❖ Results were replicated in other studies
- ❖ Biomarker evaluation procedure used was consistent across studies

5.3.4 DATA EXTRACTION

Data were collected by one reviewer from the EMA documentation and identified trial reports on the items shown in Box 5.2.

The majority of these items were not included in the list of criteria selected for data analysis, however these were considered important to aid the interpretation of the study. Data were recorded in a MS Access database.

5.3.5 DATA ANALYSIS

5.3.5.1 SUMMARY OF STUDY NUMBERS AND PATIENT NUMBERS

Data on the numbers of studies supporting each B-I-D combination were presented in a stacked bar chart and summarised as the median and range. The same approach was used for data on the numbers of patients supporting each B-I-D combination. The numbers of studies with a different rank (as described in section 5.3.5.2 below) were reported in tables and presented as percentages.

Box 5.2 Data extraction items for the analysis of evidence supporting EMA recommendations

- study name,
- characteristics of included patients,
- study EMA status (main, supportive or unclear),
- study phase,
- study design,
- blinding,
- power calculation (presence of and method),
- primary outcome (and way of its measurement),
- follow-up duration,
- total number of patients and numbers of patients in different study arms,
- treatment in each arm,
- design of biomarker evaluation,
- biomarker assessment and sample type,
- results

5.3.5.2 Assignment of ranks for different criteria

To facilitate construction of radial plots, for each study within a B-I-D combination the first five criteria (as outlined in Box 5.1) were assessed. These were scored mainly on ordinal scales (with the exception of patient number, which was measured on a continuous scale), and therefore the differences between individual ranks do not provide quantitative information. These were then plotted for each study, as described below in section 5.3.5.3. The further two criteria (whether results were replicated and the consistency of the biomarker assay used) were addressed by the number and appearance of the plots.

The assignment of ranks for each criterion was carried out as described below.

1. Population – this criterion was assessed as shown in Table 5.1. The highest rank was given to studies where the included patients were representative of all patients identified by the drug indication. It was lower when the study patients were either a subset or a wider

group (where the relevant patient population could not be analysed separately). Finally, when the study population was different or NR, the lowest rank was given.

Table 5.1 Ranks for the similarity between the study population and the indication

	Study data cover the indication exactly	Study data cover wider group and impossible to separate out relevant patients or covers subgroup of patients in the indication	Study data on population different to indication/ NR
Rank	1	0.5	0

2. Design of biomarker evaluation – this criterion was assessed as shown in Table 5.2. The highest ranks were given to studies designed to evaluate the clinical utility of a predictive biomarker. Studies providing limited information on how well a biomarker predicts patient response were given progressively lower ranks. The lowest rank was given to cases when the study design was not relevant to the predictive biomarker or NR. The study designs used here are described in Chapter 1, with the exception of:

- Non-RCT biomarker study: any non-randomised study where the biomarker evaluation is part of the study design,
- Case reports and case series: observational studies with a small number of patients included; the biomarker status is measured, however evaluation of the biomarker was NR as part of the design.

Table 5.2 Ranks for the study design

	Stratified/ biomarker strategy	RCT enrichment	RCT subgroup (prospective)	RCT subgroup (retrospective)	RCT subgroup (cross-sectional); non-RCT biomarker study	Not relevant / NR
Rank	1	0.8	0.6	0.4	0.2	0

3. Primary outcome – for this criterion objectively measured clinically relevant outcomes (such as survival and disease cure) were given the highest rank. These were followed by other clinically relevant outcomes and surrogate outcomes. The lowest rank was given to studies which did not define or report a primary outcome. The details of assigning a rank to a study are shown in Table 5.3.

Table 5.3 Ranks for the primary outcome

	Objectively measured clinically relevant outcomes (eg. overall survival, disease cure)	Other clinically relevant outcomes (eg. PFS, TTP, QOL)	Surrogate outcomes (eg. tumour response, HIV RNA level)	None/ NR
Rank	1	0.7	0.4	0

4. Sample size (with available biomarker status) is assessed on a scale from 0 (which would correspond to no patients in the study) to 1 (1000 or more patients in the study). An individual score is obtained by dividing the number of patients by 1000 for patient numbers up to 1000 and assigning a score of 1 for any number above 1000. Although this is a relatively arbitrary cut-off, it was selected as the majority of studies were smaller and therefore it provided discrimination between these. It was also chosen in preference to study power, as the power is heavily dependent on the assumptions about the biomarker and treatment effects. In addition, power was considered irrelevant for studies where the biomarker hypothesis was not primary and for observational studies.

5. Findings – for this criterion, ranks were assigned as shown in Table 5.4. For hypothesis-testing studies these were based on the p-value to indicate the level of uncertainty in the results:

- For studies comparing biomarker strategy with standard care – for the comparison between the study arms,
- For enrichment design – for the comparison between the study arms,
- For stratified design and subgroup analyses – for the biomarker by treatment interaction test

For studies where no hypothesis was tested, the rank was based on drug activity:

- Studies with at least 6 patients showing activity in ≥50% patients are given a rank of 0.4,
- Studies with at least 6 patients showing activity in <50%, but ≥30% patients are given a rank of 0.2,
- Studies with up to 5 patients are given a rank of 0.2 if there is activity in the majority of patients.

Table 5.4 Ranks for the strength of the findings supporting the biomarker

	P ≤0.01 favors biomarker positive/ treatment (enrichment)	p∈ (0.01, 0.05) favors biomarker positive/ treatment (enrichment)	p ∈ (0.05, 0.2] favors biomarker positive/ treatment (enrichment)	No hypothesis tested and ≥50% activity (n>5)	No hypothesis tested and ≥30% activity (n>5), activity in majority (n≤5)	NR, no hypothesis and activity in <30% (n>5), no activity in majority (n≤5)
Rank	1	0.8	0.6	0.4	0.2	0

The process of assigning ranks is illustrated below in Table 5.5, which shows the example of a study supporting the use of zoledronic acid in hormone receptor positive BC.

Table 5.5 Example of rank assignment - supportive study (Z-FAST) for hormone receptor positive breast cancer treatment with zoledronic acid

Criterion	Rank	Reason
1. Population	0	The study included postmenopausal women with early stage BC, while the proposed indication was for premenopausal women with early BC
2. Design of biomarker evaluation	0.8	Enrichment RCT including only women with hormone receptor positive early BC
3. Primary outcome	0.4	The primary outcome was bone mineral density, which was considered a surrogate outcome
4. Sample size	0.602	There were 602 patients with an available biomarker status in the study
5. Findings	1	At the end of the trial the difference in bone mineral density between treatment and control was significant (p<0.0001) and favored treatment with zoledronic acid

5.3.5.3 SUMMARY PLOTS

Ranks given for individual items were plotted on a radial plot – one for each study. The explanation of the labels used in the plots is provided in Figure 5.1. Within each B-I-D combination, studies using the same procedure for biomarker evaluation are all plotted using the same colour. However, the same colour used in different B-I-D combinations does not indicate identical or similar tests. The only exception is grey, which indicates that biomarker evaluation was undertaken, but the assay used was NR.

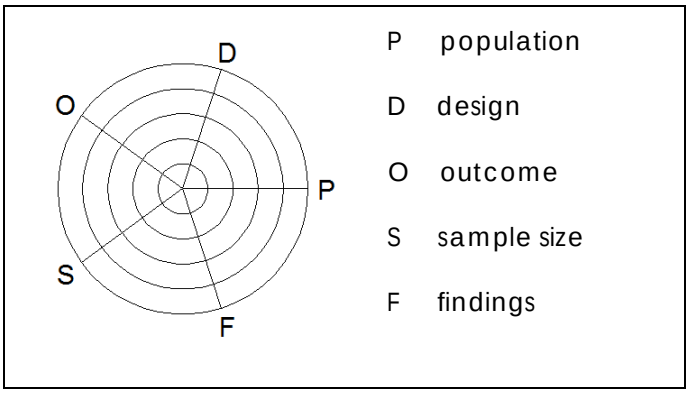


Figure 5.1 Radial plot labels

An area of the polygon was also considered as a quantitative summary across the different criteria. It was however not utilised due to the following reasons:

- The scales for all dimensions (except sample size) are ordinal and the sizes of intervals between any two ranks are meaningless. Calculating an area of the polygon would, for example suggest that a randomised enrichment study is 0.2 less than a biomarker stratified RCT.
- The dimensions illustrated in the plots are not comparable.
- The areas are dependent on the order in which different dimensions are drawn. An example is shown in Figure 5.2, where non-zero ranks were assigned to only two dimensions. When these two dimensions are neighbouring, there is a non-zero area, however when they are separated by another dimension (where the rank is zero), the area becomes zero.

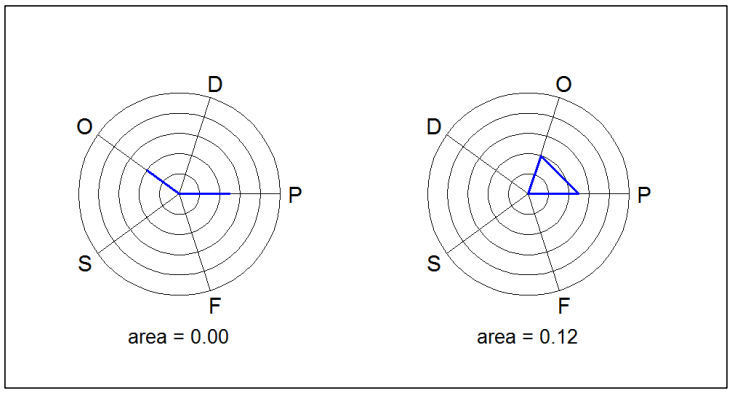


Figure 5.2 Example where area is influenced by position of different dimensions on a radial plot

5.4 FINDINGS

Based on the EMA documents 159 studies were included (reported in Appendix 12). Some studies were used more than once to support different B-I-D combinations:

- Non-overlapping subgroups of patients from Heinrich 2008 were used to support two B-I-D combinations – one with a positive recommendation[194] and one with a negative one[200]
- Data from the same patients in BOLERO-2 and Baselga 2009 were used to support two licensed B-I-D combinations that included the same drug and indication, but a different biomarker.[228]

The median number of studies per B-I-D combination was 2.5 (range zero to 19). The data for efficacy biomarkers included in B-I-D combinations without an orphan designation are presented in Figure 5.3 and in Figure 5.5 for B-I-D combinations with an orphan designation.

For B-I-D combinations where there was no orphan designation the total number of studies ranged from one to six. The number of main studies was:

- one for 17,
- two for 11 and
- three for five B-I-D combinations.

The number of supportive studies varied from one to five, as shown in Figure 5.3. In three cases the status of the studies (as main or supportive) was NR.

The total number of studies for B-I-D combinations containing orphan drugs ranged from zero to 19. For these B-I-D combinations the number of main studies was:

- one in two cases,
- two in three cases, and
- three in one case.

The number of supportive studies varied from one to 13. For four B-I-D combinations the study status as main or supportive was NR and for one B-I-D combination there were no studies.

For biomarkers predicting safety (four B-I-D combinations) there was only one study undertaken to support one B-I-D combination. In the remaining three cases the biomarker was based on the understanding of the drug metabolism.

For all studies supporting each B-I-D combination radial plots were constructed and these are reported in Appendix 13

Application of the seven criteria to the EMA dataset is discussed below and then the evidence supporting groups of B-I-D combinations is discussed.

5.4.1 Application of criteria

5.4.1.1 Population

As shown in Table 5.6, the population in studies was mostly either in agreement with, or covered a subgroup or wider group than the drug indication. In some cases where the study population was a subgroup of that identified by the indication, different studies supporting one B-I-D combination collectively covered the entire indication.

Table 5.6 Number of studies evaluating the predictive biomarker in different study populations relative to the indication

B-I-D combinations	Population in agreement with indication	Subgroup or wider group	Different or NR	Total
all	55 (35%)	92 (57%)	14 (9%)	161
efficacy non-orphan	50 (49%)	40 (39%)	12 (12%)	102
efficacy orphan	4 (7%)	52 (90%)	2 (3%)	58
safety	1 (100%)	0	0	1

The situation when the study participants were only a subgroup of the patients identified by the indication was more common in B-I-D combinations including a drug with an orphan designation. Only three studies of the 58 supporting orphan drugs included a patient group representative of the entire indication. This may be due to a large number of these studies being case reports or case series.

There were also some cases where the studies did not include a patient group in agreement with the drug indication, for example:

- one supportive study for two B-I-D combinations (Baselga 2009) – the indication was for previously treated progressive or recurrent BC, while the study included only patients with an untreated primary tumour,[228]
- three supportive studies for a refused B-I-D combination, in which case the indication was the treatment of BC in premenopausal women, while the studies included postmenopausal women.[211]

5.4.1.2 SAMPLE SIZE

The median number of patients included in all studies supporting B-I-D combinations was 88 (range 1 to 1956) and for main studies the median was 274 (range 6 to 1803). Further details of median patient numbers in identified studies for different study types and types of biomarkers are reported in Table 5.7. As expected, the numbers were much smaller for B-I-D combinations containing a drug with an orphan designation than for those without an orphan designation. Figure 5.4 shows patient numbers supporting each efficacy B-I-D combination without an orphan designation and data for B-I-D combinations including a drug with an orphan designation are presented in Figure 5.6. For safety biomarkers there was only one study which included 1956 patients.

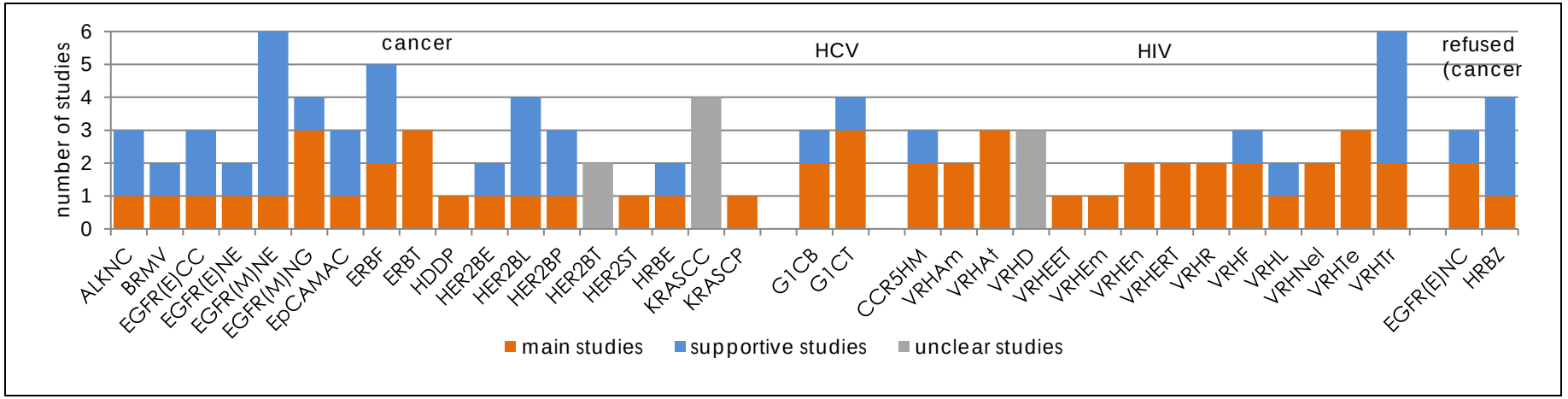
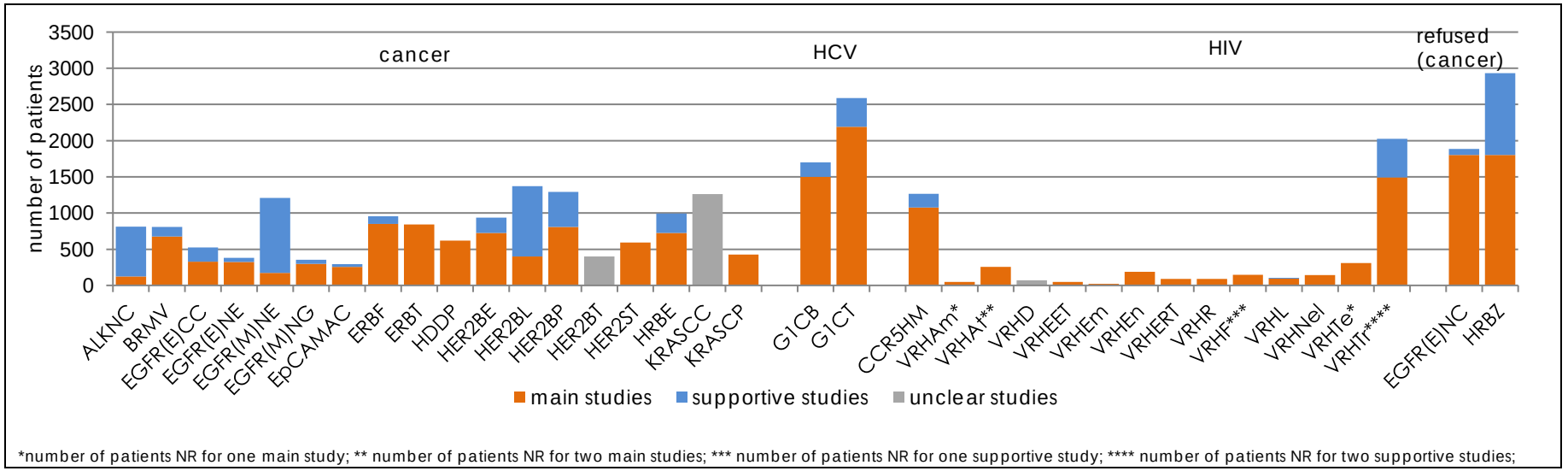


Figure 5.3 Number of studies supporting non – orphan B-I-D combinations with a biomarker predicting treatment efficacy



*number of patients NR for one main study; ** number of patients NR for two main studies; *** number of patients NR for one supportive study; **** number of patients NR for two supportive studies;

Figure 5.4 Number of patients in studies supporting non – orphan B-I-D combinations with a biomarker predicting treatment efficacy

Table 5.7 Median (range) number of patients within study types supporting B-I-D combinations

B-I-D combinations	main studies	supportive studies	all studies*
all	274 (6, 1803)	61.5 (1, 1065)	88 (1, 1956)
efficacy non-orphan	364 (22, 1803)	151 (11, 1065)	233 (11, 1803)
efficacy orphan	52 (6, 167)	1 (1, 192)	7 (1, 694)
safety	0	0	1956**

**also include studies with an unclear status; ** there was only one study with unclear status; studies where patient numbers were NR were not included in the calculation;*
medians for all studies of given type supporting a B-I-D combination; if there were no studies of a given type, the B-I-D combination was not included in the combination

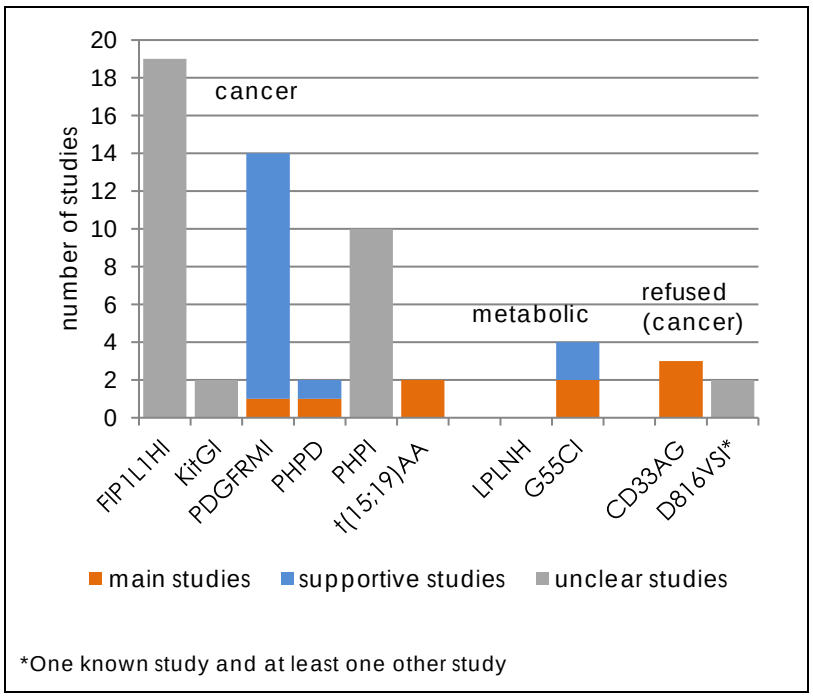


Figure 5.5 Number of studies supporting orphan B-I-D combinations with a biomarker predicting treatment efficacy

5.4.1.3 STUDY DESIGN

The identified study designs are summarised in Table 5.8. There was only one biomarker strategy design study. The design with the highest frequency in this dataset was enrichment (28%). This was followed by non-RCT studies (27%), which mostly included single-arm studies either including only patients who were biomarker positive, or irrespective of biomarker status. 25% of the identified studies evaluated the biomarker in a subgroup analysis of an RCT: 5% in a prospective, 10% in a retrospective and 10% in a cross-sectional one. There was a large number of case series and case studies (17%).

There was also one literature review and three studies where the design of the biomarker evaluation could not be identified.

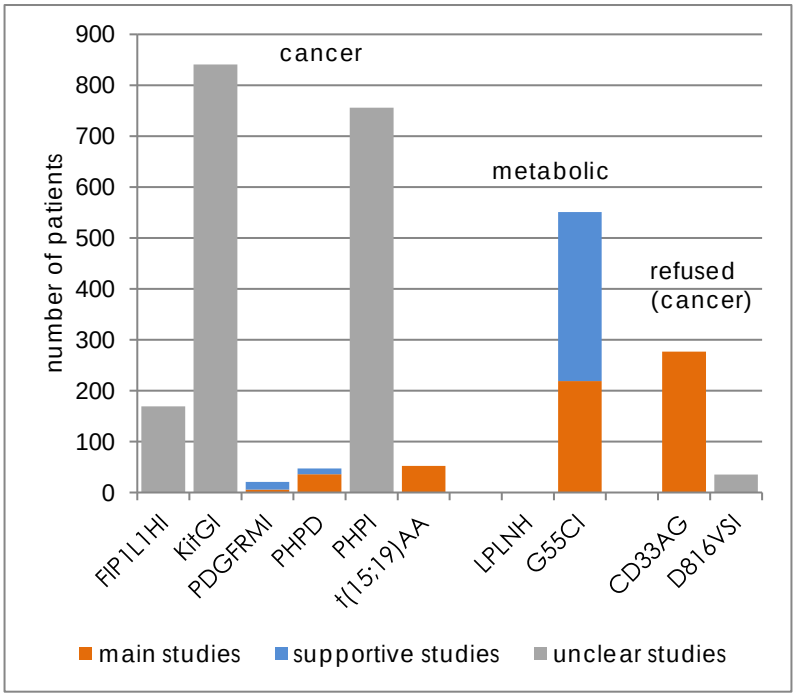


Figure 5.6 Number of patients in studies supporting orphan B-I-D combinations with a biomarker predicting treatment efficacy

Table 5.8 Number of studies evaluating the predictive biomarker in each design patients within study types supporting B-I-D combinations

B-I-D combinations	Biomarker strategy	Enrichment	Prospective RCT subgroup	Retrospective RCT subgroup	Cross-sectional RCT subgroup	Non-RCT study	Case series and analyses	Other	Total number of studies
all	1 (1%)	45 (28%)	8 (5%)	16 (10%)	16 (10%)	43 (27%)	28 (17%)	4* (2%)	161
efficacy non-orphan	0	39 (38%)	8 (8%)	16 (16%)	16 (16%)	19 (19%)	0	4* (4%)	102
efficacy orphan	0	6 (10%)	0	0	0	24 (41%)	28 (48%)	0	58
safety	1 (100%)	0	0	0	0	0	0	0	1

** includes one literature review and three studies where the design of biomarker evaluation was unclear*

For drugs without an orphan designation where the biomarker was predictive of efficacy the most frequent study design was enrichment (38%). Subgroup analyses of RCTs comprised 40% of all studies in this group, with retrospective and cross-sectional being the most common. Non-RCT studies comprised 19% of all studies.

For drugs with an orphan designation case studies and case series comprised 48% of all studies. The remaining designs were non-RCT studies (41%) and enrichment trials (10%).

For the biomarkers predicting safety there was only one biomarker strategy trial identified. This trial was a variant where the biomarker positive patients (more likely to experience an adverse event) were excluded from the biomarker strategy arm after randomisation.[29]

5.4.1.4 PRIMARY OUTCOME

As shown in Table 5.9, the primary outcome was a surrogate in 45% of studies. It was NR or not defined in 30% of studies, often reflecting the observational character of these studies. Objective clinically relevant outcomes were assessed in 7% of studies and other clinically relevant in 17% of studies.

Table 5.9 Number of studies using different types of primary outcome within study types supporting B-I-D combinations

B-I-D combinations	objective clinically relevant	other clinically relevant	surrogate	NR/ none	Total
all	11 (7%)	28 (17%)	73 (45%)	49 (30%)	161
efficacy non-orphan	9 (9%)	26 (25%)	60 (59%)	7 (7%)	102
efficacy orphan	2 (3%)	1 (2%)	13 (22%)	42 (72%)	58
safety	0	1 (100%)	0	0	1

As expected, the study outcome was generally dependent on the indication.

A primary outcome was available for 95 out of the 102 studies that supported B-I-D combinations with a non-orphan drug and a biomarker predicting efficacy. 55 of the 95 studies were carried out in a cancer indication and these assessed:

- overall survival in nine studies,
- progression free survival in 26 studies,
- tumour response in 22 studies.

There were 38 studies with an available primary outcome supporting authorised B-I-D combinations for HCV and HIV drugs. In this group all studies measured a primary outcome related to levels of the virus in blood. Although this outcome possibly does not reflect the disease mortality or morbidity, it would be difficult to measure a more relevant outcome within a population of patients suffering from a chronic condition.

For B-I-D combinations including drugs with an orphan designation data on a primary outcome were available for 16 of the 58 studies. 13 of these were undertaken in cancer and these measured:

- overall survival in two studies,
- progression free survival in one studies,
- tumour response in 10 studies.

In three studies investigating a drug for cystic fibrosis, the primary outcome was volume exhaled during the first second of a forced expiratory manoeuver (FEV1). Measurement of this surrogate outcome may again be at least partly due to the chronic character of the disease.

In one case of a study supporting a B-I-D combination with a biomarker predicting treatment safety, the occurrence of a hypersensitivity reaction (measured by patch test) was the primary outcome.

The variation in the primary outcomes assessed partially reflects the inclusion of phase III studies and those of earlier phases. It may also be associated with the difficulties in measuring more robust outcomes, such as overall survival in cases where the drug treatment may be followed by another therapy.

5.4.1.5 FINDINGS

The strength of findings is shown in Table 5.10. 50 (26%) of the studies provided evidence supporting the biomarker hypothesis and of these:

- 39 (19% of all 161 studies) had highly significant results ($p \leq 0.01$),
- 8 (5% of all 161 studies) had significant results ($p \in (0.01, 0.05]$),
- 3 (2% of all 161 studies) indicated a trend in support of the biomarker hypothesis ($p \in (0.05, 0.2]$).

In 55 studies evidence of drug activity in biomarker positive patients was shown and it was considered:

- strong in 27 (17% of all 161 studies), and
- weak in 28 (17% of all 161 studies).

In 56 studies (35%) it was considered the results did not provide sufficient support to the biomarker hypothesis, treatment activity in biomarker positive patients, or were NR.

Table 5.10 Findings of studies evaluating the predictive biomarker in each design patients within study types supporting B-I-D combinations

B-I-D combinations	Hypothesis tested: highly significant P ≤0.01	Hypothesis tested: significant p∈ (0.01, 0.05)	Hypothesis tested: trend p ∈ (0.05, 0.2)	No hypothesis; activity: strong ≥50% (n>5)	No hypothesis; activity: weak ≥30% (n>5) ≥50% (n≤5)	NR, p>0.2, activity <30% (n>5) <50% (n≤5)	Total
all	39 (19%)	8 (5%)	3 (2%)	27 (17%)	28 (17%)	56 (35%)	161
efficacy non-orphan	33 (32%)	8 (8%)	2 (2%)	12 (12%)	3 (3%)	44 (43%)	102
efficacy orphan	5 (9%)	0	1 (2%)	15 (26%)	25 (43%)	12 (21%)	58
safety	1 (100%)	0	0	0	0	0	1

For B-I-D combinations with a non-orphan drug and a biomarker predicting efficacy of the 102 studies:

- 32% had highly significant results of a hypothesis test (p≤0.01),
- 8% had significant results of a hypothesis test (p ∈ (0.01, 0.05]),
- 2% indicated a positive trend based on of a hypothesis test (p ∈ (0.01, 0.05]),
- 12% showed strong evidence of activity,
- 3% showed weak evidence of activity, and
- 43% did not provide evidence in support of the biomarker.

For B-I-D combinations with an orphan drug few of the 58 studies provided evidence based on a hypothesis test – 9% had highly significant results (p ≤ 0.01) and 2% showed a trend supporting the biomarker (p ∈ (0.01, 0.05]). The majority of the studies provided evidence of drug activity in biomarker positive patients: 26% strong and 43% weak. In 21% the results did not provide evidence in support of the biomarker.

There was one study investigating a safety biomarker and it provided highly significant results (p ≤ 0.01).

5.4.1.6 REPLICATION OF RESULTS

There were five cases of B-I-D combinations where only a single study was reported that evaluated the biomarker (shown in Appendix 13). All of these were for efficacy biomarkers in non-orphan B-I-D combinations. All of these were also authorised.

There may however be additional cases where, although multiple studies were included, these were, for example, not conducted in similar patient populations.

5.4.1.7 Consistency of the biomarker assay used

Only one type of biomarker assay was used in all studies supporting a B-I-D combination in 15 cases. In five of these the B-I-D combination was supported by a single study. In the evidence supporting eight B-I-D combinations at least two different types of biomarker assay were used. For the remaining cases it was not possible to tell, as the biomarker assay was NR for some or all of the studies supporting a B-I-D combination. There were no noticeable differences between the types of biomarker, orphan and non-orphan drugs and authorised and refused B-I-D combinations.

When the biomarker was quantitative (for example EGFR expression), the threshold was often NR. No study reported the rationale for the choice of the threshold.

5.4.2 Evidence supporting B-I-D combinations including biomarkers predicting efficacy without an orphan designation

There were 102 studies supporting 36 B-I-D combinations where the biomarker was predicting treatment efficacy and the drug did not have an orphan designation. Of these, 95 studies were identified in the documents for 34 authorised B-I-D combinations and seven for two refused ones. These are shown in Figure 5.7 and discussed in more detail below.

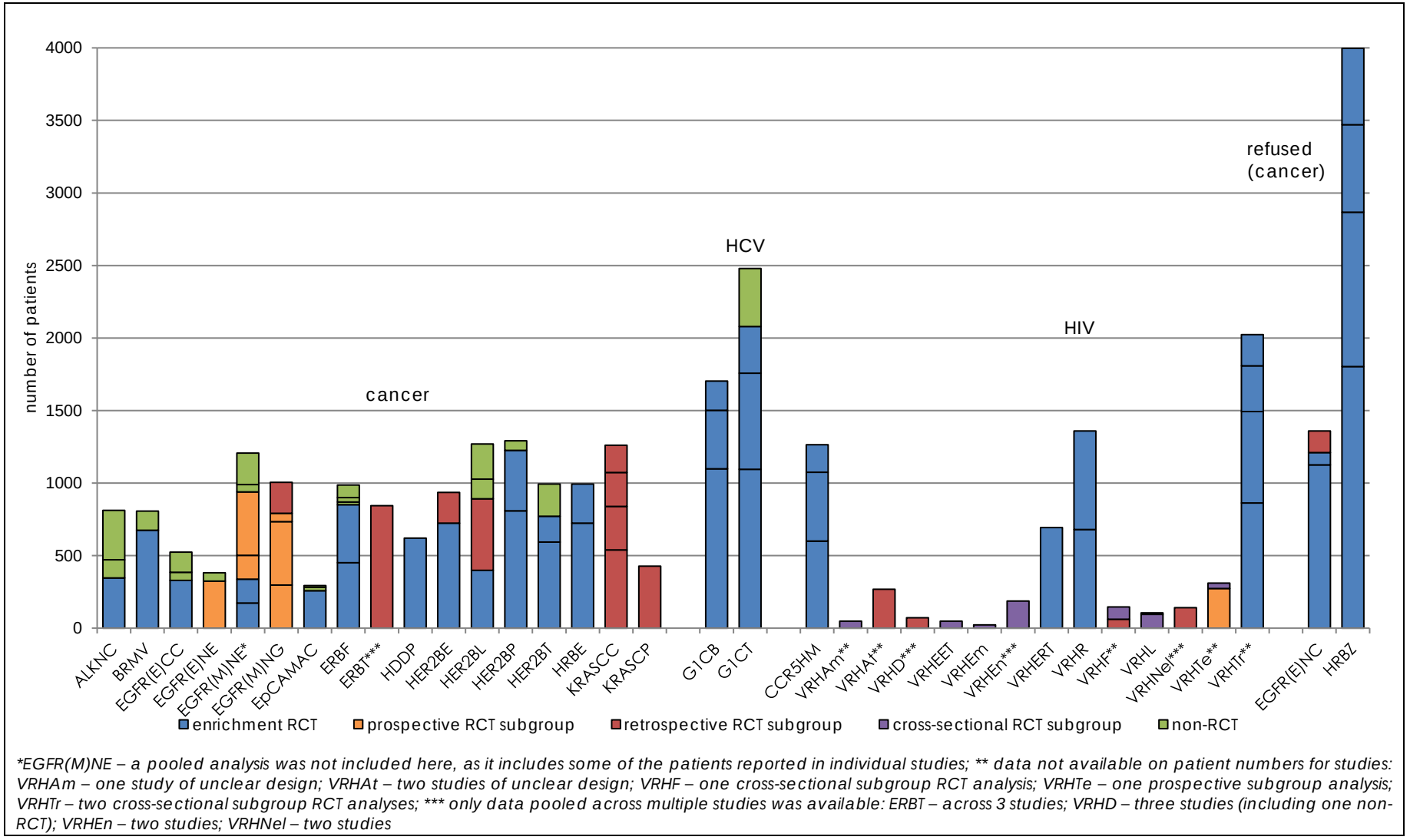
5.4.2.1 Authorised drugs

The enrichment design was the most common in this dataset. The number of patients included in these trials varied from 165 to 1099. There were also a number of subgroup analyses: prospective (sample size ranging from 57 to 437), retrospective (sample size ranging from 71 to 844) and cross-sectional (sample size ranging from 11 to 187). There were also a number of non-randomised (usually single-arm) studies with the number of patients with an available biomarker status ranging from 13 to 400.

The identified evidence is discussed below based on the best study design (according to the ranking introduced in Table 5.2) identified for each B-I-D combination.

Enrichment design

The enrichment design was used in 18 of the 34 B-I-D combinations and in 16 of these it was part of the group of main studies. In the two cases where it was not a main study:



EGFR(M)NE – a pooled analysis was not included here, as it includes some of the patients reported in individual studies; ** data not available on patient numbers for studies: VRHAm – one study of unclear design; VRHAt – two studies of unclear design; VRHF – one cross-sectional subgroup RCT analysis; VRHTe – one prospective subgroup analysis; VRHTr – two cross-sectional subgroup RCT analyses; * only data pooled across multiple studies was available: ERBT – across 3 studies; VRHD – three studies (including one non-RCT); VRHEn – two studies; VRHNel – two studies*

Figure 5.7 Numbers of patients supporting non **–** orphan B-I-D combinations with a biomarker predicting treatment efficacy by study design

- the status of studies (as main or supportive) was NR (HER2 expression – BC - trastuzumab),[102]
- the main study was a single arm study that was supported by an ongoing enrichment trial and another single arm study (ALK mutation – NSCLC - crizotinib).[171]

Examples of an enrichment design visualised using radial plots is shown in Figure 5.8.

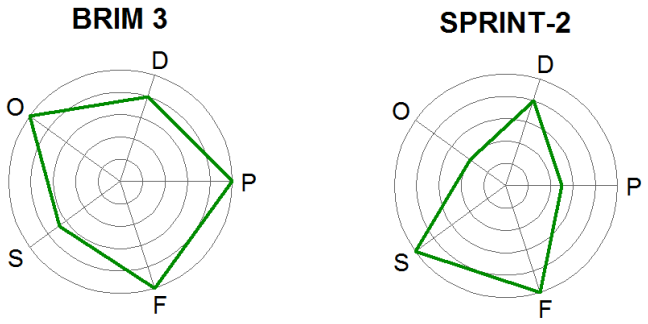


Figure 5.8 Examples of an enrichment design supporting B-I-D combinations

Where the best study had an enrichment design (18 B-I-D combinations) it was usually undertaken in a population representative of that in the indication (11 cases) or at least part of the population (6 cases). The population characteristics were NR in one case. The primary outcome was PFS in eight, OS in two and a surrogate outcome in eight cases. In 16 of the B-I-D combinations where an enrichment RCT was the best design the results were highly significant ($p \leq 0.01$) and these were also supported by other studies:

- at least one more enrichment trial (sometimes with less significant findings) and/or single arm study[102,167,175-177,182,185,186,228-230] in 12 cases,
- at least one single-arm study
- one enrichment trial, two prospective subgroup analyses, two single arm studies and a literature review[180] in one case,
- a retrospective RCT subgroup analysis and two single arm studies[231] in one case,
- a retrospective RCT subgroup analysis[228] in one case,
- in one case there were four enrichment RCTs and two cross-sectional RCT subgroup analyses.[208]
- no additional trials in two cases.[187,190]

The additional studies often showed weaker evidence in support of the predictive biomarker or treatment use in the biomarker positive patients.

For two B-I-D combinations the results of the enrichment trial they were not significant ($p > 0.2$). These were supported by either single-arm studies (which also showed no evidence of activity)[167] or an enrichment trial (with non-significant results) and single-arm studies, some of which showed activity in biomarker positives.[183]

PROSPECTIVE SUBGROUP ANALYSIS

For three B-I-D combinations the best study design was a prospective subgroup analysis and in all of these it was identified as one of the main studies. An example of this design visualised using radial plots is shown in Figure 5.9. The population in these studies was representative of at least a subgroup of that identified by the drug indication. In two cases the findings of the subgroup analysis were significant for overall survival($p \geq 0.05$)[179,181] and in one these were NR for a surrogate primary outcome.[207] In all three B-I-D combinations there were additional studies included and these were: a single arm study,[179] a retrospective subgroup analysis,[181] and a cross-sectional subgroup analysis.[207]

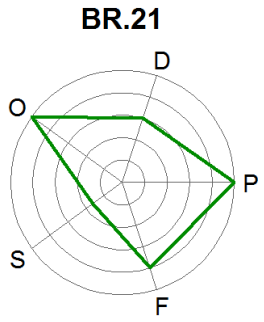


Figure 5.9 Example of a prospective subgroup analysis used to support a B-I-D combination

RETROSPECTIVE SUBGROUP ANALYSIS

In six B-I-D combinations cases a retrospective subgroup analysis (example of visualisation provided in Figure 5.10) was the best study design. In five of these it was labelled as a main study and in one the study status was not clear.[169] For four of these B-I-D combinations the population in the subgroup analysis was representative of that in the drug indication,[159,192,232,233] for one it was a subgroup[169] and in one it was not clearly reported.[205]

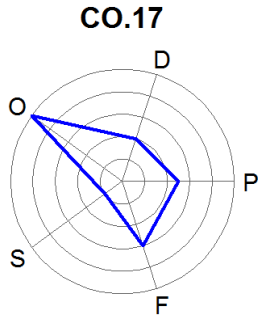


Figure 5.10 Example of a retrospective subgroup analysis used as a main study to support a B-I-D combination

In three cases there was a single retrospective subgroup analysis supporting the B-I-D combination. It one case each the for the subgroup analyses the findings were: highly significant ($p \leq 0.01$) for PFS,[192] NR for PFS,[159] neither the outcome, nor the findings were reported.[205]

In the remaining three B-I-D combinations in addition to a retrospective subgroup analysis supporting the biomarker, there were other studies: another retrospective subgroup analysis,[169] two cross-sectional subgroup analyses[203] and two studies of unreported design.[198]

CROSS-SECTIONAL SUBGROUP ANALYSIS

A cross-sectional subgroup analysis was the best design in six B-I-D combinations (an example is shown in Figure 5.11), all of which included viral resistance mutations used to predict lack of treatment efficacy in HIV. In most cases there were only studies of this design included: a single one in three[199,234,235] and two in in two cases.[204,236] In one case there was a study of unreported design in addition to the cross-sectional analysis.[197]

These analyses generally provided evidence on only a subgroup of the relevant population, as they included only patients who did not respond to, or lost response to the treatment. They usually showed high prevalence of the resistance mutations in non-responders (often above 50%).

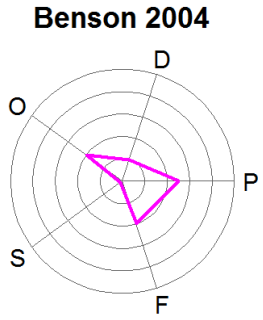


Figure 5.11 Example of a cross-sectional subgroup analysis used as a main study to support a B-I-D combination

SINGLE-ARM

There were no cases in this dataset where a single-arm study (example shown in Figure 5.12) was the best study design supporting a B-I-D combination. In only one case it was the only main study.[171]

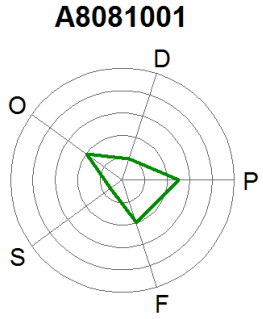


Figure 5.12 Example of a single-arm study used as a main study to support a B-I-D combination

5.4.2.2 REFUSED DRUGS

There were two cases where the B-I-D combination was given a negative recommendation. In both cases there was one main enrichment trial with significant[210] or highly significant findings[209] (as shown in Figure 5.13). These results were however not replicated in another study. In one case there were three additional enrichment studies that showed efficacy, however these were conducted in a different population (postmenopausal rather than premenopausal women).[210] In the other case there was another enrichment study and a single arm study, which did not show significant efficacy and substantial activity respectively.[209]

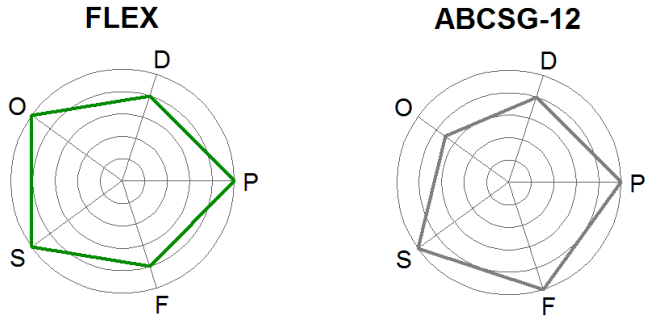


Figure 5.13 Examples of main studies supporting refused B-I-D combinations without an orphan designation

5.4.3 Evidence supporting B-I-D combinations including biomarkers predicting efficacy with an orphan designation

There were 58 studies supporting 10 B-I-D combinations where the biomarker was predicting treatment efficacy and the drug had an orphan designation. Of these, 53 studies were identified in the documents for 8 authorised B-I-D combinations and five for two refused ones. These are shown in Figure 5.14 and discussed in more detail below.

5.4.3.1 Authorised drugs

In this part of the dataset again the best study design was an enrichment trial (with sample size ranging from 55 to 694). However lower-level evidence was more frequently used and this included mainly non-RCT studies (sample size ranging from 5 to 353), case reports and case series.

Enrichment

In three cases the best study design supporting the B-I-D combination was an enrichment design. In one case two such trials were labelled as main studies and in the remaining two cases the status as main or supportive was not indicated. All of these included patients who were representative of a subgroup or a wider group compared to the population identified by the indication.

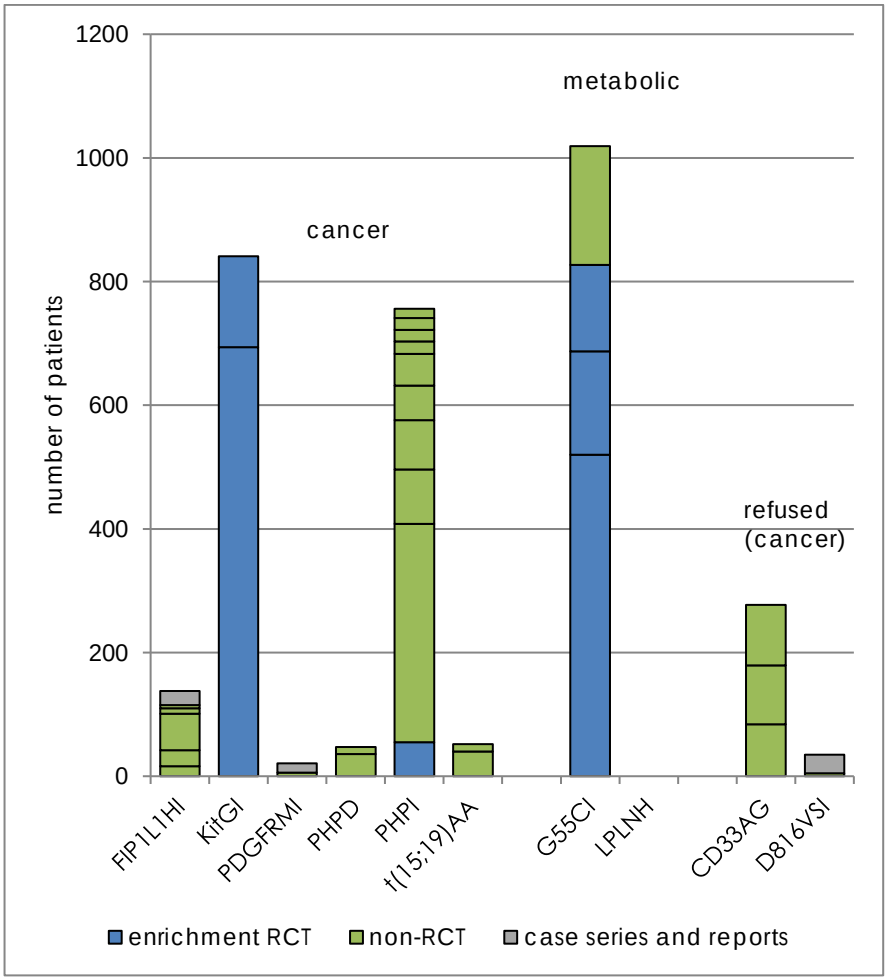


Figure 5.14 Numbers of patients supporting orphan B-I-D combinations with a biomarker predicting treatment efficacy by study design

In all three cases an enrichment trial provided strong evidence in support of the efficacy of the drug in biomarker positive patients ($p \leq 0.01$), as illustrated in Figure 5.15. The primary outcomes measured were overall survival[191] or a surrogate outcome.[165,196] These were supported by:

- another enrichment study with less significant findings,[191]
- another enrichment study with less significant findings and a single arm study,[165]
- single arm studies.[196]

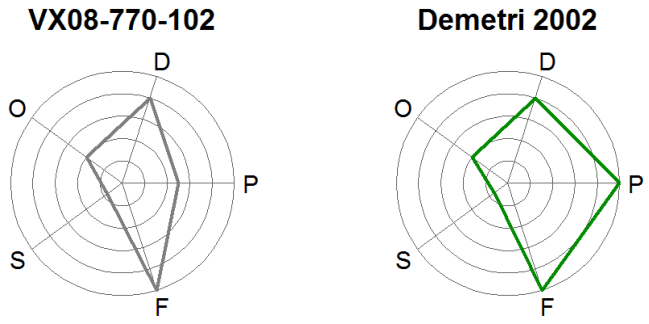


Figure 5.15 Examples of enrichment RCTs

NON-RANDOMISED STUDIES

In four cases the best design was a non-randomised study (mainly single-arm) with the biomarker forming part of the design (examples are shown in Figure 5.16). In three of these at least one non-randomised study was labelled as main and in one its status as main or supportive was not. Where reported, the primary outcome was tumour response.

The number of non-randomised studies supporting a B-I-D combination varied from one to five. In three cases at least one showed drug activity in >50%[170,184,194] and in one in >30% of patients.[195]

For two B-I-D combinations only non-randomised studies were found.[170,195] In the remaining two cases these were supported by either 13[194] or 14[184] case reports and series.

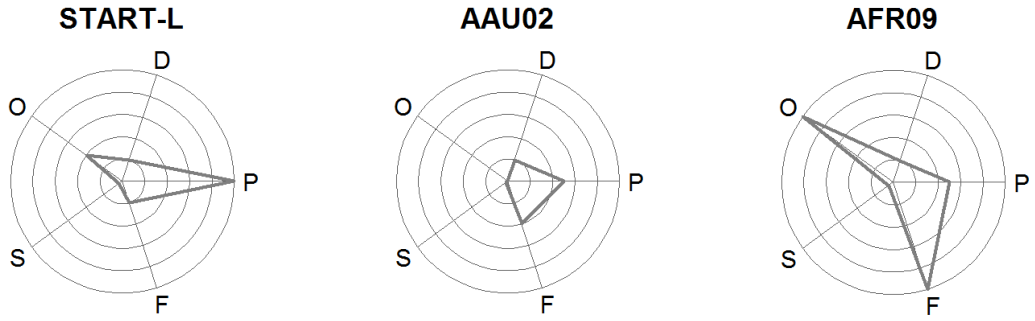


Figure 5.16 Non-randomised studies

CASE REPORTS AND SERIES

Case reports and series (examples shown in Figure 5.17), usually showing activity, were used to support more robust study designs, however these were never used on their own. These were often judged to be only partly representative of the patients identified by the drug indication.

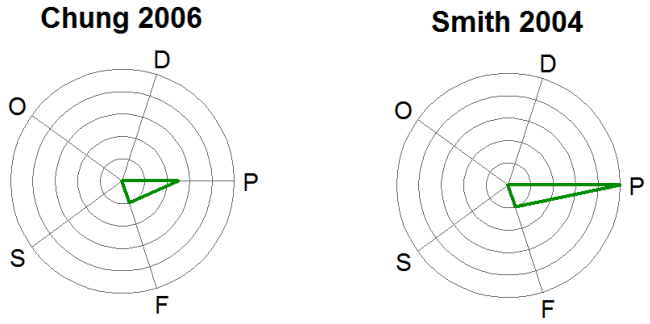


Figure 5.17 Examples of case series and reports used to support B-I-D combinations with an orphan designation

MECHANISM OF DRUG ACTION

In one case (LPL deficiency) the biomarker was based on the mechanism of drug action only and no studies investigating the biomarker were reported.[214]

5.4.3.2 REFUSED DRUGS

There were two B-I-D combinations with a negative recommendation. In both cases the best study design was a non-randomised study (an example shown below in Figure 5.18). These generally included a subset of patients identified by the proposed drug indication. All the non-randomised studies measured tumour response as their primary outcome and did not demonstrate large drug activity (<30% in all of these). In one case there was a single non-randomised study and data from 30 patients from other, unspecified studies.[200] In the other case three non-randomised studies were reported.[164]

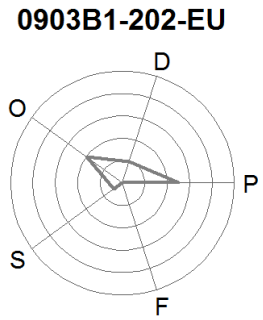


Figure 5.18 Radial plot characteristic of refused B-I-D combinations with an orphan designation

5.4.4 Evidence supporting B-I-D combinations including biomarkers predicting drug safety

There were four B-I-D combinations where the biomarker was predictive of treatment toxicity (all received a positive recommendation). For three of these there were no studies provided that evaluated the biomarker and the inclusion of the biomarker in the B-I-D combination was based on the mechanism of drug action.[174,178,193] In one case there was one study reported (it was not clear whether it was a main study)[34-36] – the PREDICT-1 trial, which evaluated HLAB*5701 in a biomarker-strategy design.[29] The patients in this trial were representative of those identified by the indication. The primary outcome was occurrence of a hypersensitivity reaction and a highly significant result was shown ($p \leq 0.01$). The radial plot for this trial is shown below in Figure 5.19.

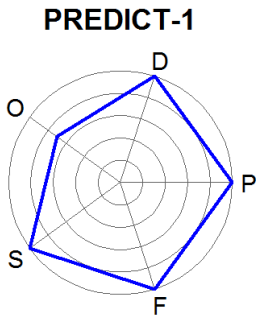


Figure 5.19 Radial plot for the PREDICT-1 trial

5.5 Discussion

This chapter investigated the evidence supporting the inclusion of predictive biomarkers in the indication or contraindication of drugs considered by the EMA. 159 studies were analysed in an attempt to identify standards of evidence sufficient for inclusion of a predictive biomarker in a drug licence. Radial plots were used to summarise the criteria selected for this chapter.

The findings of this chapter did not provide a clear picture of the evidence standard required in practice by the EMA. There were cases where similar levels of evidence supported B-I-D combinations with a positive and negative recommendation. This suggests the evidence may be considered within a given context, rather than required to meet a certain standard. This was also identified as an important factor in Chapter 4. There were however a number of issues that were identified within the evidence base.

The common use of enrichment design can be potentially problematic. Although it provides information on the efficacy of the drug within a patient population identified by

the biomarker, it does not evaluate the biomarker itself. Even if significant efficacy is shown, it is possible a wider population of patients could benefit. This was shown in the example of vemurafenib, which was originally developed to target melanoma positive for the BRAF V600E mutation. In the course of clinical research it emerged that the assay used to evaluate this biomarker and include patients into trials was classifying patients with other V600 mutations as positive. As these patients clearly benefited from treatment with vemurafenib, they were also included in the final indication.[176]

In two cases the biomarker predicted lack of efficacy (KRAS mutation) and was identified after an initial marketing authorisation. This biomarker was investigated only in retrospective subgroup analyses, some of which included only a relatively small subgroup of patients from the original study (for example 33% in the CO.17[237,238] and 23% in the EPIC[169,239] study). Although due to the relatively late stage of the drug cycle when this biomarker was identified, it was potentially difficult to investigate it in a more robust design. And in spite of the limitations in the evidence base, it appears to have become important in clinical practice.

A cross-sectional subgroup analysis was only observed in HIV studies. In this design at the end of the trial plasma HIV RNA samples from patients who had no or lost response to the treatment were evaluated for presence of viral mutations. This approach appears to be largely justified, as viral resistance mutations may be undetectable at baseline or develop later in response to the treatment.[74,75] A retrospective subgroup analysis at the end of the trial may also not be possible, as in patients responding to treatment the amount of the HIV virus in their blood falls below the level of detection.[240] However, as the studies cannot use controls (samples from patients who responded to the treatment), there is a danger that this may result in a large number of chance findings.

For orphan drugs, as expected, the population studied was smaller than for non-orphan drugs. It was however clear that in some cases patient numbers were relatively large and single-arm studies included up to 353 patients. It appears that in such cases a randomised trial would have been feasible and would have provided more robust data.

There were also four B-I-D combinations where the inclusion of the biomarker in the indication (one B-I-D combination with an efficacy biomarker) or contraindication (three B-I-D combinations with a safety biomarker) was based on the understanding of the mechanism of drug action or drug metabolism. Although clinical trials of biomarkers predicting adverse events can be challenging, the PREDICT-1 trial[29] demonstrates that it is possible in at least some cases.

All HIV studies were assigned a low score for the outcome assessed, which generally was based on plasma HIV RNA levels at a certain time point. However, as HIV is a chronic condition and on development of resistance to one treatment regimen a new one is initiated, it would be very difficult (if not impossible) to measure an outcome more directly related to morbidity or mortality. The same may also apply to certain cancer settings, where the treatment is not curative and a further line of therapy is given after development of tumour resistance.

This review attempted to explore the standards of evidence supporting EMA recommendations, however it was on occasion limited by the lack of reporting of important data in the EMA documentation. An attempt was made to identify published papers and other information sources (such as trial reports published on the drug manufacturer's website) that would supplement the information provided within EMA documents. This was however not always possible as there were no additional sources found or based on the very limited information provided by the EMA the trial could not be unambiguously identified.

Poor reporting was especially important with regard to the information on the biomarker assay. Within the EMA documentation there was usually not enough detail about the biomarker assay used and sample collection. This information was more frequently reported in published papers, however important details were often still missing.

The analysis was also limited by the fact that data collection and analysis was carried out by one reviewer only.

5.6 Conclusions

For biomarkers predicting treatment efficacy the evidence standards were not clear. In two cases of negative recommendations for non-orphan drugs, it appears that the promising results of a single study have not been replicated in another study. This however was also the case for some of the B-I-D combinations which received a positive recommendation. For drugs with an orphan designation there was little, if any, clear difference between the evidence base supporting the positive and negative recommendations.

Based on this chapter it appears that for biomarkers predicting treatment safety the study evidence requirements are minimal, as in three of the four B-I-D combinations the biomarker predicting safety was based on the understanding of the drug action and/or

metabolism. However, interestingly, this group also included the only biomarker strategy design within the entire dataset.

The analysis identified poor reporting, especially of information related to the biomarker assay and biological sample collection, as an important issue limiting the evaluation of the studies and implementation of their findings.

These findings possibly highlight the importance of the context in which the B-I-D combinations were assessed. However, there is also need for more consistent methodological and evidence standards to ensure optimal patient treatment.

CHAPTER 6. STUDY DESIGNS FOR EVALUATION OF PREDICTIVE BIOMARKERS FOR USE IN CLINICAL PRACTICE - APPLICATION OF A PUBLISHED FRAMEWORK

Author Contributions

Kinga Malottki led the design of the project, carried out data analysis and drafted the chapter
Lucinda Billingham provided advice on the design of the project, data analysis and the chapter
Jon Deeks provided advice on the design of the project, data analysis and the chapter

Abstract

Background: To ensure that biomarkers used in clinical practice are truly predictive, appropriate study designs need to be used. Chapter 5 investigated what evidence supported licensing of biomarker-indication-drug combinations in practice.

Aims: The main aim of this chapter was to assess the validity and any limitations of the framework proposed in Tajik 2013[78] for study designs for evaluation of predictive biomarkers by application of study data from Chapter 5. Based on the discrepancies between the framework and the existing data, the framework was to be modified.

Methods: The systematic review that provided a framework for study designs for stratified medicine (Tajik 2013[78]) was critically appraised. The framework proposed in this review was applied to the data from Chapter 5. Based on the comparison, the framework was modified.

Findings: The framework in Tajik 2013[78] was modified based on the dataset identified in Chapter 5 to include seven major study designs (case report, case series, single-arm, enrichment, randomise-all and biomarker-strategy), four of which were further subdivided into subclasses of design. Six additional questions that could be addressed by studies were also identified. Of the 152 studies that had sufficient data to identify their design, 86 matched those proposed in Tajik 2013.[78] For published papers authors' conclusions seemed to be mainly addressing questions possible to answer based on the study design, although there were a number of studies where conclusions beyond these appeared to be made. The study labels did not often reflect the biomarker aspect of the study.

Conclusions: The framework in Tajik 2013[78] needed to be modified to better reflect the study designs found in practice. This suggests there might be a substantial discrepancy between what designs are proposed and evaluated in the methodological literature and those identified in the dataset supporting EMA recommendations. There may be a need to apply more robust methodology to studies carried out in practice, as well as develop the methodology for certain cases, for example investigation of HIV resistance mutations. There is also a need for more transparent labelling of biomarker-based studies.

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6.1 BACKGROUND

The use of predictive biomarkers can offer substantial improvement to patient care, by identifying patients more likely to benefit from a particular treatment.[9,241] There have been some advancements in the field, however biomarker research has also seen many failures.[242] To ensure that biomarkers used in clinical practice truly offer patient benefit, appropriate study designs need to be used for their evaluation prior to implementation in clinical practice.[21,49]

Chapter 5 investigated what evidence supported licensing of biomarker-indication-drug (B-I-D) combinations in practice. Study design labels were given based on a subjective selection of published papers and theoretical concerns in one case (introducing the name "cross-sectional subgroup analysis" of an RCT). However it was still not known how this compared to the entirety of the methodological literature on evaluation of predictive biomarkers.

A systematic review of trials methodology for stratified medicine was identified (Tajik 2013[78]). This review proposed a framework which matched different study designs to the questions that can be answered within these. This framework was therefore used to compare the methodological literature with the research practice identified in Chapter 5.

6.2 AIMS

The aim of this chapter was to assess the validity and any limitations of the framework proposed in Tajik 2013[78] for study designs for evaluation of predictive biomarkers by application to study data from Chapter 5. This was undertaken to explore how the methodological literature matched the studies carried out in practice. Based on any discrepancies, the framework was to be modified to better reflect methodology used in practice. It was considered how the authors' conclusions matched the questions that the design of their study could potentially address. It was also investigated how each design was described in study publications.

6.3 METHODS

The identified systematic review (Tajik 2013[78]) was critically appraised using criteria based on the AMSTAR tool[243] that were adapted to a methodological review context (reported in section 6.4.1). Some limitations to the methodology were identified. It was however

considered that the framework proposed by this review may be relatively complete with regards to study designs proposed in the theoretical literature.

Therefore the framework proposed by Tajik 2013[78] was applied to the evidence collected in the review of evidence supporting EMA recommendations (Chapter 5).

6.3.1 Applying the framework in Tajik 2013[78] to the EMA dataset

The Tajik 2013[78] systematic review proposed a framework identifying the questions that can be addressed by each identified study design (reproduced below in Table 6.2). This framework was applied to the evidence supporting EMA recommendations identified in Chapter 5.

6.3.1.1 Study designs and questions addressed

For each study identified in Chapter 5 the design was:

- assigned according to the framework in Table 6.2 if the design matched the proposed categories,
- based on Chapter 5 if the design did not fall under any of the proposed categories.

In addition, for the category of "randomise-all" study designs a further description was retained (for example "prospective subgroup analysis"), as based on the findings of Chapter 5 there was variation within this category with possible impact on the information provided by different subtypes of this study design. Therefore it was considered that this category of study design might need refining.

Study designs that did not match the framework in Tajik 2013[78] were added to the framework and questions answered by these designs were considered. This was partially based on consideration of study authors' conclusions (explained in more detail in section 6.3.1.2 below) and partially on theoretical concerns.

For each study the questions addressed by the design were recorded. This was also summarised for each B-I-D combination and questions addressed by the main studies were highlighted.

Where relevant, the biomarker type, drug orphan status and classification of studies by the EMA as main (forming the major part of the evidence supporting a drug indication) or supportive were noted.

6.3.1.2 CONCLUSION AND STUDY LABEL REPORTING IN IDENTIFIED PUBLICATIONS

Where there were publications available matched to the identified study, the conclusions from the most recent relevant publication were extracted. It was then attempted to class these conclusions as commenting on questions 1-8 in Table 6.2 using the criteria in Table 6.1.

Table 6.1 Criteria used to class conclusions from identified publications

Question	Criteria
1-3	For each population (respectively: biomarker positive, biomarker negative and overall) words such as "effectiveness" or "efficacy" were used in the conclusions or the clinical effects were stated and no attempt was made to label the results as, for example, exploratory or suggestive.
4-5	For each population (respectively: biomarker positive, biomarker negative and overall) words such as "effectiveness" or "efficacy" were used in the conclusions or the clinical effects were stated and no attempt was made to label the results as, for example, exploratory or suggestive.
6	Biomarker effects were compared between experimental and control study group, an interaction test was mentioned, or conclusions on whether the biomarker was predictive were made.
7-8	Biomarker-based strategy was commented on compared to different treatments (control and experimental respectively) within the overall patient population.

If there were conclusions relevant to the predictive biomarker, that were not described in questions 1-8, these were considered for addition to the list of questions addressed by the different designs. If the question list was expanded, the new question was given a label (A1, A2 and so on) and it was considered whether any of the study designs addressed this question. The criteria for classifying study conclusions as addressing any of the new questions (A1, A2 and so on) were defined.

If available, the study design label reported in the matched publication was recorded and compared to that proposed by Tajik 2013[78] or, if there was none, Chapter 5.

Table 6.2 List of effects that can be assessed and questions that can be answered by the trials of each design category. Reproduced from the Tajik 2013[78] systematic review

				Biomarker-strategy		
Questions trial can answer	Single-arm*	Enrichment	Randomise-all**	With biomarker measurement in the control arm	Without biomarker measurement in the control arm	With treatment randomisation in the control arm
Treatment effects						
Q1. How does the experimental treatment compare with the control treatment in biomarker-positives?		✓	✓	✓	✓ indirect	✓
Q2. How does the experimental treatment compare with the control treatment in biomarker-negatives?			✓			✓
Q3. How does the experimental treatment compare with the control treatment in overall study population?			✓			✓
Biomarker effects						
Q4. Is the biomarker status associated with the outcome in the standard of care group? (Is the biomarker prognostic?)			✓	✓	✓ indirect	✓
Q5. Is the biomarker status associated with the outcome in the experimental treatment group?	✓		✓	✓	✓	✓
Biomarker by treatment effect						
Q6. Is the biomarker status associated with a benefit of experimental treatment? (Is the biomarker is predictive?)			✓			✓
Strategy effects						
Q7. How does the biomarker-based treatment strategy compare with the control treatment in the overall study population?			✓ indirect	✓	✓	✓
Q8. How does the biomarker-based treatment strategy compare with the experimental treatment in the overall study population?			✓ indirect			✓

* this includes biomarker positive and negative patients and the label will be modified in the following tables to reflect this; ** this would include the stratified design, prospective-retrospective framework and all types of subgroup analysis described in Chapter 1

6.4 FINDINGS

6.4.1 CRITICAL APPRAISAL OF TAJIK 2013[78] SYSTEMATIC REVIEW USING THE AMSTAR TOOL

The detailed critical appraisal of this review is reported in Appendix 14.

There were some issues in this systematic review, suggesting it could have potentially missed some of the existing trial designs. There were also some doubts to whether the proposed framework truly reflects the information that can be obtained from each trial design. It was however considered that the proposed framework might be sufficiently complete to be used within this thesis for the comparison of the most commonly discussed study methodologies with studies carried out in practice.

6.4.2 IDENTIFIED STUDY DESIGNS

The collected data for all studies and matched publications are reported in Appendix 15.

In total there were 159 studies in the dataset, however the design with respect to biomarker evaluation could be identified for 152 of these. The design of 86 studies matched the categories in the framework proposed in Tajik 2013[78]. These studies were:

- seven single-arm studies including biomarker positive and negative patients,
- 41 enrichment studies,
- 37 randomise-all studies,
- one biomarker-strategy study with biomarker measurement in the control arm (this was however modified to exclude biomarker negative patients from the biomarker strategy arm)

There were no biomarker-strategy trials without biomarker measurement in the control arm or with randomisation in the control arm.

There were 66 primary studies for which the design did not match the framework proposed by Tajik 2013[78]. These were:

- 21 case reports - detailed reports on individual patient(s) management; defined here as including five patients or less,
- five case series - small observational study without a clear design; defined here as including more than 5 patients,
- 35 single arm studies including only biomarker positive patients – similar to the single arm study in Tajik 2013[78] with narrower inclusion criteria,

- one single arm study including only biomarker negative patients – similar to the single arm study in Tajik 2013[78] with narrower inclusion criteria,
- three non-randomised comparative studies including both biomarker positive and negative patients – studies that included patients of any biomarker status that were allocated to different treatments based on clinical considerations and therefore did not provide a robust comparison between different treatment options,
- one enrichment study including biomarker negative patients only - similar to the enrichment study in Tajik 2013[78], however only biomarker negative patients (not expected to respond to treatment) were included.

The identified study designs are summarised in Figure 6.1.

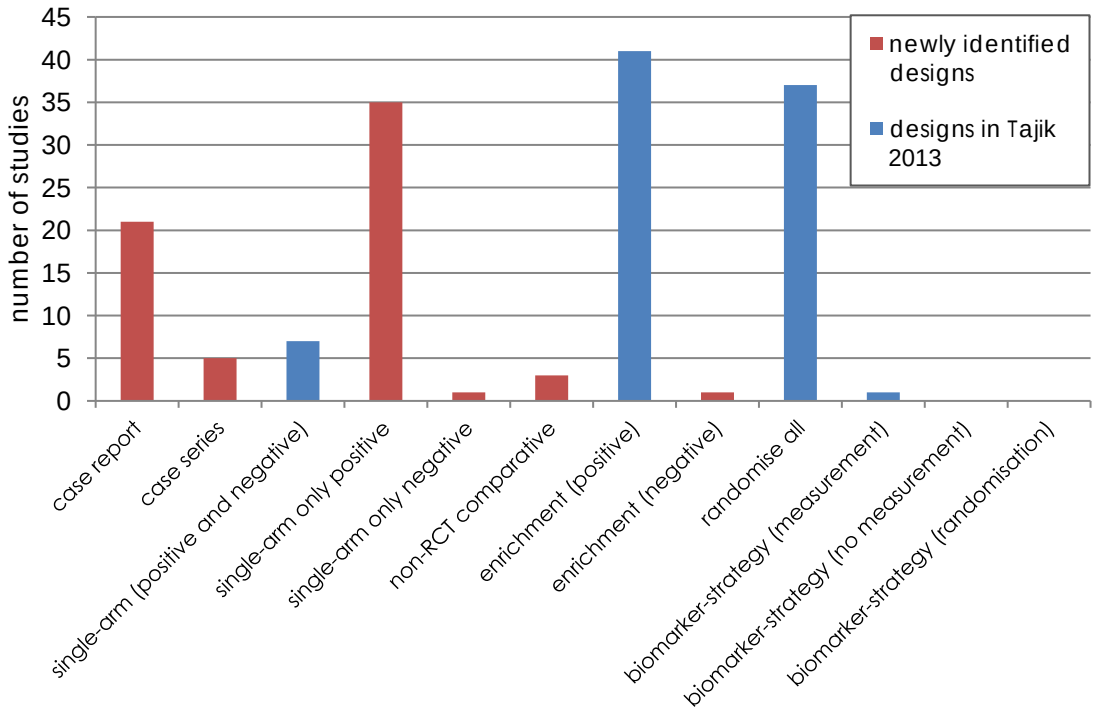


Figure 6.1 Study designs in EMA dataset according to the expanded framework proposed in Tajik 2013[78]

Further, based on the questions that could be potentially answered by different designs and the strength of evidence the following sub-types of randomise-all study design were identified:

- 11 prospective subgroup analyses – the subgroup analysis based on the biomarker was planned in the study protocol and the biomarker was measured in the beginning of the trial,
- 11 retrospective subgroup analyses - after the conclusion of the trial a biomarker subgroup of interest was identified, the biomarker status was tested (using, for example, archival tumour samples) and a subgroup analysis was performed,
- 15 cross-sectional subgroup analyses - after the conclusion of the trial a subgroup of patients (either from one treatment arm only or from multiple treatment arms) was identified based on outcome (for example lack of response to treatment) and the biomarker (or a panel of biomarkers) was assessed in this group – the final information provided was the prevalence of the biomarker in the subgroup of patients.

The cross-sectional subgroup analysis was identified only in studies investigating viral resistance mutations to predict lack of response to drugs for treatment of HIV infection, as discussed in Chapter 5. This disease setting may present a challenging situation, where a more robust trial design may be difficult to achieve. The virus with the resistance mutations may be either present at baseline in quantities below the limit of detection for the currently available tests, or develop later in response to the treatment.[74,75] A retrospective subgroup analysis at the end of the trial may however not be possible, as in patients responding to treatment the amount of the HIV virus in their blood falls below the level of detection and therefore no mutations can be detected.[240] This information may still be however useful for prediction of response to the treatment in future patients, as using the drug in the clinic may lead to the spread of the resistant virus in the patient population.[244,245]

6.4.3 Additional questions identified

As a result of expanding the list of study designs and analysis of the conclusions in identified papers, six additional questions were added (shown in Box 6.1).

Box 6.1 Additional questions identified in the analysis

A1. Does the experimental treatment show activity in biomarker-positives?

A2. Does the experimental treatment show activity in biomarker-negatives?

A3. Does the experimental treatment show activity in biomarker-unknowns?

A4. Does the experimental treatment show activity in overall study population?

A5. How does the experimental treatment compare with the control treatment in biomarker-unknowns?

A6. What is the prevalence of patients with a different biomarker status in patients not responding to treatment?

Where relevant, these new questions (A1-A6) were mapped onto the original study designs (reported in Tajik 2013[78]) and those identified in this chapter.

6.4.4 New proposed framework

Based on the newly identified designs and questions an expanded framework was proposed (shown in Table 6.3). The new questions (A1-A6) were grouped with the original questions (Q1-Q8) according to themes these addressed.

For RCT study designs where treatment effects compared to a control group could be investigated, activity was considered irrelevant, as higher level evidence on efficacy was available.

A list of all the questions included in the expanded framework is provided in Box 6.2.

6.4.5 Questions addressed by studies in the entire dataset

For 156 of the 159 studies in the dataset sufficient information was available on the design to allow evaluation of which questions were addressed within this design based on the proposed framework. These included 99 studies supporting drugs without an orphan indication (53 main studies and 46 supportive and unclear studies) and 57 studies supporting drugs with an orphan indication (9 main studies and 48 supportive and unclear studies). The high number of supportive and unclear studies in the orphan category was mainly due to a large proportion of these being case reports.

Table 6.3 Expanded framework

			single-arm				enrichment		randomise all				biomarker-strategy		
	case report‡	case series§	positive and negative	Only positive	Only negative	non-RCT comparative	positive	negative	Prospective subgroup	Retrospective subgroup	Cross-sectional subgroup	With biomarker measurement	Without biomarker measurement	With randomization in the control arm
A1	✓w	✓w	✓	✓		✓								
A2	✓w	✓w	✓		✓	✓								
A3	✓w	✓w												
A4		✓w	✓			✓								
Q1							✓		✓	✓		✓	✓i	✓
Q2								✓	✓	✓				✓
A5														✓
Q3									✓	✓				✓
Q4									✓	✓		✓	✓i	✓
Q5			✓						✓	✓		✓	✓	✓
Q6									✓	✓				✓
Q7									✓i	✓i		✓	✓	✓
Q8									✓i	✓i				✓
A6			✓			✓			✓	✓	✓			✓

✓w - weak evidence; ✓i - indirect evidence; crossed out area indicates that for RCT study designs activity was considered irrelevant, as higher level evidence on efficacy was available

When all 156 studies with known design were considered (details shown in Figure 6.2), the most frequently addressed questions were A1 and Q1 (respectively activity and effectiveness of the investigated drug in biomarker positive patients), both of which were addressed by over 60 of the 156 studies. However, effectiveness was addressed more frequently by main studies (69% of all studies addressing Q1 were classed as main), while activity was mainly within the scope of supportive and unclear studies (88% of studies addressing A1 were classed as supportive or unclear). Question A6 (biomarker prevalence in non-responders) was addressed by 38 studies, 25 (66%) of which were classed as main.

‡ Exact question depends on the type of patient(s) included in the case report

§ Exact question depends on the type of patients included in the case series

Between 20 and 30 studies addressed questions Q2-Q8 and between 52% and 64% of these were classed as main. Question A2 (activity in biomarker negative) was addressed by 12 studies, all supportive or unclear. Question A3 (activity in biomarker unknown) was addressed by one and A4 (activity in all patients) by five supportive or unclear studies. Question A5 (effectiveness in biomarker unknown) was addressed by three main and one supportive study.

Box 6.2 Questions included in the expanded framework

Treatment activity
A1. Does the experimental treatment show activity in biomarker-positives?
A2. Does the experimental treatment show activity in biomarker-negatives?
A3. Does the experimental treatment show activity in biomarker-unknowns?
A4. Does the experimental treatment show activity in overall study population?
Treatment effects
Q1. How does the experimental treatment compare with the control treatment in biomarker-positives?[78]
Q2. How does the experimental treatment compare with the control treatment in biomarker-negatives?[78]
A5. How does the experimental treatment compare with the control treatment in biomarker-unknowns?
Q3. How does the experimental treatment compare with the control treatment in overall study population?[78]
Biomarker effects
Q4. Is the biomarker status associated with the outcome in the standard of care group? (Is the biomarker prognostic?)[78]
Q5. Is the biomarker status associated with the outcome in the experimental treatment group?[78]
Biomarker by treatment effect
Q6. Is the biomarker status associated with a benefit of experimental treatment? (Is the biomarker is predictive?)[78]
Strategy effects
Q7. How does the biomarker-based treatment strategy compare with the control treatment in the overall study population?[78]
Q8. How does the biomarker-based treatment strategy compare with the experimental treatment in the overall study population?[78]
Biomarker prevalence
A6. What is the prevalence of patients with a different biomarker status in patients not responding to treatment?

Italics indicate question form the original Tajik 2013[78] framework

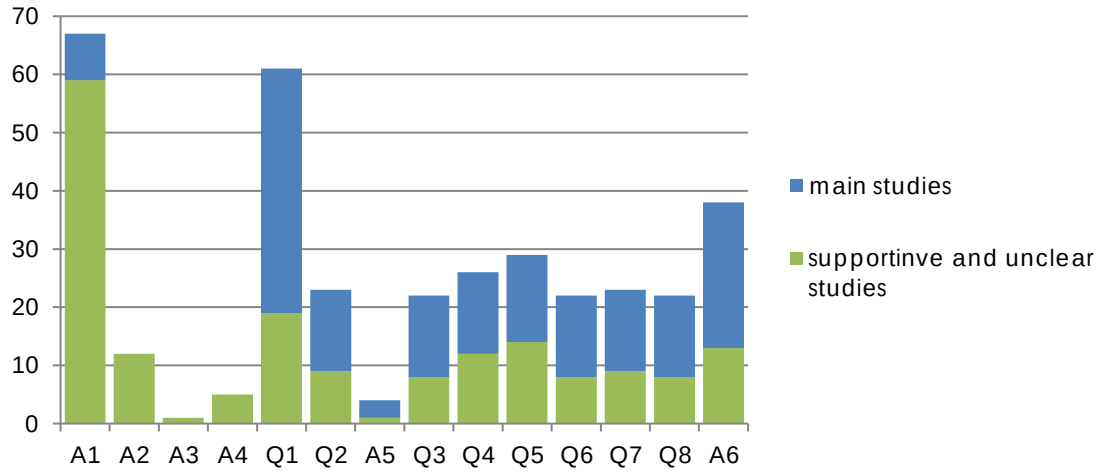


Figure 6.2 Questions addressed in all studies

When the 99 studies supporting drugs without an orphan designation were considered (shown in Figure 6.3), the most frequently addressed question was Q1 – drug effectiveness in biomarker positive patients (57 studies, 40 (70%) of which were classed as main). Question A6 (biomarker prevalence in non-responders) was addressed in 38 studies, 25 (66%) of which were main studies. There were 22 or 23 studies that addressed each of the questions Q2-Q8 (61% and 66% of these were classed as main). Activity in biomarker positive patients (A1) was addressed by 17 studies, only one of which was classed as main. Question A5 (effectiveness in biomarker unknown) was addressed by 3 main and one supportive or unclear study. Question A4 (activity in all patients) was addressed by one supportive or unclear study. Question A5 (effectiveness in biomarker unknown) was addressed by three main and one supportive or unclear study. Question A3 (activity in biomarker unknown) was not addressed by any of the studies in this dataset.

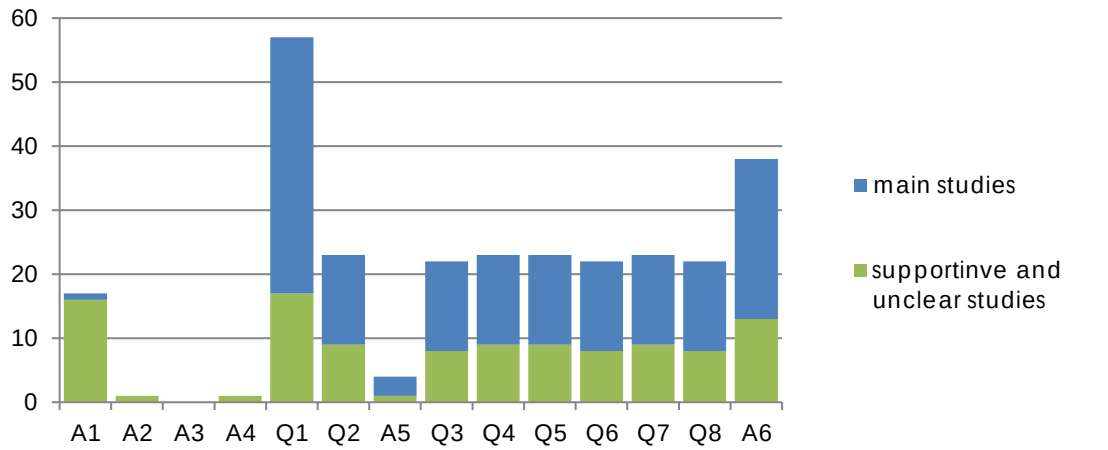


Figure 6.3 Questions addressed in studies supporting drugs without an orphan indication

When the 57 studies supporting drugs with an orphan designation were considered (shown in Figure 6.4), the most frequently addressed question was A1 (activity in biomarker positive patients) – in 50 studies (14% of which were classed as main). This was largely due to the fact that a high proportion of these studies (42% of 50 studies reporting on A1) were case reports. Question A2 (activity in biomarker negative) was addressed by 11 studies, all supportive or unclear. Again, five of these 11 studies were case reports. A maximum of six studies addressed questions A3 (one supportive or unclear study), A4 (four supportive or unclear studies), Q1 (two main and two supportive or unclear studies), Q4 (three supportive or unclear studies) and Q5 (one main and five supportive or unclear studies). A number of questions were not addressed at all for B-I-D combinations with an orphan drug: Q2, Q3, Q6-Q8, A5 and A6.

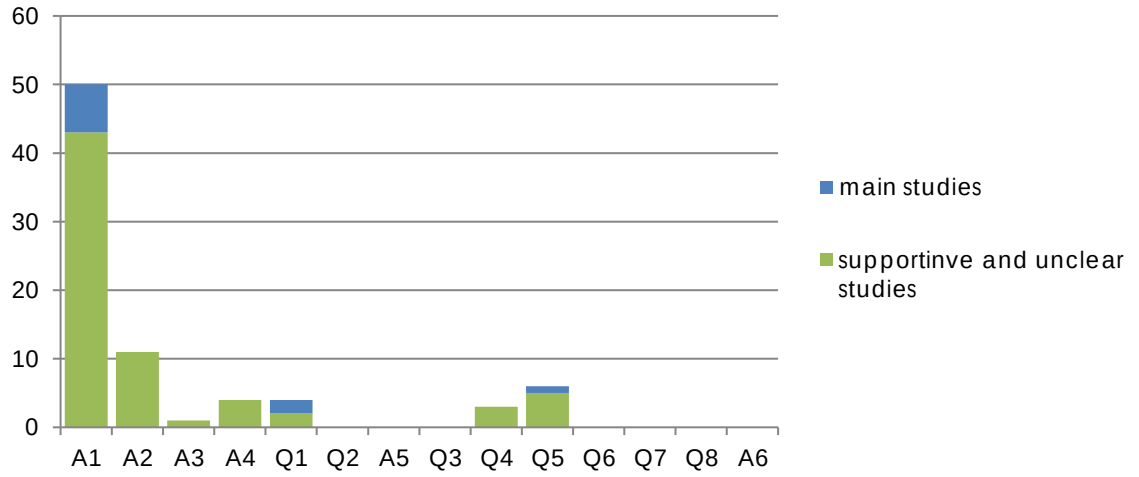


Figure 6.4 Questions addressed in studies supporting drugs with an orphan indication

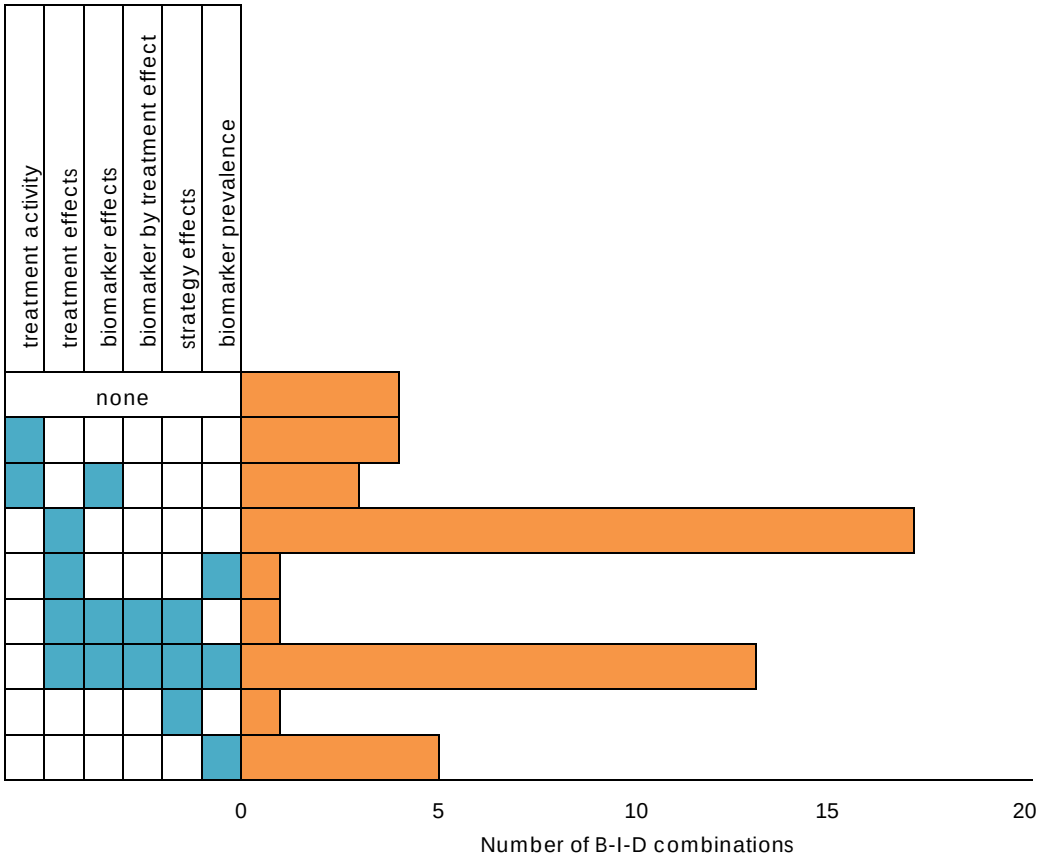
6.4.6 QUESTIONS ADDRESSED WITHIN B-I-D COMBINATIONS

Of the 50 B-I-D combinations in the EMA review (discussed in Chapter 5), sufficient information on the design of supporting studies to identify what questions were addressed was available for 49. For one B-I-D combination the study design with respect to the biomarker was unclear. Detailed information on the questions addressed within each B-I-D combination is reported in Appendix 15.

6.4.6.1 QUESTION TYPES ADDRESSED

Figure 6.5 presents the number of B-I-D combinations out of a total of 49 for which different types of questions, described in Box 6.2 were addressed by the supporting study

designs. In this analysis it was assumed that if there was information on treatment effects, treatment activity became irrelevant and was not included in the diagram. The exact questions addressed in B-I-D combinations are however reported in Figure 6.6 and Figure 6.7 below.



For categories where questions on treatment effects were addressed data on treatment activity are not considered.

Figure 6.5 Number of B-I-D combinations where different question categories were addressed by the supporting studies

In four cases there were no studies supporting the B-I-D combination and therefore none of the questions within the framework were addressed. For four B-I-D combinations only treatment activity was addressed and in 17 treatment effects. Three B-I-D combinations were supported by studies addressing only treatment activity and biomarker effects. In 13 cases all question types were addressed. In five cases the available research could only provide information on biomarker prevalence in patients not responding to treatment. The remaining three B-I-D combinations had information potentially available on a mixture of categories, one each on:

- treatment effects and biomarker prevalence in non-responders,

- all items apart from biomarker prevalence in non-responders, and
- biomarker prevalence in non-responders only.

6.4.6.2 QUESTIONS ADDRESSED

The exact combinations of questions addressed by the available evidence supporting 35 non-orphan B-I-D combinations with biomarkers predicting treatment efficacy are shown in Figure 6.6. For 13 of those the evidence available could only provide information on treatment activity or effects in biomarker positive patients. In one case the supporting studies could provide evidence on the treatment effects in biomarker positive and negative patients. For 14 B-I-D combinations information was available on all original questions proposed in Tajik 2013[78] (Q1-Q8), however not all of these provided information on the treatment effects in patients with an unknown biomarker status (A5), biomarker prevalence in patents who did not respond to treatment (A6) and the questions related to treatment activity (A1-A4). In five cases only biomarker prevalence in patents who did not respond to treatment was addressed (A6). One B-I-D combination was supported by studies addressing treatment effects in biomarker positive patients (Q1) and biomarker prevalence in patents who did not respond to treatment (A6).

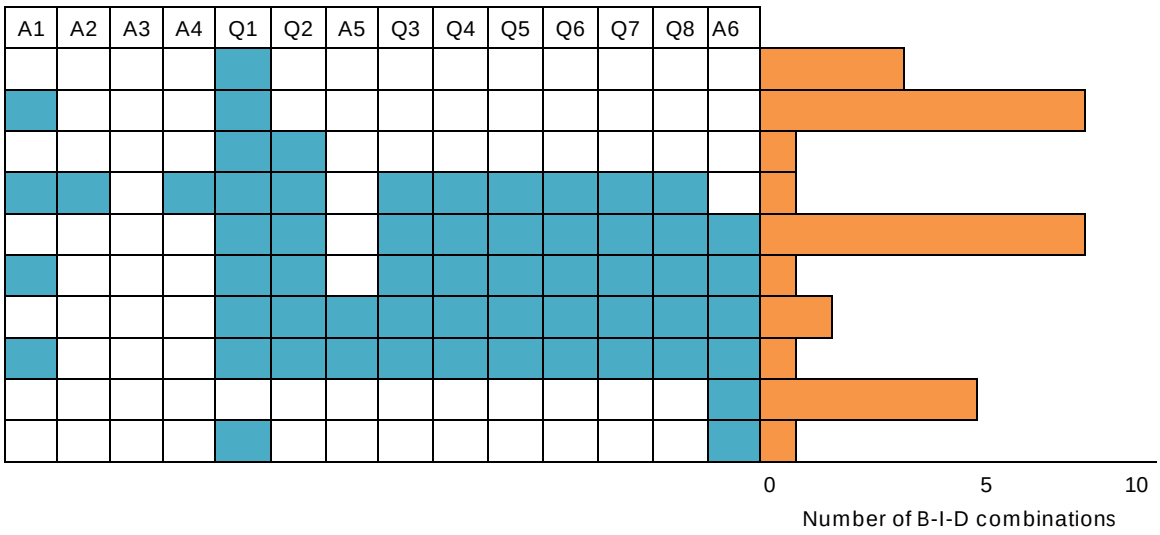


Figure 6.6 Number of non-orphan B-I-D combinations with biomarkers predicting treatment efficacy where different questions were addressed by the supporting studies

Questions addressed by studies supporting ten orphan B-I-D combinations with biomarkers predicting treatment efficacy are shown in Figure 6.7. In one case there were no studies supporting the B-I-D combination. Only treatment activity in biomarker positives (A1) was addressed in four and treatment activity (A1) end effects (Q1) in biomarker positives in

two cases. The remaining three B-I-D combinations were supported by evidence addressing a mixture of questions on treatment activity (A1, A2, A4) and biomarker effects (Q4 and Q5).

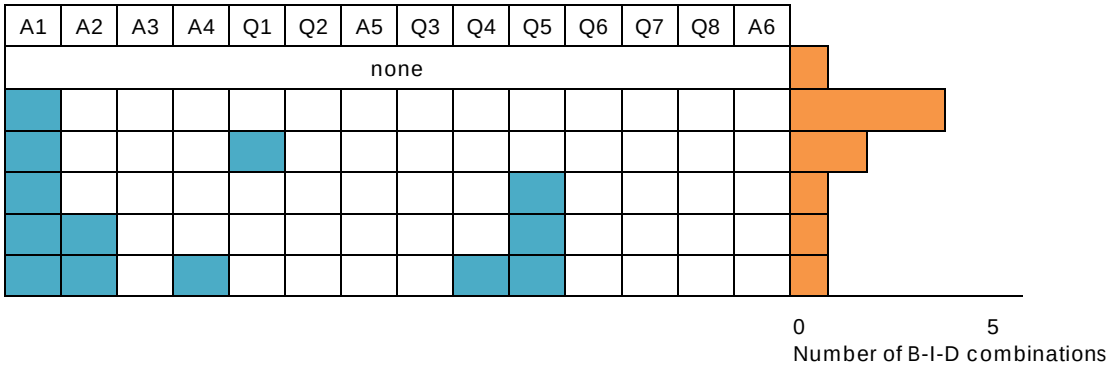


Figure 6.7 Number of orphan B-I-D combinations with biomarkers predicting treatment efficacy where different questions were addressed by the supporting studies

There were four B-I-D combinations where the biomarker was predictive of treatment safety, however study evidence was available only for one. This included one study which provided evidence on the effects of the biomarker strategy compared to the experimental treatment in the overall study population (Q8).

6.4.7 AUTHORS' CONCLUSIONS COMPARED TO PROPOSED FRAMEWORK

Author's conclusions were available for 110 studies. These were summarised according to which questions they discussed (details shown in Appendix 15). Where studies included only biomarker positive (or negative) patients the conclusions talked about treatment activity or efficacy without mentioning the biomarker, it was assumed that these conclusions referred to patients with the relevant biomarker status. The questions addressed by authors' conclusions compared to those in the proposed framework are summarised in Table 6.4.

For the questions not included in Tajik 2013[78] (A1-A6) and therefore not described in section 6.3.1.2 the following definitions were used:

- Activity (A1-A4) was considered to be commented on in the conclusions of a paper if these used the word "activity", or talked about the drug inhibiting a target. Activity was also assumed if the results were described as suggestive of efficacy or effectiveness and response to treatment was mentioned without indicating clinical relevance of the treatment.

- A5 was applied similarly to Q1-Q3, only it referred to patients with an unknown biomarker status.
- A6 was considered to be commented on if authors mentioned the prevalence of the biomarker positive in patients who did not respond to treatment.

Authors of about 30% of the non-randomised studies provided conclusions beyond activity and these included efficacy of the treatment compared to standard practice. One single arm study including only biomarker positive patients also provided conclusions on the association between the biomarker status and treatment outcome. [246] This is partly possible in the case of a continuous biomarker, where only patients with a value above a certain threshold are included, but the actual biomarker values are used for analysis. In this case, however, the biomarker used was not continuous (EGFR mutation).

Randomised enrichment trials generally only reported conclusion on the efficacy of the treatment within the biomarker-defined subgroup of patients. In one case an enrichment trial including only biomarker positive patients provided conclusions on the association between the biomarker status and treatment outcome.[247] This is probably due to the continuous nature of the biomarker (HER2 expression). In addition, four enrichment studies (all investigating HIV treatments) commented on the prevalence of the biomarker in patients failing the treatment. This was due to the fact that the studies included only patients who at the start of the study were not identified as carriers of virus with treatment resistance mutations, however they could have developed these mutations at the end of the study (as discussed in section 6.4.2 for cross-sectional subgroup analyses).

The randomise-all (prospective and retrospective) studies essentially made conclusions in agreement with the framework proposed. The only exceptions were four prospective subgroup analyses where patients with an unknown biomarker status were included as a separate subgroup and, appropriately, conclusions were made about these.

Of the five cross-sectional subgroup analyses only one commented on the biomarker prevalence in patients failing treatment. The remaining four made conclusions about the biomarker being associated with the outcome in the treatment and/or control group.

Questions A3, A4 and Q8 were not commented on by any of the study authors.

Table 6.4 Questions addressed in identified papers mapped onto the proposed framework

	single-arm						enrichment		randomise all			biomarker-strategy		
	case report** (n=21)	case series†† (n=2)	positive and negative (n=7)	Only positive (n=23)	Only negative(n=1)	non-RCT comparative (n=3)	Positive (n=31)	Negative (n=1)	Prospective subgroup (n=10)	Retrospective subgroup (n=5)	Cross-sectional subgroup (n=5)	With biomarker measurement(n=1)	Without biomarker measurement(n=0)	With randomization in the control arm (n=0)
A1	15	2	2	17		1								
A2	2				1									
A3														
A4														
Q1	7	1	3	5		2	28		5	4				
Q2								1	3	1				
A5									4					
Q3									1	1				
Q4									1					
Q5			1	1			1		2	2	1			
Q6									3	4	4			
Q7												1		
Q8														
A6							4		1		1			

Shaded areas indicate questions that can be addressed by each trial design according to the proposed framework; crossed out area indicates that for RCT study designs where activity was considered irrelevant, as higher level evidence on efficacy was available

** Exact question depends on the type of patients included in the case report (biomarker positive, negative, or unknown)

†† Exact question depends on the type of patients included in the case series (biomarker positive, negative, or unknown)

6.4.8 LABELS REPORTED IN PUBLISHED PAPERS

The labels for study designs used in papers matched to included studies are reported in Table 6.5 grouped by biomarker study design.

No reference to the biomarker was made in the study design label for the majority of the identified designs: case reports, case series, single-arm, enrichment and biomarker-strategy studies. The label usually described the study referring to characteristics such as presence or absence of control group, randomisation, or blinding. In these cases the biomarker information was usually described as part of the inclusion criteria and/or patient characteristics for a particular study.

For non-RCT comparative studies one of three labels available made a reference to the biomarker by referring to the study as molecular characterisation.[248]

Randomise-all studies were also usually described without referring to the fact that patients of any biomarker status were included. The only exception was labelled as an "*open-label trial that enrolled patients regardless of EGFR expression*".[249]

The subgroup analysis labels sometimes contained information on the fact that these were investigating biomarkers. Of the seven identified unique labels for prospective subgroup analyses two referred to biomarkers: "*predefined candidate biomarkers*"[250] and "*virologic genotyping substudy*".[251] Of the three identified unique labels for retrospective subgroup analyses one referred to biomarkers ("*biomarker analysis*"[175,252]). All four identified unique labels for cross-sectional subgroups referred to biomarkers and these were: "*retrospective genotypic and phenotypic analyses,*"[253] " *genotypic and phenotypic resistance analyses,*"[254] "*genotypic analysis*"[255] and "*resistance analysis*".[256]

The only identified study label matching the framework in Tajik 2013[78] was "single-arm study". The remaining labels ("enrichment", "randomise-all" and "biomarker-strategy") were not used. From the extended framework the labels "case report" and "case series" were used.

Table 6.5 Study labels used in published papers

Study design	Labels in papers
Case report	• *case* • *case report*
Case series	• *case series* • *cohort of patients*
Single-arm (positive and negative)	• *prospective study* • *single-arm/ open-label exploratory study* • *randomized trial*[‡‡] • *open-label, randomized study*[§§]
Single-arm positive only	• *study* (often *single-arm*) often further described as one or more of the following: *dose-escalating/ dose-escalation, expanded, Gehan two-stage design, noncomparative, open , open-label, pilot, prospective, Simon two-stage* • *clinical trial* or *prospective clinical trial* • *open-label, nonrandomized trial*
Single-arm negative only	*study*
Non-RCT comparative	• *molecular characterisation* • *study* • *study on retrospective cases*
Enrichment (positive)	• *randomised trial* or *study* often further described as one or more of the following: *clinical, double-blind, placebo-controlled, open-label, pilot, two-arm, double-dummy, parallel-group, controlled, two-by-two factorial, prospective* • *trial* with further descriptors: *clinical, active-controlled, non-inferiority*
Enrichment (negative)	*randomised, placebo-controlled, double-blind trial*
Randomise-all (prospective)	• *randomised trial* or *study* often further described as one or more of the following: *clinical, double-blind, open-label, parallel-group, placebo-controlled, postmarketing, three-arm comparison* • *clinical trial* or just *trial* prospective subgroup analyses were described as: • *correlative studies* • *exploratory analyses in patient subgroups* • *planned subgroup analyses* • *pre-planned analyses according to predefined candidate biomarkers* • *preplanned, blinded, subset analysis* • *preplanned subgroup analyses* • *virologic genotyping substudy*
Randomise-all (retrospective)	• *randomised trial* or *study* with further descriptors: *double-blind, open-label , placebo-controlled* • *open-label trial that enrolled patients regardless of EGFR expression* • *placebo-controlled study* retrospective subgroup analyses were described as: • biomarker analysis • correlative analysis • retrospective subgroup analysis
Randomise-all (cross-sectional)	• *randomised trial* or *study* with further descriptors: *2-arm, dose-ranging, double-blind, equivalence, noninferiority, open-label, prospective* • *clinical trial* cross-sectional subgroup analyses were described as: • *genotypic analysis* • *genotypic and phenotypic resistance analyses* • *resistance analysis* • *retrospective genotypic and phenotypic analyses*
Biomarker-strategy	*double-blind, prospective, randomised study*

Reported study labels are in *italics*

[‡‡] RCT compared two arms with different doses of the drug under investigation
[§§] RCT compared two arms with different doses of the drug under investigation

6.5 DISCUSSION

The framework in Tajik 2013[78] contained six trial designs that could provide information on one or more of eight questions. It was modified based on the dataset identified in Chapter 5 to include seven major study designs, four of which were further subdivided into subclasses of design. In particular lower-level designs were added (case reports and series, other types of single-arm studies and non-RCT comparative studies). Six additional questions that could be addressed by studies were also identified.

The framework proposed in Tajik 2013[78] turned out to be too narrow compared to the study designs identified in the dataset from the EMA review. As a result, it needed expanding to add study designs and questions that were addressed by studies. It also appeared to emphasise some study designs (three types of biomarker-strategy design), which seem to have very little application in practice, possibly due to practical reasons and low efficiency.[13]

It appears that some designs may be relatively common in practice without a large body of methodological literature underpinning these. This might be due to the fact that the choice of the study design may be driven, in addition to being able to obtain robust data, by factors such as practical or ethical considerations.[257] This was possibly the reason for the frequent use of study designs including only biomarker positive patients and of cross-sectional subgroup analyses. The cross-sectional subgroup analysis, although currently limited to HIV treatments, might in the future become more important in other clinical areas, such as cancer due to the better understanding of tumour heterogeneity and evolution.[258]

For some study designs the questions these could potentially address were, to some extent, situation-dependent. For example, for continuous biomarker studies including only patients who are biomarker positive can contribute information on the correlation between the biomarker and outcome. Even though only patients with a biomarker value above a certain threshold are included, within the study there is still a distribution of different biomarker values and these can be correlated with patient outcomes.

Frequently in publications study labels did not refer to the biomarker aspect of the design. The relatively widespread terms used in methodological literature ("enrichment", "randomise-all" and "biomarker-strategy")[78] were not encountered in this dataset. Biomarkers often seemed to be reported as an inclusion criterion only and not considered a major component of the study design. To some extent, subgroup analyses were an exception, as the labels for these frequently mentioned biomarkers. This might suggest the need for better labelling of studies.

The questions addressed by studies supporting B-I-D combinations were considered. For only 14 of 49 B-I-D combinations supporting studies provided information on whether the biomarker was predictive and the benefit of implementing a biomarker-based strategy. The remaining studies mainly investigated activity or effectiveness within biomarker-defined patient groups. Some studies investigated the correlation between the biomarker status and study outcome, or the biomarker prevalence in patients not responding to treatment. This could potentially reflect a strong biological basis for evaluating the treatment in biomarker positive patients and a robust biomarker test. However, it could also suggest a need for improvement in the study methodology.

It is not certain whether other published systematic reviews suggesting different frameworks were available, as systematic searches were not undertaken. Another systematic review of trials methodology for evaluation of predictive biomarkers was published as a conference abstract.[259] The data provided in the abstract were however not sufficient to utilise within this chapter and personal communication with the first author suggested the work on non-adaptive trial designs would not be published in the near future.[260]

The limitations in the methodology of the Tajik 2013[78] review may potentially impact on the results of this chapter. However, as methodological literature is concerned, these limitations may be less serious than in the case of systematic reviews evaluating clinical effects of interventions. There are very little, if any, consequences of missing some methodological papers if the information contained in them was obtained from other publications. [261] It has been in fact argued that attempting to identify all relevant papers may be redundant and represent highly inefficient use of time and resources.[262]

The EMA dataset itself may be a limited representation of research practice. It is more likely to include studies where at the design stage the evidence supporting the biomarker is much stronger and therefore some designs might not be seen. For example, the stratified design was not encountered in this dataset to evaluate any of the biomarkers, however this design has been used in multiple cases in practice. For example the trials evaluating erlotinib in NSCLC were stratified by EGFR gene copy number (MARVEL),[61] or EGFR mutation type (OPTIMAL).[263]

Another limitation of this work is that searches for the studies in the EMA dataset went up to the beginning of 2013. There may be some newer studies with a different design. One person collected the data and analysed it. This may be particularly important in summarising the conclusions of study authors', as these may have more than one interpretation.

However this piece of work was based on a relatively large sample of studies (n=159) and was carried out in a systematic way, therefore it may provide a relatively robust comparison of at least some research practice against the Tajik 2013[78] framework.

6.6 CONCLUSIONS

The framework proposed in Tajik 2013[78] needed expanding to encompass all study designs identified in the dataset from the EMA review. This suggests there might be a discrepancy between what designs are proposed and evaluated in the methodological literature and carried out in practice. There may be a need to further develop and gain better understanding of the limitations of methodology for certain cases, such as investigation of HIV resistance mutations.

It was also clear that for the majority of B-I-D combinations there was no evidence to address the question of whether the biomarker was truly predictive, or whether the implementation of the biomarker in clinical practice would result in patient benefit. This could potentially suggest that either in most cases there is a strong biological rationale not to evaluate the biomarker, or there is a need for improvement in the methodology of studies being conducted.

For published papers authors' conclusions seemed to be mainly addressing questions possible to answer based on the study design, although there were a number of studies where conclusions beyond these appeared to be made.

The study labels did not often reflect the biomarker aspect of the study. This suggests there is a need to improve the labelling of biomarker studies in practice.

CHAPTER 7. PROCEDURES FOR EVALUATION OF A PREDICTIVE BIOMARKER - A CASE STUDY OF ERCC1 IN NSCLC

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Jon Deeks contributed to the design of the review and commented on data analysis and the chapter
Richard Riley contributed to the design of the review and commented on data analysis and the chapter
Sanjay Popat contributed to the design of the review and questionnaire, commented on data analysis
Andrew Nicholson contributed to the design of the questionnaire, commented on data analysis

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Abstract

Background: Implementation of findings of predictive biomarker research in clinical practice, requires sufficient information on the appropriate procedures of biomarker evaluation. Ideally, the procedures used should be consistent across different studies, to facilitate combining their results and provide a useful tool for implementation in clinical practice applications.

Aim: The aim of this chapter is to provide a case study of how a particular predictive biomarker, ERCC1, was assessed in recent research and what motivated the choice of laboratory procedures. Thus this case study aims to identify general lessons that can be learned for the wider context of predictive biomarker research.

Methods: Searches were carried out in three databases of ongoing trials on 26.03.2013. Details of ERCC1 assessment and study design were obtained from records in these databases. In addition, questionnaires were sent to all identified studies asking for detailed information on ERCC1 evaluation procedures and the rationale for their choice.

Findings: Thirty three studies of ERCC1 in platinum-based chemotherapy of non-small-cell lung cancer were identified that were either ongoing or completed or terminated after 1st January 2007. Information was received in response to the questionnaires for 16 studies. The procedures for ERCC1 evaluation varied substantially and, where reported, (20 studies) included reverse transcriptase quantitative polymerase chain reaction (nine studies), immunohistochemistry (five studies) and other methods (six studies). In five of the identified studies ERCC1 evaluation was initially planned, but not undertaken. Even when different studies used the same assay, there was still variation in the details of the laboratory techniques and scoring systems used.

Conclusions: Large variation was found across studies in the procedures used for ERCC1 evaluation. This could potentially limit the comparability of results between different studies. To enable evidence-based clinical practice, there needs to be a generally accepted standard biomarker test as well as standardised laboratory protocol to be followed, especially in late phase studies. A consensus on and validation of the procedures to evaluate a predictive biomarker may be required in the early phase of research to achieve this. If multiple procedures for evaluation are to be used, research needs to demonstrate that these result in comparable classification of patients into biomarker positive and negative

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7.1 BACKGROUND

Chapter 2 reviewed the different frameworks for development of predictive biomarkers. One of the elements of some of these was analytical validation of the biomarker. This involved establishment of acceptable accuracy and replicability of the procedures used to evaluate these biomarkers. It also seems important that standardised procedures are used to enable combining the results of multiple studies and implementation of their findings in clinical practice. There are however reasons to believe that in practice there is little consistency in the procedures used. As shown in Chapter 5, there is often substantial variability in the procedures used for evaluation of an individual biomarker within B-I-D (Biomarker-Indication-Drug) combinations. Also, a review of published papers investigating the use of excision repair cross-complementation group 1 (ERCC1) to predict response to platinum-based chemotherapy in lung cancer found that there was large variability in the assays used.[264] This review was published in 2011, thus including relatively early ERCC1 evaluations. There is a possibility that the more recent research practice has become more harmonised.

This chapter therefore set out to investigate the reports of consistency of methods for evaluation of ERCC1 as a biomarker predictive of response to platinum-based chemotherapy in ongoing or recently completed studies in NSCLC patients. It also attempted to explore the rationale that motivated the choice of assays. This particular case was chosen in preference to any of the B-I-D combinations identified in Chapter 3, as ERCC1 was still being investigated as a potential predictive biomarker, while the B-I-D combinations contained biomarkers that have already been considered ready for implementation in clinical practice. Therefore, ERCC1 was more likely to illustrate the more recent research practice. This chapter sets out to provide a case study, from which lessons can be learned that may apply to a wider context of predictive biomarker research.

To enable a detailed discussion of the results of this case study, the background to the investigation of ERCC1 as a biomarker predictive of response to platinum-based chemotherapy will be outlined. Some of the more important procedures for ERCC1 evaluation will also be introduced below.

7.1.1 TREATMENT OF NON-SMALL-CELL LUNG CANCER

Lung cancer is one of the leading causes of cancer death globally.[264-266] Cancer registration data from England shows 34 848 new lung cancer cases diagnosed in 2011.[267] The majority of patients have NSCLC histology.[266,268] The prognosis of lung cancer patients

is generally poor,[264,268] with a five year survival of about 5% for patients with advanced NSCLC and about 15% for all patients irrespective of stage.[266] In spite of development of new, targeted treatments, platinum-based chemotherapy remains part of standard care in NSCLC.[264,268-270]

The effectiveness of platinum-based chemotherapy is however limited,[265,271] with resistance to treatment resulting in little or no patient benefit and potentially unnecessary toxicity.[272] Identification of biomarkers predictive of resistance (or lack of efficacy) to platinum-based chemotherapy could potentially result in avoiding unnecessary treatment and lead to significant improvement in patient care, as well as better allocation of healthcare resources.

7.1.2 PLATINUM-BASED CHEMOTHERAPY: MECHANISM OF ACTION AND RESISTANCE

Understanding the mechanism of action of platinum-based agents was an important step towards identification of potential biomarkers predictive of lack of treatment efficacy. Although it is still a subject of research, there is certainty that DNA is the primary target of platinum-based chemotherapy.[273] The main effects of this type of chemotherapy on cells involve preventing cell replication, interruption of cell function, and, most importantly, cell death (apoptosis).

This discussion will focus on cisplatin, as the mechanism of action of cisplatin is probably the most extensively studied and it is, to some extent, representative of the other platinum-containing agents.

As shown in Figure 7.1, after administration, cisplatin is taken up into cells, where it is converted into an active form. The activated molecule then binds strongly to DNA, but can also bind to other molecules within the cell (such as RNA and some proteins). On binding to DNA, cisplatin can create adducts, which lead to the effects outlined above. Formation of adducts is followed by activation of complex pathways (both pro-survival and pro-apoptosis),[273] which may vary for different platinum-containing agents.[272]

Resistance to platinum-containing therapy has been observed in a high proportion of patients. As illustrated in Figure 7.1, there is a wide range of proposed mechanisms of resistance and it has been suggested that it may involve several mechanisms at the same time. The major mechanisms of resistance involve:

- reduction in cisplatin uptake and/or increase in efflux from the cell,[272,273]
- increase in cisplatin inactivation, mainly by presence of proteins which react with activated cisplatin and stop it from binding to DNA,[272,273]

- various mechanisms involved in repair of damaged DNA and preventing apoptosis.[272,273]

The first two mechanisms can be, to some degree, overcome by the use of other platinum-containing agents (such as carboplatin). Enhanced repair, however, generally results in resistance to other platinum-containing drugs as well.[273]

The nucleotide excision repair (NER) pathway has been suggested as one of the major routes of platinum adduct repair. Increase in expression of NER genes has been correlated with resistance to cisplatin and other platinum-containing drugs.[272,273] The ERCC1 enzyme in particular has a role in removing cisplatin adducts.[266]

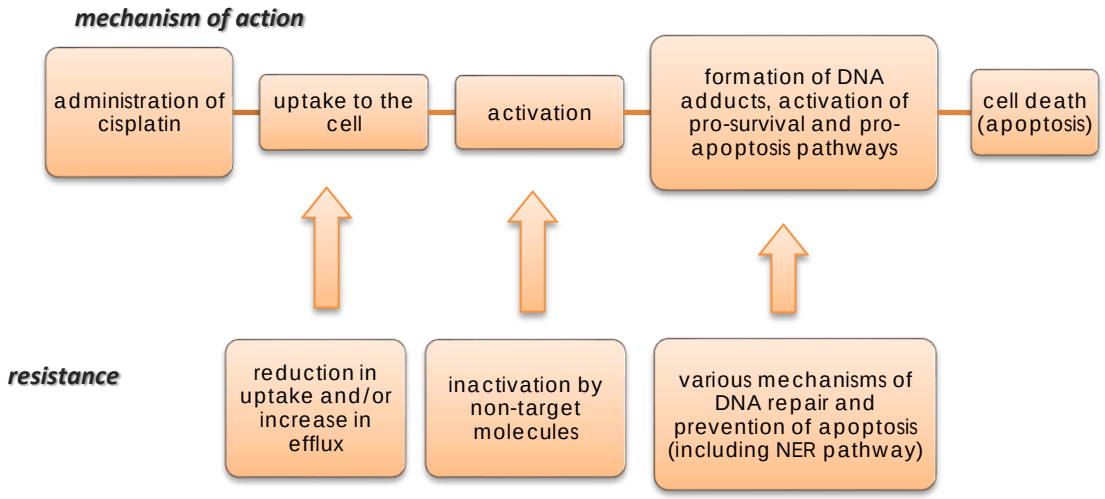


Figure 7.1 Major mechanisms of action of cisplatin chemotherapy and related resistance mechanisms

7.1.3 Investigation of ERCC1 expression in IALT Bio and 2011 re-analysis

This biological rationale, as well as evidence from *in vitro* and small retrospective studies, pointed to ERCC1 expression as a biomarker predictive of response to platinum-based chemotherapy in NSCLC. This was investigated in IALT Bio, a retrospective analysis of patients enrolled in the IALT RCT, which included 1867 patients and compared adjuvant cisplatin to observation only.[265] Paraffin-embedded tumour tissue samples from 783 patients were analysed, which constituted 42% of all patients included in the original IALT RCT.[265,274] The biomarker evaluation was undertaken by IHC using the 8F1 antibody (Neomarkers, Fremont, California, USA).[265]

IALT Bio results indicated that patients whose tumours had low levels of ERCC1 expression were benefiting from cisplatin chemotherapy both in terms of overall survival (adjusted

HR=0.65, 95% CI: 0.50, 0.86) and disease free survival (HR=0.65, 95% CI: 0.50, 0.85) compared to observation only control group. At the same time, for patients with high tumour ERCC1 expression levels there seemed to be a non-significant trend suggesting shorter overall survival with cisplatin treatment compared to control (HR=1.04, 95% CI: 0.84, 1.55). The test of interaction indicated a predictive effect of ERCC1 expression both for overall survival (p=0.009) and disease free survival (p=0.008).[265] The results of this study have largely increased interest in ERCC1 as a biomarker predictive of response to platinum-based chemotherapy.

A new retrospective analysis was undertaken in 2011 and published in 2013. It included samples from IALT Bio and two other studies. For this analysis tumour tissue samples were available for only 589 patients from the IALT RCT – about 32% of patients from the original trial. The same antibody and laboratory procedures were used, however a substantial inconsistency was observed. For patients with their biomarker status evaluated both in IALT Bio and the 2011 re-analysis:

- 34% of patients initially classed as ERCC1 negative, were classed as positive in 2011,
- 2% of patients initially classed as ERCC1 positive, were classed as negative in 2011,
- classification was consistent for 64% of patients.[271]

Based on the 2011 evaluation, ERCC1 no longer appeared predictive of overall survival (HR=0.81, 95% CI: 0.50, 1.31 for ERCC1 negative and HR=0.96, 95% CI: 0.74, 1.25 for ERCC1 positive). Furthermore, combined data from all three studies showed a trend suggesting patients classed as ERCC1 positive were more likely to benefit from cisplatin chemotherapy, although the interaction test was not significant (p=0.23).[271]

The authors of the 2011 re-analysis undertook additional laboratory work investigating the role of ERCC1, which resulted in identification of different forms of the ERCC1 protein (isoforms), with potentially only one of them having a role in cisplatin resistance. They concluded that:

> *"Currently available antibodies do not have adequate discrimination for therapeutic decision making regarding cisplatin-containing treatment in patients with NSCLC, which requires the specific detection of the unique functional isoform of ERCC1 — ERCC1-202".*[271]

7.1.4 METHODS USED FOR ERCC1 EVALUATION

The two main methods that have been used for ERCC1 evaluation are IHC and reverse transcriptase quantitative polymerase chain reaction (RTqPCR).[264,275] These are described in more detail in Appendix 16. For both assays the results will not only be influenced by the choice and performance of the assay, but also by pre- and post-analytical factors which have been introduced in Chapter 1.[276]

7.1.5 AIMS

The aims of this chapter are:

1) to identify the procedures for evaluation of ERCC1 as a biomarker predictive of response to platinum-based chemotherapy in NSCLC in studies that were ongoing, or completed or terminated on 1 January 2007 or later,
2) to investigate the details of the laboratory procedures employed in studies, and explore the rationale that motivated the choice of a specific procedure.

7.2 METHODS

As shown in Box 7.1, the systematic review was undertaken to address the first aim: to investigate the procedures for ERCC1 evaluation used in studies of platinum-based chemotherapy in NSCLC patients that were ongoing and recently completed or terminated (after 1st January 2007). ERCC1 was of interest as a predictive biomarker and not prognostic. In practice this was however difficult to ascertain, therefore it was assumed that studies investigating platinum-containing chemotherapy and measuring ERCC1 are likely to be using this biomarker as predictive. There was no limitation in terms of study design or outcomes assessed.

The second aim involved investigating the details of laboratory procedures and rationale for the choice of the methods for ERCC1 evaluation in the identified studies. This was addressed by the questionnaire.

Box 7.1 Definition of the question addressed by the systematic review

Population: patients with non-small cell lung cancer
Intervention: platinum-containing chemotherapy
Comparator: any, including none
Outcome: any
Biomarker: Excision Repair Cross-Complementation Group 1 (ERCC1) expression
Study: any ongoing study or completed/ terminated after 1st January 2007

7.2.1 SEARCHES IN ONGOING TRIALS DATABASES

Searches were undertaken on 26.03.2013. in the ClinicalTrials.gov, WHO and Controlled-Trials database. Search terms used are reported in Table 7.1 below. The full search strategies can be found in Appendix 17.

Table 7.1 Search terms used in ongoing trials databases to identify studies potentially using ERCC1 as predictive of platinum based therapy

Clinical area	Biomarker	Treatment*
Lung NSCLC	ERCC1 ERCC-1 ERCC excision AND repair customized individualized tailored personalized biomarker pharmacogenomic pharmacogenetic	cisplatin carboplatin platinum platin chemotherapy

*Terms in each column were combined using the "OR" operator; columns were then combined using the "AND" operator; *ClinicalTrials.gov only*

Results of the searches (study number, title and link to the full record) were entered into an Excel spreadsheet. Duplicates identified based on the study number were removed after comparison of entries in different databases to ensure no unique studies were removed.

7.2.2 IDENTIFICATION OF RELEVANT STUDIES

Studies meeting the criteria reported in Box 7.1 were included.

The application of the inclusion criteria proceeded in two stages. In the first stage of screening study titles were screened for inclusion by two independent reviewers. Any study that was judged by at least one reviewer as potentially meeting the inclusion criteria (above) or unclear was included in the second stage of screening. Only studies clearly not meeting the inclusion criteria were excluded at this stage. The full records of all studies included after the first stage were downloaded from the databases of ongoing trials.

In the second stage the full records of studies identified in the first stage were considered for inclusion by two independent reviewers. Studies were included if they met all inclusion criteria. An exception was made for studies only specifying the intervention as chemotherapy or systemic therapy, where all the remaining criteria were met. These were also included to avoid potential omissions of relevant studies.

Disagreements between the two reviewers regarding inclusion decisions were resolved by discussion and in two cases by seeking further information on the studies in internet searches. The reasons for all exclusions at this stage were recorded.

7.2.3 DATA EXTRACTION

Data were extracted by one reviewer into an Excel spreadsheet from records obtained from databases of ongoing trials that were downloaded on 12.04.2013. Information was collected on the items shown in Box 7.2.

7.2.4 QUESTIONNAIRE

A questionnaire was prepared in collaboration with clinical and pathology experts. It included questions related to details of laboratory methods for ERCC1 evaluation. The full questionnaire can be found in Appendix 18.

The questionnaires were sent by email to contacts for each included study, as indicated in the relevant entry in a database of clinical trials. Where there was no individual identified (in the case of two studies), contact with the sponsor or the corresponding author (for a published trial) was attempted. The questionnaire was sent on 5 August 2013. Where no reply was received, a reminder was sent on 28 January 2014.

Information received was extracted into the Excel spreadsheet for all items included in the questionnaire.

Box 7.2 Data extraction items for review of trials evaluating ERCC1

- Trial ID and acronym
- Study phase
- Study status (ongoing, completed, terminated or withdrawn)
- Study title
- Study start date and planned end date
- Interventions investigated in the study
- Role of platinum-containing chemotherapy
- Design (with respect to ERCC1)
- Planned sample size
- NSCLC stage
- Methods of obtaining tumour tissue samples (also investigated in the questionnaire)
- Methods of ERCC1 assessment (also investigated in the questionnaire)
- Primary outcome
- Study location
- Study sponsor
- Study contact details for the purpose of sending the questionnaire

7.2.5 Searches for published papers

Additional searches using Google and Google Scholar were undertaken for published papers for studies where a reply was not received and which were not ongoing. The terms used included, but were not limited to: trial number, trial acronym and trial title. These searches were undertaken during the week commencing 11 August 2014.

7.2.6 Data analysis

The data obtained were summarised narratively. For all included studies the summaries were based on the best information available. Where available, information from

questionnaires was used and in the remaining cases data extracted from databases of ongoing trials were utilised. Data on the study design with respect to the biomarker were analysed by study phase, as it was hypothesised that the design should vary depending on the phase, with designs aiming at finding a correlation between the biomarker and response to treatment occurring mainly in earlier phases. For the purpose of analysis seamless study designs were grouped according to the earlier phase component (for example a phase I/II was grouped with phase I studies).

The type of biomarker assay used was also analysed by phase, as again the highest variation in the choice of assays for ERCC1 evaluation was expected to be seen in early phase trials, where the biomarker was still in development. It was expected that the laboratory methods would become more standardised in later phase studies. In addition, data on the assays used were analysed by year of study initiation to investigate whether there is any trend suggesting that the choice of an assay may be dictated by technological developments. For each individual method where information was available from questionnaires, the details of the laboratory procedures were compared. Data on the rationale for the choice of an assay were compiled from the replies received.

7.3 FINDINGS

The numbers of studies identified in each stage of the review as well as the number of replies received are shown in Figure 7.2.

The searches identified 921 records across the three databases. After removing duplicates, 730 studies remained. Thirty three studies met the inclusion criteria.

A questionnaire was sent to a contact for each of the 33 included studies. A completed questionnaire was returned by eight respondents. In further eight cases an email was received, which included, for example, published papers or conference abstracts, as well as information that even though reported in the database of ongoing trials, ERCC1 assessment was not undertaken.

The searches for published papers did not result in identification of any publications.

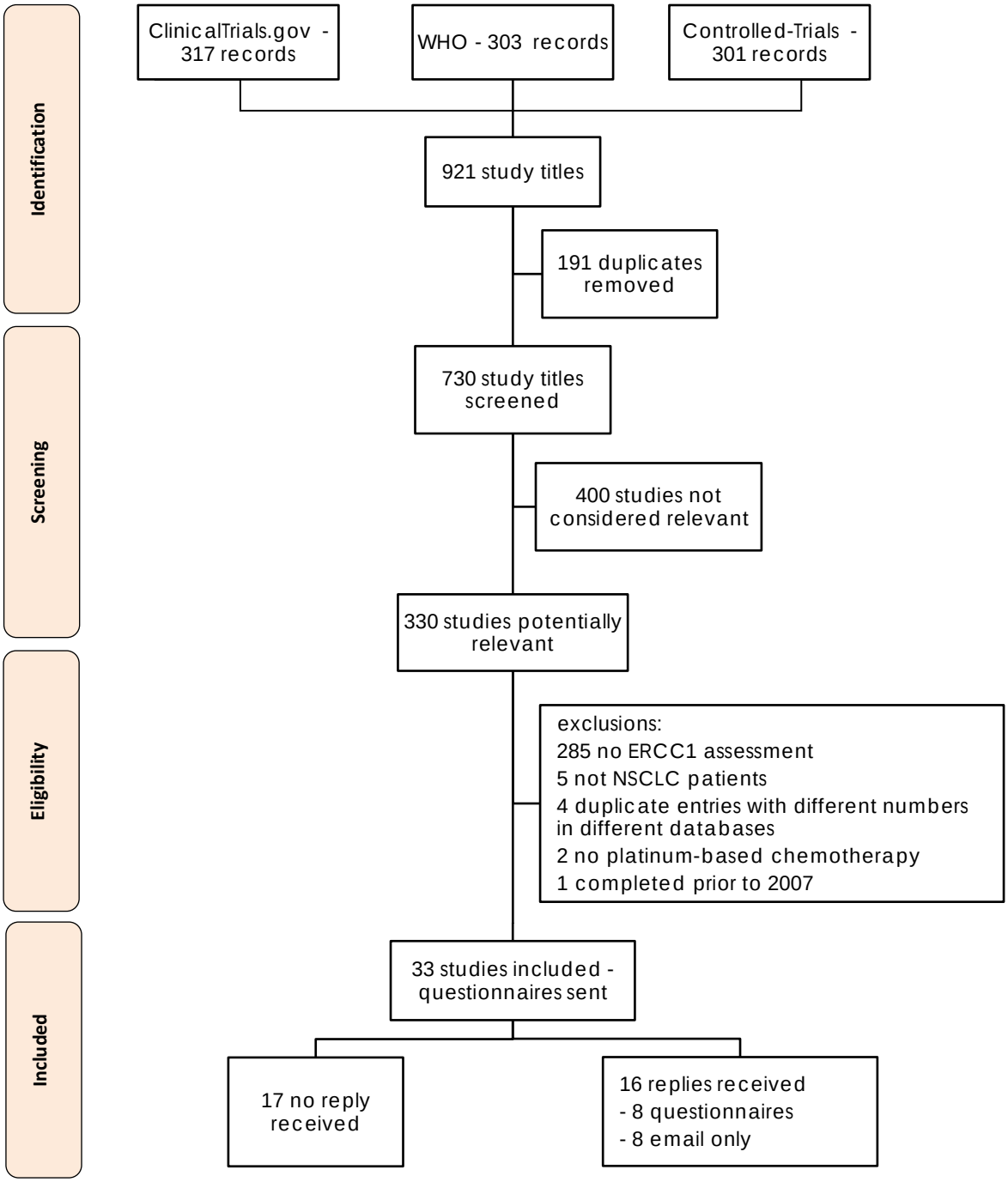


Figure 7.2 Flow diagram outlining the results of searches in ongoing trials databases, review of studies and replies received for the questionnaire

7.3.1 INCLUDED STUDIES

Of the identified 33 studies 18 were ongoing, eight completed, two terminated early and one withdrawn prior to enrolment. The status of four studies was unknown. There were 14 phase II, five phase III and two phase I studies. There was also one each: phase 0, phase I/II, phase II/III and phase IV study. The study phase was NR for eight studies. Nine studies

were conducted in Asia, eight in Europe, 13 in North America and one study included locations in both Europe and North America. For two studies the location was NR.

As shown in Figure 7.3, six studies planned to include up to 50 patients, nine between 51 and 100, 14 between 101 and 500, two between 501 and 1000 and two more than 1000 patients.

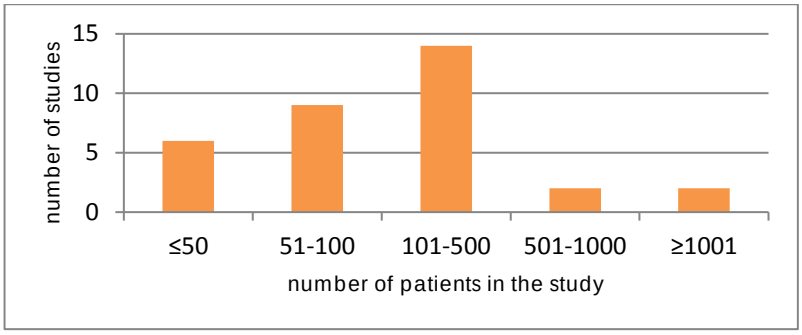


Figure 7.3 Planned numbers of patients in included studies

In 18 of the included studies the intervention was reported as containing cisplatin, in nine carboplatin, in three a platinum-containing agent and in three it was unclear.

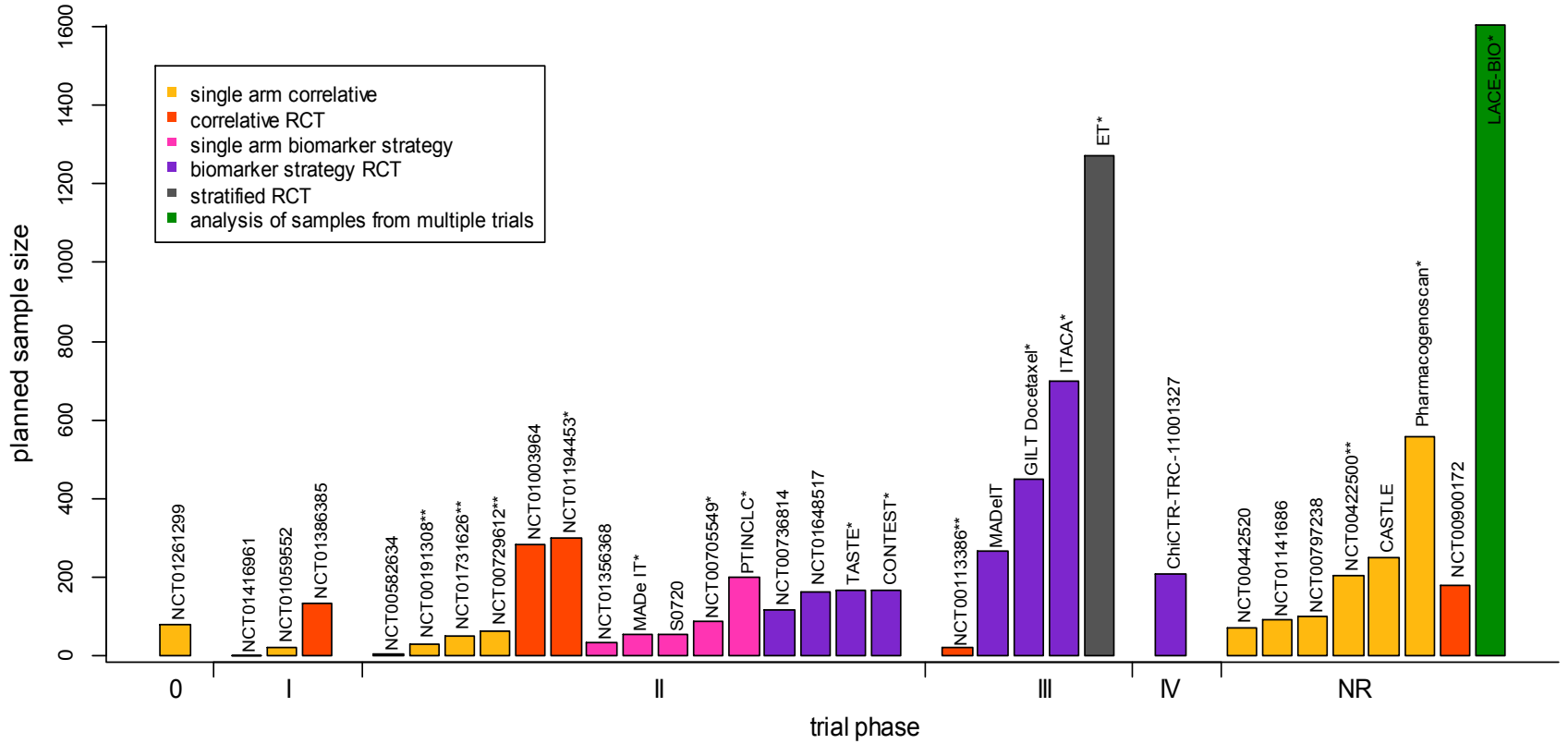
As shown in Figure 7.4, eighteen studies investigated correlation between ERCC1 status and patient outcome, without this biomarker being integral to the trial design. Fourteen used ERCC1 on its own, or in combination with other biomarkers to determine the treatment strategy (either in an RCT or single-arm trial). One RCT was stratified by ERCC1 status.

A correlative non-randomised design for ERCC1 investigation was most frequently used in early phase studies (phase 0, I and II). A relatively large proportion of phase II studies reported testing a strategy that was based on ERCC1 and in some cases also included other biomarkers. Phase III trials included one correlative RCT, one RCT stratified by ERCC1 and three RCTs using ERCC1 to select the treatment strategy. The phase IV study was biomarker-based strategy RCT.

Detailed characteristics of the included studies are reported in Table 7.3.

7.3.2 ERCC1 EVALUATION BASED ON BEST AVAILABLE INFORMATION

This section presents details of procedures for ERCC1 evaluation based on combined information from the survey and records in ongoing trials databases, which are reported in Figure 7.5.



* response to questionnaire received and ERCC1 evaluation undertaken; ** response to questionnaire received and ERCC1 evaluation not undertaken although the intention reported in an ongoing trials database was to perform a correlative analysis

Figure 7.4 Planned trial sample size and design with respect to ERCC1 by trial phase in identified studies

Table 7.2 Characteristics of included studies ordered by the study phase

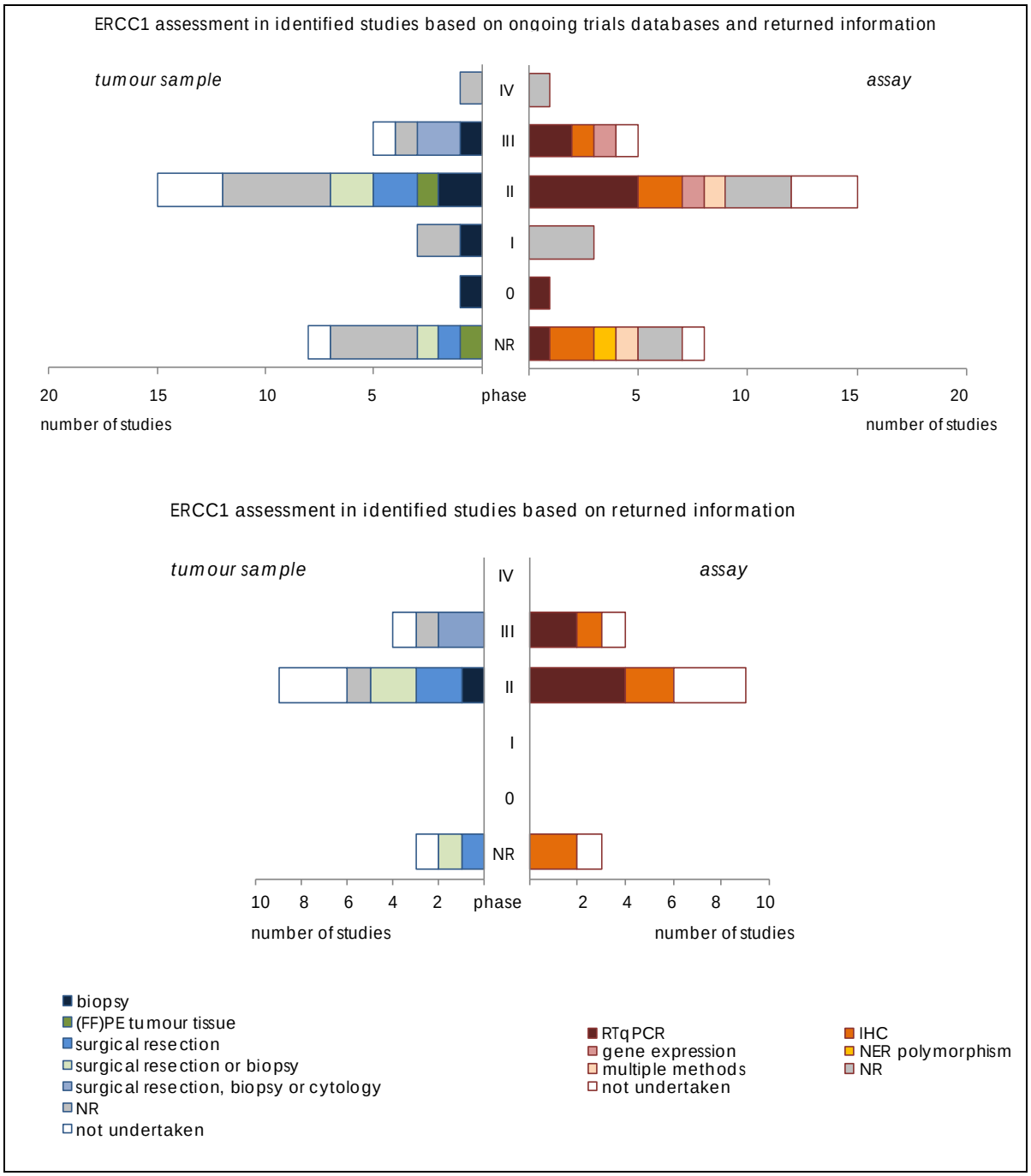
Trial ID	Trial status	Reply received	Start - end year	Type and role of chemotherapy; NSCLC stage	Design	Sample size	ERCC1 assessment method*
Phase 0							
NCT01261299[277]	Ongoing	No	2010 - 2013	Carboplatin; palliative Stage IV	single-arm correlative	80	quantitative RT-PCR
Phase I (including phase I/II)							
NCT01059552[278]	Ongoing	No	2009 - 2013	Cisplatin; unclear Stage IIIa/IIIb	single-arm correlative	22	NR
NCT01416961[279]	Withdrawn	No	2011 - 2011	Cisplatin; unclear Stage IIIa/IIIb	single-arm correlative	0	NR
NCT01386385[280]	Ongoing	No	2011 - 2016	Carboplatin; only treatment Stage III	RCT, correlative	132	NR
Phase II (including phase II/III)							
EUCTR2011-005267-24-IT (CONTEST)[281]	Ongoing	Yes (questionnaire)	2012 - 2014	Cisplatin; neoadjuvant Stage IIIa	Randomised biomarker - strategy design (using ERCC1, RRM1, EGFR, TS)	168	*RTqPCR; ERCC1 RNA level as ratio of ERCC1 gene transcripts to β-actin reference gene transcripts; cut-off 1.7*
NCT00775385 (TASTE)[282]	Ongoing	Yes (email + conference presentation)	2009 - 2014	Cisplatin; adjuvant Stage II/IIIa	Randomised biomarker - strategy design (using ERCC1 and EGFR)	165	*IHC; details NR*
NCT00792701 (S0720)[77]	Ongoing	No	2008 - 2016	Cisplatin; adjuvant Stage Ia/Ib	single-arm biomarker strategy (using ERCC1 and RRM1)	55	immunofluorescence-based automated quantitative analysis; additional available samples using RT-PCR and RTqPCR, polymorphism, ERCC1 expression at protein level; tissue microarray analysis of genes associated with DNA synthesis, damage repair, and drug efficacy
NCT01003964[283]	Ongoing	No	2009 - 2013	Cisplatin; unclear Stage IIIb/IV	RCT correlative	284	NR

Trial ID	Trial status	Reply received	Start - end year	Type and role of chemotherapy; NSCLC stage	Design	Sample size	ERCC1 assessment method*
NCT01194453[284]	Ongoing	Yes (questionnaire)	2009 - 2012	Cisplatin; first-line Stage IIIb/IV	RCT correlative	300	*RTqPCR; median value as threshold (value NR)*
NCT01356368[285]	Ongoing	No	2010 - 2013	Cisplatin; first line Stage IIIb/IV	single-arm biomarker strategy (using ERCC1, β-Tubulin and RRM1)	35	NR
NCT01731626[286]	Ongoing	Yes (email)	2013	Cisplatin;p neoadjuvant and adjuvant Stage Ib to IIIb	single-arm correlative	52	*Not undertaken*
NCT00705549[287]	Terminated	Yes (questionnaire)	2008 - 2011	Cisplatin; unclear Stage IIIb/IV	single-arm biomarker strategy (using ERCC1, BRCA1 and RRM1)	88	*RTqPCR with threshold based on the a chart analysis in >800 samples*
NCT00191308[288]	Completed	Yes (email + conference abstract)	2005 - 2010	Cisplatin; neoadjuvant Stage Ib to IIIa	single-arm, correlative	30	*not conducted due to insufficient tumor samples and "lack of scientific value"*
NCT00582634[289]	Completed	No	2004 - 2007	Cisplatin; adjuvant Stage Ib to IIIa	single-arm correlative	4	NR
NCT01781988 (PTINCLC)[290]	Ongoing	Yes (questionnaire)	2009 - 2013	Carboplatin; NR NR	Randomised biomarker-strategy design based on ERCC1, RRM1, TS and β-Tubulin	200	*IHC using ZSGB-Bio in China antibody; H-score >1 classed as ERCC1 high*
NCT01648517[291]	Ongoing	No	2012 - 2015	Carboplatin; unclear Stage IIIb/IV	Randomised biomarker-strategy design based on ERCC1 and RRM1	162	mRNA expression
NCT00736814[292]	Unknown	No	2008 - NR	Carboplatin; only treatment Stage IIIb/IV	Randomised biomarker-strategy design based on ERCC1 and RRM1	117	RT-PCR
NCT00729612[293]	Unknown	Yes (email)	2008 - 2010	Carboplatin; NR Stage IIIb/IV or recurrent	single-arm correlative	63	*Not undertaken*
NCT00215930 (MADe IT)[294]	Completed	Yes (email + publications)	2004 - 2009	Carboplatin; only treatment Stage IIIb/IV	single-arm biomarker strategy (using ERCC1 and RRM1)	53	*RTqPCR using ABI prism 7700; Perkin-Elmer, Foster City, CA; threshold: ERCC1 expression above/ below 8.7*

Trial ID	Trial status	Reply received	Start - end year	Type and role of chemotherapy; NSCLC stage	Design	Sample size	ERCC1 assessment method*
				Phase III			
EUCTR2007-007639-17-GB (ET)[295]	Ongoing	Yes (questionnaire)	2008 - 2014	Cisplatin; only treatment Stage IIIb/IV	Randomised stratified (by ERCC1)	1272	*IHC using Neomarkers (ThermoFisher) clone 8F1 antibody; threshold: Quick Score 6 and over*
EUCTR2008-001764-36-IT (ITACA)[296]	Ongoing	Yes (email + conference poster)	2008 - NR	Cisplatin; adjuvant Stage II/IIIa	Randomised biomarker - strategy design (using ERCC1 and TS)	700	*RTqPCR using 7900 ABIPRISM; values dichotomized on median value using ΔΔCT method (value NR)*
NCT00113386[297]	Terminated	Yes (email)	2005 - 2009	Cisplatin; neoadjuvant Stage IIIa	RCT correlative	19	*Terminated early due to poor accrual*
NCT00174629 (GILT Docetaxel)[298]	Completed	Yes (questionnaire)	2001 - 2007	Cisplatin; only treatment Stage IIIb/IV	Randomised biomarker - strategy design (using ERCC1)	449	*RTqPCR; threshold: median using ΔΔCT method (value 3.42)*
EUCTR2008-000617-30-DE (MADeIT)[299]	Ongoing	No	2008 - 2015	Carboplatin; only treatment Stage IIIb/IV	Randomised biomarker-strategy design based on ERCC1 and RRM1	267	ERCC1 expression at protein level
				Phase IV			
ChiCTR-TRC-11001327[300]	Ongoing	No	2010 - 2013	Cisplatin; NR Stage IIIb/IV	Randomised biomarker - strategy design, (unclear if ERCC1 used)	210	NR
				Phase NR			
NCT01294280 (LACE-BIO)[301]	Ongoing	Yes (questionnaire)	2008 - 2013	Cisplatin; adjuvant Early stage	analysis of samples from completed trials	1606	*IHC using Ab-2, clone 8F1 (Neomarkers) with H-score >1 classed positive*
NCT00900172[302]	Unknown	No	2008 - 2009	Carboplatin; unclear Stage IIIb/IV	RCT, correlative	180	Polymorphisms in ERCC-1 (…) are assessed using Taqman assays.
NCT00797238[303]	Unknown	No	2007 - 2010	platinum-based; neoadjuvant Stage III	single-arm correlative	100	NR
NCT00222404 (Pharmacogenoscan)[304]	Completed	Yes (questionnaire)	2005 - 2010	platinum-based; unclear Any stage	single-arm correlative	556	*IHC; antibody and threshold NR*

Trial ID	Trial status	Reply received	Start - end year	Type and role of chemotherapy; NSCLC stage	Design	Sample size	ERCC1 assessment method*
NCT01141686[305]	Completed	No	2009 - 2009	platinum-based; unclear Stage NR	single-arm correlative	90	FISH and IHC
NCT01574300 (CASTLE)[306]	Ongoing	No	2010 - 2017	Unclear; unclear Stage IV	single-arm correlative	250	NR
NCT00422500[307]	Completed	Yes (email)	2003 - 2010	Unclear; unclear Stage III to IV	single-arm correlative	204	*Not undertaken*
NCT00442520[308]	Completed	No	2006 - 2008	Unclear; unclear Stage NR	single-arm correlative	70	NR (SNPs in ERCC1 gene)

** information in italics is based on the returned questionnaires and emails received*



Surgical resection, biopsy or cytology includes one study where cut sections on slides were also used

Figure 7.5 ERCC1 evaluation in identified studies

The procedures for evaluation of ERCC1 varied across studies, with RTqPCR used in nine studies and IHC in five. In two studies use of multiple methods was reported:

- immunofluorescence-based automated quantitative analysis for in situ expression and (if additional samples available): RT-PCR, RTqPCR, polymorphism analysis and

tissue microarray analysis of genes associated with DNA synthesis, damage repair, and drug efficacy

- fluorescence in situ hybridization (FISH) and IHC

In one study NER polymorphism was measured. In two studies gene expression was measured, but further details were NR. In five studies (where questionnaires were returned), although initially planned, there was no ERCC1 evaluation undertaken. There was no information on the procedures used in nine studies.

The type of specimen used also varied across studies:

- three studies used surgical resection only,
- five studies used biopsy,
- cytology on its own was not used,
- three studies used surgical resection or biopsy,
- one study used surgical resection, biopsy, or cytology,
- one study used the combination of all three techniques together with cut sections on slides,
- two studies reported use of paraffin embedded specimens (of these one used formalin and one did not report the fixative),
- the type of specimen was NR in 13 cases, and
- in five studies ERCC1 evaluation was not undertaken.

There seemed to be no clear pattern indicating less variation in the procedures used in later phase trials.

It was also hypothesised that although there was no clear trend depending on the study phase, there might be some consistency in preferences for different methods depending on the time of the initiation of the study. This was however not confirmed by the available evidence (Figure 7.6).

7.3.3 ERCC1 EVALUATION BASED ON INFORMATION RECEIVED IN REPLIES TO QUESTIONNAIRES

Information was received for 16 studies. Nine of these were phase II, four phase III and for three the phase was not known. For five studies the person contacted informed that although previously planned, ERCC1 evaluation was not undertaken due to issues such as early termination of study, unavailability of sufficient samples to be tested, or funding.

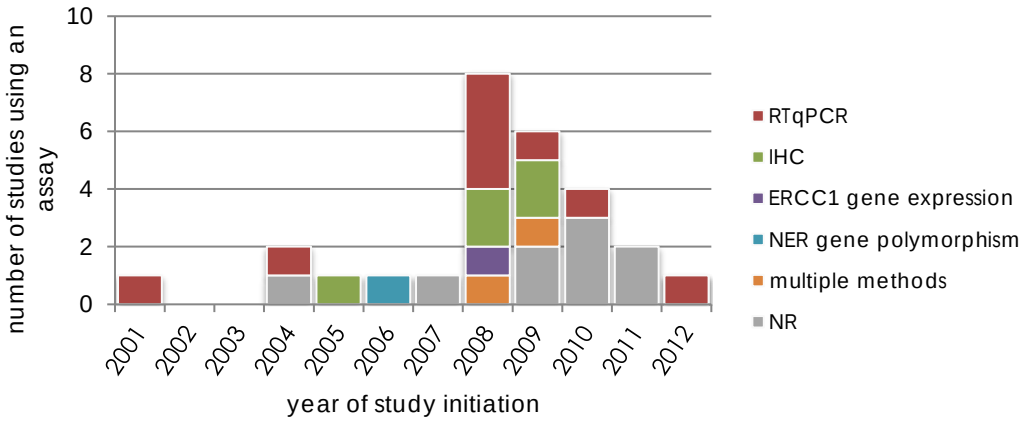


Figure 7.6 Assays for evaluation of ERCC1 expression in identified studies by date of study initiation

7.3.3.1 Objective and time of ERCC1 evaluation

The objective of ERCC1 evaluation was correlative in three studies, assignment of treatment strategy in seven and patient stratification in one (details are shown in Figure 7.4). The studies where the planned ERCC1 evaluation was not undertaken were all correlative (one phase III RCT, three phase II single-arm studies and one single-arm study where the phase was NR).

Of the eleven studies that carried out ERCC1 evaluation, it was done prospectively (prior to patients receiving treatment) in all eight studies which used the biomarker to identify the treatment strategy or stratify randomisation. Three studies used ERCC1 in a correlative analysis and all of these evaluated the biomarker retrospectively and blind to patient outcome.

7.3.3.2 ERCC1 evaluation procedures

Nine of the replies reported on the site where evaluation of ERCC1 was carried out. It was done in a central laboratory in seven studies and in an individual hospital in two. In studies assessing ERCC1 prospectively the time needed for results to be returned to the treating physician varied between a minimum of one to two days (assessment carried out in individual hospital, rather than central laboratory) to 14 days.

The details of methods used are reported in Figure 7.5. Where replies to the questionnaire were received, there was large variation in procedures for evaluation of ERCC1. RTqPCR was used in six and IHC in five studies. The type of specimen used also varied across studies:

- three studies used surgical resection only,
- one study used biopsy only,
- cytology on its own was not used,
- three studies used surgical resection or biopsy,
- one study used surgical resection, biopsy, or cytology,
- one study used the combination of all three techniques together with cut sections on slides,
- two studies did not report it.

In the five studies where more than one technique to obtain the specimen was used, biopsy was used for the majority of samples. In four of these studies biopsy was the method of obtaining 60 to 90% of tumour samples, and for one study details on the frequency of use of different techniques were NR.

In the five studies where ERCC1 was evaluated with IHC, the monoclonal 8F1 antibody was used in three studies (in 1:300 dilution in two and NR in one study), the ZSGB-Bio, China in one (in 1:50 dilution) and it was NR in one study. Ancillary methods were reported for two of these studies and did not appear similar.

For obtaining an expression score four studies used the H-score and one study used the Allred Quick Score. The thresholds for classifying patients as positive were Allred Quick Score 6 and above, and for the H-score a value of 1 was chosen as the threshold in two studies and the median value in one study (analysis undertaken retrospectively).

The percentage of patients classed as positive by IHC was 0.78 in one study, 0.6 in two studies, 0.25 in one study and NR in one study.

In the six studies which used RTqPCR it appears that each study used a different set of primers, although this could not be established with certainty due to poor reporting. β-actin on its own was used as the reference gene in four studies. In one study β-actin was used together with phosphoglycerate kinase (PGK) and in one study 18SrRNA was used. Three studies used the median as the threshold value, two selected a particular value and in one the threshold was not clearly reported. Details of RTqPCR procedures used are reported in Table 7.3. Only two studies reported the method used to calculate the quantity of ERCC1 RNA and it was the ΔΔCT method.

The percentage of patients classed as ERCC1 positive was reported for two studies using RTqPCR and was 0.6 and 0.64. In one study it was reported that as it is an ongoing prospective study, the percentage is unknown.

7.3.4 RATIONALE FOR CHOICE OF ERCC1 EVALUATION PROCEDURES

The rationale for the choice of the procedure varied across studies, as shown in

Table 7.4. The reasons provided were: experience of the laboratory, published literature, previous research experience (for example pilot study), a belief that the method of choice was superior or the limitations imposed by the type of tumour samples available. For one study it was declared that:

> *"no rationale for the present time : the AB are not isoforms specific!! only one iosoforms is functional in DNA repair and no antibody recognises it specifically".*

Table 7.3 Details of RTqPCR used in studies where information was returned

study	primers	reference gene(s)	threshold chosen
EUCTR2008-001764-36-IT (ITACA)	exons-spanning	β-actin	median using ΔΔCT method (value NR)
EUCTR2011-005267-24-IT (CONTEST)	don't know (carried out by external laboratory)	β-actin	ratio of ERCC1 to reference gene transcripts: 0.14 (low), 13.4 (high), Cut-off: 1.7
NCT00174629 (GILT Docetaxel)	designed according to their Ref Seq in https://www.ncbi.nlm.nih.gov/sites/entrez?db/gene	β-actin	median using ΔΔCT method (value 3.4)
NCT00705549	"primers have been previously described in details (Papadaki et al BR J Ca)" – paper could not be identified	β-actin and PGK	unclear ("the cut-off was based on the a chart analysis in >800 samples")
NCT01194453	primers spanning exons 7-9 of the ENST00000300853 ERCC1 transcript: 5'TCGTCTCCCGGGTGACTG 3'and5'TTCTCTTGATGCGGCGATG AG 3	β-actin	median (value NR)
NCT00215930 (MADe IT)	intron-spanning primers	housekeeping gene 18SrRNA	above/ below 8.7

Table 7.4 Rationale for the choice of method of ERCC1 assessment in studies for which information was provided

	Published literature	Previous own research experience	Laboratory experience	Believe method most appropriate	Method suitable for available samples	None	NR
EUCTR2007-007639-17-GB (ET)	✓	✓					
EUCTR2008-001764-36-IT (ITACA)			✓				
EUCTR2011-005267-24-IT (CONTEST)				✓			
NCT00174629 (GILT Docetaxel)			✓				
NCT00705549			✓	✓			
NCT00775385 (TASTE)							✓
NCT01194453					✓		
NCT01294280 (LACE-BIO)	✓						
NCT01781988 (PTINCLC)							✓
NCT00215930 (MADE IT)	✓	✓					
NCT00222404 (Pharmacogenoscan)						✓	

7.4 DISCUSSION

There were 33 studies that met our inclusion criteria, ranging from phase 0 to phase IV. Fifteen of the studies used ERCC1 as an integral part of their design: either to allocate treatment or to stratify patients.

The aim was to investigate whether the laboratory methods used for ERCC1 assessment have become more standardised since a meta-analysis published in 2011 found large variation.[264] The findings suggest that there is still large variation in both the laboratory procedures and the tumour specimens used for ERCC1 evaluation. As indicated in section 7.1, this could potentially suggest that results of trials using different methods may not be comparable, even though they appear to be evaluating the same biomarker.

In fact, some small studies have suggested that classifying patients as ERCC1 positive and negative based on either RTqPCR or IHC can lead to relatively large discrepancies.[275,309] For example, one study investigating samples from 91 patients found that there was a statistically significant correlation between the ERCC1 mRNA and protein expression

levels. However when thresholds for classification of patients as positive and negative were used, 33% of tumours ERCC1 negative by RTqPCR were IHC positive and 32% IHC-negative tumours were classed as ERCC1 positive using RTqPCR.[310] These findings possibly suggest that both techniques cannot be used interchangeably.

In this review even when the same assay was used the details of the laboratory procedures often varied. This could largely influence the comparability of results between different studies.

Three out of five studies using IHC used the 8F1 antibody (Nomarkers). The use of the same antibody is crucial, as different antibodies bind to different epitopes and can therefore have different sensitivities and specificities.[311,312] In two of the three studies using the 8F1 antibody, the dilution was reported and it was the same. Consistency is again important, as antibody dilution can influence results and has been shown to influence the proportion of cells classed as positive for other biomarkers (HER2 and p53 expression).[311]

There was also a large variation in the techniques used for RTqPCR, especially in terms of the primers used, which can have a substantial impact on the results obtained using this method.[313] Five of the six studies used β-actin as the reference standard (in one case together with another gene). The choice of a reference standard can be challenging and several publications have suggested that levels of β-actin expression can vary and may not be a good reference standard.[314,315] The thresholds chosen in individual studies also varied and, interestingly, three studies chose the median value obtained within the study. This seems to imply an assumption that half of the patients in these studies are resistant to platinum-based chemotherapy due to ERCC1 overexpression, however there seems to be no obvious reason for this assumption.

An important factor involved in comparison of different RTqPCR experiments is the yield of the reverse transcriptase reaction which copies RNA into cDNA, which can largely influence the results.[316] No information however was collected on this issue. There are numerous methods for calculation of the amount of the target RNA. Only two studies reported the method used and it was the ΔΔCT method. The threshold for classifying patients as positive did not show much variation, which is an encouraging finding, as for relatively consistent experimental procedures this would facilitate comparison of results from different laboratories.[312]

Apart from the different tests used, the methods of tumour sample collection also varied between studies. A small study using IHC for ERCC1 assessment suggested that there might be a discrepancy in classifying patients' ERCC1 expression levels depending on whether tumour tissue was obtained using biopsy or surgical resection.[317] Another study

found discrepant results depending on whether a tumour sample was obtained (using a range of methods) from the primary tumour or a metastatic site.[318]

Where reported, the proportion of patients classed as ERCC1 positive ranged from 0.25 to 0.78. This would further suggest that the procedures and criteria used in different studies for classifying ERCC1 expression levels do not produce comparable results. It is however also possible that this variation is, for example, largely due to chance or ERCC1 is expressed at different levels in different NSCLC stages.

When undertaking this review and questionnaire it was hypothesised that the highest variation in the choice of assays for ERCC1 assessment should be seen in early phase trials and higher levels of standardisation were expected for later phases. This was however not observed. There was relatively large variation in the methods chosen for ERCC1 evaluation in phase II and III trials. There was also no evidence of a trend suggesting certain methods of ERCC1 evaluation became more popular at a particular time (for example due to publication of research suggesting one method could be superior).

With regards to the rationale for the choice of a particular method, it was often motivated by experience of either the laboratory or the researchers involved, although for three studies published literature was also referred to.

As recent research suggests there may be no ERCC1 assay capable of identifying a subgroup of patients more likely to benefit from platinum-based chemotherapy.[271] This raises the issue of potentially unnecessarily enrolling patients in trials where ERCC1 is, or was integral to the trial design. This potentially resulted in suboptimal treatment of these patients and a suboptimal allocation of resources.

This systematic review is based on a relatively small sample of studies and detailed information (obtained from the questionnaire) was limited to only 16 of these. The objective here was however not quantitative in nature, but to provide an example of large discrepancies in evaluation of a potential predictive biomarker. There is no reason to believe that this example is not representative of at least some stratified medicine research. A potentially similar situation was recently identified in programmed-death ligand 1 (PD-L1) testing in NSCLC, where multiple IHC assays using different antibodies are under development for four different drugs and there is still uncertainty with regards to how well these assays predict patient response.[137]

From the perspective of reviewing evidence and implementing biomarkers in clinical practice, it would be ideal if there was one valid laboratory procedure used for biomarker evaluation in all studies. However, in practice this is unlikely to happen as the technology

in this field is rapidly developing. New laboratory procedures are being developed, which can potentially be cheaper, faster and more accurate. It is therefore not surprising that these are implemented in studies, although this review did not identify an effect of dissemination of new technologies on the choice of the laboratory procedures. This presents a challenge for implementing the findings of studies using different procedures in clinical practice, especially since, as some research on ERCC1 suggests, the results obtained using different procedures may not be comparable. Therefore there is a need for more research to ensure that procedures used to evaluate the same predictive biomarker actually stratify patients into comparable cohorts.

7.5 CONCLUSIONS

This example of ERCC1 evaluation in NSCLC highlights the need for a more structured approach to development and analytical validation of biomarkers prior to their use in clinical trials. Although on a superficial level it may appear that studies are using the same assay (for example IHC), variation in important details of the laboratory procedures may result in lack of comparability between results of different trials. If a test is to be used in clinical studies, especially later phase, ideally its accuracy should already be established. There also needs to be consensus on a standardised validated biomarker evaluation protocol to be followed in clinical trials, which would ensure there is a definitive procedure to be used in future clinical practice.

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CHAPTER 8. DISCUSSION

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8.1 MAIN FINDINGS

This thesis investigated the hypothesis that there is a mismatch between the theoretical proposals and practice of stratified medicine research, focusing on the clinical utility stage of predictive biomarker development.

The theoretical approaches were ascertained by undertaking a systematic review of frameworks for staged evaluation of predictive biomarkers (Chapter 2) and by using a published systematic review of trials methodology relevant to clinical utility (Tajik 2013[78]).

The practice of stratified medicine research was identified in a systematic review of predictive biomarkers in EMA licensing followed by an analysis of the supporting evidence (Chapter 3-5). The methodology was then compared to practice in Chapter 6.

Chapter 7 investigated the practice of stratified medicine research by focusing on the research designs and the aspects of trials related to the choice of laboratory procedures for biomarker evaluation. It undertook this through a case study of ERCC1 as predictive of response to platinum-based chemotherapy in NSCLC in studies that were ongoing or completed since 2007.

8.1.1 WHAT ARE THE STRATEGIES FOR PREDICTIVE BIOMARKER DEVELOPMENT?

Chapter 2 reviewed frameworks for staged evaluation of predictive biomarkers proposed in the literature. This was undertaken to find the most appropriate strategies that could lead to fit-for-purpose predictive biomarkers.

The systematic review identified 23 papers describing complete frameworks. This thesis proposed four models based on the stages identified and the situation to which these were applicable. The general model (model I) offered the most comprehensive approach, suggesting the following stages:

1) pre-discovery,
2) discovery,
3) analytical validation,
4) clinical validation,
5) clinical utility,
6) implementation.

The remaining three models may be considered to provide special cases of the general model. These focused on: biomarker development alongside phased drug development

(model II), development of multi-marker classifier (model III) and development of a safety biomarker when the drug is already on the market (model IV). Model III differed substantially from the remaining three, as it focused primarily on statistical issues.

Study designs appropriate for each stage often varied both between the four models and the individual frameworks within these. For some stages, such as analytical validation, there was little information on the most appropriate research methodology.

The clinical utility stage was reported in most detail and for this stage there was most agreement between frameworks. RCTs were generally considered the best choice. Depending on the situation, enrichment, stratified, or biomarker strategy designs were advocated. However, it was acknowledged that these may not always be feasible and subgroup analyses of RCTs, or even single-arm studies, may be permissible.

Few of the papers provided clear guidance on when the laboratory procedures should be finalised or the threshold of a continuous biomarker selected. When these issues were discussed, there was little agreement and some suggestions included "locking" the assay on conclusion of the analytical validation, clinical validation, or clinical utility stage.

The criteria for entry into and completion of each stage were also considered. These were rarely discussed in detail, beyond indicating that a previous stage should be completed. Interestingly, it was noted in two papers that prior to undertaking any research, the feasibility of development of a biomarker useful in addressing a given clinical problem should be established.[50,130]

8.1.2 What predictive biomarkers have been developed?

Chapter 3 investigated the predictive biomarkers included in EMA licensing. To facilitate analysis of the identified information, the idea of a B-I-D combination was introduced.

The systematic review of EMA licensing identified 49 B-I-D combinations. This was fewer than expected, given the large research effort in this area. The number of B-I-D combinations considered by the EMA each year was small, reaching a maximum of seven.

The majority of stratified medicines were found in cancer (60% of B-I-D combinations) and HIV treatments (31% of B-I-D combinations). There were also rare cases of biomarkers used in other disease areas such as HCV or cystic fibrosis.

Most of the drugs were licensed and in three cases a negative recommendation was issued by the EMA. Ten of the 49 identified B-I-D combinations involved orphan drugs,

reflecting stratification of rare diseases. In the majority of cases the biomarker was included in the indication or contraindication at the time of the first licensing of the drug.

All the identified biomarkers were molecular. In 45 B-I-D combinations the biomarker predicted drug efficacy and in four – drug safety.

8.1.3 How was the evidence considered?

Chapter 4 aimed to determine the issues that were important in decisions on licensing of a drug with predictive biomarkers in the indication or contraindication.

Text analysis of EMA documentation relevant to the B-I-D combinations identified in Chapter 3 was undertaken. Forty one criteria grouped into 13 themes were identified from the 49 cases of recommendations. The most frequently commented on were:

- theme 1: population (31 cases),
- theme 2: study design (34 cases),
- theme 7: clinical efficacy evidence (49 cases),
- theme 8: toxicity (48 cases),
- theme 9: context (40 cases),
- theme 13: pre-efficacy evidence supporting the drug (33 cases).

There were situations when some of the criteria were considered not met, however this did not preclude a positive recommendation. In some cases the study design was considered poor, yet the drug was licensed. Little attention was given to issues related to the biomarker assay (theme 10), as it was commented on in ten cases only.

Critical issues in negative recommendations were also considered. The most frequently discussed were criteria within theme 7. In such cases, the evidence supporting the drug efficacy was considered insufficient. Other critical issues were related to theme 1, 2, and 12 (pre-efficacy evidence supporting the biomarker).

One B-I-D combination (alipogene tiparvovec for LPL protein positive lipoprotein lipase deficiency) was first given a negative recommendation but after EMA reconsidered the evidence, drug authorisation followed. This was analysed in more detail in a case study. It appeared that the two opposing conclusions were based on the same evidence, however in each case a different perspective was adopted by the EMA. For the positive recommendation, more weight was given to the context: lack of any alternative

treatment and rarity of the condition. This was in line with theme 9 being amongst the most frequently considered.

8.1.4 What was the evidence?

Chapter 5 aimed to investigate the level of evidence sufficient to include a predictive biomarker in an indication or contraindication. This was attempted in an analysis of the evidence base underpinning EMA decisions. The evidence standards for B-I-D combinations that were given a positive recommendation were compared with those for refusals of marketing authorisation.

The criteria used in this chapter were based on the themes identified in Chapter 4 together with general methodological concerns and focused on the evidence relating to the biomarker. These were concerned with the:

1. population,
2. design,
3. type of primary outcome,
4. sample size, and
5. findings.

Radial plots were constructed to summarise the evidence from these five criteria. In addition, the replication of findings of a single study and the consistency of the biomarker assay within a B-I-D combination were investigated.

No clear picture was obtained of the standards required by the EMA. It appeared that the evidence was considered within a given context, rather than required to meet a certain standard. The available data were analysed in three sets of B-I-D combinations: in two of these the biomarkers predicted treatment efficacy and the drugs either did, or did not have an orphan designation; in the third, the biomarkers predicted treatment safety.

For biomarkers predicting treatment efficacy and drugs without an orphan designation, the identified evidence levels varied. In two cases of negative recommendations, it appeared that the promising results of a single study have not been replicated. However, this was also the case for some of the B-I-D combinations which received a positive recommendation. Enrichment designs constituted 38% of studies in this part of the dataset, possibly reflecting a high proportion of targeted treatments. Non-randomised designs comprised 19% of the dataset and were frequently used in addition to RCTs. Sixteen percent of the studies were retrospective subgroup analyses. Some of these

included a relatively small subgroup of patients from the original study (for example 33% in the CO.17 trial[237,238]). Cross-sectional subgroup analyses were observed relatively frequently (16%) and only in HIV. This approach is likely due to the disease-specific context, which will be discussed below.

For drugs with an orphan designation there was also little, if any, clear difference between the evidence base supporting the positive and negative recommendations. The majority of studies for these B-I-D combinations were either non-randomised (41%), mainly single-arm, or case series and reports (48%). Ten percent of the studies were enrichment RCTs. It was however clear that in some cases patient numbers were relatively large with single-arm studies including up to 353 patients.[196] It appears that in such situations a randomised trial would have been feasible and would have provided more robust evidence.

For biomarkers predicting treatment safety, the evidence requirements were minimal. In three of the four B-I-D combinations such a biomarker was based on the understanding of the drug mechanism of action or metabolism. However, in the fourth B-I-D combination, the biomarker was evaluated in a biomarker-strategy RCT.

Only 15 B-I-D combinations were supported by studies which all used the same assay to evaluate the biomarker. The details of laboratory procedures were rarely reported, both in the EMA documents and the publications matched to the identified studies.

8.1.5 How did the evidence compare to methodology?

Chapter 6 set out to compare the trials methodology suggested in Tajik 2013[78] to the evidence collected in Chapter 5. This was undertaken to assess the validity and limitations of the methodological framework proposed in the published review. The framework was also used to further evaluate the EMA dataset.

Tajik 2013[78] contained six trial designs (single-arm, enrichment, randomise-all and three variants of biomarker-strategy RCT) that could provide information on one or more of eight questions. Of the 152 studies that had sufficient data to identify their design, 86 matched those proposed in Tajik 2013.[78]

The framework was therefore modified to include seven major study designs, four of which were further divided into subtypes. In particular, lower-level designs were added (case reports and series, other types of single-arm trials and non-RCT comparative studies). Six additional questions that could be addressed by studies were also identified. The Tajik 2013[78] framework also appeared to strongly support study designs, that seemed to have little application in practice (three types of biomarker-strategy design).[13]

It was also noted that in published papers authors' conclusions seemed to be mainly addressing questions possible to answer based on the study design, although there were cases where conclusions beyond these appeared to be made. The study labels used in publications did not often reflect the biomarker aspect of the study.

The evidence supporting the 49 B-I-D combinations with available study designs was evaluated using the expanded framework. The questions addressed by these studies were considered. Information on whether the biomarker was predictive and the benefit of implementing a biomarker-based strategy was available only for 14 B-I-D combinations. In the remaining B-I-D combinations, studies mainly investigated activity or effectiveness within biomarker-defined patient groups. Some studies also investigated the correlation between the biomarker status and study outcome, or the prevalence of the biomarker in patients not responding to treatment.

8.1.6 What laboratory procedures were chosen for ERCC1 evaluation and why?

Chapter 7 described a case study of ERCC1 expression in NSCLC to predict response to platinum-based chemotherapy. It attempted to identify the reasons for and the consequences of no standardisation in laboratory methods used for biomarker evaluation.

A systematic review of trials that were either ongoing or completed since 2007 was undertaken. This was followed by a survey of trialists aiming to explore the variation in some analytical and pre-analytical factors, as well as the motivation for the choice of a particular biomarker assay.

The systematic review identified 33 trials, ranging from phase 0 to phase IV. Fifteen of the studies used ERCC1 as an integral part of their design: either in a biomarker strategy (five single-arm, nine RCTs) or stratified design (one RCT). A reply to the survey was received for 16 trials.

Large differences were identified in the laboratory procedures and the tumour specimens used for ERCC1 evaluation. The most frequently used assays were RTqPCR (nine trials) and IHC (five trials). In seven cases the assay was NR. Even in cases when the same assay was used, the details of laboratory procedures were often dissimilar. This was particularly evident for the primers used in RTqPCR. The methods of tumour tissue collection also differed between studies. The identified variation did not appear to depend on the stage of the trial or the year of its initiation.

These discrepancies were possibly reflected in the proportion of patients classed as ERCC1 positive. Based on the replies to the questionnaire, this ranged from 25% to 78%.

The rationale for the choice of a particular laboratory procedure was explored in the survey. This was often reported as based on experience of either the laboratory or the researchers involved, although for three trials published literature was also referred to.

8.2 Mismatch between theory and practice in trial design

This thesis investigated a hypothesis that there is a mismatch between the theoretical proposals and the practice of stratified medicine research. Such discrepancies were found in a number of areas.

8.2.1 Main features of the mismatch

Out of the three major trial designs proposed in the methodological literature, the enrichment design was most frequently used and comprised 28% of the studies in the EMA dataset. It was most commonly encountered in B-I-D combinations where the biomarker predicted efficacy and the drug did not have an orphan designation. A single biomarker strategy trial was identified and there were no stratified designs.

Subgroup analyses of RCTs constituted 25% of all studies in the EMA dataset and were only identified for efficacy biomarkers and drugs without an orphan designation. The majority of these were either retrospective or cross-sectional. The cross-sectional subgroup analysis was not mentioned in the theoretical literature, however it was commonly used in HIV trials.

A relatively large proportion (27%) of the identified studies were non-randomised. These mainly comprised single-arm trials investigating the drug in biomarker positive patients, a design not recognised in Tajik 2013.[78] The majority of such studies were part of the evidence base for B-I-D combinations where the drug had an orphan designation.

Another relatively large group of studies were case series and reports in which the biomarker status was evaluated. These comprised 17% of the EMA dataset, however they accounted for almost half of the studies supporting drugs with an orphan designation. Again, these were not recognised in the theoretical literature, but seemed to be frequently used in research on rare conditions.

Finally, in four B-I-D combinations (including three with the biomarker predicting safety) there were no studies that supported the biomarker. It appeared that the

recommendations were based solely on the understanding of the mechanism of drug action or metabolism. Such an approach has not been encountered in the methodological literature reviewed in Tajik 2013.[78] In addition, Chapter 2 identified two frameworks focusing on development of safety biomarkers. However, none of these proposed omission of clinical investigation of potential predictive biomarkers.[51,134]

Further investigation of stratified medicine practice was undertaken in the ERCC1 case study in Chapter 7. In spite of the lack of validation, this biomarker was often integral to the design, with one stratified and 14 biomarker strategy trials (five single-arm and nine RCTs). The relatively high frequency of biomarker strategy designs seems counterintuitive. Prior to the initiation of these trials the evidence supporting ERCC1 was relatively weak and the analytical validity of the available assays was apparently not established. In such a case a biomarker-stratified trial would have been more appropriate.[49,61]

8.2.2 Possible reasons for the mismatch

Understanding the reasons for the identified mismatch requires consideration of the fact that different scenarios may necessitate different approaches. In fact, the context in which research is undertaken was already identified as an important factor in Chapter 4 and Chapter 5.

Predictive biomarkers may be discovered at different stages of drug development:

- before clinical development,
- during clinical development, and
- after marketing authorisation.[91]

All of these result in different research designs appearing more appropriate from the perspective of a drug developer.

For biomarkers identified prior to clinical development, an enrichment design might be the most attractive, possibly limiting the scale of the necessary research. There are numerous examples of targeted treatments where such a strategy has been implemented, including trastuzumab.

Identification during clinical development may be, in some ways, the most challenging. It can possibly lead to either enrichment or stratified trials, depending on the exact time of biomarker discovery and the strength of the evidence supporting it. Such a case was however not observed in the dataset.

When biomarkers are identified after marketing authorisation, retrospective subgroup analyses using data from completed trials may be most convenient. This is exemplified by the KRAS mutation to predict the lack of response to cetuximab and panitumumab.

Selection of designs will most likely depend on financial and time constraints. A large number of designs identified in the EMA dataset may offer substantial savings compared to more informative ones. For example, an enrichment trial requires less patients overall and possibly, a shorter follow-up compared to a stratified one. The other disincentives to undertaking a stratified design are the opportunity costs of such an investment.[112] However, an enrichment trial still requires screening all the eligible patients and may therefore result in a relatively long recruitment period.[49]

From the perspective of a drug developer, prior to marketing, stratification may be actually advantageous when there is limited confidence in the biomarker. It may allow identification of a possible stratum of patients with improved response, and therefore a market where the price may be increased. If the biomarker turns out not to be predictive, it does not preclude future use of the drug in the unstratified population.[112]

When there are completed studies with available biological specimens, undertaking retrospective subgroup analyses will require much less time and resources than conducting new trials.[1] This often also corresponds to a situation when the drug is already on the market and the pharmaceutical company will likely lose future profits due to the shrinking demand for the drug and unlikely increase in its price.[112] Therefore, investment in new trials will not appear attractive. Yet, there are examples, where an introduction of a predictive biomarker post-marketing actually increased the use of a drug. For abacavir treatment of HIV, the possibility of occurrence of a hypersensitivity reaction resulted in limiting its use. Introduction of a biomarker predictive of safety (HLA-B*5701 allele) increased the use of this drug in clinical practice.[319]

Another mismatch was observed in cases of drugs with an orphan designation, where single-arm studies, case series and reports were extensively used. From the numbers of patients included in these research designs it appeared that more robust studies could have been undertaken. However practical reasons may explain the relative rarity of more adequate designs. The types of studies that were identified may be more convenient to undertake. They do not require randomisation, or blinding. Also, to ensure a sufficient number of patients for an RCT, an international trial may be required. These may be difficult to set up compared to smaller studies conducted within one country.

In addition, the majority of such studies were identified in cancer. Arguably, in this clinical area a response may be relatively easily observed based on tumour shrinkage or the drop

in the concentration of leukemic cells in the blood. Such a response would be highly unlikely without treatment. However, the establishment of the correlation between tumour response and more clinically relevant outcomes, for instance overall survival, may be problematic,[320,321] with examples of research providing mixed conclusions on the association.[322-324]

There may also be ethical reasons for the choice of a particular trial design. When there is strong biological evidence suggesting that a treatment would not benefit biomarker-negative patients, it may be unethical to expose them to possible adverse effects,[257] particularly if there are alternative treatments available. These considerations would generally result in trials including only biomarker positive patients.

In Chapter 7 a biomarker strategy design was often used where a stratified design appeared more appropriate due to the limitations in the biomarker. Possibly, ethical reasons influenced the selection of the trial methodology. In this case, the biomarker use was to result in withdrawal of the standard care and this would be ethically problematic for patients likely to benefit. A similar case was identified for HLA-B*5701 allele predicting abacavir safety. This again resulted in withdrawal of potentially beneficial treatment and therefore the most appropriate design appeared to be a biomarker strategy trial.

As noted in the methodological literature, the biological rationale for the use of the biomarker may impact on the design choice. In cases when there is strong evidence that a treatment would not benefit biomarker negative patients, there may be little reason to invest time and money in such research. This is generally the case for targeted drugs, which have been developed for patients positive for a particular biomarker. For example trastuzumab, discussed in detail in Chapter 2, was developed to bind the HER2 protein, therefore it was highly unlikely to provide benefit to patients whose tumours do not express this biomarker. Stratified and biomarker strategy designs may be used in cases where there is less biological evidence in support of the biomarker.

Biological reasons are also responsible for the cross-sectional subgroup analyses identified in the EMA dataset. These were commonly used to identify viral resistance mutations predicting lack of response to HIV treatments. These studies can only determine the prevalence of the biomarker positive patients in non-responders. Although this evidence is highly limited, it has been used to support B-I-D combinations. More robust trials may not be feasible due to either possible presence of viral resistance mutations at low, sub-detection level at baseline, or the evolution of such mutations in response to treatment.

Different designs may also appear appealing depending on who is investigating potential predictive biomarkers. Due to the nature of the EMA dataset, this discussion

predominantly focused on the perspective of pharmaceutical companies. However, for example policymakers may be more interested in the consequences of implementation of a treatment strategy using a predictive biomarker. This would naturally result in a preference for the biomarker strategy design. Academic trials may be more likely to investigate potential biomarkers for existing treatments with a weaker evidence base, leading to more stratified trials.

Furthermore, Chapter 6 identified that different designs are capable of answering different questions. Although it may appear beneficial to be able to collect as much information as possible, there are always opportunity costs of the research that may be conducted. In a proportion of cases, it may not be necessary to answer all questions. For purposes such as drug licensing the efficacy of the drug in the biomarker positive patients may be sufficient. This, of course, may result in difficulties in surmounting the so called "fourth hurdle" of reimbursement. For institutions such as the National Institute of Health and Care Excellence whether a biomarker is truly predictive could be of huge importance, as this may have implications for the cost-effectiveness of the treatment strategy.

Finally, as noted in Chapter 1, there is a relative lack of regulatory standards focusing on predictive biomarker research, possibly resulting in suboptimal trials being conducted.[91] This may also add to the observed mismatch.

8.3 Other observations

Apart from addressing the main hypothesis of this thesis, further observations were made on some issues important to this field. These are briefly discussed below.

8.3.1 Procedures for biomarker evaluation

The laboratory procedures for evaluation of predictive biomarkers rarely seemed to be considered of high importance. This was reflected by the criteria identified in Chapter 4. In addition, insufficient details were often reported in identified papers (Chapter 5) and study records (Chapter 7) to allow identification of the exact laboratory methods used.

The importance of analytical validity of biomarkers appeared insufficiently considered in methodology, research practice and interpretation. It was infrequently discussed in EMA documents in Chapter 4 and omitted in a number of frameworks in Chapter 2. Chapter 7 found that insufficiently validated biomarkers were used in clinical trials, including phase III

and IV. The choice of the assay for a clinical trial in some cases seemed to be motivated by the experience of a research group, rather than considerations of validity.

It may be argued that validation of predictive biomarkers is expensive, particularly if it requires new studies.[112] However, evaluation of biomarkers within trials may itself be costly. Also investigation of non-validated biomarkers may then result in generation of noise, rather than useful information.[325,326] This was shown in the example of ERCC1, where findings obtained using a non-validated biomarker led to unnecessary trials. Therefore, contrary to some arguments, thorough validation of a biomarker early on in the development process may in fact result in a more efficient development strategy and facilitate engagement with regulators.[112]

The issue of the biomarker threshold also seems to be rarely addressed. Little consideration was given to this topic both in papers addressing biomarker development in Chapter 2, and those reporting research in Chapter 5. As shown in Chapter 7, threshold selection can often be arbitrary. For example, some studies decided to retrospectively select a median value as the cut-off. However, there was no reason to believe that approximately 50% of patients would not respond to the treatment.

An interesting approach to selecting threshold values was proposed in the FDA framework.[47] It suggested that a grey zone may exist between the biomarker positive and negative patients. In such a grey zone, the decision on treatment cannot be based on the biomarker value, due to either inaccuracies in biomarker evaluation or uncertainty around the measurement of the clinical outcome. Based on the accuracy of available biomarker assays, for example the HercepTest, such grey zones may possibly be common. These should be recognised and taken into account in treatment decisions.

Another approach to continuous biomarkers where a trial is undertaken including patients irrespective of their biomarker values has been proposed in the literature. No threshold is selected, but patient level survival curves are developed. These could allow individual treatment decisions to be made for each patient.[327] Although this design seems to offer a more personalised approach, there may be some practical limitations to its implementation in clinical practice, including the challenges in funding such strategies.

8.3.2 Role of the wider context

One of the recurring issues identified in this thesis was the impact of the context on the research undertaken and its interpretation. This seems intuitive and is often taken into account, at least to some extent, in methodological papers. These, for example, suggest

that for targeted drugs an enrichment design might be most appropriate. However, issues such as the biomarker threshold (for continuous biomarkers) and accuracy seem to be rarely considered in the context in which the biomarker is developed. When there are few or no alternative treatments available, biomarkers that are good at ruling out patients unlikely to benefit may be appropriate. However, in situations where there are multiple options to choose from, biomarkers good at identifying patients most likely to respond may be more important.

Another issue related to the wider context is use of information from other clinical areas to inform decisions. This was observed in some cases in the EMA dataset in Chapter 4. In some cases, evidence from other indications of the same drug or other drugs with a similar mechanism of action was used. One such example involved consideration of cetuximab for treatment of EGFR expressing NSCLC. It was noted that there appeared to be no effect of KRAS mutation status on treatment efficacy, which would normally be expected in the class of EGFR targeted treatments. Therefore, together with other reasons, this led to a negative recommendation for this drug.[209] Formal incorporation of such considerations into trial designs, particularly for rare diseases, have been discussed in the theoretical literature,[328,329] however their implementation in practice may be challenging.[330]

8.3.3 Clinical areas

It appears that successful stratified medicines have been developed mainly for cancer and HIV treatment. These diseases appear to naturally lend themselves to stratification. Cancer can be driven by a wide range of somatic mutations,[27] differing between tumours.[331] These can be used as targets for new drugs.[75,332] Similarly, in HIV and a number of other viral diseases, there is a range of virus genotypes, which can be controlled by different drugs.[75]

However, there are other clinical areas where there is large variability in patient response, and thus potential need for predictive biomarkers, such as mental health.[333] Development of stratified medicines in these areas may be a difficult task.[334] These diseases are often more complex, less well understood and their diagnosis based on symptoms, rather than molecular biology.[333] This may render identification of relevant molecular biomarkers challenging. There have been some efforts to use other types of biomarkers, such as electroencephalography to predict response to treatment for major depressive disorder.[335,336] These however do not seem ready for implementation in clinical practice.

Even in the clinical areas where stratified medicines are available, resistance to treatment may often emerge by evolutionary mechanisms.[75,337] In addition, in HIV drug-resistant genotypes may be transmitted during primary infection.[244] However, these may be present in quantities below detection level, at least using standard laboratory techniques.[245] A similar problem exists in cancer where intra-tumour heterogeneity has been identified as an important problem.[338] This may arise from the both genetic and non-genetic differences between cells in the same tumour. It has also been shown that there are differences between primary tumours and metastatic sites.[331,339] As different tumour cells may be characterised by different biomarkers, these may in fact be eligible for different treatments.[339]

Combination therapy may offer profound improvements in treatment of evolving conditions and a way to address tumour and viral heterogeneity. This idea has already been implemented in HIV, where standard therapy involves drugs from different classes with different mechanisms of action.[75] Improvements compared to monotherapy have also been shown in some cancers, for example in a trial investigating a combination of nivolumab and ipilimumab in melanoma.[339] Such approaches may however require evaluation of biomarkers in multiple tumour samples, preferably from both the primary tumour and metastatic sites (if these are present). Implementation of combination therapy in cancer may also face some barriers. In particular, the high cost of cancer drugs may limit the use of combination therapy in practice. In addition, if a combination of stratified medicines is to be investigated, this may result in trials in small sub-populations.[75] As was shown in Chapter 5 and 6, the research methodology standards for such cases are relatively poor, and there may be a need to both further develop the available research designs and improve the research practice.

8.4 Strengths and weaknesses

This thesis looked at both the theory and practice and therefore the identifications of these two aspects of stratified medicine needs to be considered.

The theory was identified in two steps. First, a systematic review of frameworks for staged evaluation was undertaken in Chapter 2. This was based on broad literature searches and inclusion decisions were undertaken by two independent reviewers. However, the extraction, synthesis of data and construction of models was undertaken by one reviewer only and therefore some subjectivity might have been involved.

Secondly, the theoretical literature on trials methodology was identified in a published systematic review.[78] This was found to have some limitations. However, these were unlikely to have led to missing major designs proposed in the literature. Further, there are arguments suggesting that systematic reviews of methodology do not need to identify all relevant papers, as there is a large repetition and the required information is not quantitative in nature.[261,340]

The evidence on practice was obtained primarily from the EMA dataset and also from a case study of ERCC1 in platinum-based chemotherapy for NSCLC. Both datasets were identified in a systematic review with broad searches and two independent reviewers making inclusion decisions. However, again some limitations may be introduced by the data extraction and analysis being undertaken by one reviewer only.

The use of the EMA dataset was in some cases limited by the level of detail included in the available documentation. This was addressed by an attempt to identify published papers that matched the studies in the dataset. This was not always possible, as some of these were reported in insufficient detail and others appeared to be unpublished.

For identification of the issues considered by the EMA, documents were used that possibly do not provide a faithful record of actual discussions. These may still give some indication of the problems judged important enough to be reported.

The EMA dataset was used for comparison of the theoretical literature to real cases of stratified medicine research. This dataset was somewhat selected and may not be representative of all stratified medicine. As licensing decisions were considered, only evidence supporting relatively successful drugs and biomarkers was included. Although there were a few cases where the EMA issued a negative recommendation, these were still representative of relatively strong cases. In situations where the evidence was extremely weak, a licensing application was probably not submitted. However, if the evidence in the EMA dataset is considered to represent the stronger cases, it appears to be painting a rather negative picture of stratified medicine.

A different picture, at least with respect to trials methodology, emerged from the review of studies investigating ERCC1 in NSCLC in Chapter 7. This dataset included a high proportion of trials not sponsored by the pharmaceutical industry. It also provided a snapshot of the trials undertaken over a short period of time investigating a single predictive biomarker.

The data available from ongoing trials databases are usually limited and do not always allow an understanding of the trial methodology and laboratory procedures to be

achieved. To address this, a survey of clinical trials was undertaken. A reply was only received from 48% of trials, in spite of a reminder being sent. This is a relatively low response rate. However, the identified lack of laboratory procedure standardisation across trials is likely to provide a picture of at least some stratified medicine research.

8.5 IMPLICATIONS

In spite of the wealth of methodological literature, there seem to be serious limitations in the actual research being undertaken. There appears to be little consistency in the standards for predictive biomarker development and evaluation. The interpretation of research evidence often takes into account the wider context. This is not surprising, however better understanding and incorporation of the context into research methodology may be necessary.

The frequently used enrichment design is appropriate when the drug is very unlikely to provide benefit to biomarker negative patients. However, some doubts may exist as to whether the understanding of disease biology and drug mechanism of action is always sufficient to limit the investigation to biomarker positive patients. In addition, to use the enrichment design, acceptable analytical validity of the laboratory procedure to evaluate the biomarker needs to be established. However, these conditions do not always appear to be met. As shown by the example of BRAF mutations, the initial subgroup for which the treatment was developed proved to be narrower than that which actually benefited. This was discovered due to the inaccuracy of the assay used to evaluate the biomarker.[176] Another example is provided by trastuzumab. Research suggesting that patients with HER2 expression levels below the original threshold may also benefit led the American Society for Clinical Oncology to modify the criteria for classifying patients as positive.[341] This resulted in an increase in the proportion of patients eligible for treatment with trastuzumab.[342]

Undertaking trials using biomarkers that have not been appropriately validated may result in large numbers of patients receiving suboptimal treatments, as well as substantial loss of resources. This was investigated in the ERCC1 case study, which is likely not an isolated case. Even when the biomarker does not influence the patient flow in the study, conducting analyses using non-validated assays should be an exception, as these may result in large costs and spurious findings. These can potentially lead to unnecessary trials, as shown for ERCC1.

The use of cross-sectional subgroup analyses in HIV led to limited evidence in support of the biomarker. The findings of such studies are often supported by in vitro assays, however these may not always translate into clinical outcomes.[75] There is therefore a need to further understand and possibly improve the existing methodology that may be applicable to such situations.

The relatively low number of biomarkers predictive of safety suggests these may need to be further developed, as adverse events related to treatment are still an important problem.[10] It can be however argued that introduction of biomarkers predictive of efficacy improves the safety profile of a treatment. Assuming a random distribution of adverse events across all patients treated with a drug, the benefit-to-risk ratio will improve if a smaller number of non-responders are treated. There are cases where adverse events are more common in patients who are more likely to respond to treatment. For example, there is possibly a higher incidence of rash in patients responding to EGFR inhibitors.[343] In such cases the benefit-to-risk ratio will however be no worse, as the mean efficacy will improve.[112]

The clinical areas where predictive biomarkers have been developed are limited. There are few stratified medicines outside of cancer and viral diseases. This may be due to a number of barriers which were already discussed, however some of these concerns could potentially be addressed by more sound and transparent standards for biomarker development.

Few studies investigated the problem of patients whose biomarker status cannot be established. The reasons may be varied and include the difficulty in access to tumour tissue (for example in lung cancer) and poor quality of samples.[61,326] There were only five studies in the EMA dataset that attempted this. Presumably, such patients are often excluded from trials with a biomarker-driven design. However, these may constitute a substantial proportion of patients in clinical practice. The size of such a population is unclear, however based on a survey of laboratories evaluating EGFR mutation in NSCLC, this may be as much as 10% of all samples.[344] Some recent trials, for example FOCUS4,[82] have incorporated this subgroup into their design.

Finally, one of the observations in this thesis was the poor reporting of biomarker evaluation methods in the identified trials. These details are necessary to replicate the findings of trials and use them for secondary research and decision making in healthcare. This is particularly important, given that variation in the laboratory procedures may result in large differences in the biomarker values. Reporting guidelines may be necessary for

stratified medicine trials, possibly similar to those proposed for prognostic tumour marker studies (REMARK).[220]

8.6 PRIORITIES FOR FUTURE RESEARCH

Two major areas of priority have been identified in this thesis.

8.6.1 SYSTEM FOR BIOMARKER EVALUATION AND HIERARCHY OF EVIDENCE

No clear standards for biomarker evaluation were identified. Establishment of such standards could potentially be facilitated by consensus on the strategies for development of predictive biomarkers. Implementation of a rigid, sequential framework, as those reviewed in Chapter 2 may not be feasible. This was recognised when in 2005 the FDA published a concept paper describing such a framework. It met with criticism for being inflexible,[345] resulting in it not being implemented. However, a set of issues to be addressed within a biomarker development programme may be sufficient. Analytical validation of biomarkers and development of broad consensus on the appropriate laboratory procedures needs to take place at a time that will facilitate efficient development of stratified medicines and prevent cases such as that described in Chapter 7.

In terms of methodology for investigating clinical utility, more attention should be given to the choice of a correct trial design. An agreement on a hierarchy of evidence taking into account the context of biomarker development would be ideal.

8.6.2 BIOMARKER THRESHOLD

Issues associated with the threshold for continuous biomarkers were rarely considered. Its choice, however, may be crucial to the predictive biomarker evaluation and maximisation of patient benefit. The optimal point (or range of points) in the biomarker development process for the identification of a threshold should be established. There is also a need for clear methodological standards for the determination of a cut-off, which would take into account the uncertainties in the evaluation of biomarker values. It needs to be recognised that revisions to the threshold may be necessary in some cases. The impact of such revisions on the interpretation of previous research should also be considered.

Such decisions should ideally be undertaken by a multidisciplinary panel. However, more thought is required on the appropriate composition of such a panel. There may also be a need for a mechanism that would allow standardisation of the threshold across different research teams.

8.7 CONCLUSIONS

This thesis identified the theoretical proposals for development and evaluation of stratified medicines. It also analysed a large sample of studies that represent the research practice in this area. This was done by investigating the reasons for and the evidence underlying EMA recommendations. In addition, practice was investigated in a case study of ERCC1 for prediction of response to platinum-based chemotherapy in NSCLC.

The theory and practice were then compared and a mismatch between the two was identified. It appeared to be a result of both the practice not following some theoretical requirements, and the underdevelopment of methodology for certain situations.

Areas where further methodological developments may be necessary were identified and potential barriers to the implementation of the most appropriate methodology discussed.

The major research priorities identified in this thesis were development of a clear hierarchy of biomarker research designs and development of methodology related to the biomarker threshold.

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BIBLIOGRAPHY

1. R. M. Simon, S. Paik and D. F. Hayes, Use of archived specimens in evaluation of prognostic and predictive biomarkers, *J Natl Cancer Inst.*, 2009, 101, 21, 1446-1452

2. R. Bazell, Her-2 the Making of Herceptin, a and BGT Pogo, 2001. Detection of MMTV-like LTR Revolutionary Treatment for Breast Cancer. Random and LTR-env gene sequences in human breast house, New York, pp: 3-53. cancer, *Intl. J. Oncol*, 1998, 18, 1041-1044

3. S. Muhkerjee, The emperor of all maladies: a biography of cancer, 2011, Fourth Estate, London

4. K. Strebhardt and A. Ullrich, Paul Ehrlich's magic bullet concept: 100 years of progress, *Nature Reviews Cancer*, 2008, 8, 6, 473-480

5. Q. Ma and A. Y. Lu, Pharmacogenetics, pharmacogenomics, and individualized medicine, *Pharmacol Rev*, 2011, 63, 2, 437-459

6. U. A. Meyer, U. M. Zanger and M. Schwab, Omics and drug response, *Annu Rev Pharmacol Toxicol*, 2013, 53, 475-502

7. A. Bhathena and B. B. Spear, Pharmacogenetics: improving drug and dose selection, *Curr.Opin.Pharmacol.*, 2008, 8, 5, 639-646

8. S. Xinghua Hu, T. Foster and A. Kieffaber, Pharmacogenomics and personalized medicine: mapping future value creation, *BioTechniques*, 2005, 39, 4,

9. M. R. Trusheim, E. R. Berndt and F. L. Douglas, Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers, *Nat.Rev.Drug Discov.*, 2007, 6, 4, 287-293

10. J. C. Bouvy, M. L. De Bruin and M. A. Koopmanschap, Epidemiology of adverse drug reactions in Europe: a review of recent observational studies, *Drug Saf*, 2015, 38, 5, 437-453

11. W. K. Redekop and D. Mladsi, The faces of personalized medicine: a framework for understanding its meaning and scope, *Value Health*, 2013, 16, 6 Suppl, S4-9

12. C. J. Melief, T. van Hall, R. Arens, F. Ossendorp and S. H. van der Burg, Therapeutic cancer vaccines, *J Clin Invest*, 2015, 125, 9, 3401-3412

13. R. Simon, Roadmap for developing and validating therapeutically relevant genomic classifiers, *J Clin Oncol*, 2005, 23, 29, 7332-7341

14. G. Biomarkers Definitions Working, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, *Clin Pharmacol Ther*, 2001, 69, 3, 89-95

15. R. Simon, Validation of pharmacogenomic biomarker classifiers for treatment selection, *Cancer Biomark*, 2006, 2, 3-4, 89-96

16. R. Simon, Advances in Clinical Trial Designs for Predictive Biomarker Discovery and Validation, *Current Breast Cancer Reports*, 2009, 1, 216-221

17. D. J. Sargent, B. A. Conley, C. Allegra and L. Collette, Clinical trial designs for predictive marker validation in cancer treatment trials, *J Clin Oncol*, 2005, 23, 9, 2020-2027

18. N. A. Alymani, M. D. Smith, D. J. Williams and R. D. Petty, Predictive biomarkers for personalised anti-cancer drug use: discovery to clinical implementation, *Eur J Cancer*, 2010, 46, 5, 869-879

19. S. J. Mandrekar and D. J. Sargent, Predictive biomarker validation in practice: lessons from real trials, *Clin Trials*, 2010, 7, 5, 567-573

20. H. M. Shepard, P. Jin, D. J. Slamon, Z. Pirot and D. C. Maneval, Herceptin, *Handb Exp Pharmacol*, 2008, 181, 183-219

21. M. S. Pepe, Z. Feng, H. Janes, P. M. Bossuyt and J. D. Potter, Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design, *J Natl.Cancer Inst.*, 2008, 100, 20, 1432-1438

22. M. Baccarani, S. Pileri, J. L. Steegmann, M. Muller, S. Soverini, M. Dreyling and E. G. W. Group, Chronic myeloid leukemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, *Ann Oncol*, 2012, 23 Suppl 7, vii72-77

23. J. Bell, Redefining Disease: The Harveian Oration, Delivered Before the Fellows of the Royal College of Physicians of London on Tuesday 19 October 2010, 2010, Royal College of Physicians, London, UK

24. E. Papaemmanuil, M. Gerstung, L. Bullinger, V. I. Gaidzik, P. Paschka, N. D. Roberts, N. E. Potter, M. Heuser, F. Thol, N. Bolli, G. Gundem, P. Van Loo, I. Martincorena, P. Ganly, L. Mudie, S. McLaren, S. O'Meara, K. Raine, D. R. Jones, J. W. Teague, A. P. Butler, M. F. Greaves, A. Ganser, K. Dohner, R. F. Schlenk, H. Dohner and P. J. Campbell, Genomic Classification and Prognosis in Acute Myeloid Leukemia, *N Engl J Med*, 2016, 374, 23, 2209-2221

25. Arsenic trioxide: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000388/WC500042844.pdf, Accessed: 14.05.2013.

26. R. Mayeux, Biomarkers: potential uses and limitations, *NeuroRx*, 2004, 1, 2, 182-188

27. T. Strachan, Read, A., Human Molecular Genetics, 2010, Garland Science, New York,USA; Abingdon, UK

28. R. Kurzrock, H. M. Kantarjian, B. J. Druker and M. Talpaz, Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics, *Ann Intern Med*, 2003, 138, 10, 819-830

29. S. Mallal, E. Phillips, G. Carosi, J. M. Molina, C. Workman, J. Toma++i-ì, E. J+ñgel-Guedes, S. Rugina, O. Kozyrev, J. F. Cid, P. Hay, D. Nolan, S. Hughes, A. Hughes, S. Ryan, N. Fitch, D. Thorborn and A. Benbow, HLA-B*5701 Screening for Hypersensitivity to Abacavir, *New England Journal of Medicine*, 2008, 358, 6, 568-579

30. I. Cascorbi, Overlapping effects of genetic variation and epigenetics on drug response: challenges of pharmacoepigenomics, *Pharmacogenomics*, 2013, 14, 15, 1807-1809

31. A. de Gramont, S. Watson, L. M. Ellis, J. Rodon, J. Tabernero, A. de Gramont and S. R. Hamilton, Pragmatic issues in biomarker evaluation for targeted therapies in cancer, *Nat Rev Clin Oncol*, 2015, 12, 4, 197-212

32. D. J. Lockhart and E. A. Winzeler, Genomics, gene expression and DNA arrays, *Nature*, 2000, 405, 6788, 827-836

33. G. S. Dichter, D. Gibbs and M. J. Smoski, A systematic review of relations between resting-state functional-MRI and treatment response in major depressive disorder, *J Affect Disord*, 2015, 172, 8-17

34. Ziagen: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000252/WC500050343.pdf, Accessed: 30.04.2012.

35. Kivexa: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000581/WC500043730.pdf, Accessed: 30.04.2012.

36. Trizivir: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000338/WC500043448.pdf, Accessed: 28.07.2011.

37. Ivacaftor: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002494/WC500130696.pdf, Accessed: 24.04.2013.

38. A. Dasgupta and J. L. Sepulveda, Accurate Results in the Clinical Laboratory: A Guide to Error Detection and Correction, 2013, Newnes,

39. P. Carraro and M. Plebani, Errors in a stat laboratory: types and frequencies 10 years later, *Clin Chem*, 2007, 53, 7, 1338-1342

40. L. Jafri, A. H. Khan, F. Ghani, S. Shakeel, A. Raheem and I. Siddiqui, Error identification in a high-volume clinical chemistry laboratory: Five-year experience, *Scand J Clin Lab Invest*, 2015, 75, 4, 296-300

41. A. Balsari, P. Casalini, E. Tagliabue, M. Greco, S. Pilotti, R. Agresti, R. Giovanazzi, L. Alasio, C. Rumio, N. Cascinelli, M. I. Colnaghi and S. Menard, Fluctuation of HER2 expression in breast carcinomas during the menstrual cycle, *Am J Pathol*, 1999, 155, 5, 1543-1547

42. F. Desiere, T. S. Gutjahr and U.-P. Rohr, Developing companion diagnostics for delivering personalised medicine: opportunities and challenges, *Drug Discovery Today: Therapeutic Strategies*, 2013,

43. B. M. Heckman-Stoddard, Oncology biomarkers: discovery, validation, and clinical use, *Semin Oncol Nurs*, 2012, 28, 2, 93-98

44. N. L. Henry and D. F. Hayes, Cancer biomarkers, *Mol Oncol*, 2012, 6, 2, 140-146

45. K. Shahzad, A. Fatima, M. Cadeiras, N. Wisniewski, G. Bondar, R. Cheng, E. Reed and M. Deng, Challenges and solutions in the development of genomic biomarker panels: a systematic phased approach, *Curr Genomics*, 2012, 13, 4, 334-341

46. S. E. Taube, G. M. Clark, J. E. Dancey, L. M. McShane, C. C. Sigman and S. I. Gutman, A Perspective on Challenges and Issues in Biomarker Development and Drug and Biomarker Codevelopment, *Journal of the National Cancer Institute*, 2009, 101, 21, 1453-1463

47. Drug-Diagnostic Co-Development Concept Paper Food and Drug Administration, 2005, http://www.fda.gov/downloads/drugs/scienceresearch/researchareas/pharmacogenetics/ucm116689.pdf, Accessed: 29.08.2014.

48. R. Simon, Clinical trial designs for evaluating the medical utility of prognostic and predictive biomarkers in oncology, *Per.Med.*, 2010, 7, 1, 33-47

49. R. Simon, Clinical trials for predictive medicine, *Stat Med*, 2012, 31, 25, 3031-3040

50. C. M. Micheel, Nass, S. J., Omenn, G. S., Evolution of Translational Omics: Lessons Learned and the Path Forward, Institute of Medicine of the National Academies, 2012, http://www.ncbi.nlm.nih.gov/pubmed/24872966, Accessed: 13.10.2015.

51. T. T. Loo, C. J. Ross, J. Sistonen, H. Visscher, P. Madadi, G. Koren, M. R. Hayden and B. C. Carleton, Pharmacogenomics and active surveillance for serious adverse drug reactions in children, *Pharmacogenomics*, 2010, 11, 9, 1269-1285

52. S. Matsui, Genomic biomarkers for personalized medicine: development and validation in clinical studies, *Comput Math Methods Med*, 2013, 2013, 865980

53. Revisions of Medical Device Directives, European Comission, 2016, http://ec.europa.eu/growth/sectors/medical-devices/regulatory-framework/revision/index_en.htm, Accessed: 06.05.2016.

54. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices, European Council, 1998, http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0079&from=EN, Accessed: 03.05.2016.

55. Central authorisation of medicines, European Medicines Agency, 2011, http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/general/general_content_000109.jsp&mid=WC0b01ac0580028a47, Accessed: 22.04.2013.

56. Trastuzumab: Summary of product characteristics, European Medicines Agency, 2012, http://annonc.oxfordjournals.org/content/12/suppl_1/S57.short, Accessed: 20.12.2012.

57. How we work, European Medicines Agency, 2012, http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/general/general_content_000125.jsp&murl=menus/about_us/about_us.jsp&mid=WC0b01ac0580028a46., Accessed: 22.02.2012.

58. In Vitro Companion Diagnostic Devices Guidance for Industry and Food and Drug Administration Staff, Food and Drug Administration, 2014, http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf, Accessed: 29.08.2014.

59. S. J. Mandrekar and D. J. Sargent, Predictive biomarker validation in practice: lessons from real trials, *Clin Trials*, 2010,

60. R. Simon, Use of Predictive Biomarker Classifiers in the Design of Pivotal Clinical Trials, *http://linus.nci.nih.gov/techreport/PredictiveBiomarkerPivotalClinicalTrials.pdf*, 2007,

61. B. Freidlin, L. M. McShane and E. L. Korn, Randomized clinical trials with biomarkers: design issues, *J Natl.Cancer Inst.*, 2010, 102, 3, 152-160

62. S. J. Wang, Biomarker as a classifier in pharmacogenomics clinical trials: a tribute to 30th anniversary of PSI, *Pharm.Stat.*, 2007, 6, 4, 283-296

63. F. Cappuzzo, T. Ciuleanu, L. Stelmakh, S. Cicenas, A. Szcz+¬sna, E. b. Juh+ísz, E. Esteban, O. Molinier, W. Brugger, I. Melez+¡nek, G. l. Klingelschmitt, B. Klughammer and G. Giaccone, Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study, *The Lancet Oncology*, 2010, 11, 6, 521-529

64. M. Antoniou, A. L. Jorgensen and R. Kolamunnage-Dona, Biomarker-Guided Adaptive Trial Designs in Phase II and Phase III: A Methodological Review, *PLoS One*, 2016, 11, 2, e0149803

65. B. Freidlin and R. Simon, Adaptive signature design: an adaptive clinical trial design for generating and prospectively testing a gene expression signature for sensitive patients, *Clin Cancer Res.*, 2005, 11, 21, 7872-7878

66. X. Sun, M. Briel, J. W. Busse, E. A. Akl, J. J. You, F. Mejza, M. Bala, N. Diaz-Granados, D. Bassler, D. Mertz, S. K. Srinathan, P. O. Vandvik, G. Malaga, M. Alshurafa, P. Dahm, P. Alonso-Coello, D. M. Heels-Ansdell, N. Bhatnagar, B. C. Johnston, L. Wang, S. D. Walter, D. G. Altman and G. H. Guyatt, Subgroup Analysis of Trials Is Rarely Easy (SATIRE): a study protocol for a systematic review to characterize the analysis, reporting, and claim of subgroup effects in randomized trials, *Trials*, 2009, 10, 101

67. R. Wang, S. W. Lagakos, J. H. Ware, D. J. Hunter and J. M. Drazen, Statistics in medicine--reporting of subgroup analyses in clinical trials, *N Engl J Med*, 2007, 357, 21, 2189-2194

68. S. T. Brookes, E. Whitely, M. Egger, G. D. Smith, P. A. Mulheran and T. J. Peters, Subgroup analyses in randomized trials: risks of subgroup-specific analyses; power and sample size for the interaction test, *J Clin Epidemiol*, 2004, 57, 3, 229-236

69. K. F. Schulz and D. A. Grimes, Multiplicity in randomised trials II: subgroup and interim analyses, *Lancet*, 2005, 365, 9471, 1657-1661

70. S. J. Pocock, S. E. Assmann, L. E. Enos and L. E. Kasten, Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems, *Stat Med*, 2002, 21, 19, 2917-2930

71. P. M. Rothwell, Treating individuals 2. Subgroup analysis in randomised controlled trials: importance, indications, and interpretation, *Lancet*, 2005, 365, 9454, 176-186

72. M. Markowitz, L. N. Slater, R. Schwartz, P. H. Kazanjian, B. Hathaway, D. Wheeler, M. Goldman, D. Neubacher, D. Mayers, H. Valdez and S. McCallister, Long-term efficacy and safety of tipranavir boosted with ritonavir in HIV-1-infected patients failing multiple protease inhibitor regimens: 80-week data from a phase 2 study, *J Acquir.Immune Defic.Syndr.*, 2007, 45, 4, 401-410

73. J. D. Baxter, J. M. Schapiro, C. A. Boucher, V. M. Kohlbrenner, D. B. Hall, J. R. Scherer and D. L. Mayers, Genotypic changes in human immunodeficiency virus type 1 protease associated with reduced susceptibility and virologic response to the protease inhibitor tipranavir, *J Virol*, 2006, 80, 21, 10794-10801

74. V. A. Johnson, V. Calvez, H. F. Gunthard, R. Paredes, D. Pillay, R. W. Shafer, A. M. Wensing and D. D. Richman, Update of the drug resistance mutations in HIV-1: March 2013, *Top Antivir Med*, 2013, 21, 1, 6-14

75. C. Bock and T. Lengauer, Managing drug resistance in cancer: lessons from HIV therapy, *Nat Rev Cancer*, 2012, 12, 7, 494-501

76. P. Selaru, Y. Tang, B. Huang, A. Polli, K. D. Wilner, E. Donnelly and D. P. Cohen, Sufficiency of Single-Arm Studies to Support Registration of Targeted Agents in Molecularly Selected Patients with Cancer: Lessons from the Clinical Development of Crizotinib, *Clinical and Translational Science*, 2016, 9, 2, 63-73

77. Phase II ERCC1 and RRM1-Based Adjuvant Therapy Trial in Patients With Stage I Non-Small Cell Lung Cancer (NSCLC) (NCT00792701), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00792701, Accessed: 15.08.2013.

78. P. Tajik, A. H. Zwinderman, B. W. Mol and P. M. Bossuyt, Trial designs for personalizing cancer care: a systematic review and classification, *Clin Cancer Res*, 2013, 19, 17, 4578-4588

79. S. Kummar, P. M. Williams, C. J. Lih, E. C. Polley, A. P. Chen, L. V. Rubinstein, Y. Zhao, R. M. Simon, B. A. Conley and J. H. Doroshow, Application of molecular profiling in clinical trials for advanced metastatic cancers, *J Natl Cancer Inst*, 2015, 107, 4,

80. F. Klauschen, M. Andreeff, U. Keilholz, M. Dietel and A. Stenzinger, The combinatorial complexity of cancer precision medicine, *Oncoscience*, 2014, 1, 7, 504-509

81. J. Menis, B. Hasan and B. Besse, New clinical research strategies in thoracic oncology: clinical trial design, adaptive, basket and umbrella trials, new end-points and new evaluations of response, *Eur Respir Rev*, 2014, 23, 133, 367-378

82. R. Kaplan, T. Maughan, A. Crook, D. Fisher, R. Wilson, L. Brown and M. Parmar, Evaluating many treatments and biomarkers in oncology: a new design, *J Clin Oncol*, 2013, 31, 36, 4562-4568

83. G. Middleton, L. R. Crack, S. Popat, C. Swanton, S. J. Hollingsworth, R. Buller, I. Walker, T. H. Carr, D. Wherton and L. J. Billingham, The National Lung Matrix Trial: translating the biology of stratification in advanced non-small-cell lung cancer, *Ann Oncol*, 2015, 26, 12, 2464-2469

84. A. J. Redig and P. A. Janne, Basket trials and the evolution of clinical trial design in an era of genomic medicine, *J Clin Oncol*, 2015, 33, 9, 975-977

85. L. Billingham, K. Malottki and N. Steven, Research methods to change clinical practice for patients with rare cancers, *Lancet Oncol*, 2016, 17, 2, e70-80

86. A Screening Study to Detect BRAF V600 Mutation-Positive Patients For Enrollment Into Clinical Research Studies of Zelboraf (Vemurafenib), ClinicalTrials.gov 2016, https://clinicaltrials.gov/ct2/show/study/NCT01804140?term=NCT01804140&rank=1, Accessed: 06.05.2016.

87. ICH Topic E 8. General Considerations for Clinical Trials., European Medicines Agency, 1998, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002877.pdf, Accessed: 14.04.2016.

88. The Drug Development Process., Food and Drug Administration, 2015, http://www.fda.gov/ForPatients/Approvals/Drugs/ucm405622.htm, Accessed: 14.04.2016.

89. Guideline on Clinical Trials in Small Populations, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003615.pdf, Accessed: 14.04.2016.

90. Conditional marketing authorisation, 2016, http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000925.jsp&mid=WC0b01ac05809f843b, Accessed: 14.04.2016.

91. N. A. Meadows, A. Morrison, D. A. Brindley, A. Schuh and R. W. Barker, An evaluation of regulatory and commercial barriers to stratified medicine development and adoption, *Pharmacogenomics J*, 2015, 15, 1, 6-12

92. J. F. Pritchard, M. Jurima-Romet, M. L. Reimer, E. Mortimer, B. Rolfe and M. N. Cayen, Making better drugs: Decision gates in non-clinical drug development, *Nat Rev Drug Discov*, 2003, 2, 7, 542-553

93. G. L. Kumar and S. Badve, Milestones in the Discovery of HER2 Proto-Oncogene and Trastuzumab (Herceptin™), *Connection*, 2008, 13, 9-14

94. L. Coussens, T. L. Yang-Feng, Y. C. Liao, E. Chen, A. Gray, J. McGrath, P. H. Seeburg, T. A. Libermann, J. Schlessinger, U. Francke and et al., Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene, *Science*, 1985, 230, 4730, 1132-1139

95. Y. Yarden and A. Ullrich, Growth factor receptor tyrosine kinases, *Annu Rev Biochem*, 1988, 57, 443-478

96. D. J. Slamon, G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich and W. L. Mcguire, Human-Breast Cancer - Correlation of Relapse and Survival with Amplification of the Her-2 Neu Oncogene, *Science*, 1987, 235, 4785, 177-182

97. M. Harries and I. Smith, The development and clinical use of trastuzumab (Herceptin), *Endocr Relat Cancer*, 2002, 9, 2, 75-85

98. D. J. Slamon, W. Godolphin, L. A. Jones, J. A. Holt, S. G. Wong, D. E. Keith, W. J. Levin, S. G. Stuart, J. Udove, A. Ullrich and M. F. Press, Studies of the Her-2/Neu Proto-Oncogene in Human-Breast and Ovarian-Cancer, *Science*, 1989, 244, 4905, 707-712

99. R. M. Hudziak, J. Schlessinger and A. Ullrich, Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells, *Proc Natl Acad Sci U S A*, 1987, 84, 20, 7159-7163

100. P. Carter, L. Presta, C. M. Gorman, J. Ridgway, D. Henner, W. Wong, A. M. Rowland, C. Kotts, M. E. Carver and H. M. Shepard, Humanization of an anti-p185HER2 antibody for human cancer therapy, *Proceedings of the National Academy of Sciences*, 1992, 89, 10, 4285-4289

101. J. Baselga, Phase I and II clinical trials of trastuzumab, *Annals of Oncology*, 2001, 12, suppl 1, S49-S55

102. Trastuzumab: Assessment Report European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000278/WC500126896.pdf, Accessed: 20.12.2012.

103. J. Baselga, Clinical trials of Herceptin® (trastuzumab), *European Journal of Cancer*, 2001, 37, Supplement 1, 18-24

104. J. Baselga, D. Tripathy, J. Mendelsohn, S. Baughman, C. C. Benz, L. Dantis, N. T. Sklarin, A. D. Seidman, C. A. Hudis, J. Moore, P. P. Rosen, T. Twaddell, I. C. Henderson and L. Norton, Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer, *J Clin Oncol*, 1996, 14, 3, 737-744

105. M. A. Cobleigh, C. L. Vogel, D. Tripathy, N. J. Robert, S. Scholl, L. Fehrenbacher, J. M. Wolter, V. Paton, S. Shak, G. Lieberman and D. J. Slamon, Multinational Study of the Efficacy and Safety of Humanized Anti-HER2 Monoclonal Antibody in Women Who Have HER2-Overexpressing Metastatic Breast Cancer That Has Progressed After Chemotherapy for Metastatic Disease, *Journal of Clinical Oncology*, 1999, 17, 9, 2639-2639

106. M. D. Pegram, A. Lipton, D. F. Hayes, B. L. Weber, J. M. Baselga, D. Tripathy, D. Baly, S. A. Baughman, T. Twaddell, J. A. Glaspy and D. J. Slamon, Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment, *J Clin Oncol*, 1998, 16, 8, 2659-2671

107. Genetech, Herceptin® (Trastuzumab) Development Timeline, 2016, http://www.gene.com/media/product-information/herceptin-development-timeline, Accessed: 15.04.2016.

108. DAKO Herceptest approval letter, Food and Drug Administration, 1998, http://www.accessdata.fda.gov/cdrh_docs/pdf/P980018.pdf, Accessed: 20.05.2016.

109. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga and L. Norton, Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2, *New England Journal of Medicine*, 2001, 344, 11, 783-792

110. Herceptin : EPAR - Procedural steps taken before authorisation, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Procedural_steps_taken_before_authorisation/human/000278/WC500049817.pdf, Accessed: 24.05.2016.

111. D. F. Hayes, J. Allen, C. Compton, G. Gustavsen, D. G. B. Leonard, R. McCormack, L. Newcomer, K. Pothier, D. Ransohoff, R. L. Schilsky, E. Sigal, S. E. Taube and S. R. Tunis, Breaking a vicious cycle, *Science Translational Medicine*, 2013, 5, 196,

112. M. R. Trusheim, B. Burgess, S. X. Hu, T. Long, S. D. Averbuch, A. A. Flynn, A. Lieftucht, A. Mazumder, J. Milloy, P. M. Shaw, D. Swank, J. Wang, E. R. Berndt, F. Goodsaid and M. C. Palmer, Quantifying factors for the success of stratified medicine, *Nature Reviews Drug Discovery*, 2011, 10, 11, 817-833

113. R. H. Perlis, Translating biomarkers to clinical practice, *Mol Psychiatry*, 2011, 16, 11, 1076-1087

114. L. Jennings, V. M. Van Deerlin, M. L. Gulley and C. College of American Pathologists Molecular Pathology Resource, Recommended principles and practices for validating clinical molecular pathology tests, *Arch Pathol Lab Med*, 2009, 133, 5, 743-755

115. J. Cummings, T. H. Ward, A. Greystoke, M. Ranson and C. Dive, Biomarker method validation in anticancer drug development, *British Journal of Pharmacology*, 2008, 153, 4, 646-656

116. J. E. Dancey, K. K. Dobbin, S. Groshen, J. M. Jessup, A. H. Hruszkewycz, M. Koehler, R. Parchment, M. J. Ratain, L. K. Shankar, W. M. Stadler, L. D. True, A. Gravell and M. R. Grever, Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents, *Clinical Cancer Research*, 2010, 16, 6, 1745-1755

117. P. G. Febbo, M. Ladanyi, K. D. Aldape, A. M. De Marzo, M. E. Hammond, D. F. Hayes, A. J. Iafrate, R. K. Kelley, G. Marcucci, S. Ogino, W. Pao, D. C. Sgroi and M. L. Birkeland, NCCN Task Force report: Evaluating the clinical utility of tumor markers in oncology, *J Natl Compr Canc Netw*, 2011, 9 Suppl 5, S1-32; quiz S33

118. V. B. Kraus, B. Burnett, J. Coindreau, S. Cottrell, D. Eyre, M. Gendreau, J. Gardiner, P. Garnero, J. Hardin, Y. Henrotin, D. Heinegard, A. Ko, L. S. Lohmander, G. Matthews, J. Menetski, R. Moskowitz, S. Persiani, A. R. Poole, J. C. Rousseau, M. Todman and O. F. O. B. W. Group, Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis, *Osteoarthritis Cartilage*, 2011, 19, 5, 515-542

119. J. W. Lee, R. S. Weiner, J. M. Sailstad, R. R. Bowsher, D. W. Knuth, P. J. O'Brien, J. L. Fourcroy, R. Dixit, L. Pandite, R. G. Pietrusko, H. D. Soares, V. Quarmby, O. L. Vesterqvist, D. M. Potter, J. L. Witliff, H. A. Fritche, T. O'Leary, L. Perlee, S. Kadam and J. A. Wagner, Method validation and measurement of biomarkers in nonclinical and clinical samples in drug development: a conference report, *Pharmaceutical Research*, 2005, 22, 4, 499-511

120. J. A. Wagner, S. A. Williams and C. J. Webster, Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs, *Clinical Pharmacology & Therapeutics*, 2007, 81, 1, 104-107

121. C. Ecker, W. Spooren and D. Murphy, Developing new pharmacotherapies for autism, *Journal of Internal Medicine*, 2013, 274, 4, 308-320

122. N. Goossens, S. Nakagawa, X. Sun and Y. Hoshida, Cancer biomarker discovery and validation, *Transl Cancer Res*, 2015, 4, 3, 256-269

123. A. R. Horvath, E. Kis and E. Dobos, Guidelines for the use of biomarkers: principles, processes and practical considerations, *Scand J Clin Lab Invest Suppl*, 2010, 242, 109-116

124. H. Kaur, S. Grover and R. Kukreti, Concept of pharmacogenomics and future considerations, *CNS Neurosci Ther*, 2013, 19, 10, 842-844

125. D. Love, E. Stratton and M. Stocum, Best practices for companion diagnostic and therapeutic development: translating between the stakeholders, *New Biotechnology*, 2012, 29, 6, 689-694

126. N. Majkic-Singh, What Is a Biomarker? From Its Discovery to Clinical Application, *Journal of Medical Biochemistry*, 2011, 30, 3, 186-192

127. H. Mischak, J. P. Ioannidis, A. Argiles, T. K. Attwood, E. Bongcam-Rudloff, M. Broenstrup, A. Charonis, G. P. Chrousos, C. Delles, A. Dominiczak, T. Dylag, J. Ehrich, J. Egido, P. Findeisen, J. Jankowski, R. W. Johnson, B. A. Julien, T. Lankisch, H. Y. Leung, D. Maahs, F. Magni, M. P. Manns, E. Manolis, G. Mayer, G. Navis, J. Novak, A. Ortiz, F.

Persson, K. Peter, H. H. Riese, P. Rossing, N. Sattar, G. Spasovski, V. Thongboonkerd, R. Vanholder, J. P. Schanstra and A. Vlahou, Implementation of proteomic biomarkers: making it work, *Eur J Clin Invest*, 2012, 42, 9, 1027-1036

128. V. M. Garcia, P. A. Cassier and J. de Bono, Parallel anticancer drug development and molecular stratification to qualify predictive biomarkers: dealing with obstacles hindering progress, *Cancer Discov*, 2011, 1, 3, 207-212

129. D. R. Hodgson, R. D. Whittaker, A. Herath, D. Amakye and G. Clack, Biomarkers in oncology drug development, *Mol Oncol*, 2009, 3, 1, 24-32

130. D. Lin, Z. Hollander, A. Meredith and B. M. McManus, Searching for 'omic' biomarkers, *Can J Cardiol*, 2009, 25 Suppl A, 9A-14A

131. S. H. Cho, J. Jeon and S. I. Kim, Personalized medicine in breast cancer: a systematic review, *J Breast Cancer*, 2012, 15, 3, 265-272

132. G. S. Ginsburg and S. B. Haga, Translating genomic biomarkers into clinically useful diagnostics, *Expert Rev Mol Diagn*, 2006, 6, 2, 179-191

133. J. P. Ioannidis, A roadmap for successful applications of clinical proteomics, *Proteomics Clin Appl*, 2011, 5, 5-6, 241-247

134. K. Matheis, D. Laurie, C. Andriamandroso, N. Arber, L. Badimon, X. Benain, K. Bendjama, I. Clavier, P. Colman, H. Firat, J. Goepfert, S. Hall, T. Joos, S. Kraus, A. Kretschmer, M. Merz, T. Padro, H. Planatscher, A. Rossi, N. Schneiderhan-Marra, I. Schuppe-Koistinen, P. Thomann, J. M. Vidal and B. Molac, A generic operational strategy to qualify translational safety biomarkers, *Drug Discov Today*, 2011, 16, 13-14, 600-608

135. J. G. Lijmer, M. Leeflang and P. M. M. Bossuyt, Proposals for a Phased Evaluation of Medical Tests, In: *Medical Tests-White Paper Series* (Ed: 2009, Rockville (MD)

136. H. Newell, The Cancer Research UK Biomarker Roadmap Initiative, 2009,

137. K. M. Kerr, M.-S. Tsao, A. G. Nicholson, Y. Yatabe, I. I. Wistuba, F. R. Hirsch and o. b. o. t. I. P. Committee., PD-L1 immunohistochemistry in lung cancer: in what state is this art?, *Journal of Thoracic Oncology*, 9000, Publish Ahead of Print,

138. C. A. Altar, D. Amakye, D. Bounos, J. Bloom, G. Clack, R. Dean, V. Devanarayan, D. Fu, S. Furlong, L. Hinman, C. Girman, C. Lathia, L. Lesko, S. Madani, J. Mayne, J. Meyer, D. Raunig, P. Sager, S. A. Williams, P. Wong and K. Zerba, A prototypical process for creating evidentiary standards for biomarkers and diagnostics, *Clin Pharmacol Ther*, 2008, 83, 2, 368-371

139. A. D. Hingorani, D. A. Windt, R. D. Riley, K. Abrams, K. G. Moons, E. W. Steyerberg, S. Schroter, W. Sauerbrei, D. G. Altman, H. Hemingway and P. Group, Prognosis research strategy (PROGRESS) 4: stratified medicine research, *BMJ*, 2013, 346, e5793

140. V. C. Jordan and A. M. Brodie, Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer, *Steroids*, 2007, 72, 1, 7-25

141. G. Poste, Bring on the biomarkers, *Nature*, 2011, 469, 7329, 156-157

142. M. V. Holmes, T. Shah, C. Vickery, L. Smeeth, A. D. Hingorani and J. P. Casas, Fulfilling the promise of personalized medicine? Systematic review and field synopsis of pharmacogenetic studies, *PLoS One*, 2009, 4, 12, e7960

143. The human genome at ten, *Nature*, 2010, 464, 7289, 649-650

144. Stratified Medicine Programme, Cancer Research UK, 2011, http://www.cancerresearchuk.org/science/research/how-we-deliver-our-research/others/by-programme/stratified-medicine-programme/, Accessed: 22.04.2013.

145. Orphan Designation, European Medicines Agency, 2013, www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000029.jsp&murl=menus/regulations/regulations.jsp&mid=WC0b01ac05800240ce, Accessed: 22.04.2013.

146. European public assessment reports, European Medicines Agency, 2013, www.ema.europa.eu/ema/index.jsp?curl=/pages/medicines/landing/epar_search.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d124, Accessed: 17.01.2013.

147. Pending EC decisions, European Medicines Agency, 2013, www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/smop_search.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d127, Accessed: 17.01.2013.

148. A Giudeline on Summary of Prduct Characteristics, European Medicines Agency, 2005, http://ec.europa.eu/health/files/eudralex/vol-2/c/spcguidrev1-oct2005_en.pdf, Accessed: 05.05.2012.

149. WHO, ICD-10 Version:2010, 2010, http://apps.who.int/classifications/icd10/browse/2010/en, 'Accessed:' 12.02.2013.

150. Glivec (imatinib) EPAR website, European Medicines Agency, 2015, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000406/human_med_000808.jsp&mid=WC0b01ac058001d124, Accessed: 17.07.2015.

151. J. J. Carlson and J. A. Roth, The impact of the Oncotype Dx breast cancer assay in clinical practice: a systematic review and meta-analysis, *Breast Cancer Res Treat*, 2013, 141, 1, 13-22

152. J. E. Bibault, I. Fumagalli, C. Ferte, C. Chargari, J. C. Soria and E. Deutsch, Personalized radiation therapy and biomarker-driven treatment strategies: a systematic review, *Cancer Metastasis Rev*, 2013, 32, 3-4, 479-492

153. J. Barretina, G. Caponigro, N. Stransky, K. Venkatesan, A. A. Margolin, S. Kim, C. J. Wilson, J. Lehar, G. V. Kryukov, D. Sonkin, A. Reddy, M. Liu, L. Murray, M. F. Berger, J. E. Monahan, P. Morais, J. Meltzer, A. Korejwa, J. Jane-Valbuena, F. A. Mapa, J. Thibault, E. Bric-Furlong, P. Raman, A. Shipway, I. H. Engels, J. Cheng, G. K. Yu, J. J. Yu, P. Aspesi, M. de Silva, K. Jagtap, M. D. Jones, L. Wang, C. Hatton, E. Palescandolo, S. Gupta, S. Mahan, C. Sougnez, R. C. Onofrio, T. Liefeld, L. MacConaill, W. Winckler, M. Reich, N. X. Li, J. P. Mesirov, S. B. Gabriel, G. Getz, K. Ardlie, V. Chan, V. E. Myer, B. L. Weber, J. Porter, M. Warmuth, P. Finan, J. L. Harris, M. Meyerson, T. R. Golub, M. P. Morrissey, W. R. Sellers, R. Schlegel and L. A. Garraway, The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity (vol 483, pg 603, 2012), *Nature*, 2012, 492, 7428, 290-290

154. M. J. Garnett, E. J. Edelman, S. J. Heidorn, C. D. Greenman, A. Dastur, K. W. Lau, P. Greninger, I. R. Thompson, X. Luo, J. Soares, Q. S. Liu, F. Iorio, D. Surdez, L. Chen, R. J. Milano, G. R. Bignell, A. T. Tam, H. Davies, J. A. Stevenson, S. Barthorpe, S. R. Lutz, F. Kogera, K. Lawrence, A. McLaren-Douglas, X. Mitropoulos, T. Mironenko, H. Thi, L. Richardson, W. J. Zhou, F. Jewitt, T. H. Zhang, P. O'Brien, J. L. Boisvert, S. Price, W. Hur, W. J. Yang, X. M. Deng, A. Butler, H. G. Choi, J. Chang, J. Baselga, I. Stamenkovic, J. A. Engelman, S. V. Sharma, O. Delattre, J. Saez-Rodriguez, N. S. Gray, J. Settleman, P. A. Futreal, D. A. Haber, M. R. Stratton, S. Ramaswamy, U. McDermott and C. H. Benes, Systematic identification of genomic markers of drug sensitivity in cancer cells, *Nature*, 2012, 483, 7391, 570-U587

155. H. Janes, M. S. Pepe, P. M. Bossuyt and W. E. Barlow, Measuring the Performance of Markers for Guiding Treatment Decisions, *Annals of Internal Medicine*, 2011, 154, 4, 253-W280

156. J. Barwise and J. Etchemendy, Conditionals, In: *Language, Proof and Logic* (Ed: 2000, Seven Bridges Press, New York, London

157. Centralised Procedure, European Comission, 2006, http://ec.europa.eu/health/files/eudralex/vol-2/a/chap4rev200604_en.pdf, Accessed: 06.03.2014.

158. Guidance document for the content of the <Co->Rapporteur day 80 critical assessment report, European Medicines Agency, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004856.pdf, Accessed: 03.06.2014.

159. Toremifene: Scientific Discussion, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000091/WC500020684.pdf, Accessed: 09.05.2012.

160. Alipogene tiparvovec: Assessment Report, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002145/WC500135476.pdf, Accessed: 25.04.2013.

161. E. Barnett-Page and J. Thomas, Methods for the synthesis of qualitative research: a critical review, *BMC Med Res Methodol*, 2009, 9, 59

162. C. Carroll, A. Booth and K. Cooper, A worked example of "best fit" framework synthesis: a systematic review of views concerning the taking of some potential chemopreventive agents, *BMC Med Res Methodol*, 2011, 11, 29

163. M. N. Marshall, Sampling for qualitative research, *Fam Pract*, 1996, 13, 6, 522-525

164. Gemtuzumab ozogamicin: Refusal Assessment Report, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000705/WC500070677.pdf, Accessed: 09.03.2012.

165. Ivacaftor: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002494/WC500130766.pdf, Accessed: 23.04.2013.

166. Lapatinib: Assessment Report, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000795/WC500044960.pdf, Accessed: 24.05.2013.

167. Erbitux: Scientific Discussion, European Medicines Agency, 2004, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000558/WC500029113.pdf, Accessed: 26.03.2012.

168. N. Thatcher, A. Chang, P. Parikh, P. J. Rodrigues, T. Ciuleanu, P. J. von, S. Thongprasert, E. H. Tan, K. Pemberton, V. Archer and K. Carroll, Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer), *Lancet*, 2005, 366, 9496, 1527-1537

169. Erbitux: Assessment Report, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000558/WC500029117.pdf, Accessed: 26.03.2012.

170. Arsenic Trioxide: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000388/WC500042839.pdf, Accessed: 14.05.2013.

171. Crizotinib: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002489/WC500134759.pdf, Accessed: 03.02.2014.

172. J. M. Molina, C. Cohen, C. Katlama, B. Grinsztejn, A. Timerman, R. J. Pedro, T. Vangeneugden, D. Miralles, S. D. Meyer, W. Parys and E. Lefebvre, Safety and efficacy of darunavir (TMC114) with low-dose ritonavir in treatment-experienced patients: 24-week results of POWER 3, *J Acquir.Immune Defic.Syndr.*, 2007, 46, 1, 24-31

173. Emtricitabine: Scientific Discussion, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000533/WC500055657.pdf, Accessed: 20.08.2012.

174. Capecitabine: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000316/WC500058145.pdf, Accessed: 18.04.2012.

175. Crizotinib: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002489/WC500134761.pdf, Accessed: 27.03.2013.

176. Vemurafenib: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002409/WC500124400.pdf, Accessed: 09.05.2012.

177. Maraviroc: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000811/WC500022194.pdf, Accessed: 07.03.2012.

178. Tegafur/gimeracil/oteracil: Assessment report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001242/WC500104417.pdf, Accessed: 18.04.2012.

179. Erlotinib: Assessment Report, European Medicines Agency, 2010, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000618/WC500090680.pdf, Accessed: 18.05.2012.

180. Erlotinib: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000618/WC500117593.pdf, Accessed: 18.05.2012.

181. Gefitinib: Assessment Report, European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001016/WC500036361.pdf, Accessed: 08.10.2012.

182. Catumaxomab: Assessment Report, European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000972/WC500051808.pdf, Accessed: 02.05.2012.

183. Fulvestrant: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000540/WC500021171.pdf, Accessed: 09.05.2012.

184. Imatinib: Scientific Discussion (HES/CEL), European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion_-_Variation/human/000406/WC500022211.pdf, Accessed: 11.05.2012.

185. Victrelis: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002332/WC500109789.pdf, Accessed: 04.05.2012.

186. Telaprevir: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002313/WC500115532.pdf, Accessed: 21.03.2012.

187. Degarelix: Scientific Discussion, European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000986/WC500023256.pdf, Accessed: 29.04.2013.

188. Everolimus: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/001038/WC500130788.pdf, Accessed: 28.05.2013.

189. Pertuzumab: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002547/WC500141004.pdf, Accessed: 28.05.2013.

190. Trastuzumab (gastric cancer): Scientific discussion, European Medicines Agency, 2010, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000278/WC500074921.pdf, Accessed: 19.12.2012.

191. Imatinib: Scientific Discussion (GIST), European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000406/WC500022212.pdf, Accessed: 18.07.2012.

192. Panitumumab: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000741/WC500047707.pdf, Accessed: 22.02.2013.

193. Methylthioninium chloride: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002108/WC500107131.pdf, Accessed: 09.05.2012.

194. Imatinib for MDS/MPD: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion_-_Variation/human/000406/WC500022210.pdf, Accessed: 08.03.2012.

195. Dasatinib: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000709/WC500056995.pdf, Accessed: 30.05.2013.

196. Imatinib: Scientific Discussion (ALL), European Medicines Agency, 2007, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000406/human_med_000808.jsp&mid=WC0b01ac058001d124, Accessed: 29.05.2013.

197. Amprenavir: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000264/WC500022925.pdf, Accessed: 20.06.2012.

198. Atazanavir: Scientific Discussion, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000494/WC500056384.pdf, Accessed: 11.01.2013.

199. Darunavir: Scientific Discussion, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000707/WC500041754.pdf, Accessed: 08.03.2012.

200. Atripla: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000797/WC500028105.pdf, Accessed: 23.07.2012.

201. Enfuvirtide: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000514/WC500024739.pdf, Accessed: 21.09.2012.

202. Eviplera: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002312/WC500118803.pdf, Accessed: 31.07.2012.

203. J. C. J. Gathe, P. Ive, R. Wood, D. Sch++rmann, N. C. Bellos, E. DeJesus, A. Gladysz, C. Garris and J. Yeo, SOLO: 48-week efficacy and safety comparison of once-daily fosamprenavir /ritonavir versus twice-daily nelfinavir in naive HIV-1-infected patients, *AIDS*, 2004, 18, 11,

204. Lopinavir/ ritonavir: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion_-_Variation/human/000368/WC500039047.pdf, Accessed: 16.01.2013.

205. Nelfinavir: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000164/WC500050625.pdf, Accessed: 16.05.2013.

206. Rilpivirine: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002264/WC500118872.pdf, Accessed: 20.08.2012.

207. Tenofovir: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000419/WC500051732.pdf, Accessed: 23.05.2013.

208. Tipranavir: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000631/WC500025932.pdf, Accessed: 18.07.2012.

209. Erbitux: Assessment Report, European Medicines Agency, 2010, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000558/WC500075683.pdf, Accessed: 26.03.2012.

210. Zoledronic acid: Withdrawal Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2011/03/WC500102910.pdf, Accessed: 28.05.2013.

211. M. Gnant, B. Mlineritsch, H. Stoeger, G. Luschin-Ebengreuth, D. Heck, C. Menzel, R. Jakesz, M. Seifert, M. Hubalek, G. Pristauz, T. Bauernhofer, H. Eidtmann, W. Eiermann, G. Steger, W. Kwasny, P. Dubsky, G. Hochreiner, E. P. Forsthuber, C. Fesl and R. Greil, Adjuvant endocrine therapy plus zoledronic acid in premenopausal women with early-stage breast cancer: 62-month follow-up from the ABCSG-12 randomised trial, *The Lancet Oncology*, 2011, 12, 7, 631-641

212. D. Gaudet, J. de Wal, K. Tremblay, S. Dery, S. van Deventer, A. Freidig, D. Brisson and J. Methot, Review of the clinical development of alipogene tiparvovec gene therapy for lipoprotein lipase deficiency, *Atheroscler Suppl*, 2010, 11, 1, 55-60

213. D. Gaudet, J. Methot and J. Kastelein, Gene therapy for lipoprotein lipase deficiency, *Curr Opin Lipidol*, 2012, 23, 4, 310-320

214. Degarelix: Summary of Product Characteristics, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000986/WC500023252.pdf, Accessed: 29.04.2013.

215. G. A. Bowen, Document analysis as a qualitative research method, *Qualitative research journal*, 2009, 9, 2, 27-40

216. L. Prior, Basic Themes: Use, Production and Content, In: *Using Documents in Social Research* (Ed: L. Prior), 2003, SAGE Publications, London, Thousand Oaks, New Delhi

217. Kivexa. Procedural steps taken and scientific information after the authorisation, European Medicines Agency, 2011, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000581/human_med_000878.jsp&mid=WC0b01ac058001d124, Accessed: 30.04.2012.

218. Trizivir. Procedural steps taken and scientific information after the authorisation, European Medicines Agency, 2011, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000338/human_med_001109.jsp&mid=WC0b01ac058001d124, Accessed: 30.04.2012.

219. Ziagen. Procedural steps taken and scientific information after the authorisation. , European Medicines Agency, 2011, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000252/human_med_001179.jsp&mid=WC0b01ac058001d124, Accessed: 30.04.2012.

220. REporting recommendations for tumor MARKer prognostic studies (REMARK), *Nat Clin Prac Oncol*, 2005, 2, 8, 416-422

221. Critical Appraisal Skills Programme RCT checklist, CASP, 2013, http://media.wix.com/ugd/dded87_40b9ff0bf53840478331915a8ed8b2fb.pdf, Accessed: 08.06.2016.

222. Critical Appraisal Skills Programme RCT cohort study checklist, CASP, 2013, http://media.wix.com/ugd/dded87_e37a4ab637fe46a0869f9f977dacf134.pdf, Accessed: 08.06.2016.

223. K. Stanley, Design of Randomized Controlled Trials, *Circulation*, 2007, 115, 9, 1164-1169

224. H. J. Sterne JAC, Reeves BC A Cochrane Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT- NRSI), Version 1.0.0, 24 S, 2014, http://www.riskofbias.info, Accessed: 08.06.2016.

225. S. J. Mandrekar and D. J. Sargent, Genomic advances and their impact on clinical trial design, *Genome Med.*, 2009, 1, 7, 69

226. G. H. Guyatt, A. D. Oxman, R. Kunz, D. Atkins, J. Brozek, G. Vist, P. Alderson, P. Glasziou, Y. Falck-Ytter and H. J. Schunemann, GRADE guidelines: 2. Framing the question and deciding on important outcomes, *J Clin Epidemiol.*, 2011, 64, 4, 395-400

227. J. P. Ioannidis, Why most published research findings are false, *PLoS Med*, 2005, 2, 8, e124

228. J. Baselga, M. Campone, M. Piccart, H. A. Burris, H. S. Rugo, T. Sahmoud, S. Noguchi, M. Gnant, K. I. Pritchard, F. Lebrun, J. T. Beck, Y. Ito, D. Yardley, I. Deleu, A. Perez, T. Bachelot, L. Vittori, Z. Xu, P. Mukhopadhyay, D. Lebwohl and G. N. Hortobagyi, Everolimus in Postmenopausal Hormone-ReceptorΓÇôPositive Advanced Breast Cancer, *New England Journal of Medicine*, 2011, 366, 6, 520-529

229. J. Baselga, J. Cort+¬s, S. B. Kim, S. A. Im, R. Hegg, Y. H. Im, L. Roman, J. L. Pedrini, T. Pienkowski, A. Knott, E. Clark, M. C. Benyunes, G. Ross and S. M. Swain, Pertuzumab plus Trastuzumab plus Docetaxel for Metastatic Breast Cancer, *New England Journal of Medicine*, 2011, 366, 2, 109-119

230. J. M. Molina, P. Cahn, B. Grinsztejn, A. Lazzarin, A. Mills, M. Saag, K. Supparatpinyo, S. Walmsley, H. Crauwels, L. T. Rimsky, S. Vanveggel and K. Boven, Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial, *The Lancet*, 1916, 378, 9787, 238-246

231. D. Cameron, M. Casey, C. Oliva, B. Newstat, B. Imwalle and C. E. Geyer, Lapatinib Plus Capecitabine in Women with HER-2 Positive Advanced Breast Cancer: Final Survival Analysis of a Phase III Randomized Trial, *The Oncologist*, 2010, 15, 9, 924-934

232. E. Van Cutsem, M. Peeters, S. Siena, Y. Humblet, A. Hendlisz, B. Neyns, J. L. Canon, J. L. Van Laethem, J. Maurel, G. Richardson, M. Wolf and R. G. Amado, Open-Label Phase III Trial of Panitumumab Plus Best Supportive Care Compared With Best Supportive Care Alone in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer, *Journal of Clinical Oncology*, 2007, 25, 13, 1658-1664

233. N. A. T. A. Project, Nelfinavir: preliminary efficacy and safety data update 9/3/96, *http://www.natap.org/1998/drug/nelf2.html*, 1996,

234. N. A. Margot, J. Enejosa, A. K. Cheng, M. D. Miller, D. J. McColl and a. t. Study, Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared With Lamivudine/Zidovudine and Efavirenz in Study GS-01-934, *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 2009, 52, 2,

235. C. A. Benson, C. van der Horst, A. LaMarca, D. W. Haas, C. K. McDonald, C. R. Steinhart, J. Rublein, J. B. Quinn, E. Mondou, F. Rousseau and a. t. FTC-, A randomized study of emtricitabine and lamivudine in stably suppressed patients with HIV, *AIDS*, 2004, 18, 17,

236. J. P. Lalezari, K. Henry, M. O'Hearn, J. S. G. Montaner, P. J. Piliero, B. i. Trottier, S. Walmsley, C. Cohen, D. R. Kuritzkes, J. J. Eron, J. Chung, R. DeMasi, L. Donatacci, C. Drobnes, J. Delehanty and M. Salgo, Enfuvirtide, an HIV-1 Fusion Inhibitor, for Drug-Resistant HIV Infection in North and South America, *New England Journal of Medicine*, 2003, 348, 22, 2175-2185

237. C. S. Karapetis, S. Khambata-Ford, D. J. Jonker, C. J. O'Callaghan, D. Tu, N. C. Tebbutt, R. J. Simes, H. Chalchal, J. D. Shapiro, S. Robitaille, T. J. Price, L. Shepherd, H. J. Au, C. Langer, M. J. Moore and J. R. Zalcberg, K-ras mutations and benefit from cetuximab in advanced colorectal cancer, *N Engl.J Med*, 2008, 359, 17, 1757-1765

238. D. J. Jonker, C. J. O'Callaghan, C. S. Karapetis, J. R. Zalcberg, D. Tu, H. J. Au, S. R. Berry, M. Krahn, T. Price, R. J. Simes, N. C. Tebbutt, H. G. van, R. Wierzbicki, C. Langer and M. J. Moore, Cetuximab for the treatment of colorectal cancer, *N Engl J Med*, 2007, 357, 20, 2040-2048

239. A. F. Sobrero, J. Maurel, L. Fehrenbacher, W. Scheithauer, Y. A. Abubakr, M. P. Lutz, M. E. Vega-Villegas, C. Eng, E. U. Steinhauer, J. Prausova, H. J. Lenz, C. Borg, G. Middleton, H. Kroning, G. Luppi, O. Kisker, A. Zubel, C. Langer, J. Kopit and H. A. Burris, III, EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer, *J Clin Oncol*, 2008, 26, 14, 2311-2319

240. N. Siegfried, Personal correspondence 2015, 'Accessed:'

241. S. J. Mandrekar and D. J. Sargent, Clinical trial designs for predictive biomarker validation: one size does not fit all, *J Biopharm Stat.*, 2009, 19, 3, 530-542

242. J. P. Ioannidis, Biomarker failures, *Clin Chem*, 2013, 59, 1, 202-204

243. B. Shea, J. Grimshaw, G. Wells, M. Boers, N. Andersson, C. Hamel, A. Porter, P. Tugwell, D. Moher and L. Bouter, Development of AMSTAR: a measurement tool to assess the methodological quality of systematic reviews, *BMC Medical Research Methodology*, 2007, 7, 1, 10

244. A. M. Wensing, D. A. van de Vijver, G. Angarano, B. Asjo, C. Balotta, E. Boeri, R. Camacho, M. L. Chaix, D. Costagliola, A. De Luca, I. Derdelinckx, Z. Grossman, O. Hamouda, A. Hatzakis, R. Hemmer, A. Hoepelman, A. Horban, K. Korn, C. Kucherer, T.

Leitner, C. Loveday, E. MacRae, I. Maljkovic, C. de Mendoza, L. Meyer, C. Nielsen, E. L. Op de Coul, V. Ormaasen, D. Paraskevis, L. Perrin, E. Puchhammer-Stockl, L. Ruiz, M. Salminen, J. C. Schmit, F. Schneider, R. Schuurman, V. Soriano, G. Stanczak, M. Stanojevic, A. M. Vandamme, K. Van Laethem, M. Violin, K. Wilbe, S. Yerly, M. Zazzi, C. A. Boucher and S. Programme, Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management, *J Infect Dis*, 2005, 192, 6, 958-966

245. M. R. Jakobsen, M. Tolstrup, O. S. Sogaard, L. B. Jorgensen, P. R. Gorry, A. Laursen and L. Ostergaard, Transmission of HIV-1 drug-resistant variants: prevalence and effect on treatment outcome, *Clin Infect Dis*, 2010, 50, 4, 566-573

246. P. A. Janne, X. Wang, M. A. Socinski, J. Crawford, T. E. Stinchcombe, L. Gu, M. Capelletti, M. J. Edelman, M. A. Villalona-Calero, R. Kratzke, E. E. Vokes and V. A. Miller, Randomized Phase II Trial of Erlotinib Alone or With Carboplatin and Paclitaxel in Patients Who Were Never or Light Former Smokers With Advanced Lung Adenocarcinoma: CALGB 30406 Trial, *Journal of Clinical Oncology*, 2012, 30, 17, 2063-2069

247. Atazanavir sulphate: SUmmary of Product Characteristics, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000494/WC500056380.pdf, Accessed: 11.01.2013.

248. C. Roche-Lestienne, S. Lepers, V. Soenen-Cornu, J. E. Kahn, J. L. Lai, E. Hachulla, F. Drupt, A. L. Demarty, A. S. Roumier, M. Gardembas, M. Dib, N. Philippe, N. Cambier, S. Barete, C. Libersa, O. Bletry, P. Y. Hatron, B. Quesnel, C. Rose, K. Maloum, O. Blanchet, P. Fenaux, L. Prin and C. Preudhomme, Molecular characterization of the idiopathic hypereosinophilic syndrome (HES) in 35 French patients with normal conventional cytogenetics, *Leukemia*, 2005, 19, 5, 792-798

249. S. Khambata-Ford, C. T. Harbison, L. L. Hart, M. Awad, L. A. Xu, C. E. Horak, S. Dakhil, R. C. Hermann, T. J. Lynch and M. R. Weber, Analysis of Potential Predictive Markers of Cetuximab Benefit in BMS099, a Phase III Study of Cetuximab and First-Line Taxane/Carboplatin in Advanced NonΓÇôSmall-Cell Lung Cancer, *Journal of Clinical Oncology*, 2010, 28, 6, 918-927

250. R. Rosell, E. Carcereny, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, R. Palmero, R. Garcia-Gomez, C. Pallares, J. M. Sanchez, R. Porta, M. Cobo, P. Garrido, F. Longo, T. Moran, A. Insa, F. De Marinis, R. Corre, I. Bover, A. Illiano, E. Dansin, J. de Castro, M. Milella, N. Reguart, G. Altavilla, U. Jimenez, M. Provencio, M. A. Moreno, J. Terrasa, J. Mu+¦oz-Langa, J. Valdivia, D. Isla, M. Domine, O. Molinier, J. Mazieres, N. Baize, R. Garcia-Campelo, G. Robinet, D. Rodriguez-Abreu, G. Lopez-Vivanco, V. Gebbia, L. Ferrera-Delgado, P. Bombaron, R. Bernabe, A. Bearz, A. Artal, E. Cortesi, C. Rolfo, M. Sanchez-Ronco, A. Drozdowskyj, C. Queralt, I. de Aguirre, J. L. Ramirez, J. J. Sanchez, M. A. Molina, M. Taron and L. Paz-Ares, Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial, *The Lancet Oncology*, 2012, 13, 3, 239-246

251. J. E. Gallant, S. Staszewski, A. L. Pozniak, E. Dejesus, J. M. Suleiman, M. D. Miller, D. F. Coakley, B. Lu, J. J. Toole and A. K. Cheng, Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial, *JAMA*, 2004, 292, 2, 191-201

252. C. Bokemeyer, I. Bondarenko, J. T. Hartmann, B. F. de, G. Schuch, A. Zubel, I. Celik, M. Schlichting and P. Koralewski, Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study, *Ann Oncol*, 2011, 22, 7, 1535-1546

253. M. Maguire, D. Shortino, A. Klein, W. Harris, V. Manohitharajah, M. Tisdale, R. Elston, J. Yeo, S. Randall, F. Xu, H. Parker, J. May and W. Snowden, Emergence of resistance to protease inhibitor amprenavir in human immunodeficiency virus type 1-infected patients: selection of four alternative viral protease genotypes and influence of viral susceptibility to coadministered reverse transcriptase nucleoside inhibitors, *Antimicrob.Agents Chemother.*, 2002, 46, 3, 731-738

254. D. F. Hayes, J. A. Van Zyl, A. Hacking, L. Goedhals, W. R. Bezwoda, J. A. Mailliard, S. E. Jones, C. L. Vogel, R. F. Berris and I. Shemano, Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer, *Journal of Clinical Oncology*, 1995, 13, 10, 2556-2566

255. Study 0004, AstraZeneca, 2005, http://www.astrazenecaclinicaltrials.com/_mshost800325/content/clinical-trials/resources/pdf/9256854, Accessed: 24.08.2012.

256. Lapatinib: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000795/WC500044957.pdf, Accessed: 24.05.2013.

257. S. J. Mandrekar and D. J. Sargent, Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges, *J Clin Oncol*, 2009, 27, 24, 4027-4034

258. A. Marusyk, V. Almendro and K. Polyak, Intra-tumour heterogeneity: a looking glass for cancer?, *Nat Rev Cancer*, 2012, 12, 5, 323-334

259. M. Antoniou, A. Jorgensen and R. Kolamunnage-Dona, Evaluating statistical characteristics of biomarker-guided trial designs, *Trials*, 2015, 16, Suppl 2, O83

260. M. Antoniou, Personal communication, 2015, 'Accessed:'

261. R. J. Lilford, A. Richardson, A. Stevens, R. Fitzpatrick, S. Edwards, F. Rock and J. L. Hutton, Issues in methodological research: perspectives from researchers and commissioners, *Health Technol Assess*, 2001, 5, 8, 1-57

262. A. Booth, Cochrane or cock-eyed? how should we conduct systematic reviews of qualitative research?, *Qualitative Evidence-based Practice Conference, Taking a Critical Stance. Coventry University, May 14-16 2001*, 2001,

263. C. Zhou, Y. L. Wu, G. Chen, J. Feng, X. Q. Liu, C. Wang, S. Zhang, J. Wang, S. Zhou, S. Ren, S. Lu, L. Zhang, C. Hu, C. Hu, Y. Luo, L. Chen, M. Ye, J. Huang, X. Zhi, Y. Zhang, Q. Xiu, J. Ma, L. Zhang and C. You, Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study, *The Lancet Oncology*, 2011, 12, 8, 735-742

264. R. A. Hubner, R. D. Riley, L. J. Billingham and S. Popat, Excision repair cross-complementation group 1 (ERCC1) status and lung cancer outcomes: a meta-analysis of published studies and recommendations, *PLoS One*, 2011, 6, 10, e25164

265. K. A. Olaussen, A. Dunant, P. Fouret, E. Brambilla, F. Andre, V. Haddad, E. Taranchon, M. Filipits, R. Pirker, H. H. Popper, R. Stahel, L. Sabatier, J. P. Pignon, T. Tursz, T. Le Chevalier, J. C. Soria and I. B. Investigators, DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy, *N Engl J Med*, 2006, 355, 10, 983-991

266. D. Allingham-Hawkins, A. Lea and S. Levine, ERCC1 Expression Analysis to Guide Therapy in Non-Small Cell Lung Cancer, *PLoS Curr*, 2010, 2, RRN1202

267. Cancer Registration Statistics, England, 2011, Office For National Statistics, 2013, http://www.ons.gov.uk/ons/dcp171778_315795.pdf, Accessed: 06.05.2014.

268. CG121: The diagnosis and treatment of lung cancer, National Institute for Health and Care Excellence, 2011, http://www.nice.org.uk/nicemedia/live/13465/54199/54199.pdf, Accessed: 06.05.2014.

269. J. Vansteenkiste, D. De Ruysscher, W. E. Eberhardt, E. Lim, S. Senan, E. Felip, S. Peters and E. G. W. Group, Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, *Ann Oncol*, 2013, 24 Suppl 6, vi89-98

270. S. Peters, A. A. Adjei, C. Gridelli, M. Reck, K. Kerr, E. Felip and E. G. W. Group, Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, *Ann Oncol*, 2012, 23 Suppl 7, vii56-64

271. L. Friboulet, K. A. Olaussen, J. P. Pignon, F. A. Shepherd, M. S. Tsao, S. Graziano, R. Kratzke, J. Y. Douillard, L. Seymour, R. Pirker, M. Filipits, F. Andre, E. Solary, F. Ponsonnailles, A. Robin, A. Stoclin, N. Dorvault, F. Commo, J. Adam, E. Vanhecke, P. Saulnier, J. Thomale, T. Le Chevalier, A. Dunant, V. Rousseau, G. Le Teuff, E. Brambilla and J. C. Soria, ERCC1 Isoform Expression and DNA Repair in Non-Small-Cell Lung Cancer, *New England Journal of Medicine*, 2013, 368, 12, 1101-1110

272. C. A. Rabik and M. E. Dolan, Molecular mechanisms of resistance and toxicity associated with platinating agents, *Cancer Treatment Reviews*, 2007, 33, 1, 9-23

273. Z. H. Siddik, Cisplatin: mode of cytotoxic action and molecular basis of resistance, *Oncogene*, 2003, 22, 47, 7265-7279

274. R. Arriagada, B. Bergman, A. Dunant, T. Le Chevalier, J. P. Pignon, J. Vansteenkiste and G. International Adjuvant Lung Cancer Trial Collaborative, Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer, *N Engl J Med*, 2004, 350, 4, 351-360

275. B. Besse, K. A. Olaussen and J. C. Soria, ERCC1 and RRM1: ready for prime time?, *J Clin Oncol*, 2013, 31, 8, 1050-1060

276. C. R. Taylor and R. M. Levenson, Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II, *Histopathology*, 2006, 49, 4, 411-424

277. Clinical Study of Microdosing Carboplatin in Lung or Bladder Cancer (NCT01261299), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01261299, Accessed: 12.04.2013.

278. Treatment of Locally Advanced Non-Small Cell Lung Cancer (NSCLC) (NCT01059552), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01059552, Accessed: 12.04.2013.

279. Integration of Neutron Therapy in the Treatment Plan for Stage III Non-Small Cell Lung Cancer (NSCLC) (NCT01416961), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01416961, Accessed: 12.04.2013.

280. Veliparib With or Without Radiation Therapy, Carboplatin, and Paclitaxel in Patients With Stage III Non-Small Cell Lung Cancer That Cannot Be Removed by Surgery (NCT01386385), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01386385, Accessed: 12.04.2013.

281. A multi-center phase II randomized study of CustOmized Neoadjuvant ThErapy vs Standard chemoTherapy in non-small cell lung cancer (NSCLC) patients with resectable stage IIIA (N2) disease (CONTEST trial) (EUCTR2011-005267-24-IT), WHO, 2013, http://apps.who.int/trialsearch/Trial.aspx?TrialID=EUCTR2011-005267-24-IT, Accessed: 12.04.2013.

282. TAilored Post-Surgical Therapy in Early Stage NSCLC (TASTE) (NCT00775385), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00775385, Accessed: 12.04.2013.

283. Pharmacogenomic Study for Providing Personalized Strategy to the Treatment of Non-small Cell Lung Cancer (NSCLC) IIIB/IV (NCT01003964), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01003964, Accessed: 12.04.2013.

284. Pemetrexed Plus Cisplatin Versus Gemcitabine Plus Cisplatin for Advanced and Metastatic Non-small Cell Lung Cancer (AP/GP) (NCT01194453), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01194453, Accessed: 12.04.2013.

285. Customizing First Line Chemotherapy in Advanced Non-Small Cell Lung Cancer (NCT01356368), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01356368, Accessed: 12.04.2013.

286. Pemetrexed Disodium and Cisplatin in Treating Patients Undergoing Surgery For Stage I-III Non-Small Cell Lung Cancer (NCT01731626), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01731626, Accessed: 12.04.2013.

287. Individualized Therapy Based of Tumoral mRNA Levels of ERCC1, RRM1 and BRCA1 in Advanced Non-Small-Cell Lung Cancer (NCT00705549), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00705549, Accessed: 12.04.2013.

288. Molecular Profiling in Lung Cancer Patients (NCT00191308), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00191308, Accessed: 12.04.2013.

289. Adjuvant Cisplatin and Docetaxel in Non Small Cell Lung Cancer (NCT00582634), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00582634, Accessed: 12.04.2013.

290. Personalized Therapy in Non-small Cell Lung Cancer (PTINCLC) (NCT01781988), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01781988, Accessed: 12.04.2013.

291. Genotype-Driven Treatment of Advanced Non-small Cell Lung Cancer Based on mRNA Expression of ERCC1 & RRM1 as First-line Chemotherapy (NCT01648517), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01648517, Accessed: 12.04.2013.

292. First-Line Combination Chemotherapy in Treating Patients With Stage IIIB or Stage IV Non-Small Cell Lung Cancer That Cannot Be Removed by Surgery (NCT00736814), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00736814, Accessed: 12.04.2013.

293. Paclitaxel Albumin-Stabilized Nanoparticle Formulation and Carboplatin in Treating Patients With Stage IIIB, Stage IV, or Recurrent Non-Small Cell Lung Cancer (NCT00729612), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00729612, Accessed: 12.04.2013.

294. The 'MADe IT' Clinical Trial: Molecular Analyses Directed Individualized Therapy for Advanced Non-Small Cell Lung Cancer (NCT00215930), 2013, http://ClinicalTrials.gov/show/NCT00215930, Accessed: 12.04.2013.

295. A multicentre, randomised, phase III trial of platinum-based chemotherapy versus non-platinum chemotherapy, after ERCC1 stratification, in patients with advanced/metastatic non-small cell lung cancer - ET (EUCTR2007-007639-17-GB), WHO, 2013, http://apps.who.int/trialsearch/Trial.aspx?TrialID=EUCTR2007-007639-17-GB, Accessed: 12.04.2013.

296. Phase III Multicenter Randomized Trial Comparing Adjuvant Pharmacogenomic-Driven Chemotherapy versus Standard Adjuvant Chemotherapy in Completely Resected Stage II-IIIA Non-Small Cell Lung Cancer. - International TAilored Chemotherapy Adjuvant (EUCTR2008-001764-36-IT), WHO, 2013, http://apps.who.int/trialsearch/Trial.aspx?TrialID=EUCTR2008-001764-36-IT, Accessed: 12.04.2013.

297. Cisplatin and Docetaxel With or Without Radiation Therapy in Treating Patients Who Are Undergoing Surgery for Newly Diagnosed Stage III Non-Small Cell Lung Cancer (NCT00113386), ClinicalTrials.gov, 2013, http://www.controlled-trials.com/mrct/trial/389021, Accessed: 12.04.2013.

298. GILT Docetaxel - Non-Small Cell Lung Cancer (NCT00174629), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00174629, Accessed: 12.04.2013.

299. Randomized Phase III Multicenter Trial of RRM1 & ERCC1 Directed Customized Chemotherapy versus Standard of Care for 1st Line Treatment of Patients with Advanced Non-Small-Cell Lung Cancer - MADeIT (EUCTR2008-000617-30-DE), WHO, 2013, http://apps.who.int/trialsearch/Trial.aspx?TrialID=EUCTR2008-000617-30-DE, Accessed: 12.04.2013.

300. Randomized Phase IV Trial of Molecular Analysis-Directed Individualized Therapy in Advanced Non-Small-Cell Lung Cancer (ChiCTR-TRC-11001327), WHO, 2013, http://apps.who.int/trialsearch/Trial.aspx?TrialID=ChiCTR-TRC-11001327, Accessed: 12.04.2013.

301. Biomarkers in Predicting Response in Patients With Early-Stage Non-Small Cell Lung Cancer Previously Treated With Adjuvant Chemotherapy (NCT01294280), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01294280,

302. Study of Blood and Tissue Samples From Patients With Locally Advanced, Metastatic, or Recurrent Non-Small Cell Lung Cancer Treated With Bevacizumab, Carboplatin, and Paclitaxel (NCT00900172), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00900172, Accessed: 12.04.2013.

303. DNA Repair Genes and Outcomes in Patients With Stage III NSCLC (NCT00797238), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00797238, Accessed: 12.04.2013.

304. Pharmacogenomic Study Realized on "Non-small Cell Lung Carcinoma" (Pharmacogenos) (NCT00222404), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00222404, Accessed: 12.04.2013.

305. Non Small Cell Lung Cancer Evaluation According to Pre-determined Drug-targeting Tumor Markers (NCT01141686), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01141686, Accessed: 12.04.2013.

306. Collaborative Advanced Stage Tissue Lung Cancer (CASTLE) Network (NCT01574300), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT01574300, Accessed: 12.04.2013.

307. Longitudinal Study of Multiple Symptoms in Advanced Lung Cancer (NCT00422500), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00422500, Accessed: 12.04.2013.

308. Pharmacogenomic Study in Patients of Lung, Colorectal and Head/Neck Cancers Receiving Chemotherapy (NCT00442520), ClinicalTrials.gov, 2013, http://ClinicalTrials.gov/show/NCT00442520, Accessed: 12.04.2013.

309. A. Vilmar, J. Garcia-Foncillas, M. Huarriz, E. Santoni-Rugiu and J. B. Sorensen, RT-PCR versus immunohistochemistry for correlation and quantification of ERCC1, BRCA1, TUBB3 and RRM1 in NSCLC, *Lung Cancer*, 2012, 75, 3, 306-312

310. L. Friboulet, D. Barrios-Gonzales, F. Commo, K. A. Olaussen, S. Vagner, J. Adam, A. Goubar, N. Dorvault, V. Lazar, B. Job, B. Besse, P. Validire, P. Girard, L. Lacroix, J. Hasmats, F. Dufour, F. Andre and J. C. Soria, Molecular Characteristics of ERCC1-

Negative versus ERCC1-Positive Tumors in Resected NSCLC, *Clinical Cancer Research*, 2011, 17, 17, 5562-5572

311. In Situ Molecular Pathology and Co-expression Analyses, 2013, Academic Press, London, UK, San Diego, USA

312. R. A. Walker, Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment I, *Histopathology*, 2006, 49, 4, 406-410

313. S. A. Bustin, Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays, *J Mol Endocrinol*, 2000, 25, 2, 169-193

314. A. Radonic, S. Thulke, I. M. Mackay, O. Landt, W. Siegert and A. Nitsche, Guideline to reference gene selection for quantitative real-time PCR, *Biochem Biophys Res Commun*, 2004, 313, 4, 856-862

315. K. Dheda, J. F. Huggett, S. A. Bustin, M. A. Johnson, G. Rook and A. Zumla, Validation of housekeeping genes for normalizing RNA expression in real-time PCR, *Biotechniques*, 2004, 37, 1, 112-114, 116, 118-119

316. M. Kubista, J. M. Andrade, M. Bengtsson, A. Forootan, J. Jonak, K. Lind, R. Sindelka, R. Sjoback, B. Sjogreen, L. Strombom, A. Stahlberg and N. Zoric, The real-time polymerase chain reaction, *Mol Aspects Med*, 2006, 27, 2-3, 95-125

317. L. Taillade, F. Penault-Llorca, T. Boulet, P. Fouret, S. Michiels, E. Taranchon, G. Mountzios, P. Validire, J. Domont, P. Girard, D. Grunenwald, T. Le Chevalier and J. C. Soria, Immunohistochemichal expression of biomarkers: a comparative study between diagnostic bronchial biopsies and surgical specimens of non-small-cell lung cancer, *Ann Oncol*, 2007, 18, 6, 1043-1050

318. C. Gomez-Roca, C. M. Raynaud, F. Penault-Llorca, O. Mercier, F. Commo, L. Morat, L. Sabatier, P. Dartevelle, E. Taranchon, B. Besse, P. Validire, A. Italiano and J. C. Soria, Differential expression of biomarkers in primary non-small cell lung cancer and metastatic sites, *J Thorac Oncol*, 2009, 4, 10, 1212-1220

319. M. Lai-Goldman and H. Faruki, Abacavir hypersensitivity: a model system for pharmacogenetic test adoption, *Genet Med*, 2008, 10, 12, 874-878

320. L. Hutchinson, Tumour response, correlates of survival and clinical benefit, *Nat Rev Clin Oncol*, 2015, 12, 8, 433

321. R. Pazdur, Response rates, survival, and chemotherapy trials, *J Natl Cancer Inst*, 2000, 92, 19, 1552-1553

322. H. Imai, K. Mori, K. Wakuda, A. Ono, H. Akamatsu, T. Shukuya, T. Taira, H. Kenmotsu, T. Naito, K. Kaira, H. Murakami, M. Endo, T. Nakajima, N. Yamamoto and T. Takahashi, Progression-free survival, post-progression survival, and tumor response as surrogate markers for overall survival in patients with extensive small cell lung cancer, *Annals of Thoracic Medicine*, 2015, 10, 1, 61-66

323. M. Buyse, P. Thirion, R. W. Carlson, T. Burzykowski, G. Molenberghs, P. Piedbois and M.-A. G. Canc, Relation between tumour response to first-line chemotherapy and survival in advanced colorectal cancer: a meta-analysis, *Lancet*, 2000, 356, 9227, 373-378

324. S. J. Cohen, C. J. A. Punt, N. Iannotti, B. H. Saidman, K. D. Sabbath, N. Y. Gabrail, J. Picus, M. Morse, E. Mitchell, M. C. Miller, G. V. Doyle, H. Tissing, L. W. M. M. Terstappen and N. J. Meropol, Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer, *Journal of Clinical Oncology*, 2008, 26, 19, 3213-3221

325. J. P. Ioannidis and M. J. Khoury, Are randomized trials obsolete or more important than ever in the genomic era?, *Genome Med*, 2013, 5, 4, 32

326. M. Jenkins, A. Flynn, T. Smart, C. Harbron, T. Sabin, J. Ratnayake, P. Delmar, A. Herath, P. Jarvis, J. Matcham and P. S. I. B. S. I. Group, A statistician's perspective on biomarkers in drug development, *Pharm Stat*, 2011, 10, 6, 494-507

327. S. Matsui, R. Simon, P. Qu, J. D. Shaughnessy, Jr., B. Barlogie and J. Crowley, Developing and validating continuous genomic signatures in randomized clinical trials for predictive medicine, *Clin Cancer Res*, 2012, 18, 21, 6065-6073

328. R. J. Lilford, J. G. Thornton and D. Braunholtz, Clinical trials and rare diseases: a way out of a conundrum, *BMJ*, 1995, 311, 7020, 1621-1625

329. S. B. Tan, K. B. Dear, P. Bruzzi and D. Machin, Strategy for randomised clinical trials in rare cancers, *BMJ*, 2003, 327, 7405, 47-49

330. L. Billingham, K. Malottki and N. Steven, Small sample sizes in clinical trials: a statistician's perspective, *Clinical Investigation*, 2012, 2, 7, 655-657

331. M. S. Lawrence, P. Stojanov, P. Polak, G. V. Kryukov, K. Cibulskis, A. Sivachenko, S. L. Carter, C. Stewart, C. H. Mermel, S. A. Roberts, A. Kiezun, P. S. Hammerman, A. McKenna, Y. Drier, L. Zou, A. H. Ramos, T. J. Pugh, N. Stransky, E. Helman, J. Kim, C. Sougnez, L. Ambrogio, E. Nickerson, E. Shefler, M. L. Cortes, D. Auclair, G. Saksena, D. Voet, M. Noble, D. DiCara, P. Lin, L. Lichtenstein, D. I. Heiman, T. Fennell, M. Imielinski, B. Hernandez, E. Hodis, S. Baca, A. M. Dulak, J. Lohr, D. A. Landau, C. J. Wu, J. Melendez-Zajgla, A. Hidalgo-Miranda, A. Koren, S. A. McCarroll, J. Mora, R. S. Lee, B. Crompton, R. Onofrio, M. Parkin, W. Winckler, K. Ardlie, S. B. Gabriel, C. W. Roberts, J. A. Biegel, K. Stegmaier, A. J. Bass, L. A. Garraway, M. Meyerson, T. R. Golub, D. A. Gordenin, S. Sunyaev, E. S. Lander and G. Getz, Mutational heterogeneity in cancer and the search for new cancer-associated genes, *Nature*, 2013, 499, 7457, 214-218

332. C. Sawyers, Targeted cancer therapy, *Nature*, 2004, 432, 7015, 294-297

333. S. Kapur, A. G. Phillips and T. R. Insel, Why has it taken so long for biological psychiatry to develop clinical tests and what to do about it?, *Molecular Psychiatry*, 2012, 17, 12, 1174-1179

334. G. Schumann, E. B. Binder, A. Holte, E. R. de Kloet, K. J. Oedegaard, T. W. Robbins, T. R. Walker-Tilley, I. Bitter, V. J. Brown, J. Buitelaar, R. Ciccocioppo, R. Cools, C. Escera, W. Fleischhacker, H. Flor, C. D. Frith, A. Heinz, E. Johnsen, C. Kirschbaum, T. Klingberg,

K. P. Lesch, S. Lewis, W. Maier, K. Mann, J. L. Martinot, A. Meyer-Lindenberg, C. P. Muller, W. E. Muller, D. J. Nutt, A. Persico, G. Perugi, M. Pessiglione, U. W. Preuss, J. P. Roiser, P. M. Rossini, J. K. Rybakowski, C. Sandi, K. E. Stephan, J. Undurraga, E. Vieta, N. van der Wee, T. Wykes, J. M. Haro and H. U. Wittchen, Stratified medicine for mental disorders, *Eur Neuropsychopharmacol*, 2014, 24, 1, 5-50

335. D. V. Iosifescu, S. Greenwald, P. Devlin, D. Mischoulon, J. W. Denninger, J. E. Alpert and M. Fava, Frontal EEG predictors of treatment outcome in major depressive disorder, *Eur Neuropsychopharmacol*, 2009, 19, 11, 772-777

336. M. Arns, G. Bruder, U. Hegerl, C. Spooner, D. M. Palmer, A. Etkin, K. Fallahpour, J. M. Gatt, L. Hirshberg and E. Gordon, EEG alpha asymmetry as a gender-specific predictor of outcome to acute treatment with different antidepressant medications in the randomized iSPOT-D study, *Clinical Neurophysiology*, 2016, 127, 1, 509-519

337. C. Holohan, S. Van Schaeybroeck, D. B. Longley and P. G. Johnston, Cancer drug resistance: an evolving paradigm, *Nat Rev Cancer*, 2013, 13, 10, 714-726

338. P. L. Bedard, A. R. Hansen, M. J. Ratain and L. L. Siu, Tumour heterogeneity in the clinic, *Nature*, 2013, 501, 7467, 355-364

339. J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J. J. Grob, C. L. Cowey, C. D. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P. F. Ferrucci, A. Hill, J. Wagstaff, M. S. Carlino, J. B. Haanen, M. Maio, I. Marquez-Rodas, G. A. McArthur, P. A. Ascierto, G. V. Long, M. K. Callahan, M. A. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L. M. Rollin, C. Horak, F. S. Hodi and J. D. Wolchok, Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma, *New England Journal of Medicine*, 2015, 373, 1, 23-34

340. B. Andrew, Cochrane or cock-eyed? how should we conduct systematic reviews of qualitative research?, 2001, 'Accessed:'

341. A. C. Wolff, M. E. Hammond, D. G. Hicks, M. Dowsett, L. M. McShane, K. H. Allison, D. C. Allred, J. M. Bartlett, M. Bilous, P. Fitzgibbons, W. Hanna, R. B. Jenkins, P. B. Mangu, S. Paik, E. A. Perez, M. F. Press, P. A. Spears, G. H. Vance, G. Viale, D. F. Hayes, O. American Society of Clinical and P. College of American, Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update, *Arch Pathol Lab Med*, 2014, 138, 2, 241-256

342. H. Nitta, B. D. Kelly, C. Allred, S. Jewell, P. Banks, E. Dennis and T. M. Grogan, The assessment of HER2 status in breast cancer: the past, the present, and the future, *Pathology International*, 2016, 66, 6, 313-324

343. A. Jaka, A. Gutierrez-Rivera, N. Ormaechea, J. Blanco, A. La Casta, C. Sarasqueta, A. Izeta and A. Tuneu, Association between EGFR gene polymorphisms, skin rash and response to anti-EGFR therapy in metastatic colorectal cancer patients, *Experimental Dermatology*, 2014, 23, 10, 751-753

344. M. Westwood, M. Joore, P. Whiting, T. van Asselt, B. Ramaekers, N. Armstrong, K. Misso, J. Severens and J. Kleijnen, Epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutation testing in adults with locally advanced or metastatic non-small cell lung

cancer: a systematic review and cost-effectiveness analysis, *Health Technol Assess*, 2014, 18, 32, 1-166

345. K. H. Carver, Companion diagnostics: evolving FDA regulation and issues for resolution. In Vitro Diagnostics: The Complete Regulatory Guide., Washington DC: Food and Drug Law Institute, 2010, https://www.cov.com/~/media/files/corporate/publications/2010/01/companion-diagnostics---evolving-fda-regulation-and-issues-for-resolution.pdf, Accessed: 21.06.2016.

APPENDICES

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APPENDIX 1. ABBREVIATIONS AND ACRONYMS

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aCML	atypical chronic myelogenous (or myeloid or myelocytic) leukemia
ADME	absorption, distribution, metabolism and excretion
AIDS	acquired immune deiciency syndrome
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukaemia
ART	anti retroviral therapy
AUC	area under the curve
BC	breast cancer
B-I-D	Biomarker-Indication-Drug
BM	biomarker
BSC	best supportive care
CAT	Committee for Advanced Therapies
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CHMP	Committee for Human Medicinal Products
CI	confidence interval
CML	chronic myelogenous (or myeloid or myelocytic) leukemia
CMML	chronic myelomonocytic leukaemia
CNS	central nervous system
CR	complete response
DAVG4	time weighted mean change in plasma HIV-1 RNA (log10 copies/ml) from baseline to week 4
DAVG24	time weighted mean change in plasma HIV-1 RNA (log10 copies/ml) from baseline to week 24
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
ECOG PS	Eastern Cooperative Oncology Group performance status
EGFR	epidermal growth factor receptor
EMA	European Medicines Agency
EPAR	European Public Assessment Report

ERCC1	Excision Repair Cross-Complementation Group 1
FDA	Food and Drug Administration
FISH	fluorescence in situ hybridization
FFPE	formalin fixed paraffin embedded
FOLFOX-4	chemotherapy regimen comprising folinic acid (leucovorin), fluorouracil, and oxaliplatin
GWAS	genome wide association study
HAART	highly active antiretroviral therapy
HER2	human epidermal growth factor receptor 2
HIV	human immunodeficiency virus
HLA-B	the human leukocyte antigen, class I B
HMG	high mobility group
HR	hazard ratio
IHC	immunohistochemistry
IOM	Institute of Medicine
iv.	inravenous
KRAS	Kirsten rat sarcoma viral oncogene homolog
mg	miligrams
mRNA	messenger RNA
N	number
NA	not available
N/A	not applicable
NER	nucleotide excision repair
NNRTI	non-nucleoside reverse-transcriptase inhibitors
NPV	negative predictive value (of a test)
NRTI	nucleoside reverse-transcriptase inhibitors
NR	not reported
NSCLC	non-small cell lung cancer

OD	once daily
OS	overall survival
PCR	polymerase chain reaction
PD	progressive disease
PDGFR	Platelet-derived growth factor receptor
PDGFRα	Platelet-derived growth factor receptor alpha
PE	paraffin embedded
PFS	progression free survival
PI	protease inhibitor
PPV	positive predictive value (of a test)
PR	partial response
RCT	randomised controlled trial
RNA	ribonucleic acid
ROC	receiver operator characteristic
RT-PCR	reverse transcriptase polymerase chain reaction
RTqPCR	reverse transcriptase quantitative polymerase chain reaction
s.c.	subcutaneous
SD	standard deviation (for continuous data)
SD	stable disease (in context of assessment of tumour response)
SE	standard error
TTP	time to progression
WHO	World Health Organisation

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APPENDIX 2. GLOSSARY

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Adaptive trial design - includes an opportunity for modification of specified aspects of the "design and hypotheses based on analysis of data (usually interim data) from subjects in the study"[1]

Basket trials - investigate a single drug in a range of diseases; often utilised as discovery tools

Biomarker – accorfing to US National Institute of Health workshop: *"a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."*[2]

Biomarker strategy design - patients are included in the trial irrespective of their biomarker status, patients are randomised to receive biomarker-determined therapy or therapy independent of the biomarker status; outcomes in both arms are compared

Case report – detailed report on individual patient(s) management, here defined as including five patients or less

Case series - small observational study without a clear design, here defined as including more than five patients

Diagnostic biomarker - "used in people with signs or symptoms to aid assessing whether they have a condition."[3]

Efficacy biomarker – biomarker predictive of treatment efficacy

Enrichment (or targeted) design - only biomarker positive patients are included in the trial – they are randomised to a different treatment and the effect is assessed in biomarker positive patients only.

Factual statement – usually referred to as a positive statement, however this term will not be used to avoid confusion; describes a fact without providing any value judgement

ICD10 classification-

Main study (EMA) – studies labelled as "main" within the EMA documentation generally form the major part of the evidence supporting a drug indication; these are often accompanied by studies labelled as "supportive"

Necessary condition – if A is a necessary condition for B to happen, if the condition A is not met, B will not happen ($\sim A \rightarrow \sim B$)

Non-RCT biomarker study - any non-randomised study where the biomarker evaluation is part of the study design

Normative statement – provides a value judgement

Orphan drug – possessing a designation granted by the EMA to drugs intended for the treatment of a life-threatening or chronically debilitating condition which is either affecting no more than 5 in 10,000 people in the EU or when the revenue is unlikely to cover the investment in drug development.[4]

Predictive biomarker – used: (1) for prediction of patient outcome (either in terms of efficacy or safety), and (2) in the context of a particular treatment.

Prognostic biomarker - "associated with a differential outcome regardless of the therapy given, even if choice of therapy is available".[5]

Prospective-retrospective design – using specimens and dara from a completed RCT; a protocol should be developed to test the biomarker hypothesis prior to any analysis of archived specimens

RCT subgroup analysis (cross-sectional) - patients are included in the trial irrespective of their biomarker status, after conclusion of the trial a subgroup of patients (either from one treatment arm only or from multiple treatment arms) is identified based on outcome (for example lack of response to treatment) and the biomarker (or a panel of biomarkers) is assessed in this group – the final information provided is the prevalence of the biomarker in the subgroup of patients

RCT subgroup analysis (prospective) - patients are included in the trial irrespective of their biomarker status, a subgroup analysis based on the biomarker is planned in the study protocol and the biomarker is measured in the beginning of the trial

RCT subgroup analysis (retrospective) - patients are included in the trial irrespective of their biomarker status, after the conclusion of the trial a biomarker subgroup of interest is identified, the biomarker status is tested (using, for example, archival tumour samples) and a subgroup analysis is performed

Safety biomarker – in this thesis: biomarker predictive of treatment safety

Scientific Discussion (EMA) – document discussing the properties and clinical evidence supporting a drug in detail; published on EMA website

Single-arm biomarker strategy study – equivalent to the biomarker-strategy arm of the biomarker-strategy RCT

Single-arm study including only biomarker positive patients – equivalent to one arm of the enrichment design

Single arm study including patients irrespective of biomarker status - all patients are included irrespective of biomarker status and all receive the experimental treatment; the

biomarker is measured in all included patients to identify a subgroup responding to the treatment

Stratified design - patients are included in the trial irrespective of their biomarker status and randomisation is stratified by the biomarker status; the analysis plan involves the use of the biomarker information

Stratified medicine – approach where "a patient can be found to be similar to a cohort that has historically exhibited a differential therapeutic response using a biomarker that has been correlated to that differential response."[6]

Sufficient condition – if A is a sufficient condition for B to happen, if the condition A is met, B will happen ($A \rightarrow B$)

Summary of Product Characteristics (EMA) – document that sets out the position of the drug obtained in the assessment process and summarises its properties and clinical use together with the clinical trial evidence that was considered by the EMA;[7] published on EMA website

Supportive study (EMA) – studies labelled as "supportive" within the EMA documentation often accompany main studies supporting a drug indication and may be less robust, still ongoing without mature data, or reporting on the effectiveness of the drug in a slightly different population

Umbrella trials - investigate multiple drugs associated with multiple predictive biomarkers; patients are assigned to the appropriate treatment based on their biomarker status

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APPENDIX 3. CASE STUDY OF HER2 EXPRESSION TO PREDICT RESPONSE TO TRASTUZUMAB IN BREAST CANCER

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3.1. HER2 DISCOVERY

In the early 1980s information started to emerge on the involvement in cancer of a family of proteins called erbB epidermal growth factor receptor tyrosine kinases. The first member of this family to be discovered was the EGFR.[8] It was established that apart from stimulating cell growth and proliferation, activation of EGFRs in tumour cells may help them to evade the immune system, which normally would destroy the cancerous cells.[9]

In vitro and *in vivo* experiments showed that DNA from certain neuroblastoma and glioblastoma cell lines was capable of inducing cancer in non-cancerous tissues.[10] By the mid-1980s these cell lines were discovered to contain a new member of the erbB protein family, which was called "p185", reflecting its relative molecular mass of 185,000. Because of its similarity to EGFR, p185 was thought to also perform the role of a growth factor receptor. The hypothetical gene coding for this protein was named "*neu*". It was speculated that the gene has a normal counterpart (proto-oncogene), which, on mutation may become oncogenic.[11]

Further research, published in 1985, led to the identification of the DNA sequence coding p185. The protein was found in a range of non-cancerous human tissues. The ligand for p185 was unknown. However, based on structural similarity, this protein was considered to be closely related to the human EGFR and was thus called "human EGF receptor 2", or HER2 for short.[12,13]

A year after the publication of the HER2/*neu* sequence, another paper demonstrated that a particular point mutation is sufficient to turn the proto-oncogenic HER2 into its oncogenic version.[14]

3.2. CLINICAL SIGNIFICANCE OF HER2

The first large-scale study exploring the clinical significance of HER2 was published in 1987. It analysed tumour tissue samples from 189 women with breast cancer. HER2 amplification (increase in the number of gene copies) was evaluated in 103 primary breast tumours using Southern blotting. The gene was amplified in 19 (18%) of the cases and it appeared to be correlated with lymph node involvement. The number of HER2 gene copies varied from two to over 20. To investigate HER2 further, 86 breast tumours from patients with node-positive disease were used. These were considered more likely to include cases of HER2 amplification. In this group of patients the HER2 gene was amplified in 34 (40%) patients. The correlation of HER2 amplification with the number of nodes involved appeared even stronger. When data on survival and time to relapse for the 86 node

positive patients was considered, it was found that HER2 amplification appeared to be a very good prognostic factor – second only to the number of nodes involved. Importantly, the vast majority of HER2 genes evaluated for amplification appeared normal – mutation was detected in three cases only.[15]

It was thus proposed that HER2 amplification (potentially leading to an increase in the protein product) results in a more aggressive breast cancer, at least in node-positive patients.[15] Subsequent attempts at replication of these findings in further small studies by other teams led to mixed results.[8]

A large retrospective study, including 526 patients with breast cancer was then undertaken by scientists involved in the original prognostic study. In a multivariate analysis it found that in 345 node-positive patients HER2 amplification (evaluated using Southern blotting) was an independent predictor of both relapse and overall survival. Again, the only superior predictive factor was the number of involved nodes. For the remaining 181 patients with node-negative disease HER2 amplification was not a significant prognostic factor.[16]

To confirm the biological rationale for this association, further investigation was undertaken into the relationship between HER2 gene amplification and expression at the level of RNA (evaluated using Northern blotting) and protein (evaluated using both immunohistochemistry (IHC) and Western blotting). Sufficient samples were available from 187 patients to evaluate them using all four laboratory techniques. This was done without knowledge of the results of the other assays. A strong correlation between the results obtained using all four laboratory techniques was found. However, in18 cases there was clear HER2 over-expression measured both at RNA and protein level, but no gene amplification. The results obtained for RNA levels and Western blotting were less convincing.[16]

The suggestion that increased expression of the HER2 proto-oncogene (non-mutated gene) may be sufficient to drive cancer was then confirmed in *in vitro* experiments.[17] It was later shown that overexpression of HER2 also appears to help tumour cells evade the immune system.[18]

3.3. DRUG DISCOVERY

Although several groups attempted to use monoclonal antibodies to inhibit growth of cell lines overexpressing HER2,[8] it was the Genetech research that lead to a successful compound.[8]

In 1989 human tumour cells overexpressing HER2 were treated with a number of different mouse antibodies directed against the extracellular part of the HER2 protein. In particular, one of these (muMAb 4D5) led to the most extensive *in vitro* inhibition of tumour proliferation and showed high specificity for HER2. It also appeared that this antibody sensitised tumour cells to control by the immune system.[19] Other studies followed, exploring a range of antibodies to HER2. One such study investigated 10 mouse monoclonal antibodies[20] and again the most promising appeared to be muMAb 4D5. This antibody was selected by Genetech for further development.[9] Interestingly, one of the antibodies also investigated at that time was muMAb 2C4, later developed into pertuzumab.[21]

3.4. PRE-CLINICAL DEVELOPMENT OF MUMAB 4D5 AND PHASE I TRIAL

Discovery of the muMAb 4D5 antibody was followed by *in vivo* proof of concept studies. In one such study mice with human breast and ovarian tumour xenografts overexpressing HER2 were used. The animals were injected with a range of muMAb 4D5 doses and a control antibody inactive against HER2. Ten days after initiation of treatment, the weight of the tumours was shown to decrease with an increase in dose of the antibody. It was also shown, using a radioactive iodine–labelling, that the muMAb 4D5 localised to the tumour tissue overexpressing HER2.[9]

As muMAb 4D5 was a molecule of mouse origin, it was likely that using it in human patients would result in an immune reaction – the production of human anti-mouse antibodies. This was confirmed in a phase I trial including 12 patients with HER2 overexpressing breast and ovarian tumours. It showed that muMAb 4D5 was well tolerated and localised to tumour tissue, but a human anti-mouse response was observed, limiting the therapeutic use of muMAb 4D5.[9]

3.5. PRE-CLINICAL DEVELOPMENT OF THE HUMANISED ANTIBODY

To address the issue of development of the immune reaction, a series of humanised antibodies were prepared and investigated in *in vitro* experiments. An antibody labelled huMAb 4D5-8 showed the highest affinity for HER2 and one of the best inhibitions of cell proliferation. It was also shown to have little effect on a cell line obtained from normal tissue, thus promising limited adverse events.[22] HuMAb 4D5-8 later became known as trastuzumab, or Herceptin.[8]

Further animal research showed that trastuzumab appeared to have synergistic interactions with certain chemotherapeutic drugs such as cisplatin, docetaxel and cyclophosphamide. It was also demonstrated to have an acceptable safety profile when administered over a long period to a range of animals including primates.[8] The research into this drug was therefore advanced to phase I clinical trials.

3.6. PHASE I TRIALS OF TRASTUZUMAB

Three phase I trials of trastuzumab were undertaken, as shown in Table 5. Recruitment to the first one opened in June 1992 and closed for the last one in March 1993. All of these trials included patients with refractory metastatic breast cancer whose tumour was overexpressing HER2 (although the assay used to evaluate the biomarker was not reported). The earliest trial (Ho407g) investigated a single dose of the drug. The following two trials investigated a weekly dose schedule of trastuzumab either as monotherapy (Ho452g),[23] or in combination with cisplatin (Ho453g).[24]

Table 5 Phase I trials of trastuzumab

Study	Ho407g[8,9,23-25]	Ho452g[8,9,23-25]	Ho453g[8,9,23-25]
Number of patients	16	17	15
Inclusion criteria	Refractory metastatic breast cancer, HER2 positive		
HER2 evaluation	NR	NR	NR
Design	Open-label single-arm including only biomarker positive (details of dose escalation NR)		
Recruitment of first-last patient	1 June 1992 - 27 July 1992	9 November 1992 – 4 March 1993	6 October 1992 – 26 October 1992
Treatment	trastuzumab (single dose 10-500 mg iv.)	trastuzumab (weekly dose 10-500 mg iv.)	trastuzumab (weekly dose 10-500 mg iv.) + cisplatin (50 or 100 mg/m^2)
Outcomes assessed	Safety, maximum tolerated dose, pharmacokinetics		

iv. intravenous; NR – not reported

These studies showed that administration of trastuzumab was safe. A dose limiting toxicity was not reached.[25] The half-life of the drug was established to be 8.3 days.[8] There was also no evidence of development of anti-trastuzumab immune response. Encouragingly, four patients in the combination trial with cisplatin demonstrated objective response to treatment.[9]

Based on these results a dose schedule for phase II trials was established. It was to involve a 250 mg loading dose followed by 100 mg weekly dose. This was expected to ensure that for over 90% of patients, the target blood serum concentration anticipated to provide clinical benefit would be exceeded.[26] Such a schedule was also considered appropriate due to the safety profile of the drug.[9]

3.7. PHASE II TRIALS OF TRASTUZUMAB

The phase II trials are shown in Table 6. These recruited patients between March 1993 and September 1996 and evaluated trastuzumab as monotherapy(Baselga 1996[26] and Cobleigh 1999[27]) or in combinations with cisplatin (Pegram 1998[28]). The largest and final phase II trial introduced a new dose schedule based on body mass.[27]

Table 6 Phase II trials of trastuzumab

Study	Baselga 1996[26]	Pegram 1998[28]	Cobleigh 1999[27]
Number of patients	46	39	222
Inclusion criteria	Metastatic breast cancer, HER2 positive	Metastatic breast cancer, resistant to chemotherapy, HER2 positive	Metastatic breast cancer, resistant to chemotherapy, HER2 positive
HER2 evaluation	Tumour tissue - IHC using muMAb 4D5; Threshold: ≥25% cells staining positive	Tumour tissue – IHC using muMAb 4D5; Threshold: >10% cells staining 2+ (light to moderate) or 3+ (moderate to strong)	Tumour tissue - IHC using muMAb 4D5 and CB11*; Threshold: >10% cells staining 2+ (light to moderate) or 3+ (moderate to strong)
Design	Open-label single-arm including only biomarker positive		
Recruitment of first-last patient	15 March 1993 - 14 June 1994[23]	31 March 1993 – 25 May 1994[23]	12 June 1995 - 25 September 1996[23]
Treatment	Trastuzumab (iv. 250 mg initial dose followed by 100 mg weekly for 10 weeks); possibility of subsequent maintenance if no progression	Trastuzumab (iv. 250 mg initial dose followed by 100 mg weekly for 8 weeks) + cisplatin (75 mg/m^2 on day 1, 29 and 57); possibility of subsequent maintenance if no progression	Trastuzumab (iv. 4 mg/kg initial dose followed by 2 mg/kg weekly until progression); on progression allowed: continuation of treatment, increase in dose to 4 mg/kg, discontinuation, additional treatment
Major outcomes assessed	overall response duration of response TTP	overall response duration of response TTP	overall response duration of response TTP Overall survival
Follow-up	NR, 43 patients were assessable at day 77	NR; 37 patients were assessable at day 70	NR
Main results	At day 77 (n=43): 1 CR, 4 PR, 16 SD, 22 PD For non-PD patients given maintenance therapy median TTP=5.1 months (range NR)	At day 70 (n=37): 0 CR, 8 PR, 10 SD, 19 PD; For non-PD patients given maintenance therapy median TTP=5.3 months (range 1.6, 18)	Tumour response (n=213): Investigator assessment: 9 CR, 37 PR, 74 SD, 93 PD; independent committee assessment: 8 CR and 26 PR, NR SD, NR PD; Median time to treatment failure = 5.4 months (range 0, 27.4 months) TTP NR Median overall survival 13 months (range 0, >30)

IHC – immunohistochemistry, iv. intravenous; NR – not reported, TTP – time to progression, CR – complete response, PR – partial response, SD – stable disease, PD – progressive disease
*The reason why and how these were combined was not reported

These trials were all single-arm and evaluated the drug only in HER2 IHC positive patients. The IHC assay utilised the muMAb 4D5 antibody across all three trials. However, in the

largest trial an additional antibody was used (CB11*).[27] The threshold for considering patients as HER2 positive was also changed from ≥25% of cells staining positive in the first trial[26] to >10% cells staining either lightly (referred to as 2+) or strongly (referred to as 3+) in the following two.[27,28]

The percentage of patients in the monotherapy trials who responded to treatment (either CR or PR) was 11%[26] and 15% (or 21% when assessed by investigators).[27] 19% of the patients responded to the combination of trastuzumab with cisplatin.[28] The median time to loss of response ranged from 5.1 to 5.4 months. In the combination therapy trial the median overall survival was 13 months (range 0, >30 months). The drug was also shown to be relatively safe and anti-trastuzumab antibodies were not detected.[26-28]

3.1. DEVELOPMENT OF COMMERCIAL ASSAY (HERCEPTEST)

In December 1996 Genetech started a partnership with a diagnostics company (DAKO) to develop a commercial HER2 expression assay - HercepTest.[29,30]

A number of reproducibility studies were also undertaken, as shown in Table 7.

HercepTest was also compared to the assay used in clinical trials (IHC, antibody NR) in a study using breast cancer specimens not obtained in any of the trastuzumab trials. It was assumed that concordance between the two assays lower than 75% would be unacceptable. 1190 tumour specimens were evaluated using the clinical trial assay. All positive specimens (n=274) and an equal number of negative specimens selected randomly were then evaluated using HercepTest. Results of the study are summarised in Table 8, showing a 79% (95% CI: 76, 82%) concordance between the two tests for the dichotomous classification. Based on the data the sensitivity of HercepTest was 0.79 (95%CI: 0.73, 0.83) and specificity of 0.78 (95% CI: 0.73, 0.83).[31] There were however relatively large discrepancies with regard to the 2+/3+ staining.

* The reason why and how these were combined was not reported

Table 7 Reproducibility studies undertaken in development of HercepTest

Study type	Details
Intra-run	Five specimens were examined three times each in a random order. Both manual and automated laboratory procedures were used and the investigators were unaware of which specimen they were evaluating. There were no discordant results obtained with regards to classifying samples as positive or negative.[31]
Inter-run	Five specimens were examined in multiple runs (number NR) in a random order. The investigators were blind to which specimen they were evaluating. The reproducibility was described as satisfactory (further details NR).[31]
Inter-laboratory	40 fresh specimens were evaluated by 6 laboratories using both manual and automated laboratory procedures. Three of the laboratories produced invalid results. The reasons for this were available in two cases, where the exact laboratory procedures were not followed. As a result, the kit labelling was improved. The agreement between the results obtained by the three laboratories which provided valid results ranged from 82 to 90% for the dichotomous (positive/ negative) classification, corresponding to 15 discrepant results. A further 12 discrepant results were produced for the 2+ and 3+ classification.[31]
Lot-to-lot	Three different lots of HercepTest were used in a single laboratory to evaluate three cell lines with known levels of HER2 expression, two breast tumours expressing HER2 and HER2 negative tonsil tissue. All three lots produced identical results for the cell lines and tonsil tissue. Two lots produced identical results for the tumour tissues. One lot produced one discrepant result for the breast cancer sample.[31]

Table 8 Study evaluating HercepTest accuracy in 574 tumour tissue samples[31]

			Clinical trial assay		
			negative		positive
			0 and 1+	2+	3+
Hercep Test	negative	0 and 1+	215	50	8
	positive	2+	53	57	16
		3+	6	36	107

To confirm the accuracy of HercepTest, another study was undertaken using 168 breast tumour specimens which had been previously characterised using five different methods of HER2 evaluation, including IHC. The results are shown in Table 9, indicating 85% concordance (95% CI: 78, 89%), a sensitivity of 0.6 (95% CI: 0.5, 0.7) and a specificity of 1 (95% CI: 0.95, 1). No information was available on the 2+ and 3+ classification.[31]

Table 9 Study evaluating HercepTest accuracy in 168 well characterised tumour samples[31]

		Reference classification	
		negative	positive
HercepTest	negative	99	26
	positive	0	43

In 1998 HercepTest was approved by the FDA as "an aid in the assessment of patients for whom Herceptin (trastuzumab) treatment is being considered."[29,30]

3.2. PHASE III TRIAL

A randomised phase III trial (known as H0648g) recruited 469 HER2 positive patients with progressive metastatic breast cancer between June 1995 and March 1997. Their biomarker status was determined using IHC (antibody was NR) where 2+ or 3+ staining in >10% of cells was classed as positive.[32] The details of this trial are shown in Table 10. It is also included in the datasets in Chapter 5 and Chapter 6 in the main text.

The trial demonstrated that trastuzumab added to chemotherapy was superior to chemotherapy alone. It also appeared that the benefit was higher in the group of patients treated with trastuzumab and paclitaxel compared to trastuzumab and anthracycline + cyclophosphamide. The authors also noted that patients with a 3+ IHC score seemed to be obtaining more benefit than those with 2+. 63 patients in the trial suffered from cardiac dysfunction and 51 of these were treated with trastuzumab. The mechanism of cardiotoxicity was however unclear.[32]

3.1. MARKETING AUTHORISATION

The FDA considered trastuzumab in a fast-track process and as a result it was approved in 1998 in combination with paclitaxel for first-line treatment of HER2 positive metastatic breast cancer patients and as a single agent for second and third line therapy.[30]

An application was submitted to the EMA in the beginning of 1999 and was approved in the middle of 2000 in a similar indication to the FDA.[33]

Table 10 Phase III trial of trastuzumab

Study	Slamon 2001[32]
Number of patients	469 (235 trastuzumab + chemotherapy, 234 chemotherapy)
Inclusion criteria	Progressive metastatic breast cancer, no prior chemotherapy for metastatic disease, HER2 positive
HER2 evaluation	Tumour tissue – IHC; antibody NR; Threshold: >10% cells staining positive
Design	Enrichment; open-label[24]
Recruitment of first-last patient	12 June 1995 – 7 March 1997[30]
Treatment	• Trastuzumab (iv. 4 mg/kg initial dose followed by 2 mg/kg weekly until progression) + chemotherapy (as below); on progression allowed: continuation of treatment, increase in dose to 4 mg/kg, discontinuation, additional treatment • Chemotherapy: anthracycline (doxorubicin 600 mg/m^2 or epirubicin 75 mg/m^2) + cyclophosphamide (600 mg/m^2) or paclitaxel (175 mg/m^2) if patients received previous anthracycline once every three weeks for a minimum of six cycles
Major outcomes assessed	Primary: TTP Secondary: • overall response • duration of response • time to treatment failure • overall survival
Follow-up	NR, median time in the trial for chemotherapy 25 weeks (range 1, 131), chemotherapy + trastuzumab 40 weeks (range 1, 127)
Main results	Intention to treat analysis:* Median TTP: trasatuzumab + chemotherapy 7.4 months, chemotherapy 4.6 months, p<0.001 Overall response: trasatuzumab + chemotherapy 50%, chemotherapy 32%, p<0.001 Median duration of response: trasatuzumab + chemotherapy 9.1 months, chemotherapy 6.1 months, p<0.001 Median time to treatment failure: trasatuzumab + chemotherapy 6.9 months, chemotherapy 4.5 months, p<0.001 overall survival: : trasatuzumab + chemotherapy 25.1 months, chemotherapy 20.3 months, p=0.046 (including patients who switched from chemotherapy alone to trastuzumab + chemotherapy due to treatment failure)

IHC – immunohistochemistry, iv. intravenous; NR – not reported, TTP – time to progression
*hazard ratios were NR

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APPENDIX 4. SEARCH STRATEGIES FOR SYSTEMATIC REVIEW OF FRAMEWORKS OF STAGED EVALUATION OF PREDICTIVE BIOMARKERS

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4.1. MEDLINE

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>
Carried out on 07.01.2015.
Search Strategy:
---
1 phased.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (5315)
2 hierarchical.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (37558)
3 staged.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (16475)
4 model.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1423241)
5 approach.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (878336)
6 evaluation.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1253214)
7 1 or 2 or 3 (59295)
8 4 or 5 or 6 (3245155)
9 7 and 8 (21584)
10 guideline$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (301661)
11 framework$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (152603)
12 roadmap.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1961)
13 10 or 11 or 12 (449540)
14 9 or 13 (469139)
15 Biological Markers/ (175450)
16 Tumor Markers, Biological/ (93623)
17 Biomarkers, Pharmacological/ (1201)
18 Individualized Medicine/ (5941)
19 Drug Screening Assays, Antitumor/ (21186)
20 Pharmacogenetics/ (9628)
21 Toxicogenetics/ (649)
22 Genetic Markers/ (47655)
23 Gene Expression Profiling/ (91749)
24 Marker$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (719775)
25 Biomarker$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (115125)
26 Classifier$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (11772)
27 Predict*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1093899)

28 24 or 25 or 26 (781406)
29 27 and 28 (118237)
30 Individuali?ed Medicine.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (6218)
31 Pharmacogen*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (15569)
32 Stratified Medicine.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (104)
33 Personali?ed Medicine.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (5065)
34 precision medicine.mp. (284)
35 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 29 or 30 or 31 or 32 or 33 or 34 (493363)
36 14 and 35 (11376)

4.2. EMBASE

Database: Embase <1974 to 2015 January 05>
Search Strategy:
---
1 phased.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (6113)
2 hierarchical.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (39712)
3 staged.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (21720)
4 model.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (2625154)
5 approach.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (1059104)
6 evaluation.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (1344948)
7 1 or 2 or 3 (67476)
8 4 or 5 or 6 (4585673)
9 7 and 8 (27878)
10 guideline$.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (431448)
11 framework$.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (162544)
12 roadmap.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (2292)
13 10 or 11 or 12 (587261)
14 9 or 13 (612687)
15 Biological Markers/ (137099)
16 Tumor Markers, Biological/ (49703)
17 Biomarkers, Pharmacological/ (260)
18 Individualized Medicine/ (10590)
19 Drug Screening Assays, Antitumor/ (139987)
20 Pharmacogenetics/ (14531)
21 Toxicogenetics/ (833)
22 Genetic Markers/ (30004)
23 Gene Expression Profiling/ (60233)
24 Marker$.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (831692)

25 Biomarker$.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (162357)
26 Classifier$.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (12908)
27 Predict*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (1282207)
28 24 or 25 or 26 (900565)
29 27 and 28 (139228)
30 Individuali?ed Medicine.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (559)
31 Pharmacogen*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (26595)
32 Stratified Medicine.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (174)
33 Personali?ed Medicine.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] (14881)
34 precision medicine.mp. (327)
35 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 29 or 30 or 31 or 32 or 33 or 34 (537243)
36 14 and 35 (15182)

4.3. INTERNET

Searches were carried out 29.07.2015 using google.co.uk and a combination of terms for the field of stratified medicine and for frameworks:

stratified medicine terms	frameworks terms
stratified medicine	framework
predictive biomarker	guideline
pharmacogenomics	phased evaluation
	roadmap

Websites of the EMA and FDA were also searched using the terms for stratified medicine

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APPENDIX 5. DATA EXTRACTION FORM FOR SYSTEMATIC REVIEW OF FRAMEWORKS FOR STAGED EVALUATION OF PREDICTIVE BIOMARKERS

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Guideline *author and year*

Ref ID *in main database (in frameworks database)*

Clinical context *that the paper refers to*

Basis *background information on what lead to development of the framwork (for example experience, consensus conference)*

Geographic context *country/ area of framework origin*

Stage id	Stage name	Aim	Description	Actions	Minimum requirement for entry into stage	Relevant study designs	Criteria for stage completion	Comments
preferably from paper, but can be assigned based on text interpretation	*as provided in paper, unless not given - in this case a name summarising the stage*	*aim of the stage*	*description of the work undertaken within the stage*	*brief outline of pieces of work undertaken within each stage to allow easy comparison across different frameworks*	*what:* • *work needs to be completed,* • *information needs to be available, or* • *criteria have to be met* *prior to stage entry*	*what research designs are reported as appropriate to be undertaken within this stage*	*what:* • *work needs to be completed,* • *information needs to be available, or* • *criteria have to be met* *to conclude the stage is completed*	*any relevant comments*

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APPENDIX 6. PAPERS FOR WHICH FULL TEXT COULD NOT BE OBTAINED IN THE SYSTEMATIC REVIEW OF FRAMEWORKS FOR STAGED EVALUATION OF PREDICTIVE BIOMARKERS

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1. Anonymous, FDA issues pharmacogenomics data submission guidance, Expert Review of Molecular Diagnostics, 2005, 5, 3, 275-277

2. P. C. Bank, J. J. Swen and H. J. Guchelaar, Pharmacogenetic biomarkers for predicting drug response, Expert Review of Molecular Diagnostics, 2014, 14, 6, 723-735

3. G. De Meyer and F. Shapiro, Biomarker development: The road to clinical utility, Current Drug Discovery, 2003, MAY, 23-27

4. G. Ferber, Biomarkers and proof of concept, Methods & Findings in Experimental & Clinical Pharmacology, 2002, 24 Suppl C, 35-40

5. F. W. Frueh and B. Quinn, Molecular diagnostics clinical utility strategy: A six-part framework, Expert Review of Molecular Diagnostics, 2014, 14, 7, 777-786

6. P. Gadkari, Challenges in analytical method development for drug products, Pharma Times, 2012, 44, 1, 22-24

7. M. W. Linder, Pharmacogenetics in the practice of laboratory medicine, Molecular Diagnosis, 1999, 4, 4, 365-379

8. S. Little, Pharmacogenomics in drug development and regulatory decision-making: The genomic data submission proposal, European Pharmaceutical Contractor, 2004, SPRING, 22-24

9. S. M. Mariani, Conference report - breast cancer markers: what next? Highlights from the Annual Meeting of the American Society of Clinical Pathology; September 18-21, 2003; New Orleans, Louisiana, Medgenmed [Computer File]: Medscape General Medicine, 2003, 5, 4, 20

10. R. A. Miller and D. S. Spellman, Mass spectrometry-based biomarkers in drug development, Advances in Experimental Medicine & Biology, 2014, 806, 341-359

11. S. Piperaki, Personalized medicine and patient-centred care, Pharmakeftiki, 2012, 24, 3, 63-67

12. A. D. Roses, Genome-wide screening for drug discovery and companion diagnostics, Expert Opinion on Drug Discovery, 2007, 2, 4, 489-501

13. D. W. Wright, S. Wan, N. Shublaq, S. J. Zasada and P. V. Coveney, From base pair to bedside: molecular simulation and the translation of genomics to personalized medicine, Wiley interdisciplinary reviews. Systems biology & medicine, 2012, 4, 6, 585-598

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APPENDIX 7. PAPERS EXCLUDED BASED ON FULL TEXT IN THE SYSTEMATIC REVIEW OF FRAMEWORKS FOR STAGED EVALUATION OF PREDICTIVE BIOMARKERS

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7.1. THE PAPERS DID NOT DISCUSS PREDICTIVE BIOMARKERS

1. Draft guidance for industry. ANDAs: Pharmaceutical Solid Polymorphism. Chemistry, Manufacturing and Controls Information, *Journal of Generic Medicines*, 2005, 2, 3, 264-269

2. S. V. Aksenov, B. Church, A. Dhiman, A. Georgieva, R. Sarangapani, G. Helmlinger and I. G. Khalil, An integrated approach for inference and mechanistic modeling for advancing drug development, *FEBS Letters*, 2005, 579, 8, 1878-1883

3. M. A. Albert, Biomarkers and heart disease, *Journal of Clinical Sleep Medicine*, 2011, 7, 5 Suppl, S9-11

4. K. C. Anderson, A. L. Hannah, R. Pazdur and A. T. Farrell, A strategic framework for novel drug development in multiple myeloma, *British Journal of Haematology*, 2007, 138, 2, 153-159

5. G. Biomarkers Definitions Working, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, *Clinical Pharmacology & Therapeutics*, 2001, 69, 3, 89-95

6. R. M. Califf, Benefit assessment of therapeutic products: The Centers for Education and Research on Therapeutics, *Pharmacoepidemiology and Drug Safety*, 2007, 16, 1, 5-16

7. P. J. Goebell, S. L. Groshen and B. J. Schmitz-Drager, Guidelines for development of diagnostic markers in bladder cancer, *World Journal of Urology*, 2008, 26, 1, 5-11

8. M. N. Lassere, The Biomarker-Surrogacy Evaluation Schema: a review of the biomarker-surrogate literature and a proposal for a criterion-based, quantitative, multidimensional hierarchical levels of evidence schema for evaluating the status of biomarkers as surrogate endpoints, *Statistical Methods in Medical Research*, 2008, 17, 3, 303-340

9. J. P. Lehner, R. S. Epstein and T. Salimi, Integrating new approaches for clinical development: Translational research and relative effectiveness, *Journal of Comparative Effectiveness Research*, 2012, 1, 1 SUPPL.1, 15-21

10. X. J. Lou, S. M. Belkowski, J. M. Dixon, B. Hertzog, D. Horowitz, S. I. Ilyin, D. Lawrence, D. Polkovitch, M. Towers and M. R. D'Andrea, Strategies of biomarker discovery for drug development, In: (Ed: 2006, Bentham Science Publishers B.V.,

11. N. L. C. Members, G. L. Myers, R. H. Christenson, M. Cushman, C. M. Ballantyne, G. R. Cooper, C. M. Pfeiffer, S. M. Grundy, D. R. Labarthe, D. Levy, N. Rifai and P. W. Wilson, National Academy of Clinical Biochemistry Laboratory Medicine Practice guidelines: emerging biomarkers for primary prevention of cardiovascular disease, *Clinical Chemistry*, 2009, 55, 2, 378-384

12. D. M. O'Hara, Y. Xu, Z. Liang, M. P. Reddy, D. Y. Wu and V. Litwin, Recommendations for the validation of flow cytometric testing during drug development: II assays, *Journal of Immunological Methods*, 2011, 363, 2, 120-134

13. M. Otto, R. Bowser, M. Turner, J. Berry, J. Brettschneider, J. Connor, J. Costa, M. Cudkowicz, J. Glass, O. Jahn, S. Lehnert, A. Malaspina, L. Parnetti, A. Petzold, P. Shaw, A. Sherman, P. Steinacker, S. Sssmuth, C. Teunissen, H. Tumani, A. Wuolikainen and A. Ludolph, Roadmap and standard operating procedures for biobanking and discovery of neurochemical markers in ALS, *Amyotrophic Lateral Sclerosis*, 2012, 13, 1, 1-10

14. M. S. Pepe, R. Etzioni, Z. Feng, J. D. Potter, M. L. Thompson, M. Thornquist, M. Winget and Y. Yasui, Phases of biomarker development for early detection of cancer, *Journal of the National Cancer Institute*, 2001, 93, 14, 1054-1061

15. J. Peris-Vicente, E. Ochoa-Aranda, S. Carda-Broch and J. Esteve-Romero, Validation of human clinical genetic tests, *Mini-Reviews in Medicinal Chemistry*, 2014, 14, 10, 831-836

16. L. K. Seymour, A. H. Calvert, M. W. Lobbezoo, E. A. Eisenhauer and G. Giaccone, Design and conduct of early clinical studies of two or more targeted anticancer therapies: Recommendations from the task force on Methodology for the Development of Innovative Cancer Therapies, *European Journal of Cancer*, 2013, 49, 8, 1808-1814

17. F. D. Sistare, F. Dieterle, S. Troth, D. J. Holder, D. Gerhold, D. Andrews-Cleavenger, W. Baer, G. Betton, D. Bounous, K. Carl, N. Collins, P. Goering, F. Goodsaid, Y. Z. Gu, V. Guilpin, E. Harpur, A. Hassan, D. Jacobson-Kram, P. Kasper, D. Laurie, B. S. Lima, R. Maclulaitis, W. Mattes, G. Maurer, L. A. Obert, J. Ozer, M. Papaluca-Amati, J. A. Phillips, M. Pinches, M. J. Schipper, K. L. Thompson, S. Vamvakas, J. M. Vidal, J. Vonderscher, E. Walker, C. Webb and Y. Yu, Towards consensus practices to qualify safety biomarkers for use in early drug development, *Nature Biotechnology*, 2010, 28, 5, 446-454

18. P. Timmerman, C. Herling, D. Stoellner, B. Jaitner, S. Pihl, K. Elsby, N. Henderson, B. Barroso, S. Fischmann, A. Companjen, A. Versteilen, S. Bates, C. Kingsley, U. Kunz and F. European Bioanalysis, European Bioanalysis Forum recommendation on method establishment and bioanalysis of biomarkers in support of drug development, *Bioanalysis*, 2012, 4, 15, 1883-1894

19. C. Q. Zhu, W. Shih, C. H. Ling and M. S. Tsao, Immunohistochemical markers of prognosis in non-small cell lung cancer: A review and proposal for a multiphase approach to marker evaluation, *Journal of Clinical Pathology*, 2006, 59, 8, 790-800

7.2. THE PAPERS DID NOT DISCUSS A FRAMEWORK FOR STAGED EVALUATION

1. Researcher outlines cost-effectiveness framework for pharmacogenomics-based therapies, *Formulary*, 2001, 36, 7, 522+531-532

2. Framework for a fully powered risk engine, *Nature Genetics*, 2005, 37, 11, 1153

3. Opinion: Pharmacogenetics-based therapeutic recommendations - Ready for clinical practice?, *Pharmacogenomics*, 2005, 6, 6, 553

4. ICH efforts to improve processes, *WHO Drug Information*, 2006, 20, 4, 267-268

5. European Medicines Agency. Reflection paper on co-development of pharmacogenomic biomarkers and Assays in the context of drug development. Draft., 2010, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/07/WC500094445.pdf, accessed: 20.12.2015.

6. Policy-ready science, *Nature Genetics*, 2011, 43, 8, 721

7. European Medicines Agency. Reflection paper on methodological issues associated with pharmacogenomic biomarkers in relation to clinical development and patient selection, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500108672.pdf, accessed: 20.12.2015.

8. Technology Strategy Board. Stratified Medicine in the UK. Vision and Roadmap, 2011, https://connect.innovateuk.org/documents/2843120/3724280/Stratified+Medicines+Roadmap.pdf/fbb39848-282e-4619-a960-51e3a16ab893, accessed: 10.11.2015.

9. European Medicines Agency. Workshop on pharmacogenomics: from science to clinical care, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/Report/2012/11/WC500134968.pdf, accessed: 10.11.2015.

10. Association of the British Pharmaceutical Industry. An experimental medicine model to support a stratified medicine approach – points to consider, 2013, http://www.abpi.org.uk/_layouts/download.aspx?sourceurl=/our-work/library/medical-disease/Documents/An%20experimental%20medicine%20model%20to%20support%20a%20stratified%20medicine%20approach%20-%20points%20to%20consider.pdf, accessed:

11. Food and Drug Administration. Guidance ofr Industry: Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling, 2013, http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm337169.pdf, accessed: 08.11.2015.

12. Erratum: Pharmacogenomic characterization of gemcitabine response-a framework for data integration to enable personalized medicine (Pharmacogenetics and Genomics (2014) 24 (329)), *Pharmacogenetics and Genomics*, 2014, 24, 6, 329

13. J. A. Agundez, Interview: Perspective on the use of genomic biomarkers in the clinical setting, *Pharmacogenomics*, 2014, 15, 4, 419-422

14. J. A. Agundez, G. Esguevillas, G. Amo and E. Garcia-Martin, Clinical practice guidelines for translating pharmacogenomic knowledge to bedside. Focus on anticancer drugs, *Frontiers in Pharmacology*, 2014, 5, 188

15. M. A. Albert, Biomarkers and heart disease, *Journal of Clinical Sleep Medicine*, 2011, 7, 5, S9-S11

16. J. Albrethsen, The first decade of MALDI protein profiling: a lesson in translational

biomarker research, *Journal of Proteomics*, 2011, 74, 6, 765-773

17. C. A. Altar, D. Amakye, D. Bounos, J. Bloom, G. Clack, R. Dean, V. Devanarayan, D. Fu, S. Furlong, L. Hinman, C. Girman, C. Lathia, L. Lesko, S. Madani, J. Mayne, J. Meyer, D. Raunig, P. Sager, S. A. Williams, P. Wong and K. Zerba, A prototypical process for creating evidentiary standards for biomarkers and diagnostics, *Clinical Pharmacology & Therapeutics*, 2008, 83, 2, 368-371

18. S. Altmae, F. J. Esteban, A. Stavreus-Evers, C. Simon, L. Giudice, B. A. Lessey, J. A. Horcajadas, N. S. Macklon, T. D'Hooghe, C. Campoy, B. C. Fauser, L. A. Salamonsen and A. Salumets, Guidelines for the design, analysis and interpretation of 'omics' data: Focus on human endometrium, *Human Reproduction Update*, 2014, 20, 1, 12-28

19. D. G. Altman and R. D. Riley, Primer: an evidence-based approach to prognostic markers, *Nature Clinical Practice Oncology*, 2005, 2, 9, 466-472

20. AMS, The Academy of Medical Sciences. Realising the potential of stratified medicine, 2013, https://www.acmedsci.ac.uk/viewFile/51e915f9f09fb.pdf, accessed: 22.12.2015.

21. U. Amstutz and B. C. Carleton, Pharmacogenetic testing: time for clinical practice guidelines, *Clinical Pharmacology & Therapeutics*, 2011, 89, 6, 924-927

22. S. Amur, F. W. Frueh, L. J. Lesko and S. M. Huang, Integration and use of biomarkers in drug development, regulation and clinical practice: A US regulatory perspective, *Biomarkers in Medicine*, 2008, 2, 3, 305-311

23. K. C. Anderson and R. N. DuBois, Overview of the AACR clinical and translational cancer research Think Tank meeting, *Clinical Cancer Research*, 2012, 18, 3, 607-611

24. R. Anderson, New MRC guidance on evaluating complex interventions, *Bmj*, 2008, 337, 7676, 944-945

25. A. Anonychuk, G. Beastall, S. Shorter, R. Kloss-Wolf and P. Neumann, A framework for assessing the value of laboratory diagnostics. [French, English], *Healthcare Management Forum*, 2012, 25, SUPPL. 3, S4-S19

26. Anonymous, Genetics and drugs, *FDA Consumer*, 2004, 38, 1, 7

27. Anonymous, Personalized medicine, *FDA Consumer*, 2005, 39, 4, 4

28. Anonymous, FDA releases final guidance for pharmacogenomic data, *Pharmacogenomics*, 2005, 6, 3, 209

29. S. S. Ansher and R. Scharf, The Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute: Industry collaborations in new agent development, In: (Ed: 2001, New York Academy of Sciences,

30. A. R. Baer, D. Collyar, M. L. Smith and J. Bendell, Cancer genomics: Conducting exemplary trials with biospecimen and biomarker components, *Journal of Oncology Practice*, 2010, 6, 3, 164-167

31. R. W. Barker, D. A. Brindley and A. Schuh, Establish good genomic practice to guide medicine forward, *Nature Medicine*, 2013, 19, 5, 530

32. G. Bartlett, J. Antoun and N. K. Zgheib, Theranostics in primary care: pharmacogenomics tests and beyond, *Expert Review of Molecular Diagnostics*, 2012, 12, 8, 841-855

33. R. C. Bast Jr, H. Lilja, N. Urban, D. L. Rimm, H. Fritsche, J. Gray, R. Veltri, G. Klee, A. Allen, N. Kim, S. Gutman, M. A. Rubin and A. Hruszkewycz, Translational crossroads for biomarkers, *Clinical Cancer Research*, 2005, 11, 17, 6103-6108

34. D. Bedognetti, J. M. Balwit, E. Wang, M. L. Disis, C. M. Britten, L. G. Delogu, S. Tomei, B.

A. Fox, T. F. Gajewski, F. M. Marincola and L. H. Butterfield, SITC/iSBTc Cancer Immunotherapy Biomarkers Resource Document: online resources and useful tools - a compass in the land of biomarker discovery, *Journal of Translational Medicine*, 2011, 9, 155

35. T. Behrens, N. Bonberg, S. Casjens, B. Pesch and T. Bruning, A practical guide to epidemiological practice and standards in the identification and validation of diagnostic markers using a bladder cancer example, *Biochimica et Biophysica Acta - Proteins and Proteomics*, 2014, 1844, 1 PART A, 145-155

36. D. A. Bennett and M. D. Waters, Applying biomarker research, *Environmental Health Perspectives*, 2000, 108, 9, 907-910

37. A. S. Berghoff, H. Stefanits, A. Woehrer, H. Heinzl, M. Preusser and J. A. Hainfellner, Clinical neuropathology practice guide 3-2013: Levels of evidence and clinical utility of prognostic and predictive candidate brain tumor biomarkers, *Clinical Neuropathology*, 2013, 32, 3, 148-158

38. F. Bertucci and D. Birnbaum, Breast cancer genomics: real-time use, *Lancet Oncology*, 2007, 8, 12, 1045-1047

39. J. P. Boissel, R. Kahoul, E. Amsallem, F. Gueyffier, M. Haugh and F. H. Boissel, Towards personalized medicine: Exploring the consequences of the effect model-based approach, *Personalized Medicine*, 2011, 8, 5, 581-586

40. J. Boltze, C. Ayata, D. C. Wagner and N. Plesnila, Preclinical phase III trials in translational stroke research call for collective design of framework and guidelines, *Stroke*, 2014, 45, 2, 357

41. M. Boulton and J. Dally, Biomarkers in oncology drug development, *Regulatory Rapporteur*, 2010, 7, 4, 5-9

42. K. Bourzac, The right roadmap for personalized tests, *Cancer Discovery*, 2012, 2, 6, 478

43. A. Boussioutas and D. Taupin, Towards a molecular approach to gastric cancer management, *Internal Medicine Journal*, 2001, 31, 5, 296-303

44. M. Boyer, M. S. Tsao, P. Janne, S. Ramalingam, S. Pitman Lowenthal and M. Alam, Preparing for tomorrow: molecular diagnostics and the changing nonsmall cell lung cancer landscape, *Asia-Pacific Journal of Clinical Oncology*, 2014, 10 Suppl 2, 2-10

45. G. Brauers, Regulatory Aspects of Pharmacogenomics in Clinical Drug Development, Master-Thesis, 2007, http://dgra.de/deutsch/studiengang/master-thesis/2007-Georg-Brauers-Regulatory-Aspects-of-Pharmacogenomics-in-Clinical-Drug-Developm?nav=studiengang, accessed:

46. K. Broich, M. Weiergraber and H. Hampel, Biomarkers in clinical trials for neurodegenerative diseases: regulatory perspectives and requirements, *Progress in Neurobiology*, 2011, 95, 4, 498-500

47. C. M. Bromley, S. Close, N. Cohen, R. Favis, B. Fijal, F. Gheyas, W. Liu, C. Lopez-Correa, A. Prokop, J. B. Singer, A. Snapir, A. Tchelet, D. Wang, D. Goldstaub and G. Industry Pharmacogenomics Working, Designing pharmacogenetic projects in industry: practical design perspectives from the Industry Pharmacogenomics Working Group, *Pharmacogenomics Journal*, 2009, 9, 1, 14-22

48. N. E. Broude, A cancer roadmap, *Expert Review of Molecular Diagnostics*, 2005, 5, 2, 121-122

49. N. S. Buckholtz, Perspective: in search of biomarkers, *Nature*, 2011, 475, 7355, S8

50. A. J. Buckler, L. Bresolin, N. R. Dunnick and D. C. Sullivan, Quantitative imaging test approval and biomarker qualification: Interrelated but distinct activities, *Radiology*, 2011,

259, 3, 875-884

51. A. J. Buckler, T. T. Liu, E. Savig, B. E. Suzek, D. L. Rubin and D. Paik, Quantitative imaging biomarker ontology (QIBO) for knowledge representation of biomedical imaging biomarkers, *Journal of Digital Imaging*, 2013, 26, 4, 630-641

52. B. L. Bukaveckas, Pharma, clinicians and the lab come together over personalized medicine, *Personalized Medicine*, 2007, 4, 1, 105-108

53. T. Burt and S. Dhillon, Pharmacogenomics in early-phase clinical development, *Pharmacogenomics*, 2013, 14, 9, 1085-1097

54. R. M. Califf, Defining the balance of risk and benefit in the era of genomics and proteomics, *Health Affairs*, 2004, 23, 1, 77-87

55. P. M. A. Calverley and S. I. Rennard, Application of management guidelines to the assessment of new medicines: Driving us forward or holding us back?, *European Respiratory Journal*, 2004, 23, 6, 793-794

56. G. Campbell, Some statistical and regulatory issues in the evaluation of genetic and genomic tests, *Journal of Biopharmaceutical Statistics*, 2004, 14, 3, 539-552

57. B. Carmeli, P. Casali, A. Goldbraich, A. Goldsteen, C. Kent, L. Licitra, P. Locatelli, N. Restifo, R. Rinott, E. Sini, M. Torresani and Z. Waks, Evicase: an evidence-based case structuring approach for personalized healthcare, *Studies in Health Technology & Informatics*, 2012, 180, 604-608

58. A. Carracedo, J. M. Butler, L. Gusmao, A. Linacre, W. Parson, L. Roewer and P. M. Schneider, Update of the guidelines for the publication of genetic population data, *Forensic Science International: Genetics*, 2014, 10, 1, A1-A2

59. F. X. Castellanos, A. Di Martino, R. C. Craddock, A. D. Mehta and M. P. Milham, Clinical applications of the functional connectome, *NeuroImage*, 2013, 80, 527-540

60. K. E. Caudle, T. E. Klein, J. M. Hoffman, D. J. Muller, M. Whirl-Carrillo, L. Gong, E. M. McDonagh, K. Sangkuhl, C. F. Thorn, M. Schwab, J. A. Agundez, R. R. Freimuth, V. Huser, M. T. Lee, O. F. Iwuchukwu, K. R. Crews, S. A. Scott, M. Wadelius, J. J. Swen, R. F. Tyndale, C. M. Stein, D. Roden, M. V. Relling, M. S. Williams and S. G. Johnson, Incorporation of pharmacogenomics into routine clinical practice: the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline development process, *Current Drug Metabolism*, 2014, 15, 2, 209-217

61. D. Chalmers, D. Nicol, M. Otlowski and C. Critchley, Personalised medicine in the genome era, *Journal of Law & Medicine*, 2013, 20, 3, 577-594

62. R. Chen and M. Snyder, Promise of personalized omics to precision medicine, *Wiley interdisciplinary reviews. Systems biology & medicine*, 2013, 5, 1, 73-82

63. R. K. Chetty, J. S. Ozer, A. Lanevschi, I. Schuppe-Koistinen, D. McHale, J. S. Pears, J. Vonderscher, F. D. Sistare and F. Dieterle, A Systematic approach to preclinical and clinical safety biomarker qualification incorporating bradford hill's principles of causality association, *Clinical Pharmacology and Therapeutics*, 2010, 88, 2, 260-262

64. R. L. Chisholm, The opportunities and challenges of implementing genomics-informed personalized medicine, *Clinical Pharmacology and Therapeutics*, 2013, 94, 2, 181-182

65. J. Cohen, Dearth of clinically useful diagnostics limits growth of personalized medicine, *Expert Review of Clinical Pharmacology*, 2011, 4, 5, 527-529

66. J. P. Cohen, Overcoming regulatory and economic challenges facing pharmacogenomics, *New Biotechnology*, 2012, 29, 6, 751-756

67. N. Cohen and T. Frangiosa, Challenges, opportunities, and evolving landscapes in

pharmacogenomics and personalized medicine: An industry perspective, In: (Ed: 2008, Humana Press, 999 Riverview Drive, Suite 208, Totowa NJ 07512-1165, United States

68. W. A. Colburn, Optimizing the use of biomarkers, surrogate endpoints, and clinical endpoints for more efficient drug development, *Journal of Clinical Pharmacology*, 2000, 40, 12 Pt 2, 1419-1427

69. E. Costello and J. Neoptolemos, Enhancing the translation of cancer biomarkers, *British Journal of Surgery*, 2011, 98, 8, 1039-1040

70. K. R. Crews, J. K. Hicks, C. H. Pui, M. V. Relling and W. E. Evans, Pharmacogenomics and individualized medicine: translating science into practice, *Clinical Pharmacology & Therapeutics*, 2012, 92, 4, 467-475

71. J. L. Cummings, S. J. Banks, R. K. Gary, J. W. Kinney, J. M. Lombardo, R. R. Walsh and K. Zhong, Alzheimer's disease drug development: translational neuroscience strategies, *Cns Spectrums*, 2013, 18, 3, 128-138

72. M. E. Curran and S. Platero, Conference Scene: Diagnostics and personalized medicine: Observations from the World Companion Diagnostics Summit, *Pharmacogenomics*, 2011, 12, 4, 465-470

73. P. L. Daly and R. C. Becker, Pharmacogenetics of antiplatelet therapy, *Current Atherosclerosis Reports*, 2014, 16, 5,

74. J. E. Dancey, P. L. Bedard, N. Onetto and T. J. Hudson, The genetic basis for cancer treatment decisions, *Cell*, 2012, 148, 3, 409-420

75. K. Das, M. F. Mohd Omar, C. W. Ong, S. Bin Abdul Rashid, B. K. Peh, T. C. Putti, P. H. Tan, K. S. Chia, M. Teh, N. Shah, R. Soong and M. Salto-Tellez, TRARESA: a tissue microarray-based hospital system for biomarker validation and discovery, *Pathology*, 2008, 40, 5, 441-449

76. R. L. Davis and M. J. Khoury, A public health approach to pharmacogenomics and gene-based diagnostic tests, *Pharmacogenomics*, 2006, 7, 3, 331-337

77. P. M. Dean, Chemical genomics: a challenge for de novo drug design, *Molecular Biotechnology*, 2007, 37, 3, 237-245

78. V. H. Deneer and R. H. van Schaik, Evidence based drug dosing and pharmacotherapeutic recommendations per genotype, *Methods in Molecular Biology*, 2013, 1015, 345-354

79. E. P. Diamandis, Cancer biomarkers: Can we turn recent failures into success?, *Journal of the National Cancer Institute*, 2010, 102, 19, 1462-1467

80. B. Doble, A. Harris, D. M. Thomas, S. Fox and P. Lorgelly, Multiomics medicine in oncology: assessing effectiveness, cost-effectiveness and future research priorities for the molecularly unique individual, *Pharmacogenomics*, 2013, 14, 12, 1405-1417

81. E. Drucker and K. Krapfenbauer, Pitfalls and limitations in translation from biomarker discovery to clinical utility in predictive and personalised medicine, *EPMA J*, 2013, 4, 1, 7

82. G. E., From biomarkers to diagnostics: The road to success. White paper., 2015, http://www.quintiles.com/library/white-papers/from-biomarkers-to-diagnostics--the-road-to-success, accessed: 21.12.2015.

83. E. Edelman and C. Eng, A practical guide to interpretation and clinical application of personal genomic screening, *BMJ (Online)*, 2009, 339, 7730, 1136-1140

84. F. Ehmann, L. Caneva and M. Papaluca, European Medicines Agency initiatives and perspectives on pharmacogenomics, *British Journal of Clinical Pharmacology*, 2014, 77, 4, 612-617

85. F. Ehmann, M. Papaluca Amati, T. Salmonson, M. Posch, S. Vamvakas, R. Hemmings, H. G. Eichler and C. K. Schneider, Gatekeepers and enablers: How drug regulators respond to a challenging and changing environment by moving toward a proactive attitude, *Clinical Pharmacology and Therapeutics*, 2013, 93, 5, 425-432

86. P. E. Empey, Genetic predisposition to adverse drug reactions in the intensive care unit, *Critical Care Medicine*, 2010, 38, 6 SUPPL., S106-S116

87. B. J. Evans, Distinguishing product and practice regulation in personalized medicine, *Clinical Pharmacology and Therapeutics*, 2007, 81, 2, 288-293

88. A. W. Evers, M. M. Rovers, J. A. Kremer, J. A. Veltman, J. A. Schalken, B. R. Bloem and A. J. van Gool, An integrated framework of personalized medicine: from individual genomes to participatory health care, *Croatian Medical Journal*, 2012, 53, 4, 301-303

89. G. Farrugia and R. M. Weinshilboum, Challenges in implementing genomic medicine: The mayo clinic center for individualized medicine, *Clinical Pharmacology and Therapeutics*, 2013, 94, 2, 204-206

90. A. A. Flynn, Pharmacogenetics: Practices and opportunities for study design and data analysis, *Drug Discovery Today*, 2011, 16, 19-20, 862-866

91. R. A. Freeman, Personalized medicine and therapeutic decision-making in oncology: A commentary on key environmental issues, *Journal of Oncology Pharmacy Practice*, 2011, 17, 3, 295-297

92. F. W. Frueh, R. A. Salerno, L. J. Lesko and R. D. Hockett, 4th US FDA-drug information association pharmacogenomics workshop, held 10-12 December, 2007, *Pharmacogenomics*, 2009, 10, 1, 111-115

93. S. Gaisser, M. Dreiling, J. Hartig, M. M. Hopkins, J. Ryan and D. Ibarreta, Building effective institutional frameworks to support pharmacogenetic research: An international empirical analysis, *International Journal of Biotechnology*, 2008, 10, 6, 552-569

94. S. Gaisser, E. Vignola-Gagne, B. Husing, C. Enzing and T. van der Valk, EU policies in personalized medicine-related technologies, *Personalized Medicine*, 2009, 6, 1, 93-102

95. N. Gharani, M. A. Keller, C. B. Stack, L. M. Hodges, T. J. Schmidlen, D. E. Lynch, E. S. Gordon and M. F. Christman, The Coriell personalized medicine collaborative pharmacogenomics appraisal, evidence scoring and interpretation system, *Genome Med*, 2013, 5, 10, 93

96. D. Ghosh and L. M. Poisson, "Omics" data and levels of evidence for biomarker discovery, *Genomics*, 2009, 93, 1, 13-16

97. K. M. Giacomini, S. W. Yee, M. J. Ratain, R. M. Weinshilboum, N. Kamatani and Y. Nakamura, Pharmacogenomics and patient care: One size does not fit all, *Science Translational Medicine*, 2012, 4, 153,

98. G. S. Ginsburg and N. M. Kuderer, Comparative effectiveness research, genomics-enabled personalized medicine, and rapid learning health care: a common bond, *Journal of Clinical Oncology*, 2012, 30, 34, 4233-4242

99. G. S. Ginsburg and J. J. McCarthy, Transforming the practice of medicine using genomics, *Clinical Cases in Mineral and Bone Metabolism*, 2009, 6, 1, 25-28

100. M. Gion and A. S. C. Fabricio, New frontiers in tumor marker studies: from biobanking to collaboration in translational research, *International Journal of Biological Markers*, 2011, 26, 2, 73-74

101. S. Girish, S. W. Martin, M. C. Peterson, L. K. Zhang, H. Zhao, J. Balthasar, R. Evers, H. Zhou, M. Zhu, L. Klunk, C. Han, E. G. Berglund, S. M. Huang and A. Joshi, AAPS workshop

report: Strategies to address therapeutic protein-drug interactions during clinical development, *AAPS Journal*, 2011, 13, 3, 405-416

102. J. J. Goldberger and A. E. Buxton, Personalized medicine vs guideline-based medicine, *JAMA*, 2013, 309, 24, 2559-2560

103. F. Goodsaid and F. W. Frueh, Implementing the U.S. FDA guidance on pharmacogenomic data submissions, *Environmental & Molecular Mutagenesis*, 2007, 48, 5, 354-358

104. D. Gurwitz, Applying pharmacogenomics in drug development: Call for collaborative efforts, *Drug Development Research*, 2004, 62, 2, 71-75

105. A.-B. Halim, Biomarkers in drug development: a useful tool but discrepant results may have a major impact, 2011, accessed:

106. S. T. Hall, N. Abbott, G. Schmith and C. Brazell, Pharmacogenetics in drug development: Regulatory and clinical considerations, *Drug Development Research*, 2004, 62, 2, 102-111

107. T. Hampton, Researchers draft guidelines for clinical use of pharmacogenomics, *JAMA*, 2006, 296, 12, 1453-1454

108. D. F. Hayes, Contribution of biomarkers to personalized medicine, *Breast Cancer Research*, 2010, 12, SUPPL. 4,

109. D. F. Hayes, OMICS-based personalized oncology: if it is worth doing, it is worth doing well!, *BMC Medicine*, 2013, 11, 221

110. D. F. Hayes, From genome to bedside: are we lost in translation?, *Breast*, 2013, 22 Suppl 2, S22-26

111. D. F. Hayes, J. Allen, C. Compton, G. Gustavsen, D. G. Leonard, R. McCormack, L. Newcomer, K. Pothier, D. Ransohoff, R. L. Schilsky, E. Sigal, S. E. Taube and S. R. Tunis, Breaking a vicious cycle, *Science Translational Medicine*, 2013, 5, 196, 196cm196

112. D. F. Hayes, J. Allen, C. Compton, G. Gustavsen, D. G. B. Leonard, R. McCormack, L. Newcomer, K. Pothier, D. Ransohoff, R. L. Schilsky, E. Sigal, S. E. Taube and S. R. Tunis, Breaking a vicious cycle, *Science Translational Medicine*, 2013, 5, 196,

113. D. F. Hayes, R. C. Bast, C. E. Desch, H. Fritsche, Jr., N. E. Kemeny, J. M. Jessup, G. Y. Locker, J. S. Macdonald, R. G. Mennel, L. Norton, P. Ravdin, S. Taube and R. J. Winn, Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers, *Journal of the National Cancer Institute*, 1996, 88, 20, 1456-1466

114. D. F. Hayes, B. Trock and A. L. Harris, Assessing the clinical impact of prognostic factors: When is 'statistically significant' clinically useful?, *Breast Cancer Research and Treatment*, 1998, 52, 1-3, 305-319

115. M. Herder, Patents & the progress of personalized medicine: biomarkers research as lens, *Annals of Health Law*, 2009, 18, 2, 187-229, 188 p. preceding i

116. A. D. Hingorani, D. A. Van Der Windt, R. D. Riley, K. Abrams, K. G. M. Moons, E. W. Steyerberg, S. Schroter, W. Sauerbrei, D. G. Altman, H. Hemingway, A. Briggs, N. Brunner, P. Croft, J. Hayden, P. Kyzas, N. Malats, G. Peat, P. Perel, I. Roberts and A. Timmis, Prognosis research strategy (PROGRESS) 4: Stratified medicine research, *BMJ (Online)*, 2013, 346, e5793,

117. L. M. Hinman, K. M. Carl, B. B. Spear, R. A. Salerno, R. L. Becker, B. M. Abbott, J. F. Kelly, E. Mansfield, R. G. Katz, C. Harper, S. P. Day, M. A. Pacanowski and W. Pignato, Development and regulatory strategies for drug and diagnostic co-development, *Pharmacogenomics*, 2010, 11, 12, 1669-1675

118. M. A. Hlatky, Framework for evaluating novel risk markers, *Annals of Internal Medicine*, 2012, 156, 6, 468-469

119. C. J. Hoban, From the lab to the clinic: Integration of pharmacogenics into clinical development, *Pharmacogenomics*, 2002, 3, 4, 429-436

120. J. Hodgson and A. Marshall, Pharmacogenomics: will the regulators approve?, *Nature Biotechnology*, 1998, 16, 3, 243-246

121. W. Hoffmann, J. Krafczyk-Korth, H. Vlzke, K. Fendrich and H. K. Kroemer, Towards a unified concept of individualized medicine, *Personalized Medicine*, 2011, 8, 2, 111-113

122. R. S. Huang and E. R. Gamazon, Translating pharmacogenomics discoveries into the clinic: an implementation framework, *Genome Medicine*, 2013, 5, 10, 94

123. P. Hunter, Research and practice coming together. the advent of personalized medicine is bringing clinical research and practice closer together, *EMBO Reports*, 2012, 13, 2, 109-112

124. I. Ieiri, What are barriers to pharmacogenomics (PGx) clinical uptake?, *Drug Metabolism and Pharmacokinetics*, 2012, 27, 3, 279

125. S. Ignjatovic and N. Majkic-Singh, Biomarkers of diseases: An evidence-based approach, *Jugoslovenska Medicinska Biohemija*, 2006, 25, 3, 227-233

126. J. P. A. Ioannidis and M. J. Khoury, Are randomized trials obsolete or more important than ever in the genomic era?, *Genome Medicine*, 2013, 5, 4,

127. A. M. Issa, Evaluating the value of genomic diagnostics: implications for clinical practice and public policy, *Advances in Health Economics & Health Services Research*, 2008, 19, 191-206

128. K. K. Jain, Personalised medicine for cancer: from drug development into clinical practice, *Expert Opinion on Pharmacotherapy*, 2005, 6, 9, 1463-1476

129. H. Janes, M. D. Brown, Y. Huang and M. S. Pepe, An approach to evaluating and comparing biomarkers for patient treatment selection, *The International Journal of Biostatistics*, 2014, 10, 1, 99-121

130. H. Janes, M. S. Pepe, P. M. Bossuyt and W. E. Barlow, Measuring the performance of markers for guiding treatment decisions, *Annals of Internal Medicine*, 2011, 154, 4, 253-260

131. H. Janes, M. S. Pepe and Y. Huang, A framework for evaluating markers used to select patient treatment, *Medical Decision Making*, 2014, 34, 2, 159-167

132. D. J. Johann Jr and T. D. Veenstra, Multiple biomarkers in molecular oncology, *Expert Review of Molecular Diagnostics*, 2007, 7, 3, 223-225

133. J. A. Johnson and L. H. Cavallari, Pharmacogenetics and cardiovascular disease--implications for personalized medicine, *Pharmacological Reviews*, 2013, 65, 3, 987-1009

134. M. R. Johnson, N. C. Tan, P. Kwan and M. J. Brodie, Newly diagnosed epilepsy and pharmacogenomics research: a step in the right direction?, *Epilepsy & Behavior*, 2011, 22, 1, 3-8

135. Y. Joly, Personalized medicine in developing countries: A roadmap to personalized innovation, *Current Pharmacogenomics and Personalized Medicine*, 2011, 9, 3, 156-158

136. Y. Joly, G. Koutrikas, A. M. Tasse, A. Issa, B. Carleton, M. Hayden, M. J. Rieder, E. Ramos-Paque and D. Avard, Regulatory approval for new pharmacogenomic tests: a comparative overview, *Food & Drug Law Journal*, 2011, 66, 1, 1-24, i

137. W. Kaplan, V. Wirtz, A. Mantel-Teeuwisse, P. Stolk, B. Duthey and R. Laing, Stratified medicine and pharmacogenomics, In: *Priority medicines for Europe and the World 2013*

update (Ed: 2013, World Health Organization,

138. D. A. Katz, From bench to bedside: a diagnostics framework for pharmacogenetics research, *Molecular Genetics & Metabolism*, 2002, 77, 1-2, 57-60

139. M. J. Khoury, R. J. Coates, M. L. Fennell, R. E. Glasgow, M. T. Scheuner, S. D. Schully, M. S. Williams and S. B. Clauser, Multilevel research and the challenges of implementing genomic medicine, *Journal of the National Cancer Institute. Monographs*, 2012, 2012, 44, 112-120

140. K. Kieburtz and C. W. Olanow, Translational experimental therapeutics: The translation of laboratory-based discovery into disease-related therapy, *Mount Sinai Journal of Medicine*, 2007, 74, 1, 7-14

141. D. C. Kim, X. Wang, C. R. Yang and J. X. Gao, A framework for personalized medicine: prediction of drug sensitivity in cancer by proteomic profiling, *Proteome Science*, 2012, 10 Suppl 1, S13

142. G. D. Kitsios and D. M. Kent, Personalised medicine: Not just in our genes, *BMJ (Online)*, 2012, 344, 7859,

143. T. B. Knudsen and G. P. Daston, MIAME guidelines [1], *Reproductive Toxicology*, 2005, 19, 3 SPEC. ISS., 263

144. N. Kozauer and R. Katz, Regulatory innovation and drug development for early-stage Alzheimer's disease, *New England Journal of Medicine*, 2013, 368, 13, 1169-1171

145. W. K. Kraft and S. A. Waldman, Bridging the therapeutic continuum of drug development, regulation and use, *Expert Review of Clinical Pharmacology*, 2008, 1, 1, 1-3

146. E. H. Krekels, J. N. van den Anker, P. Baiardi, M. Cella, K. Y. Cheng, D. M. Gibb, H. Green, A. Iolascon, E. M. Jacqz-Aigrain, C. A. Knibbe, G. W. Santen, R. H. van Schaik, D. Tibboel and O. E. Della Pasqua, Pharmacogenetics and paediatric drug development: issues and consequences to labelling and dosing recommendations, *Expert Opinion on Pharmacotherapy*, 2007, 8, 12, 1787-1799

147. R. L., IHC Bio-Marker, Development, Testing and Commercialization in the United States: A Personal Perspective, 2008, http://www.dako.com/28827_2008_conn12_ihc_bio-marker_development_and_commercialization_in_us_richards.pdf, accessed: 20.12.2015.

148. T. Layloff, M. Nasr, R. Baldwin, M. Caphart, H. Drew, J. Hanig, C. Hoiberg, S. Koepke, G. Lunn, J. T. MacGregor, Y. Mille, E. Murphy, L. Ng, R. Rajagopalan, E. Sheinin, M. Smela, M. Welschenbach, H. Winkle and R. Williams, The FDA regulatory methods validation program for new and abbreviated new drug applications, *Pharmaceutical Technology*, 2000, 24, 1, 30-42

149. K. N. Lazaridis and G. M. Petersen, Genomics, genetic epidemiology, and genomic medicine, *Clinical Gastroenterology and Hepatology*, 2005, 3, 4, 320-328

150. T. Lecroq and L. F. Soualmia, Managing large-scale genomic datasets and translation into clinical practice, *Yearbook of Medical Informatics*, 2014, 9, 1, 212-214

151. H. Ledford, Translational research: 4 ways to fix the clinical trial, *Nature*, 2011, 477, 7366, 526-528

152. C. K. Lee, S. J. Lord, A. S. Coates and R. J. Simes, Molecular biomarkers to individualise treatment: Assessing the evidence, *Medical Journal of Australia*, 2009, 190, 11, 631-636

153. R. T. Lee, P. Yang, H. Greenlee, S. Bauer-Wu, L. G. Balneaves and S. Zick, Personalized integrative oncology: targeted approaches for optimal outcomes: the 11th international conference of the society for integrative oncology, *Integrative Cancer Therapies*, 2015, 14, 1, 98-105

154. S. Lejeune and D. Lacombe, Towards personalized medicine in the EU: What is needed to facilitate the complex international clinical research?, *Personalized Medicine*, 2013, 10, 8, 849-857

155. L. J. Lesko, Personalized medicine: elusive dream or imminent reality?, *Clinical Pharmacology & Therapeutics*, 2007, 81, 6, 807-816

156. L. J. Lesko and S. Schmidt, Individualization of drug therapy: history, present state, and opportunities for the future, *Clinical Pharmacology & Therapeutics*, 2012, 92, 4, 458-466

157. L. J. Lesko and J. Woodcock, Pharmacogenomic-guided drug development: Regulatory perspective, *Pharmacogenomics Journal*, 2002, 2, 1, 20-24

158. L. J. Lesko and J. Woodcock, Translation of pharmacogenomics and pharmacogenetics: A regulatory perspective, *Nature Reviews Drug Discovery*, 2004, 3, 9, 763-769

159. F. Leung, E. P. Diamandis and V. Kulasingam, Ovarian cancer biomarkers: current state and future implications from high-throughput technologies, *Advances in Clinical Chemistry*, 2014, 66, 25-77

160. J. Li, K. B. Kelm and Z. Tezak, Regulatory perspective on translating proteomic biomarkers to clinical diagnostics, *Journal of Proteomics*, 2011, 74, 12, 2682-2690

161. E. T. Liu, Mechanism-derived gene expression signatures and predictive biomarkers in clinical oncology, *Proceedings of the National Academy of Sciences of the United States of America*, 2005, 102, 10, 3531-3532

162. A. Loboda, M. Nebozhyn, C. Cheng, R. Vessey, P. Huang, H. Dai and J. W. Watters, Biomarker discovery: identification of a growth factor gene signature, *Clinical Pharmacology & Therapeutics*, 2009, 86, 1, 92-96

163. D. Loughnot, Potential interactions of the Orphan Drug Act and pharmacogenomics: A flood of orphan drugs and abuses?, *American Journal of Law and Medicine*, 2005, 31, 2-3, 365-380

164. J. Lunshof and G. De Wert, Pharmacogenomics, drug development, and ethics: Some points to consider, *Drug Development Research*, 2004, 62, 2, 112-116

165. R. Madabushi, L. Lesko and J. Woodcock, Regulatory Perspectives on Biomarker Development, *Predictive Approaches in Drug Discovery and Development: Biomarkers and In Vitro/In Vivo Correlations*, 2012, 11, 231

166. A. S. Maisel, K. Nakao, P. Ponikowski, W. F. Peacock, M. Yoshimura, T. Suzuki, T. Tsutamoto, G. S. Filippatos, Y. Saito, Y. Seino, N. Minamino, Y. Hirata, M. Mukoyama, T. Nishikimi and R. Nagai, Japanese-Western consensus meeting on biomarkers, *International Heart Journal*, 2011, 52, 5, 253-265

167. M. Maliepaard, C. Nofziger, M. Papaluca, I. Zineh, Y. Uyama, K. Prasad, C. Grimstein, M. Pacanowski, F. Ehmann, S. Dossena and M. Paulmichl, Pharmacogenetics in the evaluation of new drugs: a multiregional regulatory perspective, *Nature Reviews. Drug Discovery*, 2013, 12, 2, 103-115

168. V. G. Manolopoulos, Pharmacogenomics and adverse drug reactions in diagnostic and clinical practice, *Clinical Chemistry & Laboratory Medicine*, 2007, 45, 7, 801-814

169. R. March, K. Cheeseman and M. Doherty, Pharmacogenetics--legal, ethical and regulatory considerations, *Pharmacogenomics*, 2001, 2, 4, 317-327

170. C. Marchio, M. Dowsett and J. S. Reis-Filho, Revisiting the technical validation of tumour biomarker assays: how to open a Pandora's box, *BMC Medicine*, 2011, 9, 41

171. N. J. Markward, Quantifying genomic variation at the individual level: putting the

"person" in personalized medicine, *AMIA .. 2007, Annual Symposium Proceedings/AMIA Symposium.*, 1041

172. S. Marsh and T. Van Rooij, Challenges of incorporating pharmacogenomics into clinical practice, *Gastrointestinal Cancer Research*, 2009, 3, 5, 206-207

173. P. Martin, False Positive?: The Clinical and Commercial Development of Pharmacogenetics, 2006, accessed:

174. B. Matharoo-Ball, A. K. Miles, C. S. Creaser, G. Ball and R. Rees, Serum biomarker profiling in cancer studies: a question of standardisation?, *Veterinary & Comparative Oncology*, 2008, 6, 4, 224-247

175. S. Matsui, R. Simon, P. Qu, J. D. Shaughnessy, Jr., B. Barlogie and J. Crowley, Developing and validating continuous genomic signatures in randomized clinical trials for predictive medicine, *Clinical Cancer Research*, 2012, 18, 21, 6065-6073

176. K. McGeechan, P. Macaskill, L. Irwig, G. Liew and T. Y. Wong, Assessing new biomarkers and predictive models for use in clinical practice: A clinician's guide, *Archives of Internal Medicine*, 2008, 168, 21, 2304-2310

177. R. A. McKinnon, M. B. Ward and M. J. Sorich, Pharmacogenomics in a global world: A roadmap for Australia, prospects and challenges, *Current Pharmacogenomics and Personalized Medicine*, 2013, 11, 4, 257-259

178. F. J. McMahon and T. R. Insel, Pharmacogenomics and Personalized Medicine in Neuropsychiatry, *Neuron*, 2012, 74, 5, 773-776

179. L. M. McShane, M. M. Cavenagh, T. G. Lively, D. A. Eberhard, W. L. Bigbee, P. M. Williams, J. P. Mesirov, M. Y. Polley, K. Y. Kim, J. V. Tricoli, J. M. Taylor, D. J. Shuman, R. M. Simon, J. H. Doroshow and B. A. Conley, Criteria for the use of omics-based predictors in clinical trials: explanation and elaboration, *BMC Medicine*, 2013, 11, 220

180. D. Melzer, R. L. Zimmern, D. E. Detmer and T. Ling, Regulatory options for pharmacogenetics, *Pharmacogenomics*, 2003, 4, 5, 527-530

181. C. P. Milne and R. Zuckerman, Biopharmaceutical industry perspectives on the business prospects for personalized medicine, *Personalized Medicine*, 2011, 8, 5, 541-550

182. H. Mischak, A. Vlahou, P. G. Righetti and J. J. Calvete, Putting value in biomarker research and reporting, *Journal of Proteomics*, 2014, 96, A1-A3

183. D. A. Morrow and N. R. Cook, Determining decision limits for new biomarkers: Clinical and statistical considerations, *Clinical Chemistry*, 2011, 57, 1, 1-3

184. D. A. Mrazek and C. Lerman, Facilitating clinical implementation of pharmacogenomics, *JAMA*, 2011, 306, 3, 304-305

185. K. Mullane, R. J. Winquist and M. Williams, Translational paradigms in pharmacology and drug discovery, *Biochemical Pharmacology*, 2014, 87, 1, 189-210

186. G. L. Myers, N. Rifai, R. P. Tracy, W. L. Roberts, R. W. Alexander, L. M. Biasucci, J. D. Catravas, T. G. Cole, G. R. Cooper, B. V. Khan, M. M. Kimberly, E. A. Stein, K. A. Taubert, G. R. Warnick, P. P. Waymack, Cdc and Aha, CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: report from the laboratory science discussion group, *Circulation*, 2004, 110, 25, e545-549

187. P. M. Nadkarni and M. Wiepert, Translating pharmacogenomics discoveries into clinical practice: The role of curated databases, *Pharmacogenomics*, 2005, 6, 5, 451-454

188. T. Nakazawa, S. Kai, M. Kawai, E. Maki, F. Sagami, H. Onodera, S. Kitajima and T. Inoue, "Points to consider" regarding safety assessment of biotechnology-derived

pharmaceuticals in non-clinical studies (English translation), *The Journal of toxicological sciences*, 2004, 29, 5, 497-504

189. H. Newell, Biomarkers in clinical trials: Overview of roadmaps for PD/ prognostic/predictive biomarkers, 2015, http://www.ecmcnetwork.org.uk/sites/default/files/Newell%20Biomarkers%20ECMC%20Jan%202016.pdf, accessed: 14.12.2015.

190. P. Newton, Upgrading pharmaceutical test methods using a contract research organization, *Pharmaceutical Technology North America*, 2002, 26, 7, 30-40

191. P. C. Ng, S. S. Murray, S. Levy and J. C. Venter, An agenda for personalized medicine, *Nature*, 2009, 461, 7265, 724-726

192. C. P. O'Brien, S. E. Taylor, J. J. O'Leary and S. P. Finn, Molecular testing in oncology: Problems, pitfalls and progress, *Lung Cancer*, 2014, 83, 3, 309-315

193. P. Oestreicher, 4th annual pharmacogenomics and medicine lectures, *Pharmacogenomics*, 2001, 2, 3, 291-296

194. P. Oestreicher, Individualizing therapeutic decision making in early-stage breast cancer, *ONS Connect*, 2007, 22, 8 Suppl, 41-42

195. G. O'Hurley, E. Sjostedt, A. Rahman, B. Li, C. Kampf, F. Ponten, W. M. Gallagher and C. Lindskog, Garbage in, garbage out: A critical evaluation of strategies used for validation of immunohistochemical biomarkers, *Molecular Oncology*, 2014, 8, 4, 783-798

196. G. S. Omenn, Strategies for plasma proteomic profiling of cancers, *Proteomics*, 2006, 6, 20, 5662-5673

197. S. F. Oon, S. R. Pennington, J. M. Fitzpatrick and R. W. Watson, Biomarker research in prostate cancer--towards utility, not futility, *Nature Reviews Urology*, 2011, 8, 3, 131-138

198. M. Ostman, Product specifications setting and review process for new drug entities, *Drug Information Journal*, 1996, 30, 1, 109-112

199. Y. Otsubo, A. Ishiguro and Y. Uyama, Regulatory perspective on remaining challenges for utilization of pharmacogenomics-guided drug developments, *Pharmacogenomics*, 2013, 14, 2, 195-203

200. V. Ozdemir, B. Williams-Jones, D. M. Cooper, T. Someya and B. Godard, Mapping translational research in personalized therapeutics: from molecular markers to health policy, *Pharmacogenomics*, 2007, 8, 2, 177-185

201. F. Palau, Personalized medicine in rare diseases, *Personalized Medicine*, 2012, 9, 2, 137-141

202. A. C. Patel, Clinical relevance of target identity and biology: Implications for drug discovery and development, *Journal of Biomolecular Screening*, 2013, 18, 10, 1164-1185

203. N. W. Paul and A. D. Roses, Pharmacogenetics and pharmacogenomics: Recent developments, their clinical relevance and some ethical, social, and legal implications, *Journal of Molecular Medicine*, 2003, 81, 3, 135-140

204. P. R. O. Payne and C. B. Marsh, Towards a "4I" approach to personalized healthcare, *Clinical and Translational Medicine*, 2012, 1, 14,

205. K. A. Phillips, S. Y. Liang, S. Van Bebber, A. Afable-Munsuz, S. J. Knight, M. Kuppermann, U. Ladabaum, J. Walsh, E. Elkin, J. Haas, M. Hassett, N. Kulin, D. Marshall and N. Ponce, Challenges to the translation of genomic information into clinical practice and health policy: Utilization, preferences and economic value, *Current Opinion in Molecular Therapeutics*, 2008, 10, 3, 260-266

206. K. A. Phillips and S. L. Van Bebber, Regulatory perspectives on pharmacogenomics: a review of the literature on key issues faced by the United States Food and Drug Administration, *Medical Care Research & Review*, 2006, 63, 3, 301-326

207. K. A. Phillips, S. L. Van Bebber and A. M. Issa, Initial development of an evidence base for personalized medicine's translation to clinical practice and health policy, *Personalized Medicine*, 2006, 3, 4, 411-414

208. M. S. Phillips, A. K. Hibi, T. van Rooij, A. C. Smith, S. James, S. Marsh, N. Laplante, M. P. Dube and J. C. Tardif, Genome Quebec & Montreal Heart Institute Pharmacogenomics Centre: A translational pharmacogenomics platform - From R&D to the clinic, *Pharmacogenomics*, 2008, 9, 10, 1391-1396

209. C. Piana, L. Surh, S. Furst-Recktenwald, A. Iolascon, E. M. Jacqz-Aigrain, I. Jonker, R. Russo, R. H. van Schaik, J. Wessels and O. E. Della Pasqua, Integration of pharmacogenetics and pharmacogenomics in drug development: implications for regulatory and medical decision making in pediatric diseases, *Journal of Clinical Pharmacology*, 2012, 52, 5, 704-716

210. F. Pignatti, F. Ehmann, R. Hemmings, B. Jonsson, M. Nuebling, M. Papaluca-Amati, Posch and G. Rasi, Cancer drug development and the evolving regulatory framework for companion diagnostics in the european union, *Clinical Cancer Research*, 2014, 20, 6, 1458-1468

211. M. Pirmohamed and D. A. Hughes, Pharmacogenetic tests: the need for a level playing field, *Nature Reviews. Drug Discovery*, 2013, 12, 1, 3-4

212. M. Plebani, Lessons from controversy: Biomarkers evaluation, *Clinical Chemistry and Laboratory Medicine*, 2013, 51, 2, 247-248

213. R. J. Plumridge, Pharmacoeconomics: Challenges in Developing the Fourth Dimension of Drug Evaluation, *Journal of Pharmacy Practice and Research*, 2003, 33, 4, 287-289

214. K. Prasad, Role of regulatory agencies in translating pharmacogenetics to the clinics, *Clinical Cases in Mineral and Bone Metabolism*, 2009, 6, 1, 29-34

215. A. S. Ptolemy, Biomarker diversity, validation and clinical translation: Tenets for emergent personalized medicine initiatives, *Current Pharmacogenomics and Personalized Medicine*, 2011, 9, 4, 286-298

216. X. Pu, Y. Ye and X. Wu, Development and validation of risk models and molecular diagnostics to permit personalized management of cancer, *Cancer*, 2014, 120, 1, 11-19

217. M. Qattan, C. Demonacos and M. Krstic-Demonacos, Roadmap to personalized medicine, *Croatian Medical Journal*, 2012, 53, 4, 294-297

218. B. Quinn, Payers and the assessment of clinical utility for companion diagnostics, *Clinical Pharmacology & Therapeutics*, 2010, 88, 6, 751-754

219. D. F. Ransohoff, How to improve reliability and efficiency of research about molecular markers: roles of phases, guidelines, and study design, *Journal of Clinical Epidemiology*, 2007, 60, 12, 1205-1219

220. M. J. Ratain, Incorporating biomarkers into drug labeling, *Clinical Advances in Hematology and Oncology*, 2014, 12, 8, 525-527

221. M. Ratner, FDA pharmacogenomics guidance sends clear message to industry, *Nature Reviews Drug Discovery*, 2005, 4, 5, 359

222. M. V. Relling and T. E. Klein, CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network, *Clinical Pharmacology &*

Therapeutics, 2011, 89, 3, 464-467

223. M. V. Relling and T. E. Klein, CPIC: Clinical pharmacogenetics implementation consortium of the pharmacogenomics research network, *Clinical Pharmacology and Therapeutics*, 2011, 89, 3, 464-467

224. R. Ricafort, Tumor markers in infancy and childhood, *Pediatrics in Review*, 2011, 32, 7, 306-308

225. R. A. Salerno and L. J. Lesko, Pharmacogenomic data: FDA voluntary and required submission guidance, *Pharmacogenomics*, 2004, 5, 5, 503-505

226. R. A. Salerno and L. J. Lesko, Pharmacogenomics in drug development and regulatory decision-making: The genomic data submission (GDS) proposal, *Pharmacogenomics*, 2004, 5, 1, 25-30

227. D. R. Savage, U. S. Food and A. Drug, FDA guidance on pharmacogenomics data submission, *Nature Reviews. Drug Discovery*, 2003, 2, 12, 937-938

228. C. W. Schmidt, NIH roadmap for medical research, *Environmental Health Perspectives*, 2004, 112, 3, A165-A166

229. G. Schumann, E. B. Binder, A. Holte, E. R. de Kloet, K. J. Oedegaard, T. W. Robbins, T. R. Walker-Tilley, I. Bitter, V. J. Brown, J. Buitelaar, R. Ciccocioppo, R. Cools, C. Escera, W. Fleischhacker, H. Flor, C. D. Frith, A. Heinz, E. Johnsen, C. Kirschbaum, T. Klingberg, K. P. Lesch, S. Lewis, W. Maier, K. Mann, J. L. Martinot, A. Meyer-Lindenberg, C. P. Muller, W. E. Muller, D. J. Nutt, A. Persico, G. Perugi, M. Pessiglione, U. W. Preuss, J. P. Roiser, P. M. Rossini, J. K. Rybakowski, C. Sandi, K. E. Stephan, J. Undurraga, E. Vieta, N. van der Wee, T. Wykes, J. M. Haro and H. U. Wittchen, Stratified medicine for mental disorders, *European Neuropsychopharmacology*, 2014, 24, 1, 5-50

230. R. A. Scolyer and J. F. Thompson, Biospecimen banking: the pathway to personalized medicine for patients with cancer, *Journal of Surgical Oncology*, 2013, 107, 7, 681-682

231. A. Serretti, M. Kato and J. L. Kennedy, Pharmacogenetic studies in depression: a proposal for methodologic guidelines, *Pharmacogenomics Journal*, 2008, 8, 2, 90-100

232. R. Shah, B. Darne, D. Atar, E. Abadie, K. F. Adams and F. Zannad, Pharmacogenomics in cardiovascular clinical trials, *Fundamental and Clinical Pharmacology*, 2004, 18, 6, 705-708

233. R. R. Shah, Pharmacogenetics in drug regulation: promise, potential and pitfalls, *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences*, 2005, 360, 1460, 1617-1638

234. J. A. Shaw and M. E. Cooper, Guidelines and their use in clinical practice, *Diabetes & Vascular Disease Research*, 2014, 11, 1, 3-4

235. J. Shin, S. R. Kayser and T. Y. Langaee, Pharmacogenetics: from discovery to patient care, *American Journal of Health-System Pharmacy*, 2009, 66, 7, 625-637

236. A. R. Shuldiner, M. V. Relling, J. F. Peterson, J. K. Hicks, R. R. Freimuth, W. Sadee, N. L. Pereira, D. M. Roden, J. A. Johnson, T. E. Klein, G. Pharmacogenomics Research Network Translational Pharmacogenetics Program, A. R. Shuldiner, M. Vesely, S. W. Robinson, N. Ambulos, Jr., S. A. Stass, M. D. Kelemen, L. A. Brown, T. I. Pollin, A. L. Beitelshees, R. Y. Zhao, R. E. Pakyz, K. Palmer, T. Alestock, C. O'Neill, K. Maloney, A. Branham, D. Sewell, M. V. Relling, K. Crews, J. Hoffman, S. Cross, C. Haidar, D. Baker, J. K. Hicks, G. Bell, F. Greeson, A. Gaur, U. Reiss, A. Huettel, C. Cheng, A. Gajjar, A. Pappo, S. Howard, M. Hudson, C. H. Pui, S. Jeha, W. E. Evans, U. Broeckel, R. B. Altman, L. Gong, M. Whirl-Carrillo, T. E. Klein, W. Sadee, K. Manickam, K. M. Sweet, P. J. Embi, D. Roden, J. Peterson, J. Denny, J. Schildcrout, E. Bowton, J. Pulley, M. Beller, J. Mitchell, I. Danciu, L. Price, N. L. Pereira, R.

Weinshilboum, L. Wang, J. A. Johnson, D. Nelson, M. Clare-Salzler, A. Elsey, B. Burkley, T. Langaee, F. Liu, D. Nessl, H. J. Dong, L. Lesko, R. R. Freimuth and C. G. Chute, The Pharmacogenomics Research Network Translational Pharmacogenetics Program: overcoming challenges of real-world implementation, *Clinical Pharmacology & Therapeutics*, 2013, 94, 2, 207-210

237. R. Simon, Genomic biomarkers in predictive medicine: an interim analysis, *EMBO Mol Med*, 2011, 3, 8, 429-435

238. D. R. J. Singer and A. Marsh, Challenges and solutions for personalizing medicines, *Health Policy and Technology*, 2012, 1, 1, 50-57

239. E. Singer, Personalized medicine prompts push to redesign clinical trials, *Nature Medicine*, 2005, 11, 5, 462

240. F. D. Sistare and J. J. DeGeorge, Promise of new translational safety biomarkers in drug development and challenges to regulatory qualification, *Biomarkers in Medicine*, 2011, 5, 4, 497-514

241. J. Smeds, L. D. Miller, J. Bjohle, P. Hall, S. Klaar, E. T. Liu, Y. Pawitan, A. Ploner and J. Bergh, Gene profile and response to treatment, *Annals of Oncology*, 2005, 16, SUPPL. 2, ii195-ii202

242. C. M. Sturgeon, B. R. Hoffman, D. W. Chan, S. L. Ch'ng, E. Hammond, D. F. Hayes, L. A. Liotta, E. F. Petricoin, M. Schmitt, O. J. Semmes, G. Soletormos, E. Van Der Merwe and E. P. Diamandis, National academy of clinical biochemistry laboratory medicine practice guidelines for use of tumor markers in clinical practice: Quality requirements, *Clinical Chemistry*, 2008, 54, 8, e1-e10

243. A. Surendiran, S. Pradhan and C. Adithan, Role of pharmacogenomics in drug discovery and development, *Indian Journal of Pharmacology*, 2008, 40, 4, 137-143

244. L. C. Surh, M. A. Pacanowski, S. B. Haga, S. Hobbs, L. J. Lesko, S. Gottlieb, M. Papaluca-Amati, S. D. Patterson, A. R. Hughes, M. J. Kim, S. L. Close, M. Mosteller, I. Zineh, B. Dechairo and N. A. Cohen, Learning from product labels and label changes: how to build pharmacogenomics into drug-development programs, *Pharmacogenomics*, 2010, 11, 12, 1637-1647

245. E. Tambuyzer, Towards a framework for personalized healthcare: Lessons learned from the field of rare diseases, *Personalized Medicine*, 2010, 7, 5, 569-586

246. L. Tang and X. H. Zhou, A general framework of marker design with optimal allocation to assess clinical utility, *Statistics in Medicine*, 2013, 32, 4, 620-630

247. W. Tang, Z. Hu, H. Muallem and M. L. Gulley, Clinical implementation of RNA signatures for pharmacogenomic decision-making, *Pharmacogenomics and Personalized Medicine*, 2011, 4, 1, 95-107

248. S. E. Taube, Biomarkers in oncology: Trials and tribulations, In: *Biomarkers in Brain Disease* (Ed: S. Lovestone), 2009, Blackwell Publishing Inc., 350 Main Street, Malden MA 02148, United States

249. C. A. Thompson, Regulations, economics hindering adoption of personalized medicine, *American Journal of Health-System Pharmacy*, 2011, 68, 5, 372-374

250. W. L. Trepicchio, D. Essayan, S. T. Hall, G. Schechter, Z. Tezak, S. J. Wang, D. Weinreich and R. Simon, Designing prospective clinical pharmacogenomic (PG) trials: Meeting report on drug development strategies to enhance therapeutic decision making, *Pharmacogenomics Journal*, 2006, 6, 2, 89-94

251. L. D. True, Methodological requirements for valid tissue-based biomarker studies that can be used in clinical practice, *Virchows Archiv*, 2014, 464, 3, 257-263

252. M. R. Trusheim, B. Burgess, S. X. Hu, T. Long, S. D. Averbuch, A. A. Flynn, A. Lieftucht, A. Mazumder, J. Milloy, P. M. Shaw, D. Swank, J. Wang, E. R. Berndt, F. Goodsaid and M. C. Palmer, Quantifying factors for the success of stratified medicine, *Nature Reviews Drug Discovery*, 2011, 10, 11, 817-833

253. R. S. Tuma, Biomarker developers face big hurdles, *Journal of the National Cancer Institute*, 2008, 100, 7, 456-457, 461

254. R. Valdes Jr, M. W. Linder and S. A. Jortani, What is next in pharmacogenomics? Translating it to clinical practice, *Pharmacogenomics*, 2003, 4, 4, 499-505

255. D. L. Veenstra, J. A. Roth, L. P. Garrison Jr, S. D. Ramsey and W. Burke, A formal risk-benefit framework for genomic tests: Facilitating the appropriate translation of genomics into clinical practice, *Genetics in Medicine*, 2010, 12, 11, 686-693

256. P. Vicini, M. R. Gastonguay and D. M. Foster, Model-based approaches to biomarker discovery and evaluation: a multidisciplinary integrated review, *Critical Reviews in Biomedical Engineering*, 2002, 30, 4-6, 379-418

257. H. Volzke, C. O. Schmidt, S. E. Baumeister, T. Ittermann, G. Fung, J. Krafczyk-Korth, W. Hoffmann, M. Schwab, H. E. Meyer zu Schwabedissen, M. Dorr, S. B. Felix, W. Lieb and H. K. Kroemer, Personalized cardiovascular medicine: concepts and methodological considerations, *Nature Reviews Cardiology*, 2013, 10, 6, 308-316

258. N. Von Ahsen and M. Oellerich, Chip-based genotyping: Translation of pharmacogenetic research to clinical practice, *Clinical Chemistry*, 2007, 53, 7, 1186-1187

259. J. A. Wagner, Strategic approach to fit-for-purpose biomarkers in drug development, *Annual Review of Pharmacology & Toxicology*, 2008, 48, 631-651

260. J. A. Wagner, Biomarkers: principles, policies, and practice, *Clinical Pharmacology & Therapeutics*, 2009, 86, 1, 3-7

261. S. A. Waldman and A. Terzic, The roadmap to personalized medicine, *Clinical and translational science*, 2008, 1, 2, 93

262. S. A. Waldman and A. Terzic, Patient-centric clinical pharmacology advances the path to personalized medicine, *Biomarkers in Medicine*, 2011, 5, 6, 697-700

263. S. J. Wang, N. Cohen, D. A. Katz, G. Ruano, P. M. Shaw and B. Spear, Retrospective validation of genomic biomarkers - What are the questions, challenges and strategies for developing useful relationships to clinical outcomes - Workshop summary, *Pharmacogenomics Journal*, 2006, 6, 2, 82-88

264. D. G. Warnock and C. C. Peck, A roadmap for biomarker qualification, *Nature Biotechnology*, 2010, 28, 5, 444-445

265. P. Weeke and D. M. Roden, Applied pharmacogenomics in cardiovascular medicine, In: (Ed: 2014, Annual Reviews Inc., 4139 El Camino Way, P.O. Box 10139, Palo Alto CA 94306, United States

266. R. L. Weiss, The long and winding regulatory road for laboratory- developed tests, *American Journal of Clinical Pathology*, 2012, 138, 1, 20-26

267. R. J. Willke, W. Crown, M. Del Aguila, M. J. Cziraky, Z. M. Khan and R. Migliori, Melding regulatory, pharmaceutical industry, and U.S. payer perspectives on improving approaches to heterogeneity of treatment effect in research and practice, *Value in Health*, 2013, 16, 6 Suppl, S10-15

268. J. Woodcock, Janet Woodcock discusses the FDA and the drug development process, *Drug Discovery Today*, 2004, 9, 13, 548-550

269. J. Woodcock, Molecular medicine: how, what, and when?, *Clinical Pharmacology &*

Therapeutics, 2007, 82, 4, 376-378

270. J. Woodcock, The prospects for "personalized medicine" in drug development and drug therapy, *Clinical Pharmacology and Therapeutics*, 2007, 81, 2, 164-169

271. J. Woodcock, Perspective: The human genome and translational research: How much evidence is enough?, *Health Affairs*, 2008, 27, 6, 1616-1618

272. E. Zerhouni, Medicine. The NIH Roadmap, *Science*, 2003, 302, 5642, 63-72

273. R. L. Zimmern, Testing challenges: Evaluation of novel diagnostics and molecular biomarkers, *Clinical Medicine, Journal of the Royal College of Physicians of London*, 2009, 9, 1, 68-73

274. I. Zineh and S. M. Huang, Biomarkers in drug development and regulation: A paradigm for clinical implementation of personalized medicine, *Biomarkers in Medicine*, 2011, 5, 6, 705-713

275. I. Zineh and L. J. Lesko, Pharmacogenetics in medicine: Barriers, critical factors and a framework for dialogue, *Personalized Medicine*, 2009, 6, 4, 359-361

276. K. C. Zoon, Future directions in cancer research: impact of the completion of the human genome, *Toxicologic Pathology*, 2004, 32 Suppl 1, 1-2

277. J. Zou, M. W. Zheng, G. Li and Z. G. Su, Advanced systems biology methods in drug discovery and translational biomedicine, *BioMed Research International*, 2013, 2013, 742835

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APPENDIX 8. DETAILED SUMMARY OF FRAMEWORK MODELS II-IV

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8.1. MODEL II (ALONGSIDE PHASED DRUG EVALUATION)

8.1.1. IDENTIFIED FRAMEWORKS

Three papers were identified that matched model II (alongside phased drug evaluation), as shown in Table 11.[34-36] These were published in 2009[35,36] or 2011[34] and included four[34,35] or six stages.[36] Two of these focused on predictive biomarkers in cancer[34,35] and one on –omics biomarkers in cardiovascular disease.[36] The authors were based in the UK[34,35] or Canada.[36] None of the papers reported the basis for the frameworks.

Table 11 Frameworks matched to model II

Framework	Stages (number)	Scope	Country/ region	Basis
Garcia 2011[34]	4	cancer; parallel drug and predictive biomarker development	UK	NR
Hodgson 2009[35]	4	predictive biomarkers in cancer	UK	NR
Lin 2009[36]	6	cardiovascular disease; -omics biomarkers	Canada	NR

NR – not reported

As shown in Table 2.10, all included frameworks matched well the stages in the proposed model. The only exception was that an implementation stage was only present in one paper.[36]

Table 12 Labels and stage numbers in identified frameworks matched to model II

Stage	Garcia 2011[34]	Hodgson 2009[35]	Lin 2009[36]
Pre-clinical	(1) Preclinical discovery and analytical assay validation	(1) Pre-clinical	(1-3) (1) Discovery (2) Internal validation (3) External validation
phase I trial	(2) Phase I trial clinical qualification	(2) Phase 1 (biomarker validation)	(4) clinical trial (phase I, II)
phase II trial	(3) Phase II trial clinical qualification	(3) Phase 2 trial against comparator in biomarker +ve and -ve patients	(4) Clinical trial (phase I, II)
phase III trial	(4) Phase III trial clinical qualification	(4) Phase 3 Preparation for commercial launch	(5) Large clinical trial (phase III)
implementation			(6) Continued surveillance

8.1.2. STUDY DESIGNS AND OUTCOMES OF EACH STAGE

Table 14 presents the proposed study designs and outcomes of each of the stages identified for this model.

Pre-clinical

The suggested study designs for the pre-clinical stage were reported in two papers and these were: literature reviews,[35,36] pre-clinical models (details NR),[35] case-control studies and data-mining.[36] One framework suggested initial discovery study should be replicated for external validation.[36] Apart from identification of the candidate biomarker,[35,36] it was suggested this stage should provide understanding of the biomarker biology and assess performance characteristics of the assay.[36]

Phase I trials

The design of biomarker evaluation in phase I trials was not reported. However, two frameworks suggested that at this stage either the "clinical usefulness",[34] or the evaluability and prevalence of the biomarker should be established.[35]

Phase II trials

Two papers provided information on phase II trials.[34,35] One paper suggested either conducting an RCT (stratified design or randomised discontinuation design for prolonged stable disease), or deciding a study may not be needed if sufficient evidence is available from a phase I expansion cohort. This should provide evidence on the usefulness of the biomarker in a clinical setting and reproducibility, validity and variability of the assay.[34] The second paper suggested conducting a study to evaluate the benefit/risk ratio in all patients and biomarker-defined subgroups without specifying the design.[35]

Phase III trials

Again, the same two papers reported on the details of phase III trials.[34,35] Only one of these dentified relevant study designs

- RCT with biomarker-based inclusion criteria for biomarkers with high predictive value (most likely referring to an enrichment design), or
- stratified RCT otherwise.[34]

The outcome of this stage was demonstration of biomarker utility – according to two papers[34,35] and not reported in one.[36] One paper also proposed that at this stage the biomarker assay should be finalised.[35]

Implementation

None of the papers provided details of the implementation stage.

8.1.3. CRITERIA FOR ENTRY AND COMPLETION OF STAGES

As shown in Table 45, the criteria for entry to some of the stages were included in two papers,[34,35] and for completion in one paper for one stage.[34]

It was suggested that to enter the pre-clinical stage some understanding of the drug mechanism of action is required.[34]

There was no information on phase I trial phase. For entry into phase II trial *"data on biomarker evaluability and prevalence and estimate of effect size"* was required in one paper.[35] To complete phase II another paper suggested clinical validity should be demonstrated.[34]

Prior to a phase III trial one paper proposed clinical validity of the assay needs to be shown.[34] No information on the implementation stage was available.

Table 45 Model II criteria for stage entry and completion

	Garcia 2011[34]		Hodgson 2009[35]		Lin 2009[36]	
Stage	Entry	Completion	Entry	Completion	Entry	Completion
Pre-clinical	some understanding of how the drug kills tumour cells	NR	NR	NR	NR	NR
Phase I trial	NR	NR	NR	NR	NR	NR
Phase II trial	NR	clinical validity shown	*"data on biomarker evaluability and prevalence and estimate of effect size"*	NR	NR	NR
Phase III trial	clinical validity of the assay	NR	NR	NR	NR	NR
implementation	N/A	N/A	N/A	N/A	NR	NR

N/A – not applicable; NR – not reported;

Table 14 Model II study designs and outcomes assessed within studies

Stage	Garcia 2011[34] Study design	Garcia 2011[34] Outcomes	Hodgson 2009[35] Study design	Hodgson 2009[35] Outcomes	Lin 2009[36] Study design	Lin 2009[36] Outcomes
Pre-clinical	NR	NR	review or pre-clinical models	predictive hypothesis	NR directly, likely refers to case-control study; Also data- and literature-mining techniques replicate initial study in a new cohort - external validation	• identify candidate biomarkers; • understand biomarker biology • parameters for selection of biomarker candidates - use with cost-effectiveness and potential impact on management to select biomarkers for development • *"assay performance characteristics, and clinical validation"* showing *"link of the biomarker with a biological process or clinical end point"*
Phase I trial	NR	Biomarker clinically useful (identifies likely to respond);	NR	evaluability and prevalence of biomarker	NR	NR
Phase II trial	depending on NPV and PPV: • may not be needed - large expansion cohort (phase I) and reliable established assay • PPV and NPV low: RCT (new drug vs. placebo) stratified by biomarker • randomised discontinuation (if drug results in prolonged stable disease) - after treatment randomise to drug or placebo	Biomarker clinically useful (identifies likely to respond); reproducibility, validity and variability of assay by testing multiple baseline samples	NR	*examine the benefit/risk ratio in all potential patients within an existing indication and compare it with the benefit/ risk ratio in a sub-set of patients as defined by the candidate predictive biomarker.*	NR	NR
Phase III trial	RCT • biomarker-based inclusion - high predictive value • stratified - less previous evidence	Biomarker can select patients most likely to benefit	NR	• clinical utility of biomarker • assay converted to final format	NR	NR
implementation	N/A	N/A	N/A	N/A	NR	NR

N/A – not applicable; NR – not reported; RCT – randomised controlled trial; PPV – positive predictive value; NPV – negative predictive value

8.2. MODEL III (MULTI-MARKER CLASSIFIER)

8.2.1. IDENTIFIED FRAMEWORKS

There were seven papers[37-43] that described frameworks matched to model III (multi-marker classifier). Five of these were published since 2011, however two were older (published in 2005[43] and 2006[38]). These are described in more detail in Table 47. The number of major stages varied between two and five and four of the frameworks also contained from four to 16 sub-stages.[38,40-42] None of the frameworks was focused exclusively on predictive biomarkers. One of the frameworks specified the clinical area for which it was intended as cancer.[43] Three papers reported the basis of the proposed framework and this was: literature searches,[39] a committee with members from a variety backgrounds (including governmental, regulatory and clinical),[40] and experience from an observational study.[42] Four of the papers were written by authors based in the USA,[38-40,42,43] one in Korea[37] and one in Japan. [41]

Table 47 Frameworks matched to model III

Framework	Stages (number)	Scope	Country / region	Basis
Cho 2012[37]	5	predictive and prognostic biomarkers	Korea	NR
Ginsburg 2006[38]	5 (+ 7 sub-stages)	genomic biomarkers	USA	NR
Ioannidis 2011[39]	5	proteomic biomarkers	USA	literature searches (details NR)
IOM 2012[40]	2 (+ 9 sub-stages)	-omics tests to guide patient treatment	USA	*"IOM committee was convened to help clarify questions about how to effectively develop omics-based tests to enable progress toward improving patient outcomes; with support from NCI, FDA, the Centers for Disease Control and Prevention, the U.S. Department of Veterans Affairs, the American Society for Clinical Pathology, and the College of American Pathologists"*
Matsui 2013[41]	3 (+ 4 sub-stages)	multigene classifiers; mainly predictive, but also prognostic	Japan	NR
Shahzad 2012[42]	2 (+ 16 sub-stages)	any gene expression biomarker panel; systems biology approach	USA	experience from an observational study (CARGO)
Simon 2005[43]	5	cancer; multigene expression classifiers (mainly prognostic)	USA	NR

NR – not reported;

The order of stages in the identified frameworks was generally in agreement with that proposed for model III with one exception (Ginsburg 2006[38]), as shown in Table 2.11. All frameworks included stages corresponding to identification of candidate biomarkers, prediction model development, external validation and clinical utility. The pre-discovery stage was not included in two[41,43] and internal validation in one framework.[38] Implementation was only included in two frameworks.[38,39]

8.2.2. STUDY DESIGNS AND OUTCOMES OF EACH STAGE

Pre-discovery

As shown in Table 48, the study designs in the pre-discovery phase reflected the different sources of data to be later used for discovery. Where reported, these were: cell-line experiments,[37] genetic association studies[38] and a pilot of a multicentre study.[42] The outcomes of this phase generally included: formulation of a question to be addressed,[42] collection of data (of sufficient quality) from experiments,[37-40] a validated discovery platform[38,39] and a protocol for a multicentre study.[42]

Table 48 Model III: pre-discovery stage

Framework	Study design	outcomes
Cho 2012[37]	Cell line experiments to collect genomic or genetic characteristics and patterns of drug activity	Normalised data
Ginsburg 2006[38]	Genetic association studies	Collect clinical and biological data; validate discovery platform
Ioannidis 2011[39]	NR	Discovery tools - refine old or develop new: • *characterisation and improvement of analytical validity* • data quality control • standardisation of laboratory procedures and database annotations
IOM 2012[40]	NR	Quality controlled data collected
Matsui 2013[41]	N/A	N/A
Shahzad 2012[42]	Pilot multicentre study	Formulated question of interest protocol for multicentre study and logistics including training needs (modified after pilot study)
Simon 2005[43]	N/A	N/A

N/A – not applicable; NR – not reported;

Table 49 Labels and stage numbers in identified frameworks matched to model III

stage	Cho 2012[37]	Ginsburg 2006[38]	Ioannidis 2011[39]	IOM 2012[40]	Matsui 2013[41]	Shahzad 2012[42]	Simon 2005[43]
pre-discovery	(1-2) (1) Data collection (2) Quality control/ pre-processing	(1-2) (1) Biomarker discovery (2) Clinical and biological data collection	(1) (1) Analytical tools	(1.1.1) (1) Discovery and Test Validation Stage (1.1)Discovery Phase (1.1.1) Step 1: Data Quality Control		(1.1-1.2) (1) phased approach (1.1) Clinical phenotype consensus definition (1.2) Establishment of study logistics (1.2.1) initial protocol (1.2.2) feasibility studies (1.2.3) pilot studies (1.2.4) problem identification (1.2.5) trouble shooting (1.2.6) protocol modification (1.2.7) individual training (1.2.8) new protocol	
identification of candidate biomarkers	(3) (3) Identification of candidate biomarkers	(1, 2.1) (1) Biomarker discovery (2.1) Biomarker validation	(2) (2) Clinically oriented discovery	(1.1.2) (1.1.2) Step 2: Computational Model Development and Cross-Validation	(1.1) (1)Developing genomic signatures (1.1) gene screening	(1.3) (1.3) Candidate gene discovery	(1) (1) developing a genomic classifier
prediction model development	(4) (4) Construction of prediction model	(3) (3) Predictive model development	(2) (2) Clinically oriented discovery	(1.1.2) (1.1.2) Step 2: Computational Model Development and Cross-Validation	(1.2 - 2.1) (1.2) ranking and selection (2) prediction analysis (2.1) development of predictor	(1.4-1.5.1) (1.4) Differential Gene List Validation/ Verification (1.5) Molecular Classifier Algorithm Development (1.5.1) identification of classifier genes and cutoff	(1) (1) developing a genomic classifier

stage	Cho 2012[37]	Ginsburg 2006[38]	Ioannidis 2011[39]	IOM 2012[40]	Matsui 2013[41]	Shahzad 2012[42]	Simon 2005[43]
internal validation	(4) Construction of prediction model		(3) Validation	(1.1.2) Step 2: Computational Model Development and Cross-Validation	(2.2) Clinical validation of predictors	(1.5.2) Independent Testing of Selected Classifier Genes	(2-3) (2) Internal validation of a classifier in developmental studies (3) Evaluating if classifier is superior to existing prognostic factors
external validation	(4) Construction of prediction model	(5) Implementation (5.1) Development of diagnostic test	(3) Validation	(1.1.3 - 1.2) (1.1.3) Step 3: Confirmation on an Independent Dataset (1.1.4) Step 4: Release of Data, Code, and the Fully Specified Computational Procedures to the Scientific Community (1.2) Test Validation Phase (1.2.1) Analytical Validatiton (1.2.3) Clinical/ Biological Validation (1.2.4) implementation of the new test in the workflow and quality management system of the CLIA-certified laboratory	(2.2) Clinical validation of predictors	(1.6) External Classifier Validation/ Testing	(4) Translation of platforms and demonstrating assay reproducibility
clinical utility	(5) Independent validation of prediction	(4) Decision support tool development	(4) Clinical application	(2) Evaluation for Clinical Utility and Use Stage	(3) Biomarker-Based Clinical Trials for	(2) Comparison against standard of care & personalized use	(5) Independent validation of genomic classifier

stage	Cho 2012[37]	Ginsburg 2006[38]	Ioannidis 2011[39]	IOM 2012[40]	Matsui 2013[41]	Shahzad 2012[42]	Simon 2005[43]
					Assessing Clinical Utility		
implementation		(5) Implementation (5.2) Health professional and public education (5.3) Development of clinical guidelines (5.4) Regulatory oversight in laboratories (5.5) Cost-effectiveness (5.6) Privacy	(4-5) (4) Clinical application (5) Post-clinical appraisal				

Identification of candidate biomarkers

The study designs suggested for this stage are shown in Table 50. These were mainly focused around different statistical techniques, such as two-sample t-tests, variant t-tests, empirical Bayes methods,[37] or variety of pattern recognition techniques[38]). One paper suggested two different approaches that can be used on their own or in combination: non-hypothesis driven and hypothesis driven discovery.[42] Another paper suggested that the findings should be replicated in a new study and biological plausibility studies carried out to investigate the relationship between the biomarker and the outcome.[38]

Table 50 Model III: identification of candidate biomarkers

Framework	Study design	outcomes
Cho 2012[37]	Data analysis (various statistical approaches possible)	candidate genes
Ginsburg 2006[38]	• data analysis of genetic association study • replication of genetic association studies; • biological plausibility studies of biological rationale for the biomarkers	candidate markers
Ioannidis 2011[39]	Transparent statistical analysis (details NR)	multi-marker profiles
IOM 2012[40]	NR	NR
Matsui 2013[41]	Multiple approaches possible; most popular: separate statistical tests for each gene to test the null hypothesis of no association	identification of genes
Shahzad 2012[42]	Analysis of data from a multicentre study: • non-hypothesis-driven, "whole-genome wide approach" Bioinformatics can be used to identify differentially expressed genes "in relationship to the concurrent phenotype of interest" • hypothesis-driven, knowledge-based approach (can involve a literature review) - "focusing on known genes " • both approaches can be combined Laboratory techniques: array-based non-supervised approach may require additional assays (eg. RT-PCR) to confirm findings	identification of genes
Simon 2005[43]	NR	NR

NR – not reported;

Prediction model development

As shown in Table 51, a variety of statistical models have been suggested here including linear discriminant analysis, support vector machines, Bayesian regression, partial least squares, principal component regression.[37] Three papers identify two major tasks within this stage:

3) selection of biomarkers to be included in the classifier (feature selection) and

4) construction of the prediction model.[40,41,43]

The IOM 2012 framework suggested using approaches for development of the classifier (split sample and cross-validation) which correspond to what other papers describe as internal validation of the model.[40]

Table 51 Model III: prediction model development stage

Framework	Study design	outcomes
Cho 2012[37]	Classification methods (various approaches possible)	multivariate prediction model
Ginsburg 2006[38]	Methods include classical biostatistical methods and pattern recognition techniques	predictive model
Ioannidis 2011[39]	Transparent statistical analysis (details NR)	multi-marker profiles
IOM 2012[40]	• feature selection (based on data analysis and prior knowledge) • development of model by: ✓ splitting data into training and testing set, or ✓ cross-validation	development of model
Matsui 2013[41]	• Selection of genes for clinical platform (based on magnitude of association or effect size and possibly biological understanding) • Prediction model construction - statistical models: univariate models; hierarchical mixture models	prediction model
Shahzad 2012[42]	Use validated genes to identify classifier (details NR)	classifier
Simon 2005[43]	Developing a classifier: • establish type of statistical model (linear classifiers are usually sufficient) • feature selection - number small enough to be used in practice	classifier

Internal validation

Internal validation, as shown in Table 52, was often described as involving two main approaches:

- Split-sample - where the available sample of patients is divided into a training set (used for model development) and a separate test set (used for testing the performance of the classifier),[40-43]
- Cross-validation – where statistical techniques using a single set of patients for both development and validation of the classifier are implemented;[37,39-43]

One paper referred to cross-validation as less preferred and performed in the situation of low availability of patient samples.[40]

Generally, the aim of internal validation was to assess the performance of the classifier. The details of statistical measures used for these purpose were reported in two frameworks and these were mainly measures of accuracy such as classification error rate,[37,43] AUC ROC,[37] Sen, Spe, PPV and NPV.[43]

Table 52 Model III: internal validation stage

Framework	Study design	outcomes
Cho 2012[37]	various statistical techniques including: leave-one-out approach, random splitting	Assessment of the model performance using measures such as: • classification error rate, • AUC ROC, • product of posterior classification probabilities, • misclassification-penalized posterior.
Ginsburg 2006[38]	N/A	N/A
Ioannidis 2011[39]	statistical techniques: internal cross-validation or bootstrapping methods	estimates of classification accuracy
IOM 2012[40]	Internal validation integrated into model development: • training set/test set approach – preferred (*"two distinct datasets, (…) each composed of independent samples that have been collected and processed by different investigators at different institutions"*) • cross-validation – limited availability of samples (*"using a single dataset, by dividing the data into multiple segments, and iteratively fitting the model to all but one segment and then evaluating its performance on the remaining segment"*)	Evaluation of model performance
Matsui 2013[41]	*"typically using validation techniques such as split-sample or cross-validation"*	*"predictive accuracy for the study population from which the predictor was built"*
Shahzad 2012[42]	Two approaches: • *"collected patient samples are typically randomly split into separate training and validation sets. (…) genes identified in the training set and are tested in a validation set to estimate the degree of misclassification"* • *"leave-one-out cross validation (…) involves using a single observation from the original sample as the validation data, and the remaining observations as the training data. This is repeated such that each observation in the sample is used once as the validation data."*	degree of misclassification
Simon 2005[43]	two approaches to internal validation: • split-sample validation - "partitioning the set of samples into a training set and a test set" • cross-validation – *"based on repeated model development and testing on random data partitions (..) many variants of cross-validation and bootstrap resampling for classification problems"*	• estimate overall error rate • for split-sample validation possible to estimate characteristics such as sensitivity, specificiy, PPV and NPV

N/A – not applicable; AUC – area under the curve; ROC – receiver operator characteristic, PPV – positive predictive value; NPV – negative predictive value;

External validation

As shown in Table 53, the stages mapped to external validation all seem to identify this stage with conducting a new study on an independent sample of patients. In addition some of the frameworks advocated this should be a large-scale[39] and/or multicentre

study,[37,40] involving a diverse population,[39] relevant to the intended use of the test.[40,41] In some frameworks it was also proposed that the analysis should be blind to patient outcomes and ideally performed by investigators not involved in model development.[39,40] One paper also suggested that collection and processing of biological samples should be carried out by a laboratory that was not involved in model development.[40] According to one framework this should be a study testing a hypothesis that the classifier score distinguishes the phenotype in question.[42]

The outcomes of this stage generally involve showing the adequate performance of the classifier, which in some papers is defined in terms of test accuracy,[38,41,42] precision[38,42] and reproducibility.[38,42,43] Two papers also mentioned showing the generalisability of the test.[39,40] One paper suggested specifying the type of the analyte to be used.[38] Another advised investigation of the influence of specimen handling on the results of the test (including issues such as "*operator-to-operator variation, run-to-run variation, lot-to-lot variation of reagents, plate-to-plate variation, and section-to-section variation of the plates used to run the test*").[42] One paper postulated a prototype test platform should be developed at this stage,[38] while another two suggested standardisation of the test.[40,43] Investigation of cost-effectiveness of the biomarker use was also mentioned at this stage.[38]

One paper proposed conducting a separate analytical laboratory study, where the test is used on samples with known biomarker values aiming to quantify the test characteristics (such as analytical Sen, Spe and limit of detection).[40]

One framework also suggested that at the end of external validation, the data and computer code used for the classifier should be made available to the scientific community or at least the regulators to provide independent verification of the results obtained.[40]

Clinical utility

The clinical utility stage reported in Table 54 generally involved carrying out a new study, with the exception of one framework which only recommended testing the classifier in comparison to the standard of care without providing further details.[42]

The remaining papers advocated a clinical trial, usually a randomised one.[37,39-41,43] The suggested designs differed depending on the situation and, where reported, these were:

- enrichment,
- biomarker-strategy and

- prospective subgroup analysis of an RCT, and
- prospective-retrospective.[40,41,43]

Table 53 Model III: external validation stage

Framework	Study design	outcomes
Cho 2012[37]	multicentre validation (details NR)	performance of statistical model tested
Ginsburg 2006[38]	study in other assembled populations and/or biological samples	• develop prototype platform • retest the predictor • *"development of an easy, cost-effective, accurate and reliable test assay"* • precision and reproducibility • *"analytical problems (…) such as the type of controls to use, turnaround time, throughput, detection limit, accuracy, quantification, on-site availability and portability, cost of replicate analysis, and normalization."* • specify type of analyte to be used
Ioannidis 2011[39]	large-scale studies performed by *"many diverse teams in various populations"* Ideally *"analysis (…) by different investigators than those involved in the original analysis"*	• adequate classification performance • show generalisability
IOM 2012[40]	• 'analytical laboratory studies on samples with known biomarker values; *"using specimens comparable to* [those] *on which the test will eventually be used, with known or expected characteristics related to the test being validated. If necessary, an alternative to using a limited supply of valuable clinical specimens with known or expected test results is the use of control materials that will provide known or expected test results and can be spiked into negative clinical specimens"* • *study "using an independent set of samples not used in the generation" of the model and ideally "blinded to any outcome or phenotypic data"; "independent specimen and clinical dataset must be relevant to the intended use" of the test "Ideally, the specimens (…) will have been collected at a different point in time, at different institutions, from a different patient population, with samples processed in a different laboratory"* • *"data and meta-data used for development of the candidate omics-based test should be made available"* with computer code and fully specified computational development procedures for of the candidate omics-based test – to scientific community or at least the FDA	• quantify technical variations of a test performed on patient specimens or spiked control materials • test characteristics including: *"accuracy, precision, reproducibility, linearity, reportable range, analytical sensitivity and specificity, and limit of detection"* • defined computational procedures and data management procedures • two possible levels of evidence: ✓ Lower Level: *"Independent sets of specimens and clinical data collected at a single institution using carefully controlled protocols, with samples from the same patient population"* ✓ Higher Level: *"Independent sets of specimens and clinical data collected at multiple institutions"* • broad applicability of test • *"verification of results by the scientific community"* • standard operating procedure

Framework	Study design	outcomes
Matsui 2013[41]	*"using an independent set of samples, possibly from a more relevant population for clinical application of the predictor."*	• predictive accuracy (usually proportion of correct classification, sensitivity and specificity) • *"establish that the predictive accuracy is statistically higher than that expected when there is no relationship between genomic data and the clinical variable"*
Shahzad 2012[42]	Study in independent patient sample to test the pre-specified hypothesis that the classifier score distinguishes phenotype in question	• establish test reproducibility and demonstrate acceptable precision • may include *"documentation of diagnostic performance across thresholds and description of correlations to clinical variables"* • *"effect of different variables associated with the specimen handling process"* on test results
Simon 2005[43]	New study designed to show reproducibility (inter- and intra-laboratory)	• standardized assay • evaluation of reproducibility

NR – not reported;

Some papers indicated there may be a possibility of another study design as an alternative to a randomised trial. These were an observational study[39] and a single arm study including only biomarker positive patients where the event rate is very low.[43]

This stage should lead to establishing the clinical utility[37,39-41,43] and a fully developed, standardised classifier.[37,39] It should also provide information on how feasible is using the classifier in clinical practice.[41]

Implementation

Only two papers reported any details of implementation,[38,39] as shown in Table 55. Both of these considered this stage appropriate for investigation of cost-effectiveness. One of the papers suggested at this stage issues such as education, policy and regulation of the test should be considered.[38] The other one included audit of the actual use in practice and cost-utility of the test.[39]

8.2.3. CRITERIA FOR ENTRY AND COMPLETION OF STAGES

Five of the six papers reported on at least some of the criteria for entry into a stage[37,39-43] as shown in Table 56.

For pre-discovery it was proposed by one paper that the phenotype of interest should be defined (for example the outcome to be predicted)[42]. In another paper it was suggested that prior to embarking on any classifier research it should be considered whether it is possible to develop a classifier to address a given problem (for example in terms of the required sensitivity and specificity).[40]

For entry into the discovery stage it was proposed that adequate quality [40] or adequately normalised data[37] should be available and study infrastructure and logistics established.[42]

Table 54 Model III (multi-marker classifier): clinical utility stage

Framework	Study design	outcomes
Cho 2012[37]	RCT	• usefulness of prediction model • developed assay
Ginsburg 2006[38]	clinical trial (details NR)	NR
Ioannidis 2011[39]	observational or randomised studies	• possible refinement or expansion of applications • "incremental value over standard information" (usually reclassification), impact on decision-making • clinical standardization, possible commercialization of test • impact on surrogate (non-clinical or clinical) and "hard" outcomes
IOM 2012[40]	clinical study design consulted with regulators, depends on intended use of the test and availability of "*appropriate archived specimens*": • prospective–retrospective (especially when prospective not feasible) • prospective trial with test that "does not direct patient management": ✓ subgroup analysis – biomarker results can be generated at start or end of trial ✓ stratified • prospective trial with test that "does direct patient management" ✓ enrichment – "*clinical utility of some of the test-designated categories is already established or assumed and need not be re-evaluated*" ✓ biomarker strategy– "*fully defined and validated omics-based test*"; direct assessment of utility	Test evaluated for clinical use
Matsui 2013[41]	RCTs: • biomarker strategy, • enrichment - if "*compelling biological evidence for believing that biomarker-negative patients will not benefit*" • Randomize-All Designs ✓ with a single, completely specified biomarker (prospective subgroup analysis) - no compelling evidence biomarker negative will not benefit; ✓ more complex designs with biomarker development and validation	establish that: • biomarker actionable in clinical practice • use of the biomarker leads to improved outcome in patients and patient benefit
Shahzad 2012[42]	test classifier compared with standard care (details NR)	clinical acceptance
Simon 2005[43]	preferably prospective clinical trials: • biomarker-strategy, • enrichment • single arm including only biomarker positive (low event rate) • possibly prospective-retrospective • prospective subgroup analysis of RCT and splitting type I error (little faith in classifier)	establish patient benefit from using the classifier

Table 55 Model III (multi-marker classifier): implementation stage

Framework	Study design	outcomes
Cho 2012[37]	N/A	N/A
Ginsburg 2006[38]	• education of health professionals and public on the use of genomic biomarkers • Include genomic biomarkers in clinical guidelines • regulation of tests and oversight of laboratory standards • cost-effectiveness analysis • develop policy to regulate patient privacy	• *implement biomarker into clinical practice* • cost-effectiveness
Ioannidis 2011[39]	cost-effectiveness analyses Audit of: • appropriate use • improved outcomes • *"cost-utility based on actual use"*	• cost-utility • test adopted to clinical practice • monitoring of test use
IOM 2012[40]	N/A	N/A
Matsui 2013[41]	N/A	N/A
Shahzad 2012[42]	N/A	N/A
Simon 2005[43]	N/A	N/A

N/A – not applicable; NR – not reported;

For entry into prediction model construction the requirements provided were a manageable number of candidate genes[37] and data of sufficient quality.[40]

To initiate internal validation, it was claimed a model needs to be completed.[40,41] For external validation, complete model[40,41] and satisfactory results from internal validation were required.[40,43]

To initiate investigation of clinical utility two papers required completion of discovery and validation of the model.[39,40] One of these also highlighted that at this point the classifier needs to be *"locked-down"*. [40]

None of the papers reported the requirements for entry into the implementation stage.

Only one paper reported the criteria for completion of one stage (internal validation in the proposed model) and it was: *"A candidate omics-based test should be defined precisely, including the molecular measurements, the computational procedures, and the intended clinical use of the test"*.[40]

Table 56 Entry criteria for stages of model III (multi-marker classifier)

stage	Cho 2012[37]	Ginsburg 2006[38]	Ioannidis 2011[39]	IOM 2012[40]	Matsui 2013[41]	Shahzad 2012[42]	Simon 2005[43]
pre-discovery	NR	NR	NR	considering if potential classifier *"has a reasonable chance of demonstrating clinical validity and utility. For example, the sensitivity and specificity needed, particularly in light of the prevalence of the condition in the population"*	N/A	consensus on phenotype definition	N/A
identification of candidate biomarkers	adequate data normalisation	NR	NR	data of adequate quality	NR	established multicentre infrastructure and logistics for sample processing	NR
prediction model development	candidate genes limited to "a few hundred"	NR	NR	data of adequate quality	NR	NR	NR
internal validation	NR	N/A	NR	model developed	*"a completely specified genomic signature is needed. Complete specification (…) includes not only the list of component genes, but also the mathematical form used to combine genomic data for the genes used in the signature, weights for the relative importance of the genes, and cut-off values when making classification."*	NR	NR
external validation	NR	NR	NR	*"discovery and confirmation of a candidate omics-based test"*	Same as internal validation	NR	satisfactory internal validaty
clinical utility	NR	NR	discovery and validation	*"fully defined, validated, and locked-down clinical test"*	NR	NR	NR
implementation	N/A	NR	NR	N/A	N/A	N/A	N/A

N/A – not applicable; NR – not reported;

8.3. MODEL IV (SAFETY)

8.3.1. IDENTIFIED FRAMEWORKS

Two papers published within the last five years were identified that described a framework that matched model IV (safety), shown in Table 57. One proposed four,[44] and the other - five stages[45]. Both of these papers appeared to focus on a situation when drugs are already on the market when new safety biomarkers are identified. One focused on biomarkers for prediction of adverse events in a paediatric population,[45] and the other on identification of a range of biomarkers relevant to safety.[44] Both papers were based on the experience and practice of organisations that aim to discover and develop safety biomarkers – one based in Canada[45] and one in the EU.[44]

Table 57 Frameworks matched to model IV (safety)

Framework	Stages (number)	Scope	Country/ region	Basis
Loo 2012[45]	5	prediction of paediatric adverse events	Canada	experience of Canadian Pharmacogenomics Network for Drug Safety (adverse drug reactions *"surveillance network that predominantly operates within Canadian pediatric teaching hospitals"*)
Matheis 2011[44]	4	adverse event biomarkers (predictive, diagnostic, monitoring)	EU	proposed by Safer and Faster Evidence-based Translation consortium, *"a public–private partnership comprising 20 partners from the pharmaceutical industry, small–medium enterprises, academic institutions and clinical units of excellence with representatives from the European Medicines Agency (EMA) as external observers and advisors. It operates under the framework of the EU Innovative Medicines Initiative Joint Undertaking"*[44]

Neither of the two papers described a framework that exactly matched the stages proposed for model IV, as shown in Table 2.12. Some of activities corresponding to the stages in model IV were mentioned in one paper outside of the sequence of stages for the framework.[44]

The unique characteristic of this model is that it appears to suggest progression into investigation of biomarker utility immediately after preclinical investigation and analytical validation.

8.3.2. STUDY DESIGNS AND OUTCOMES OF EACH STAGE

The study designs and outcomes relevant to each stage are reported in Table 60.

Surveillance

For this stage one paper suggested recruitment of patients with adverse events and matched controls.[45] Although this stage was not explicitly identified in the other paper, it did propose that a dedicated biobank should be established.[44] None of the papers discussed the outcomes of this stage.

Table 58 Labels and stage numbers in identified frameworks matched to model IV (safety)

	Loo 2010[45]	Matheis 2011[44]
surveillance	(1) Active surveillance, patient recruitment and collection of data and biomaterial	(NR as stage, but setting up a dedicated biobank discussed)
discovery	(2) Identification of gene variants and replication of findings	(1) Candidate biomarker identification
pre-clinical	(3) Pharmacokinetic and functional validation	(NR as stage, but referred to as pre-requisite to stage 2)
analytical validation		(2) Exploratory phase
clinical utility	(4) Prospective clinical studies to evaluate diagnostic utility	(3) Confirmatory phase
implementation	(5) Determination of the cost-effectiveness of diagnostic testing	(4) Submit for regulatory approval

Discovery

The discovery stage was based on a case-control study approach in one framework, utilising candidate gene approach supplemented by GWAS and followed by a replication study in a different population.[45] The other paper did not provide extensive detail on this stage and advocated the use of a literature review, databases and a biobank.[44] None of the papers discussed the outcomes of this stage.

Pre-clinical

Pre-clinical mechanism investigation was described in one framework and was based on *in vitro* and animal model studies.[45] None of the papers discussed outcomes of this stage.

Analytical validation

This stage was described in one framework only and involved a small study in healthy subjects and patients comparing the biomarker test under investigation to the gold standard. The suggested outcomes to be assessed were the performance of the test

(sensitivity, specificity, PPV, NPV, ROC AUC, or partial ROC AUC), the biological variability of the biomarker and stability of the analyte after sampling.[44]

Clinical utility

Studies addressing clinical utility of the biomarker were described as either:

- a prospective clinical trial aiming to establish the utility of the predictive biomarker in preventing adverse events,[45] or
- a study in a large patient population ("proof of performance") aiming to establish biomarker performance and threshold. [44]

Implementation

There was no information on the study designs or outcomes of the implementation stage.

8.3.3. CRITERIA FOR ENTRY AND COMPLETION OF STAGES

These criteria were described only in Matheis 2011 for two stages,[44] as shown in Table 60.

Entry into the analytical validation stage was to be based on pre-clinical and clinical evidence supporting the biomarker and discussion of the validation protocol with regulatory agencies. Completion of this stage was to be based on "assay acceptance criteria" defined prior to undertaking the validation and, if applicable, in accordance with regulatory guidance. For biomarker utility only entry criteria were described and these were presentation of the results of analytical validation to regulatory agencies.

Table 59 Entry and completion criteria for stages of model IV (safety) papers

	Loo 2010[45]		Matheis 2011[44]	
Stage	entry	completion	entry	completion
surveillance	NR	NR	NR	NR
discovery	NR	NR	NR	NR
pre-clinical	NR	NR	NR	NR
analytical validation	N/A	N/A	biomarker "*supported by solid scientific evidence and a clear rationale based on available preclinical and clinical data*" study protocol "*discussed with regulatory agencies* "	assay acceptance criteria "*defined in the standard validation procedure and are, if applicable, in accordance with EMA and FDA guidelines*"
clinical utility	NR	NR	results of exploratory studies presented to regulatos	NR
implementation	NR	NR	NR	NR

N/A – not applicable; NR – not reported;

Table 60 Model IV (safety) study designs and outcomes assessed within studies

	Loo 2010[45]		Matheis 2011[44]	
Stage	Study design	Outcomes assessed	Study design	Outcomes assessed
surveillance	Recruitment (multisite) of patients with adverse events and matched controls; collection of clinical data and biological samples	NR	NR for stage (set up biobank)	NR
discovery	Splitting patient sample into: (1) discovery: case-control study - usually patients from one area (homogeneity) - candidate gene approach - key genes involved in drug biotransformation or toxicity - GWAS– possibly complementary strategy - not limited by *a priori* set of genes; generates a larger number of false-positives; needs large sample size to identify true positives, (2) replication in a different population - ensures generalisability and limits false positives	NR	mainly literature review, databases and biobank	NR
pre-clinical	pharmacokinetic and functional studies to support relevance of identified genes in the mechanism of drug toxicity: *in vitro* assays (overexpression or knock-down of associated gene expression) or animal models	NR	NR	NR
analytical validation	N/A	N/A	proof of translation - *"conducted with small groups of healthy subjects or patients (…) for testing the translational value of selected biomarker candidates in comparison to current gold standards"*	Selection of best biomarkers based on: • sensitivity, specificity, PPV, NPV, • biomarker variability in healthy volunteers and patients, • impact of covariates (e.g. age, gender)on biomarker values • *in vivo* stability biomarker metabolism and circadian rhythm effects)," • *"stability after sampling"* • *"characteristics of the intended assay"* • ROC AUC, or partial ROC AUC
clinical utility	prospective clinical trial where appropriate	utility in preventing adverse events	proof of performance - in large patient populations	• biomarker threshold • biomarker performance
implementation	NR	NR	NR	NR

N/A – not applicable; NR – not reported;

APPENDIX 9. BIOMARKERS EXCLUDED IN THE SECOND STAGE OF SCREENING WITH REASONS FOR EXCLUSION.

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Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
3β-Hydroxy-**Δ5**-C27-steroid oxidoreductase deficiency	defines disease	Digestive System Diseases Metabolism, Inborn Errors	*Treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ5-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults. Treatment must be initiated and monitored by an experienced hepatologist or a paediatric hepatologist in the case of paediatric patients*	cholic acid (Orphacol)
activated protein C (APC) resistance	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of Zoely. Should any of the conditions appear for the first time during Zoely use, the medicinal product should be stopped immediately. (...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant). ...*	nomegestrol acetate / estradiol (Zoely)
activated protein C (APC) resistance	ICD10	Contraception	*Female contraception* *EVRA is intended for women of fertile age. The safety and efficacy has been established in women aged 18 to 45 years* *Contraindications:* *EVRA should not be used in the presence of one of the following disorders. If one of these disorders occurs during the use of EVRA, EVRA must be discontinued immediately(...)* *● Possible hereditary predisposition for venous or arterial thrombosis, such as activated protein C (APC-) resistance, antithrombin-III deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinemia, and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	norelgestromin / ethinyl estradiol (EVRA)
activated protein C (APC) resistance	ICD10	Contraception	*Oral contracepon.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of IOA. Should any of the conditions appear for the first time during IOA use, the medicinal product should be stopped immediately. (...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (IOA)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
adverse cytogenetics	associated with another treatment	NR	*treatment of acute myeloid leukaemia (AML) in elderly patients who have one or more of the following: adverse cytogenetics, secondary AML, ≥ 70 years old or significant co-morbidities and are therefore not considered suitable for intensive chemotherapy. Safety and efficacy have been assessed in studies of patients ≥ 65 years old (see section 5.1)*	Clofarabine (Evoltra)
ALT	diagnostic	Hepatitis C, Chronic	*Adult patients with histologically proven chronic hepatitis C who are positive for HCV antibodies or HCV RNA and have elevated serum alanine aminotransferase (ALT) without liver decompensation. The efficacy of Interferon-alfa-2a in the treatment of hepatitis C is enhanced when combined with ribavirin. Alpheon should be given alone mainly in case of intolerance or contra-indication to ribavirin.*	recombinant human interferon-alfa-2a (Alpheon)
ALT	diagnostic	Hepatitis B, Chronic	*Baraclude is indicated for the treatment of chronic hepatitis B virus (HBV) infection (see section 5.1) in adults with:* *§ compensated liver disease and evidence of active viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active inflammation and/or fibrosis.* *§ decompensated liver disease (see section 4.4)* *For both compensated and decompensated liver disease, this indication is based on clinical trial data in nucleoside naive patients with HBeAg positive and HBeAg negative HBV infection. With respect to patients with lamivudine-refractory hepatitis B, see sections 4.4 and 5.1.*	Entecavir (Baraclude)
ALT	diagnostic	Hepatitis B, Chronic	*Hepsera is indicated for the treatment of chronic hepatitis B in adults with:* *• compensated liver disease with evidence of active viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active liver inflammation and fibrosis* *• decompensated liver disease.*	adefovir dipivoxil (Hepsera)
ALT	diagnostic	Hepatitis B, Chronic	*Treatment of adult patients with chronic hepatitis B associated with evidence of hepatitis B viral replication (presence of DNA of hepatitis B virus (HBV-DNA) and hepatitis B antigen (HBeAg), elevated alanine aminotransferase (ALT) and histologically proven active liver inflammation and/or fibrosis.*	interferon alfa-2b (IntronA; Viraferon)
ALT	diagnostic	Hepatitis B, Chronic	*Zeffix is indicated for the treatment of chronic hepatitis B in adults with:* *- ƒcompensated liver disease with evidence of active viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active liver inflammation and/or fibrosis. Initiation of lamivudine treatment should only be considered when the use of an alternative antiviral agent with a higher genetic barrier is not available or appropriate (see in section 5.1).* *- decompensated liver disease in combination with a second agent without cross-resistance to lamivudine (see section 4.2).*	Lamivudine (Lamivudine Teva; Zeffix)
ALT	diagnostic	Hepatitis B, Chronic	*Pegasys is indicated for the treatment of HBeAg-positive or HBeAg-negative chronic hepatitis B in adult patients with compensated liver disease and evidence of viral replication, increased ALT and histologically verified liver inflammation and/or fibrosis (see sections 4.4 and 5.1).*	peginterferon alfa-2a (Pegasys)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
ALT	diagnostic	Hepatitis C, Chronic	*Tritherapy:* *Rebetol in combination with boceprevir and peginterferon alfa-2b is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection in adults patients (18 years of age and older) with compensated liver disease who are previously untreated or who have failed previous therapy.* *Please refer to peginterferon alfa -2b and boceprevir SmPCs when using Rebetol in combination with these medicines.* *Bitherapy:* *Rebetol is indicated for the treatment of chronic hepatitis C virus infection in adults, children 3 years of age and older and adolescents and must only be used as part of a combination regimen with peginterferon alfa-2b or interferon alfa-2b. Rebetol monotherapy must not be used.* *Please refer to interferon alfa-2b and peginterferon alfa-2b SmPCs when using Rebetol in combination with these medicines.* *There is no safety or efficacy information on the use of Rebetol with other forms of interferon (i.e., not alfa-2b).* *Previously untreated (naïve) patients* *Adult patients (18 years of age or older): Rebetol is indicated for:* *• tritherapy - in combination with peginterferon alfa-2b and boceprevir for the treatment of adult patients with chronic hepatitis C genotype 1 infection with compensated liver disease.* *• bitherapy - in combination with interferon alfa-2b or peginterferon alfa-2b, for the treatment of adult patients with chronic hepatitis C, not previously treated, without liver decompensation, with elevated alanine aminotransferase (ALT), who are positive for hepatitis C viral ribonucleic acid (HCV-RNA).* *• bitherapy – for the treatment of CHC infection in combination with peginterferon alfa-2b for patients with compensated cirrhosis and/or clinically stable HIV co-infection (see section 4.4).* *Bitherapy* *Paediatric patients (children 3 years of age and older and adolescents): Rebetol is indicated, in a combination regimen with peginterferon alfa-2b or interferon alfa-2b, for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, not previously treated, without liver decompensation, and who are positive for HCV-RNA. When deciding to not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Previously treated patients* *Adult patients: Rebetol is indicated for:* *• tritherapy - in combination with peginterferon alfa-2b and boceprevir for the treatment of adult patients having CHC genotype 1 infection with compensated liver disease.* *• bitherapy - in combination with peginterferon alfa-2b, for the treatment of patients with chronic hepatitis C who have failed previous treatment with interferon alpha (pegylated or nonpegylated) alone or in combination with ribavirin (see section 5.1).* *• bitherapy - in combination with interferon alfa-2b, for the treatment of patients with chronic*	Ribavirin (Rebetol; Ribavirin BioPartners; Ribavirin Mylan (previously Ribavirin Three Rivers); Ribavirin Teva; Ribavirin Teva Pharma B.V.)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			hepatitis C who have previously responded (with normalisation of ALT at the end of treatment) to interferon alfa monotherapy but who have subsequently relapsed.	
ALT	diagnostic	Hepatitis B, Chronic	*Sebivo is indicated for the treatment of chronic hepatitis B in adult patients with compensated liver disease and evidence of viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active inflammation and/or fibrosis.* *Initiation of Sebivo treatment should only be considered when the use of an alternative antiviral agent with a higher genetic barrier to resistance is not available or appropriate.* *See section 5.1 for details of the study and specific patient characteristics on which this indication is based.*	Telbivudine (Sebivo)
ALT	diagnostic	Hepatitis B, Chronic	*Viread (...) indicated for the treatment of chronic hepatitis B (see section 5.1) in adults for whom a solid dosage form is not appropriate with:* *· compensated liver disease, with evidence of active viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active inflammation and/or fibrosis* *· decompensated liver disease (see sections 4.4, 4.8 and 5.1).* *Viread (..) also indicated for the treatment of chronic hepatitis B in adolescents 12 to < 18 years of age for whom a solid dosage form is not appropriate with:* *· compensated liver disease and evidence of immune active disease, i.e. active viral replication, persistently elevated serum ALT levels and histological evidence of active inflammation and/or fibrosis (see sections 4.4, 4.8 and 5.1).*	tenofovir disoproxil fumarate (Viread)
ALT	diagnostic	Hepatitis B, Chronic	*Zeffix is indicated for the treatment of chronic hepatitis B in adults with:* *ƒ compensated liver disease with evidence of active viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active liver inflammation and/or fibrosis. Initiation of lamivudine treatment should only be considered when the use of an alternative antiviral agent with a higher genetic barrier is not available or appropriate (see in section 5.1).* *ƒ decompensated liver disease in combination with a second agent without cross-resistance to lamivudine (see section 4.2).*	Lamivudine (Zeffix)
ALT	diagnostic	Prostatic Neoplasms	*Contraindications:* *· Hypersensitivity to cabazitaxel, to other taxanes, or to any excipients of the formulation including polysorbate 80.* *· Neutrophil counts less than 1,500/mm3.* *· Hepatic impairment (bilirubin ≥1 x ULN, or AST and/or ALT≥1.5 × ULN).* *· Concomitant vaccination with yellow fever vaccine (see section 4.5).*	Cabazitaxel (Jevtana)
ALT	diagnostic	Hypertension, Pulmonary	*Contraindications:* *(...)* *Elevated aminotransferases prior to initiation of treatment (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) > 3 x ULN)...*	sitaxentan sodium (Thelin)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
ALT	diagnostic	Hypertension, Pulmonary Scleroderma, Systemic	*Contraindications:* *(...) Baseline values of liver aminotransferases, i.e., aspartate aminotransferases (AST) and/or alanine aminotransferases (ALT), greater than 3 times the upper limit of normal (see section 4.4)...*	bosentan monohydrate (Tracleer)
ALT	diagnostic	Hypertension, Pulmonary	*Contraindications:* *(...) Baseline values of hepatic aminotransferases (aspartate aminotransferases (AST) and/or alanine aminotransferases (ALT))>3xULN (see sections 4.2 and 4.4)...*	Ambrisentan (Volibris)
ALT	diagnostic	HIV Infections	*Contraindications;* *(...) Patients with severe hepatic impairment (Child-Pugh C) or pre-treatment ASAT or ALAT > 5 ULN until baseline ASAT/ALAT are stabilised < 5 ULN...*	Nevirapine (Viramune; Nevirapine Teva)
antibodies against IgA	diagnostic	Immunologic Deficiency Syndromes Guillain-Barre Syndrome Bone Marrow Transplantation Purpura, Thrombocytopenic, Idiopathic Mucocutaneous Lymph Node Syndrome	*Contraindications:* *Hypersensitivity to the active substance or to any of the excipientslisted in section 6.1 (see alsosection 4.4).* *Hypersensitivity to human immunoglobulins, especially in patients with antibodies against IgA.* *Patients with hyperprolinaemia.*	human normal immunoglobulin (ivig) (Privigen)
anti-dsDNA	monitoring	Lupus Erythematosus, Systemic	*Benlysta is indicated as add-on therapy in adult patients with active, autoantibody-positive systemic lupus erythematosus (SLE) with a high degree of disease activity (e.g positive anti-dsDNA and low complement) despite standard therapy (see section 5.1).*	Belimumab (Benlysta)
antiphospholipid antibodies	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of Zoely. Should any of the conditions appear for the first time during Zoely use, the medicinal product should be stopped immediately. (...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (Zoely)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
antithrombin-III deficiency	ICD10	Contraception	*Female contraception* *EVRA is intended for women of fertile age. The safety and efficacy has been established in women aged 18 to 45 years* *Contraindications:* *EVRA should not be used in the presence of one of the following disorders. If one of these disorders occurs during the use of EVRA, EVRA must be discontinued immediately (...)* *● Possible hereditary predisposition for venous or arterial thrombosis, such as activated protein C (APC-) resistance, antithrombin-III deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinemia, and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	norelgestromin / ethinyl estradiol (EVRA)
antithrombin-III deficiency	defines disease	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of IOA. Should any of the conditions appear for the first time during IOA use, the medicinal product should be stopped immediately. (...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (IOA)
antithrombin-III-deficiency	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of Zoely. Should any of the conditions appear for the first time during Zoely use, the medicinal product should be stopped immediately. (...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (Zoely)
AST	diagnostic		*Contraindications:* *· Hypersensitivity to cabazitaxel, to other taxanes, or to any excipients of the formulation including polysorbate 80.* *· Neutrophil counts less than 1,500/mm3.* *· Hepatic impairment (bilirubin ≥1 x ULN, or AST and/or ALT≥1.5 × ULN).* *· Concomitant vaccination with yellow fever vaccine (see section 4.5).*	Cabazitaxel (Jevtana)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
AST	diagnostic	Hypertension, Pulmonary	*Contraindications:* *(...) Elevated aminotransferases prior to initiation of treatment (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) > 3 x ULN)...*	sitaxentan sodium (Thelin)
AST	diagnostic	Hypertension, Pulmonary Scleroderma, Systemic	*Contraindications:* *(...) Baseline values of liver aminotransferases, i.e., aspartate aminotransferases (AST) and/or alanine aminotransferases (ALT), greater than 3 times the upper limit of normal (see section 4.4)…*	bosentan monohydrate (Tracleer)
AST	diagnostic	Hypertension, Pulmonary	*Contraindications:* *(...) Baseline values of hepatic aminotransferases (aspartate aminotransferases (AST) and/or alanine aminotransferases (ALT))>3xULN (see sections 4.2 and 4.4).*	Ambrisentan (Volibris)
AST	diagnostic	HIV Infections	*Contraindications;* *(…) Patients with severe hepatic impairment (Child-Pugh C) or pre-treatment ASAT or ALAT > 5 ULN until baseline ASAT/ALAT are stabilised < 5 ULN…*	Nevirapine (Viramune; Nevirapine Teva)
autoantibody-positive	diagnostic	Lupus Erythematosus, Systemic	*Benlysta is indicated as add-on therapy in adult patients with active, autoantibody-positive systemic lupus erythematosus (SLE) with a high degree of disease activity (e.g positive anti-dsDNA and low complement) despite standard therapy (see section 5.1).*	Belimumab (Benlysta)
B-cell	ICD10	Leukemia, Lymphocytic, Chronic, B-Cell	*MabCampath is indicated for the treatment of patients with B-cell chronic lymphocytic leukaemia (BCLL) for whom fludarabine combination chemotherapy is not appropriate.*	Alemtuzumab (MabCampath)
B-cell	ICD10	Lymphoma, Non-Hodgkin	*MabThera is indicated for the treatment of patients with CD20 positive diffuse large B cell nonHodgkin's lymphoma in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy.*	Rituximab (MabThera)
B-cell	ICD10	Lymphoma, Follicular	*[90Y]-radiolabelled Zevalin is indicated as consolidation therapy after remission induction in previously untreated patients with follicular lymphoma. The benefit of Zevalin following rituximab in combination with chemotherapy has not been established.* *[90Y]-radiolabelled Zevalin is indicated for the treatment of adult patients with rituximab relapsed or refractory CD20+ follicular B-cell non-Hodgkin's lymphoma (NHL).*	ibritumomab tiuxetan (Zevalin)
B-cell	ICD10	Lymphoma, Non-Hodgkin	*Pixuvri is indicated as monotherapy for the treatment of adult patients with multiply relapsed or refractory aggressive Non-Hodgkin B-cell Lymphomas (NHL). The benefit of pixantrone treatment has not been established in patients when used as fifth line or greater chemotherapy in patients who are refractory to last therapy*	pixantrone dimaleate (Pixuvri)
BMI	defines disease	Obesity	*As an adjunct to diet and exercise for the treatment of obese patients (BMI ≥ 30 kg/m2), or overweight patients (BMI > 27 kg/m2) with associated risk factor(s), such as type 2 diabetes or dyslipidaemia (see section 5.1)*	Rimonabantrimonabant (Acomplia)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
BMI	defines disease	Obesity	*alli is indicated for weight loss in adults who are overweight (body mass index, BMI >= 28 kg/m2) and should be taken in conjunction with a mildly hypocaloric, lower-fat diet*	Orlistat (Alli (previously Orlistat GSK))
C1 inhibitor deficiency	ICD10	Angioedemas, Hereditary	*Firazyr is indicated for symptomatic treatment of acute attacks of hereditary angioedema (HAE) in adults (with C1-esterase-inhibitor deficiency).*	Icatibant (Firazyr)
C1 inhibitor deficiency	ICD10	Angioedema	*for use as replacement treatment in acute attacks of angioedema in patients with congenital C1 inhibitor activity deficiency*	recombinant human C1 inhibitor (Rhucin)
C1 inhibitor deficiency	ICD10	Angioedemas, Hereditary	*Ruconest is indicated for treatment of acute angioedema attacks in adults with hereditary angioedema (HAE) due to C1 esterase inhibitor deficiency.*	conestat alfa (Ruconest)
carcinoembryonic antigen	non-therapeutic	Radionuclide Imaging Colorectal Neoplasms	*CEA-Scan is indicated only in patients with histologically-demonstrated carcinoma of the colon or rectum for imaging of recurrence and/or metastases. CEA-Scan is employed for diagnostic use only, in the above mentioned patients, as an adjunct to standard non-invasive imaging techniques, such as ultrasonography or CT scan, in the following situations:* *Patients with evidence of recurrence and/or metastatic carcinoma of the colon or rectum, who are undergoing an evaluation for extent of disease, such as prior to surgical resection and/or other therapy, or* *Patients with suspected recurrence and/or metastatic carcinoma of the colon or rectum in association with rising levels of carcinoembryonic antigen (CEA).*	Arcitumomab (CEA-Scan)
CD 30+	diagnostic	Hodgkin Disease	*ADCETRIS is indicated for the treatment of adult patients with relapsed or refractory CD30+ Hodgkin lymphoma (HL):* *1. following autologous stem cell transplant (ASCT) or* *2. following at least two prior therapies when ASCT or multi-agent chemotherapy is not a treatment option*	brentuximab vedotin (Adcetris)
CD20+	diagnostic	Lymphoma, Non-Hodgkin	*MabThera is indicated for the treatment of patients with CD20 positive diffuse large B cell nonHodgkin's lymphoma in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy.*	Rituximab (MabThera)
CD20+	diagnostic	Lymphoma, Follicular	*[90Y]-radiolabelled Zevalin is indicated as consolidation therapy after remission induction in previously untreated patients with follicular lymphoma. The benefit of Zevalin following rituximab in combination with chemotherapy has not been established.* *[90Y]-radiolabelled Zevalin is indicated for the treatment of adult patients with rituximab relapsed or refractory CD20+ follicular B-cell non-Hodgkin's lymphoma (NHL).*	ibritumomab tiuxetan (Zevalin)
CD4	monitoring	Sarcoma, Kaposi	*For treatment of AIDS-related Kaposi's sarcoma (KS) in patients with low CD4 counts (< 200 CD4 lymphocytes/mm3) and extensive mucocutaneous or visceral disease.* *Caelyx may be used as first-line systemic chemotherapy, or as second line chemotherapy in AIDS-*	doxorubicin hydrochloride (Caelyx)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			KS patients with disease that has progressed with, or in patients intolerant to, prior combination systemic chemotherapy comprising at least two of the following agents: a vinca alkaloid, bleomycin and standard doxorubicin (or other anthracycline).	
CD4	non-therapeutic	Mumps Rubella Immunization Measles	*Contraindications:* *(…) Humoral or cellular (primary or acquired) immunodeficiency, including hypogammaglobulinemia and dysgammaglobulinemia and AIDS, or symptomatic HIV infection or an age-specific CD4+ T lymphocyte percentage <25% (see section 4.4). In severely immunocompromised individuals inadvertently vaccinated with measles-containing vaccine, measles inclusion body encephalitis, pneumonitis, and fatal outcome as a direct consequence of disseminated measles vaccine virus infection have been reported...*	virus, live attenuated, measles, virus, live attenuated, mumps, virus, live attenuated, rubella (M-M-RVAXPRO)
CD4	non-therapeutic	Mumps Chickenpox Rubella Immunization Measles	*Contraindications:* *(…) Humoral or cellular (primary or acquired) immunodeficiency, including hypogammaglobulinemia and dysgammaglobulinemia and AIDS, or symptomatic HIV infection or a CDC Class 2 or higher or an age-specific CD4+ T-lymphocyte percentage <25% (see section 4.4). In severely immunocompromised individuals inadvertently vaccinated with measles-containing vaccine, measles inclusion body encephalitis, pneumonitis, and fatal outcome as a direct consequence of disseminated measles vaccine virus infection have been reported...*	virus, live attenuated, measles, virus, live attenuated, mumps, virus, live attenuated, rubella, virus, live attenuated, varicella (Proquad)
CD4	monitoring	HIV Infections	*SUSTIVA is indicated in antiviral combination treatment of human immunodeficiency virus-1 (HIV-1) infected adults, adolescents and children 3 years of age and older. SUSTIVA has not been adequately studied in patients with advanced HIV disease, namely in patients with CD4 counts < 50 cells/mm3,or after failure of protease inhibitor (PI) containing regimens. Although cross-resistance of efavirenz with PIs has not been documented, there are at present insufficient data on the efficacy of subsequent use of PI based combination therapy after failure of regimens containing SUSTIVA. For a summary of clinical and pharmacodynamic information, see section 5.1.*	Efavirenz (Stocrin; Sustiva; Efavirenz Teva)
Child-Pugh score	diagnostic	HIV Infections	*Contraindications:* *(…) Patients with moderate or severe (Child-Pugh B or C) hepatic impairment…*	Tipranavir (Aptivus)
Child-Pugh score	diagnostic	Angina, Unstable Acute Coronary	*Contraindications:* *(…) Severe hepatic impairment (Child Pugh class C).*	Prasugrel (Efient)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
		Syndrome Myocardial Infarction		
Child-Pugh score	diagnostic	HIV Infections	*Contraindications;* *(…) Patients with severe hepatic impairment (Child-Pugh C) or pre-treatment ASAT or ALAT > 5 ULN until baseline ASAT/ALAT are stabilised < 5 ULN…*	Nevirapine (Viramune; Nevirapine Teva)
Child-Pugh score	diagnostic	Hypertension, Pulmonary	*Contraindications:* *(...) Mild to severe hepatic impairment (Child-Pugh Class A-C)…*	sitaxentan sodium (Thelin)
Child-Pugh score	diagnostic	Hypertension, Pulmonary Scleroderma, Systemic	*Contraindications:* *(...) Moderate to severe hepatic impairment, i.e.,Child-Pugh class Bor C (see section 5.2)…*	bosentan monohydrate (Tracleer)
Child-Pugh score	diagnostic	Hepatitis C, Chronic	*Contraindications:* *(…) HCV/HIV patients with cirrhosis and a Child-Pugh score ≥ 6…*	peginterferon alfa-2b (PegIntron; ViraferonPeg)
Child-Pugh score	diagnostic	Hepatitis B, Chronic	*Contraindications:* *(…) Initiation of Pegasys is contraindicated in HIV-HCV patients with cirrhosis and a Child-Pugh score ≥ 6, except if only due to indirect hyperbilirubinemia caused by drugs such as atazanavir and indinavir...*	peginterferon alfa-2a (Pegasys)
congenital factor IX deficiency	ICD10	Hemophilia B	*Treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency)*	nonacog alfa (BeneFIX)
congenital factor VIII deficiency	ICD10	Hemophilia A	*Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).* *ADVATE does not contain von Willebrand Factor in harmacologically effective quantities and is therefore not indicated in von Willebrand disease.*	octocog alfa (Advate)
creatine phosphokinase elevation	diagnostic	Dyslipidemias	*Contraindications:* *(…) Personal history of myopathy and/or rhabdomyolysis with statins and/or fibrates or confirmed creatine phosphokinase (CK) elevation above 5 times the upper limit of normal (ULN) under previous statin treatment (see section 4.4).*	fenofibrate / pravastatin (Pravafenix)
CYP2D6	metabolic	HIV Infections	*Contraindications:* *(…) Agenerase with ritonavir must not be co-administered with medicinal products with narrow therapeutic windows that are highly dependent on CYP2D6 metabolism, e.g. flecainide and propafenone (see section 4.5)…*	Amprenavir (Agenerase)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
CYP3A	metabolic	HIV Infections	*Contraindications:* *(…) Co-administration of APTIVUS with low dose ritonavir, with active substances that are highly dependent on CYP3A for clearance, and for which elevated plasma concentrations are associated with serious and/or life-threatening events, is contraindicated...*	Tipranavir (Aptivus)
CYP3A	metabolic	HIV Infections	*Contraindications:* *(...) Eviplera should not be co-administered with the following medicinal products as significant decreases in rilpivirine plasma concentrations may occur (due to CYP3A enzyme induction or gastric pH increase), which may result in loss of therapeutic effect of Eviplera...*	emtricitabine / rilpivirine / tenofovir disoproxil (Eviplera)
CYP3A	metabolic	Hepatitis C, Chronic	*Contraindications:* *(…) Concomitant administration with active substances that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events. These active substances include alfuzosin, amiodarone, bepridil, quinidine, astemizole, terfenadine, cisapride, pimozide, ergot derivatives (dihydroergotamine, ergonovine, ergotamine, methylergonovine), lovastatin, simvastatin, atorvastatin, sildenafil or tadalafil (only when used for treatment of pulmonary arterial hypertension) and orally administered midazolam or triazolam. Concomitant administration with Class Ia or III antiarrhythmics, except for intravenous lidocaine (see section 4.5).* *Concomitant administration of INCIVO with active substances that strongly induce CYP3A e.g. rifampicin, St John's wort (Hypericum perforatum), carbamazepine, phenytoin and phenobarbital and thus may lead to lower exposure and loss of efficacy of INCIVO...*	Telaprevir (Incivo)
CYP3A	metabolic	HIV Infections	*Contraindications:* *(…) Kaletra contains lopinavir and ritonavir, both of which are inhibitors of the P450 isoform CYP3A. Kaletra should not be co-administered with medicinal products that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with serious and/or life threatening events. These medicinal products include...*	lopinavir / ritonavir (Kaletra)
CYP3A4	metabolic	HIV Infections	*Contraindications:* *(...) Agenerase must not be administered concurrently with medicinal products with narrow therapeutic windows that are substrates of cytochrome P450 3A4 (CYP3A4). Co-administration may result in competitive inhibition of the metabolism of these medicinal products and create the potential for serious and/or life-threatening adverse events such as cardiac arrhythmia (e.g. amiodarone, bepridil, quinidine, terfenadine, astemizole, cisapride, pimozide), respiratory depression and /or prolonged sedation (e.g. oral triazolam and oral midazolam (for caution on parenterally administered midazolam, see section 4.5)) or peripheral vasospasm or ischaemia and ischaemia of other tissues, including cerebral or myocardial ischaemia (e.g. ergot derivatives)...*	Amprenavir (Agenerase)
CYP3A4	metabolic	HIV Infections	*Contraindications:* *(…) Co-administration with terfenadine, astemizole, cisapride, midazolam, triazolam, pimozide, bepridil, or ergot alkaloids (for example, ergotamine, dihydroergotamine, ergonovine, and*	efavirenz / emtricitabine / tenofovir

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			methylergonovine). Competition for cytochrome P450 (CYP) 3A4 by efavirenz could result in inhibition of metabolism and create the potential for serious and/or life-threatening adverse reactions (for example, cardiac arrhythmias, prolonged sedation or respiratory depression) (see section 4.5)...	disoproxil (Atripla)
CYP3A4	metabolic	Peripheral Vascular Diseases Acute Coronary Syndrome	*Contraindications:* *(...) Co-administration of ticagrelor with strong CYP3A4 inhibitors (e.g., ketoconazole, clarithromycin, nefazodone, ritonavir, and atazanavir) is contraindicated, as co-administration may lead to a substantial increase in exposure to ticagrelor (see section 4.4 and 4.5)...*	Ticagrelor (Possia)
factors VII, VIII or IX	ICD10	Hemophilia B Thrombasthenia Factor VII Deficiency Hemophilia A	*NovoSeven is indicated for the treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups:* *• in patients with congenital haemophilia with inhibitors to coagulation factors VIII or IX > 5 Bethesda Units (BU)* *• in patients with congenital haemophilia who are expected to have a high anamnestic response to factor VIII or factor IX administration* *• in patients with acquired haemophilia* *• in patients with congenital FVII deficiency* *• in patients with Glanzmann's thrombasthenia with antibodies to GP IIb – IIIa and/or HLA, and with past or present refractoriness to platelet transfusions.*	eptacog alfa (activated) (NovoSeven)
G6PD deficiency	ICD10	Hyperuricemia	*Contraindications:* *Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.* *G6PD deficiency and other cellular metabolic disorders known to cause haemolytic anaemia. Hydrogen peroxide is a by-product of the conversion of uric acid to allantoin. In order to prevent possible haemolytic anaemia induced by hydrogen peroxide, rasburicase is contraindicated in patients with these disorders.*	Rasburicase (Fasturtec)
G6PD deficiency	ICD10	Methemoglobinemia	*Contraindications:* *· Hypersensitivity to the active substance, or to any other thiazine dyes* *· Patients with Glucose-6-phosphate dehydrogenase deficiency (G6PD) due to the risk of haemolytic anaemia* *· Patients with sodium nitrite-induced methaemoglobinaemia* *· Patients with methaemoglobinaemia due to chlorate poisoning* *· Deficiency in NADPH reductase.*	Methylthioninium chloride (Methylthioninium chloride Proveblue)
genetic testing (familial lipoprotein lipase deficiency)	defines disease	Hyperlipoproteinemia Type I	*Glybera is indicated for adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions. The diagnosis of LPLD has to be confirmed by genetic testing. The indication is restricted to patients with detectable levels of LPL protein (see section 4.4).*	alipogene tiparvovec (Glybera)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
genotype 1	associated with another treatment	Hepatitis C, Chronic	*Tritherapy:* *Rebetol in combination with boceprevir and peginterferon alfa-2b is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection in adults patients (18 years of age and older) with compensated liver disease who are previously untreated or who have failed previous therapy.* *Please refer to peginterferon alfa -2b and boceprevir SmPCs when using Rebetol in combination with these medicines.* *Bitherapy:* *Rebetol is indicated for the treatment of chronic hepatitis C virus infection in adults, children 3 years of age and older and adolescents and must only be used as part of a combination regimen with peginterferon alfa-2b or interferon alfa-2b. Rebetol monotherapy must not be used.* *Please refer to interferon alfa-2b and peginterferon alfa-2b SmPCs when using Rebetol in combination with these medicines.* *There is no safety or efficacy information on the use of Rebetol with other forms of interferon (i.e., not alfa-2b).* *Previously untreated (naïve) patients* *Adult patients (18 years of age or older): Rebetol is indicated for:* *• tritherapy - in combination with peginterferon alfa-2b and boceprevir for the treatment of adult patients with chronic hepatitis C genotype 1 infection with compensated liver disease.* *• bitherapy - in combination with interferon alfa-2b or peginterferon alfa-2b, for the treatment of adult patients with chronic hepatitis C, not previously treated, without liver decompensation, with elevated alanine aminotransferase (ALT), who are positive for hepatitis C viral ribonucleic acid (HCV-RNA).* *• bitherapy – for the treatment of CHC infection in combination with peginterferon alfa-2b for patients with compensated cirrhosis and/or clinically stable HIV co-infection (see section 4.4).* *Bitherapy* *Paediatric patients (children 3 years of age and older and adolescents): Rebetol is indicated, in a combination regimen with peginterferon alfa-2b or interferon alfa-2b, for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, not previously treated, without liver decompensation, and who are positive for HCV-RNA. When deciding to not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Previously treated patients* *Adult patients: Rebetol is indicated for:* *• tritherapy - in combination with peginterferon alfa-2b and boceprevir for the treatment of adult patients having CHC genotype 1 infection with compensated liver disease.* *• bitherapy - in combination with peginterferon alfa-2b, for the treatment of patients with chronic hepatitis C who have failed previous treatment with interferon alpha (pegylated or nonpegylated) alone or in combination with ribavirin (see section 5.1).* *• bitherapy - in combination with interferon alfa-2b, for the treatment of patients with chronic*	Ribavirin (Rebetol; Ribavirin BioPartners; Ribavirin Mylan (previously Ribavirin Three Rivers); Ribavirin Teva; Ribavirin Teva Pharma B.V.)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			hepatitis C who have previously responded (with normalisation of ALT at the end of treatment) to interferon alfa monotherapy but who have subsequently relapsed.	
genotype 1	associated with another treatment	Hepatitis C, Chronic	*Ribavirin Teva is indicated for the treatment of chronic hepatitis C virus (HCV) infection in adults, children 3 years of age and older and adolescents and must only be used as part of a combination regimen with interferon alfa-2b. Ribavirin monotherapy must not be used.* *There is no safety or efficacy information on the use of Ribavirin with other forms of interferon (i.e., not alfa-2b).* *Naïve patients* *Adult patients: Ribavirin Teva is indicated, in combination with interferon alfa-2b, for the treatment of adult patients with all types of chronic hepatitis C except genotype 1, not previously treated, without liver decompensation, with elevated alanine aminotransferase (ALT), who are positive for hepatitis C viral ribonucleic acid HCV-RNA (see section 4.4).* *Children 3 years of age and older and adolescents: Ribavirin Teva is intended for use, in a combination regimen with interferon alfa2b, for the treatment of children and adolescents 3 years of age and older, who have all types of chronic hepatitis C except genotype 1, not previously treated, without liver decompensation, and who are positive for HCV-RNA.* *When deciding to not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Previous treatment failure patients* *Adult patients: Ribavirin Teva is indicated, in combination with interferon alfa-2b, for the treatment of adult patients with chronic hepatitis C who have previously responded (with normalisation of ALT at the end of treatment) to interferon alpha monotherapy but who have subsequently relapsed. (see* *section 5.1).*	Ribavirin (Ribavirin BioPartners; Ribavirin Mylan (previously Ribavirin Three Rivers); Ribavirin Teva; Ribavirin Teva Pharma B.V.)
GH receptor mutations	defines disease	Laron Syndrome	*For the long-term treatment of growth failure in children and adolescents from 2 to 18 years with severe primary insulin-like growth factor-1 deficiency (Primary IGFD).* *Severe Primary IGFD is defined by:* *· height standard deviation score £ –3.0 and* *· basal IGF-1 levels below the 2.5th percentile for age and gender and* *· GH sufficiency.* *· Exclusion of secondary forms of IGF-1 deficiency, such as malnutrition, hypothyroidism, or chronic treatment with pharmacologic doses of anti-inflammatory steroids.* *Severe Primary IGFD includes patients with mutations in the GH receptor (GHR), post-GHR signaling pathway, and IGF-1 gene defects; they are not GH deficient, and therefore, they cannot be expected to respond adequately to exogenous GH treatment. It is recommended to confirm the diagnosis by conducting an IGF-1 generation test.*	Mecasermin (Increlex)
H5N1 subtype of	non-therapeutic	Influenza, Human	*Active immunisation against H5N1 subtype of Influenza A virus.* *This indication is based on immunogenicity data from healthy subjects from the age of 18 years*	Influenza virus surface

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
Influenza A virus		Immunization Disease Outbreaks	*onwards following administration of two doses of the vaccine containing A/turkey/Turkey/1/05 (H5N1)-like strain (see section 5.1).*	antigens*, inactivated: A/Viet Nam/1194/2004 (H5N1) – like strain used (NIBRG-14) * produced in eggs (Aflunov)
HBeAg	doesn't distinguish	Hepatitis B, Chronic	*Baraclude is indicated for the treatment of chronic hepatitis B virus (HBV) infection (see section 5.1) in adults with:* *§ compensated liver disease and evidence of active viral replication, persistently elevated serum alanine aminotransferase (ALT) levels and histological evidence of active inflammation and/or fibrosis.* *§ decompensated liver disease (see section 4.4)* *For both compensated and decompensated liver disease, this indication is based on clinical trial data in nucleoside naive patients with HBeAg positive and HBeAg negative HBV infection. With respect to patients with lamivudine-refractory hepatitis B, see sections 4.4 and 5.1.*	Entecavir (Baraclude)
HBeAg	diagnostic	Hepatitis B, Chronic	*Treatment of adult patients with chronic hepatitis B associated with evidence of hepatitis B viral replication (presence of DNA of hepatitis B virus (HBV-DNA) and hepatitis B antigen (HBeAg), elevated alanine aminotransferase (ALT) and histologically proven active liver inflammation and/or fibrosis.*	interferon alfa-2b (IntronA; Viraferon)
HBeAg	doesn't distinguish	Hepatitis B, Chronic	*Pegasys is indicated for the treatment of HBeAg-positive or HBeAg-negative chronic hepatitis B in adult patients with compensated liver disease and evidence of viral replication, increased ALT and histologically verified liver inflammation and/or fibrosis (see sections 4.4 and 5.1).*	peginterferon alfa-2a (Pegasys)
HBV-DNA	non-therapeutic	Immunization, Passive Hepatitis B Liver Transplantation	*Prevention of hepatitis B virus (HBV) re-infection in HBV-DNA negative patients ≥ 6 months after liver transplantation for hepatitis B induced liver failure.* *Zutectra is indicated in adults only.* *The concomitant use of adequate virostatic agents should be considered, if appropriate, as standard of hepatitis B re-infection prophylaxis*	human hepatitis B immunoglobulin (Zutectra)
HBV-DNA	diagnostic	Hepatitis B, Chronic	*Treatment of adult patients with chronic hepatitis B associated with evidence of hepatitis B viral replication (presence of DNA of hepatitis B virus (HBV-DNA) and hepatitis B antigen (HBeAg), elevated alanine aminotransferase (ALT) and histologically proven active liver inflammation and/or fibrosis.*	interferon alfa-2b (IntronA; Viraferon)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
HCV antibodies	diagnostic	Hepatitis C, Chronic	*Adult patients with histologically proven chronic hepatitis C who are positive for HCV antibodies or HCV RNA and have elevated serum alanine aminotransferase (ALT) without liver decompensation. The efficacy of Interferon-alfa-2a in the treatment of hepatitis C is enhanced when combined with ribavirin. Alpheon should be given alone mainly in case of intolerance or contra-indication to ribavirin.*	recombinant human interferon-alfa-2a (Alpheon)
HCV genotype	prognostic	Hepatitis C, Chronic	*Adult patients:* *Viraferon is indicated for the treatment of adult patients with chronic hepatitis C who have elevated transaminases without liver decompensation and who are positive for serum HCV-RNA or anti-HCV (see section 4.4).* *The best way to use Viraferon in this indication is in combination with ribavirin.* *Chidren and adolescents:* *Viraferon is intended for use, in a combination regimen with ribavirin, for the treatment of children and adolescents 3 years of age and older, who have chronic hepatitis C, not previously treated, without liver decompensation, and who are positive for serum HCV-RNA. The decision to treat should be made on a case by case basis, taking into account any evidence of disease progression such as hepatic inflammation and fibrosis, as well as prognostic factors for response, HCV genotype and viral load. The expected benefit of treatment should be weighed against the safety findings observed for paediatric subjects in the clinical trials (see sections 4.4, 4.8 and 5.1).*	interferon alfa-2b (Viraferon)
HCV genotype	associated with another treatment	Hepatitis C, Chronic	*Adults (tritherapy):* *ViraferonPeg in combination with ribavirin and boceprevir (tritherapy) is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection in adult patients (18 years of age and older) with compensated liver disease who are previously untreated or who have failed previous therapy (see section 5.1).* *Please refer to the ribavirin and boceprevir Summary of Product Characteristics (SmPCs) when ViraferonPeg is to be used in combination with these medicines.* *Adults (bitherapy and monotherapy):* *ViraferonPeg is indicated for the treatment of adult patients (18 years of age and older) with CHC who are positive for hepatitis C virus RNA (HCV-RNA), including patients with compensated cirrhosis and/or co-infected with clinically stable HIV (see section 4.4).* *ViraferonPeg in combination with ribavirin (bitherapy) is indicated for the treatment of CHC infection in adult patients who are previously untreated including patients with clinically stable HIV coinfection and in adult patients who have failed previous treatment with interferon alpha (pegylated or nonpegylated) and ribavirin combination therapy or interferon alpha monotherapy (see section 5.1).* *Interferon monotherapy, including ViraferonPeg, is indicated mainly in case of intolerance or contraindication to ribavirin.* *Please refer to the ribavirin SmPC when ViraferonPeg is to be used in combination with ribavirin.* *Paediatric population (bitherapy):* *ViraferonPeg is indicated in a combination regimen with ribavirin for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, previously untreated,*	peginterferon alfa-2b (ViraferonPeg)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			without liver decompensation, and who are positive for HCV-RNA. *When deciding not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Please refer to the ribavirin SmPC for capsules or oral solution when ViraferonPeg is to be used in combination with ribavirin.*	
HCV RNA	diagnostic	Hepatitis C, Chronic	*Adult patients with histologically proven chronic hepatitis C who are positive for HCV antibodies or HCV RNA and have elevated serum alanine aminotransferase (ALT) without liver decompensation. The efficacy of Interferon-alfa-2a in the treatment of hepatitis C is enhanced when combined with ribavirin. Alpheon should be given alone mainly in case of intolerance or contra-indication to ribavirin.*	recombinant human interferon-alfa-2a (Alpheon)
HCV RNA	diagnostic	Hepatitis C, Chronic	*Treatment of patients of 18 years and older with chronic hepatitis and serum markers for hepatitis C virus (HCV) infection e.g. those who have elevated serum transaminase levels without decompensated liver disease and who are positive for serum HCV-RNA (see section 4.4). Consideration should be given to current official guidance on the appropriate use of interferons for the treatment of patients with chronic hepatitis C.* *Interferon alfacon-1 should be given alone mainly in case of intolerance or contraindication to ribavirin.*	interferon alfacon-1 (Infergen)
HCV RNA	diagnostic	Hepatitis C, Chronic	*Before initiating treatment with IntronA, consideration should be given to the results from clinical trials comparing IntronA with pegylated interferon (see section 5.1).* *Adult patients IntronA is indicated for the treatment of adult patients with chronic hepatitis C who have elevated transaminases without liver decompensation and who are positive for hepatitis C virus RNA (HCV RNA) (see section 4.4).* *The best way to use IntronA in this indication is in combination with ribavirin.* *Children 3 years of age and older and adolescents IntronA is indicated, in a combination regimen with ribavirin, for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, not previously treated, without liver decompensation, and who are positive for HCV-RNA.* *When deciding not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain* *The decision to treat should be made on a case by case basis (see section 4.4)*	interferon alfa-2b (IntronA; Viraferon)
HCV RNA	diagnostic	Hepatitis C, Chronic	*Pegasys is indicated for the treatment of chronic hepatitis C in adult patients who are positive for serum HCV-RNA, including patients with compensated cirrhosis and/or co-infected with clinically stable HIV (see section 4.4).* *The optimal way to use Pegasys in patients with chronic hepatitis C is in combination with ribavirin. The combination of Pegasys and ribavirin is indicated in naive patients and patients who have failed previous treatment with interferon alpha (pegylated or non-pegylated) alone or in*	peginterferon alfa-2a (Pegasys)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			combination therapy with ribavirin. *Monotherapy is indicated mainly in case of intolerance or contraindication to ribavirin*	
HCV RNA	diagnostic	Hepatitis C, Chronic	*Adults (tritherapy):* *PegIntron in combination with ribavirin and boceprevir (tritherapy) is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection in adult patients (18 years of age and older) with compensated liver disease who are previously untreated or who have failed previous therapy (see section 5.1).* *Please refer to the ribavirin and boceprevir Summary of Product Characteristics (SmPCs) when PegIntron is to be used in combination with these medicines.* *Adults (bitherapy and monotherapy):* *PegIntron is indicated for the treatment of adult patients (18 years of age and older) with CHC who are positive for hepatitis C virus RNA (HCV-RNA), including patients with compensated cirrhosis and/or co-infected with clinically stable HIV (see section 4.4).* *PegIntron in combination with ribavirin (bitherapy) is indicated for the treatment of CHC infection in adult patients who are previously untreated including patients with clinically stable HIV co-infection and in adult patients who have failed previous treatment with interferon alpha (pegylated or nonpegylated) and ribavirin combination therapy or interferon alpha monotherapy (see section 5.1).* *Interferon monotherapy, including PegIntron, is indicated mainly in case of intolerance or contraindication to ribavirin.* *Please refer to the ribavirin SmPC when PegIntron is to be used in combination with ribavirin.* *Paediatric population (bitherapy):* *PegIntron is indicated in a combination regimen with ribavirin for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, previously untreated, without liver decompensation, and who are positive for HCV-RNA.* *When deciding not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Please refer to the ribavirin SmPC for capsules or oral solution when PegIntron is to be used in combination with ribavirin.*	peginterferon alfa-2b (PegIntron; ViraferonPeg)
HCV RNA	diagnostic	Hepatitis C, Chronic	*Tritherapy:* *Rebetol in combination with boceprevir and peginterferon alfa-2b is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection in adults patients (18 years of age and older) with compensated liver disease who are previously untreated or who have failed previous therapy.* *Please refer to peginterferon alfa -2b and boceprevir SmPCs when using Rebetol in combination with these medicines.* *Bitherapy:* *Rebetol is indicated for the treatment of chronic hepatitis C virus infection in adults, children 3 years of age and older and adolescents and must only be used as part of a combination regimen with peginterferon alfa-2b or interferon alfa-2b. Rebetol monotherapy must not be used.*	Ribavirin (Rebetol; Ribavirin BioPartners; Ribavirin Mylan (previously Ribavirin Three Rivers); Ribavirin Teva;

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			Please refer to interferon alfa-2b and peginterferon alfa-2b SmPCs when using Rebetol in combination with these medicines. *There is no safety or efficacy information on the use of Rebetol with other forms of interferon (i.e., not alfa-2b).* *Previously untreated (naïve) patients* *Adult patients (18 years of age or older): Rebetol is indicated for:* *• tritherapy - in combination with peginterferon alfa-2b and boceprevir for the treatment of adult patients with chronic hepatitis C genotype 1 infection with compensated liver disease.* *• bitherapy - in combination with interferon alfa-2b or peginterferon alfa-2b, for the treatment of adult patients with chronic hepatitis C, not previously treated, without liver decompensation, with elevated alanine aminotransferase (ALT), who are positive for hepatitis C viral ribonucleic acid (HCV-RNA).* *• bitherapy – for the treatment of CHC infection in combination with peginterferon alfa-2b for patients with compensated cirrhosis and/or clinically stable HIV co-infection (see section 4.4).* *Bitherapy* *Paediatric patients (children 3 years of age and older and adolescents): Rebetol is indicated, in a combination regimen with peginterferon alfa-2b or interferon alfa-2b, for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, not previously treated, without liver decompensation, and who are positive for HCV-RNA. When deciding to not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Previously treated patients* *Adult patients: Rebetol is indicated for:* *• tritherapy - in combination with peginterferon alfa-2b and boceprevir for the treatment of adult patients having CHC genotype 1 infection with compensated liver disease.* *• bitherapy - in combination with peginterferon alfa-2b, for the treatment of patients with chronic hepatitis C who have failed previous treatment with interferon alpha (pegylated or nonpegylated) alone or in combination with ribavirin (see section 5.1).* *• bitherapy - in combination with interferon alfa-2b, for the treatment of patients with chronic hepatitis C who have previously responded (with normalisation of ALT at the end of treatment) to interferon alfa monotherapy but who have subsequently relapsed.*	Ribavirin Teva Pharma B.V.)
HCV-RNA	diagnostic	Hepatitis C, Chronic	*Adults (tritherapy):* *ViraferonPeg in combination with ribavirin and boceprevir (tritherapy) is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 infection in adult patients (18 years of age and older) with compensated liver disease who are previously untreated or who have failed previous therapy (see section 5.1).* *Please refer to the ribavirin and boceprevir Summary of Product Characteristics (SmPCs) when ViraferonPeg is to be used in combination with these medicines.* *Adults (bitherapy and monotherapy):*	peginterferon alfa-2b (ViraferonPeg)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			ViraferonPeg is indicated for the treatment of adult patients (18 years of age and older) with CHC who are positive for hepatitis C virus RNA (HCV-RNA), including patients with compensated cirrhosis and/or co-infected with clinically stable HIV (see section 4.4). *ViraferonPeg in combination with ribavirin (bitherapy) is indicated for the treatment of CHC infection in adult patients who are previously untreated including patients with clinically stable HIV coinfection and in adult patients who have failed previous treatment with interferon alpha (pegylated or nonpegylated) and ribavirin combination therapy or interferon alpha monotherapy (see section 5.1).* *Interferon monotherapy, including ViraferonPeg, is indicated mainly in case of intolerance or contraindication to ribavirin.* *Please refer to the ribavirin SmPC when ViraferonPeg is to be used in combination with ribavirin.* *Paediatric population (bitherapy):* *ViraferonPeg is indicated in a combination regimen with ribavirin for the treatment of children 3 years of age and older and adolescents, who have chronic hepatitis C, previously untreated, without liver decompensation, and who are positive for HCV-RNA.* *When deciding not to defer treatment until adulthood, it is important to consider that the combination therapy induced a growth inhibition. The reversibility of growth inhibition is uncertain. The decision to treat should be made on a case by case basis (see section 4.4).* *Please refer to the ribavirin SmPC for capsules or oral solution when ViraferonPeg is to be used in combination with ribavirin.*	
HER2	does not identify subgroups	Breast Neoplasms	*Bevacizumab in combination with paclitaxel is indicated for first-line treatment of adult patients with metastatic breast cancer. For further information as to human epidermal growth factor receptor 2 (HER2) status, please refer to section 5.1.* *Bevacizumab in combination with capecitabine is indicated for first-line treatment of adult patients with metastatic breast cancer in whom treatment with other chemotherapy options including taxanes or anthracyclines is not considered appropriate. Patients who have received taxane and anthracyclinecontaining regimens in the adjuvant setting within the last 12 months should be excluded from treatment with Avastin in combination with capecitabine. For further information as to HER2 status,* *please refer to section 5.1.*	Bevacizumab (Avastin)
HER2	associate with other treatment	Breast Neoplasms	*TAXOTERE in combination with doxorubicin and cyclophosphamide is indicated for the adjuvant treatment of patients with:* *· operable node-positive breast cancer* *· operable node-negative breast cancer* *For patients with operable node-negative breast cancer, adjuvant treatment should be restricted to patients eligible to receive chemotherapy according to internationally established criteria for primary therapy of early breast cancer (see section 5.1).* *TAXOTERE in combination with doxorubicin is indicated for the treatment of patients with locally advanced or metastatic breast cancer who have not previously received cytotoxic therapy for this condition.*	Docetaxel (Docefrez; Docetaxel Teva; Docetaxel Winthrop; Docetaxel Winthrop; Taxotere; Docetaxel

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			TAXOTERE monotherapy is indicated for the treatment of patients with locally advanced or metastatic breast cancer after failure of cytotoxic therapy. Previous chemotherapy should have included an anthracycline or an alkylating agent. *TAXOTERE in combination with trastuzumab is indicated for the treatment of patients with metastatic breast cancer whose tumours over express HER2 and who previously have not received chemotherapy for metastatic disease.* *TAXOTERE in combination with capecitabine is indicated for the treatment of patients with locally advanced or metastatic breast cancer after failure of cytotoxic chemotherapy. Previous therapy should have included an anthracycline.*	Accord; Docetaxel Kabi; Docetaxel Mylan)
histology, tumour	disease subtype	Soft Tissue Sarcoma	*Soft tissue sarcoma (STS)* *Votrient is indicated for the treatment of adult patients with selective subtypes of advanced Soft Tissue Sarcoma (STS) who have received prior chemotherapy for metastatic disease or who have progressed within 12 months after (neo) adjuvant therapy.* *Efficacy and safety has only been established in certain STS histological tumour subtypes (see section 5.1).*	Pazopanib (Votrient)
homozygous	prognostic	Hypercholesterolemia	*Kynamro was expected to be used to treat patients with an inherited disease causing high blood cholesterol levels, called familial hypercholesterolaemia. It was initially expected to be used to treat two closely related forms of the disease called 'severe heterozygous' and 'homozygous' familial hypercholesterolaemia. During the assessment of Kynamro, the indication was restricted to patients with homozygous familial hypercholesterolaemia only.*	mipomersen sodium (Kynamro)
IgA antibodies	diagnostic	Immunologic Deficiency Syndromes Guillain-Barre Syndrome Bone Marrow Transplantation Purpura, Thrombocytopenic, Idiopathic Mucocutaneous Lymph Node Syndrome	*Contraindications:* *(…) Hypersensitivity to human immunoglobulins, especially in patients with antibodies against IgA…*	human normal immunoglobulin (Flebogamma DIF)
IgA antibodies	diagnostic	Immunologic Deficiency Syndromes Guillain-Barre Syndrome	*Contraindications:* *Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.* *Hypersensitivity to human immunoglobulins, especially in patients with antibodies against IgA.*	human normal immunoglobulin (ivig) (Kiovig)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
		Bone Marrow Transplantation Purpura, Thrombocytopenic, Idiopathic Mucocutaneous Lymph Node Syndrome		
IgE	diagnostic	Asthma	*Xolair is indicated in adults, adolescents and children (6 to <12 years of age).* *Xolair treatment should only be considered for patients with convincing IgE (immunoglobulin E) mediated asthma (see section 4.2).* *Adults and adolescents (12 years of age and older)* *Xolair is indicated as add-on therapy to improve asthma control in patients with severe persistent allergic asthma who have a positive skin test or in vitro reactivity to a perennial aeroallergen and who have reduced lung function (FEV1 <80%) as well as frequent daytime symptoms or night-time awakenings and who have had multiple documented severe asthma exacerbations despite daily highdose inhaled corticosteroids, plus a long-acting inhaled beta2-agonist.* *Children (6 to <12 years of age)* *Xolair is indicated as add-on therapy to improve asthma control in patients with severe persistent allergic asthma who have a positive skin test or in vitro reactivity to a perennial aeroallergen and frequent daytime symptoms or night-time awakenings and who have had multiple documented severe asthma exacerbations despite daily high-dose inhaled corticosteroids, plus a long-acting inhaled beta2-agonist.*	Omalizumab (Xolair)
IGF-1	diagnostic	Laron Syndrome	*For the long-term treatment of growth failure in children and adolescents from 2 to 18 years with severe primary insulin-like growth factor-1 deficiency (Primary IGFD).* *Severe Primary IGFD is defined by:* *· height standard deviation score £ –3.0 and* *· basal IGF-1 levels below the 2.5th percentile for age and gender and* *· GH sufficiency.* *· Exclusion of secondary forms of IGF-1 deficiency, such as malnutrition, hypothyroidism, or chronic treatment with pharmacologic doses of anti-inflammatory steroids.* *Severe Primary IGFD includes patients with mutations in the GH receptor (GHR), post-GHR signaling pathway, and IGF-1 gene defects; they are not GH deficient, and therefore, they cannot be expected to respond adequately to exogenous GH treatment. It is recommended to confirm the diagnosis by conducting an IGF-1 generation test.*	Mecasermin (Increlex)
IGF-1	associated with another treatment	Acromegaly	*Treatment of patients with acromegaly who have had an inadequate response to surgery and/or radiation therapy and in whom an appropriate medical treatment with somatostatin analogues did not normalize IGF-I concentrations or was not tolerated.*	Pegvisomant (Somavert)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
IGF-1 gene defects	disease subtype	Laron Syndrome	*For the long-term treatment of growth failure in children and adolescents from 2 to 18 years with severe primary insulin-like growth factor-1 deficiency (Primary IGFD).* *Severe Primary IGFD is defined by:* *· height standard deviation score £ –3.0 and* *· basal IGF-1 levels below the 2.5th percentile for age and gender and* *· GH sufficiency.* *· Exclusion of secondary forms of IGF-1 deficiency, such as malnutrition, hypothyroidism, or chronic treatment with pharmacologic doses of anti-inflammatory steroids.* *Severe Primary IGFD includes patients with mutations in the GH receptor (GHR), post-GHR signaling pathway, and IGF-1 gene defects; they are not GH deficient, and therefore, they cannot be expected to respond adequately to exogenous GH treatment. It is recommended to confirm the diagnosis by conducting an IGF-1 generation test.*	Mecasermin (Increlex)
N-acetylglutamate synthase primary deficiency	defines disease	Amino Acid Metabolism, Inborn Errors Propionic Acidemia	*Carbaglu is indicated in treatment of* *• hyperammonaemia due to N-acetylglutamate synthase primary deficiency.* *• hyperammonaemia due to isovaleric acidaemia.* *• hyperammonaemia due to methymalonic acidaemia.* *• hyperammonaemia due to propionic acidaemia.*	carglumic acid (Carbaglu)
non-Q wave	ICD10	Angina, Unstable Myocardial Infarction	*INTEGRILIN is intended for use with acetylsalicylic acid and unfractionated heparin.* *INTEGRILIN is indicated for the prevention of early myocardial infarction in adults presenting with unstable angina or non-Q-wave myocardial infarction, with the last episode of chest pain occurring within 24 hours and with electrocardiogram (ECG) changes and/or elevated cardiac enzymes. Patients most likely to benefit from INTEGRILIN treatment are those at high risk of developing myocardial infarction within the first 3-4 days after onset of acute angina symptoms including for instance those that are likely to undergo an early PTCA (Percutaneous Transluminal Coronary Angioplasty) (see section 5.1).*	Eptifibatide (Integrilin)
NSTEMI	disease subtype	Angioplasty, Transluminal, Percutaneous Coronary Acute Coronary Syndrome	*Angiox is indicated as an anticoagulant in adult patients undergoing percutaneous coronary intervention (PCI), including patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI.* *Angiox is also indicated for the treatment of adult patients with unstable angina/non-ST segment elevation myocardial infarction (UA/NSTEMI) planned for urgent or early intervention.* *Angiox should be administered with aspirin and clopidogrel.*	Bivalirudin (Angiox)
NSTEMI	disease subtype	Peripheral Vascular Diseases Acute Coronary Syndrome	*Brilique, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in adult patients with Acute Coronary Syndromes (unstable angina, non ST elevation Myocardial Infarction [NSTEMI] or ST elevation Myocardial Infarction [STEMI]); including patients managed medically, and those who are managed with percutaneous coronary intervention (PCI) or coronary artery by-pass grafting (CABG)…*	Ticagrelor (Brilique)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
NSTEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary Syndrome Myocardial Infarction	*Clopidogrel is indicated in adults for the prevention of atherothrombotic events in:* *· Patients suffering from myocardial infarction (from a few days until less than 35 days), ischaemic stroke (from 7 days until less than 6 months) or established peripheral arterial disease.* *· Patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy…*	Clopidogrel (Clopidogrel 1A Pharma; Clopidogrel Acino; Clopidogrel Hexal; Clopidogrel ratiopharm GmbH; Clopidogrel Zentiva (previously Clopidogrel Winthrop); Iscover)
NSTEMI	disease subtype	Peripheral Vascular Diseases Stroke Myocardial Infarction	*Clopidogrel is indicated in:* *• Adult patientssuffering from myocardial infarction (from a few days until less than 35 days), ischaemic stroke (from 7 days until less than 6 months) or established peripheral arterial disease.* *• Adult patientssuffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy.*	clopidogrel besilate (Clopidogrel Apotex; Grepid)
NSTEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary Syndrome Myocardial Infarction Atrial Fibrillation	*Adult patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy*	clopidogrel hydrogen sulphate (Clopidogrel BMS; Clopidogrel Teva (hydrogen sulphate); Plavix - non-generic; Zyllt)
NSTEMI	disease subtype	Peripheral Vascular	*Adult patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave*	clopidogrel hydrochloride

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
		Diseases Stroke Acute Coronary Syndrome Myocardial Infarction	*myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy.*	(Clopidogrel HCS; Clopidogrel Teva Generics B.V.)
NSTEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary Syndrome Myocardial Infarction	*Clopidogrel is indicated in:* *• Adult patients suffering from myocardial infarction (from a few days until less than 35 days), ischaemic stroke (from 7 days until less than 6 months) or established peripheral arterial disease.* *• Adult patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA)* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy.*	clopidogrel hydrobromide (Clopidogrel Teva Pharma B.V.)
NSTEMI	disease subtype	Acute Coronary Syndrome Myocardial Infarction	*DuoPlavin is indicated for the prevention of atherothrombotic events in adult patients already taking both clopidogrel and acetylsalicylic acid (ASA). DuoPlavin is a fixed-dose combination medicinal product for continuation of therapy in:* *· Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction) including patients undergoing a stent placement following percutaneous coronary intervention* *· ST segment elevation acute myocardial infarction in medically treated patients eligible for thrombolytic therapy…*	clopidogrel / acetylsalicylic acid (DuoCover; DuoPlavin)
NSTEMI	disease subtype	Angina, Unstable Acute Coronary Syndrome Myocardial Infarction	*Efient, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in patients with acute coronary syndrome (i.e. unstable angina, non-ST segment elevation myocardial infarction [UA/NSTEMI] or ST segment elevation myocardial infarction [STEMI]) undergoing primary or delayed percutaneous coronary intervention (PCI)…*	Prasugrel (Efient)
NSTEMI	disease subtype	Peripheral Vascular Diseases Acute Coronary Syndrome	*Possia, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in adult patients with Acute Coronary Syndromes (unstable angina, non ST elevation Myocardial Infarction [NSTEMI] or ST elevation Myocardial Infarction [STEMI]); including patients managed medically, and those who are managed with percutaneous coronary intervention (PCI) or coronary artery by-pass grafting (CABG)…*	Ticagrelor (Possia)
Philadelphia chromosome	ICD10	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	*Glivec is indicated for the treatment of· adult and paediatric patients with newly diagnosed Philadelphia chromosome (bcr-abl) positive (Ph+) chronic myeloid leukaemia (CML) for whom bone marrow transplantation is not considered as the first line of treatment.*	Imatinib (Glivec)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			· adult and paediatric patients with Ph+ CML in chronic phase after failure of interferon-alpha therapy, or in accelerated phase or blast crisis.	
Philadelphia chromosome	ICD10	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	*SPRYCEL is indicated for the treatment of adult patients with:* *§ newly diagnosed Philadelphia chromosome positive (Ph+) chronic myelogenous leukaemia (CML) in the chronic phase.*	Dasatinib (Sprycel)
Philadelphia chromosome	ICD10	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	*Tasigna is indicated for the treatment of adult patients with newly diagnosed Philadelphia chromosome positive chronic myelogenous leukaemia (CML) in the chronic phase.*	Nilotinib (Tasigna)
post-GH receptor pathway mutations	defines disease	Laron Syndrome	*For the long-term treatment of growth failure in children and adolescents from 2 to 18 years with severe primary insulin-like growth factor-1 deficiency (Primary IGFD).* *Severe Primary IGFD is defined by:* *· height standard deviation score £ –3.0 and* *· basal IGF-1 levels below the 2.5th percentile for age and gender and* *· GH sufficiency.* *· Exclusion of secondary forms of IGF-1 deficiency, such as malnutrition, hypothyroidism, or chronic treatment with pharmacologic doses of anti-inflammatory steroids.* *Severe Primary IGFD includes patients with mutations in the GH receptor (GHR), post-GHR signaling pathway, and IGF-1 gene defects; they are not GH deficient, and therefore, they cannot be expected to respond adequately to exogenous GH treatment. It is recommended to confirm the diagnosis by conducting an IGF-1 generation test.*	Mecasermin (Increlex)
protease inhibitor (PI) experienced	associated with another treatment	HIV Infections	*Agenerase, in combination with other antiretroviral agents, is indicated for the treatment of protease inhibitor (PI) experienced HIV-1 infected adults and children above the age of 4 years. Agenerase capsules should normally be administered with low dose ritonavir as a pharmacokinetic enhancer of amprenavir (see sections 4.2 and 4.5). The choice of amprenavir should be based on individual viral resistance testing and treatment history of patients (see section 5.1).* *The benefit of Agenerase boosted with ritonavir has not been demonstrated in PI naïve patients (see section 5.1)*	Amprenavir (Agenerase)
protease inhibitor (PI) experienced	associated with another treatment	HIV Infections	*APTIVUS, co-administered with low dose ritonavir, is indicated for combination antiretroviral treatment of HIV-1 infection in highly pre-treated adults and adolescents 12 years of age or older with virus resistant to multiple protease inhibitors. APTIVUS should only be used as part of an active combination antiretroviral regimen in patients with no other therapeutic options.* *This indication is based on the results of two phase III studies, performed in highly pre-treated adult patients (median number of 12 prior antiretroviral agents) with virus resistant to protease inhibitors and of one phase II study investigating pharmacokinetics, safety and efficacy of APTIVUS in mostly treatment-experienced adolescent patients aged 12 to 18 years (see section 5.1).* *In deciding to initiate treatment with APTIVUS, co-administered with low dose ritonavir, careful*	Tipranavir (Aptivus)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			consideration should be given to the treatment history of the individual patient and the patterns of mutations associated with different agents. Genotypic or phenotypic testing (when available) and treatment history should guide the use of APTIVUS. Initiation of treatment should take into account the combinations of mutations which may negatively impact the virological response to APTIVUS, co-administered with low dose ritonavir (see section 5.1).	
protein C deficiency	ICD10	Protein C Deficiency Purpura Fulminans	*CEPROTIN is indicated in purpura fulminans and coumarin-induced skin necrosis in patients with severe congenital protein C deficiency. Furthermore CEPROTIN is indicated for short-term prophylaxis in patients with severe congenital protein C deficiency if one or more of the following conditions are met:* *· surgery or invasive therapy is imminent* *· while initiating coumarin therapy* *· when coumarin therapy alone is not sufficient* *· when coumarin therapy is not feasible.*	human protein C (Ceprotin)
protein C deficiency	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of Zoely. Should any of the conditions appear for the first time during Zoely use, the medicinal product should be stopped immediately(...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (Zoely)
protein C deficiency	ICD10	Contraception	*Female contraception* *EVRA is intended for women of fertile age. The safety and efficacy has been established in women aged 18 to 45 years* *Contraindications:* *EVRA should not be used in the presence of one of the following disorders. If one of these disorders occurs during the use of EVRA, EVRA must be discontinued immediately (...)* *● Possible hereditary predisposition for venous or arterial thrombosis, such as activated protein C (APC-) resistance, antithrombin-III deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinemia, and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	norelgestromin / ethinyl estradiol (EVRA)
protein C deficiency	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications*	nomegestrol acetate / estradiol (IOA)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			for ethinylestradiol containing COCs are considered applicable to the use of IOA. Should any of the conditions appear for the first time during IOA use, the medicinal product should be stopped immediately(...) *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	
protein S deficiency	ICD10	Contraception	*Female contraception* *EVRA is intended for women of fertile age. The safety and efficacy has been established in women aged 18 to 45 years* *Contraindications:* *EVRA should not be used in the presence of one of the following disorders. If one of these disorders occurs during the use of EVRA, EVRA must be discontinued immediately(...)* *● Possible hereditary predisposition for venous or arterial thrombosis, such as activated protein C (APC-) resistance, antithrombin-III deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinemia, and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	norelgestromin / ethinyl estradiol (EVRA)
protein S deficiency	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of Zoely. Should any of the conditions appear for the first time during Zoely use, the medicinal product should be stopped immediately(...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (Zoely)
protein S deficiency	ICD10	Contraception	*Oral contraception.* *Contraindications:* *COCs should not be used in the presence of any of the conditions listed below. As no epidemiological data are yet available with 17β-estradiol containing COCs, the contraindications for ethinylestradiol containing COCs are considered applicable to the use of IOA. Should any of the conditions appear for the first time during IOA use, the medicinal product should be stopped immediately. (...)* *• Hereditary or acquired predisposition for venous or arterial thrombosis, such as activated protein C (APC) resistance, antithrombin-III-deficiency, protein C deficiency, protein S deficiency, hyperhomocysteinaemia and antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant)...*	nomegestrol acetate / estradiol (IOA)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
QTc interval	prognostic	Thyroid Neoplasms	*Contraindications:* *(...)Patients with a QTc interval over 480 msec.* *• Concomitant use of vandetanib with the following medicinal products known to also prolong the QTc interval and / or induce Torsades de pointes: Arsenic, cisapride, erythromycine intravenous (IV), toremifene, mizolastine, moxifloxacine, Class IA and III antiarrhythmics (see section 4.5).*	Vandetanib (Caprelsa)
rheumatoid factor	does not identify a subgroup	Arthritis, Juvenile Rheumatoid	*Treatment of polyarthritis (rheumatoid factor positive or negative) and extended oligoarthritis in children and adolescents from the age of 2 years who have had an inadequate response to, or who have proved intolerant of, methotrexate.* *Treatment of psoriatic arthritis in adolescents from the age of 12 years who have had an inadequate response to, or who have proved intolerant of, methotrexate.* *Treatment of enthesitis-related arthritis in adolescents from the age of 12 years who have had an inadequate response to, or who have proved intolerant of, conventional therapy.* *Enbrel has not been studied in children aged less than 2 years*	Etanercept (Enbrel)
serum CA 125	non-therapeutic	Radionuclide Imaging Ovarian Neoplasms	*Positive diagnosis of relapsing ovarian adenocarcinoma when serum CA 125 is increased without positive results of ultrasound or computerised tomography scan.*	Igovomab (Indimacis 125)
STEMI	disease subtype	Angioplasty, Transluminal, Percutaneous Coronary Acute Coronary Syndrome	*Angiox is indicated as an anticoagulant in adult patients undergoing percutaneous coronary intervention (PCI), including patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI.* *Angiox is also indicated for the treatment of adult patients with unstable angina/non-ST segment elevation myocardial infarction (UA/NSTEMI) planned for urgent or early intervention.* *Angiox should be administered with aspirin and clopidogrel.*	Bivalirudin (Angiox)
STEMI	disease subtype	Peripheral Vascular Diseases Acute Coronary Syndrome	*Brilique, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in adult patients with Acute Coronary Syndromes (unstable angina, non ST elevation Myocardial Infarction [NSTEMI] or ST elevation Myocardial Infarction [STEMI]); including patients managed medically, and those who are managed with percutaneous coronary intervention (PCI) or coronary artery by-pass grafting (CABG)…*	Ticagrelor (Brilique)
STEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary Syndrome Myocardial Infarction	*Clopidogrel is indicated in adults for the prevention of atherothrombotic events in:* *· Patients suffering from myocardial infarction (from a few days until less than 35 days), ischaemic stroke (from 7 days until less than 6 months) or established peripheral arterial disease.* *· Patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy...*	Clopidogrel (Clopidogrel 1A Pharma; Clopidogrel Acino; Clopidogrel Hexal; Clopidogrel ratiopharm GmbH;

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
				Clopidogrel Zentiva (previously Clopidogrel Winthrop); Iscover)
STEMI	disease subtype	Peripheral Vascular Diseases Stroke Myocardial Infarction	*Clopidogrel is indicated in:* *• Adult patientssuffering from myocardial infarction (from a few days until less than 35 days), ischaemic stroke (from 7 days until less than 6 months) or established peripheral arterial disease.* *• Adult patientssuffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy.*	clopidogrel besilate (Clopidogrel Apotex; Grepid)
STEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary Syndrome Myocardial Infarction Atrial Fibrillation	*Adult patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy*	clopidogrel hydrogen sulphate (Clopidogrel BMS; Clopidogrel Teva (hydrogen sulphate); Plavix; Zyllt)
STEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary Syndrome Myocardial Infarction	*Adult patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA).* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy.*	clopidogrel hydrochloride (Clopidogrel HCS; Clopidogrel Teva Generics B.V.)
STEMI	disease subtype	Peripheral Vascular Diseases Stroke Acute Coronary	*Clopidogrel is indicated in:* *• Adult patients suffering from myocardial infarction (from a few days until less than 35 days), ischaemic stroke (from 7 days until less than 6 months) or established peripheral arterial disease.* *• Adult patients suffering from acute coronary syndrome:* *- Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave*	clopidogrel hydrobromide (Clopidogrel Teva Pharma B.V.)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
		Syndrome Myocardial Infarction	*myocardial infarction), including patients undergoing a stent placement following percutaneous coronary intervention, in combination with acetylsalicylic acid (ASA)* *- ST segment elevation acute myocardial infarction, in combination with ASA in medically treated patients eligible for thrombolytic therapy.*	
STEMI	disease subtype	Acute Coronary Syndrome Myocardial Infarction	*DuoPlavin is indicated for the prevention of atherothrombotic events in adult patients already taking both clopidogrel and acetylsalicylic acid (ASA). DuoPlavin is a fixed-dose combination medicinal product for continuation of therapy in:* *· Non-ST segment elevation acute coronary syndrome (unstable angina or non-Q-wave myocardial infarction) including patients undergoing a stent placement following percutaneous coronary intervention* *· ST segment elevation acute myocardial infarction in medically treated patients eligible for thrombolytic therapy…*	clopidogrel / acetylsalicylic acid (DuoCover; DuoPlavin)
STEMI	disease subtype	Angina, Unstable Acute Coronary Syndrome Myocardial Infarction	*Efient, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in patients with acute coronary syndrome (i.e. unstable angina, non-ST segment elevation myocardial infarction [UA/NSTEMI] or ST segment elevation myocardial infarction [STEMI]) undergoing primary or delayed percutaneous coronary intervention (PCI)…*	Prasugrel (Efient)
STEMI	disease subtype	Myocardial Infarction	*Metalyse is indicated in adultsfor the thrombolytic treatment of suspected myocardial infarction with persistent ST elevation or recent left Bundle Branch Block within 6 hours after the onset of acute myocardial infarction (AMI) symptoms.*	Tenecteplase (Metalyse; Tenecteplase Boehringer Ingelheim Pharma GmbH & Co. KG)
STEMI	disease subtype	Peripheral Vascular Diseases Acute Coronary Syndrome	*Possia, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in adult patients with Acute Coronary Syndromes (unstable angina, non ST elevation Myocardial Infarction [NSTEMI] or ST elevation Myocardial Infarction [STEMI]); including patients managed medically, and those who are managed with percutaneous coronary intervention (PCI) or coronary artery by-pass grafting (CABG).*	Ticagrelor (Possia)
STEMI	disease subtype	Myocardial Infarction	*Rapilysin is indicated for the thrombolytic treatment of suspected myocardial infarction with persistent ST elevation or recent left Bundle Branch Block within 12 hours after the onset of acute myocardial infarction AMI symptoms.*	Reteplase (Rapilysin)
T2 lesion	monitoring	Multiple Sclerosis	*Gilenya is indicated as single disease modifying therapy in highly active relapsing remitting multiple sclerosis for the following adult patient groups:* *- Patients with high disease activity despite treatment with a beta-interferon.* *These patients may be defined as those who have failed to respond to a full and adequate course*	fingolimod hydrochloride (Gilenya)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
			(normally at least one year of treatment) of beta-interferon. Patients should have had at least 1 relapse in the previous year while on therapy, and have at least 9 T2-hyperintense lesions in cranial MRI or at least 1 Gadolinium-enhancing lesion. A "non-responder" could also be defined as a patient with an unchanged or increased relapse rate or ongoing severe relapses, as compared to the previous year. *or* *- Patients with rapidly evolving severe relapsing remitting multiple sclerosis defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing lesions on brain MRI or a significant increase in T2 lesion load as compared to a previous recent MRI.*	
T2 lesion	monitoring	Multiple Sclerosis	*TYSABRI is indicated as single disease modifying therapy in highly active relapsing remitting multiple sclerosis for the following patient groups:* *• Adult patients aged 18 years and over with high disease activity despite treatment with a betainterferon.* *These patients may be defined as those who have failed to respond to a full and adequate course (normally at least one year of treatment) of beta-interferon. Patients should have had at least 1 relapse in the previous year while on therapy, and have at least 9 T2-hyperintense lesions in cranial Magnetic Resonance Image (MRI) or at least 1 Gadolinium-enhancing lesion. A "nonresponder" could also be defined as a patient with an unchanged or increased relapse rate or ongoing severe relapses, as compared to the previous year.* *or* *• Adult patients aged 18 years and over with rapidly evolving severe relapsing remitting multiple sclerosis defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing lesions on brain MRI or a significant increase in T2 lesion load as compared to a previous recent MRI.*	Natalizumab (Tysabri)
take up technetium [99mTc]-labelled biphosphonates on bone scan	diagnostic	Pain Cancer	*Quadramet is indicated for the relief of bone pain in patients with multiple painful osteoblastic skeletal metastases which take up technetium (99mTc)-labelled biphosphonates on bone scan. The presence of osteoblastic metastases which take up technetium (99mTc)-labelled biphosphonates should be confirmed prior to therapy.*	samarium [¹⁵³Sm] lexidronam pentasodium (Quadramet)
T-cell	ICD10	Precursor T-Cell Lymphoblastic Leukemia-Lymphoma	*Nelarabine is indicated for the treatment of patients with T-cell acute lymphoblastic leukaemia (TALL) and T-cell lymphoblastic lymphoma (T-LBL) whose disease has not responded to or has relapsed following treatment with at least two chemotherapy regimens.* *Due to the small patient populations in these disease settings, the information to support these indications is based on limited data.*	Nelarabine (Atriance)
T-cell	ICD10	Lymphoma, T-Cell, Cutaneous	*Targretin capsules are indicated for the treatment of skin manifestations of advanced stage cutaneous T-cell lymphoma (CTCL) patients refractory to at least one systemic treatment*	Bexarotene (Targretin)

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
T-cell	ICD10	Lymphoma, T-Cell	*treatment of adult patients with peripheral T-cell lymphoma (PTCL) (nodal, other extranodal and leukaemic/disseminated) who have progressed after at least one prior therapy*	Pralatrexate (Folotyn)
T-cell	ICD10	Lymphoma, Non-Hodgkin	*treatment of adults with peripheral T-cell lymphoma that no longer responds to or has come back after at least two previous therapies*	Romidepsin (Istodax)
tetrahydrobiopterin deficiency	defines disease	Phenylketonurias	*Kuvan is indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients of 4 years of age and over with phenylketonuria (PKU) who have been shown to be responsive to such treatment (see section 4.2).* *Kuvan is also indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients with tetrahydrobiopterin (BH4) deficiency who have been shown to be responsive to such treatment (see section 4.2).*	Sapropterin (Kuvan)
Tg	non-therapeutic	Thyroid Neoplasms	*Thyrogen is indicated for use with serum thyroglobulin (Tg) testing with or without radioiodine imaging for the detection of thyroid remnants and well-differentiated thyroid cancer in post-thyroidectomy patients maintained on hormone suppression therapy (THST).* *Low risk patients with well-differentiated thyroid carcinoma who have undetectable serum Tg levels on THST and no rh (recombinant human) TSH-stimulated increase of Tg levels may be followed-up by assaying rh TSH-stimulated Tg levels.* *Thyrogen is indicated for pre-therapeutic stimulation in combination with a range of 30 mCi (1.1 GBq) to 100 mCi (3.7 GBq) radioiodine for ablation of thyroid tissue remnants in patients who have undergone a near-total or total thyroidectomy for well-differentiated thyroid cancer and who do not have evidence of distant metastatic thyroid cancer (see section 4.4).*	thyrotropin alfa (Thyrogen)
viral resistance	associated with another treatment	HIV Infections	*Viramune is indicated in combination with other anti-retroviral medicinal products for the treatment of HIV-1 infected adults, adolescents, and children of any age (see section 4.4).* *Most of the experience with Viramune is in combination with nucleoside reverse transcriptase inhibitors (NRTIs). The choice of a subsequent therapy after Viramune should be based on clinical experience and resistance testing (see section 5.1).*	Nevirapine (Viramune; Nevirapine Teva)
viral resistance	associated with another treatment	HIV Infections	*Viramune is indicated in combination with other anti-retroviral medicinal products for the treatment of HIV-1 infected adults, adolescents, and children of any age (see section 4.4).* *Most of the experience with Viramune is in combination with nucleoside reverse transcriptase inhibitors (NRTIs). The choice of a subsequent therapy after Viramune should be based on clinical experience and resistance testing (see section 5.1).*	Nevirapine (Viramune)
virus serotype	non-therapeutic	Rotavirus Infections Immunization	*RotaShield is indicated for active immunisation of infants aged 6 weeks to 30 weeks for prevention of severe clinical manifestations of gastro-enteritis caused by rotavirus serotypes 1, 2, 3 and 4 of group A.*	rotavirus serotype 1 reassortant, rotavirus serotype 2 reassortant, rotavirus

Biomarker	Exclusion reason	Therapeutic area	Indication/ Contraindication (if relevant) text	Drug
				serotype 3 rhesus, rotavirus serotype 4 reassortant (Rotashield)
α-L-iduronidase deficiency	defines disease	Mucopolysaccharidosis I	*Aldurazyme is indicated for long-term enzyme replacement therapy in patients with a confirmed diagnosis of Mucopolysaccharidosis I (MPS I; α-L-iduronidase deficiency) to treat the nonneurological manifestations of the disease (see section 5.1).*	Laronidase (Aldurazyme)
Δ4-3-Oxosteroid-**5β**-reductase deficiency	defines disease	Digestive System Diseases Metabolism, Inborn Errors	*Treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ5-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults. Treatment must be initiated and monitored by an experienced hepatologist or a paediatric hepatologist in the case of paediatric patients*	cholic acid (Orphacol)

APPENDIX 10. ABBREVIATIONS FOR B-I-D COMBINATIONS

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B-I-D combination	Biomarker	Indication	Drug
ALKNC	ALK	Non-Small-Cell Lung Carcinoma	crizotinib
BRMV	BRAF	Melanoma	vemurafenib
CCR5HM	CCR5 tropism	HIV	maraviroc
CD33AG	CD-33	Acute Myeloid Leukemia	gemtuzumab ozogamicin
D816VSI	D816V mutation in c-Kit	Systemic Mastocytosis	imatinib
DPDCC	DPD deficiency	Colorectal Neoplasms, Colonic Neoplasms, Stomach Neoplasms, Breast Neoplasms	capecitabine
DPDST	DPD deficiency	Stomach Neoplasms	tegafur/ gimeracil/ oteracil
EGFR(E)CC	EGFR expression	Colorectal Neoplasms	cetuximab
EGFR(E)NC	EGFR expression	Non-Small-Cell Lung Carcinoma	cetuximab
EGFR(E)NE	EGFR expression	Non-Small-Cell Lung Carcinoma	erlotinib
EGFR(M)NE	EGFR mutation	Non-Small-Cell Lung Carcinoma	erlotinib
EGFR(M)NG	EGFR mutation	Non-Small-Cell Lung Carcinoma	gefitinib
EpCAMAC	EpCAM expression	Ascites Cancer	catumaxomab
ERBF	oestrogen receptor	Breast Neoplasms	fulvestrant
ERBT	oestrogen receptor	Breast Neoplasms	toremifene
FIP1L1HI	FIP1L1-PDGFRα rearrangement	Hypereosinophilic Syndrome	imatinib
G1CB	genotype 1 HCV	Chronic Hepatitis C	bocepravir
G1CT	genotype 1 HCV	Chronic Hepatitis C	telaprevir
G55CI	G551D	Cystic Fibrosis	ivacaftor
HDDP	hormone dependent	Prostatic Neoplasms	degarelix
HER2BE	HER2 expression	Breast Neoplasms	everolimus
HER2BL	HER2 expression	Breast Neoplasms	lapatinib
HER2BP	HER2 expression	Breast Neoplasms	pertuzumab
HER2BT	HER2 expression	Breast Neoplasms	trastuzumab
HER2ST	HER2 expression	Stomach Neoplasms	trastuzumab
HLAHA	HLA-B*5701 allele	HIV	abacavir
HRBE	hormone receptor	Breast Neoplasms	everolimus
HRBZ	hormone receptor	Breast Neoplasms	zoledronic acid
KitGI	Kit (CD 117) positive	Gastrointestinal Stromal Tumors	imatinib
KRASCC	KRAS mutation	Colorectal Neoplasms	cetuximab
KRASCP	KRAS mutation	Colorectal Neoplasms	panitumumab
LPLNH	LPL protein	Hyperlipoproteinemia Type I	alipogene tiparvovec
NMMc	NADPH	Methaemoglobinaemia	methylthioninum chloride
PDGFRMI	PDGFR gene re-arrangements	Myelodysplastic-Myeloproliferative Diseases	imatinib
PHPD	Philadelphia chromosome	Precursor Cell Lymphoblastic Leukemia-Lymphoma	dasatinib

B-I-D combination	Biomarker	Indication	Drug
PHPI	Philadelphia chromosome	Precursor Cell Lymphoblastic Leukemia-Lymphoma	imatinib
t(15;19)AA	t(15;17) translocation and/or PML/RAR-α gene	Acute Promyelocytic Leukemia	arsenic trioxide
VRHAm	viral resistance	HIV	amprenavir
VRHAt	viral resistance	HIV	atazanavir
VRHD	viral resistance	HIV	darunavir
VRHEET	viral resistance	HIV	efavirenz/ emtricitabine/ tenofovir disoproxil
VRHEm	viral resistance	HIV	emtricitabine
VRHEn	viral resistance	HIV	enfuvirtide
VRHERT	viral resistance	HIV	emticitabine/ rilpivirine h/ tenofovir df
VRHF	viral resistance	HIV	fosamprenavir
VRHL	viral resistance	HIV	lopinavir/ ritonavir
VRHNel	viral resistance	HIV	nelfinavir
VRHR	viral resistance	HIV	rilpivirine
VRHTe	viral resistance	HIV	tenofovir
VRHTr	Viral resistance	HIV	tipranavir

APPENDIX 11. FINAL DATA COLLECTION TABLE FOR TEXT ANALYSIS OF EMA DOCUMENTS

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B-I-D combination	
ref	
drug	
biomarker	
indication	
disease area	
orphan designation	
biomarker (efficacy/ toxicity)	
authorised	
comments	
population in studies in accordance with the population identified by the drug indication - text to support item	
comments	
condition*	
design of the biomarker/ treatment evaluation adequate - text to support item	
comments	
condition*	
primary outcome appropriate - text to support item	
comments	
condition*	
sample size adequate - text to support item	
comments	
condition*	
sufficient proportion of patients with biomarker status available - text to support item	
comments	
condition*	
representativeness of the subgroup of patients with the biomarker status available - text to support item	
comments	
condition*	
evidence supporting the drug (irrespective of biomarker) based on the results for the primary outcome in studies - text to support item	
comments	
condition*	
evidence supporting the biomarker based on the results for the primary outcome in studies - text to support item	
comments	
condition*	
evidence supporting the drug (irrespective of biomarker) based on the results for any outcomes - text to support item	
comments	

condition*	
evidence supporting the biomarker based on the results for any outcomes - text to support item	
comments	
condition*	
evidence supporting the drug (irrespective of biomarker) based on the results for secondary outcomes - text to support item	
comments	
condition*	
evidence supporting the biomarker based on the results for secondary outcomes - text to support item	
comments	
condition*	
evidence was sufficient to conclude that drug (irrespective of biomarker) provides clinical benefit - text to support	
comments	
condition*	
evidence was sufficient to conclude that biomarker provides clinical benefit - text to support	
comments	
condition*	
toxicity acceptable	
comments	
condition*	
drug addressed an unmet clinical need	
comments	
condition*	
novel mechanism of action/ new active substance	
comments	
condition*	
ammendments to trials acceptable	
comments	
condition*	
availability of biomarker test on the market/ clear guidelines for test	
comments	
condition*	
imbalances between groups in trials acceptable	
comments	
condition*	
consistency of results across subgroups (other than the biomarker)	
comments	

condition*	
sufficient long term data provided	
comments	
condition*	
benefit-risk balance	
comments	
condition*	
level of possible bias acceptable	
comments	
condition*	
supporting evidence from drugs with similar mechanism	
comments	
condition*	
evidence from pharmacology suppporting biomarker	
comments	
condition*	
evidence from previous studies (for example indication is being broadened etc.)	
comments	
condition*	
evidence from pre-clinical/ in vitro studies to support biomarker	
comments	
condition*	
no further data required	
comments	
condition*	
quality of the product acceptable	
comments	
condition*	
preclinical studies provide adequate information	
comments	
condition*	
no cross resistance with other available treatments observed	
comments	
condition*	
sufficient evidence to support selected dose and/or duration of treatment	
comments	
condition*	
sufficient evidence to support the B-I-D compared with other available treatment options	

comments	
condition*	
evidence from mechanism of action to support biomarker	
comments	
condition*	
more acceptable route/ mode of delivery	
comments	
condition*	
ADME/ pharmacokinetics characterised sufficiently	
comments	
condition*	
statistical approach to data analysis adequate	
comments	
condition*	
biomarker prognostic of poor outcome	
comments	
condition*	
biomarker test accurate	
comments	
condition*	
???	
comments	
condition*	
conclusions supported by published meta-analysis or other literature sources	
comments	
condition*	
information on how biomarker was assessed in studies sufficient	
comments	
condition*	
correlation between expression level and effect	
comments	
condition*	
results reproduced	
comments	
condition*	

* Possible values: met, partly, not met, discussed only, not discussed

Original criteria

New criteria

APPENDIX 12. SUMMARY OF STUDIES SUPPORTING B-I-D COMBINATIONS

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ALK mutation – NSCLC – crizotinib

Study	Design	Population	Interventions	Biomarker measurement	Results
A8081001[46-49] EMA status: main Phase I/II N=170* Status: ongoing	Biomarker evaluation: non-randomised only positive design: single arm; two stages: (1) dose escalation, (2) response in a population selected based on the first stage follow-up: 120 days primary outcome: Objective Response Rate (the percent of patients in the Response Evaluable (RE) population achieving a confirmed CR or confirmed PR according to RECIST)	dose escalation part: advanced malignancies; second part (based on response in first): ALK-rearrangement positive patients with advanced NSCLC	A) crizotinib 250 mg orally twice daily in continuous 4-week cycles; to be taken approximately 12 hours apart B) crizotinib 250 mg orally twice daily in continuous 3-week cycles; to be taken approximately 12 hours apart	samples: unstained slides from formalin-fixed, paraffin embedded (FFPE) tumor samples method: "analyzed prospectively by means of FISH with the use of an ALK break-apart (or split-signal) probe. (…) Reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays for specific EML4-ALK fusions and immunohistochemical analyses for ALK protein were performed retrospectively on a subgroup of FFPE tumor samples." (70 of 82 samples (85%) were confirmed to be FISH-positive by a central laboratory)[48] threshold: FISH-positive if more than 15% of scored tumor cells had split ALK 5′ and 3′ probe signals or had isolated 3′ signals group / N / BM+ / BM- A / 125 / 125 / 0 B / 35 / 0 / 35	121 pre-treated ALK+ patients,** day 120: 3 patients had confirmed CRs, and 70 patients had confirmed PRs, for an investigator-assessed ORR of 60.3% (95% CI: 51.0%, 69.1%) Data for ALK- patients NR

* NSCLC patients only

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>A8081005[49,50]
EMA status: supportive

Phase II

N=340*

Status: ongoing</td><td>Biomarker evaluation: non-randomised only positive

design: single arm

follow-up: 120 days

primary outcome: Objective Response Rate (details NR)</td><td>locally advanced or metastatic ALK-positive NSCLC who have received at least 1 prior chemotherapy regimen</td><td>Crizotinib 250 mg orally twice daily</td><td>samples: NR

method: NR

threshold: NR

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>340</td><td>340</td><td>0</td></tr></table></td><td>156 patients of 340 responded: 4 CR, 152 PR</td></tr>
<tr><td>A8081007[49]

EMA status: supportive

Phase III

N= 347

Status: ongoing</td><td>Biomarker evaluation: enrichment

design: randomised 1:1, stratified by ECOG performance status, brain metastases, and prior (yes, no) EGFR TKI treatment

follow-up: NR

primary outcome: PFS (based on Independent Radiology Review)</td><td>ALK-positive, advanced NSCLC patients who received only one prior platinum-based chemotherapy regimen</td><td>A) crizotinib starting dose of 250 mg twice daily
B) standard care chemotherapy pemetrexed (500 mg/m2, on Day 1 of every cycle) or docetaxel (75 mg/m2, on Day 1 of every cycle)</td><td>samples: NR

method: NR

threshold: NR

<table><tr><td>group</td><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>A</td><td>173</td><td>173</td><td>0</td></tr><tr><td>B</td><td>174</td><td>174</td><td>0</td></tr></table></td><td>PFS (number of patients included in analysis NR): 7.7 (95% CI: 6.0, 8.8) and 3.0 (95% CI: 2.6, 4.3) months in crizotinib and chemotherapy arm, respectively: HR 0.487 (95% CI: 0.371, 0.638, p-value <0.0001)</td></tr>
</table>

* the largest number of patients identified in the EMA data

BRAF V600 mutation – melanoma - vemurafenib

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>BRIM 3 (NO25026)[51,52]

EMA status: main

Phase III

N=675

Status: ongoing (modified for efficacy*)</td>
<td>Biomarker evaluation: enrichment

design: randomised 1:1; stratified by cancer stage, ECOG PS, region and serum lactate dehydrogenase level
follow-up: median 3.8 months for vemurafenib and 2.3 months for dacarbazine
primary outcome: overall survival (time from randomization to death from any cause)</td>
<td>adults with unresectable previously untreated stage IIIC or IV melanoma</td>
<td>A) vemurafenib 960 mg twice daily orally; dose reductions for adverse effects prespecified; discontinued on progression (unless investigator and sponsor consider continuation beneficial)
B) dacarbazine 1000 mg per square m of body surface area by iv infusion for 3 weeks; dose reductions for adverse effects prespecified; discontinued on progression (unless investigator and sponsor consider continuation beneficial)</td>
<td>samples: tumour tissue (details NR)

method: Cobas 4800 BRAF V600E Mutation Test†, Roche Molecular Systems (real time PCR); in one of 5 central laboratories

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>337</td><td>337</td><td>0</td></tr>
<tr><td>B</td><td>338</td><td>338</td><td>0</td></tr>
</table>
</td>
<td>"Study was stopped after an interim analysis for efficacy. The protocol was amended in January 2011 to allow patients in the dacarbazine group to cross over to vemurafenib. Data used in SciD is from October 2011."
Overall survival (with censoring at crossover): HR = 0.62 (95%CI 0.49, 0.77); p<0.0001; favours vemurafenib
Overall survival (without censoring at crossover): HR = 0.67 (95% CI 0.54, 0.84); p=0.0003</td>
</tr>
<tr>
<td>NP22657[53,54]

EMA status: supportive

Phase II

N=132

Status: completed</td>
<td>Biomarker evaluation: single arm only positive

design: uncontrolled (details NR)
follow-up: NR (data for median PFS 6.1 months)
primary outcome: best overall response rate (assessed by independent review committee using RECIST criteria)</td>
<td>adults previously treated metastatic melanoma</td>
<td>Vemurafenib 960 mg twice daily orally; until progression or withdrawal from study</td>
<td>samples: tumour tissue (details NR)

method: Roche CoDx BRAF mutation assay

threshold: N/A

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>132</td><td>132</td><td>0</td></tr>
</table>
</td>
<td>Best overall response rate: response was observed in 69 patients - 52% of all patients in the study (95% CI: 43, 61). The reported responses were 3 CR, 66 PR and 39 SD.</td>
</tr>
</table>

* Protocol was amended in January 2011 after an interim analysis demonstrated superiority of vemurafenib: patients in dacarbazine group crossed over to vemurafenib; data used in Scientific Discussion from October 2011

† The test was found to identify patients with V600E mutation; retrospective sequencing studies have shown it also detects V600D and V600K with lower sensitivity

CCR5 – HIV- maraviroc

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>MOTIVATE 1[55,56]

EMA status:
main

Phase III

N=601

Status:
completed</td>
<td>Biomarker evaluation:
enrichment

design: 3-arm RCT; double blind; randomisation 2:2:1; stratified by use of enfuviritide and HIV-1 RNA level at screening (log10 - transformed copies per ml)

follow-up: 48 weeks

primary outcome: mean change in levels of HIV-1 RNA from baseline to week 48</td>
<td>age ≥ 16 years; CCR5 tropic; experienced drugs from 3 different categories</td>
<td>All with optimised background therapy:
A) maraviroc 300 mg (or 150 mg if the optimised background therapy included delavirdine); oral; once daily (plus placebo once daily)
B) maraviroc 300 mg (or 150 mg if the optimised background therapy included delavirdine); oral; twice daily (total daily dose 600 mg or 300 mg)
C) placebo twice daily</td>
<td>samples: blood

method: using a phenotypic assay (Trofile, Monogram Biosciences)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>241</td><td>241</td><td>0</td></tr>
<tr><td>B</td><td>240</td><td>240</td><td>0</td></tr>
<tr><td>C</td><td>120</td><td>120</td><td>0</td></tr>
</table>
</td>
<td>only combined results for MOTIVATE 1 and 2 available: maraviroc 300mg twice daily (n=426) -1.84; optimised background (n=209) -0.78; difference -1.05 (97.5%CI: -1.33, -0.78); p<0.0001</td>
</tr>
<tr>
<td>MOTIVATE 2[55,56]

EMA status:
main

Phase III

N=474

Status:
completed</td>
<td>Biomarker evaluation:
enrichment

design: 3-arm RCT; double blind; randomisation 2:2:1; stratified by use of enfuviritide and HIV-1 RNA level at screening

follow-up: 48 weeks

primary outcome: mean change in levels of HIV-1 RNA from baseline to week 48 (log10 - transformed copies per ml)</td>
<td>age ≥ 16 years; CCR5 tropic; experienced drugs from 3 different categories</td>
<td>All with optimised background therapy:
A) maraviroc 300 mg (or 150 mg if the optimised background therapy included delavirdine); oral; once daily (plus placebo once daily)
B) maraviroc 300 mg (or 150 mg if the optimised background therapy included delavirdine); oral; twice daily (total daily dose 600 mg or 300 mg)
C) placebo twice daily</td>
<td>samples: blood

method: using a phenotypic assay (Trofile, Monogram Biosciences)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>186</td><td>186</td><td>0</td></tr>
<tr><td>B</td><td>194</td><td>194</td><td>0</td></tr>
<tr><td>C</td><td>94</td><td>94</td><td>0</td></tr>
</table>
</td>
<td>only combined results for MOTIVATE 1 and 2 available: maraviroc 300mg twice daily (n=426) -1.84; optimised background (n=209) -0.78; difference -1.05 (97.5%CI: -1.33, -0.78); p<0.0001</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Saag 2009[55,57]

EMA status: supportive

Phase IIb

N=190

Status: completed</td>
<td>Biomarker evaluation: enrichment (negative)

design: 3-arm RCT; double blind; randomisation 1:1:1

follow-up: 24 weeks

primary outcome: change in levels of HIV-1 RNA from baseline to week 24 (log10 - transformed copies per ml)</td>
<td>age ≥ 16 years; CXCR4, dual of mixed tropic HIV-1 infection; at least 1 previous treatment</td>
<td>All with optimised background therapy:
A) maraviroc 300 mg once daily; 150 mg if concomitant treatment with potent CYP3A4 inhibitors; oral)
B) maraviroc 300 mg twice daily; 150 mg if concomitant treatment with potent CYP3A4 inhibitors (total daily dose 600 mg or 300 mg); oral
C) placebo NR</td>
<td>samples: blood

method: Trofile assay, Monogram Biosciences

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>63</td><td>0</td><td>63</td></tr>
<tr><td>B</td><td>63</td><td>0</td><td>63</td></tr>
<tr><td>C</td><td>64</td><td>0</td><td>64</td></tr>
</table>
</td>
<td>only reported that no harm was seen compared to placebo</td>
</tr>
</table>

CD-33 expression – AML – gemtuzumab ozogamicin

Study	Design	Population	Interventions	Biomarker measurement	Results
0903B1-201-US/CA[58,59] EMA status: main Phase II N=84 Status: completed	Biomarker evaluation: single arm only positive design: uncontrolled Simon 2-stage follow-up: NR; median overall survival 4.9 months (for all three studies) primary outcome: complete remission (defined as 1) absence of leukemic blasts in peripheral blood; 2) ≤5% leukemic blasts in the bone marrow measured in bone marrow aspirates or biopsy samples; 3) peripheral blood counts with hemoglobin at least 9 mg/dL, absolute neutrophil count at least 1500/microL, and platelets at least 100,000/mictoL; and 4) red blood cell transfusion independence for at least 2 weeks and platelet transfusion independence for at least 1 week. Determination of remission was evaluated approximately 28 days after last dose of gemtuzumab ozogamicin)	CD33 positive; untreated first recurrence of AML; 18 years or older; initial complete remission of at least 6 months	gemtuzumab ozogamicin two doses of 9 mg/m2 as 2-hour intravenous infusion with 14-28 days between doses	samples: bone marrow (details NR) method: analysis of bone marrow aspirates and immunotyping threshold: N/A N: 84; BM+: 84; BM-: 0	complete remission was achieved in 14 patients

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>0903B1-202-EU[58,59]

EMA status: main

Phase II

N=95

Status: completed</td><td>Biomarker evaluation: single arm only positive

design: uncontrolled (details NR)

follow-up: NR; median overall survival 4.9 months (for all three studies)

primary outcome: complete remission (defined as in 0903B1-201-US/CA)</td><td>CD33 positive; untreated first recurrence of AML; 18 years or older; initial complete remission of at least 6 months; prior hematopoietic stem cell transplant allowed</td><td>gemtuzumab ozogamicin two doses of 9 mg/m2 as 2-hour intravenous infusion with 14-28 days between doses</td><td>samples: bone marrow (details NR)

method: analysis of bone marrow aspirates and immunotyping

threshold: N/A

CD33 was assessed in all patients and only positive patients were included in the study

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>95</td><td>95</td><td>0</td></tr></table></td><td>complete remission was achieved in 13 patients</td></tr>
<tr><td>0903B1-203-US/EU[58,59]

EMA status: main

Phase II

N=98

Status: completed</td><td>Biomarker evaluation: single arm only positive

design: uncontrolled Simon 2-stage

follow-up: NR; median overall survival 4.9 months (for all three studies)

primary outcome: complete remission (defined as in 0903B1-201-US/CA)</td><td>CD33 positive; AML in first relapse; age 60 years or older with initial remission of at least 3 months</td><td>gemtuzumab ozogamicin two doses of 9 mg/m2 as 2-hour intravenous infusion with 14-28 days between doses</td><td>samples: bone marrow (details NR)

method: analysis of bone marrow aspirates and immunotyping

threshold: N/A

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>98</td><td>98</td><td>0</td></tr></table></td><td>complete remission was achieved in 8 patients</td></tr>
</table>

D816V mutation in c-Kit - systemic mastocytosis - imatinib

Study	Design	Population	Interventions	Biomarker measurement	Results
Heinrich 2008[60,61] EMA status: unclear Phase II N=5 Status: completed	Biomarker evaluation: subgroup (retrospective) design: single-arm "exploratory", "proof of concept" follow-up: NR primary outcome: tumour response based on blood counts and bone marrow analyses	patients with systemic mastocytosis; age ≥15 years	imatinib 400 mg daily with escalation to 300 or 400 mg twice daily if no significant improvement after 4 to 8 weeks of therapy	samples: pathology specimens method: PCR amplification of genomic DNA for KIT (exons 9, 11, 13 and 17); screened for mutations using denaturating, high-pressure liquid chromatography (WAVE, Transgenomic, Inc.) threshold: N/A N: 5; BM+: 3-4*; BM-: 0-2	NR
other studies[60] EMA status: unclear Phase N/A N=30 Status: completed	Biomarker evaluation: NR design: a collection of patient data from studies; unclear if a systematic review follow-up: NR primary outcome: NR	unclear: systemic mastocytosis; age NR	imatinib (details NR)	samples: NR method: NR threshold: N/A N: 30; BM+: 3; BM-: 0-27†	NR

DPD deficiency - colon, colorectal gastric and breast cancer - capecitabine

No studies – based on mechanism of action/ metabolism[62]

* number of patients with mutation inconsitent based on the study report: "Activating point mutations in exon 17 of KIT were found in four cases of systemic mastocytosis (D816V, three cases; D816T, one case; Fig. 3) and one case of mast cell leukemia (D816V). No specimens were available for analysis from the other three patients with systemic mastocytosis." (however only 5 patietns with systemic mastocytosis were included in the study)

† EMA document reports 3 positive patients; not clear if the remaining are negative or unknown and if 3 positive patietns include patietns from Heinrich 2008

DPD deficiency - gastric cancer - tegafur/ gimeracil/ oteracil: mechanism of action

No studies – based on mechanism of action/ metabolism[63]

EGFR expression – colorectal cancer - cetuximab

Study	Design	Population	Interventions	Biomarker measurement	Results
BOND[64,65] EMA status: main Phase II N=329 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; randomised 2:1; stratified by Karnofsky PS, previous treatment (w/ or w/out oxaliplatin) and centre (minimisation) follow-up: NR primary outcome: rate of confirmed radiologic tumour response assessed by independent review committee; using modified WHO criteria: CR - complete disappearence of all measurable elsions w/out appearance of new ones; PR: at least 50% reduction in volume (sum of products of largest perpendicular diameters) of bidimentionally measurable lesions and absence of progression in other lesions, no appearance of new ones; SD: reduction in volume less than 50% or increase less than 25%, no new lesions; PD: increase in volume over 25% and appearance of new lesions	stage IV colorectal adenocarcinoma; progression on irinotecan	A) cetuximab (iv infusion 400 mg/m2 at day 1; followed by weekly infusions of 250 mg/m2; premedication w/ histamine-receptor antagonist at least before first dose; until disease progression or unacceptable toxicity) + irinotecan B) cetuximab monotherapy (iv infusion 400 mg/m2 at day 1; followed by weekly infusions of 250 mg/m2; premedication w/ histamine-receptor antagonist at least before first dose; until disease progression or unacceptable toxicity)	samples: paraffin-embedded tumour specimen method: immunohistochemistry at a central location in Germany - EGFR diagnostic kit (Dako Cytomation) threshold: NR group / N / BM+ / BM- A / 218 / 217 / 1 B / 111 / 110 / 1	objective response rates: combination (n=218) 22.9% (95%CI: 17.5, 29.1); monotherapy (n=111) 10.8% (95%CI: 5.7, 18.1)

Study	Design	Population	Interventions	Biomarker measurement	Results
IMCL CP02-0141[64,66] EMA status: supportive Phase II N=57 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: NR primary outcome: response rate (details NR)	stage IV colorectal cancer; progression w/in 6 months of completing irinotecan therapy	cetuximab monotherapy (iv over 1-2 hours weekly for 6 weeks; treatment repeated in absence of disease progression or unacceptable toxicity; dose NR)	samples: tumour; details NR method: NR threshold: NR N: 57; BM+: 57; BM-: 0	objective esponse rate (CR+PR): (n=57) 8.8% (95% CI: 2.9, 19.3)
IMCL CP02-9923[64,67] EMA status: supportive Phase II N=138 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: NR primary outcome: : response rate (details NR)	advanced colorectal carcinoma; disease progression at any time after receiving irinotecan	cetuximab (iv; day 1: 10 min test dose => no grade 4 anaphylactic reaction => loading dose over 2 h; maintanence over 1 hour on days 8, 15, 22, 29 and 36; repeat courses every 6 weeks if no progression or unacceptable toxicity; dose NR) + irinotecan	samples: method: threshold: N/A N: 138; BM+: 138; BM-: 0	objective esponse rate (CR+PR): (n=138) 15.2% (95% CI: 9.7, 22.3)

EGFR expression – NSCLC - cetuximab

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>CA225099[68-70]

EMA status:
main

Phase III

N=676

Status:
completed</td>
<td>Biomarker evaluation: subgroup (retrospective)

design: RCT; open-label; randomised 1:1; stratified by site; ECOG PS (0 or 1) and intended taxane; initially designed as phase II study (10 months after accrual initiation protocol amended to phase III and accrual increased from 300 to 660 patients)

follow-up: NR

primary outcome: PFS based on assessment by independent radiologic review committee</td>
<td>adult; wet stage IIB or IV NSCLC or recurrent after chemo- or radiotherapy NSCLC</td>
<td>A) cetuximab (iv infusion starting dose 400 mg/m2 over 2 h - day 1; from day 8 at 250 mg/m2 over 1h once a week; until progression or untolerable toxicity) + chemotherapy (carboplatin + taxane: paclitaxel or docetaxel)
B) chemotherpay (carboplatin + taxane: paclitaxel or docetxel)</td>
<td>samples: formalin-fixed, paraffin-embedded tissue samples, as blocks or 5-micro meter - thick unstained sections from most recent diagnostic tumour biopsy available

method: immunohistochemistry using PharmDx Kit (Dako, Carpinteria, CA)

threshold: one or more tumour cells showed staining

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>338</td><td>66</td><td>11</td><td>261</td></tr>
<tr><td>B</td><td>338</td><td>65</td><td>6</td><td>267</td></tr>
</table>
</td>
<td>(n=676) HR=0.902 (95%CI: 0.761, 1.069)
Subgroup NR</td>
</tr>
<tr>
<td>FLEX[68,71]

EMA status:
main

Phase III

N=1125

Status:
completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; randomised 1:1; stratified by ECOG PS (0-1 vs 2) and tumour stage (wet IIIB vs IV)

follow-up: median 23.8 months in both groups

primary outcome: overall survival from time of randomisation until death; calculated in months</td>
<td>adults; EGFR+ advanced (stage wet IIIB or IV) NSCLC; chemotherapy naïve</td>
<td>A) cetuximab (iv infusion at starting dose 400 mg/m2 over 2 hours on day 1; from day 8 at 250 mg/m2 over 1h once a week; premedication with antihistamine on day 1 and recommended on other; until progression) + chemotherapy (cisplatin + vinorelbine)
B) chemotherapy (cisplatin + vinorelbine)</td>
<td>samples: tumour tissue (details NR)

method: immunohistochemistry (DakoCytomation pharmDxTM immunohistochemistry kit, Dako, Glostrup, Denmark)

threshold: evidence of EGFR expression in at least one positively stained tumour cell

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>557</td><td>557</td><td>0</td></tr>
<tr><td>B</td><td>568</td><td>568</td><td>0</td></tr>
</table>
</td>
<td>(n=1125) HR=0.871 (95% CI: 0.762, 0.996) (stratified by ECOG PS and tumour stage)</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Rosell 2007[68,72]

EMA status: supportive

Phase II

N=86

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; randomised 1:1

follow-up: NR

primary outcome: best overall response rate (based on number of patients achieving a PR or CR as best overall response)</td>
<td>adult; wet stage IIB or IV EGFR-expressing NSCLC; no prior chemotherapy</td>
<td>A) cetuximab (iv infusion starting dose 400 mg/m2 over 2 h on day 1; from day 8 at 250 mg/m2 over 1h once a week; premedication with antihistamine; until progression or unacceptable toxicity) + chemotherapy (cisplatin + vinorelbine)
B) chemotherapy (cisplatin + vinorelbine)</td>
<td>samples: primary tumour and/or metastases (details NR)

method: immunohistochemistry (Dako EGFR pharmDx Kit)

threshold: at least 1% of malignant cells were EGFR detectable with respest to positive control

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>43</td><td>43</td><td>0</td></tr>
<tr><td>B</td><td>43</td><td>43</td><td>0</td></tr>
</table>
</td>
<td>best overall response rate: cetuximab (n=43) 34.9% (95%CI: 21.0, 50.9), standard (n=43) 27.9 (95%CI: 15.3, 43.7); OR=1.38 (95%CI: 0.55, 3.46)</td>
</tr>
</table>

EGFR expression – NSCLC - erlotinib

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>BR.21[73-75]

EMA status: main

Phase III

N=731

Status: completed</td>
<td>Biomarker evaluation: subgroup (prospective) - participation in "correlative studies" was optional

design: RCT; double blind randomised 2:1 (erlotinib:placebo); stratified using dynamic minimisation by centre, number of prior regimens, prior platinum therapy, best response to prior therapy and ECOG PS

follow-up: NR

primary outcome: overall survival (details NR)</td>
<td>adults; stage IIIB or IV NSCLC after the failure of one or two prior chemotherapy regimens</td>
<td>Background best supportive care:
A) erlotinib 150 mg orally once daily
B) placebo once daily</td>
<td>samples: cancer tissue from diagnostic or resection specimens - paraffin blocks or 10-20 unstained slides

method: immunohistochemistry in a central laboratory that used Dako EGFR PharmDx kits (DakoCytomation)

threshold: positivity - more than 10% of cells staining at any intensity for EGFR

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>488</td><td>117</td><td>93</td><td>278</td></tr>
<tr><td>B</td><td>243</td><td>67</td><td>48</td><td>128</td></tr>
</table>
</td>
<td>Overall OS: HR = 0.73 (95% CI: 0.60-0.87, p=0.001); median erlotinib 6.7 months (95% CI: 5.5, 7.8 months), placebo 4.7 months (95% CI: 4.1, 6.3 months)
EGFR+ (n=185) OS: HR = 0.68 (95% CI: 0.5, 0.9); median erlotinib 8.6 months (95% CI: NR), placebo 3.7 months (95% CI: NR)
EGFR- (n=141) OS: HR = 0.93 (95% CI: 0.6, 1.4); median erlotinib 5.0 months (95% CI: NR), placebo 5.4 months (95% CI: NR)
EGFR unmeasured (n=405) OS: HR = 0.77 (95% CI: 0.6, 1.0); median erlotinib 6.3 months (95% CI: NR), placebo 5.5 months (95% CI: NR)</td>
</tr>
<tr>
<td>Perez-Soler 2004[73,76]

EMA status: main

Phase II

N=57

Status: supportive</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm (details NR)

follow-up: NR

primary outcome: response rate (percentage of patients with partial or complete response according to WHO criteria determined by an investigator)</td>
<td>adults, stage IIIB or IV NSCLC after the failure of prior platinum-based chemotherapy</td>
<td>erlotinib 150 mg/ day orally in the morning; dose increase to 200 mg/day or reduction due to toxicity allowed; minimum treatment for 8 weeks; maximum 52 weeks with potential to continue beyond 52 weeks if potentially beneficial</td>
<td>samples: tumour specimen (details NR)

method: immunohistochemistry (details NR)

threshold: positivity - more than 10% of cells staining for EGFR

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>57</td><td>57</td><td>0</td></tr>
</table>
</td>
<td>"2 achieved a CR and 5 had a best response of PR as determined by both WHO and RECIST, for an objective response rate of 12.3% (95% CI, 5.1 – 23.7%). Twenty-two patients had SD (38.6%) and 28 patients (49.1%) had progressive disease (PD)"</td>
</tr>
</table>

EGFR mutation – NSCLC - erlotinib

Study	Design	Population	Interventions	Biomarker measurement	Results
EURTAC[77,78] EMA status: main Phase III N=173 Status: ongoing	Biomarker evaluation: enrichment design: RCT; open-label; centrally randomised 1:1; stratified by type of EGFR mutation and ECOG PS follow-up: median 18.9 for erlotinib and 14.4 for chemotherapy primary outcome: PFS (time from randomisation to the date when disease progression was first observed or death occurred)	adults with stage IIIB (with pleural effusion) or IV NSCLC; not previously treated for metastatic disease	A) erlotinib 150 mg daily; orally B) standard chemotherapy 3 week iv cycles: (75 mg/m2 cisplatin + 75 mg/m2 docetaxel on day 1) or (75 mg/m2 cisplatin on day 1 + 1250 mg/m2 gemcitabine on day 1 and 8) or (if ineligible for cisplatin: AUC6 + 75 mg/m2 docetaxel on day 1 or AUC5 +1000g/m2 gemcitabine on day 1 and 8)	samples: tumour specimens from original biopsy obtained before any treatment was given method: Sanger sequencing (exons 19 and 21); confirmed by PCR: exon 19 deletions with FAM-labelled primer in an ABI prism 3130 DNA analyser (Applied Biosystems) and L868R mutations in exon 21 with FAM MGB-labelled probe for the wild-type and VIC MGB-labelledprobe for the mutant sequence threshold: N/A group / N / BM+ / BM- A / 86 / 86 / 0 B / 87 / 87 / 0	interim analysis: PFS HR = 0.42 (95% CI 0.27-0.64, p<0.0001); median PFS erlotinib; 9.7 months chemotherapy 5.2 months

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>CALGB30406[77, 79,80]

EMA status: supportive

Phase II

N=181

Status: completed</td>
<td>Biomarker evaluation: subgroup (prospective)

design: RCT (details NR)

follow-up: median 38 months

primary outcome: PFS (time from randomisation to disease progression or death)</td>
<td>adults with primary lung adenocarcinoma; stage IIIB with pleural effusion or IV; no prior chemotherapy, erlotinib or other EGFR targeted drugs; radiation and surgery had to be completed at least 3 weeks prior to enrollment; never or light smokers</td>
<td>A) erlotinib monotherapy 50 mg/ day until disease progression or unacceptable toxicity; dose reductions for toxicity allowed to 100 or 50 mg/day
B) erlotinib (as erlotinib group) + chemotherapy (up to 6 cycles of paclitaxel 200 mg/m2 every 21 days and carboplatin AUC 6 every 21 days - standard dose reductions allowed for toxicity)</td>
<td>samples: cancer tissue (details NR)

method: using a sensitive heteroduplex method coupled with enzymatic digestion; positive findings were independently verified and subjected to sequencing

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>81</td><td>33</td><td>44</td><td>4*</td></tr>
<tr><td>B</td><td>100</td><td>33</td><td>54</td><td>13</td></tr>
</table>
</td>
<td>Overall (median?) PFS: erlotinib 6.7 months; [80% CI, 4.7 to 8.2 months]); chemotherapy plus erlotinib 6.0 months; [80% CI, 5.6-7.3 months])
with EGFR mutations: (median?) PFS: erlotinib 16.4 months [80% CI, 12.1 to 23.8]; chemotherapy + erlotinib 17.2 months [80% CI, 11.1 to 27.6]
no EGFR mutation: NR
unknown EGFR mutation status: NR</td>
</tr>
<tr>
<td>Laskin 2009[77,81]

EMA status: supportive

Phase II

N=65

Status: completed</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm (details NR)

follow-up: 8 weeks

primary outcome: non-progression at 8 weeks (detaile NR)</td>
<td>"stage IIIB/IV NSCLC; no prior chemo; ECOG ≤2; at least 2 of the following 4 criteria: women, never-smokers, Southeast Asian origin, adenocarcinoma and/or BAC"</td>
<td>erlotinib oral 150 mg/ day until disease progression</td>
<td>samples: pre-treatment tumour samples (probably fresh frozen biopsy)

method: sequencing using "traditional methods" (details NR)

threshold: N/A

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>65</td><td>19</td><td>32</td><td>14</td></tr>
</table>
</td>
<td>52 patients had not progressed after 8 weeks NR for EGFR mutation subgroups</td>
</tr>
</table>

* 17 patients had insufficient tissue material for analysis

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>OPTIMAL[77,82]

EMA status: supportive

Phase III

N=165

Status: ongoing</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; randomised 1:1 by dynamic minimisation (Mini Randomisation software, v. 1.5); stratified by EGFR mutation type, histological subtype and smoking status

follow-up: NR

primary outcome: PFS (time from randomisation to first confirmed disease progression or death from any cause)</td>
<td>adults with advanced or recurrent stage IIIB-IV NSCLC; no previous systemic therapy for advanced disease</td>
<td>A) erlotinib oraly; 150 mg/day until disease progression or unacceptable toxicity
B) chemotherapy up to 4 cycles of platinum-based chemotherapy (iv gemcitabine 1000 mg/m2 on day 1 and 8 + iv carboplatin AUC = 5 on day 1)</td>
<td>samples: tumour sample - fresh or paraffin embedded

method: PCR-based direct sequencing (exon 19 deletion or exon 21 L858R point mutation)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>83</td><td>83</td><td>0</td></tr>
<tr><td>B</td><td>82</td><td>82</td><td>0</td></tr>
</table>
</td>
<td>median PFS was erlotinib 13.1 months, carb/gem 4.6 months; PFS HR = 0.16 [95% CI, 0.10 to 0.26]; p <0.0001</td>
</tr>
<tr>
<td>Paz-Ares 2010[77]

EMA status: supportive

Phase N/A

N=1809

Status: N/A</td>
<td>Biomarker evaluation: NR

design: literature review (details NR)

follow-up: NR

primary outcome: median PFS</td>
<td>NSCLC; details unclear - not only first line therapy</td>
<td>A) erlotinib 150 mg/ day orally (12 studies)
B) gefitinb 250 mg/day orally or 500 mg/day orally (39 studies)
C) chemotherapy: platinum-based/ docetaxel/ standard (9 studies)</td>
<td>samples: tumour tissue (details NR)

method: variety of techniques (details NR)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>365</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>1069</td><td>NR</td><td>NR</td></tr>
<tr><td>C</td><td>375</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>For patients treated predominantly in the first-line setting, median PFS was erlotinib (57%* of all patients in review) 12.5 months (range 10.0 – 16.0 months), gefitinib (57%* of all patients in review) 9.9 months (range 9.0 – 10.9 months), chemotherapy (95%* of all patients in review) 6.0 months (range 4.5 – 6.7 months)</td>
</tr>
</table>

* unclear if analysis includes only data for patients treated with first line therapy or studies wher most patients were receiving first-line treatment

Study	Design	Population	Interventions	Biomarker measurement	Results
Rosell 2009[77,83] EMA status: supportive Phase NR N=217 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm; patients screened prospectively for EGFR mutations; patients with mutations were considered for erlotinib treatment follow-up: median 14 months (range, 1 to 42) primary outcome: NR	stage IIIB with pleural effusion or IV NSCLC*	erlotinib 150 mg/ day until disease progression or intolerable adverse events	samples: cancer tissue from original biopsy before any treatment - either paraffin-embedded (2060 of 2105 screened) or fresh specimens method: exon 19 deletions by length analysis after PCR amplification with FAM-labeled primer in an ABI Prism 3130 DNA Analyzer (Applied Biosystems); exon 21 point mutations in codon 858 with 5' nuclease PCR assay (TaqMan) using FAM and VIC MGB-labeled probes for the wild type sequence; all mutants were confirmed by DNA sequencing threshold: N/A N: 217; BM+: 217; BM-: 0	median PFS (n = 217) 14.0 months (95% CI, 11.2 to 16.7)

* participating centres included more samples from patietns likely to have EGFR mutations (women, never smokers, adenocarcinoma) - possible bias/confounding?

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>SATURN[77]

EMA status: supportive

Phase III

N=889

Status: completed</td>
<td>Biomarker evaluation: subgroup (prospective)

design: RCT; double-blind; randomised 1:1; stratified (minimisation) by EGFR expression, disease stage, ECOG PS, chemotherapy regimen, smoking status and region

follow-up: median 11.4 months for erlotinib and 11.5 for placebo

primary outcome: PFS (tumour response classified by RECIST 1.0 criteria; scans every 6 weeks for 48 weeks and every 12 weeks thereafter)</td>
<td>maintenance in locally advanced or metastatic patients stable after 4 cycles of platinum-based chemotherapy</td>
<td>A) erlotinib 150 mg oraly once daily until disease progression, unacceptable toxicity or death
B) placebo until disease progression, unacceptable toxicity or death</td>
<td>samples: tumour tissue (details NR)

method: using DNA lysates from macrodissected or microdissected tissue samples with minimum tumour-cell content 60%; exons 18-21 of EGFR amplified using PCR (nested primers) and sequenced

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>438</td><td>22</td><td>199</td><td>217</td></tr>
<tr><td>B</td><td>451</td><td>27</td><td>189</td><td>235</td></tr>
</table>
</td>
<td>Overall NR
EGFR mutation + (n=49): from the clinical cut-off for overall survival (May 17, 2009): PFS HR 0.23 ([95% CI, 0.12 to 0.45]; log-rank p < 0.0001); median erlotinib 46.1 weeks [95% CI, 33.7 to 59.6]; placebo 13.0 weeks [95% CI, 11.6 to 21.3]
EGFR mutation - (n=388): from the clinical cut-off for overall survival (May 17, 2009): HR = 0.78 ([95% CI: 0.64; 0.96], p=0.0182); median PFS erlotinib 12.0 weeks (95% CI, 10.9 to 12.7 weeks), placebo 8.9 weeks (95% CI, 6.3 to 11.4 weeks)
EGFR mutation unknown: NR</td>
</tr>
</table>

EGFR mutation – NSCLC - gefitinib

Study	Design	Population	Interventions	Biomarker measurement	Results
INTEREST[84,85] EMA status: main Phase III N=1466 Status: completed	Biomarker evaluation: subgroup (prospective) design: RCT; open-label; randomised 1:1 with dynamic balancing with respect to histology (adenocarcinoma vs other), performance status, previous platinum chemotherapy (refractory vs non-refractory), previous paclitaxel (refractory vs non-refractory vs none), number of previous regimens (one vs two), smoking history (ever vs never), and study site follow-up: NR primary outcome: overall survival from the date of randomisation to the date of death due to any cause or last date that the patient was known to be alive	patients with locally advanced or metastatic NSCLC that progressed or recurred after at least one previous platinum-based chemotherapy (up to two), no previous therapy with an EGFR tyrosine kinase inhibitor	A) gefitinib 250 mg per day orally; until disease progression, unacceptable toxic effects, or patient or physician request to discontinue treatment B) docetaxel 75 mg/m² in a 1-h infusion every 3 weeks (could be reduced to 60 mg/m² to reduce toxic eff ects); until disease progression, unacceptable toxic effects, or patient or physician request to discontinue treatment	samples: paraffin-embedded archival diagnostic tumour tissue method: "direct gene sequencing of exons 18–21 of chromosome seven. Patients were positive if we detected a mutation in the EGFR gene in both the forward and reverse directions in at least one of the three independent PCR products derived from the tumour DNA"; testing performed by "approved commercial laboratories" threshold: N/A group / N / BM+ / BM- / BM? A / 733 / 22 / 119 / 529 B / 733 / 22 / 134 / 577	Overall OS (primary - per protocol analysis): gefitinib (n=723) vs docetaxel (n=710) HR = 1.020 (95% CI: 0.905, 1.150, p=0.7332) EGFR mutation +: OS (primary - per protocol analysis): gefitinib (n=22) vs docetaxel (n=22) HR = 0.832 (95% CI: 0.414, 1.670, p=0.6043) EGFR mutation -: OS (primary - per protocol analysis): gefitinib (n=119) vs docetaxel (n=134) HR = 1.015 (95% CI: 0.776, 1.327, p=0.9131) EGFR mutation unknown: OS (primary - per protocol analysis): gefitinib (n=592) vs docetaxel (n=572) HR = 1.027 (95% CI: 0.904, 1.167, p=0.6808)

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>IPASS[84,86]

EMA status:
main

Phase III

N=1217

Status:
completed</td>
<td>Biomarker evaluation:
subgroup (prospective)

design:RCT; open-label; non-inferiority; randomised 1:1; using dynamic balancing with respect to performance

follow-up: median 5.6 months

primary outcome: PSS (from the date of randomization to the earliest sign of disease progression, as determined by means of the RECIST)</td>
<td>first-line treatment adults in East Asia with stage IIIB or IV NSCLC (adenocarcinoma, including bronchoalveolar carcinoma), nonsmokers (had smoked <100 cigarettes in their lifetime) or former light smokers (had stopped smoking at least 15 years previously and had a total of ≤10 pack-years of smoking), no previous chemotherapy or biologic or immunologic therapy</td>
<td>A) gefitinib 250 mg per day, administered orally; until disease progression, unacceptable toxic effects, request by patient or physician, serious noncompliance with protocol
B) chemotherapy (up to 6 3-week cycles: on day 1 paclitaxel 200 mg/m2 of body-surface area (iv over 3-hours) followed immediately by carboplatin (AUC 5.0 or 6.0 mg/ ml per minute, iv over 15 to 60 minutes); same discontinuation reasons)</td>
<td>samples: tumour tissue(details NR)

method: analysed at two central laboratories to determine biomarker status; patients were "considered to be positive for the EGFR mutation if 1 of 29 EGFR mutations was detected with the use of the amplification refractory mutation system (ARMS) and the DxS EGFR29 mutation-detection kit" details of mutations assessed NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>609</td><td>132</td><td>91</td><td>386</td></tr>
<tr><td>B</td><td>608</td><td>129</td><td>85</td><td>394</td></tr>
</table>
</td>
<td>Overall PFS: gefitinib (n=609) vs carboplatin + pacliaxel (n=608) HR = 0.741 (95% CI: 0.651, 0.845, p<0.0001)
EGFR mutation+: PFS: gefitinib (n=132) vs carboplatin + pacliaxel (n=129) HR = 0.482 (95% CI: 0.362, 0.642, p<0.0001)
EGFR mutation-: PFS: gefitinib (n=91) vs carboplatin + pacliaxel (n=85) HR = 2.853 (95% CI: 2.048, 3.975, p<0.0001)
EGFR mutation unknown: PFS: gefitinib (n=386) vs carboplatin + pacliaxel (n=394) HR = 0.684 (95% CI: 0.579, 0.808, p<0.0001)
EGFR mutation known: PFS: gefitinib (n=223) vs carboplatin + pacliaxel (n=214) HR = 0.853 (95% CI: 0.690, 1.055, p=0.1426)</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>ISEL[84,87,88]

EMA status:
main

Phase III

N=1692

Status:
completed</td>
<td>Biomarker evaluation:
subgroup (retrospective)

design: RCT; double-blind; randomised 2:1 by minimisation

follow-up: median follow-up 7.2 months

primary outcome: overall survival from the date of randomisation to the date of a patient's death; participants alive at data cutoff were censored in the analysis at the last time they were known to be alive</td>
<td>patients with locally advanced or metastatic NSCLC that was not curable with surgery or radiotherapy, who had received one or two previous chemotherapy regimens and were refractory to (recurrent or progressive disease within 90 days of the last chemotherapy dose) or intolerant of their latest chemotherapy regimen</td>
<td>A) gefitinib 250 mg/day until unacceptable toxic effects occurred, consent was withdrawn, or the patient was no longer deriving clinical benefit
B) placebo</td>
<td>samples: tumour sample (details NR)

method: "analyzed primarily by DNA sequencing of exons 18 to 24, and secondarily using the amplification refractory mutation system (ARMS) assay (allelespecific polymerase chain reaction [PCR]) to detect the exon 21 L858R point mutation and the most common exon 19 deletion (del G2235-A2249). Patients were mutation positive if a mutation in the EGFR gene was detected either by ARMS or by gene sequencing in both forward and reverse directions in at least two independent PCR products from tumor DNA. Sequence alterations detected in more than one amplicon were considered true mutations"

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>1126</td><td>21</td><td>132</td><td>973</td></tr>
<tr><td>B</td><td>562</td><td>5</td><td>57</td><td>500</td></tr>
</table>
</td>
<td>Overall OS: HR = 0.89 (95% CI: 0.77, 1.02, p=0.0871); favours gefitinib
EGFR mutation + OS: 10 deaths (seven of 21 patients receiving gefitinib; three of five patients receiving placebo)
EGFR mutation OS: 130 deaths (93 of 132 patients receiving gefitinib; 37 of 57 patients receiving placebo)
EGFR mutation unknown: NR</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>V-15-32[84,89]

EMA status: supportive

Phase III

N=490

Status: completed</td>
<td>Biomarker evaluation: subgroup (prospective)

design: RCT; open-label; noninferiority; randomisation stratified by sex, performance status, histology, and study site

follow-up: median 21 months

primary outcome: overall survival "from date of random assignment to date of death as a result of any cause, or data were censored at the last date the patient was known to be alive"</td>
<td>apanese patients with pretreated, locally advanced/metastatic (stages IIIB to IV) or recurrent NSCLC</td>
<td>A) gefitinib 250 mg/day orally until disease progression, intolerable toxicity, or discontinuation for another reason
B) docetaxel every 3 weeks as a 1-hour intravenous infusion of 60 mg/m2 until disease progression, intolerable toxicity, or discontinuation for another reason</td>
<td>samples: tumour tissue (details NR)

method: direct sequencing of exon 18 to 21 of chromosome 7

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>245</td><td>NR*</td><td>NR</td></tr>
<tr><td>B</td><td>244</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>Overall study failed to meet non-inferiority objective; NR for EGFR mutation determined subgroups</td>
</tr>
</table>

* 31 (54.4%) of 57 patients in whom mutation was measured (in both groups) had EGFR mutation–positive tumors

EpCAM expression – cancer ascities - catumaxomab

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>Heiss 2010[90,91]

EMA status: main

Phase II/III

N=258

Status: completed</td><td>Biomarker evaluation:

design: RCT; open-label; randomised 2:1; stratified by cancer (ovarian vs. not ovarian) and country

follow-up: 7 months

primary outcome: puncture-free survival (time to first need for therapeutic puncture or death after treatment, whichever occurred first)</td><td>adult malignant ascites secondary to epithelial cancer, requiring paracentesis</td><td>A) catumaxomab (6 hour intraperitoneal infusion on day 0, 3, 7 and 10 at 10, 20, 50 and 150 microgrograms) + paracentesis
B) paracentesis only</td><td>samples: ascites fluid (details NR)

method: immunohistochemistry: anti-EpCAM antibody HO-3 (the parental antibody of catumaxomab; TRION Pharma, Munich, Germany)

threshold: at least 400 EpCAM+ cells per 10^6 cells

<table><tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr><tr><td>A</td><td>170</td><td>170</td><td>0</td></tr><tr><td>B</td><td>88</td><td>88</td><td>0</td></tr></table></td><td>median puncture-free survival: catumaxomab 46 days (95% CI: 35, 53), control 11 days (95% CI: 9, 16), p (log-rank test) < 0.0001</td></tr>
<tr><td>Burges 2007[90,92]

EMA status: supportive

Phase I/II

N=23

Status: completed</td><td>Biomarker evaluation: non-randomised only positive

design: single-arm; dose escalation

follow-up: 28 days after last infusion

primary outcome: NR</td><td>female adult patients with malignant ascites due to ovarian carcinoma</td><td>catumaxomab (6 hour intraperitoneal infusion on day 0, 3, 6, 9 and 13; at 5 to 250 microgrograms)</td><td>samples: ascites fluid (details NR)

method: immunohistochemistry: anti-EpCAM antibody HO-3 (the parental antibody of catumaxomab; TRION Pharma, Munich, Germany)

threshold: more than 400 EpCAM+ cells per 10^6 cells

<table><tr><th>N</th><th>BM+</th><th>BM-</th></tr><tr><td>23</td><td>23</td><td>0</td></tr></table></td><td>necessity for peritoneal puncture (28+/- 4 days from start of last infusion) - 1 patient needed puncture; MTD was established at 200 micrograms</td></tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
Ruf 2010[90,93] EMA status: supportive Phase II N=13 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm pharmacokinetic (details NR) follow-up: NR primary outcome: loacal and systemic catumaxomab concentrations	ascites due to cancer (no criteria for prior treatments)	catumaxomab (6 hour intraperitoneal infusion on day 0, 3, 6 or 7 and 10; at 10, 20, 50 and 150 microgrograms)	samples: ascites fluid (details NR) method: immunohistochemistry (details NR) threshold: at least 400 EpCAM+ cells per 10^6 cells N: 23; BM+: 23; BM-: 0	primary outcome data NR; need for peritoneal puncture: 5 patients; not necessary in 7 and one died without need of puncture

oestrogen receptor – breast cancer - fulvestrant

Study	Design	Population	Interventions	Biomarker measurement	Results
0020[94-96] EMA status: main Phase III N=451 Status: completed	Biomarker evaluation: enrichment* design: RCT; open-label; superiority study with retrospective switch to non-inferiority after no significant difference was shown follow-up: median 15.1 months (for trial 0020 and 0021) primary outcome: TTP (number of days from randomisation until disease progression or death from any cause)	postmenopausal women with locally advanced or metastatic breast cancer with objective evidence of disease progression or recurrence on adjuvant endocrine therapy or following first-line endocrine therapy for advanced disease	A) fulvestrant 250 mg as a once-monthly intra-muscular injection (5 ml) until disease progression or withdrawal from trial (due to toxicity, non-complience or withdrawal of consent) B) anastrozole 1 mg once daily orally until disease progression or withdrawal from trial (due to toxicity, non-complience or withdrawal of consent)	samples: NR method: NR threshold: NR group A: N 222, BM+ 156, BM- 15, BM? 51 group B: N 229, BM+ 173, BM- 19, BM? 37	overall TTP HR = 0.98 (95%CI: 0.80, 1.21, p=0.84) favours fulvestrant NR for oestrogen receptor expressing only

* included patients with evidence of tumour hormone sensitivity: prior sensitivity to hormonal therapy or known estrogen receptor or progesterone receptor positivity

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>0021[94-96]

EMA status: main

Phase III

N= 400

Status: completed</td>
<td>Biomarker evaluation: enrichment*

design: RCT; double-blind; superiority study with retrospective switch to non-inferiority after no significant difference was shown

follow-up: median 15.1 months (for trial 0020 and 0021)

primary outcome: TTP (number of days from randomisation until disease progression or death from any cause)</td>
<td>postmenopausal women with locally advanced or metastatic breast cancer with objective evidence of disease progression or recurrence on adjuvant endocrine therapy or following first-line endocrine therapy for advanced disease</td>
<td>A) fulvestrant 250 mg as a once-monthly intra-muscular injection (2 x 2.5 ml) until disease progression or withdrawal from trial (due to toxicity, non-complience or withdrawal of consent)
B) anastrozole 1 mg once daily orally until disease progression or withdrawal from trial (due to toxicity, non-complience or withdrawal of consent)</td>
<td>samples: NR

method: NR

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>206</td><td>170</td><td>23</td><td>13</td></tr>
<tr><td>B</td><td>194</td><td>156</td><td>22</td><td>16</td></tr>
</table>
</td>
<td>Overall TTP HR = 0.92 (95% CI: 0.74, 1.14, p=0.43), favours fulvestrant
NR for oestrogen receptor expressing only</td>
</tr>
<tr>
<td>0004[94,97]

EMA status: supportive

Phase II

N=19

Status: completed</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm; 2 parts: part I - 1 month with assessments at day 0, 1, 3, 7, 10, 14, 21 and 28; part II - 6 months with assessment once a month

follow-up: 6 months

primary outcome: partial or complete response (details NR)</td>
<td>postmenopausal women with advanced breast cancer who have relapsed on tamoxifen therapy</td>
<td>fulvestrant once a month by intramuscular injection: part I: 1 ml (50 mg); part II: either 2 ml (100mg) + 5 x 5 ml (250 mg) or 6 x 5 ml (250 mg)</td>
<td>samples: NR

method: NR

threshold: NR

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>19</td><td>19</td><td>0</td></tr>
</table>
</td>
<td>among patients who received the 250 mg dose (n=19) 7 had PR and 6 SD for at least 6 months</td>
</tr>
</table>

* included patients with evidence of tumour hormone sensitivity: prior sensitivity to hormonal therapy or known estrogen receptor or progesterone receptor positivity

Study	Design	Population	Interventions	Biomarker measurement	Results
O-15-22[94] EMA status: supportive Phase II N=30 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: NR primary outcome: objective tumour response (details NR)	postmenopausal women who relapsed on tamoxifen or toremifene after initial response	fulvestrant (details NR)	samples: NR method: NR threshold: NR N: 30; BM+: 30; BM-: 0	objective tumour response in 23.3% of patients
SAKK[94,98] EMA status: supportive Phase II N=86 Status: ongoing	Biomarker evaluation: non-randomised only positive* design: single-arm (details NR) follow-up: NR primary outcome: initially objective response, duration of response, time to progression and time to treatment failure; after interim analysis changed to clinical benefit (clinical benefit - objective response (complete or partial) or stable disease for at least 24 weeks)	postmenopausal women with metastatic breast cancer and objective evidence of disease progression after at least 12 weeks of treatment with aromatase inhibitors	fulvestrant 250 mg in one 5-ml intramuscular injection every 28 (+/- 3) days until disease progression, withdrawal due to unacceptable toxicity or withdrawal of consent	samples: NR method: NR threshold: NR N: 86; BM+: 73; BM-: 2; BM?: 11	(n=32) 6% PR 28% SD, 66% PD; and clinical benefit in 34% of patients

* evidence of tumour hormone sensitivity: estrogen receptor and/or progesterone receptor expression, at least 12 months of previous hormonal therapy before relapse or stabilisation for at least 3 months during endocrine therapy

oestrogen receptor – breast cancer - toremifene

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>Gershanovich 1997[99,100]

EMA status: main

Phase III

N=463

Status: completed</td><td>Biomarker evaluation: unclear

design: RCT; open-label (details NR)

follow-up: median 20.5 months

primary outcome: response rate and TTP (response defined according to WHO criteria for measurable disease and ECOG criteria for non-measurable but evaluable bone disease; time to progression NR)</td><td>postmenopausal women with "previously untreated, inoperable, primary, residual, metastatic or recurrent breast cancer"</td><td>A) toremifene 60 mg tablet once a day; for at least 2 months; could be discontinued due to intolerable toxicity, rapid disease progression or non-complience
B) toremifene 240 mg: 2 60 mg tablets twice a day; for at least 2 months; could be discontinued due to intolerable toxicity, rapid disease progression or non-complience
C) tamoxifen 40 mg tablet once a day; for at least 2 months; could be discontinued due to intolerable toxicity, rapid disease progression or non-complience</td><td>samples: tumour (details NR)

method: NR

threshold: ER concentration at least 10 fmol/mg protein or ER unknown

<table><tr><td>group</td><td>N</td><td>BM+</td><td>BM-</td><td>BM?</td></tr><tr><td>A</td><td>157</td><td>48</td><td>5</td><td>104</td></tr><tr><td>B</td><td>157</td><td>50</td><td>2</td><td>105</td></tr><tr><td>C</td><td>149</td><td>44</td><td>6</td><td>99</td></tr></table></td><td rowspan="2">overall; only pooled estimate across all main studies reported; response rate (toremifene 60 mg vs. tamoxifen) was -0.8% (90% CI: -4.8, 3.2 %; p=0.744); TTP HR = 0.91 (90% CI: 0.81, 1.02, p=0.158); OS HR = 1.00 (90% CI: 0.86, 1.16, p=0.966)

oestrogen receptor positive; only pooled estimate across all main studies reported; response rate (toremifene 60 mg vs. tamoxifen) was -3.4% (90% CI: -9.0, 2.1 %; p=0.312); TTP HR = 0.93 (90% CI: 0.80, 1.08, p=0.407); OS HR = 1.06 (90% CI: 0.97, 1.29, p=0.651)
oestrogen receptor negative; NR</td></tr>
<tr><td>Hayes 1995[99,101]

EMA status: main

Phase III

N=648

Status: completed</td><td>Biomarker evaluation: unclear

design: RCT; open label; equivalence trial; stratified by presence of bone metastases (yes vs. no); originally two identical trials by the same sponsor; results were combined and analysed as a single study

follow-up: NR

primary outcome: NR</td><td>postmenopausal or perimenopausal women with metastatic (apart from brain) breast cancer; previous tamoxifen allowed</td><td>A) toremifene one 60 mg tablet a day orally till breast cancer progression, intolerable toxicity, serious incurrent ilness or patient non-complience
B) toremifene one 200 mg tablet a day orally till breast cancer progression, intolerable toxicity, serious incurrent ilness or patient non-complience
C) tamoxifen two 10 mg tablets a day orally till breast cancer progression, intolerable toxicity, serious incurrent ilness or patient non-complience</td><td>samples: tumour (details NR)

method: NR

threshold: ER concentration at least 10 fmol/mg protein or ER unknown

<table><tr><td>group</td><td>N</td><td>BM+</td><td>BM-</td><td>BM?</td></tr><tr><td>A</td><td>221</td><td>147</td><td>14</td><td>60</td></tr><tr><td>B</td><td>212</td><td>123</td><td>16</td><td>73</td></tr><tr><td>C</td><td>215</td><td>130</td><td>21</td><td>64</td></tr></table></td></tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
Pyrhonen 1997[99,102] EMA status: main Phase III N=415 Status: completed	Biomarker evaluation: subgroup (prospective) design: RCT; double-blind; stratified by wheather the patients had measurable disease follow-up: median 25.2 months primary outcome: reponse rate and TTP (responses evaluated according to the WHO criteria adopted by the UICC, response was accepted only if confirmed at two consecutive evaluations 2 months apart; time to progression (TTP) "was defined as the time between randomization and onset of relapse or disease progression ")	postmenoupousal metastatic or recurrent breast cancer; prior adjuvant therapy allowed	A) toremifene 60 mg tablet orally once daily for at least 2 months; until disease progression or adverse events precluding use of the drug B) tamoxifen 40 mg tablet orally once daily for at least 2 months; until disease progression or adverse events precluding use of the drug	samples: tumour (details NR) method: NR threshold: NR group / N / BM+ / BM- / BM? A / 214 / 117 / 4 / 93 B / 201 / 114 / 3 / 84	oestrogen receptor unknown; only pooled estimate across all main studies reported; response rate (toremifene 60 mg vs. tamoxifen) was -2.5% (90% CI: -3.2, 8.3 %; p=0.469); TTP HR = 0.87 (90% CI: 0.74, 1.06, p=0.267); OS HR = 0.86 (90% CI: 0.67, 1.09, p=0.294)

FIP1L1-PDGFRα rearrangement - HES/CEL - imatinib

Study	Design	Population	Interventions	Biomarker measurement	Results
Cervetti 2005[103,104] EMA status: unclear Phase N/A N=2 Status: completed	Biomarker evaluation: none design: case report follow-up: 12 months and 13 years primary outcome: N/A	adult with HES	A) imatinib 100 mg/day B) interferon-α 5 MUI/day three times a week	samples: bone marrow (details NR) method: NR threshold: N/A group / N / BM+ / BM- A / 1 / 1 / 0 B / 1 / 1 / 0	complete hematological response (duration ≥12 months) on imatinib; NR for interferon-α

Study	Design	Population	Interventions	Biomarker measurement	Results
Chung 2006[103,105] EMA status: unclear Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 2 months primary outcome: N/A	adult with HES	imatinib 100 mg/day and prednisolone gradually discontinued over 2 months	samples: NR method: single-step RT-PCR and nested PCR threshold: N/A N: 1; BM+: 1*; BM-: 0	complete hematological response (duration ≥2 months)
Cools 2003[103] EMA status: unclear Phase N/A N=17 Status: completed	Biomarker evaluation: subgroup (retrospective) design: non-randomised comparative (details NR) follow-up: NR primary outcome: N/A	adults with HES	A) imatinib 100-400 mg/day (patients with symptomatic disease) B) other (details NR)	samples: blood (details NR) method: nested PCR threshold: N/A A: N 11, BM+ 5, BM- 5, BM? 1 B: N 6, BM+ 4, BM- 2, BM? 0	Overall 9 complete hematological responses (duration 3-16 months), 1 transient response, 1 no response FIP1L1-PDGFRA positive 5 complete hematological responses (duration 3-9 months) FIP1L1-PDGFRA negative 3 complete hematological responses (duration 3-16 months), 1 transient, 1 none FIP1L1-PDGFRA unknown 1 complete hematological response (duration 3 months)
Frickhofen 2004[103,106] EMA status: unclear Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: approximately 10 months primary outcome: N/A	adult with CEL	imatinib 200 mg/day	samples: blood (details NR) method: nested RT-PCR (as in Cools 2003) threshold: N/A N: 1; BM+: 1; BM-: 0	complete hematological response (duration ≥248 days)

* patient negative using single-step RT-PCR and weakly positive using nested PCR

Study	Design	Population	Interventions	Biomarker measurement	Results
Imashuku 2005[103,107] EMA status: unclear Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 62 weeks primary outcome: N/A	adult with HES	imatinib 100 mg/day increased to 200 mg/day	samples: NR method: FISH (details NR) threshold: N/A N: 1; BM+: 0; BM-: 1	1 transient hematological response (duration 30 weeks)
Klion 2004[103,108] EMA status: unclear Phase N/A N=7 Status: completed	Biomarker evaluation: none design: case series follow-up: 6 months primary outcome: N/A	adults with HES	Imatinib single daily oral dose of 400 mg; possibility of interruption and restarting at a lower dose due to adverse events	samples: peripheral blood (details NR) method: nested PCR (details NR) threshold: N/A N: 7; BM+: 7; BM-: 0	7 complete hematological responses (duration 1-3 months)
La Starza 2005[103,109] EMA status: unclear Phase N/A N=26 Status: completed	Biomarker evaluation: subgroup (retrospective) design: non-randomised comparative (details NR) follow-up: ranging from 6 to 204 months; no data for 2 patients primary outcome: NR	20 patients with HES/CEL; 6 who did not satisfy WHO criteria; one patient was 10 years old (other were adults)	A) imatinib 100-600 mg/day + other treatments (including steroids, interferon-alpha, hydroxyurea) B) other including steroids, interferon-α, hydroxyurea	samples: NR method: interphase FISH (details NR) threshold: N/A A: N 12; BM+ 7; BM- 5 B: N 14; BM+ 3; BM- 11	Overall imatinib: 8 complete hematological responses (duration 2-25 months), 3 none; other: NR FIP1L1-PDGFRA positive imatinib: 6 complete hematological responses (duration 2-11 months); other: NR FIP1L1-PDGFRA negative imatinib: 2 complete hematological responses (duration 19-25 months), 3 none; other: NR

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>Malagola 2004[103,110]

EMA status: unclear

Phase N/A

N=1

Status: completed</td><td>Biomarker evaluation: none

design: case report

follow-up: 120 days

primary outcome: N/A</td><td>adult with CEL</td><td>imatinib 100 mg/day increased by 100 mg/day/week to 400 mg/day</td><td>samples: NR

method: RT-PCR (as in Martinelli 2004)

threshold: N/A

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>1</td><td>1</td><td>0</td></tr></table></td><td>1 complete hematological responses (duration ≥120 days)</td></tr>
<tr><td>Martinelli 2004[103,111]

EMA status: unclear

Phase N/A

N=1

Status: completed</td><td>Biomarker evaluation: none

design: case report

follow-up: 17 months

primary outcome: N/A</td><td>adult with HES</td><td>imatinib 600 mg/day</td><td>samples: peripheral blood and bone marrow

method: RT-PCR (as in Cools 2003) done retrospectively on diagnostic sample

threshold: N/A

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>1</td><td>1</td><td>0</td></tr></table></td><td>1 complete hematological response (duration ≥17 months)</td></tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Martinelli 2006[103,112]

EMA status: unclear

Phase II

N=59

Status: completed</td>
<td>Biomarker evaluation: subgroup (prospective)

design: single-arm (details NR)

follow-up: median 4 months (range 2-39 months)

primary outcome: NR</td>
<td>patients with HES, age ≥17 years</td>
<td>imatinib 100/day increased by 100 mg/day each week up to 400 mg/day and continued for at least 4 weeks in case of no response and for period 'beneficial for patient' if response; first year: dose adjustments for adverse events; later: at investigator discretion</td>
<td>samples: bone marrow (details NR)

method: nested RT PCR as in Cools 2003

threshold: N/A

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>59</td><td>23</td><td>36</td></tr></table></td>
<td>Overall 26 complete hematological responses (duration 1-44 months), 9 partial response, 22 none, 2 unknown, 4 NR
FIP1L1-PDGFRA positive 23 complete hematological responses (duration 1-44 months), 4 NR
FIP1L1-PDGFRA negative 3 complete hematological responses (duration 1-44 months), 9 partial response, 22 none, 2 unknown</td>
</tr>
<tr>
<td>Muller 2006 [103,113]

EMA status: unclear

Phase N/A

N=2

Status: completed</td>
<td>Biomarker evaluation: none

design: case report

follow-up: 16 and 21 months

primary outcome: N/A</td>
<td>adults with HES</td>
<td>imatinib 100 mg every 2 days to 600 mg/day</td>
<td>samples: peripheral blood, bone marrow

method: NR

threshold: N/A

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>2</td><td>1</td><td>1</td></tr></table></td>
<td>FIP1L1-PDGFRA positive: 1 complete hematological responses (duration ≥21 months)
FIP1L1-PDGFRA negative: 1 complete hematological responses (duration ≥16 months)</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Musial 2005 [103,114]

EMA status: unclear

Phase N/A

N=1

Status: completed</td>
<td>Biomarker evaluation: none

design: case report

follow-up: 6 months

primary outcome: N/A</td>
<td>adult with HES</td>
<td>imatinib 100 mg/day for the first 3 months and 100 mg every other day afterwards</td>
<td>samples: NR

method: NR

threshold: N/A

<table><tr><th>N</th><th>BM+</th><th>BM-</th></tr><tr><td>1</td><td>1</td><td>0</td></tr></table></td>
<td>1 complete hematological responses (duration ≥6 months)</td>
</tr>
<tr>
<td>Musto 2004 [103]

EMA status: unclear

Phase N/A

N=4

Status: completed</td>
<td>Biomarker evaluation: none

design: case report

follow-up: 10 months

primary outcome: N/A</td>
<td>adult with HES</td>
<td>imatinib 100 mg/day (possible escalation to 400 to 800 mg/day)</td>
<td>samples: NR

method: NR

threshold: N/A

<table><tr><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr><tr><td>4</td><td>1</td><td>0</td><td>3</td></tr></table></td>
<td>FIP1L1-PDGFRA positive: 1 complete hematological response (duration ≥9 months)
FIP1L1-PDGFRA unknown: 2 complete hematological responses (duration ≥12 months), 1 none</td>
</tr>
<tr>
<td>Roche-Lestienne 2005[103,115]

EMA status: unclear

Phase N/A

N=35

Status: completed</td>
<td>Biomarker evaluation: subgroup (retrospective)

design: non-randomised comparative (details NR)

follow-up: NR

primary outcome: NR</td>
<td>adults with HES</td>
<td>A) imatinib 100-200 mg/day
B) other (details NR)</td>
<td>samples: blood (dtails NR)

method: nested PCR (as in Cools 2003)

threshold: N/A

<table><tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr><tr><td>A</td><td>9</td><td>4</td><td>5</td></tr><tr><td>B</td><td>26</td><td>2</td><td>24</td></tr></table></td>
<td>Data only for imatinib available
FIP1L1-PDGFRA positive: 4 complete hematological responses (duration 2 months and NR)
FIP1L1-PDGFRA negative: 1 complete hematological response (duration NR), 4 none</td>
</tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
Rose 2004[103] EMA status: unclear Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 1 year primary outcome: N/A	adult with HES	imatinib 200 mg/day	samples: blood (details NR) method: nested PCR (as in Cools 2003) threshold: N/A N: 1; BM+: 1; BM-: 0	1 complete hematological response (duration ≥12 months)
Rotoli 2004[103] EMA status: unclear Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: primary outcome: N/A	adult with HES	imatinib 200 mg/day reduced to 100 mg/day after 2 weeks	samples: NR method: "cytogenetics, FISH and molecular analysis" threshold: N/A N: 1; BM+: 1; BM-: 0	1 complete hematological response (duration ≥17 months)
Smith 2004[103,116] EMA status: unclear Phase N/A N=3 Status: completed	Biomarker evaluation: none design: case report follow-up: 4 - 8 months primary outcome: N/A	adults with HES/CEL	imatinib 400-600/day	samples: blood (details NR) method: nested PCR (details NR) threshold: N/A N: 3; BM+: 2; BM-: 1	FIP1L1-PDGFRA positive: 2 complete hematological responses (duration 7-8 months) FIP1L1-PDGFRA negative: 1 complete hematological response (duration 4 months followed by relapse)

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Vanderberghe 2004[103,117]

EMA status: unclear

Phase N/A

N=17

Status: completed</td>
<td>Biomarker evaluation: subgroup (retrospective)

design: non-randomised comparative (details NR)

follow-up: NR

primary outcome: N/A</td>
<td>adults with idiopathic HES/CEL</td>
<td>A) imatinib initial dose of 100 mg/day followed by a tapered maintenance dose
B) other treatment including corticosteroids, hydroxyurea, bone marrow transplant, interferon-α, etc.</td>
<td>samples: blood or bone marrow

method: RT-PCR (details NR)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>5</td><td>4</td><td>1</td></tr>
<tr><td>B</td><td>12</td><td>4</td><td>8</td></tr>
</table>
</td>
<td>FIP1L1-PDGFRA positive: 4 complete hematological response (duration 4 months and NR); other: 1 complete, 2 partial, 1 NR
FIP1L1-PDGFRA negative: 1 no hematological response; other: 3 complete, 3 partial, 1 stable, 1 none</td>
</tr>
<tr>
<td>Wolf 2004[103]

EMA status: unclear

Phase N/A

N=1

Status: completed</td>
<td>Biomarker evaluation: none

design: case report

follow-up: 18 months

primary outcome: N/A</td>
<td>adult with HES</td>
<td>imatinib 100 mg/day reduced to 100 mg once a week and increased to 100 mg/day</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>1</td><td>0</td><td>1</td></tr>
</table>
</td>
<td>1 complete hematological response (duration ≥24 months)</td>
</tr>
</table>

genotype 1 – HCV - boceprevir

Study	Design	Population	Interventions	Biomarker measurement	Results
RESPOND-2[118,119] EMA status: main Phase III N=403 Status: completed	Biomarker evaluation: enrichment design: RCT; double-blind; 1:2:2; stratified by previous response (nonresponse vs. relapse) and HCV subgenotype (1a vs. 1b) follow-up: 72 weeks primary outcome: sustained virologic response (undetectable HCV RNA levels (measured with the use of the TaqMan 2.0 assay, Roche Diagnostics) for 24 weeks after the completion of therapy)	HCV with responsiveness to interferon and either "nonresponse" (decrease in HCV RNA level, but detectable) or relapse	A) boceprevir 4 weeks: peginterferon alfa-2b + ribavirin; 24 weeks: boceprevir (oral 800 mg 3 times daily) + peginterferon alfa-2b + ribavirin; => nothing OR if HCV RNA detectable from week 8 onwards - 32 weeks: peginterferon alfa-2b + ribavirin + placebo B) boceprevir 4 weeks: peginterferon alfa-2b + ribavirin; => 44 weeks: boceprevir (oral 800 mg 3 times daily) + peginterferon alfa-2b + ribavirin C) placebo 4 weeks: peginterferon alfa-2b + ribavirin => 44 weeks: peginterferon alfa-2b + ribavirin + placebo All treatments discontinued if at HCV RNA was detectable at week 12	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 162 / 162 / 0 B / 161 / 161 / 0 C / 80 / 80 / 0	sustained virologic response was: in placebo group 17 (21.3%), guided therapy (n=162) 95 (58.6%; p<0.0001 for difference with placebo) and fixed therapy (n=161) 107 (66.5%; p<0.0001 for difference with placebo)

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>SPRINT-2[118,120]

EMA status: main

Phase III

N=1099

Status: completed</td>
<td>Biomarker evaluation: enrichment*

design: RCT; double-blind; randomised 1:1:1; two cohorts based on self-reported ethnicity (black vs. non black) and stratified by HCV RNA level (maximum 400,000 IU per ml vs more)

follow-up: 72 weeks

primary outcome: sustained virologic response (undetectable HCV RNA levels (measured with the use of the TaqMan 2.0 assay, Roche Diagnostics) for 24 weeks after the completion of therapy)</td>
<td>adult, chronic HCV, no prior treatment</td>
<td>A) boceprevir 4 weeks: peginterferon alfa-2b + ribavirin; 24 weeks: boceprevir (oral 800 mg 3 times daily) + peginterferon alfa-2b + ribavirin; => nothing OR if HCV RNA detectable from week 8 onwards - 20 weeks: peginterferon alfa-2b + ribavirin + placebo
B) boceprevir 4 weeks: peginterferon alfa-2b + ribavirin; => 44 weeks: boceprevir (oral 800 mg 3 times daily) + peginterferon alfa-2b + ribavirin
C) placebo 4 weeks: peginterferon alfa-2b + ribavirin => 44 weeks: peginterferon alfa-2b + ribavirin + placebo

All treatments discontinued if at HCV RNA was detectable at week 24</td>
<td>samples: blood plasma

method: Trugene assay (Bayer Diagnostics) for purposes of randomization and by sequencing of the nonstructural 5B (NS5B) region (Virco) for subsequent analyses

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>368</td><td>358</td><td>0</td><td>10</td></tr>
<tr><td>B</td><td>366</td><td>354</td><td>0</td><td>12</td></tr>
<tr><td>C</td><td>363</td><td>348</td><td>0</td><td>15</td></tr>
</table>
</td>
<td>sustained virologic response was: in placebo group 137 (37.7%), guided therapy (n=368) 233 (63.3%; p<0.0001 for difference with placebo) and fixed therapy (n=366) 242 (66.1%; p<0.0001 for difference with placebo)</td>
</tr>
<tr>
<td>P05685 [118,121]

EMA status: supportive

Phase III

N= 201

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 1:2

follow-up: 3.5 years

primary outcome: sustained virologic response (undetectable HCV RNA by TaqMan 2.0 assay (Roche Diagnostics))</td>
<td>adults with HCV who failed previous treatmen</td>
<td>A) boceprevir 4 weeks: peginterferon alfa-2a + ribavirin; => 44 weeks peginterferon alfa-2a + ribavirin + bocepravir (oral; 800 mg 3 times a day); treatment stopped if no response at 12 weeks
B) placebo 4 weeks: peginterferon alfa-2a + ribavirin; => 44 weeks peginterferon alfa-2a + ribavirin + placebo; treatment stopped if no response at 12 weeks</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>134</td><td>134</td><td>0</td></tr>
<tr><td>B</td><td>67</td><td>67</td><td>0</td></tr>
</table>
</td>
<td>sustained virologic response was: in placebo group 14 (20.9%), boceprevir 86 (64.2%; p<0.0001 for difference with placebo)</td>
</tr>
</table>

* patients with HCV genotype 1 or patietns for whom it could not be determined

genotype 1 – HCV - telaprevir

Study	Design	Population	Interventions	Biomarker measurement	Results
ADVANCE[122,123] EMA status: main Phase III N=1095 Status: completed	Biomarker evaluation: enrichment design: RCT; double-blind; stratified according to genotype 1 subtype (a, b or unknown) and baseline viral load (> or < 800000 IU/ml) follow-up: 72 weeks primary outcome: sustained viral response (proportion of patients who had undetectable plasma HCV RNA 24 weeks after the last planned dose of study treatment)	genotype 1 HCV, chronic hepatitis C, age 18-70 years	A) telaprevir (oral; 750 mg every 8 hours w/ food) for 12 weeks + peginterferon-ribavirin; extended rapid virologic responders followed by 12 weeks of peginterferon-ribavirin; for non-responders - by 36 weeks of peginterferon-ribavirin B) telaprevir (oral; 750 mg every 8 hours w/ food) for 8 weeks and placebo for 4 weeks + peginterferon-ribavirin; extended rapid virologic responders followed by 12 weeks of peginterferon-ribavirin; for non-responders - by 36 weeks of peginterferon-ribavirin C) placebo + peginterferon-ribavirin for 12 weeks; peginterferon-ribavirin for 36 weeks	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 365 / 365 / 0 B / 365 / 365 / 0 C / 365 / 365 / 0	sustained viral response: telaprevir 12 weeks vs placebo: OR = 3.95 (95% CI: 2.87, 5.45); for 8 weeks vs. placebo: OR = 2.92 (95%CI: 2.14, 3.99)

Study	Design	Population	Interventions	Biomarker measurement	Results
ILLUMINATE[122,124] EMA status: main Phase III N=540 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; non inferiority; only patients with rapid virologic response at week 4 and 12 were randomised at week 20 of the study; non-resoponders non-randomly allocated follow-up: 48-72 weeks primary outcome: sustained virologic response (undetectable HCV RNA level at the end of treatment phase 24 weeks after the last planned dose of study medication)	chronic infection with genotype 1 HCV; age 18-70 years; treatment naïve;	A) telaprevir (orally; 750 mg every 8 hours) with peginterferon and ribavirin for 12 weeks + rapid virologic response: 12 weeks of peginterferon and ribavirin B) telaprevir (orally; 750 mg every 8 hours) with peginterferon and ribavirin for 12 weeks + rapid virologic response: 36 weeks of peginterferon and ribavirin C) telaprevir* (orally; 750 mg every 8 hours) with peginterferon and ribavirin for 12 weeks + no rapid virologic response: 36 weeks of peginterferon and ribavirin	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 162 / 162 / 0 B / 160 / 160 / 0 C / 118 / 118 / 0	sustained virologic response at week 24: (12 week vs 36 week extra treatment for reponders) OR = 1.62 (95%CI: 0.77, 3.38) favours 12 week extra treatment; sustained virologic response was acheved by 76 patients in the non-randomised arm

* non-random allocation

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>REALIZE[122,125]

EMA status: main

Phase III

N=663

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; stratified by viral load (> or < 800,000 IU/ml) and previous response to treatment (no response, partial response, relapse); randomised 2:2:1

follow-up: 72 weeks

primary outcome: sustained virologic response (undetectable plasma HCV RNA 24 weeks after the last planned dose of study drug)</td>
<td>genotype 1 HCV; previous treatment with peginterferon and ribavirin (at least 80% of intended dose); no SVR; age 10-70</td>
<td>A) telaprevir (orally; 750 mg every 8 hours) + peginterferon-ribavirin for 12 weeks => placebo+peginterferon-ribavirin for 4 weeks => peginterferon-ribavirin for 32 weeks
B) placebo with peginterferon-ribavirin for 4 weeks => telaprevir (delayed) (orally; 750 mg every 8 hours) + peginterferon-ribavirin for 12 weeks => peginterferon-ribavirin for 32 weeks
C) placebo with peginterferon-ribavirin for 16 weeks => peginterferon-ribavirin for 32 weeks</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>266</td><td>266</td><td>0</td></tr>
<tr><td>B</td><td>264</td><td>264</td><td>0</td></tr>
<tr><td>C</td><td>133</td><td>133</td><td>0</td></tr>
</table>
</td>
<td>SVR - difference from placebo: teleprevir vs. placebo: 46.8% (95% CI: 36.8, 56.7%); teleprevir (delayed) vs. placebo: 49.8% (95%CI: 39.9, 59.7%)</td>
</tr>
<tr>
<td>EXTEND[122,126]

EMA status: supportive

Phase NR

N=400

Status: ongoing</td>
<td>Biomarker evaluation: non-randomised only positive

design: prospective cohort study including patients who had been treated with telaprevir in Phase 2 studies

follow-up: 3 years

primary outcome: 1) Proportion maintaining undetectable HCV RNA after achieving SVR on telaprevir; 2) Change in HCV variants with decreased sensitivity to telaprevir over time in subjects failing to achieve SVR following telaprevir</td>
<td>NR</td>
<td>A) telaprevir patients who achieved SVR
B) telaprevir patients without SVR</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>NR</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>SVR patients - interim analysis (based on 123 patients) - none of the patients follwed up for a median 22.13 months (range: 5.1 to 35.2 months) had a relapse; patients without SVR - NR</td>
</tr>
</table>

G551D mutation – cystic fibrosis - ivacaftor

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>VX08-770-102[127,128]

EMA status: main

Phase III

N=167

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 1:1, stratified according to age (<18 years vs. ≥18 years) and pulmonary function (<70% vs. ≥70% of the predicted FEV1)

follow-up: 24 and 48 weeks

primary outcome: absolute change from baseline in percent predicted FEV1 measured at week 24</td>
<td>Cystic fibrosis patients of both sexes aged 12 years and older; G551D-CFTR mutation in at least 1 allele</td>
<td>A) ivacaftor 150 mg every 12 hours for 48 weeks (24 weeks + 24 weeks of an extension period); recommended that subjects remain on stable medication regimens for their CF
B) placebo recommended that subjects remain on stable medication regimens for their CF</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>84</td><td>84</td><td>0</td></tr>
<tr><td>B</td><td>83</td><td>82</td><td>1*</td></tr>
</table>
</td>
<td>adjusted mean absolute change from baseline through week 24 in percent predicted FEV1 (n=83 ivacaftor, n=78 placebo) was greater in the ivacaftor group (10.39%) than the placebo group (-0.18%); a difference in favour to ivacaftor of 10.58% (95% CI: 8.57, 12.59)</td>
</tr>
<tr>
<td>VX08-770-103[127]

EMA status: main

Phase III

N=52

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 1:1; FEV1 severity strata

follow-up: 24 and 48 weeks

primary outcome: absolute change from baseline in percent predicted FEV1 measured at week 24</td>
<td>aged 6 to 11 years with cystic fibrosis and the G551D mutation in at least 1 allele</td>
<td>A) ivacaftor 150 mg every 12 hours for 48 weeks (24 weeks + 24 weeks of an extension period)
B) placebo (details NR)</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+†</th><th>BM-</th></tr>
<tr><td>A</td><td>26</td><td>25-26</td><td>0-1</td></tr>
<tr><td>B</td><td>26</td><td>25-26</td><td>0-1</td></tr>
</table>
</td>
<td>at week 24 the mean absolute change (n=52) was also greater in the ivacaftor group (12.58%) than the placebo group (0.13%) with an estimated treatment difference for ivacaftor versus placebo of 12.45% (95% CI: 6.56, 18.34)</td>
</tr>
</table>

* one patient was found to be G551D mutation negative after inclusion (included in data analysis)
† one patient was found not to have G551D mutation; unclear from which arm

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>VX08-770-104[127]

EMA status: supportive

Phase II

N=140

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 4:1

follow-up: 16 weeks

primary outcome: mean absolute change from baseline in percent predicted FEV1 measured at Week 16</td>
<td>Cystic fibrosis; age 12 years and older who were homozygous for the F508del mutation in the CFTR gene and who had FEV1 ≥40% predicted</td>
<td>A) ivacaftor 150 mg every 12 hours
B) placebo (details NR)</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>NR</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>primary outcome at week 16 (n=NR) 1.5 percentage points in the ivacaftor and -0.2 percentage points in the placebo group; estimated treatment difference was 1.7 percentage points (95% CI: -0.6, 4.1, p=0.15)</td>
</tr>
<tr>
<td>VX08-770-105[127]

EMA status: supportive

Phase NR

N=192

Status: ongoing</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm extension study

follow-up: approximately 96 weeks

primary outcome: NR</td>
<td>patients who completed treatment in studies 102 or 103 were eligible</td>
<td>ivacaftor 150 mg every 12 hours</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>192</td><td>192</td><td>0</td></tr>
</table>
</td>
<td>study 102:
initially ivacafor: improvement in percent predicted FEV1 was maintained after additional 24 and 48 weeks of treatment with ivacaftor in study 105, i.e. the mean absolute change in percent predicted FEV1 (SD) was 10.3 (9.31) and 9.5 (10.13), respectively.
Initially placebo: improvement in percent predicted FEV1: 10.0 (9.52) and 8.0 (8.14) at weeks 24 and 48, respectively
study 103:
initially ivacafor: mean absolute change from baseline in percent predicted FEV1 (SD) of 10.1 (14.18) at week 24
initially placebo: improvement in percent predicted FEV1 was 7.5 (10.90) at week 24</td>
</tr>
</table>

Hormone dependency – prostate cancer - degarelix

Study	Design	Population	Interventions	Biomarker measurement	Results
FE 200486 CS21[129,130] EMA status: main Phase III N=610* Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; randomised 1:1:1; stratified by geographical region and body weight; non-inferiority follow-up: 12 months primary outcome: Cumulative probability of testosterone ≤ 0.5 ng/mL at any monthly measurement from 28 to 364 days	adult patients with histologically confirmed adenocarcinoma of the prostate, all stages, requiring androgen ablation treatment, including patients with rising PSA after having undergone curative prostatectomy or radiotherapy; serum testosterone > 1.5ng/mL; Previous or current hormonal management of prostate cancer was not allowed, except in patients having undergone localized therapy of curative intent in which neoadjuvant or adjuvant hormonal therapy for ≤ 6 months was accepted	A) degarelix s.c. starting dose of 240 mg (given as two ×3 mL injections) and thereafter 12 monthly (every 28 days) maintenance doses of 80 mg (one 4 mL injection of 20 mg/mL) B) degarelix s.c. starting dose of 240 mg (given as two ×3 mL injections) and thereafter 12 monthly (every 28 days) maintenance doses of 160 mg (40 mg/mL) C) leuprolide 12-monthly (every 28 days) i.m. injections 7.5 mg (given as one injection of ≈ 1 mL	samples: NR method: NR threshold: NR group / N / BM+ / BM- A / 207 / NR / NR B / 202 / NR / NR C / 201 / NR / NR	for 80mg maintenance dose (n=207): 5 T>0.5 ng/mL, 202 censored (97.2%, 95% CI: 93.5;98.8%); for 160 mg maintenance 3 T>0.5 ng/mL, 199 censored (98.3%, 95% CI: 94.8;99.4%), for leuprolide 7 T>0.5 ng/mL, 194 censored (96.4%, 95% CI: 92.5;98.2%)

* 620 randomised, but 10 did not receive allocated treatment

HER2 expression (negative) – breast cancer - everolimus

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>BOLERO-2[131,132]

EMA status: main

Phase III

N=724

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 2:1; stratified by presence of visceral metastasis and previous sensitivity to endocrine therapy

follow-up: median 17.7 months

primary outcome: PFS based on radiographic studies assessed by local investigators</td>
<td>"ER-positive, human epidermal growth factor receptor type 2 (HER2) – nonamplified advanced breast cancer whose disease was refractory to previous letrozole or anastrozole"</td>
<td>A) everolimus oral (10 mg daily) + exemestane (25 mg daily) until disease progression, unacceptable toxicity, or withdrawal of consent
B) placebo + exemestane (25 mg daily) until disease progression, unacceptable toxicity, or withdrawal of consent</td>
<td>samples: NR

method: by protein or gene analysis (details NR)

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>485</td><td>483</td><td>0</td><td>2*</td></tr>
<tr><td>B</td><td>239</td><td>239</td><td>0</td><td>0</td></tr>
</table>
</td>
<td>median PFS: everolimus 7.82 months (95% CI: 6.93, 8.48), placebo 3.19 months (95% CI: 2.76, 4.14), HR = 0.45 (95% CI: 0.38, 0.54, p<0.0001), favours everolimus</td>
</tr>
<tr>
<td>Baselga 2009[131,133]

EMA status: supportive

Phase II

N=270

Status: completed</td>
<td>Biomarker evaluation: subgroup (retrospective)

design: RCT; double-blind; randomised 1:1

follow-up: 16 weeks

primary outcome: objective response rate ‘‘assessed with modified WHO criteria by clinical palpation (monthly), ultrasound (monthly), and bidirectional mammography (months 2 and 4)"</td>
<td>"women aged 18 years or older with postmenopausal, histologically confirmed, ER-positive (assessed locally), untreated, stage M0 breast cancer who had a primary palpable tumor greater than 2 cm in diameter by imaging and who were candidates for mastectomy or breast conserving surgery"</td>
<td>A) everolimus 10 mg + letrozole 2.5 mg for 16 weeks - last dose taken within 24 hours of surgery (could be discontinued in less than 16 weeks for progressive disease or on patient or investigator request)
B) placebo + letrozole 2.5 mg for 16 weeks - last dose taken within 24 hours of surgery (could be discontinued in less than 16 weeks for progressive disease or on patient or investigator request)</td>
<td>samples: baseline core biopsy

method: NR

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>138</td><td>12</td><td>95</td><td>31</td></tr>
<tr><td>B</td><td>132</td><td>14</td><td>91</td><td>27</td></tr>
</table>
</td>
<td>Overall objective response rate (1) by ultrasound everolimus (n=129) 77 (59.7%, 95% CI: 51.2, 68.2), placebo (n=122) 58 (47.5%, 95% CI: 8.7, 56.4), Chi-squared p=0.0268; (2) by palpitation everolimus (n=129) 86 (66.7%, 95% CI: 58.5, 74.8), placebo (n=122) 67 (54.9%, 95% CI: 46.1, 63.7), Chi-squared p=0.0283

results NR based on HER2 status</td>
</tr>
</table>

* missing status

HER2 expression – breast cancer - lapatinib

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>EGF100151[134-136]

EMA status: main

Phase III

N=399

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; randomised 1:1 (permuted blocks of 6), stratified by disease stage and the presence or absence of visceral disease

follow-up: NR

primary outcome: TTP (time from randomization to disease progression (defined according to RECIST criteria, modified to include lesions that were 15 to 19 mm in diameter as assessed by methods other than spiral CT) or death due to breast cancer)</td>
<td>"locally advanced breast cancer (a T4 primary tumor and stage IIIB or IIIC disease) or metastatic breast cancer that had progressed after treatment with regimens that included an anthracycline, a taxane, and trastuzumab"; no previous capecitabine</td>
<td>A) lapatinib 1250 mg daily, 1 hour before or after breakfast, on a continuous basis + capecitabine 2000 mg/m2 in two divided doses on days 1 through 14 of a 21-day cycle until investigator identified disease progression or unacceptable toxicity; possiblility of temporarily stopping or termination due to toxicity
B) capecitabine 2500 mg/m2 in two divided doses on days 1 through 14 of a 21-day cycle until investigator identified disease progression or unacceptable toxicity</td>
<td>samples: tumour (details NR)

method: immunohistochemical analysis or immunohistochemical analysis with gene amplification on fluorescence in situ hybridization at local institution

threshold: 3+ staining by IHC only or 2+ by IHC + FISH

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>198</td><td>197</td><td>1</td></tr>
<tr><td>B</td><td>201</td><td>201</td><td>0</td></tr>
</table>
</td>
<td>at the end of enrollment TTP: median lapatinb (n=198) 27.1 weeks, capecitabine only (n=201) 18.6 weeks; HR = 0.57 (95% CI: 0.43, 0.77, p=0.00013) favours lapatinib</td>
</tr>
<tr>
<td>EGF103659 and French ATU[134,137]

EMA status: supportive

Phase IV

N=3330

Status: ongoing</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm (details NR)

follow-up: NR

primary outcome: clinical benefit</td>
<td>advanced or metastatic BC, progression (by modified RECIST) after prior therapy, including all of the following: anthracycline, taxane, and trastuzumab alone or in combination with other therapy</td>
<td>lapatinib + capecitabine (details NR)</td>
<td>samples: tumour (details NR)

method: immunohostochemistry or FISH (reported for EGF103659 only)

threshold: +3 by IHC or FISH positive (reported for EGF103659 only)

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>3330</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>Reported only for patients with brain metastases (n=137): 3 CR, 21 PR, 56 SD, 14 PD, 43 unknown</td>
</tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
EGF105084[134,138] EMA status: supportive Phase II N=242 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm; wo cohorts: A: ECOG PS 0 to 1 and 1 or 2 prior trastuzumab regimens; B: ECOG PS 2 and/or >2 prior trastuzumab regimens follow-up: NR primary outcome: central nervous system (CNS) objective response (Brain MRIs obtained every 8 weeks; either CR or PR (≥50% reduction in the volumetric sum of all CNS lesions), and no progression of extra-CNS disease)	breast cancer with new and/or progressive brain metastases after completion of whole-brain radiotherapy or stereotactic radiosurgery; prior treatment w/ trastuzumab	lapatinib monotherapy (750 mg twice a day)	samples: cancer tissue (details NR) method: immunohistochemistry or fluorescence in situ hybridization threshold: 3+ immunohistochemistry or evidence of gene amplification by fluorescence in situ hybridization N: 242; BM+: 242; BM-: 0	16/242 experienced ≥ 50% volumetric reduction in an extension study (combination therapy)10/49 experienced ≥ 50% volumetric reduction

Study	Design	Population	Interventions	Biomarker measurement	Results
EGF30001 [134] EMA status: supportive Phase III N=580 Status: completed	Biomarker evaluation: subgroup (retrospective) design: RCT; double-blind; stratified by stage and sites of metastatic disease follow-up: NR primary outcome: TTP (time from random assignment until disease progression or death because of disease under study)	advanced breast cancer (stage III or IV) previously untreated in the metastatic setting; either HER2-negative (per the enrolling site) or HER2-unknown breast cancer	A) lapatinib oral 1,500 mg daily + paclitaxel 175 mg/m2 iv every 3 weeks (for up to six cycles) until disease progression, withdrawal as a result of toxicity, or withdrawal of consent B) placebo + paclitaxel 175 mg/m2 iv every 3 weeks (for up to six cycles) until disease progression, withdrawal as a result of toxicity, or withdrawal of consent	samples: tumor specimens from pretreatment or archived, paraffin-embedded breast cancer tissue method: "HER2 gene amplification status was analyzed by PathVision FISH (Abbott Laboratories, Abbott Park, IL), and HER-2 protein expression status was analyzed by Dako HercepTest IHC (Dako, Carpinteria, CA)." threshold: FISH positive or IHC 3+ if FISH status was unknown group / N / BM+ / BM- / BM? A / 291 / 49 / 202 / 40 B / 288 / 37 / 204 / 47	HER2+ median TTP: lapatinib (n=52) 8.1 weeks (IQR: 4.6, 12.9), control (n=39) 5.8 weeks (IQR: 4.6, 8.3), HR=0.57 (95%CI: 0.34, 0.90, p=0.011) favours lapatinib; HER2+ (ref 319): median TTP: lapatinib (n=49) 36.4 weeks (IQR NR), control (n=37) 25.1 weeks (IQR NR), HR=0.53 (95%CI: 0.31, 0.89, p=0.005) favours lapatinib HER2- NR HER2 unknown NR

HER2 expression – breast cancer - pertuzumab

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>CLEOPATRA[139,140]

EMA status: main

Phase III

N=808

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 1:1, stratified by geographic region and prior treatment (prior adjuvant or neoadjuvant chemotherapy vs. none)

follow-up: median 19.3 months

primary outcome: PFS (“time from randomization to the first documented radiographic evidence of progressive disease according to (…) RECIST (version 1.0) or death from any cause within 18 weeks after the last independent assessment of tumors")</td>
<td>"HER2-positive metastatic breast cancer who had not received chemotherapy or biologic therapy for their metastatic disease"</td>
<td>A) pertuzumab (loading dose 840 mg -> 420 mg every 3 weeks) + trastuzumab (loading dose 8 mg/kg -> 6 mg/kg every 3 weeks) + docetaxel (75 mg/m2 every 3 weeks, could be increased to 100 mg/m2) until disease progression or unacceptable toxicity
B) placebo + trastuzumab (loading dose 8 mg/kg -> 6 mg/kg every 3 weeks) + docetaxel (75 mg/m2 every 3 weeks, could be increased to 100 mg/m2) until disease progression or unacceptable toxicity</td>
<td>samples: cancer tissue (details NR)

method: confirmed centrally, by immunohistochemistry or fluorescence in situ hybridization

threshold: IHC 3+ or FISH amplification ratio ≥2.0

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>402</td><td>402</td><td>0</td></tr>
<tr><td>B</td><td>406</td><td>406</td><td>0</td></tr>
</table>
</td>
<td>median PFS: pertuzumab (n=402) 18.5 months (95% CI: 15, 23), placebo (n=406) 12.4 months (95% CI: 10, 13), HR = 0.62 (95% CI: 0.51, 0.75, p<0.0001), favours pertuzumab</td>
</tr>
<tr>
<td>Baselga 2010[139,141]

EMA status: supportive

Phase II

N=66

Status: completed</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm; Simon's two-stage design

follow-up: 28 weeks

primary outcome: objective response rate (ORR) and/or the clinical benefit rate (CBR) (ORR; confirmed CR or PR; CBR: total number of objective responses plus SD > 6 months; determined according to RECIST)</td>
<td>HER2+ metastatic breast cancer who received ≤ 3 chemotherapy lines before study entry and had lately progressed on trastuzumab</td>
<td>pertuzumab (loading dose 840 mg iv on day 2 -> following cycles 420 mg) + trastuzumab according to the same dose schedule as before study entry; for 8 cycles, but could be continued afterwards</td>
<td>samples: NR

method: NR

threshold: NR

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>66</td><td>66</td><td>0</td></tr>
</table>
</td>
<td>ORR was 24.2% (16 patients - based on paper); CBR was 50%</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>NeoSphere[139,142]

EMA status: supportive

Phase II

N=417

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; centrally randomised (1:1:1:1) "with the adaptive randomisation method and stratified by operable, locally advanced, and inflammatory breast cancer, and by positivity for oestrogen or progesterone receptors"

follow-up: NR

primary outcome: pathological CR in the breast (absence of invasive neoplastic cells at microscopic examination of the primary tumour at surgery; remaining in-situ lesions allowed)</td>
<td>adult women with HER2-positive, operable (T2–3, N0–1, M0), locally advanced (T2–3, N2–3, M0 or T4a–c, any N, M0), or inflammatory (T4d, any N, M0) breast cancer with primary tumours larger than 2 cm in diameter, no previous cancer therapy</td>
<td>A) pertuzumab 4 iv cycles: pertuzumab (840 mg, followed by 420 mg every 3 weeks) + docetaxel (75 mg/m², escalating, if tolerated, to 100 mg/m² every 3 weeks)
B) trastuzumab 4 iv cycles: trastuzumab (every 3 weeks at 8 mg/kg (cycle 1), followed by 6 mg/kg) + docetaxel (75 mg/m², escalating, if tolerated, to 100 mg/m² every 3 weeks)
C) trastuzumab 4 iv cycles: trastuzumab (every 3 weeks at 8 mg/kg (cycle 1), followed by 6 mg/kg) + docetaxel (75 mg/m², escalating, if tolerated, to 100 mg/m² every 3 weeks) + pertuzumab (840 mg, followed by 420 mg every 3 weeks)
D) trastuzumab 4 iv cycles: trastuzumab (every 3 weeks at 8 mg/kg (cycle 1), followed by 6 mg/kg) + pertuzumab (840 mg, followed by 420 mg every 3 weeks)</td>
<td>samples: tumour (details NR)

method: HER2 immunohistochemistry and fluorescence or chromogenic in-situ hybridisation

threshold: HER2 immunohistochemistry 3+ or 2+ and positive for fluorescence or chromogenic in-situ hybridisation

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>96</td><td>96</td><td>0</td></tr>
<tr><td>B</td><td>107</td><td>107</td><td>0</td></tr>
<tr><td>C</td><td>107</td><td>107</td><td>0</td></tr>
<tr><td>D</td><td>107</td><td>107</td><td>0</td></tr>
</table>
</td>
<td>"The results of the analysis of the primary endpoint demonstrate very similar pCR [pathological complete response] rates in the treatment arms A (T+D [trastuzumab + docetaxel]: 29%) and D (Ptz+D [pertuzumab + docetaxel]: 24.0%)"</td>
</tr>
</table>

HER2 expression – breast cancer - trastuzumab

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>H0648g[24,32]

EMA status: unclear

Phase III

N=469

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label (details NR)

follow-up: "median time in the study was 40 weeks (range, 1 to 127) in the group given chemotherapy plus trastuzumab, as compared with 25 weeks (range, 1 to 131) in the group given chemotherapy alone"

primary outcome: TTP (evaluation for response at weeks 8 and 20 and then at 12-week intervals by members of an independent response-evaluation committee unaware of treatment assignments; disease progression was defined as an increase of more than 25 % in the dimensions of any measurable lesion)</td>
<td>Metastatic breast cancer with no prior therapy for metastatic disese</td>
<td>A) trastuzumab (iv; loading dose 4 mg/kg, followed by 2 mg/kg once a week, until evidence of disease progression) + chemotherapy (as in chemotherapy group)
B) chemotherapy anthracycline (doxorubicin 60 mg/m2 or epirubicin 75 mg /m2) + cyclophosphamide (600 mg/m2) if no previous anthracycline, or paclitaxel (175 mg/m2) otherwise; every 3 weeks for 6 cycles; additional cycles at investigator's discretion</td>
<td>samples: tumour tissue (details NR)

method: IHC at a central laboratory

threshold: "moderate staining (…) (a score of 2+) or more than moderate staining (referred to as a score of 3+) in more than 10 percent of tumor cells"

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>235</td><td>235</td><td>0</td></tr>
<tr><td>B</td><td>234</td><td>234</td><td>0</td></tr>
</table>
</td>
<td>only data for patients treated with paclitaxel was considered: median TTP: trastuzumab + paclitaxel (n=89) 7.4 months, paclitaxel (n=89) 4.6 months, p=0.0001</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>H0649g[24,27]

EMA status: unclear

Phase III

N=222

Status: completed</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm (details NR)

follow-up: median 12.8 months

primary outcome: objective tumor response (at baseline, week 8, 16, and 24, and every 12 weeks; by independent response evaluation committee (blind to treatment))</td>
<td>progressive metastatic breast cancer after one or two cytotoxic chemotherapy regimens for metastatic disease</td>
<td>trastuzumab iv; loading dose 4 mg/kg, followed by weekly 2 mg/kg over 90 minutes; if infusion was well tolerated, shortened to 30 minutes; on disease progression investigator could continue at 2 mg/kg, increase to 4 mg/kg of discontinue treatment</td>
<td>samples: tumor tissue, collected either at the time of primary diagnosis or at recurrence

method: IHC by a core research pathology laboratory using 4D5 and CB11 murine monoclonal anti-HER2 antibodies (staining meeting threshold from at least one of the antibodies required)

threshold: 2+ or 3+ overexpression observed in over
10% of the tumor cells

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>222</td><td>222</td><td>0</td></tr>
</table>
</td>
<td>overall response rate (n=222) ORR = 34 (15%) (95%CI: 11, 21%)</td>
</tr>
</table>

HER2 expression – stomach cancer - trastuzumab

Study	Design	Population	Interventions	Biomarker measurement	Results
ToGA[143,144] EMA status: main Phase III N=594 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; randomised 1:1 by use of a randomised block design with block sizes of four patients, via a central interactive voice recognition system; stratified according to ECOG PS, chemotherapy regimen, extent of disease, primary cancer site, and measurability of disease follow-up: median 17.1 months in chemotherapy and 18.6 months in chemotherapy + trastuzumab arm primary outcome: overall survival (time from randomisation until death from any cause)	inoperable locally advanced, recurrent, or metastatic adenocarcinoma of the stomach or gastro-oesophageal junction	A) trastuzumab (iv; loading dose 8 mg/kg over 90 min(on day 1) followed by 6 mg/kg over 30 min every 3 weeks) + chemotherapy (as in chemotherapy group) B) chemotherapy 6 3-week cycles: capecitabine (1000 mg/m2 orally 2x day for 14 days) or fluorouracil (800 mg/m2/day iv over 5 days) + cisplatin (80 mg/m2 iv over 2 hours) - chosen at the investigator's discretion	samples: tumour (details NR) method: centrally tested with immunohistochemistry (HercepTest, Dako, Denmark]) and fluorescence in-situ hybridisation (FISH; HER2 FISH pharmDx, Dako); new set of immunohistochemistry scoring criteria were developed that are specific for gastric cancer (in attached Word document) threshold: 3+ on immunohistochemistry or FISH positive (HER2:CEP17 ratio ≥2) group / N / BM+ / BM- A / 298 / 298 / 0 B / 296 / 296 / 0	overall survival: hazard ratio = 0.74; 95% CI (95% CI: 0.60-0.91), p = 0.0046; favours trastuzumab

HLA-B*5701 allele – HIV - abacavir

Study	Design	Population	Interventions	Biomarker measurement	Results
PREDICT-1[145-148] EMA status: unclear Phase IV N=1956 Status: completed	Biomarker evaluation: biomarker strategy design: RCT; double blind; stratified by self reported race (white vs non-white), history of antiretroviral therapy, and intention to commence a new reverse-transcriptase inhibitor between the day of screening visit and day 1 of trial follow-up: 6 weeks primary outcome: rate of clinically diagnosed hypersensitivity reaction (no predefined criteria); rate of immunologically confirmed hypersensitivity reaction to abacavir (clinically diagnosed reaction that was confirmed by a positive result on epicutaneous patch testing 6 to 10 weeks after clinical diagnosis)	HIV positive patients eligible for abacavir treatments	A) abacavir (with prior HLA-B*5701 screening) B) abacavir (no prior screening)	samples: blood samples collected from all patients during the evaluation period method: using DNA-sequence–based typing (central laboratory) and a sequence-specific oligonucleotide probe method (Laboratory Corporation of America), with additional DNA sequencing for patients for whom the probe results were positive threshold: N/A group / N / BM+ / BM- A / 980 / 55* / 925 B / 976 / 54 / 922	NR in EMA documents, based on trial report: clinically diagnosed hypersensitivity reaction OR = 0.40 (95% CI: 0.25, 0.62) favours screening; immunologically confirmed OR = 0.03 (95% CI: 0.00, 0.19) favours screening;

* 55 positive patients from screening group excluded from the study after randomisation

Hormone receptor expression – breast cancer - everolimus

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>BOLERO-2[131,132]

EMA status: main

Phase III

N=724

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; randomised 2:1; stratified by presence of visceral metastasis and previous sensitivity to endocrine therapy

follow-up: median 17.7 months

primary outcome: PFS based on radiographic studies assessed by local investigators</td>
<td>"ER-positive, human epidermal growth factor receptor type 2 (HER2) – nonamplified advanced breast cancer whose disease was refractory to previous letrozole or anastrozole"</td>
<td>A) everolimus oral (10 mg daily) + exemestane (25 mg daily) until disease progression, unacceptable toxicity, or withdrawal of consent
B) placebo + exemestane (25 mg daily) until disease progression, unacceptable toxicity, or withdrawal of consent</td>
<td>samples: NR

method: by protein or gene analysis (details NR)

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+*</th><th>BM-</th></tr>
<tr><td>A</td><td>485</td><td>485</td><td>0</td></tr>
<tr><td>B</td><td>239</td><td>239</td><td>0</td></tr>
</table>
</td>
<td>median PFS: everolimus 7.82 months (95% CI: 6.93, 8.48), placebo 3.19 months (95% CI: 2.76, 4.14), HR = 0.45 (95% CI: 0.38, 0.54, p<0.0001), favours everolimus</td>
</tr>
<tr>
<td>Baselga 2009[131,133]

EMA status: supportive

Phase II

N=270

Status: completed</td>
<td>Biomarker evaluation: enrichment)

design: RCT; double-blind; randomised 1:1

follow-up: 16 weeks

primary outcome: objective response rate ‘‘assessed with modified WHO criteria by clinical palpation (monthly), ultrasound (monthly), and bidirectional mammography (months 2 and 4)"</td>
<td>"women aged 18 years or older with postmenopausal, histologically confirmed, ER-positive (assessed locally), untreated, stage M0 breast cancer who had a primary palpable tumor greater than 2 cm in diameter by imaging and who were candidates for mastectomy or breast conserving surgery"</td>
<td>A) everolimus 10 mg + letrozole 2.5 mg for 16 weeks - last dose taken within 24 hours of surgery (could be discontinued in less than 16 weeks for progressive disease or on patient or investigator request)
B) placebo + letrozole 2.5 mg for 16 weeks - last dose taken within 24 hours of surgery (could be discontinued in less than 16 weeks for progressive disease or on patient or investigator request)</td>
<td>samples: baseline core biopsy

method: NR

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+†</th><th>BM-</th></tr>
<tr><td>A</td><td>138</td><td>138</td><td>0</td></tr>
<tr><td>B</td><td>132</td><td>132</td><td>0</td></tr>
</table>
</td>
<td>objective response rate (1) by ultrasound everolimus (n=129) 77 (59.7%, 95% CI: 51.2, 68.2), placebo (n=122) 58 (47.5%, 95% CI: 8.7, 56.4), Chi-squared p=0.0268; (2) by palpitation everolimus (n=129) 86 (66.7%, 95% CI: 58.5, 74.8), placebo (n=122) 67 (54.9%, 95% CI: 46.1, 63.7), Chi-squared p=0.0283</td>
</tr>
</table>

* Based on data for estrogen receptor only; 523 patients in total were progesterone receptor positive (further detail NR)
† Based on data for estrogen receptor only

Hormone receptor expression – breast cancer - zoledronic acid

Study	Design	Population	Interventions	Biomarker measurement	Results
ABCSG-12[149,150] EMA status: main Phase III N=1803 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; "computer-generated adaptive randomisation method to assign treatment groups via an automated telephone service. Patients were randomly assigned (in a 1:1:1:1 ratio on the basis of Pocock and Simon's minimisation method for a two-by-two factorial design)" follow-up: median 53 months primary outcome: DFS (time from randomisation to the first occurrence of any of the following: a local or regional recurrence, contralateral breast cancer, distant metastasis, second primary carcinoma, and death from any cause")	premenopausal women with stage I or II oestrogen-receptor-positive and/or progesterone-receptor-positive breast cancer	A) zoledronic acid 4 mg iv every 6 months + goserelin 3.6 mg subcutaneously every 28 days + tamoxifen 20 mg per day orally for 3 years B) zoledronic acid 4 mg iv every 6 months + goserelin 3.6 mg subcutaneously every 28 days + anastrozole 1 mg per day orally for 3 years C) goserelin 3.6 mg subcutaneously every 28 days + tamoxifen 20 mg per day orally for 3 years D) goserelin 3.6 mg subcutaneously every 28 days + anastrozole 1 mg per day orally for 3 years	samples: NR method: NR threshold: NR group / N / BM+ / BM- A / 450 / 450 / 0 B / 450 / 450 / 0 C / 450 / 450 / 0 D / 453 / 453 / 0	zoledronic acid reduced the risk of DFS events by 34% vs. control, HR = 0.66 (95% CI: 0.48, 0.90, p=0.008); 5-year DFS 92.9% zoledronic acid and 89.1% control (data for median 62 months follow-up confirm)

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>E-ZO-FAST[149,151]

EMA status: supportive

Phase III

N=527

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; stratified according to postmenopausal status (postmenopausal vs. recently menopausal), baseline T-score, and previous adjuvant chemotherapy

follow-up: NR

primary outcome: percentage change from baseline in the lumbar spine bone mineral density measured by DEXA scan at 12 months</td>
<td>"postmenopausal or recently menopausal from ovarian-ablative treatments and had resected stage I to stage IIIa HR+ [hormone receptor+] EBC [early breast cancer], no clinical or radiologic evidence of recurrent or metastatic disease disease", baseline ECOG PS =<2, and LS and TH BMD T-scores >= –2.0</td>
<td>A) zoledronic acid (immediate) iv 4 mg for 15 minutes every 6 months + oral calcium suppl. 500 mg + multivitamin tablet w/ vitamin D (400-800 IU) daily + 2.5 mg letrozole daily for 5 years or until disease progression
B) zoledronic acid (delayed) iv 4 mg for 15 minutes every 6 months if: BMD T-score decreased to <–2.0 at either LS or TH, any clinical fracture, or an asymptomatic fracture at 36-month evaluation + treatment as immediate group</td>
<td>samples: NR

method: NR

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>263</td><td>263</td><td>0</td></tr>
<tr><td>B</td><td>264</td><td>264</td><td>0</td></tr>
</table>
</td>
<td>bone mineral density NR in EMA documents;
DFS favoured the immediate group, but results were not statistically significant</td>
</tr>
<tr>
<td>Z-FAST[149,152]

EMA status: supportive

Phase III

N=602

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; stratified by baseline T score and receipt of adjuvant chemotherapy

follow-up: NR

primary outcome: percentage change in LS BMD (L1–L4) from baseline to 12 months "using either Hologic (Hologic, Bedford, MA) or Lunar (GE Medical Systems Lunar Corporation, Madison, WI) dual-energy x-ray absorptiometry (DEXA) devices"</td>
<td>postmenopausal women with early-stage hormone receptor–positive breast cancer</td>
<td>A) zoledronic acid (immediate) iv 4 mg for 15 minutes every 6 months + oral calcium suppl. 1000-1200 mg + multivitamin tablet w/ vitamin D (400-800 IU) daily + 2.5 mg letrozole daily for 5 years or until disease progression
B) zoledronic acid (delayed) iv 4 mg for 15 minutes every 6 months if: BMD T-score decreased to <–2.0 at either LS or TH, any clinical fracture, or an asymptomatic fracture at 36-month evaluation + treatment as immediate group</td>
<td>samples: NR

method: NR

threshold: NR

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>301</td><td>301</td><td>0</td></tr>
<tr><td>B</td><td>301</td><td>301</td><td>0</td></tr>
</table>
</td>
<td>bone mineral density NR in EMA documents;
DFS favoured the immediate group, but results were not statistically significant</td>
</tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
ZO-FAST[149,153] EMA status: supportive Phase III N=1065 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; stratified according to adjuvant chemotherapy, baseline T-score, and menopausal status (recently vs established postmenopausal) follow-up: NR primary outcome: percentage change in spine bone mineral density at 12 months "using either Hologic (Hologic, Bedford, Mass), Lunar (GE Medical Systems Lunar Corporation, Madison, Wis), or Norland (Norland, Fort Atkinson, Wis) dual-energy x-ray absorptiometry (DXA) devices"	postmenopausal women w/ estrogen receptor-positive early breast cancer and baseline lumbar spine and total hip T-scores above 22.0	A) zoledronic acid (immediate) immediate iv 4 mg for 15 minutes every 6 months + oral calcium suppl. 500 mg + multivitamin tablet w/ vitamin D (400-800 IU) daily + 2.5 mg letrozole daily for 5 years or until disease progression B) zoledronic acid (delayed) iv 4 mg for 15 minutes every 6 months if 1) spine or hip T-score decreased to < 22.0; 2) nontraumatic clinical fracture or 3) asymptomatic fracture discovered at month-36 visit + treatment as immediate group	samples: NR method: NR threshold: NR group / N / BM+ / BM- A / 532 / 532 / 0 B / 533 / 533 / 0	Upfront treatment resulted in a significant increase in bone mineral density in lumbar spine to month 36 after which it remained stable; in the delayed group BMD in lumbar spine decreased and only returned to values close to baseline in the last part of the study; secondary outcome: DFS HR = 0.591 (95% CI: 0.381, 0.917)

Kit (CD 117) – GIST - imatinib

Study	Design	Population	Interventions	Biomarker measurement	Results
Blanke 2008b[154,155] EMA status: unclear Phase III N= 694 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; stratified by Zubrod performance status and disease status (measurable vs. nonmeasurable) follow-up: median 4.5 years primary outcome: PFS and overall survival	unresectable GIST; no known brain metastases	A) imatinib 400 mg orally once daily; dose reduction or interruption due to toxicity allowed B) imatinib 400 mg orally twice daily; dose reduction or interruption due to toxicity allowed	samples: tumour (details NR) method: immunohistochemistry with DAKO (Carpenteria, CA) antibody threshold: N/A group / N / BM+ / BM- A / 345 / 345 / 0 B / 349 / 349 / 0	NR

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Demetri 2002[154,156]{#176

EMA status: unclear

Phase II

N=147

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; open-label; no stratification; blocking with block size of 4

follow-up: median 288 days

primary outcome: objective response according to standard Southwest Oncology Group criteria; based solely on CT or MRI</td>
<td>unresectable and/or metastatic GIST</td>
<td>A) imatinib 400 mg/day orally once daily with food with possible increase to 600 mg/day if tumour progressed
B) imatinib 600 mg/day orally once daily with food</td>
<td>samples: tumour biopsy

method: immunohistochemistry using polyclonal rabbit antiserum (A4502, Dako)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>73</td><td>71-73*</td><td>0-2</td></tr>
<tr><td>B</td><td>74</td><td>72-74</td><td>0-2</td></tr>
</table>
</td>
<td>best tumour response: for both groups 1 CR, 98 PR, 23 SD, 18 PD, 5 not evaluable, 2 unknown</td>
</tr>
</table>

* Two patients were later found to be biomarker negative

KRAS mutation – colorectal cancer - cetuximab

Study	Design	Population	Interventions	Biomarker measurement	Results
CO.17[157-159] EMA status: unclear Phase III N=572 Status: completed	Biomarker evaluation: subgroup (retrospective) design: RCT stratified by centre and ECOG PS; central randomisation by minimisation 1:1 follow-up: median 14.6 months primary outcome: OS (time from randomisation to death from any cause)	EGFR expressing advanced colorectal cancer; prior lack of response to a fluoropyrimidine, irinotecan and oxaliplatin or contraindications to these	A) cetuximab (iv loading dose 400 mg/m2 over 120 min followed by 250 mg/m2 over 60 min once a week until disease progression or unacceptable toxicity) + supportive care B) supportive care (details NR)	samples: FFPE tumour tissue samples from specimens collected at diagnosis; if tumour blocks unavailable - unstained slides were retrieved method: in a "blinded fashion" by members of BMS Department of Clinical Biomarkers-Oncology; DNA extraction using QIAmp DNA Mini Kit (Qiagen), PCR, primer extension sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems); reactions were run on 3730x1 DNA Analyzer (Applied Biosystems), DNA sequence analysis with Mutation Surveyor v2.61 (SoftGenetics) along with visual inspection of each sample trace threshold: N/A group / N / BM+ / BM- / BM? A / 287 / 54 / 38 / 195 B / 285 / 57 / 39 / 189	Overall overal survival: (n=572): HR=0.77 (95%CI: 0.64, 0.92); in KRAS-evaluable patients (n=188): HR=0.75 (95%CI: 0.537, 1.036) KRAS mutant overal survival : (n=77): HR=0.79 (95%CI: 0.476, 1.322); KRAS wild-type overal survival: (n=111): HR=0.74 (95%CI: 0.479, 1.154); no data for patients with unknown KRAS status

Study	Design	Population	Interventions	Biomarker measurement	Results
CRYSTAL[157,160,161] EMA status: unclear Phase III N=1198* Status: completed	Biomarker evaluation: subgroup (retrospective) design: RCT with 1:1 randomisation; stratified by ECOG PS and region follow-up: NR (median 19.9 and 18.6 months†) primary outcome: PFS (time from randomisation to disease progression or death from any cause within 60 days after the last tumour assessment or after randomisation)	EGFR expressing metastatic adenocarcinoma of colon or rectum, not resectable for curative purposes; no previous exposure to anti-EGFR or irionotecan treatment	A) cetuximab on day 1 (400 mg/m2 120 min iv infusion) followed by 250 mg/m2 once a week + FOLFIRI (as in FOLFIRI group) till progression, unacceptable toxicity or withdrawal B) FOLFIRI every 14 days: irinotecan (30-90 min iv 180mg/m2) + racemic or L-leucovorin (120 min iv 400 or 200 mg/m2 respect) + fluorouracil in a bolus (400 mg/m2) followed (46 hrs iv 2400 mg/m2) till progression, unacceptable toxicity or withdrawal	samples: paraffin-embedded tumour biopsy specimens method: PCR clamping and melting curve method (LightMix k-ras Gly12 assay, TIB MOLBIOL); KRAS mutation in codons 12 and 13 threshold: N/A group / N / BM+ / BM- / BM? A / 599 / 105 / 172 / 322 B / 599 / 87 / 176 / 336	Overall PFS: (n=1198): HR = 0.851 (95%CI: 0.726, 0.998); in KRAS-evaluable patients (n=540): HR=0.822 (95%CI: 0.645, 1.048) favours cetuximab KRAS mutant PFS: (n=192): HR = 1.069 (95%CI: 0.710, 1.610); favours FOLFIRI KRAS wild-type PFS: (n=348): HR = 0.684 (95%CI: 0.501, 0.934); favours cetuximab no data for patients with unknown KRAS status

* 1198 were treated and reported, but 1217 reported as randomised
† Based on OS in groups

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>EPIC[157,162]

EMA status: unclear

Phase III

N=1298

Status: completed</td>
<td>Biomarker evaluation: subgroup (retrospective)

design: RCT randomised 1:1; atratified by ECOG PS and study site

follow-up: NR (median 10.7 and 10.0 months*)

primary outcome: OS (monitored every 3 months)</td>
<td>EGFR expressing metastatic colorectal cancer; failure within 6 months of the last dose of first-line fluoropyrimidine and oxaliplatin for metastatic disease; no previous irinotecan and anti-EGFR allowed</td>
<td>A) cetuximab 400mg/m2 (iv over 2 hrs) then 250 mg/m2 (iv over 1 hr) weekly; antihistamine premed; + irinotecan 350mg/m2 (or 300 for patients at least 70, w/ PS 2 or prior abdominal/pelvic irradiation) (iv 90 min) every 3 wks til progression/ unacceptable toxicity
B) irinotecan 350mg/m2 (or 300 for patients at least 70, w/ PS 2 or prior abdominal/pelvic irradiation) (iv 90 min) every 3 wks til progression/ unacceptable toxicity</td>
<td>samples: tumour (details NR)

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>648</td><td>49</td><td>97</td><td>502</td></tr>
<tr><td>B</td><td>650</td><td>59</td><td>95</td><td>496</td></tr>
</table>
</td>
<td>Overall overal survival: (n=1298): HR=0.98 (95%CI: 0.85, 1.11); favours cetuximab; overal survival in KRAS-evaluable patients (n=300): HR=1.25 (95%CI: 0.947, 1.660); favours irinotecan
KRAS mutant overal survival: (n=108): HR=1.28 (95%CI: 0.813, 2.005); favours irinotecan
KRAS wild-type overal survival: (n=202): HR=1.29 (95%CI: 0.894, 1.846); favours irinotecan
no data for patients with unknown KRAS status</td>
</tr>
<tr>
<td>OPUS[157,163,164]

EMA status: unclear

Phase II

N=338

Status: completed</td>
<td>Biomarker evaluation: subgroup (retrospective)

design: RCT randomised 1:1, stratified by ECOG PS

follow-up: NR (median 7.2 months†)

primary outcome: best confirmed overall response rate (assessed by independent review committee using modified WHO criteria)</td>
<td>first-occurrence of a nonresectable, EGFR expressing metastatic colorectal cancer; no prior treatment with EGFR targeted therapy or chemotherapy</td>
<td>A) cetuximab (initial dose 400 mg/m2 for 2 hours, and 250 mg/m2 weekly) + FOLFOX-4; until progressive disease or unacceptable toxicity
B) FOLFOX-4: oxaliplatin 85 mg/m2 on day 1, infused during 2 hours; LV 200 mg/m2, infused during 2 hours, followed by FU as a 400 mg/m2 intravenous bolus then a 600 mg/m2 infusion during 22 hours on days 1 and 2; until progressive disease or unacceptable toxicity</td>
<td>samples: FFPE tumour
method: PCR clamping and melting curve technique in one-step Lightcycler PCR reaction (Light- Mix, k-ras Gly12; TIB MOLBIOL, Berlin, Germany); mutation in codons 12 and 13;

threshold: N/A

KRAS mutation was assessed in 233 (69%) of the 338 patients in the study.

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>170</td><td>52</td><td>61</td><td>57</td></tr>
<tr><td>B</td><td>168</td><td>47</td><td>73</td><td>48</td></tr>
</table>
</td>
<td>Overall overal response rate (CR+PR): cetuximab (n=169) 78 (46.2%, 95%CI: 38.5, 54.0); FOLFOX-4 (n=168) 67 (39.9%, 95%CI: 32.4, 47.7); p=0.243; in KRAS-evaluable patients: cetuximab (n=113) 54 (47.8%, 95%CI: 38.3, 57.4); FOLFOX-4 (n=120) 50 (41.7%, 95%CI: 32.7, 51.0); p=0.390;
KRAS mutant overal response rate (CR+PR): cetuximab (n=52) 17 (32.7%, 95%CI: 20.3, 47.1); FOLFOX-4 (n=47) 23 (48.9%, 95%CI: 34.1, 63.9); p=0.106;
KRAS wild-type overal response rate (CR+PR): cetuximab (n=61) 37 (60.7%, 95%CI: 47.3, 72.9); FOLFOX-4 (n=73) 27 (37.0%, 95%CI: 26.0, 49.1); p=0.011
no data for patients with unknown KRAS status</td>
</tr>
</table>

* Based on OS reported in each group
† Median PFS in both groups

KRAS mutation – colorectal cancer - cetuximab

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>20020408[165-167]

EMA status:
main

Phase III

N=463

Status:
completed</td>
<td>Biomarker evaluation:
subgroup (retrospective)

design: RCT; open-label (due to expected skin toxicity with panitumumab); randomised 1:1. stratified by ECOG status and region

follow-up: median approximately 35 weeks (range 15 to 76)

primary outcome: PFS from random assignment until radiologic progression (blinded central assessment) or death</td>
<td>adults with metastatic colorectal adenocarcinoma; disease progression on or within 6 months of last administration o chemotherapy</td>
<td>A) panitumumab 6mg/mk every 2 weeks as 60-minute iv infusion + best supportive care until disease progression or unacceptable toxicity
B) best supportive care (BSC) "best palliative care per investigator excluding antineoplastic agents"</td>
<td>samples: archived formalin fixed paraffin embedded tumour sections (mainly from resection of primary tumour)

method: validated KRAS mutation kit (DxS Ltd, Manchester UK) that identifies 7 mutations in codons 12 and 13 (Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys, Gly13Asp) using allele-speciffic real-time PCR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>231</td><td>124</td><td>84</td><td>23</td></tr>
<tr><td>B</td><td>232</td><td>119</td><td>100</td><td>13</td></tr>
</table>
</td>
<td>overall At median follow–up of approximately 20 weeks, 193 patients in the panitumumab and 208 patients in the BSC alone group had disease progression or died due to any reasons; improvement with panitumumab; p<0.0001, stratified log–rank test
wild-type KRAS median PFS in panitumumab 16.0 weeks, BSC 8.0 weeks; HR=0.49, 95% CI: 0.37, 0.65; Stratified log-rank test p<0.0001
mutant KRAS median PFS in panitumumab 8.0 weeks, BSC 8.0 weeks; HR=1.07, 95% CI: 0.77, 1.48</td>
</tr>
</table>

LPL protein expression - familial lipoprotein lipase deficiency - alipogene tiparvovec

No studies – based on mechanism of action/ metabolism[168]

NADPH reductase deficiency - acquired methaemoglobinaemia – methylthionium chloride

No studies – based on mechanism of action/ metabolism[169]

PDGFR gene re-arrangements - MDS/MPD - imatinib

Study	Design	Population	Interventions	Biomarker measurement	Results
Heinrich 2008[61,170] EMA status: main Phase II N=7* Status: completed	Biomarker evaluation: subgroup (retrospective) design: single-arm; "exploratory", "proof of concept" follow-up: NR primary outcome: tumor response based on blood counts and bone marrow analyses	age at least 15 years; myeloproliferative disease	imatinib 400 mg daily with escalation to 300 or 400 mg twice daily if no significant improvement after 4 to 8 weeks of therapy	samples: NR method: protein expression of imatinib sensitive TKs IHC at local hospitals for PDGFRA and PDGFRB and confirmed by a central laboratory (Institute of Pathology, Basel, Switzerland) threshold: N/A N: 7; BM+: 3; BM-: 3; BM?: 1	overall haematological response: 3 CR, 1 PR, 1 PD, 2 unknown PDGFR positive 2 CR, 1 PR PDGFR negative 1 PD, 1 unknown PDGFR unknown 1 CR
Apperley 2002[170,171] EMA status: supportive Phase N/A N=2 Status: completed	Biomarker evaluation: none design: case report follow-up: 13 and 15 months primary outcome: N/A	both were adults with myeloproliferative disease (PDGFRβ positive)	imatinib 400 mg daily; route NR	samples: blood, bone marrow or both (details NR) method: RNA reverse-transcribed and tested for ETV6-PDGFRB fusion by single-step reverse-transcriptase PCR and hemi-nested RT-PCR (limit of detection 10^{-5}) threshold: N/A N: 2; BM+: 2; BM-: 0	both patients achieved complete hematological and cytogenetic response

* study investigated imatinib in a range of cancers positive for biomarkers possibly associated with response; only seven with MPS/MPD are included here

Study	Design	Population	Interventions	Biomarker measurement	Results
Cortes 2003[170,172] EMA status: supportive Phase NR N= Status: completed	Biomarker evaluation: NR design: single-arm; study of imatinib including patients with AML or high risk MDS who failed previous chemotherapy or were not eligible for chemotherapy; low-risk MDS eligible regardless of treatment history follow-up: median 14 weeks (range 6-42) in 7 atypical CML (aCML) patients; NR for chronic myelomonocytic leukaemia (CMML) patients primary outcome: NR	adults with aCML and CMML	imatinib single daily oral dose of 400mg	samples: NR method: NR threshold: N/A N: 10; BM+: 0; BM-: 10	none of the patients achieved hematological or cytogenetic response
Garcia 2003[170,173] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 1 year primary outcome: N/A	adult with aCML (PDGFRβ positive)	imatinib 400 mg daily; route and duration NR	samples: peripheral blood smear method: RT-PCR analysis using specific primers flanking predicted breakpoints, confirmed by sequencing (H4-PDGFRβ) threshold: N/A N: 1; BM+: 1; BM-: 0	complete hematological and cytogenetic response

Study	Design	Population	Interventions	Biomarker measurement	Results
Grand 2004[170,174] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: approximately 5 months primary outcome: N/A	adult with a myeloproliferative disorder (PDGFRβ positive)	imatinib 400 mg daily (route NR); reduced to 300 mg daily due to grade 4 neutropenia	samples: NR method: two color FISH, RT-PCR and by "characterising the genomic breakpoints" threshold: N/A N: 1; BM+: 1; BM-: 0	partial hematological response; data on cytogenetic not available
Levine 2005[170,175] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 18 months primary outcome: N/A	adult with CMML (PDGFRβ positive)	imatinib 400 mg daily; oral	samples: NR method: FISH (details NR) threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and cytogenetic response
Magnusson 2002[170,176] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 6 months primary outcome: N/A	adult with CMML (PDGFRβ positive)	imatinib 400 mg daily; route NR	samples: blood (details NR) method: RT-PCR (details NR) threshold: N/A N: 1; BM+: 1; BM-: 0	NR

Study	Design	Population	Interventions	Biomarker measurement	Results
Pardanani 2002[170,177] EMA status: supportive Phase NR N=2* Status: completed	Biomarker evaluation: none design: single-arm (details NR) follow-up: first patient >12 weeks; second patient 4 weeks primary outcome: (1) symptomatic improvement; (2) decrease in the peripheral eosinophil count by at least 50%	adults with chronic myeloid disease	imatinib started at 100-400 mg daily orally (at discretion of treating investigator); if no response at lower dose - patients treated with 400 mg daily	samples: peripheral blood or bone marrow mononuclear cells as well as purified eosinophil cell fractions method: Genomic DNA isolated from samples was used in the mutational analysis. Direct sequencing using an ABI 377 Prism DNA sequencer (Applied Biosystems). threshold: N/A N: 2; BM+: 0; BM-: 2	patient 1: complete haematologic and major cytogenetic response; patient 2: no haematologic or cytogenetic response
Pitini 2007[170,178] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 12 months primary outcome: N/A	adult with CMML (PDGFRβ positive)	imatinib 400 mg daily (route NR)	samples: bone marrow (details NR) method: southern blot analysis of DNA using a genomic PDGFBR probe (gene rearrangement) threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and cytogenetic response
Safley 2004[170,179] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 7 months primary outcome: N/A	adult with aCML (PDGFRα rearrangement)	imatinib 100 mg daily (route NR)	samples: bone marrow (details NR) method: nested RT-PCR (details NR) threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and data not available for cytogenetic response

* Study also included 5 patients with HES

Study	Design	Population	Interventions	Biomarker measurement	Results
Trempat 2003[170,180] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: approximately 12 weeks primary outcome: N/A	adult with aCML (PDGFRα rearrangement)	imatinib 400 mg daily (route NR)	samples: NR method: FISH (details NR) threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and partial cytogenetic response
Vizmanos 2004[170,181] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: approximately 18 months primary outcome: N/A	adult with MPD (PDGFRβ positive)	imatinib 400 mg stopped due to intolerance and started again at 200 mg daily raised to 400 mg daily; route NR	samples: NR method: Southern Analysis of PDGFRB: "DNA digested with HindIII, BamHI, EcoRI, and BglII, blotted using standard conditions and hybridized with an 813-bp alphaP-dCTP-labeled PDGFRB intron 10 probe obtained by amplification by PCR with primers PD3-C and PD3-D from normal human genomic DNA." threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and cytogenetic response
Wilkinson 2003[170,182] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: 7 months primary outcome: N/A	2 year old with MPD (PDGFRβ positive)	imatinib (details NR)	samples: NR method: single step RT-PCR for detection of PDE4DIP-PDGFRB and reciprocal PBGFRB-PDE4DIP fusions threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and major cytogenetic response

Study	Design	Population	Interventions	Biomarker measurement	Results
Wittman 2004[170,183] EMA status: supportive Phase N/A N=1 Status: completed	Biomarker evaluation: none design: case report follow-up: approximately 11 months primary outcome: N/A	2 year old with aCML (PDGFRβ positive)	imatinib 200 mg daily; route NR	samples: bone marrow (details NR) method: RT-PCR (details NR) threshold: N/A N: 1; BM+: 1; BM-: 0	complete haematologic and cytogenetic response

Philadelphia chromosome – ALL – dasatinib

Study	Design	Population	Interventions	Biomarker measurement	Results
START-L[184-187] EMA status: main Phase II N=36* Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm; details NR follow-up: NR primary outcome: Co-primary endpoints: major haematologic response (MaHR) rate and overall haematologic response (OHR) (“determined from hematologic laboratory values, bone marrow cytology and cytogenetics, and extramedullary disease”)	≥18 years; (1) Ph+ (or BCR-ABL+) lymphoid blast phase CML w/ primary or acquired resistance to imatinib or intolerant to imatinib or (2) Ph+ ALL previously treated with standard induction or consolidation chemotherapy and had progressed or not responded to imatinib at a dose of ≥ 600 mg/day (or 400 mg if intolerant to 600 mg)	dasatinib at oral dose of 70 mg twice a day; d ose modifications allowed for management of disease progression or toxicity	samples: NR method: NR threshold: N/A N: 36; BM+: 36; BM-: 0	MaHR rate 42% (15/36) in the total population; 38% (13/34) in the imatinib-resistant, and 100% (2/2) in imatinib-intolerant; OHR rate was 47% (17/36) in the total population, and 44% (15/34) and 100% (2/2) in the imatinib-resistant and imatinib-intolerant

* Also included CML patients (NR here)

Study	Design	Population	Interventions	Biomarker measurement	Results
CA180002[184,188,189] EMA status: supportive Phase I N=11* Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm; dose-escalation: 3 patients "enrolled per cohort and followed for 4 weeks on study drug. If one DLT [dose limiting toxicity] was observed, that dose cohort was expanded to 6 subjects. Dose escalation continued as long as there was < 1/3 of subjects in a cohort with a DLT" follow-up: "minimum of 30 days after the last dose of study therapy or until recovery from all toxic effects, whichever was longer" primary outcome: NR	≥14 years; Philadelphia chromosome - positive ALL; hematologic resistance or intolerance to imatinib	dasatinib doses ranged from 15 mg/day to 240 mg/day until progression of disease or development of intolerable toxicity	samples: NR method: NR threshold: N/A N: 11; BM+: 11; BM-: 0	Major hematologic response (n=NR) 50%, Major cytogenetic response (n=NR) 80%

* Also included CML patients (NR here)

Philadelphia chromosome – ALL – imatinib

Study	Design	Population	Interventions	Biomarker measurement	Results
0109[190,191] EMA status: unclear Phase II N=56* Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm; preliminary investigation follow-up: NR primary outcome: sustained hematologic response lasting at least 4 weeks: (1) complete hematologic response; (2) complete marrow response; or (3) partial response (fewer than 15% blasts in peripheral blood and bone marrow)	≥18 years; morphologically confirmed diagnosis of relapsed or refractory Ph+ ALL	imatinib 400 or 600 mg daily for 24 weeks and then continued indefinitely if the investigator judged further treatment to be of benefit; no concomitant anticancer drugs were to be administered	samples: method: threshold: N/A N: 56; BM+: 56; BM-: 0	initial dose of 400 mg daily - no haematological responses initial dose of 600 mg daily - sustained haematological responses in 12 (26%) patients. Four (33%) of them achieved sustained complete hematologic response
0114[190,192] EMA status: unclear Phase NR N=353† Status: unclear	Biomarker evaluation: non-randomised only positive design: single-arm; providing expanded access to imatinib until it is commercially available; unclear from which studies patients came follow-up: NR primary outcome: NR	adult patients with relapsed/ refractory Ph+ ALL	imatinib orally 600 mg daily (permitted escalation up to max 400 mg twice a day)	samples: NR method: NR threshold: N/A N: 353; BM+: 353; BM-: 0	proportions of patients without progression at 12 months was estimated 12.4% (95% CI: 6, 19); median TTP 3.2 months (95% CI: 3, 4)

* Included CML patients as well – only ALL reported here
† Included CML patients as well – only ALL reported here

Study	Design	Population	Interventions	Biomarker measurement	Results
03001[190,193] EMA status: unclear Phase I N=20* Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm pilot dose-escalation study follow-up: 101 to 349 days primary outcome: NR	Philadelphis chromosome–positive ALL who did not respond to standard induction or consolidation chemotherapy or relapsed after therapy	Imatinib successive dose cohorts ranging from 300 to 1000 mg: orally once daily, except for 800 and 1000-mg - administered twice daily in 400 and 500-mg doses; hydroxyurea was permitted (max 7 days)	samples: NR method: NR threshold: N/A N: 20; BM+: 20; BM-: 0	complete haematological response 4 (20%), marrow response 10 (50%)
AAU02[190] EMA status: unclear Phase II N=19† Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: NR primary outcome: NR	Philadelphis chromosome–positive ALL (relapsed and *de-novo*)	A) relapsed: imatinib 600 mg + idarubicin (12 mg/m2 iv) + cytarabine (200 mg/m2 iv) + vincristine (2 mg iv) + oral prednisone (40 mg/m2) B) de-novo: "Protocol LALA 94" including imatinib 600 mg	samples: NR method: NR threshold: N/A group A: N 7, BM+ 7, BM- 0 group B: N 12, BM+ 12, BM- 0	"Among 22 evaluable patients, combined imatinib and chemotherapy induction resulted in 14 (64%) complete haematological responses and all but 1 patient achieved a major cytogenetic response. There were 7 (88%) complete haematological responses (CHR) among 9 evaluable patients with CML-LBC and relapsed Ph+ALL and 7 (58%) CHR among 12 de-novo Ph+ ALL. Major cytogenetic responses were seen in all newly diagnosed Ph+ ALL patients enrolled in the study. (…) The one-year overall survival rate was 61.1 ± 13.5 %."

* Included CML patients as well – only ALL reported here
† Included CML patients as well – only ALL reported here

Study	Design	Population	Interventions	Biomarker measurement	Results
ADE04[190,194] EMA status: unclear Phase III N=88* Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm; two cohorts (concurrent and alternating schedule) follow-up: NR primary outcome: NR	Ph+ALL with minimal residual disease after induction therapy or stem cell transplant	A) imatinib 400-600 mg daily alternated with chemotherapy (cyclophosphamide 1000 mg/m2 iv, Ara-C 75mg/m2 iv, oral 6-mercaptopurine 60 mg/m2, methotrexate 15 mg i.th) B) imatinib 400-600 mg daily concurrent to chemotherapy (cyclophosphamide 1000 mg/m2 iv, Ara-C 75mg/m2 iv, oral 6-mercaptopurine 60 mg/m2, methotrexate 15 mg i.th)	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 43 / 43 / 0 B / 45 / 45 / 0	"Co-administration of imatinib with induction phase II resulted in a complete remission in 43 (95%) out of 45 patients and was superior to the alternating administration of chemotherapy and imatinib in terms of inducing PCR negativity for bcr-abl transcripts (52% versus 19%, p=0.01)."
ADE10[190,195] EMA status: unclear Phase II N=55† Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; details NR follow-up: NR primary outcome: rate of hematologic remission after induction therapy	newly diagnosed Philadelphia chromosome-positive/BCR-ABL+ acute lymphoblastic leukemia (ALL); age ≥55 years	A) 5-day prerandomisation chemotherapy with dexamethasone and cyclophosphamide -> 28 days of single-agent oral imatinib at a daily dose of 600 mg B) 5-day prerandomisation chemotherapy with dexamethasone and cyclophosphamide -> standard induction chemotherapy	samples: method: threshold: N/A group / N / BM+ / BM- A / 28 / 28 / 0 B / 27 / 27 / 0	"Response to induction was significantly superior in the front-line imatinib arm, as compared with chemotherapy induction (p=0.003): - Twenty-six of the 27 evaluable patients achieved a CR (96.3%) and one patient a PR (3.7%). One patient was not evaluated at this time point but, like the PR patient, achieved a CR after consolidation cycle C1; - Thirteen (50%) of the 26 evaluable patients enrolled in the induction chemotherapy group achieved CR, two patients achieved a PR (7.7%). Nine patients (34.6%) were refractory and 2 patients died during chemotherapy induction; no patient failed imatinib induction"

* Included CML patients as well – only ALL reported here
† Included CML patients as well – only ALL reported here

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>AFR09[190,196]

EMA status: unclear

Phase II

N=51

Status: completed</td>
<td>Biomarker evaluation: non-randomised only positive

design: controlled non-randomised; open-label; using historical controls

follow-up: NR

primary outcome: overall survival (details NR)</td>
<td>age ≥55 years; previously untreated Philadelphia chromosome+ ALL</td>
<td>A) prephase with steroids -> induction chemotherapy without imatinib -> Irrespective of response consolidation/salvage imatinib 600 mg daily and steroids
B) chemotherapy: no steroid prephase -> similar induction + random allocation to vindesine vs vincristine -> consolidation/ salvage w/ mitoxantrone and cytarabine -> interferon alpha for 3 months -> late consolidation with vincristine, doxorubicin and dexamethasone</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>30</td><td>30</td><td>0</td></tr>
<tr><td>B</td><td>21</td><td>21</td><td>0</td></tr>
</table>
</td>
<td>"The projected overall survival is 68% at 1 year vs. 43% in the control group (p=0.001, log-rank test)"</td>
</tr>
<tr>
<td>AIT04[190]

EMA status: unclear

Phase II

N=19

Status: completed</td>
<td>Biomarker evaluation: non-randomised only positive

design: single-arm; pilot study (details NR)

follow-up: NR

primary outcome: NR</td>
<td>age >60 years; Philadelphia chromosome+ ALL patients</td>
<td>imatinib 800 mg in combination w/ steroids</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>19</td><td>19</td><td>0</td></tr>
</table>
</td>
<td>"All 18 evaluable patients achieved haematological CR and 3/18 (17%) had a complete molecular response but with detectable though small numbers of p190 BCR/ABL copies by quantitative RT-PCR"</td>
</tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
AJP01[190,197] EMA status: unclear Phase II N=80 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: median 26.7 months primary outcome: complete remission rate (details NR)	newly diagnosed Philadelphia chromosome + ALL, age ≥15 and ≤64 years, ECOG PS between 0 and 3; adequate liver, kidney and heart function	imatinib 600mg from day 8 to 63 w/ daunorubicin, cyclophosphamide, vincristine and prednisolone; consolidation	samples: NR method: NR threshold: N/A N: 80; BM+: 80; BM-: 0	complete remission in 77 (96.2%) patients, resistant disease in one, early death in two; relapse in 20 patients (26%) after median remission of 5.2 months
AUS01[190] EMA status: unclear Phase II N=32 Status: completed	Biomarker evaluation: : non-randomised only positive design: single-arm (details NR) follow-up: median 2 years (range 4–36 months) primary outcome: NR	Philadelphia chromosome + ALL (details NR)	8 induction-consolidation courses alternating hyper-CVAD (cyclophosphamide 300mg/m2 on days 1-3; vincristine 2mg day 4 and 11; doxorubicine 50 mg/m2 day 4 and dexamethasone 40 mg daily on days 1-4 and 11-14) with high dose methotrexate and ara-C, concurrently with 400 mg imatinib daily on days 1 to 14; higher doses of imatinib during consolidation phase	samples: NR method: NR threshold: N/A N: 32; BM+: 32; BM-: 0	Complete molecular remission: 3 of 27 newly diagnosed patients after hyper-CVAD and imatinib alone; Complete molecular remission in 2 of 5 refractory Ph+ ALL patients after hyper-CVAD and imatinib alone

t(15;17) translocation and/or PML/RAR-**α gene –** APL - arsenic trioxide

Study	Design	Population	Interventions	Biomarker measurement	Results
97-66[198,199] EMA status: main Phase I/II N=12 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: NR primary outcome: NR	APL relapse from or resistance to standard antileukaemic therapy (including all-trans-retinoic acid) (included 2 children)	arsenic trioxide 10 mg escalated to 15 mg as iv infusion over 2-4 hours once a day; later changed to 0.15 mg/kg/day until no visible blasts and promyelocytes in bone marrow	samples: NR method: t(15;17) by FISH and PML-RAR-alpha by reverse-transcription PCR threshold: N/A N: 12; BM+: 12; BM-: 0	11 of the 12 patients had complete clinical remission after treatment; median duration of remission was >5 months; 1 year OS was 75% and 18 month OS was 67%; relapse free survival at 1 year: 55% and 18 months 36%
PLRXAS01[198,200] EMA status: main Phase II N=40 Status: completed	Biomarker evaluation: non-randomised only positive design: single-arm (details NR) follow-up: median 17.1 months primary outcome: NR	relapsed and/or refractory APL (previous treatment including all-trans retinoic acid); adults and children	arsenic trioxide 0.15 mg/kg daily until bone marrow remission or substantial toxicity observed; up to maximum of 60 doses	samples: blood or bone marrow mononuclear cells (details NR) method: "by conventional cytogenetics showing t(15;17), by positive RT-PCR assay for PML/RAR-alpha, or by fluorescence in situ hybridization (FISH) analysis that showed evidence of RAR-alpha or PML translocations"; all PCRs in a central laboratory threshold: N/A N: 40; BM+: 40; BM-: 0	34 of 40 patients achieved a complete clinical response; overall survival at 1 year 70%, at 18 months 66%; relapse-free survival at 1 year 71%, 18 months 58%

Viral resistance – HIV infection - amprenavir

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>PROAB3004[201,202]

EMA status: main

Phase III

N=229

Status: completed</td><td>Biomarker evaluation: NR

design: single-arm; initially designed as randomised controlled double-blind, but later ammended to single-arm

follow-up: 48 weeks

primary outcome: proportion of patients with plasma viral load below the threshold (10,000 and 400 copies/mL of HIV-1 RNA)</td><td>aged 4-18 years; HIV-1 infection and a viral load of ≥400 copies/mL; requiring protease inhibitor-containing therapy</td><td>amprenavir ≥13 years of age, with a weight of ≥50 kg 1200 mg twice daily, otherwise 20 mg/kg twice daily oral capsules (or solution if unable to swallow)</td><td>samples: NR

method: NR

threshold: N/A

<table><tr><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>229</td><td>NR</td><td>NR</td></tr></table></td><td>NR for biomarker</td></tr>
<tr><td>PROAB3006[201,203]

EMA status: main

Phase III

N=504

Status: completed</td><td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label; non-inferiority (details NR)

follow-up: 48 weeks

primary outcome: success rate - number of patietns with HIV-1 RNA plasma levels below the limit of setection (400 copies/ml)</td><td>protease inhibitor - naïve, nucleoside reverse transcriptase inhibitor experienced patients</td><td>A) amprenavir 1200 mg twice daily + background nucleoside reverse transcriptase inhibitor
B) indinavir 800 mg three times daily + background nucleoside reverse transcriptase inhibitor</td><td>samples: blood plasma (details NR)

method: amplified by RT-PCR; sequencing with Applied Biosystems 377 sequencer

threshold: N/A

<table><tr><td>group</td><td>N</td><td>BM+</td><td>BM-</td><td>BM?</td></tr><tr><td>A</td><td>254</td><td>25</td><td>23</td><td>206</td></tr><tr><td>B</td><td>250</td><td>0</td><td>0</td><td>250</td></tr></table></td><td>in amprenavir failures: 19% had I50V mutation, 21% I54L/M, 6%I84V, 15% V32I + I47V</td></tr>
</table>

Viral resistance – HIV infection - atazanavir

Study	Design	Population	Interventions	Biomarker measurement	Results
AI424009[204] EMA status: main Phase NR N=85 Status: completed	Biomarker evaluation: NR design: RCT; details NR follow-up: NR primary outcome: NR	antiretroviral experienced adult patients, HIV-1 infected, no history of AIDS-defining diagnoses within 4 weeks prior to randomisation	A) atazanavir 400 mg/day + saquinavir 1200 mg/ day + 2NRTIs (based on phenotypic susceptibility or if phenotypic results unavailable two previously untried) B) atazanavir 600 mg/day + saquinavir 1200 mg/ day + 2NRTIs (based on phenotypic susceptibility or if phenotypic results unavailable two previously untried) C) ritonavir 400 mg + saquinavir 400 mg + 2NRTIs (based on phenotypic susceptibility or if phenotypic results unavailable two previously untried)	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 34 / NR / NR B / 28 / NR / NR C / 28 / NR / NR	"No reliable interpretation of this study could be made since a high rate of premature discontinuation was observed"
AI424043[204] EMA status: main Phase NR N=290 Status: completed	Biomarker evaluation: NR design: RCT; open-label; non-inferiority (details NR) follow-up: 24 weeks primary outcome: time-Averaged-Difference estimate for the change from baseline in HIV RNA level through week 24	HIV-infected patients who had failed prior antiretroviral treatment(s) including one PI	A) atazanavir + 2 nucleoside analogs B) lopinavir + ritonavir + 2 nucleoside analogs	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 144 / NR / NR B / 146 / NR / NR	NR for biomarker

AI424045[204,205] EMA status: main Phase NR N=358 Status: completed	Biomarker evaluation: subgroup (retrospective) design: RCT; open-label; non-inferiority (details NR) follow-up: 48 weeks primary outcome: Time-Averaged-Difference (TAD) estimate for the change from baseline in HIV RNA level through 48 weeks	antiretroviral experienced patients >16 years, who had virological failure on two or more HAART regimens that included at least one drug from each class: PI, NNRTI, NRTI	A) atazanavir (300 mg/ day) boosted with low dose of ritonavir B) atazanavir (400 mg/ day) in combination with saquinavir C) lopinavir/ritonavir	samples: method: threshold: N/A group / N / BM+ / BM- A / 120 / NR / NR B / 115 / NR / NR C / 123 / NR / NR	<4 protease gene mutations 10, 46, 54, 82, 84, and 90: TAD HIV RNA Level Change From Baseline (log10 c/ml) at week 48: ATV300/RTV (n=84) - LPV/RTV (n=88): 0.06 (95% CI: -0.17, 0.28); ATV400/SQV (n=72) - LPV/RTV (n=88): 0.33 (95% CI: 0.08, 0.57) ≥4 protease gene mutations 10, 46, 54, 82, 84, and 90: TAD HIV RNA Level Change From Baseline (log10 c/ml) at week 48: ATV300/RTV (n=6) - LPV/RTV (n=7): 0.71 (95% CI: 0.13, 1.30); ATV400/SQV (n=11) - LPV/RTV (n=7): 0.59 (95% CI: -0.10, 1.28) virologic failure - known genotype (n=35) - emergent mutations in >20%: M36, M46, I54, A71, V82 virologic failure -unknown genotype (n=35) - emergent mutations in 10-20%: L10, I15, K20, V32, E35, S37, F53, I62, G73, I84, L90

Viral resistance – HIV infection - darunavir

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>POWER 1, 2, 3[206]

EMA status: unclear

Phase NR

N=1097

Status: completed</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: analysis of pooled data from POWER 1, 2 (randomised) and 3 (pooled data from two non-randomised studies)

follow-up: 24 weeks

primary outcome: ≥1 log10 decrease in viral load, viral load > 50 copies per ml; change in log10 viral load at week 10</td>
<td>adults; treatment experienced; in combination with ritonavir</td>
<td>A) darunavir/ ritonavir at doses of 400/100 mg per day, 800/100 mg per day, 400/100 mg twice daily or 600/100 mg twice daily
B) investigator-selected PI-based regimen</td>
<td>samples: NR

method: Antivirogram (phenotypic) and VirtualPhenotype (genotypic)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>964</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>133</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>V11I mutation change in log10 viral load at week 24 ((n) mean(SE)): darunavir (n=40) -1.18 (0.21); control (n=11) -0.77 (0.34)
V32I mutation change in log10 viral load at week 24 ((n) mean(SE)): darunavir (n=36) -0.82 (0.22); control (n=23) -0.39 (0.18)
I47V mutation change in log10 viral load at week 24 ((n) mean(SE)): darunavir (n=51) -1.00 (0.18); control (n=20) -0.30 (0.15)
I54L mutation change in log10 viral load at week 24 ((n) mean(SE)): darunavir (n=27) -1.19 (0.26); control (n=16) -0.75 (0.32);
I54M mutation change in log10 viral load at week 24 ((n) mean(SE)): darunavir (n=31) -0.66 (0.21); control (n=14) -0.24 (0.20)</td>
</tr>
</table>

Viral resistance – HIV infection - efavirenz / emtricitabine / tenofovir disoproxil

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Gallant 2006[207-209]

EMA status: main

Phase III

N=517

Status: completed</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label; non-inferiority study; randomised 1:1

follow-up: 144 weeks (extended from 48 weeks)

primary outcome: HIV RNA levels of < 400 copies per milliliter through week 48; defined according to an FDA algorithm</td>
<td>adult patietns who have not received prior antiretroviral treatment</td>
<td>A) once daily: efavirenz (600 mg) (or nevirapine 200 mg twice daily if CNS toxicity) + tenofovir DF (300 mg) + emtricitabine (200 mg) as separate components
B) twice daily: efavirenz (600 mg) (or nevirapine 200 mg twice daily if CNS toxicity) + fixed dose zidovudine (300 mg) + lamivudine (150 mg)</td>
<td>samples: blood (details NR)

method: at baseline sequencing with GeneSeq Assay; post-baseline: PhenoSense GT Assay

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>258</td><td>19</td><td>0</td><td>236</td></tr>
<tr><td>B</td><td>259</td><td>29</td><td>0</td><td>228</td></tr>
</table>
</td>
<td>resistant patients developed mutations: efavirenz + emtricitabine + tenofovir disoproxil fumarate (analysed n=19): K103N (n=8), K101E (n=3), G190A/S (n=2), Y188C/H (n=1), V108I (n=1), P225H (n=0), M184V/I (n=2), K65R (n=0), thymidine analogue associated mutations (n=0); Efavirenz + lamivudine/zidovudine (analysed n=29): K103N (n=18), K101E (n=3), G190A/S (n=4), Y188C/H (n=2), V108I (n=1), P225H (n=2), M184V/I (n=10), K65R (n=0), thymidine analogue associated mutations (n=2)</td>
</tr>
</table>

Viral resistance – HIV infection - emtricitabine

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>Benson 2004[210,211]

EMA status: main

Phase III

N=440

Status: completed</td>
<td>Biomarker evaluation:

design: RCT; open-label; equivalence trial; randomised 2:1; stratified by PI and NNRTI componenet and plasma HIV-1 RNA level at entry

follow-up: 48 weeks

primary outcome: plasma HIV-1 RNA level at week 48 (virologic failure) using Roch AMPLICOR HIV-1 MONITOR test with a limit of detection 400 copies/ml and UltraSensitive assay (limit of detection 50 copies/ml) (Roche, USA)
(virologic failure - HIV-1 RNA > 400 copies/ml)</td>
<td>adults on a stable treatment containing lamivudine, an NRTI and a PI or NNRTI</td>
<td>A) substitution of lamivudine 150mg twice a day with emtricitabine 200mg every day as part of stable triple combination ART for 48 weeks
B) continuation of lamivudine 150mg twice a day as part of stable triple combination ART for 48 weeks</td>
<td>samples: plasma HIV-1 RNA

method: nested PCR + dideoxy sequencing using ABI 377 sequencing system (Applied Biosystems Inc., USA) using labelled dye terminators following standard techniques; if insufficient baseline plasma HIV-1 RNA a modified amplification procedure was performed (details NR)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr>
<tr><td>A</td><td>294</td><td>16</td><td>2</td><td>276</td></tr>
<tr><td>B</td><td>146</td><td>3</td><td>1</td><td>142</td></tr>
</table>
</td>
<td>"complete or at least partial (around M184) sequence analysis of baseline isolates was obtained for 23/34 virological failures (19/23 in the emtricitabine arm and 4/11 in the lamivudine arm). In the emtricitabine subset, the M184V/I mutation was present in 17/19 (89.5%) isolates at baseline. In the lamivudine subset, the M184V mutation was present in 3/4 (75%) isolates at baseline. Genotypic data were available for 33/34 patients at the time of virological failure. Two emtricitabine and the onelamivudine patients with wild type virus at M184 at baseline had developed the M184V mutation."</td>
</tr>
</table>

Viral resistance – HIV infection - enfuvirtide

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>TORO 1[212,213]

EMA status: main

Phase III

N=501

Status: completed</td><td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label; randomised 2:1 using an adaptive randomisation scheme, stratified by plasma HIV-RNA level and use of newly approved or investigational drugs in the optimised background regimen

follow-up: 48 weeks

primary outcome: change from baseline to week 24 in plasma HIV-1 RNA level measured on logarythmic scale with Amplicor HIV-1 Monitor, version 1.5, Roche</td><td>prior treatment with (but no mention of failure) and/or documented resistance to all 3 classess of drugs</td><td>A) enfuvirtide 90 mg twice daily by subcutaneous injection to the abdomen, upper arm or anterior aspect of thigh (first by study personel, then patient) + optimised background therapy
B) optimised background 3-5 antiretroviral drugs selected prior to randomisation; changes allowed in case of protocol-defined failure or toxicity</td><td>samples: NR

method: genotypic resistance testing by ViroLogic, San Francisco

threshold: N/A

<table><tr><td>group</td><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>A</td><td>332</td><td>NR</td><td>NR</td></tr><tr><td>B</td><td>169</td><td>NR</td><td>NR</td></tr></table></td><td rowspan="2">enfuvirtide patients with virological failure in Toro 1 and Toro 2 after 24 weeks of therapy: almost all (185/187, 99 %) had substitutions in gp41 aa 36-45; NR for optimised background therapy</td></tr>
<tr><td>TORO 2[212,214]

EMA status: main

Phase III

N=512

Status: completed</td><td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label;

follow-up: 48 weeks

primary outcome: change from baseline to week 24 in plasma HIV-1 RNA level measured on logarythmic scale with Amplicor HIV-1 Monitor, version 1.5, Roche</td><td>prior treatment with (but no mention of failure) and/or documented resistance to all 3 classess of drugs</td><td>A) enfuvirtide 90 mg twice daily by subcutaneous injection to the abdomen, upper arm or anterior aspect of thigh (first by study personel, then patient) + optimised background therapy
B) optimised background 3-5 antiretroviral drugs selected prior to randomisation; changes allowed in case of protocol-defined failure or toxicity</td><td>samples: NR

method: genotypic resistance testing by ViroLogic, San Francisco

threshold: N/A

<table><tr><td>group</td><td>N</td><td>BM+</td><td>BM-</td></tr><tr><td>A</td><td>341</td><td>NR</td><td>NR</td></tr><tr><td>B</td><td>171</td><td>NR</td><td>NR</td></tr></table></td></tr>
</table>

Viral resistance – HIV infection - emtricitabine / rilpivirine / tenofovir disoproxil

Study	Design	Population	Interventions	Biomarker measurement	Results
ECHO[215,216] EMA status: main Phase III N=694 Status: completed	Biomarker evaluation: enrichment design: RCT; double-blind; non-inferiority; randomised 1:1; stratified by screening viral load follow-up: 100 weeks primary outcome: percentage of patients with confirmed response at week 48	HIV-1 infected adults not previously treated with antiretroviral drugs	A) once daily:* 25 mg rilpivirine + 300 mg tenofovir disoproxil fumate + 200 mg emtricatabine for 96 weeks B) once daily: 600 mg efavirenz + 300 mg tenofovir disoproxil fumate + 200 mg emtricatabine for 96 weeks	samples: NR method: viral genotyping by Virco BVBA (Mechelen, Belgium) with Virco TYPE HIV-1 assay threshold: N/A group / N / BM+ / BM- / BM? A / 346 / 29† / 11 / 342 B / 344 / 8 / 5 / 331	< 50 copies/mL: rilpivirine 82.9%, control 82.8%; non-inferiority at the 12% margin was met
THRIVE[215,217] EMA status: main Phase III N=680 Status: completed	Biomarker evaluation: enrichment design: RCT; double-blind; non-inferiority; randomised 1:1; stratified by viral load and background therapy follow-up: 100 weeks primary outcome: percentage of patients who received at least one dose of study drug with virological response at 48 weeks	antiretroviral naïve HIV-1 infected adults	A) once daily:‡ rilpivirine 25 mg + investigator selected background (tenofovir DF + emtricitabine or zidovudine + lamivudine or abacavir + lamivudine) for 96 weeks B) once daily: efavirenz 600 mg + investigator selected background (tenofovir DF + emtricitabine or zidovudine + lamivudine or abacavir + lamivudine) for 96 weeks	samples: NR method: by Virco (Mechelen, Belgium) using VircoTYPE HIV-1 assay threshold: N/A group / N / BM+ / BM- / BM? A / 340 / 15§ / 7 / 318 B / 340 / 8 / 7 / 325	< 50 copies/mL: rilpivirine 85.6% , control 81.7%; the primary endpoint of non-inferiority at the 12% margin was met

* patients had to take drugs twice daily due to double-dummy design
† resistance mutations were assessed in 40 of 45 patients with virological failure in rilpivirine group and 13 out of 19 patietns in efavirenz group; exact numbers positive for mutations unclear
‡ patients had to take drugs twice daily due to double-dummy design
§ resistance mutations were assessed in 22 of 27 patients with virological failure in rilpivirine group and 15 out of 20 patietns in efavirenz group; exact numbers positive for mutations unclear

Viral resistance – HIV infection - fosamprenavir calcium

Study	Design	Population	Interventions	Biomarker measurement	Results
APV 30003[218] EMA status: main Phase III N=315 Status: completed	Biomarker evaluation: subgroup (retrospective) design: RCT; open-label; non-inferiority follow-up: 48 weeks primary outcome: average area under the curve minus baseline (AAUCMB) in log10 plasma HIV-1	antiretroviral experienced, failure on a prior PI	A) fosamprenavir calcium 700mg twice a day + ritonavir 100mg twice a day + two active RTIs B) fosamprenavir calcium 1400mg OD + ritonavir 200mg OD + two active RTIs C) lopinavir 400mg/ritonavir 100mg twice a day + two active RTIs	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 105 / NR / NR B / 107 / NR / NR C / 103 / NR / NR	In virologic sub-study 58% (19/33) versus 25% (7/28) patients acquired resistance mutations in the fosamprenavir and lopinavir arm respectively; protease resistance-associated mutations: fosamprenavir 17/39 (44%) vs lopinavir 19/33 (58%)); majority of protease mutations in the fosamprenavir arm were mutations previously associated with amprenavir resistance (I50V n=3, I54L n=7, I54M n=3, I84V n=11, V32I n=3, I47V n=5) or associated accessory mutations (L10F n=9, L10I n=3, L33F n=8, M46I/L n=10, A71V/T n=3, V82I n=4)
SOLO[218,219] EMA status: main Phase III N=660 Status: completed	Biomarker evaluation: subgroup (cross-sectional) design: RCT; open-label; stratified according to plasma HIV-1 RNA level at screening; non-inferiority follow-up: 48 weeks primary outcome: proportion of patients with plasma HIV-1 RNA levels < 400 copies/ml at 48 weeks using the Roche Amplicor HIV-1 Monitor test version 1.5 (Roche Diagnostics, Basel, Switzerland)	antiretroviral-naïve HIV-1 infected patients	A) fosamprenavir 1400 mg OD with ritonavir 200 mg OD + abacavir 300 mg twice a day + lamivudine 150 mg BID B) nelfinavir 1250 mg twice a day + abacavir 300 mg twice a day + lamivudine 150 mg twice a day	samples: NR method: NR threshold: N/A group / N / BM+ / BM- A / 322 / NR / NR B / 327 / NR / NR	"No genotypic or phenotypic amprenavir resistance was detected in virus from 32 patients failing fosamprenavir boosted OD. A significantly higher proportion of nelfinavir treated patients acquired primary or secondary mutations (27/54 (50 %; p < 0.001). Treatment emergent NRTI resistance was significantly less frequent with fosamprenavir boosted (4/32; 13 %) compared to nelfinavir treated patients (31/54; 57 %) (p < 0.001)."

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>NEAT[218,220]

EMA status: supportive

Phase III

N=249

Status: completed</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label; randomised 2:1; non-inferiority

follow-up: 48 weeks

primary outcome: proportion of patients with plasma HIV-1 RNA levels < 400 copies/ml at 48 weeks “measured using the Roche Amplicor HIV-1 Ultrasensitive Monitor test (version 1.5, ultrasensitive limit of quantification = 50 c/mL). Samples with vRNA >75,000 c/mL were retested using the Roche Amplicor HIV-1 Monitor test (version 1.5, standard assay, limit of quantification = 400 copies/mL)."</td>
<td>ARV-naive HIV-infected at least 13 years of age (or 18 years of age according to local requirements) with plasma HIV-1 RNA (vRNA) of at least 5000 c/mL</td>
<td>A) fosamprenavir calcium 1400 mg twice a day
B) nelfinavir 1250 mg twice a day</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>166</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>83</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>NR for biomarker</td>
</tr>
</table>

Viral resistance – HIV infection - lopinavir / ritonavir

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>M98-863[221]

EMA status: main

Phase III

N=635

Status: ongoing</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; double-blind; equivalence

follow-up: 48 weeks (24 week results available)

primary outcome: proportion of patients with plasma HIV RNA levels below the limit of quantification (< 400 copies/ml) at week 24 and time until loss of virologic response through week 48</td>
<td>Antiretroviral naïve patients (>12 years) with viral load above 400 copies/ml</td>
<td>A) lopinavir / ritonavir 400 mg/100 mg twice a day with stavudine and lamivudine; after week 24 patients received nelfinavir at 1250 mg twice a day or 750 mg three times a day
B) nelfinavir 750 mg three times a day in combination with stavudine and lamivudine; after week 24 patients received nelfinavir at 1250 mg twice a day or 750 mg three times a day</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>326</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>327</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>“The absence of detection of any mutation is noteworthy (0/31 (0%) versus 21/64 (33%) in lopinavir/ ritonavir and nelfinavir arms respectively"</td>
</tr>
<tr>
<td>M97-720[221]

EMA status: supportive

Phase I/II

N=100

Status: completed</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; patients blind to the dose; dose ranging

follow-up: 204 weeks

primary outcome: NR</td>
<td>Antiretroviral naive HIV infected patients</td>
<td>A) lopinavir/ritonavir 200/100 twice a day or 400/100 mg twice a day + at day 22 stavudine/lamivudine
B) lopinavir/ritonavir 400/100 mg twice a day + stavudine/lamivudine or 400/200 mg BID + stavudine/lamivudine</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>32</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>68</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>genotypic resistance testing available from 11 of the 16 subjects who lost of virologic response at or prior to week 204 (in 5 subjects the results unavailable due to a low number of viral copies); none exhibited genotypic resistance to lopinavir; confirmed by phenotypic resistance testing; 3 subjects - M184V mutation in reverse transcriptase</td>
</tr>
</table>

Viral resistance – HIV infection - nelfinavir

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>505[222]

EMA
status:
main

Phase III

N=93

Status:
complete
d</td><td>Biomarker evaluation: subgroup (retrospective)

design: RCT; double-blind for 4 weeks, after which placebo patients switched to one of the active dose groups; details NR

follow-up: NR

primary outcome: NR</td><td>NR</td><td>A) nelfinavir 500 mg three times a day
B) nelfinavir 750 mg three times a day
C) placebo</td><td>samples: NR

method: NR

threshold: N/A

<table><tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr><tr><td>A</td><td>NR</td><td>NR</td><td>NR</td></tr><tr><td>B</td><td>NR</td><td>NR</td><td>NR</td></tr><tr><td>C</td><td>NR</td><td>NR</td><td>NR</td></tr></table></td><td rowspan="2">"The on-therapy incidence of the D30N substitution was estimated from assay of 16-week samples from 142 randomly selected patients who had received monotherapy in study 505 or combination therapy in study 511. The substitution was detected in 18/32 monotherapy patients, but in only 2/22 and 1/27 on 500 and 750 mg t.i.d. regimens with zidovudine and lamivudine. Mutations associated with other protease inhibitor treatments were not seen in any of the 142 patients."</td></tr>
<tr><td>511[222,223]

EMA
status:
main

Phase III

N=297

Status:
complete
d</td><td>Biomarker evaluation: subgroup (retrospective)

design: RCT; double-blind; randomisation stratified based on CD4 cell counts

follow-up: 24 weeks (+ 6 month blinded extension)

primary outcome: quantitative plasma HIV RNA levels and CD4 cell counts; HIV RNA levels measured by branched chain DNA assay (bDNA; Chiron Corporation, Emeryville, California, USA) with lower limit of quantification 500 copies/ml; CD4 cell counts NR</td><td>antiretroviral-naive HIV-positive patients at least 13 years old; plasma HIV RNA at least 15 000 copies/m</td><td>A) nelfinavir 500 mg three times a day in combination with zidovudine 200 mg three times a day and lamivudine 150 mg twice a day twice daily
B) nelfinavir 750 mg three times a day in combination with zidovudine 200 mg three times a day and lamivudine 150 mg twice a day twice daily
C) placebo identical capsules to active treatment in combination with zidovudine 200 mg three times a day and lamivudine 150 mg twice a day twice daily</td><td>samples: NR

method: NR

threshold: N/A

<table><tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr><tr><td>A</td><td>97</td><td>NR</td><td>NR</td></tr><tr><td>B</td><td>99</td><td>NR</td><td>NR</td></tr><tr><td>C</td><td>101</td><td>NR</td><td>NR</td></tr></table></td></tr>
</table>

Viral resistance – HIV infection - rilpivirine

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr><td>ECHO[215,216]

EMA status: main

Phase III

N=694

Status: completed</td><td>Biomarker evaluation: enrichment

design: RCT; double-blind; non-inferiority; randomised 1:1; stratified by screening viral load

follow-up: 100 weeks

primary outcome: percentage of patients with confirmed response at week 48</td><td>HIV-1 infected adults not previously treated with antiretroviral drugs</td><td>A) once daily:* 25 mg rilpivirine + 300 mg tenofovir disoproxil fumate + 200 mg emtricatabine for 96 weeks
B) once daily: 600 mg efavirenz + 300 mg tenofovir disoproxil fumate + 200 mg emtricatabine for 96 weeks</td><td>samples: NR

method: viral genotyping by Virco BVBA (Mechelen, Belgium) with Virco TYPE HIV-1 assay

threshold: N/A

<table><tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr><tr><td>A</td><td>346</td><td>29†</td><td>11</td><td>342</td></tr><tr><td>B</td><td>344</td><td>8</td><td>5</td><td>331</td></tr></table></td><td><50 copies/mL: rilpivirine 82.9%, control 82.8%; non-inferiority at the 12% margin was met</td></tr>
<tr><td>THRIVE[215,217]

EMA status: main

Phase III

N=680

Status: completed</td><td>Biomarker evaluation: enrichment

design: RCT; double-blind; non-inferiority; randomised 1:1; stratified by viral load and background therapy

follow-up: 100 weeks

primary outcome: percentage of patients who received at least one dose of study drug with virological response at 48 weeks</td><td>antiretroviral naïve HIV-1 infected adults</td><td>A) once daily:‡ rilpivirine 25 mg + investigator selected background (tenofovir DF + emtricitabine or zidovudine + lamivudine or abacavir + lamivudine) for 96 weeks
B) once daily: efavirenz 600 mg + investigator selected background (tenofovir DF + emtricitabine or zidovudine + lamivudine or abacavir + lamivudine) for 96 weeks</td><td>samples: NR

method: by Virco (Mechelen, Belgium) using VircoTYPE HIV-1 assay

threshold: N/A

<table><tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th><th>BM?</th></tr><tr><td>A</td><td>340</td><td>15§</td><td>7</td><td>318</td></tr><tr><td>B</td><td>340</td><td>8</td><td>7</td><td>325</td></tr></table></td><td><50 copies/mL: rilpivirine 85.6% , control 81.7%; the primary endpoint of non-inferiority at the 12% margin was met</td></tr>
</table>

* patients had to take drugs twice daily due to double-dummy design
† resistance mutations were assessed in 40 of 45 patients with virological failure in rilpivirine group and 13 out of 19 patietns in efavirenz group; exact numbers positive for mutations unclear
‡ patients had to take drugs twice daily due to double-dummy design
§ resistance mutations were assessed in 22 of 27 patients with virological failure in rilpivirine group and 15 out of 20 patietns in efavirenz group; exact numbers positive for mutations unclear

Viral resistance – HIV infection - tenofovir disoproxil fumarate

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>902[224,225]

EMA status: main

Phase II

N= 189

Status: completed</td>
<td>Biomarker evaluation: subgroup analysis (prospective)

design: double blind RCT randomised 2:2:2:1; after 24 weeks placebo patients were crossed-over to tenofovir 300 mg

follow-up: 48 weeks

primary outcome: co-primary: time weighted mean change in plasma HIV-1 RNA [log10 copies/ml] from baseline to weeks 4 (DAVG4) and 24 (DAVG24) using the Ultrasensitive HIV-1 Monitor Test (LLQ, 50 copies/ml) (Roche)"[226])</td>
<td>patients on a stable antiretroviral regimen (no more than 4 antiretroviral agents) for 8 weeks prior to enrolment; Antiretroviral experienced patients (> 4 years) HIV RNA ≥ 400 and ≤ 100,000 copies/ml</td>
<td>A) tenofovir disoproxil fumarate 75 mg a day + stable antiretroviral therapy
B) tenofovir disoproxil fumarate 150 mg a day + stable antiretroviral therapy
C) tenofovir disoproxil fumarate 300 mg a day + stable antiretroviral therapy
D) placebo + stable antiretroviral therapy</td>
<td>samples: NR

method: NR

threshold: N/A

Viral genotype was measured at baseline in all patients

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>54</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>51</td><td>NR</td><td>NR</td></tr>
<tr><td>C</td><td>56</td><td>NR</td><td>NR</td></tr>
<tr><td>D</td><td>28</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>"for patients receiving tenofovir DF in addition to their existing regimen, a comparable decline in HIV-RNA was observed by week 48 whatever the genotype measured at baseline (-0.62 log10 copies/ml DAVG48). In particular, a comparable virologic response was observed between patients resistant to or susceptible to zidovudine (-0.57 log10 copies/ml versus -0.61 log10 copies/ml, DAVG48, respectively)." 4 patients developed K65R mutation (resistance to tenofovir)</td>
</tr>
</table>

Study	Design	Population	Interventions	Biomarker measurement	Results
903[224,227] EMA status: main Phase III N= 602* Status: completed	Biomarker evaluation: subgroup analysis (cross-sectional) design: randomised 1:1, double-blind, stratified by baseline HIV-1 RNA and CD4 cell count; aiming to establish equivalence between study follow-up: 144 weeks primary outcome: proportion of patients with HIV RNA levels <400 copies/mL at week 48 using Roche Amplicor HIV-1 Monitor viral load assay (version 1.0 and version 1.5 [depending on the study site], Indianapolis, Ind) (lower limit of quantification, 400 copies/mL);	antiretroviral naïve patients; Plasma HIV-1 RNA levels > 5 000 copies/ml at screening	A) tenofovir disoproxil fumarate 300 mg every day + corresponding placebo + 150 mg twice daily of lamivudine and 600 mg/day of efavirenz (400 mg/day nevirapine could be substituted for efavirenz if intolerable neuropsychiatric toxicity) B) stavudine 40mg twice daily (or 30 mg twice daily if weight <60 kg) + corresponding placebo + 150 mg twice daily of lamivudine and 600 mg/day of efavirenz (400 mg/day nevirapine could be substituted for efavirenz if intolerable neuropsychiatric toxicity)	samples: blood plasma (details NR) method: "Genotypic analyses (Virtual Phenotype26: Virco, Mechelen, Belgium) included the first 400 amino acids of the reverse transcriptase coding sequence and phenotypic analyses (PhenoSense HIV27: Virologic, South San Francisco, Calif) included susceptibility to tenofovir and all other licensed NRTIs and NNRTIs. All resistance assays were performed and analyzed in a blinded fashion"[228] threshold: N/A group / N / BM+ / BM- A / 299 / 17 / 5 B / 301 / 11 / 4	Patients with virologic rebound or suboptimal therapy - tenofovir (n=22): 14 Any NNRTI Resistance Mutation†, 9 K103N, 8 M184V/I, 9 Other NRTI Resistance‡, 7 K65R, 5 none detected Patients with virologic rebound or suboptimal therapy - control (n=15): 10 Any NNRTI Resistance Mutation, 6 K103N, 8 M184V/I, 3 Other NRTI Resistance, 2 K65R, 4 none detected

* Only 600 patients were analysed
† L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/L/H, or G190A/S/E/Q in RT
‡ M41L, A62V, K65R, D67N, T69D/N, K70R, L74V/I, V75T, F77L, Y115F, F116Y, Q151M, M184V, L210W, T215Y/F, or K219Q/E/N in RT

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>907[224,226]

EMA
status:
main

Phase III

N= 552*

Status:
complete
d</td>
<td>Biomarker evaluation: subgroup analysis (prospective)

design: randomised 2:1; 24 weeks as double blind and 24 weeks as open-label

follow-up: 48 weeks

primary outcome: time-weighted average change in HIV-1 RNA level from baseline to week 24 (DAVG24) (details NR)</td>
<td>patients on a stable antiretroviral regimen (no more than 4 antiretroviral agents) for 8 weeks prior to enrolment; Antiretroviral experienced patients (> 4 years) HIV RNA ≥ 400 and ≤ 100,000 copies/ml</td>
<td>A) tenofovir disoproxil fumarate 300 mg every day + stable antiretroviral therapy
B) placebo + stable antiretroviral therapy</td>
<td>samples: blood plasma

method: reverse transcriptase PCR from plasma HIV-1 RNA (Vircogen, Virco, Mechelen, Belgium)

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>368</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>184</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>virological substudy: ‘‘The highest reduction in viral load was observed in patients without zidovudine resistance but with the M184V mutation among all genotypic groups at week 24 (-0.97 log10 DAVG24, p <0.0001) and at week 48 (-0.90 log10 DAVG48, p <0.0001). The difference with other groups was not statistically relevant. Patients with K65R mutation at baseline did not respond to tenofovir DF (+ 0.12 log10 mean DAVG24). Treatment with tenofovir DF resulted in infrequent development of resistance to tenofovir, as only 8/274 patients (3%) developed the K65R mutation by week 48." 5 patients developed K65R mutation (resistance to tenofovir) after treatment with tenofovir''</td>
</tr>
</table>

* one pateint had no post-baseline data and was excluded from analysis

Viral resistance – HIV infection - tipranavir

Study	Design	Population	Interventions	Biomarker measurement	Results
RESIST 1[229-231] EMA status: main Phase III N=630 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; follow-up: 48 weeks primary outcome: virologic response (proportion of patients with a reduction in the HIV-1 load of at least 1 log after 24 weeks)	HIV-positive, multiple antiviral drug experienced patients	A) tipranavir 500 mg + ritonavir 200 mg twice daily with optimised background therapy B) investigator-selected, ritonavir-boosted standard of care PI with optimised background therapy	samples: NR method: TruGene method, version 1.0; less than 3 mutations at codons 33, 82, 84 or 90 threshold: N/A group / N / BM+ / BM- A / 313 / 313 / 0 B / 317 / 317 / 0	virologic response at week 48: tipranavir (n=311) 103 patients achieved, standard care (n=309) 49 achieved; weighted difference 16.8% (95% CI: 10.3, 23.2%, p<0.0001)
RESIST 2[229,230,232] EMA status: main Phase III N=863 Status: completed	Biomarker evaluation: enrichment design: RCT; open-label; randomised 1:1; stratified by preselected PI and the use of enfuvirtide follow-up: 48 weeks primary outcome: virologic response (proportion of patients with a reduction in the HIV-1 load of at least 1 log after 24 weeks)	HIV-positive, multiple antiviral drug experienced patients	A) tipranavir 500 mg + ritonavir 200 mg twice daily with optimised background therapy B) investigator-selected, ritonavir-boosted standard of care PI with optimised background therapy	samples: NR method: Virtual Phenotype, version 3.6 (Virco), for European countries and the HIV-1 genotyping method, version 1.0 (TruGene), for Latin America version 1.0; less than 3 mutations at codons 33, 82, 84 or 90 threshold: N/A group / N / BM+ / BM- A / 435 / 435 / 0 B / 428 / 428 / 0	virologic response at week 24 (NR for week 48): tipranavir (n=435) 177 patients achieved, standard care (n=428) 76 achieved; weighted difference 22.3% (95% CI: 16.4, 28.1%, p<0.0001)

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>1182.4[229,233]

EMA status: supportive

Phase II

N=81

Status: completed</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label; randomised 1:1:1

follow-up: 96 weeks

primary outcome: NR</td>
<td>single PI-experienced HIV-1 patients</td>
<td>A) tipranavir 500 mg + ritonavir 100 mg twice daily with background therapy
B) tipranavir 1250 mg + ritonavir 100 mg twice daily with background therapy
C) saquinavir 400 mg + ritonavir 400 mg twice daily with background therapy</td>
<td>samples: blood (details NR)

method: TruGene 6.0 and 7.0 [Bayer] or Virtual Phenotype assays (version 3.6; [VIRCO])

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>C</td><td>NR</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>NR</td>
</tr>
<tr>
<td>1182.51[229,234]

EMA status: supportive

Phase II

N=315

Status: completed</td>
<td>Biomarker evaluation:

design: RCT; open-label; included patietns excluded from RESIST 1 and 2

follow-up: 24 weeks

primary outcome: pharmacokinetics</td>
<td>HIV-positive, multiple antiviral drug experienced patients</td>
<td>A) tipranavir 500mg and ritonavir 200 mg twice daily and optimised non-PI background
B) lopinavir 400 mg + ritonavir 100 mg+ optimised non-PI background + from week 2 tipranavir 500mg and ritonavir 100mg twice daily
C) amprenavir 600 mg + ritonavir 100 mg+ optimised non-PI background + from week 2 tipranavir 500mg and ritonavir 100mg twice daily
D) saquinavir 1000 mg + ritonavir 100 mg+ optimised non-PI background + from week 2 tipranavir 500mg and ritonavir 100mg twice daily</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>C</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>D</td><td>NR</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>72 patients were included in resistance testing, results NR</td>
</tr>
</table>

<table>
<tr><th>Study</th><th>Design</th><th>Population</th><th>Interventions</th><th>Biomarker measurement</th><th>Results</th></tr>
<tr>
<td>1182.52[229,235]

EMA status: supportive

Phase II

N=216

Status: completed</td>
<td>Biomarker evaluation: enrichment

design: RCT; double-blind; dose-optimisation study

follow-up: up to 32 weeks

primary outcome: viral load reduction after 2 weeks</td>
<td>similar: HIV-positive, multiple antiviral drug experienced patients</td>
<td>A) tipranavir 500 mg + ritonavir 100 mg twice daily
B) tipranavir 500 mg + ritonavir 200 mg twice daily
C) tipranavir 750 mg + ritonavir 200 mg twice daily</td>
<td>samples: NR

method: NR

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>73</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>72</td><td>NR</td><td>NR</td></tr>
<tr><td>C</td><td>71</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>viral load reduction after 2 weeks - the difference "was not statistically significant between the three treatment arms, a trend towards a dose-effect could be observed"</td>
</tr>
<tr>
<td>Markowitz 2007[229,236]

EMA status: supportive

Phase II

N=41

Status: completed</td>
<td>Biomarker evaluation: subgroup (cross-sectional)

design: RCT; open-label; details NR

follow-up: 80 weeks

primary outcome: viral load change from baseline and the proportion of patients with a viral load,400 and ,50 copies/mL</td>
<td>HIV-positive, multiple antiviral drug experienced patients</td>
<td>A) tipranavir 1200mg + 100 mg ritonavir twice daily + background therapy
B) tipranavir 2400mg + 200 mg ritonavir twice daily + background therapy</td>
<td>samples: NR

method: initially Visible Genetics Trugene Assay (samples up to week 48), and then by VIRCO NV. The Affymetrix Gene Chip Method (Affymetrix, Santa Clara, CA) was used for measurement before study initiation

threshold: N/A

<table>
<tr><th>group</th><th>N</th><th>BM+</th><th>BM-</th></tr>
<tr><td>A</td><td>19</td><td>NR</td><td>NR</td></tr>
<tr><td>B</td><td>22</td><td>NR</td><td>NR</td></tr>
</table>
</td>
<td>NR</td>
</tr>
</table>

APPENDIX 13. RADIAL PLOTS

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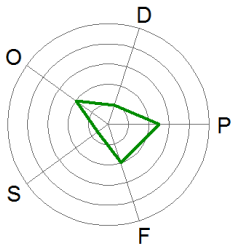
13.1. EFFECTIVENESS BIOMARKERS (NON-ORPHAN DRUGS)

Biomarker: ALK gene rearrangement
Indication: NSCLC (previously treated, advanced)
Drug: crizotinib
Status: authorised

B-I-D: ALKNC

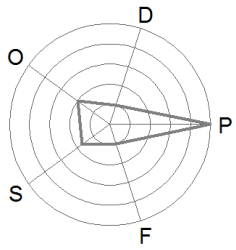
Main study

A8081001

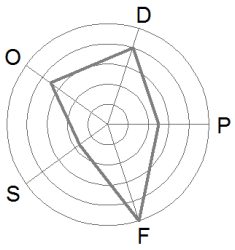


Supportive studies

A8081005



A8081007



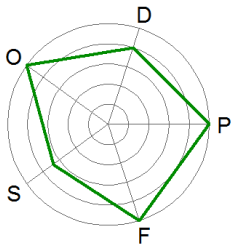
Both ongoing at time of inclusion

Biomarker: BRAF V600 mutation
Indication: unresectable or metastatic melanoma
Drug: vemurafenib
Status: authorised

B-I-D: BRMV

Main study

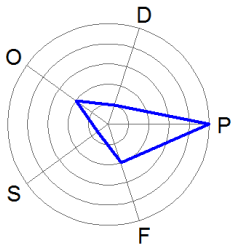
BRIM 3



ongoing at time of inclusion

Supportive study

NP22657

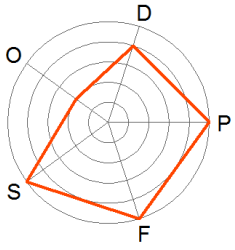


Biomarker: CCR5 tropism
Indication: HIV (treatment-experienced)
Drug: maraviroc
Status: authorised

B-I-D: CCR5HM

Main studies

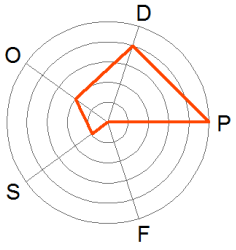
MOTIVATE 1 and 2



Only combined results of both MOTIVATE studies available for F

Supportive study

Saag 2009



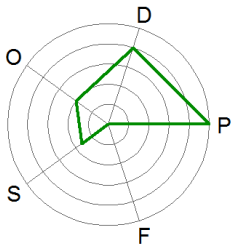
Study in biomarker negative patients

Biomarker: EGFR expression
Indication: colorectal neoplasms
Drug: cetuximab
Status: authorised

B-I-D: EGFR(E)CC

Main study

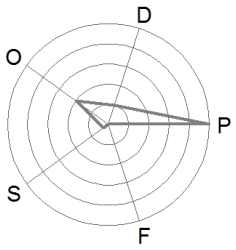
BOND



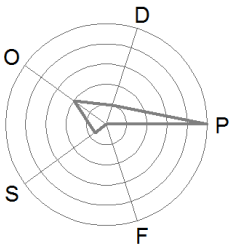
Study compares monotherapy with combination therapy

Supportive studies

IMCL CP02-0141



IMCL CP02-9923

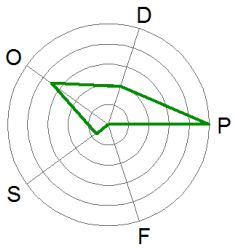


Biomarker: EGFR expression
Indication: NSCLC
Drug: cetuximab
Status: refused

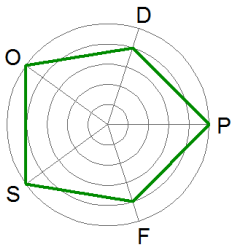
B-I-D: EGFR(E)NC

Main studies

CA225099

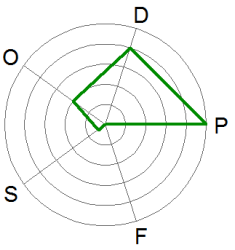


FLEX



Supportive study

Rosell 2007

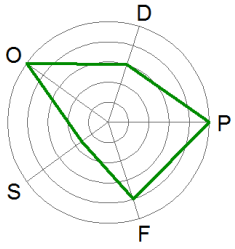


Biomarker: EGFR expression
Indication: NSCLC
Drug: erlotinib
Status: authorised

B-I-D: EGFR(E)NE

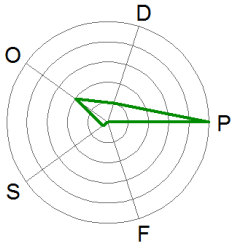
Main study

BR.21



Supportive study

Perez-Soler 2004



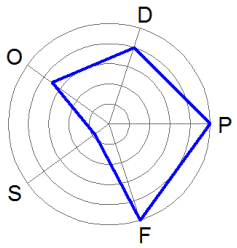
Biomarker: EGFR mutation
Indication: NSCLC
Drug: erlotinib
Status: authorised

B-I-D: EGFR(M)NE

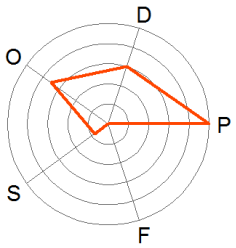
Main study

Supportive studies

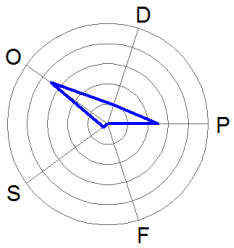
EURTAC



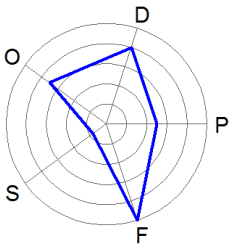
CALGB30406



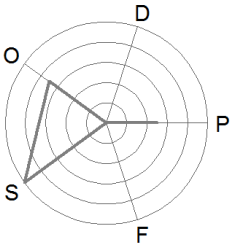
Laskin 2009



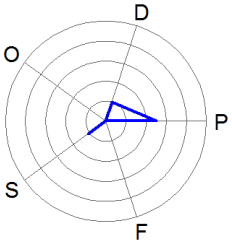
OPTIMAL



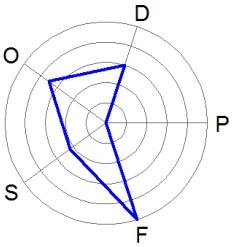
Paz-Ares 2010



Rosell 2009



SATURN



Paz-Ares 2010 was a literature review comparing erlotinib with gefitinib

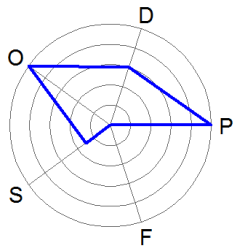
Biomarker: EGFR mutation
Indication: NSCLC
Drug: geftinib
Status: authorised

B-I-D: EGFR(M)NG

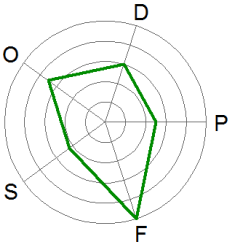
Main studies

Supportiv

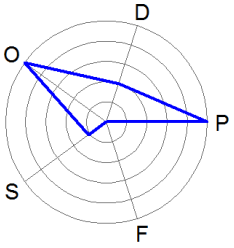
INTEREST



IPASS



ISEL



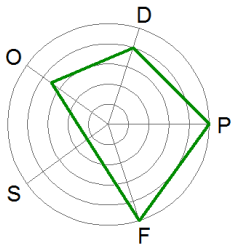
Biomarker: EpCAM expression
Indication: cancer ascites
Drug: catumaxomab
Status: authorised

B-I-D: EpCAMAC

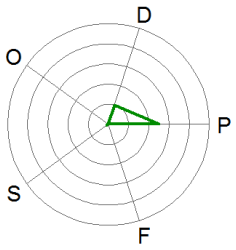
Main study

Supportive studies

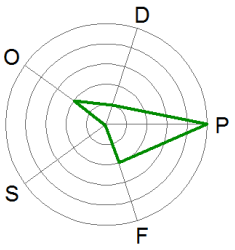
Heiss 2010



Burges 2007



Ruf 2010



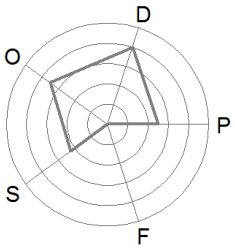
Biomarker: oestrogen receptor expression
Indication: breast neoplasms
Drug: fulvestrant
Status: authorised

B-I-D: ERBF

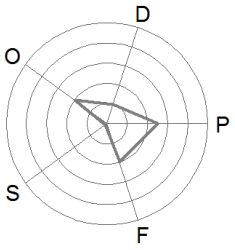
Main studies

Supportive studies

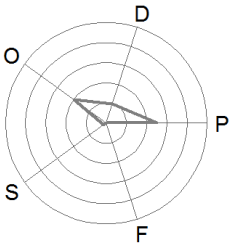
0020



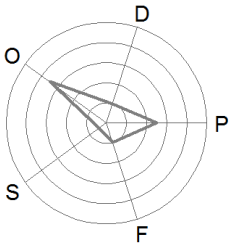
0004



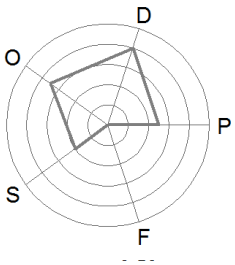
O-15-22



SAKK



0021

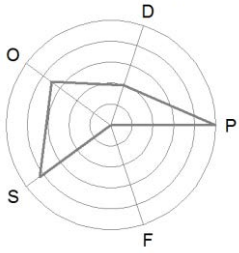


Biomarker: oestrogen receptor expression
Indication: breast neoplasms
Drug: toremifene
Status: authorised

B-I-D: ERBT

Main studies

Gershanovich 1997, Hayes 1995, Pyrhonen 1997



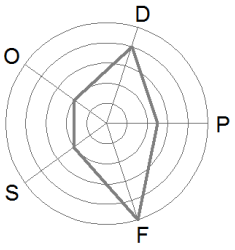
Only combined data from three main studies available for biomarker

Biomarker: genotype 1
Indication: chronic hepatitis C
Drug: bocepravir
Status: authorised

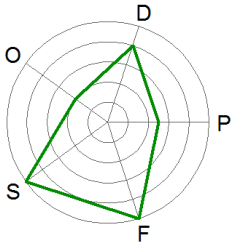
B-I-D: G1CB

Main studies

RESPOND-2

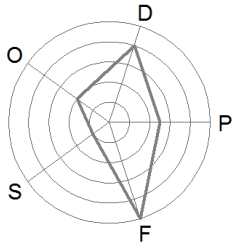


SPRINT-2



Supportive study

P05685

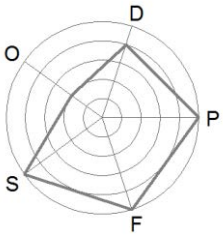


Biomarker: genotype 1
Indication: chronic hepatitis C
Drug: telaprevir
Status: authorised

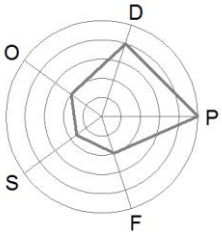
B-I-D: G1CT

Main studies

ADVANCE

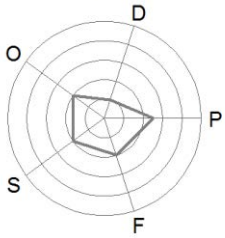


ILLUMINATE

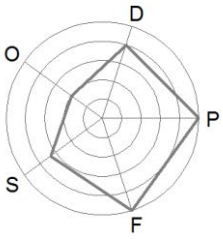


Supportive study

EXTEND



REALIZE

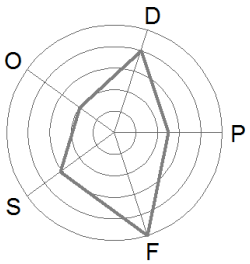


Biomarker: hormone dependency
Indication: prostatic neoplasms
Drug: degarelix
Status: authorised

B-I-D: HDDP

Main study

FE 200486 CS21

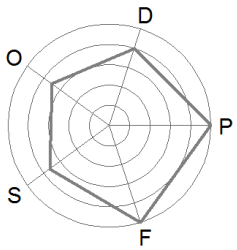


Biomarker: HER2 expression
Indication: breast neoplasms
Drug: everolimus
Status: authorised

B-I-D: HER2BE

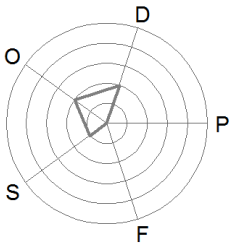
Main study

BOLERO-2



Supportive study

Baselga 2009

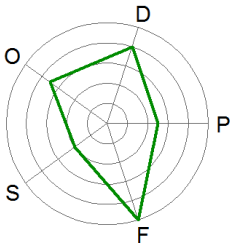


Biomarker: HER2 expression
Indication: breast neoplasms
Drug: lapatinib
Status: authorised

B-I-D: HER2BL

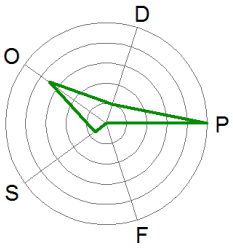
Main study

EGF100151

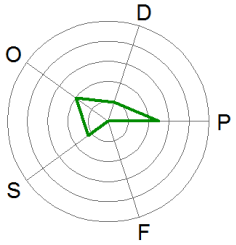


Supportive studies

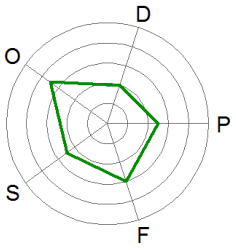
EGF103659 and French ATU



EGF105084



EGF30001



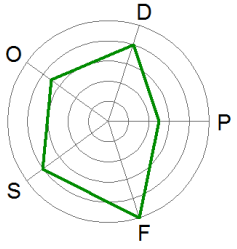
EGF103659 and French ATU were ongoing

Biomarker: HER2 expression
Indication: breast neoplasms
Drug: pertuzumab
Status: authorised

B-I-D: HER2BP

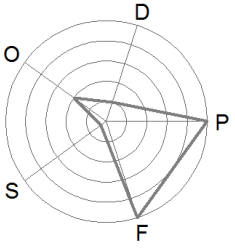
Main study

CLEOPATRA

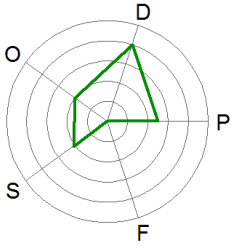


Supportive studies

Baselga 2010



NeoSphere

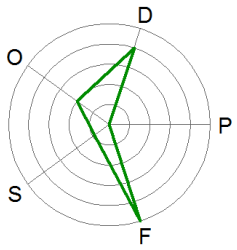


Biomarker: HER2 expression
Indication: breast neoplasms
Drug: trastuzumab
Status: authorised

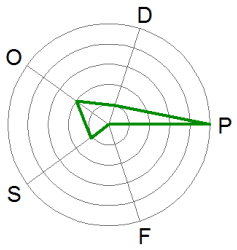
B-I-D: HER2BT

Unclear studies

H0648g



H0649g

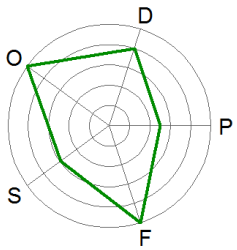


Biomarker: HER2 expression
Indication: stomach neoplasms
Drug: trastuzumab
Status: authorised

B-I-D: HER2ST

Main study

ToGA

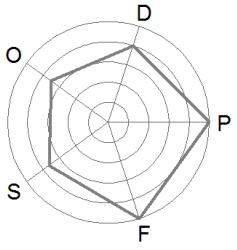


Biomarker: Hormone receptor expression
Indication: breast neoplasms
Drug: everolimus
Status: authorised

B-I-D: HRBE

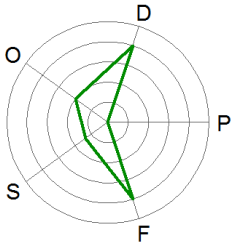
Main study

BOLERO-2



Supportive study

Baselga 2009

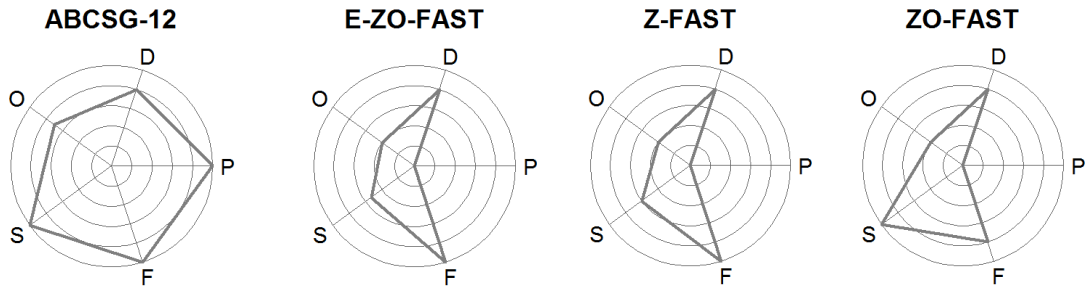


Biomarker: Hormone receptor expression
Indication: breast neoplasms
Drug: zoledronic acid
Status: pending => withdrawn (prior to refusal)

B-I-D: HRBZ

Main study

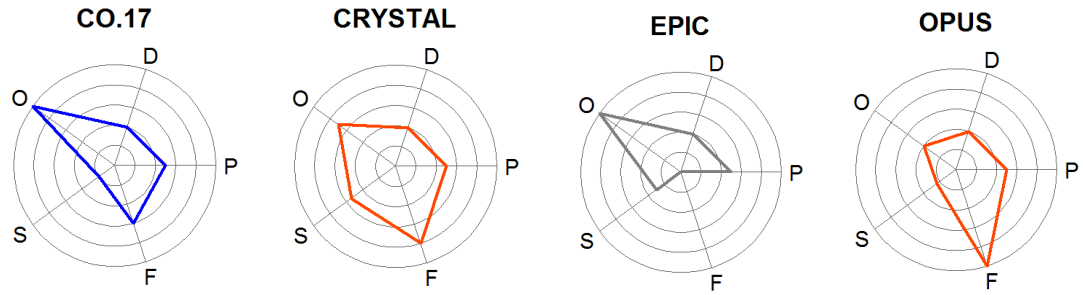
Supportive studies



Biomarker: KRAS mutation
Indication: colorectal cancer (metastatic)
Drug: cetuximab
Status: authorised

B-I-D: KRASCC

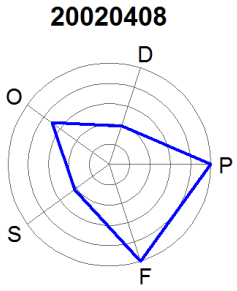
Unclear studies



Biomarker: KRAS mutation
Indication: colorectal cancer (metastatic)
Drug: panitumumab
Status: authorised

B-I-D: KRASCP

Main study

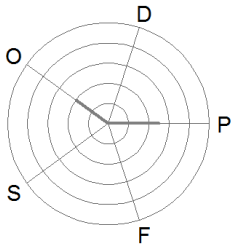


Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: amprenavir
Status: withdrawn

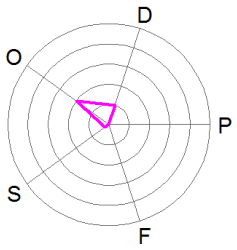
B-I-D: VRHAm

Main studies

PROAB3004



PROAB3006

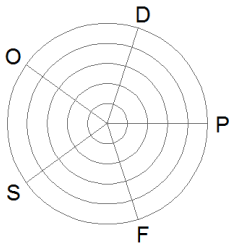


Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: atazanavir
Status: authorised

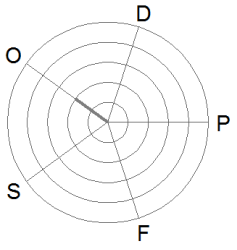
B-I-D: VRHAt

Main studies

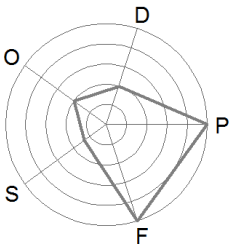
AI424009



AI424043



AI424045



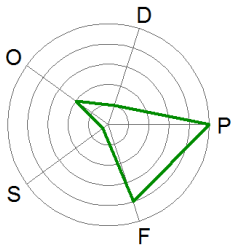
retrospective analysis in AI424045, as administration of treatment in many cases did not result in undetectable virus

Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: darunavir
Status: authorised

B-I-D: VRHD

Unclear studies

POWER 1, 2, 3

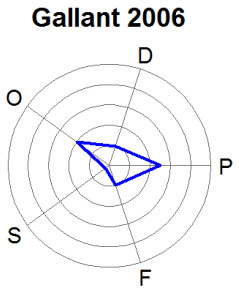


Information relevant to biomarker assessment was only provided for all three POWER studies together

Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: efavirenz / emtricitabine / tenofovir disoproxil
Status: authorised

B-I-D: VRHEET

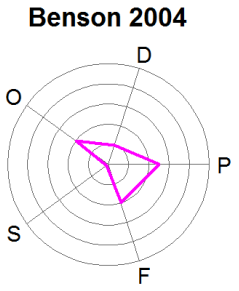
Main study



Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: emtricitabine
Status: authorised

B-I-D: VRHEm

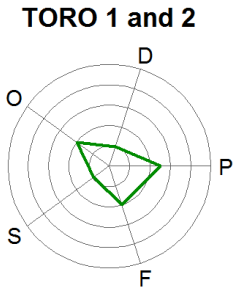
Main study



Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: enfuvirtide
Status: authorised

B-I-D: VRHEn

Main studies



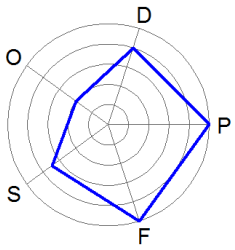
Information relevant to biomarker assessment was only provided for two TORO studies together

Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: emticitabine/ rilpivirine/ tenofovir
Status: authorised

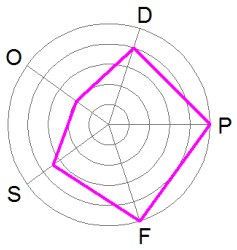
B-I-D: VRHERT

Main studies

ECHO



THRIVE

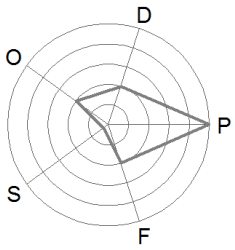


Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: fosamprenavir
Status: authorised

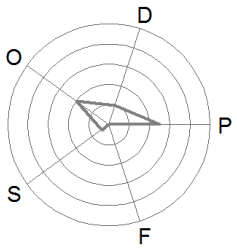
B-I-D: VRHF

Main studies

APV 30003

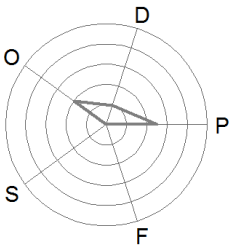


SOLO



Supportive study

NEAT



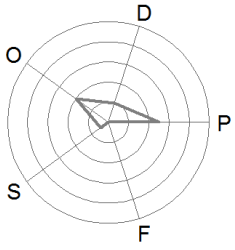
retrospective analysis in APV 30003, as administration of treatment in many cases did not result in undetectable virus

Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: lopinavir/ ritonavir
Status: authorised

B-I-D: VRHAmL

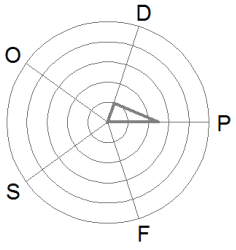
Main study

M98-863



Supportive study

M97-720

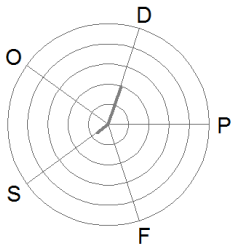


Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: nelfinavir
Status: withdrawn

B-I-D: VRHNel

Main studies

505 and 511



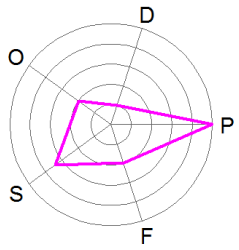
Information relevant to biomarker assessment was only provided for two studies together; retrospective analysis, as administration of nelfinavir in most cases did not result in undetectable virus

Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: rilpivirine
Status: authorised

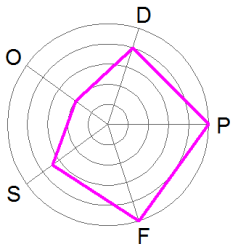
B-I-D: VRHR

Main studies

ECHO



THRIVE

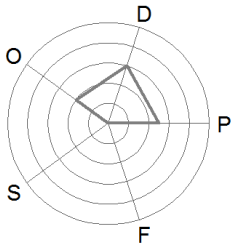


Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: tenofovir
Status: authorised

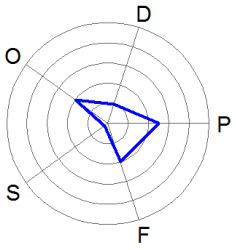
B-I-D: VRHTe

Main studies

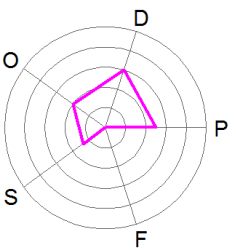
902



903



907



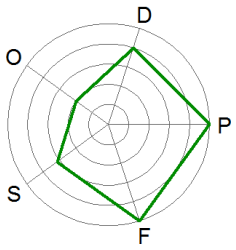
Biomarker: viral resistance mutations
Indication: HIV-1 infection
Drug: tipranavir
Status: authorised

B-I-D: VRHTr

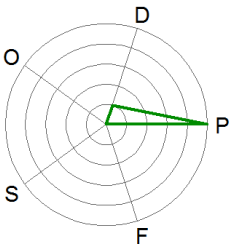
Main studies

Supportive studies

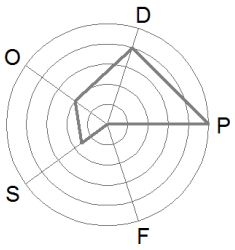
RESIST 1



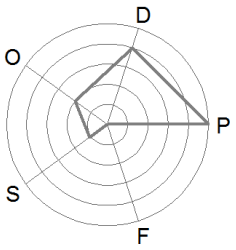
1182.4



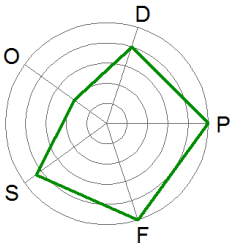
1182.51



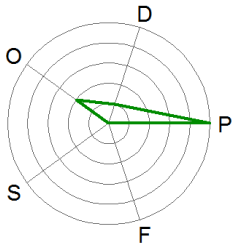
1182.52



RESIST 2



Markowitz 2007



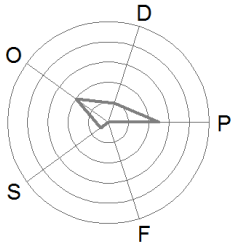
RESIST studies were ongoing

13.2. EFFECTIVENESS BIOMARKERS (ORPHAN DRUGS)

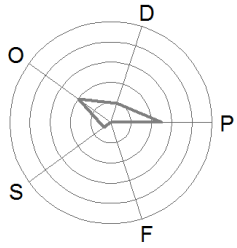
Biomarker: CD-33 B-I-D: CD33AG
Indication: AML (first relapse, not candidates for other intensive re-induction chemotherapy)
Drug: gemtuzumab ozogamicin
Status: refused

Main studies

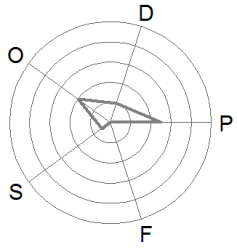
0903B1-202-EU



0903B1-201-US/CA



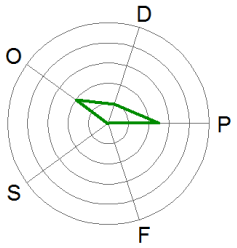
0903B1-203-US/EU



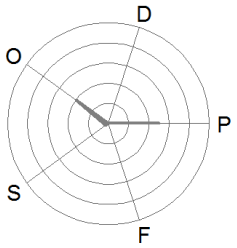
Biomarker: D816V mutation in c-Kit B-I-D: D816VSI
Indication: systemic mastocytosis (adults)
Drug: imatinib
Status: withdrawn prior to refusal

Unclear studies

Heinrich 2008



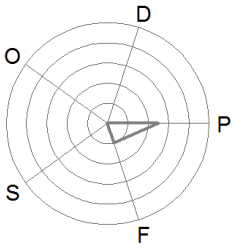
other studies



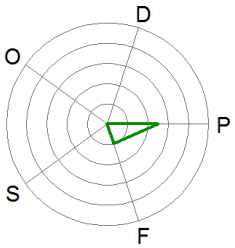
Biomarker: FIP1L1-**PDGFRa rearrangement** B-I-D: FIP1L1HI
Indication: HES/CEL
Drug: imatinib
Status: authorized

Unclear studies

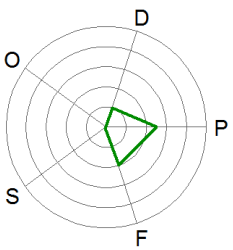
Cervetti 2005



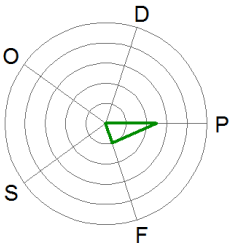
Chung 2006

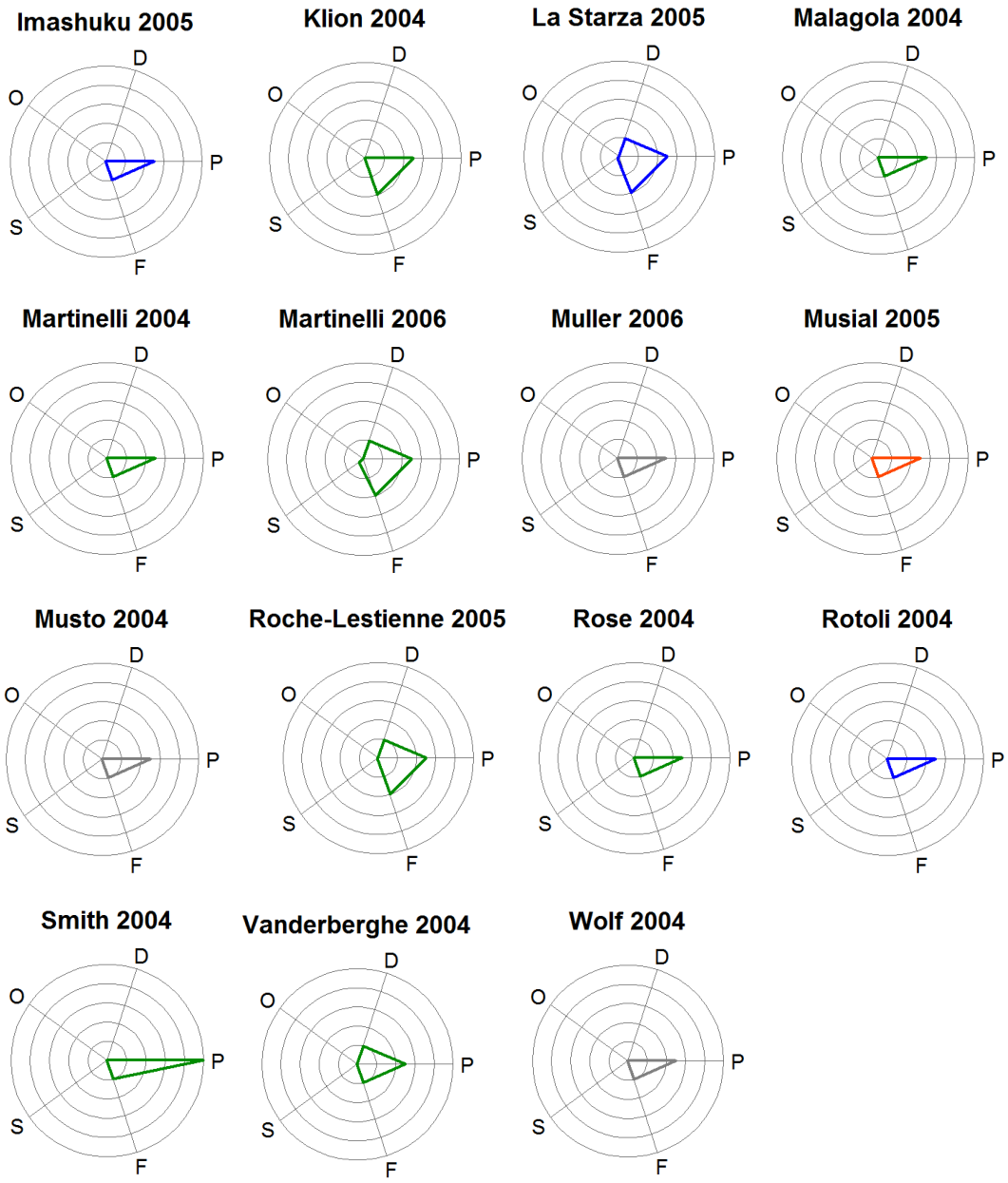


Cools 2003



Frickhofen 2004

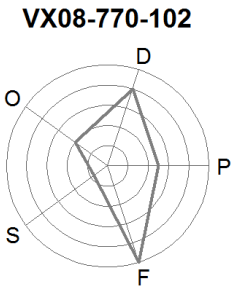


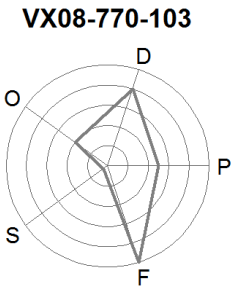

Imashuku 2005
Klion 2004
La Starza 2005
Malagola 2004
Martinelli 2004
Martinelli 2006
Muller 2006
Musial 2005
Musto 2004
Roche-Lestienne 2005
Rose 2004
Rotoli 2004
Smith 2004
Vanderberghe 2004
Wolf 2004
D
O
P
S
F

Biomarker: G551D mutation
Indication: cystic fibrosis
Drug: ivacaftor
Status: authorised

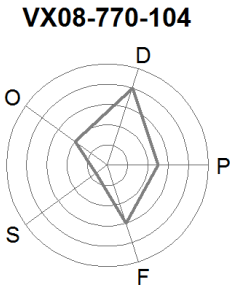
B-I-D: G55CI

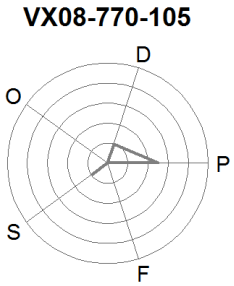
Main studies





Supportive studies



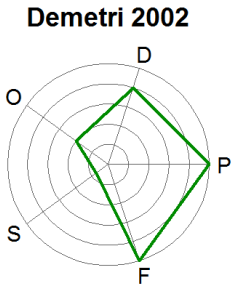


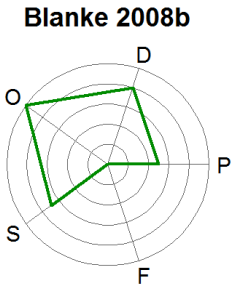
VX08-770-105 was ongoing

Biomarker: Kit (CD 117) mutation positive
Indication: gastrointestinal stromal tumors
Drug: imatinib
Status: authorised

B-I-D: KitGI

Unclear studies





Biomarker: LPL protein expression
Indication: hyperlipoproteinemia type I
Drug: alipogene tiparvovec
Status: authorized

B-I-D: LPLNH

N/A

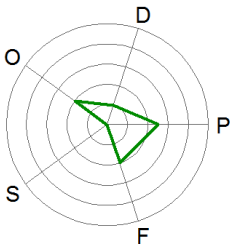
Biomarker: PDGFR gene re-arrangements
Indication: myelodysplastic-myeloproliferative disease
Drug: imatinib
Status: authorised

B-I-D: PDGFRMI

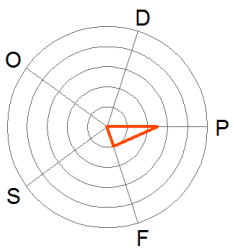
Main study

Supportive studies

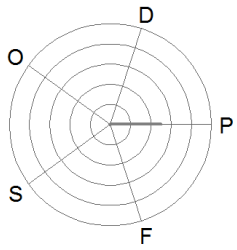
Heinrich 2008



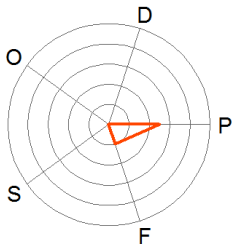
Apperley 2002



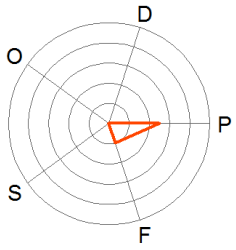
Cortes 2003



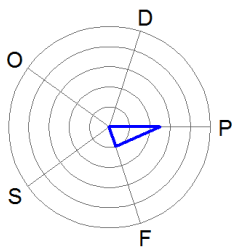
Garcia 2003



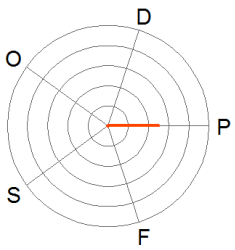
Grand 2004



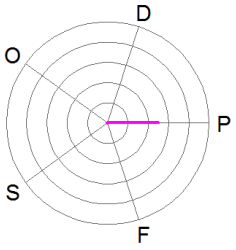
Levine 2005



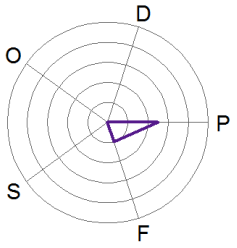
Magnusson 2002



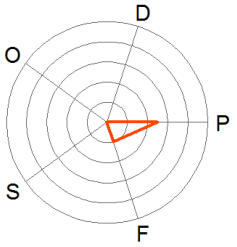
Pardanani 2002



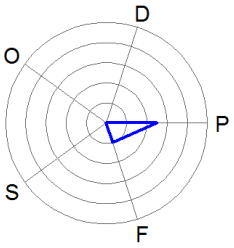
Pitini 2007



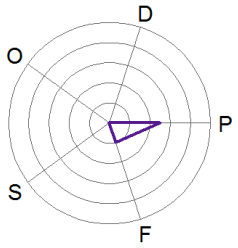
Safley 2004



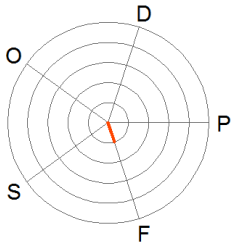
Trempat 2003



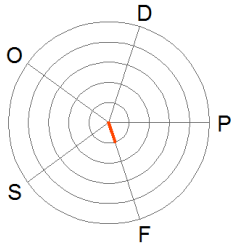
Vizmanos 2004



Wilkinson 2003



Wittman 2004

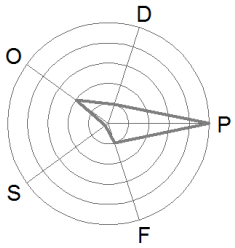


Biomarker: Philadelphia chromosome
Indication: precursor cell lymphoblastic leukemia-lymphoma
Drug: dasatinib
Status: authorised

B-I-D: PHPD

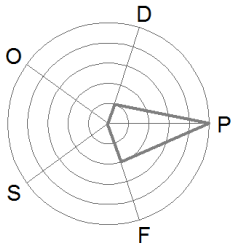
Main study

START-L



Supportive study

CA180002

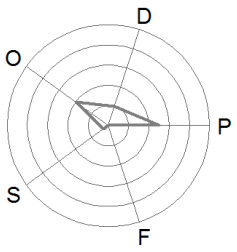


Biomarker: Philadelphia chromosome
Indication: precursor cell lymphoblastic leukemia-lymphoma
Drug: imatinib
Status: authorised

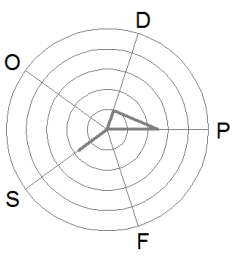
B-I-D: PHPI

Unclear studies

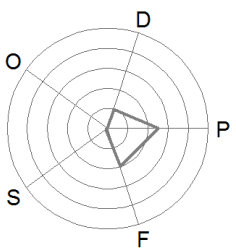
0109



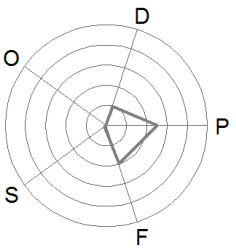
0114



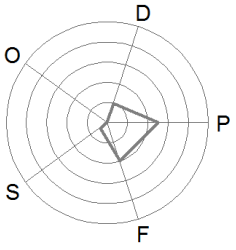
03001



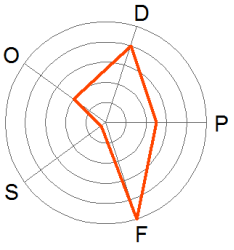
AAU02



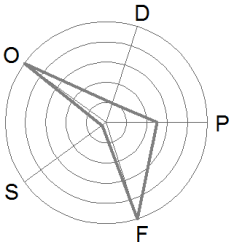
ADE04



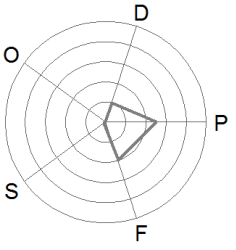
ADE10



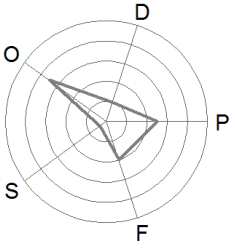
AFR09



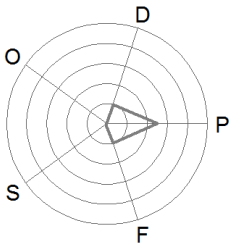
AIT04



AJP01



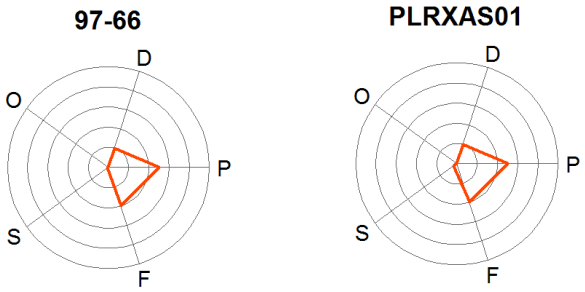
AUS01



Biomarker: t(15;17) translocation and/or PML/RAR-α gene
Indication: acute promyelocytic leukemia
Drug: arsenic trioxide
Status: authorised

B-I-D: t(15;19)AA

Main studies



13.3. SAFETY BIOMARKERS

Biomarker: DPD deficiency Indication: colorectal, colonic, stomach, breast neoplasms Drug: capecitabine Status: authorised	B-I-D: DPDCC

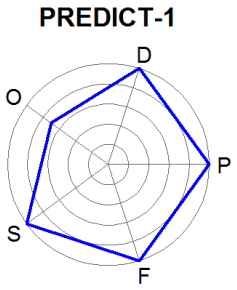
N/A

Biomarker: DPD deficiency Indication: stomach neoplasms Drug: tegafur/ gimeracil/ oteracil Status: authorized	B-I-D: DPDST

N/A

Biomarker: HLA-B*5701 allele Indication: HIV-1 infection Drug: abacavir Status: authorized	B-I-D: HLAHA

Unclear study



Biomarker: NADPH reductase deficiency Indication: Methaemoglobinaemia Drug: methylthioninum chloride Status: authorised	B-I-D: NMMc

N/A

APPENDIX 14. CRITICAL APPRAISAL OF TAJIK 2013 SYSTEMATIC REVIEW

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It was reported in the Introduction of this review that it is a *"systematic review of literature on trial designs for evaluating biomarkers for treatment selection."*[237] This scope, however, seemed to be inconsistent with the inclusion criteria in the Materials and Methods, which were looking at identification and validation of biomarkers: *"methodologic articles that described one or more trial designs for identification and/or validation of prognostic or predictive biomarkers for treatment selection"*[237]. It was not clear whether this paper used the terms "validation" and "evaluation" interchangeably. It was however judged that the findings of the review would be directly applicable to the questions addressed by this chapter.

1. *Was an 'a priori' design provided?*

It was not reported whether this review followed a pre-defined protocol. Elements of methodology used were reported.

2. *Was there duplicate paper selection and data extraction?*

Abstracts were screened by one reviewer, with a second reviewer checking 400 out of 2056 references (19.5%). This checking gave a 99% agreement. There was no information on how many of these were references missed by the reviewer assessing all abstracts. There was no information on whether full texts were assessed for inclusion by one or two reviewers. There was also no information on whether data extraction was carried out by one or two reviewers.

3. *Was a comprehensive literature search performed?*

Given the topic, the search strategy in MEDLINE/PubMed (reporting of database searched inconsistent) and EMBASE was relatively narrow. Although the paper states that the review question included both prognostic and predictive biomarkers, the search strategy does not include terms specific for prognostic biomarkers. It was also focused on genomic and genetic biomarkers and there are no terms for, as an example, proteomic or imaging biomarkers, although these would also be relevant.

It is not clear whether MEDLINE or PubMed was searched. The text of the paper and supplemental methods state that MEDLINE was searched (via PubMed), while Figure 1 in the paper reports that PubMed was searched.

In the MEDLINE/PubMed (?) search strategy, restriction of the MeSH term "Clinical Trials as Topic" to major headings can potentially lead to missing relevant papers. For example a paper describing a number of relevant trial designs[238] would have been identified if the

MEDLINE/PubMed searches using the term "Clinical Trials as Topic" were not limited to major headings. There are also other MeSH terms which could have been used in the searches, such as "Individualized Medicine" or "Tumour Markers, Biological".

Similar concerns apply to EMBASE. For example the index terms "clinical trial (topic)" or "personalized medicine" could have been used.

Searches in Cochrane Methodology Register and MathSciNet were relatively wide.

The searches were supplemented by checking citations in included papers.

It is therefore possible that some relevant papers that could have contributed additional information were missed.

4. Was the status of the publication used as an inclusion criterion?

The publication status was not an inclusion criterion. The searches were not limited to oncology and no language restrictions were applied. However the limitation of included papers to methodological papers potentially missed some methodology developed and published alongside a study, as for example relating to development of continuous signatures in clinical trials.[239]

5. Was a list of papers (included and excluded) provided?

Included papers were reported in Supplementary Table 1. A list of excluded papers was not provided, neither were detailed reasons for exclusion of papers.

6. Were the characteristics of the included papers provided?

The authors only provided the citations of the included papers. Some additional information could, for example, include what study designs were described within each paper.

7. Was the scientific quality of the included papers assessed and documented?

The assessment of scientific quality is not applicable here, as the papers describe different study methodologies.

8. Was the scientific quality of the included papers used appropriately in formulating conclusions?

Not applicable in this context.

9. Were the methods used to combine the information in the papers appropriate?

The framework used for summarising the different study designs was based on the "patient-intervention-comparator components of patient flow". As this review is looking at

trial designs for evaluation (and possibly identification) of biomarkers, it seems inappropriate that whether the biomarker was part of study hypothesis was not part of the framework. This is particularly evident in the category of "randomise-all" designs where trials designed to prospectively test a biomarker hypothesis (with randomisation stratified by the biomarker and testing for biomarker by treatment interaction) have been grouped together with *post hoc* subgroup analysis or an adaptive signature design.

Another issue here is that although according to the methods of this review study designs for different stages of biomarker development were included, there was no recognition of this fact in the framework proposed. There was no indication of whether the identified designs are suitable for biomarker identification, evaluation/validation, or both.

Also, since the searches were narrow and did not identify all relevant papers, selecting the label used for each category based on vote-counting may not be representative of the most frequent practice.

There is no information on what the authors planned to do in cases of disagreement on a certain issue between different methodological papers.

10. Was the likelihood of publication bias assessed?

No, however this issue is probably not applicable here, unless it is considered that for example certain arguments or opinions are less likely to be published.

11. Was the conflict of interest stated?

It was stated that "no potential conflicts of interests were disclosed".

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APPENDIX 15. STUDY DATA FROM EMA REVIEW MATCHED TO THE TAJIK 2013 FRAMEWORK

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Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
ALK mutation – NSCLC – crizotinib					✓				✓									
A8081001	*Crizotinib is well tolerated with rapid, durable responses in patients with ALK-positive NSCLC. There seems to be potential for ongoing benefit after initial disease progression in this population, but a more formal definition of ongoing benefit in this context is needed.*[46]	Q1	single-arm study	single-arm (only positive)	✓													
A8081005	NA	NA	NA	single-arm (only positive)	✓													
A8081007	NA	NA	NA	enrichment					✓									
BRAF V600 mutation – melanoma - vemurafenib					✓				✓									
BRIM 3	*Vemurafenib produced improved rates of overall and progression-free survival in patients with previously untreated melanoma with the BRAF V600E mutation.*[52]	Q1	randomized clinical trial	enrichment					✓									
NP22657	NA	NA	NA	single-arm (only positive)	✓													
CCR5 tropism – HIV – maraviroc									✓	✓								
MOTIVATE 1	*Maraviroc, as compared with placebo, resulted in significantly greater suppression of HIV-1 and greater increases in CD4 cell counts at 48 weeks in previously treated patients with R5 HIV-1 who were receiving OBT [optimised background therapy].*[56§§§§§§§§§§§§§§§]	Q1	randomized, double-blind, placebo-controlled study	enrichment					✓									
MOTIVATE 2			randomized, double-blind, placebo-controlled study	enrichment					✓									

§§§§§§§§§§§§§§§ Both studies reported in one paper

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Saag 2009	*In this exploratory study involving extensively treatment-experienced patients with advanced, non-R5 HIV-1 infection, neither superiority nor noninferiority was statistically demonstrated for either maraviroc dosage compared with placebo at 24 weeks of treatment.*[57]	Q2	randomized, placebo-controlled, double-blind trial	enrichment (biomarker negative)						✓							
CD-33 expression – AML – gemtuzumab ozogamicin					✓												
0903B1-201-US/CA	*In conclusion, GO [gemtuzumab ozogamicin], which is an antibody-targeted chemotherapy, provides an effective and relatively well tolerated treatment option for patients with CD33-positive AML in recurrence.*[58]**********	A1	open-label, single-arm study	single-arm (only positive)	✓												
0903B1-202-EU			open-label, single-arm study	single-arm (only positive)	✓												
0903B1-203-US/EU			open-label, single-arm study	single-arm (only positive)	✓												
DPD deficiency – Colorectal, colonic, stomach and breast neoplasms – capecitabine																	
None	N/A	N/A	N/A	N/A													
DPD deficiency – stomach neoplasms - tegafur / gimeracil / oteracil																	
None	N/A	N/A	N/A	N/A													

********** All three studies reported in one paper

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
EGFR expression - Colorectal Neoplasms – cetuximab					✓				✓									
BOND	*there was no apparent relationship between the efficacy of cetuximab and the level of EGFR in the tumor (…) Only patients with immunohistochemical evidence of EGFR expression were included in our study, and therefore whether patients without EGFR expression would benefit from cetuximab is unknown*[240]	Q1	open-label, randomized trial	enrichment					✓									
IMCL CP02-0141	NA	NA	NA	single-arm (only positive)	✓													
IMCL CP02-9923	NA	NA	NA	single-arm (only positive)	✓													
EGFR expression – NSCLC – cetuximab									✓	✓		✓	✓	✓	✓	✓	✓	✓
CA225099	"This trial did not show a significant difference in PFS"[69] "In conclusion, the results of this correlative study of cetuximab plus chemotherapy in NSCLC do not show that any of the biomarkers analyzed have a statistically significant effect on cetuximab benefit" [70]	Q3, Q6	open-label trial that enrolled patients regardless of EGFR expression (correlative analysis)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
FLEX	*The FLEX trial showed that overall survival is prolonged with the EGFR targeted antibody cetuximab added to chemotherapy in patients with advanced non-small-cell lung cancer (…) In conclusion, cetuximab added to platinum-based chemotherapy can be regarded as a new standard first-line treatment option for patients with EGFR-expressing advanced non-small-cell lung cancer."*[71]	Q1	open-label trial	enrichment					✓									
Rosell 2007	*to our knowledge, this is the first randomized trial demonstrating that combination treatment with cetuximab plus cisplatin/vinorelbine enhances the response rate and improves PFS and survival time with an acceptable safety profile compared with cisplatin/vinorelbine alone. To validate these results a large, randomized phase III trial of first-line Erbitux in lung cancer (FLEX) (…) is underway in patients with EGFR-expressing stage IIIB or IV NSCLC.*[72]	Q1	open-label, randomized pilot study	enrichment					✓									
EGFR expression – NSCLC – erlotinib					✓				✓	✓		✓	✓	✓	✓	✓	✓	✓
BR.21	*In summary, multivariate analysis revealed that expression of EGFR and an increased number of copies of EGFR [measured by FISH], but not mutations in EGFR, were associated with responsiveness to erlotinib but not with increased survival. Our results suggest that mutational analysis is not necessary to identify patients in whom treatment with EGFR inhibitors is appropriate.*[76]	Q6	clinical trial (correlative studies)	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Perez-Soler 2004	*In conclusion, this study indicates that erlotinib is an active and well-tolerated agent for the treatment of relapsing NSCLC and supports the continued clinical development of this promising agent*[241]	A1	single-arm, open-label study (Gehan two-stage design)	single-arm (only positive)	✓													
EGFR mutation – NSCLC – erlotinib					✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	
EURTAC	*The EURTAC results reinforce the feasibility of upfront genotyping of patients and the improved outcomes attained with therapy directed against a known target. Taken together with the findings of the OPTIMAL study, our results suggest a benefit in PFS with first-line erlotinib in a European population and confirm those improvements attained with EGFR targeted agents in Asian patients, thus strengthening the rationale for routine baseline tissue-based assessment of EGFR mutations in patients with NSCLC.*[83]	Q1	open-label randomised trial	enrichment					✓									
CALGB30406 ††††††††††††††	*Patients with NSCLC whose tumors harbour EGFR mutations derive the greatest degree of benefit from first-line EGFR TKI therapy (…). Improving PFS of patients with EGFR-mutant NSCLC treated with erlotinib remains a critical therapeutic challenge.*[80]	Q5	randomized trial	single arm	✓	✓		✓						✓				
Laskin 2009	*Clinical selection of pts enriches the EGFR mutation positive and KRAS mutation negative population and leads to high rates of non-progression*[82]	A1	clinical trial	single-arm (only positive)	✓													

†††††††††††††† Patients randomised to erlotinib monotherapy or erlotinib + chemotherapy

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
OPTIMAL	*The OPTIMAL study provides the first conclusive evidence that erlotinib provides superior overall response rate and progression-free survival versus platinum doublet chemotherapy as first-line treatment in Asian patients whose tumours harbour activating mutations of EGFR. The results of this study have practice-changing implications and provide justification for widespread implementation of routine EGFR mutation testing in advanced NSCLC.*[242]	Q1	open-label, randomised trial	enrichment					✓									
Paz-Ares 2010	*This extensive review of the literature has shown that NSCLC associated with EGFR mutations presents as a distinct disease that is dependent on hyperactivated EGFR for survival. Because of this, it is not surprising that blockade of EGFR TK activity appears to be the most effective treatment for this subgroup of NSCLC.*[243]	Q1	in-depth review of the published literature/ pooled analysis	literature review														
Rosell 2009	*We evaluated the feasibility of large-scale screening for EGFR mutations in such patients and analyzed the association between the mutations and the outcome of erlotinib treatment…* *In conclusion, screening for EGFR mutations is warranted in women with lung cancer, in those who have never smoked, and in those with nonsquamous tumors. Large-scale screening of patients for EGFR mutations, with subsequent customization of erlotinib, is feasible and improves the outcome*[79]	Q5	prospective study	single-arm (only positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
SATURN	*PFS benefit with erlotinib was noted in both EGFR mutation-positive and EGFR wild-type subgroups, with those with EGFR mutation-positive tumours obtaining the greatest benefit from erlotinib. Overall survival was also significantly longer with erlotinib than with placebo in the intention to treat population. The PFS benefit seen for patients with EGFR mutation-positive tumours did not translate into an equally impressive overall survival benefit, probably due to the high degree of censoring and the 67% cross-over rate to second-line EGFR TKI therapy in the placebo group for this population*[78]	Q1, Q2, Q3	randomised, placebo-controlled study (pre-planned analyses according to predefined candidate biomarkers)	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
EGFR mutation – NSCLC – gefitinib									✓	✓		✓	✓	✓	✓	✓	✓	✓
INTEREST	*We detected no difference in overall survival between gefitinib and docetaxel irrespective of a patient's EGFR protein expression, EGFR gene mutation, or K-Ras gene mutation status (treatment by biomarker interaction test was not significant for any biomarker*[86]	Q6	open-label, phase III trial (exploratory analyses in patient subgroups)	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
IPASS	*Our findings suggest that, whenever possible, EGFR-mutation status should be determined before the initial treatment of pulmonary adenocarcinoma (…) The presence of an EGFR mutation was a robust predictor of improved progression-free survival with gefitinib, as compared with carboplatin–paclitaxel, and of the benefit of gefitinib with respect to the objective response rate, indicating that patients in whom an EGFR mutation has been identified will benefit most from first-line therapy with gefitinib.*[89]	Q1, Q6	randomized, open-label, parallel-group study (planned subgroup analyses)	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
ISEL	*In agreement with previously published reports, patients with EGFR mutations had higher response rates with gefitinib, compared with patients without EGFR mutations.*[85]	Q5	placebo-controlled study (biomarker analysis)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
V-15-32	*Although the patient numbers were too small for firm conclusions, the biomarker data from this study suggest that EGFR mutation–positive or EGFR FISH–positive patients have a greater response to both gefitinib and docetaxel compared with EGFR mutation– or FISH–negative patients*[244]	Q4, Q5	randomized, open-label, postmarketing clinical study (preplanned subgroup analyses)	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
EpCAM expression – CancerAscites - Catumaxomab					✓				✓									
Heiss 2010	*In conclusion, treatment with four i.p.* [intraperitoneal] *doses of C + P* [catumaxomab + paracentesis] *demonstrated clinically relevant benefits in patients with recurrent malignant ascites due to carcinomas of different origin. Positive trends in OS together with its demonstrated efficacy against tumor cells in the peritoneal cavity support the antitumor activity of catumaxomab and suggest that it could be even more effective if used at an earlier stage in the treatment of epithelial cancers.* [91]	Q1	two-arm, randomized, open-label, study	enrichment					✓									
Baumann 2011	NR	N/A	N/A	none ‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡														
Burges 2007	*In conclusion, the i.p.* [intraperitoneal] *application of catumaxomab induced effective tumor cell destruction in malignant ascites, substantially decreased ascites accumulation, and reduced the necessity for paracentesis. Thus, i.p. infusion of catumaxomab represents a targeted tumor therapy within the peritoneal cavity associated with a substantial improvement of symptoms related to malignant ascites.*[92]	Q1	dose-escalating study	single-arm (only positive)	✓													

‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡ Biomarker measurement not reported; partly based on the fact that a high proportion of ovarian tumours are EpCAM+ (prevalence of biomarker positive patients in patient population)

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Oestrogen receptor expression – breast neoplasms – fulversant					✓				✓									
0020	*Fulvestrant was tolerated well and was at least as effective as anastrozole in the second-line treatment of patients with ABC. This new hormonaltherapy may provide a valuable treatment option for ABC in postmenopausal women.*[96§§§§§§§§§§§§§§§§]	Q1	open-label, randomized, parallel-group trial	enrichment					✓									
0021			double blind, double-dummy, randomized, parallel-group trial	enrichment					✓									
0004	NA	NA	NA	single-arm (only positive)	✓													
O-15-22	NA	NA	NA	single-arm (only positive)	✓													
SAKK	*In conclusion, by inducing a CB* [clinical benefit] *in 30% of patients with hormone receptor-positive tumors having received prior steroidal and nonsteroidal AI* [aromatase inhibitor] *and most of them having also been exposed to tamoxifen, fulvestrant emerges as an interesting and potentially important player in the sequential endocrine treatment of ABC* [advanced breast cancer].[98]	A1	open noncomparative study	single-arm (only positive)	✓													

§§§§§§§§§§§§§§§§ Both studies reported in one paper

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Oestrogen receptor expression – breast neoplasms – toremifene									✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gershanovich 1997	*Toremifene 60 mg daily is safe and effective treatment for postmenopausal women with advanced ER* [oestrogen receptor] *positive or ER unknown breast cancer.*[100]	Q1, A5	randomized, open label study (NR)	randomise-all (prospective subgroup)					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hayes 1995	*response rates, times to progression, and overall survival for patients on each arm were superior for ER* [oestrogen receptor] *-positive patients when compared with those whose tumors were ER-negative (…) In this study, we have demonstrated that TOR* [toremifene] *has similar efficacy and toxicities as those of its parent compound in postmenopausal patients with metastatic, hormone receptor-positive (or -unknown) breast cancer.*[245]	Q1, Q2, A5	randomized three-arm comparison of tamoxifen and two doses of toremifene (NR)	randomise-all (prospective subgroup)					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pyrhonen 1997	*the results of this double blind trial suggest that TOR* [toremifene] *(60 mg day) and TAM* [tamoxifen] *(40 mg day) are equally effective in the treatment of advanced ER* [oestrogen receptor] *-positive or ER-unknown breast cancer in post-menopausal patients*[102]	Q1, A5	double-blind, parallel group, randomized clinical study (NR)	randomise-all (prospective subgroup)					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
FIP1L1-PDGFRα rearrangement – HES/CEL - imatinib					✓	✓		✓					✓	✓				
Cervetti 2005	*This experience sustains not only the clinical role of imatinib in HES patients, but also its molecular activity. Indeed, notwithstanding clinical beneficial activity of IFN-alpha in HES, any molecular remission has been reported with this treatment. The molecular response achieved by the second patient with imatinib would support the usefulness of a molecular target-tailored therapy even in this haematological disorder.*[104]	Q1, A1	a case	case report (biomarker positive)	✓													
Chung 2006	*Imatinib selectively inhibits ABL, PDGFR and KIT tyrosine kinases. FIP1L1-PDGFRA is particularly sensitive to imatinib and a low initial dose is therefore appropriate. The FIP1L1-PDGFRA syndrome may lead to death, either from end-organ (particularly cardiac) damage or from transformation to acute leukaemia. The striking response to imatinib therapy means that its correct identification and treatment is of critical importance to the patient.*[105]	Q1, A1	case report	case report (biomarker positive)	✓													
Cools 2003	*demonstrates that FIP1L1-PDGFRA is the therapeutic target of imatinib in the hypereosinophilic syndrome*[246]	A1	study	non-RCT (positive and negative; two different treatments each)	✓	✓		✓					✓	✓				

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Frickhofen 2004	*In summary, these findings demonstrate that all patients with persistent eosinophilia and a diagnosis of HES or CEL should be tested for fusion genes involving PDGFR and FGFR1. Imatinib will control most diseases with rearranged PDGFR-A or PDGFR-B genes*[106]	Q1	case report	case report (biomarker positive)	✓													
Imashuku 2005	*Here we have described the case of an HES patient with increased numbers of mast cells in his bone marrow and CSS-like features who responded well to imatinib therapy, regardless of the negative molecular and cytogenetic findings. The molecular mechanisms involving genes such as PDGFRA, PDGFRB, c-KIT, and perhaps even unidentified kinases involved in the development of HES should be systematically characterized in order to further clarify the heterogeneity of HES and to identify imatinib-sensitive cases.*[107]	A2	case report	case report (biomarker negative)		✓												
Klion 2004	*The FIP1L1-PDGFRA fusion tyrosine kinase is over 100 times more sensitive to imatinib mesylate than is bcr-abl (...), explaining the dramatic clinical and hematologic response in HES to imatinib mesylate doses as low as 100 mg weekly.*[108]	Q1, A1	cohort of patients	case series (all positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
La Starza 2005	*Finally, five of 16 patients with HES who were FIP1L1/PDGFRA-negative underwent imatinib mesylate treatment. Two patients (...) achieved hematologic remission with peripheral eosinophil count normalization, confirming previous observations of succesful imatinib therapy in a subgroup of FIP1L1/PDGFRA-negative CEL.*[109]	Q1	study on retrospective cases	non-RCT (positive and negative; two different treatments)	✓	✓		✓					✓	✓				
Malagola 2004	*This case report confirms that imatinib is highly effective in cases of CEL, carrying the rearrangements of FIP1L1-PDGFR-alpha. Assessing the long term benefit of the treatment and the possibility of eradicating the mutated clone will require a much longer follow up.*[110]	Q1	case report	case report (biomarker positive)	✓													
Martinelli 2004	*we believe that imatinib treatment might be curative*[111]	A1	case report	case report (biomarker positive)	✓													
Martinelli 2006	*In conclusion, we have confirmed that imatinib is the treatment of choice for patients with FIP1L1-PDGFRα-rearranged HES/CEL, since almost all patients achieve and maintain complete hematologic, clinical and molecular remissions, chronic treatment is well tolerated, and responses are stable over time at doses as low as 100 mg daily.*[247]	Q1	prospective study	single-arm	✓	✓		✓						✓				

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Muller 2006	*Particularly exceptional is the activity of FIP1L1–PDGFRA as the first gain-of-function gene. Its tyrosine kinase receptor activity displays the molecular basis for efficiency of treatment with the inhibitor of this receptor family, imatinib mesylate. Approximately two thirds (in our cumulative data, 31/48 [65%]) of patients with HES express the fusion gene, with mostly very good responses to the specific therapy. However, also FIP1L1–PDGFRA negative patients have often been reported to respond favourably to treatment with imatinib mesylate."*[113]	A1, A2	case report	case report (biomarker positive and negative)	✓	✓												
Musial 2005	*In HES with FIP1L1-PDGFRA fusion gene, imatinib offers an advantage beyond symptomatic, molecular and laboratory improvement. Our observation provides evidence for the postulated organ function improvement with imatinib therapy*[114]	Q1	NR	case report (biomarker positive)	✓													
Musto 2004	*NA*	NA	NA	case report (biomarker positive and unknown)	✓		✓											

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Roche-Lestienne 2005	*In the present study, clinical improvement and complete hematological remission under imatinib was observed in all six F/P* [FIP1L1-PDGFRA] *patients, confirming previous results. By contrast, only one of the five treated F/P-negative patients (no. 16) responded to imatinib, which may be indicative of a deregulated TK activity in this patient, although this remains putative.*[115]	Q1	molecular characterization	non-RCT (positive and negative; two different treatments)	✓	✓		✓					✓	✓				
Rose 2004	*Analysis of the FIP1L1-PDGFRA fusion transcript appears very helpful in HES, in order to identify patients with FIP1L1-PDGFRA fusion transcripts with a high probability of responding to Imatinib. Although some HES patients without FIP1LPDGFRA transcript may respond to imatinib.*[248]	A1	case report	case report (biomarker positive)	✓													
Rotoli 2004	*NA*	NA	NA	case report (biomarker positive)	✓													
Smith 2004	*In conclusion, cytogenetic and molecular genetic analyses are probably indicated in all patients who meet the criteria for idiopathic HES, especially since they may define defects that result in activation of molecular pathways for which we now have inhibitors.*[116]	A1	case report	case report (biomarker positive and negative)	✓	✓												

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Vanderberghe 2004	[in patients with FIP1L1-PDGFRα rearrangement] *The gratifying clinical, hematological and molecular responses with imatinib in this setting are unprecedented, but more cases and longer follow-up will be required to resolve definitively whether imatinib can induce long-term molecular remission in this disease."*[117]	A1	case series	case series (positive and negative; two different treatments)	✓	✓												
Wolf 2004	*NA*	NA	NA	case report (biomarker negative)		✓												
genotype 1 - HCV **–** boceprevir									✓									
RESPOND-2	*Our data show that the addition of boceprevir to peginterferon–ribavirin therapy leads to high rates of sustained virologic response among patients in whom prior treatment had failed.*[119]	Q1	NA	enrichment					✓									
SPRINT-2	*As compared with peginterferon alfa-2b–ribavirin therapy alone, the addition of boceprevir significantly increased the rate of a sustained virologic response among previously untreated black and nonblack patients infected with HCV genotype 1, including those with a decrease of less than 1 log10 IU per milliliter in the HCV RNA level at week 4. Among nonblack patients, the combination therapy with boceprevir was associated with a relative increase of approximately 70% in the rates of sustained virologic response over standard therapy.*[120]	Q1	randomized, placebo-controlled study	enrichment					✓									
P05685	NA	NA	NA	enrichment					✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
genotype 1 - HCV – telaprevir					✓				✓									
ADVANCE	*These results confirm earlier studies and showed a significant increase in the rate of sustained virologic response among patients with HCV genotype 1 infection who are treated with a regimen combining peginterferon alfa-2a and ribavirin with telaprevir for 12 or 8 weeks, followed by peginterferon–ribavirin alone, for a total of 24 or 48 weeks of therapy, as compared with a standard regimen of peginterferon – ribavirin alone for 48 weeks.*[123]	Q1	randomized, double-blind, placebo-controlled trial	enrichment					✓									
ILLUMINATE	*We found that a 24-week treatment regimen of peginterferon–ribavirin, with telaprevir added for the first 12 weeks, was noninferior to a 48-week regimen of peginterferon–ribavirin, with telaprevir added for the first 12 weeks in patients with chronic infection with HCV genotype 1 who have not received treatment previously and who had an extended rapid virologic response.*[124]	Q1	randomized study	enrichment					✓									
REALIZE	*Telaprevir in combination with peginterferon alfa-2a plus ribavirin significantly improved the rates of sustained virologic response for patients who had received previous therapy for HCV infection.*[125]	Q1	randomized, double-blind, placebo-controlled study	enrichment					✓									
EXTEND	*NA*	NA	NA	single-arm (only positive)	✓													
G551D mutation – cystic fibrosis – ivacaftor					✓				✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
VX08-770-102	*In this randomized, placebo-controlled trial, administration of ivacaftor, an oral CFTR potentiator, was associated with significant improvements in primary and secondary end points in persons with cystic fibrosis who had at least one copy of the G551D-CFTR mutation*[249]	Q1	randomized, double-blind, placebo-controlled trial	enrichment					✓									
VX08-770-103	*NA*	NA	NA	enrichment					✓									
VX08-770-104	NA	NA	NA	enrichment					✓									
VX08-770-105	NA	NA	NA	single-arm (only positive)	✓													
HER2 expression********** – breast cancer - everolimus									✓	✓		✓	✓	✓	✓	✓	✓	✓
BOLERO-2	*Everolimus combined with an aromatase inhibitor improved progression-free survival in patients with hormone-receptor–positive advanced breast cancer previously treated with nonsteroidal aromatase inhibitors.*[133]	Q1	randomized trial	enrichment					✓									
Baselga 2009	*NR*[250]	N/A	randomized, double-blind, placebo-controlled trial (NR)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
HER2 expression – breast cancer - lapatinib					✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
EGF100151	*Overall, these updated analyses continue to support the clinical benefit and safety of lapatinib in patients with HER-2+ MBC [metastatic breast cancer].*[136]	Q1	randomized trial	enrichment					✓									

********** HER2 expression negative is the target population – for consistency reported as positive in table

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
EGF103659 and French ATU	*NA*	NA	NA	single-arm (only positive)	✓													
EGF105084	*lapatinib is associated with regressions of CNS metastases in patients who have progressed despite trastuzumab and radiotherapy. Additional activity was seen when capecitabine was added to lapatinib.*[137]	A1	open-label study	single-arm (only positive)	✓													
EGF30001	*This study demonstrated that the primary activity of lapatinib in breast cancer patients is mediated through HER-2 inhibition. Other than a higher response rate (influenced by the HER-2–positive subset), no clinically relevant antitumor activity was demonstrated when lapatinib was used in the heterogeneous population of patients with advanced breast cancer with locally defined HER-2–negative or HER-2–untested tumors. Conversely, in a preplanned, blinded, subset analysis of patients with centrally defined HER-2–positive tumors, lapatinib plus paclitaxel resulted in a clinically significant 11-week increase in median TTP as well as significant increases in ORR, CBR, and EFS. Although the data are not yet mature and differences did not achieve statistical significance, median OS was longer in patients receiving lapatinib. Therefore, this combination seems to be active as first-line therapy for HER-2–positive breast cancer.*[138]	Q1, Q2, A5	randomized, double-blind, placebo-controlled trial (preplanned, blinded, subset analysis)	randomise-all (prospective subgroup)					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
HER2 expression – breast cancer – pertuzumab					✓				✓									
CLEOPATRA	*We found that the combination of the anti-HER2 monoclonal antibodies pertuzumab and trastuzumab with docetaxel as first-line therapy prolonged progression-free survival in patients with HER2-positive metastatic breast cancer.*[141]	Q1	randomized, double-blind, placebo controlled trial	enrichment					✓									
Baselga 2010	*The combination of pertuzumab and trastuzumab is well tolerated and shows encouraging results in patients with HER2-positive breast cancer with documented progression on trastuzumab as prior therapy. The observed AEs [adverse events] were generally mild or moderate*[149]	A1	open-label, single-arm, Simon two-stage study	single-arm (only positive)	✓													
NeoSphere	*Data obtained from NeoSphere supported the conduct and informed the design of an ongoing adjuvant trial with pertuzumab (NCT01358877), and illustrated the potential of the neoadjuvant approach in new drug development.*[251]	Q1	randomised, multicentre, open-label study	enrichment					✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
HER2 expression – breast cancer – trastuzumab					✓				✓									
H0648g	*The results of this phase 3 clinical trial indicate that trastuzumab, when added to conventional chemotherapy, can benefit patients with metastatic breast cancer that overexpresses HER2. As compared with the best available standard chemotherapy, concurrent treatment with trastuzumab and first-line chemotherapy was associated with a significantly longer time to disease progression, a higher rate of response, a longer duration of response, and improved overall survival. If confirmed in additional studies of patients with HER2-positive metastatic breast cancer, our results may affect treatment of this disease.*[27]	Q1	clinical trial	enrichment					✓									
H0649g	*In summary, this study supports the use of rhuMAb HER2 [trastuzumab] for women with HER2-overexpressing metastatic breast cancer. The benefits of this therapy, durable objective responses, and favorable toxicity profile indicate that rhuMAb HER2 is an important new treatment option for women who have tumors that overexpress HER2.*[252]	Q1	NA	single-arm (only positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
HER2 expression – stomach cancer – trastuzumab									✓									
ToGA	*In patients with advanced gastric or gastro-oesophageal junction cancer, addition of trastuzumab to chemotherapy significantly improved overall survival compared with chemotherapy alone. Furthermore, an exploratory, post-hoc analysis showed that trastuzumab plus chemotherapy substantially improved overall survival in patients with high expression of HER2 protein (immunohistochemistry 2+ and FISH positive or immunohistochemistry 3+) compared with patients with low expression of HER2 protein (immunohistochemistry 0 or 1+ and FISH positive).*[253]	Q1, Q5	open-label, randomised controlled trial	enrichment					✓									
HLA-B*5701 allele – HIV - abacavir††††††††††††††††													✓			✓		✓
PREDICT-1	*The results of the PREDICT-1 study show that prospective HLA-B*5701 screening can reduce the incidence of hypersensitivity reaction to abacavir. (…) Prospective HLA-B*5701 screening, as shown in the PREDICT-1 study, may therefore be broadly useful, although the cost-effectiveness of the test will depend on several estimates that vary among populations and health care settings as well as the availability of appropriate laboratory assays.*[148]	Q7	double-blind, prospective, randomized study	biomarker-strategy with biomarker measurement in the control arm (modified) ‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡					× §§§				✓	×		✓		✓

†††††††††††††††† Prediction of adverse events

‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡ Biomarker negative patients (carriers of HLA-B*5701 allele) were excluded from the strategy arm

§§§§§§§§§§§§§§§§ × indicates the study did not address this question due to design modification from that in the framework

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
hormone dependent – prostate cancer - degarelix																		
FE 200486 CS21	NA	NA	NA	unclear														
hormone receptor expression – breast cancer – everolimus									✓									
BOLERO-2	*we report a phase 3 trial in patients with HR [hormone receptor]-positive advanced breast cancer showing that the addition of everolimus to endocrine therapy results in an improved clinical outcome*[133]	Q1	randomized trial	enrichment					✓									
Baselga 2009	*this study showed that everolimus significantly increased the efficacy of letrozole in the treatment of newly diagnosed, ER [oestrogen receptor]-positive breast cancer in terms of both clinical and antiproliferative response.*[250]	Q1	randomized, double-blind, placebo-controlled trial	enrichment					✓									
hormone receptor expression – breast cancer – zoledronic acid					✓				✓									
ABCSG-12	*On the basis of the results of this study, combination of zoledronic acid with adjuvant endocrine therapy (ovarian suppression plus tamoxifen) should be considered for premenopausal women with low-or-moderate-risk, early-stage, hormone-receptor positive breast cancer*[254]	Q1	randomised, controlled, open-label, two-by-two factorial, trial	enrichment					✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
E-ZO-FAST	*These 12-month E-ZO-FAST results add to the considerable clinical experience of letrozole-ZOL combination therapy in the adjuvant BC setting. Administering ZOL immediately with adjuvant letrozole in postmenopausal women with BC protects and maintains BMD with an acceptable safety profile. Further insight on the role of ZOL in treating AIBL is expected from longer follow-up of patients from this (E-ZO-FAST) and its companion studies (Z-FAST and ZO-FAST).*[152]	Q1	open-label randomized study	enrichment					✓									
Z-FAST	*Zoledronic acid initiated with the beginning of aromatase inhibitor therapy substantially prevents bone loss at 36 months of therapy*[255]	Q1	open-label study, randomized	enrichment					✓									
ZO-FAST **********	*Longer follow-up is needed to determine whether the bone loss observed in the delayed group can be stabilized or restored to baseline values with the subsequent administration of zoledronic acid. The 2-year, 3-year, and 5-year results of this trial and other ongoing clinical trials are necessary to further assess the impact on BMD loss and fracture rates in patients with early stage breast cancer who are receiving long-term adjuvant AIs.*[151]	Q1	open-label, randomized study	Single-arm	✓													

********** RCT comparing two different strategies using the same drug

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Kit (CD 117) mutation – gastrointestinal stromal tumours – imatinib					✓													
Blanke 2008b ††††††††††††††††	*This trial confirms the effectiveness of imatinib as primary systemic therapy for patients with incurable GIST but did not show any advantage to higher dose treatment. It appears reasonable to initiate therapy with 400 mg daily and to consider dose escalation on progression of disease.*[155]	Q1	Randomized open-label clinical trial	Single-arm	✓													
Demetri 2002 ‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡	*Imatinib induced a sustained objective response in more than half of patients with an advanced unresectable or metastatic gastrointestinal stromal tumor. Inhibition of the KIT signal-transduction pathway is a promising treatment for advanced gastrointestinal stromal tumors, which resist conventional chemotherapy.*[256]	A1	open-label, randomized trial	Single-arm	✓													

†††††††††††††††† RCT comparing two different strategies using the same drug

‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡‡ RCT comparing two different strategies using the same drug

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Kit (D816V) mutation – systemic mastocytosis – imatinib					✓									✓				
Heinrich 2008	*In this study, one patient with systemic mastocytosis had a favorable response to imatinib and was found to have a novel imatinib-sensitive KIT mutation (D816T). This result is consistent with other reports of favorable responses of systemic mastocytosis patients with imatinib-sensitive kinase mutations to imatinib treatment. Therefore, molecular characterization of systemic mastocytosis cases may be useful in identifying patients for treatment with imatinib or other KIT kinase inhibitors.*[61]	A1	single-arm/ open-label exploratory study	single-arm	✓									✓				
other studies	*NA*	NA	NA	collection of patient data														

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
KRAS mutation§§§§§§§§§§§§§§§§§§ - colorectal cancer - cetuximab									✓	✓		✓	✓	✓	✓	✓	✓	✓
CO.17	*Our findings show that the mutation status of the K-ras gene is associated with overall survival among patients with advanced colorectal cancer who are being treated with cetuximab after previous chemotherapy has failed. Treatment with cetuximab as compared with supportive care alone was associated with almost a doubling of the median overall and progression-free survival among patients with wild-type K-ras tumors. There was no significant survival benefit from cetuximab, however, among patients with tumors that had K-ras mutations. (…) Our analysis identified a biomarker that would effectively exclude a clinically significant proportion of patients with colorectal cancer — those with tumors bearing K-ras mutations (42%) — from receipt of a therapy offering little prospect of a benefit. Nevertheless, there were also patients with wild-type K-ras tumors who did not have a response to cetuximab and in whom the tumor rapidly progressed. Additional reliable and easily measured biomarkers are clearly needed to improve the identification of patients who will benefit from treatment with cetuximab.*[158]	Q1, Q2, Q5, Q6	randomized trial (correlative analyses)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓

§§§§§§§§§§§§§§§§§§ KRAS wild type as biomarker positive

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
CRYSTAL	*This trial provides confirmation that, as compared with FOLFIRI alone, cetuximab plus FOLFIRI reduces the risk of progression of metastatic colorectal cancer when used as the first-line treatment and that this benefit is seen mainly in patients with wild-type–KRAS tumors.*[161]	Q1	randomized, open-label, multicentre study (retrospective subgroup analysis)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
EPIC	NA	NA	NA	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
OPUS	*These results confirm the efficacy of cetuximab plus FOLFOX-4 in the first-line treatment of patients with KRAS wild-type mCRC and confirm KRAS mutation status as an effective predictive biomarker.*[163]	Q1, Q6	randomized study (biomarker analysis)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
KRAS mutation********** - colorectal cancer - panitumumab									✓	✓		✓	✓	✓	✓	✓	✓	✓
20020408	*Panitumumab monotherapy efficacy in mCRC is confined to patients with WT KRAS tumors. KRAS status should be considered in selecting patients with mCRC as candidates for panitumumab monotherapy.*[257]	Q1, Q6	randomized trial (biomarker analyses)	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
LPL protein expression - familial lipoprotein lipase deficiency - alipogene tiparvovec																		
None																		
NADPH reductase deficiency - acquired methaemoglobinaemia – methylthionium chloride																		
None																		

********** KRAS wild type as biomarker positive

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
PDGFR gene re-arrangements					✓	✓								✓				
Heinrich 2008	*imatinib treatment in this study was associated with favourable outcomes in patients with (…) myeloproliferative disorders, (…) adding to data showing the therapeutic efficacy of imatinib in tumour types with genomic mechanisms of activating imatinib sensitive tyrosine kinases*[61]	Q1	single-arm/ open-label exploratory study	single-arm	✓									✓				
Apperley 2002	*Imatinib mesylate induces durable responses in patients with chronic myeloproliferative diseases associated with activation of PDGFRB*[171]	A1	NA	single-arm (only positive)	✓													
Cortes 2003	*imatinib did not achieve a significant clinical response among patients with (…) atypical CML, or CMML without PDGFR fusion genes.*[172]	A2	study	single-arm (only negative)		✓												
Garcia 2003	*Our results demonstrate the efficiency of imatinib in the treatment of patients displaying the translocation involving H4 and PDGFBR genes. (…) Hence, the observed positive response strongly suggests that inhibition of PDGFBR activity may also be effective in other myeloproliferative diseases involving this tyrosine kinase receptor.*[173]	Q1	NR	case report (positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Grand 2004	*Imatinib, a known inhibitor of PDGFRB, blocked the growth of patient colony-forming unit, granulocyte macrophage in vitro and produced a clinically significant response before relapse and subsequent death with imatinib-resistant disease.*[174]	A1	NR	case report (positive)	✓													
Levine 2005	*Treatment with imatinib resulted in rapid, complete and sustained hematologic and cytogenetic response.*[175]	A1	case report	case report (positive)	✓													
Magnusson 2002	*These results clearly demonstrate (…) inhibitory effect of STI571 [imatinib] against leukemic cells harbouring a PDGFBR fusion oncogene, in a clinically relevant situation*[176]	A1	NR	case report (positive)	✓													
Pardanani 2002	*imatinib is clearly an active agent in HES and, as demonstrated in this report.*[177]	A1	case	case report (negative)		✓												
Pitini 2007	*Our case demonstrates many typical forms of myeloproliferative disorders with translocation t(5;12)(q33;p13), involvement of PDGFRB and response to STI571 [imatinib] treatment. (…) there is clearly a need for further studies of STI571 in this setting to confirm these promising initial results.*[178]	A1	case report	case report (positive)	✓													
Safley 2004	*Continuing observation and future cytogenetic and molecular analysis of the patient's bone marrow will be required to determine whether treatment has truly resulted in a durable hematologic and cytogenetic response.*[179]	A1	case	case report (positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Trempat 2003	*The results of the present study expand the list of Glivec targets and have direct clinical implication. In addition to myeloproliferative diseases bearing ABL or PDGFRB rearrangements, this molecule is also effective in CML-like disorders with fusion genes involving PDGFRA as demonstrated in the present study.* [180]	Q1	case	case report (positive)	✓													
Vizmanos 2004	*After treatment with imatinib, the patient achieved hematological and cytogenetical remission, but NIN-PDGFRB mRNA remained detectable by reverse-transcription-PCR.*[181]	A1	NR	case report (positive)	✓													
Wilkinson 2003	*we have shown that the t(1;5)(q23;q33) targets PDGFRB and that complete remission can be achieved with imatinib in a heavily pretreated patient with progressive disease*[182]	A1	case	case report (positive)	✓													
Wittman 2004	*Imatinib mesylate proved to be highly effective in treating our patient's leukemia. (…) More experience is needed to know whether the efficacy of this agent for t(5;12)(q33;p13) disorders will similarly be affected by stage of disease and type of leukemia [as with CML].*[183]	A1	case report	case report (positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
Philadelphia chromosome – ALL - dasatinib					✓													
START-L	*Dasatinib is highly active and produces hematologic and cytogenetic responses*[186] (conclusions from the START programme including studies in mainly CML and some AML patients)	A1	open-label, single-arm, study	single-arm (only positive)	✓													
CA180002	*Our results demonstrate that dasatinib has clinical activity in all stages of imatinib-resistant CML and Ph-positive ALL, including resistance caused by BCR-ABL gene mutation*[185]	A1	open-label, dose-escalation study	single-arm (only positive)	✓													
Philadelphia chromosome – ALL - imatinib					✓				✓									
0109	Our current study provides the basis for investigations aimed at identifying combination therapies most appropriate in the various clinical settings of Ph+ acute leukemias[192]	A1	open-label, nonrandomized trial	single-arm (only positive)	✓													
0114	*The interpretation of the results of the EAP should take into account that only limited efficacy and safety data were collected, and that the length of follow-up was directly related to, and limited by, the date when imatinib became commercially available. Despite these limitations, it is encouraging that TTP and OS were similar to the previously published results of the phase II studies which led to the worldwide regulatory approval of the drug*[258]	A1	open-label, nonrandomized trials	single-arm (only positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
03001	*This study demonstrates that STI571 as a single agent is well tolerated and has substantial activity against acute leukemias characterized by the BCRABL fusion protein*[191]	A1	dose-escalating pilot study	single-arm (only positive)	✓													
AAU02	NA	NA	NA	single-arm (only positive)	✓													
ADE04	*our study strongly suggests that schedules based on the simultaneous administration of imatinib and cytotoxic agents should form the basis for prospective, comparative studies aimed at improving the pretransplantation molecular response during firstline treatment of Ph+ ALL*[197]	A1	prospective clinical trial	Single-arm (only positive)	✓													
ADE10	*This randomized trial demonstrates a strikingly superior response to remission induction with single agent imatinib compared with multiagent chemotherapy in newly diagnosed Ph+ALL, as well as markedly more rapid hematopoietic recovery, a significantly lower frequency of SAEs, fewer early deaths, and better adherence to subsequent consolidation therapy*[196]	Q1	prospective, randomized trial	enrichment					✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
AFR09	*Although this study is encouraging, the long-term fate of patients treated according to this protocol is unknown. Clearly, there is room for substantial improvement in the field of Ph+ ALL in the elderly. The optimal dose of imatinib, the proper duration of treatment, the value of chemotherapy given in addition to imatinib, the contribution of new tyrosine kinase inhibitors and the place, if any, of stem cell transplantation still remain to be determined*[194]	A1	non-randomized study	single-arm (only positive)	✓													
AIT04	NA	NA	NA	single-arm (only positive)	✓													
AJP01	NR; the main objective of this study was to identify associated with resistance to imatinib[193]	N/A	NR	single-arm (only positive)	✓													
AUS01	NA	NA	NA	single-arm (only positive)	✓													
t(15;17) translocation and/or PML/RAR-**α gene** - acute promyelocytic leukaemia - arsenic trioxide					✓													
97-66	*In summary, arsenic trioxide can induce a complete remission in patients with APL who have relapsed after extensive prior therapy. This drug causes partial but incomplete cytodifferentiation of leukemic cells, followed by caspase activation and induction of apoptosis. The striking degree of activity of arsenicals in this disease, plus their lack of specificity for APL-specific proteins, suggests that they may warrant further study as therapy for other neoplastic diseases*[200]	A1	NR	single-arm (only positive)	✓													

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
PLRXAS01	*In summary, the results of this study establish ATO as a highly effective therapy for patients with APL despite prior therapy with retinoids and chemotherapy. Moreover, responses have proven to be durable for at least 18 months in over half the patients who achieved CR.*[259]	Q1	expanded study	single-arm (only positive)	✓													
viral resistance mutations – HIV - amprenavir																		✓
PROAB3004	NA	NA	NA	unclear														
PROAB3006	*In summary, the role of the I50V mutation in conferring resistance to APV has been confirmed in a large phase III study. Three additional viral protease genotypes characterized by the development of substitutions I54L, I54M, V32I + I47V, and I84V, which may occur with concomitant accessory mutations (e.g., M46I/L, L33F, L10F), evolved in response to APV and generally conferred lower levels of APV resistance. Each of these four genotypes conferred little or no cross-resistance to other PIs. Finally, the significant association between preexisting viral resistance to NRTIs subsequently administered in the PI/NRTI combination regimen and the emergence of protease mutations, emphasizes the importance of optimising treatment regimens to ensure that the virus is susceptible to as many components as possible.*[203]	Q5, Q6	clinical trial (retrospective genotypic and phenotypic analyses)	randomise-all (cross-sectional)														✓
viral resistance mutations – HIV – atazanavir									✓	✓		✓	✓	✓	✓	✓	✓	✓
AI424009	*NA*	NA	NA	unclear														

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
AI424043	NA	NA	NA	unclear														
AI424045	NA	NA	NA	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
viral resistance mutations – HIV – darunavir																		✓
POWER 1	NA	NA	NA	randomise-all (cross-sectional)														✓
POWER 2	NA	NA	NA	randomise-all (cross-sectional)														✓
POWER 3	NA	NA	NA	randomise-all (cross-sectional)														✓
viral resistance mutations – HIV – efavirenz / emtricitabine / tenofovir disoproxil																		✓
Gallant 2006	*Consistent with other studies in ARV-naïve subjects, NNRTI-R was found to be the most clinically relevant form of transmitted resistance with regard to risk of virologic failure on an EFV-containing regimen. This finding highlights the importance of baseline genotyping when initiation of ARV therapy is being considered, especially when considering an NNRTI-containing regimen. Nevertheless, it was notable that a small proportion of patients enrolled in Study 934 (approximately 2%) entered the study with evidence of genotypic and phenotypic resistance to PIs. Therefore, resistance testing is warranted for all ARV-naïve subjects.*[101]	Q6	prospective, randomized, noninferiority study (genotypic and phenotypic resistance analyses)	randomise-all (cross-sectional)														✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
viral resistance mutations – HIV – emtricitabine																		✓
Benson 2004	NR[97]	N/A	randomized, open-label equivalence trial (genotypic analysis)	randomise-all (cross-sectional)														✓
viral resistance mutations – HIV – enfuvirtide																		✓
TORO 1	NA	NA	NA	randomise-all (cross-sectional)														✓
TORO 2	NA	NA	NA	randomise-all (cross-sectional)														✓
viral resistance mutations – HIV – emtricitabine / rilpivirine / tenofovir disoproxil									✓									
ECHO	*The most prevalent treatment-emergent NNRTI RAMs [resistance associated mutations] were consistent with data from TMC278-C204 and THRIVE[217]*	A6	randomised, double-blind, double-dummy, active-controlled trial	enrichment					✓									
THRIVE	*Consistent with reports from the phase 2b TMC278-C204 trial, E138K was the most prevalent NNRTI [non-nucleoside reverse transcriptase inhibitor] RAM [resistance-associated mutation] in the rilpivirine group and K103N was in the efavirenz group, whereas M184I/V were the most prevalent N(t)RTI [nucleoside/nucleotide reverse transcriptase inhibitor] RAMs in both groups[260]*	A6	randomised, double-blind, double-dummy, non-inferiority trial	enrichment					✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
viral resistance mutations – HIV – fosamprenavir									✓	✓		✓	✓	✓	✓	✓	✓	✓
APV 30003	NA	NA	NA	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
SOLO	NR[220]	N/A	randomized, open-label study (NR)	randomise-all (cross-sectional)														✓
NEAT	The spectrum of mutations selected by 908 is consistent with that observed with the active moiety, APV, and is distinct from that observed with most other PIs. Subjects experiencing virologic failure on NFV developed protease mutations, selecting the common NFV resistance–associated mutations including D30N and L90M.[261]	A6	randomized, open-label 2-arm study (NR)	randomise-all (cross-sectional)														✓
viral resistance mutations – HIV – lopinavir / ritonavir																		✓
M98-863	NA	NA	NA	randomise-all (cross-sectional)														✓
M97-720	NA	NA	NA	randomise-all (cross-sectional)														✓
viral resistance mutations – HIV – nelfinavir									✓	✓		✓	✓	✓	✓	✓	✓	✓
505	NA	NA	NA	randomise-all (retrospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
511	NA	NA	NA	unclear														

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
viral resistance mutations – HIV – rilpivirine									✓									
ECHO	*The most prevalent treatment-emergent NNRTI RAMs [resistance associated mutations] were consistent with data from TMC278-C204 and THRIVE*[217]	A6	randomised, double-blind, double-dummy, active-controlled trial	enrichment					✓									
THRIVE	*Consistent with reports from the phase 2b TMC278-C204 trial, E138K was the most prevalent NNRTI [non-nucleoside reverse transcriptase inhibitor] RAM [resistance-associated mutation] in the rilpivirine group and K103N was in the efavirenz group, whereas M184I/V were the most prevalent N(t)RTI [nucleoside/nucleotide reverse transcriptase inhibitor] RAMs in both groups*[260]	A6	randomised, double-blind, double-dummy, non-inferiority trial	enrichment					✓									
viral resistance mutations – HIV – tenofovir									✓	✓		✓	✓	✓	✓	✓	✓	✓
902	NA	NA	NA	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]													
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
903	*treatment failure was uncommon. The development of the K65R mutation was less common than resistance to efavirenz or lamivudine. This mutation appears to be the only pathway to tenofovir resistance among treatment-naive patients, analogous to observations in treatment-experienced patients. The K65R mutation was observed in 8 patients (1 patient after week 48) failing therapy in the tenofovir DF group through 144 weeks, which represents less than 3% of the total number of patients treated or 17% of those experiencing virologic failure in the tenofovir DF group.*[228]	Q6	prospective, randomized, double-blind study (resistance analysis)	randomise-all (cross-sectional)														✓
907	*Given the low percentage of patients who achieved undetectable HIV-1 RNA levels in both the tenofovir DF and placebo groups, resistance mutations continued to develop. After 24 weeks, there was a trend toward development of fewer additional nucleoside resistance mutations in the tenofovir DF group compared with the placebo-treated group, but it did not achieve statistical significance. Despite ongoing viral replication in nearly 80% of patients receiving tenofovir DF, the K65R mutation, selected by tenofovir in vitro, was seen in only 3% of patients through week 48.*[227]	A6	randomized, double-blind, placebo-controlled study (virologic genotyping substudy)	randomise-all (prospective subgroup)					✓	✓		✓	✓	✓	✓	✓	✓	✓
viral resistance mutations – HIV – tipranavir									✓									✓
RESIST 1	NR[230,231]	N/A	randomised, open-label trial	enrichment					✓									

Study[i]	Authors' conclusions		Study design label (paper)	Study design label	Questions addressed with study designs[ii]												
	Quote	Summary			A1	A2	A3	A4	Q1	Q2	A5	Q3	Q4	Q5	Q6	Q7	Q8	A6
RESIST 2	NR[230,232]	N/A	randomised, open-label trial	enrichment					✓									
1182.4	NR[233]	N/A	dose-ranging trial (NR)	randomise-all (cross-sectional)														✓
1182.51	NA	NA	NA	enrichment					✓									
1182.52	NA	NA	NA	enrichment					✓									
Markowitz 2007	*Furthermore, patient viral isolates with reduced susceptibility had the emergence of the V82T mutation combined with an L33 (I for V) codon mutation on treatment with TPV. This suggests that reduced susceptibility to TPV may occur when these 2 mutations are present along with at least 10 other mutations. The presence of single PI mutations at codon 46, 82, 84, or 90 did not seem to influence the virologic response to TPV.[236]*	Q6	open-label, randomized study (NR)	randomise-all (2 different doses) (cross-sectional)														✓

[i] Names of main studies are in bold

[ii] For B-I-D combinations the questions addressed by main studies are underlined

APPENDIX 16. LABORATORY METHODS FOR ERCC1 EVALUATION

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16.1. IMMUNOHISTOCHEMISTRY

IHC measures ERCC1 expression at protein level. In this method an antibody is used that binds to the ERCC1 protein. The part of the protein to which the antibody binds is known as an epitope. Antibody-epitope binding results in formation of an antibody-ERCC1 complex. Further steps are then undertaken to visualise the complex.

The main antibody in use appears to be 8F1 (Neomarkers, Fremont, California, USA), however others are commercially available.[262] A number of systems exist for quantification of results of IHC experiments, and some of these are described here.

In quantifying the results of IHC assessments are usually undertaken of:

- staining (or reactivity) - aiming to measure the proportion of cells which stain positive using a given antibody,[263]
- intensity - usually based on a microscopic examination of the stained tissue in comparison with positive controls with a known range of expression levels.[263-265]

It is often expressed on a scale from 0 to 3 corresponding to no, weak, moderate and strong expression respectively.[266,267] A number of scoring systems have been introduced that take into account both the proportion of cells staining and intensity. Two of these will be discussed here: the H-score and the Quick Score.

The H-score, which was originally introduced in the context of oestrogen receptor expression, but was adapted to a wider range of biomarkers. This score can be summarised as:[263,268]

$$H - score = \sum_{i=0}^{3} p_i \times i$$

Where: i – intensity score, p_i – percentage of cells with a given intensity

The H-score has a value between 0 and 300, where 0 indicates no cell staining and 300 indicates 100% of tumour cells staining at strong intensity (intensity score of 3).[263,268]

The Quick Score was introduced partly to overcome some of the complexities in calculating the H-score. It involves the addition of a score obtained based on the percentage of cells staining positive (proportion score) and an overall intensity score:[263]

$$Quick\ Score = A + B$$

Where: A – proportion score (assigned based on proportion of cells staining positive); B – intensity score (assigned based on overall staining intensity)

A number of modifications of the Quick Score were suggested, which assign different possible values to the proportion score and the intensity score. One of the more popular versions is the Allred Quick Score, where the possible values for the proportion score range from 0 to 5 and for the intensity score from 0 to 3 (as shown in Table 61), giving a total score ranging from 0 to 8.[263,269]

Table 61 Values of proportion score and intensity score used for calculation of the Allred Quick Score for IHC

proportion score	proportion of cells staining positive
0	0
1	$< \frac{1}{100}$
2	$\frac{1}{100}$ to $\frac{1}{10}$
3	$\frac{1}{10}$ to $\frac{1}{3}$
4	$\frac{1}{3}$ to $\frac{2}{3}$
5	$> \frac{2}{3}$

intensity score	overall staining intensity
0	none
1	weak
2	intermediate
3	strong

16.2. REVERSE TRANSCRIPTASE QUANTITATIVE POLYMERASE CHAIN REACTION

RTqPCR measures ERCC1 expression at RNA level.[270] As RNA cannot be directly used in a PCR reaction, reverse transcriptase is first used to obtain a complementary DNA (cDNA) copy of the RNA molecules extracted from tumour tissue.[271] The quantity of cDNA obtained in this step may vary depending on reaction conditions. As the quantity of cDNA needs to reflect the quantity of RNA present in the sample, this point is of extreme importance.[272]

Afterwards, primers are used to generate new DNA strands containing the target sequence. These primers are short DNA sequences designed to match regions bordering on the target sequence.[271] The main points of the laboratory procedure can be summarised in the following (normally automated) steps, which constitute one cycle of PCR:

1) Separation of DNA strands by heating the solution,
2) Binding of primers to DNA strands enabled by cooling the solution,
3) Synthesis of new DNA starting from the bound primer enabled by increasing temperature (to a level below that in step 1).[271,272]

The procedure is then repeated for a number of cycles by changing the temperature. In a 100% efficient PCR reaction the quantity of copies of the sequence of interest is increased 2^n fold, where n is the number of cycles.[271] In a less efficient experiment this

increase is somewhat lower, but still progresses exponentially until one of the critical components of the reaction is used up or largely depleted, leading to below-exponential increase and eventually the reaction stops.[272]

In quantitative PCR the reaction mix contains a dye which becomes fluorescent when it is either bond to double stranded DNA or a target sequence.[271] Based on the fluorescence level, the quantity of product is continuously monitored.[270,273] In the initial cycles, this quantity is below a pre-determined threshold of detection. The cycle (often labelled C_T) at which the product first reaches the detection threshold is proportional to the quantity of original cDNA copies.[271,272]

As the threshold level is influenced by the instrument settings, each quantitative PCR experiment needs to include a control (or a reference standard) relative to which the expression level of the target protein can be established.[272] The expression level is therefore normalised to the reference standard, which also enables comparisons between different tissue samples. The choice of a reference standard is not straightforward, as ideally it should be expressed at constant levels in all tissues and at all developmental stages.[274] For this reason, housekeeping genes[275,276] (which need to be expressed in all cells[270]), such as glyceraldehyde 3-phosphate dehydrogenase and β-actin have been widely used in practice.[274]

When multiple PCR reactions are run at the same time in the same reaction either labelling of primers or sequence-specific probes are utilised. These labels become fluorescent when incorporated into a copy of the target DNA sequence. As probes or labelled primers directed at different DNA sequences emit different coloured fluorescence, the quantity of multiple DNA sequences can be measured at the same time.[272]

This relative expression of a gene compared to a reference standard can be calculated:

$$\frac{N_A}{N_B} = K_{RS} \frac{\eta_B (1 + E_B)^{C_{TB}-1}}{\eta_A (1 + E_A)^{C_{TA}-1}}$$

Where: A – target gene, B – reference gene, N_A, N_B – numbers of mRNA molecules of gene A or B present in the sample, K_{RS} – relative sensitivity of detection of the genes, η_A, η_B – yields of cDNA synthesis from mRNA for gene A and B, E_A, E_B – efficiencies of PCR, CT_A, CT_B – cycle at which product reaches threshold of detection (C_T-1 used to account for single cDNA strand in first cycle)

In most cases more than one sample is used and for two samples and under the assumption that K_{RS}, η_A, η_B, E_A and E_B values are constant, this leads to a much simpler formula:[272]

$$quantity\ of\ target = \frac{(1+E_B)^{C_{TB1}-C_{TB2}}}{(1+E_A)^{C_{TA1}-C_{TA2}}}$$

Where C_{TA1}, C_{TA2}, C_{TB1}, C_{TB2} ate the C_T values obtained for samples 1 and 2

With a further assumption that PCR efficiency is 100% this simplifies to the ΔΔCT method: [272,277290,295]

$$quantity\ of\ target = \frac{2^{C_{TB1}-C_{TB2}}}{2^{C_{TA1}-C_{TA2}}} = 2^{(C_{TB1}-C_{TB2})-(C_{TA1}-C_{TA2})} = 2^{\Delta\Delta CT}$$

Which has been relatively frequently used in practice.[22,27]

APPENDIX 17. SEARCH STRATEGIES FOR ERCC1 SYSTEMATIC REVIEW

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Search strategies used in ongoing trials databases to identify studies assessing ERCC1 expression are shown below for each database separately.

ClinicalTrials.gov (317 hits):

(lung OR NSCLC) AND (customized OR individualized OR tailored OR personalized OR biomarker OR ERCC1OR ERCC OR ERCC-1 OR (excision AND repair) OR pharmacogenomic OR pharmacogenetic) AND (cisplatin OR carboplatin OR platinum OR platin OR chemotherapy)

WHO (303 hits):

Lung AND customized OR Lung AND individualized OR Lung AND tailored OR Lung AND personalized OR Lung AND biomarker OR Lung AND ERCC1 OR Lung AND ERCC-1 OR Lung AND ERCC OR Lung AND excision AND repair OR Lung AND pharmacogenomic OR Lung AND pharmacogenetic OR NSCLC AND customized OR NSCLC AND individualized OR NSCLC AND tailored OR NSCLC AND personalized OR NSCLC AND biomarker OR NSCLC AND ERCC1 OR NSCLC AND ERCC-1 OR NSCLC AND ERCC OR NSCLC AND excision AND repair OR NSCLC AND pharmacogenomic OR NSCLC AND pharmacogenetic

(WHO portal does not recognise brackets; operator priority: NOT, AND, OR)

Controlled-Trials (301 hits):

(lung OR NSCLC) AND (customized OR individualized OR tailored OR personalized OR biomarker OR ERCC1 OR ERCC OR ERCC-1 OR (excision AND repair) OR pharmacogenomic OR pharmacogenetic)

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APPENDIX 18. QUESTIONNAIRE ON TRIALS EVALUATING ERCC1

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ERCC1 Trial Review

Trial Name:
Trial Co-ordinator or Name and email address of person responding to questionnaire:
Laboratory Lead:
Chief Investigator:

Is the ERCC1 Assessment carried out prospectively-ie prior to patients receiving treatment?

Yes ☐
No ☐

If no - please describe

If not prospective, is the ERCC1 assessment carried out by staff blind to the patient's outcome?

Yes ☐
No ☐

What type(s) of specimen are you using?-Please tick the appropriate box(es) and provide the approximate percentage of different specimen types (if known).

		Percentage
Surgical resection	☐	_____
Biopsy	☐	_____
Cytology	☐	_____

Please give details of the procedure for collection and storage of the specimen

_____
_____

Where is the ERCC1 testing done? Please tick the appropriate box(es)

Individual hospital ☐
Central laboratory ☐
Other ☐

If other please specify?
How long does it take to get the ERCC1 results back to the treating physician?

_____

What method are you using for measuring ERCC1 expression? Please tick the appropriate box(es)

IHC ☐
RTqPCR ☐
Other ☐

If other please specify?

Please give full details of the scoring system used to measure ERCC1
Staining score _____
Intensity score _____
H-score _____
If possible, please provide an estimate of the proportion of patients classified as ERCC1 positive in your study

_____

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Midlands Hub

If using immunohistochemistry
Which antibody are you using (i.e. name of company/clone)?
In what dilution?
Are you using any ancillary techniques? (e.g. microwave pretreatment)
What threshold are you using to categorise a patient as ERCC1 high vs low?

_____
_____
_____

If using RTqPCR
What strategy are you using *(i.e. primers, exons covered, endogenous control? e.g. primers spanning exons 8-10 of the ENST00000300853 ERCC1 transcript: 5' CTGACCACCGTGAAGTCA 3' and 5' AAGGGCTCGTGCAGGAC 3'; B2M as endogenous control)*

_____
_____
_____

What threshold are you using to categorise a patient as ERCC1 high vs low? *(e.g. patients with dCt values between B2M and ERRC1 >7 are considered ERCC1 low, while patients with dCT values <4 are considered ERCC1 high; Patients wich dCT values between 4-7 are considered uncertain or unclassified. This latter group represents 20% of our population)*.

_____
_____
_____
_____

Please state the rationale for the choice of ERCC1 assessment method

_____
_____
_____

From Professor Lucinda Billingham, (l.j.billingham@bham.ac.uk) University of Birmingham, UK
Dr Sanjay Popat, Imperial College and Royal Marsden Hospital, London, UK

Thank you very much

REFERENCES

1. Adaptive Design Clinical Trials for Drugs and Biologics. Draft Guidance for Industry. , Food and Drug Administration, 2010, http://www.fda.gov/downloads/Drugs/.../Guidances/ucm201790.pdf, Accessed: 22.07.2016.

2. G. Biomarkers Definitions Working, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, *Clin Pharmacol Ther*, 2001, 69, 3, 89-95

3. M. S. Pepe, Z. Feng, H. Janes, P. M. Bossuyt and J. D. Potter, Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design, *J Natl.Cancer Inst.*, 2008, 100, 20, 1432-1438

4. Orphan Designation, European Medicines Agency, 2013, www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000029.jsp&murl=menus/regulations/regulations.jsp&mid=WC0b01ac05800240ce, Accessed: 22.04.2013.

5. D. J. Sargent, B. A. Conley, C. Allegra and L. Collette, Clinical trial designs for predictive marker validation in cancer treatment trials, *J Clin Oncol*, 2005, 23, 9, 2020-2027

6. M. R. Trusheim, E. R. Berndt and F. L. Douglas, Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers, *Nat.Rev.Drug Discov.*, 2007, 6, 4, 287-293

7. A Giudeline on Summary of Prduct Characteristics, European Medicines Agency, 2005, http://ec.europa.eu/health/files/eudralex/vol-2/c/spcguidrev1-oct2005_en.pdf, Accessed: 05.05.2012.

8. M. Harries and I. Smith, The development and clinical use of trastuzumab (Herceptin), *Endocr Relat Cancer*, 2002, 9, 2, 75-85

9. H. M. Shepard, P. Jin, D. J. Slamon, Z. Pirot and D. C. Maneval, Herceptin, *Handb Exp Pharmacol*, 2008, 181, 183-219

10. C. Shih, L. Padhy, M. Murray and R. A. Weinberg, Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts, *Nature*, 1981, 290, 261 - 264

11. A. L. Schechter, D. F. Stern, L. Vaidyanathan, S. J. Decker, J. A. Drebin, M. I. Greene and R. A. Weinberg, The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen, *Nature*, 1984, 312, 5994, 513-516

12. L. Coussens, T. L. Yang-Feng, Y. C. Liao, E. Chen, A. Gray, J. McGrath, P. H. Seeburg, T. A. Libermann, J. Schlessinger, U. Francke and et al., Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene, *Science*, 1985, 230, 4730, 1132-1139

13. Y. Yarden and A. Ullrich, Growth factor receptor tyrosine kinases, *Annu Rev Biochem*, 1988, 57, 443-478

14. D. B. Weiner, J. Liu, J. A. Cohen, W. V. Williams and M. I. Greene, A Point Mutation in the Neu Oncogene Mimics Ligand Induction of Receptor Aggregation, *Nature*, 1989, 339, 6221, 230-231

15. D. J. Slamon, G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich and W. L. Mcguire, Human-Breast Cancer - Correlation of Relapse and Survival with Amplification of the Her-2 Neu Oncogene, *Science*, 1987, 235, 4785, 177-182

16. D. J. Slamon, W. Godolphin, L. A. Jones, J. A. Holt, S. G. Wong, D. E. Keith, W. J. Levin, S. G. Stuart, J. Udove, A. Ullrich and M. F. Press, Studies of the Her-2/Neu Proto-Oncogene in Human-Breast and Ovarian-Cancer, *Science*, 1989, 244, 4905, 707-712

17. R. M. Hudziak, J. Schlessinger and A. Ullrich, Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells, *Proc Natl Acad Sci U S A*, 1987, 84, 20, 7159-7163

18. R. M. Hudziak, G. D. Lewis, M. R. Shalaby, T. E. Eessalu, B. B. Aggarwal, A. Ullrich and H. M. Shepard, Amplified expression of the HER2/ERBB2 oncogene induces resistance to tumor necrosis factor alpha in NIH 3T3 cells, *Proc Natl Acad Sci U S A*, 1988, 85, 14, 5102-5106

19. R. M. Hudziak, G. D. Lewis, M. Winget, B. M. Fendly, H. M. Shepard and A. Ullrich, p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor, *Molecular and Cellular Biology*, 1989, 9, 3, 1165-1172

20. B. M. Fendly, M. Winget, R. M. Hudziak, M. T. Lipari, M. A. Napier and A. Ullrich, Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product, *Cancer Res*, 1990, 50, 5, 1550-1558

21. A. Gschwind, O. M. Fischer and A. Ullrich, The discovery of receptor tyrosine kinases: targets for cancer therapy, *Nat Rev Cancer*, 2004, 4, 5, 361-370

22. P. Carter, L. Presta, C. M. Gorman, J. Ridgway, D. Henner, W. Wong, A. M. Rowland, C. Kotts, M. E. Carver and H. M. Shepard, Humanization of an anti-p185HER2 antibody for human cancer therapy, *Proceedings of the National Academy of Sciences*, 1992, 89, 10, 4285-4289

23. J. Baselga, Phase I and II clinical trials of trastuzumab, *Annals of Oncology*, 2001, 12, suppl 1, S49-S55

24. Trastuzumab: Assessment Report European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000278/WC500126896.pdf, Accessed: 20.12.2012.

25. J. Baselga, Clinical trials of Herceptin® (trastuzumab), *European Journal of Cancer*, 2001, 37, Supplement 1, 18-24

26. J. Baselga, D. Tripathy, J. Mendelsohn, S. Baughman, C. C. Benz, L. Dantis, N. T. Sklarin, A. D. Seidman, C. A. Hudis, J. Moore, P. P. Rosen, T. Twaddell, I. C. Henderson and L. Norton, Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer, *J Clin Oncol*, 1996, 14, 3, 737-744

27. M. A. Cobleigh, C. L. Vogel, D. Tripathy, N. J. Robert, S. Scholl, L. Fehrenbacher, J. M. Wolter, V. Paton, S. Shak, G. Lieberman and D. J. Slamon, Multinational Study of the Efficacy and Safety of Humanized Anti-HER2 Monoclonal Antibody in Women Who Have HER2-Overexpressing Metastatic Breast Cancer That Has Progressed After Chemotherapy for Metastatic Disease, *Journal of Clinical Oncology*, 1999, 17, 9, 2639-2639

28. M. D. Pegram, A. Lipton, D. F. Hayes, B. L. Weber, J. M. Baselga, D. Tripathy, D. Baly, S. A. Baughman, T. Twaddell, J. A. Glaspy and D. J. Slamon, Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment, *J Clin Oncol*, 1998, 16, 8, 2659-2671

29. Genetech, Herceptin® (Trastuzumab) Development Timeline, 2016, http://www.gene.com/media/product-information/herceptin-development-timeline, Accessed: 15.04.2016.

30. G. L. Kumar and S. Badve, Milestones in the Discovery of HER2 Proto-Oncogene and Trastuzumab (Herceptin™), *Connection*, 2008, 13, 9-14

31. DAKO Herceptest approval letter, Food and Drug Administration, 1998, http://www.accessdata.fda.gov/cdrh_docs/pdf/P980018.pdf, Accessed: 20.05.2016.

32. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga and L. Norton, Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2, *New England Journal of Medicine*, 2001, 344, 11, 783-792

33. Herceptin : EPAR - Procedural steps taken before authorisation, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Procedural_steps_taken_before_authorisation/human/000278/WC500049817.pdf, Accessed: 24.05.2016.

34. V. M. Garcia, P. A. Cassier and J. de Bono, Parallel anticancer drug development and molecular stratification to qualify predictive biomarkers: dealing with obstacles hindering progress, *Cancer Discov*, 2011, 1, 3, 207-212

35. D. R. Hodgson, R. D. Whittaker, A. Herath, D. Amakye and G. Clack, Biomarkers in oncology drug development, *Mol Oncol*, 2009, 3, 1, 24-32

36. D. Lin, Z. Hollander, A. Meredith and B. M. McManus, Searching for 'omic' biomarkers, *Can J Cardiol*, 2009, 25 Suppl A, 9A-14A

37. S. H. Cho, J. Jeon and S. I. Kim, Personalized medicine in breast cancer: a systematic review, *J Breast Cancer*, 2012, 15, 3, 265-272

38. G. S. Ginsburg and S. B. Haga, Translating genomic biomarkers into clinically useful diagnostics, *Expert Rev Mol Diagn*, 2006, 6, 2, 179-191

39. J. P. Ioannidis, A roadmap for successful applications of clinical proteomics, *Proteomics Clin Appl*, 2011, 5, 5-6, 241-247

40. C. M. Micheel, Nass, S. J., Omenn, G. S., Evolution of Translational Omics: Lessons Learned and the Path Forward, Institute of Medicine of the National Academies, 2012, http://www.ncbi.nlm.nih.gov/pubmed/24872966, Accessed: 13.10.2015.

41. S. Matsui, Genomic biomarkers for personalized medicine: development and validation in clinical studies, *Comput Math Methods Med*, 2013, 2013, 865980

42. K. Shahzad, A. Fatima, M. Cadeiras, N. Wisniewski, G. Bondar, R. Cheng, E. Reed and M. Deng, Challenges and solutions in the development of genomic biomarker panels: a systematic phased approach, *Curr Genomics*, 2012, 13, 4, 334-341

43. R. Simon, Roadmap for developing and validating therapeutically relevant genomic classifiers, *J Clin Oncol*, 2005, 23, 29, 7332-7341

44. K. Matheis, D. Laurie, C. Andriamandroso, N. Arber, L. Badimon, X. Benain, K. Bendjama, I. Clavier, P. Colman, H. Firat, J. Goepfert, S. Hall, T. Joos, S. Kraus, A. Kretschmer, M. Merz, T. Padro, H. Planatscher, A. Rossi, N. Schneiderhan-Marra, I. Schuppe-Koistinen, P. Thomann, J. M. Vidal and B. Molac, A generic operational strategy to qualify translational safety biomarkers, *Drug Discov Today*, 2011, 16, 13-14, 600-608

45. T. T. Loo, C. J. Ross, J. Sistonen, H. Visscher, P. Madadi, G. Koren, M. R. Hayden and B. C. Carleton, Pharmacogenomics and active surveillance for serious adverse drug reactions in children, *Pharmacogenomics*, 2010, 11, 9, 1269-1285

46. A. T. Shaw, B. Y. Yeap, B. J. Solomon, G. J. Riely, J. Gainor, J. A. Engelman, G. I. Shapiro, D. B. Costa, S. H. Ou, M. Butaney, R. Salgia, R. G. Maki, M. Varella-Garcia, R. C. Doebele, Y. J. Bang, K. Kulig, P. Selaru, Y. Tang, K. D. Wilner, E. L. Kwak, J. W. Clark, A. J. Iafrate and D. R. Camidge, Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis, *The Lancet Oncology*, 2011, 12, 11, 1004-1012

47. E. L. Kwak, Y. J. Bang, D. R. Camidge, A. T. Shaw, B. Solomon, R. G. Maki, S. H. Ou, B. J. Dezube, P. A. J+ñnne, D. B. Costa, M. Varella-Garcia, W. H. Kim, T. J. Lynch, P. Fidias, H. Stubbs, J. A. Engelman, L. V. Sequist, W. Tan, L. Gandhi, M. Mino-Kenudson, G. C. Wei, S. M. Shreeve, M. J. Ratain, J. Settleman, J. G. Christensen, D. A. Haber, K. Wilner, R. Salgia, G. I. Shapiro, J. W. Clark and A. J. Iafrate, Anaplastic Lymphoma Kinase Inhibition in NonGÇôSmall-Cell Lung Cancer, *New England Journal of Medicine*, 2010, 363, 18, 1693-1703

48. An Investigational Drug, PF-02341066, Is Being Studied In Patients With Advanced Non-Small Cell Lung Cancer With A Specific Gene Profile Involving The Anaplastic

Lymphoma Kinase (ALK) Gene, 2013, http://clinicaltrials.gov/ct2/show/NCT00932451?term=A8081005&rank=1, Accessed: 23.04.2013.

49. Crizotinib: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002489/WC500134759.pdf, Accessed: 03.02.2014.

50. Ivacaftor: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002494/WC500130696.pdf, Accessed: 24.04.2013.

51. Vemurafenib: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002409/WC500124400.pdf, Accessed: 09.05.2012.

52. P. B. Chapman, A. Hauschild, C. Robert, J. B. Haanen, P. Ascierto, J. Larkin, R. Dummer, C. Garbe, A. Testori, M. Maio, D. Hogg, P. Lorigan, C. Lebbe, T. Jouary, D. Schadendorf, A. Ribas, S. J. O'Day, J. A. Sosman, J. M. Kirkwood, A. M. M. Eggermont, B. Dreno, K. Nolop, J. Li, B. Nelson, J. Hou, R. J. Lee, K. T. Flaherty and G. A. McArthur, Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation, *New England Journal of Medicine*, 2011, 364, 26, 2507-2516

53. A Study of RO5185426 in Previously Treated Patients With Metastatic Melanoma (Protocol), Roche, 2012, http://www.rochetrials.com/trialDetailsGet.action?studyNumber=NP22657, Accessed: 23.04.2012.

54. A Study of RO5185426 in Previously Treated Patients With Metastatic Melanoma (Results), Roche, 2011, http://www.rochetrials.com/studyResultGet.action?studyResultNumber=NP22657&diseaseCategoryId=58,

55. Maraviroc: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000811/WC500022194.pdf, Accessed: 07.03.2012.

56. R. M. Gulick, J. Lalezari, J. Goodrich, N. Clumeck, E. Dejesus, A. Horban, J. Nadler, B. Clotet, A. Karlsson, M. Wohlfeiler, J. B. Montana, M. McHale, J. Sullivan, C. Ridgway, S. Felstead, M. W. Dunne, R. E. Van Der and H. Mayer, Maraviroc for previously treated patients with R5 HIV-1 infection, *N Engl.J Med*, 2008, 359, 14, 1429-1441

57. M. Saag, J. Goodrich, G. Fatkenheuer, B. Clotet, N. Clumeck, J. Sullivan, M. Westby, R. E. Van Der and H. Mayer, A double-blind, placebo-controlled trial of maraviroc in treatment-experienced patients infected with non-R5 HIV-1, *J Infect.Dis*, 2009, 199, 11, 1638-1647

58. R. A. Larson, E. L. Sievers, E. A. Stadtmauer, B. Lowenberg, E. H. Estey, H. Dombret, M. Theobald, D. Voliotis, J. M. Bennett, M. Richie, L. H. Leopold, M. S. Berger, M. L. Sherman, M. R. Loken, J. J. van Dongen, I. D. Bernstein and F. R. Appelbaum, Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients

with CD33-positive acute myeloid leukemia in first recurrence, *Cancer*, 2005, 104, 7, 1442-1452

59. Gemtuzumab ozogamicin: Refusal Assessment Report, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000705/WC500070677.pdf, Accessed: 09.03.2012.

60. Withdrawal letter (Glivec), European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/Other/2010/01/WC500059831.pdf, Accessed: 23.07.2012.

61. M. C. Heinrich, H. Joensuu, G. D. Demetri, C. L. Corless, J. Apperley, J. A. Fletcher, D. Soulieres, S. Dirnhofer, A. Harlow, A. Town, A. McKinley, S. G. Supple, J. Seymour, S. L. Di, O. A. van, R. Herrmann, Z. Nikolova and A. G. McArthur, Phase II, open-label study evaluating the activity of imatinib in treating life-threatening malignancies known to be associated with imatinib-sensitive tyrosine kinases, *Clin Cancer Res*, 2008, 14, 9, 2717-2725

62. Capecitabine: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000316/WC500058145.pdf, Accessed: 18.04.2012.

63. Tegafur/gimeracil/oteracil: Assessment report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001242/WC500104417.pdf, Accessed: 18.04.2012.

64. Erbitux: Scientific Discussion, European Medicines Agency, 2004, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000558/WC500029113.pdf, Accessed: 26.03.2012.

65. H. Piessevaux, M. Buyse, R. W. De, H. Prenen, M. Schlichting, C. E. Van and S. Tejpar, Radiological tumor size decrease at week 6 is a potent predictor of outcome in chemorefractory metastatic colorectal cancer treated with cetuximab (BOND trial), *Ann Oncol*, 2009, 20, 8, 1375-1382

66. Phase II Study of Cetuximab in Patients With Irinotecan-Refractory, Stage IV Colorectal Cancer, National Cancer Institute, 2009, http://cancer.gov/clinicaltrials/search/view?cdrid=68731&version=healthprofessional#AlternateTitle_CDR0000068731, Accessed: 04.04.2012.

67. Phase II Study of Cetuximab and Irinotecan in Patients With Refractory Advanced Colorectal Cancer (Summary Last Modified 08/2000), National Cancer Institute, 2000, https://clinicaltrials.gov/ct2/show/NCT00005076, Accessed: 04.04.2012.

68. Erbitux: Assessment Report, European Medicines Agency, 2010, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000558/WC500075683.pdf, Accessed: 26.03.2012.

69. T. J. Lynch, T. Patel, L. Dreisbach, M. McCleod, W. J. Heim, R. C. Hermann, E. Paschold, N. O. Iannotti, S. Dakhil, S. Gorton, V. Pautret, M. R. Weber and D. Woytowitz, Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-

cell lung cancer: results of the randomized multicenter phase III trial BMS099, *J Clin Oncol*, 2010, 28, 6, 911-917

70. S. Khambata-Ford, C. T. Harbison, L. L. Hart, M. Awad, L. A. Xu, C. E. Horak, S. Dakhil, R. C. Hermann, T. J. Lynch and M. R. Weber, Analysis of Potential Predictive Markers of Cetuximab Benefit in BMS099, a Phase III Study of Cetuximab and First-Line Taxane/Carboplatin in Advanced NonGÇôSmall-Cell Lung Cancer, *Journal of Clinical Oncology*, 2010, 28, 6, 918-927

71. R. Pirker, J. R. Pereira, A. Szczesna, P. J. von, M. Krzakowski, R. Ramlau, I. Vynnychenko, K. Park, C. T. Yu, V. Ganul, J. K. Roh, E. Bajetta, K. O'Byrne, M. F. de, W. Eberhardt, T. Goddemeier, M. Emig and U. Gatzemeier, Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial, *Lancet*, 2009, 373, 9674, 1525-1531

72. R. Rosell, G. Robinet, A. Szczesna, R. Ramlau, M. Constenla, B. C. Mennecier, W. Pfeifer, K. J. O'Byrne, T. Welte, R. Kolb, R. Pirker, A. Chemaissani, M. Perol, M. R. Ranson, P. A. Ellis, K. Pilz and M. Reck, Randomized phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in EGFR-expressing advanced non-small-cell lung cancer, *Ann Oncol*, 2008, 19, 2, 362-369

73. Erlotinib: Assessment Report, European Medicines Agency, 2010, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000618/WC500090680.pdf, Accessed: 18.05.2012.

74. F. A. Shepherd, J. Rodrigues Pereira, T. Ciuleanu, E. H. Tan, V. Hirsh, S. Thongprasert, D. Campos, S. Maoleekoonpiroj, M. Smylie, R. Martins, M. van Kooten, M. Dediu, B. Findlay, D. Tu, D. Johnston, A. Bezjak, G. Clark, P. Santab+írbara and L. Seymour, Erlotinib in Previously Treated NonGÇôSmall-Cell Lung Cancer, *New England Journal of Medicine*, 2005, 353, 2, 123-132

75. M. S. Tsao, A. Sakurada, J. C. Cutz, C. Q. Zhu, S. Kamel-Reid, J. Squire, I. Lorimer, T. Zhang, N. Liu, M. Daneshmand, P. Marrano, G. da Cunha Santos, A. Lagarde, F. Richardson, L. Seymour, M. Whitehead, K. Ding, J. Pater and F. A. Shepherd, Erlotinib in Lung Cancer GÇö Molecular and Clinical Predictors of Outcome, *New England Journal of Medicine*, 2005, 353, 2, 133-144

76. R. í. Perez-Soler, A. Chachoua, L. A. Hammond, E. K. Rowinsky, M. Huberman, D. Karp, J. Rigas, G. M. Clark, P. Santab+írbara and P. Bonomi, Determinants of Tumor Response and Survival With Erlotinib in Patients With NonGÇöSmall-Cell Lung Cancer, *Journal of Clinical Oncology*, 2004, 22, 16, 3238-3247

77. Erlotinib: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000618/WC500117593.pdf, Accessed: 18.05.2012.

78. R. Rosell, E. Carcereny, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, R. Palmero, R. Garcia-Gomez, C. Pallares, J. M. Sanchez, R. Porta, M. Cobo, P. Garrido, F. Longo, T. Moran, A. Insa, F. De Marinis, R. Corre, I. Bover, A. Illiano, E. Dansin, J. de Castro, M. Milella, N. Reguart, G. Altavilla, U. Jimenez, M. Provencio, M. A. Moreno, J. Terrasa, J.

Mu+¦oz-Langa, J. Valdivia, D. Isla, M. Domine, O. Molinier, J. Mazieres, N. Baize, R. Garcia-Campelo, G. Robinet, D. Rodriguez-Abreu, G. Lopez-Vivanco, V. Gebbia, L. Ferrera-Delgado, P. Bombaron, R. Bernabe, A. Bearz, A. Artal, E. Cortesi, C. Rolfo, M. Sanchez-Ronco, A. Drozdowskyj, C. Queralt, I. de Aguirre, J. L. Ramirez, J. J. Sanchez, M. A. Molina, M. Taron and L. Paz-Ares, Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial, *The Lancet Oncology*, 2012, 13, 3, 239-246

79. P. A. Janne, X. Wang, M. A. Socinski, J. Crawford, T. E. Stinchcombe, L. Gu, M. Capelletti, M. J. Edelman, M. A. Villalona-Calero, R. Kratzke, E. E. Vokes and V. A. Miller, Randomized Phase II Trial of Erlotinib Alone or With Carboplatin and Paclitaxel in Patients Who Were Never or Light Former Smokers With Advanced Lung Adenocarcinoma: CALGB 30406 Trial, *Journal of Clinical Oncology*, 2012, 30, 17, 2063-2069

80. P. A. Janne, X. F. Wang, M. A. Socinski, J. Crawford, M. Capelletti, M. J. Edelman, M. A. Villalona-Calero, R. Kratzke, E. E. Vokes and V. A. Miller, Randomized phase II trial of erlotinib (E) alone or in combination with carboplatin/paclitaxel (CP) in never or light former smokers with advanced lung adenocarcinoma: CALGB 30406, *J Clin Oncol*, 2010, 28, 15_suppl, 7503

81. J. J. Laskin, T. Pugh, C. Jackson, M. Sutcliffe, D. Ionescu, B. Melonsky, C. Ho, S. Sun and e. al, Transcriptome-wide mutation discovery in patients in a phase II clinical trial of first-line erlotinib for clinically selected patients with advanced non-small cell lung cancer, *J Clin Oncol*, 2009, 27, 15_suppl, 8102

82. C. Zhou, Y. L. Wu, G. Chen, J. Feng, X. Q. Liu, C. Wang, S. Zhang, J. Wang, S. Zhou, S. Ren, S. Lu, L. Zhang, C. Hu, C. Hu, Y. Luo, L. Chen, M. Ye, J. Huang, X. Zhi, Y. Zhang, Q. Xiu, J. Ma, L. Zhang and C. You, Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study, *The Lancet Oncology*, 2011, 12, 8, 735-742

83. R. Rosell, T. Moran, C. Queralt, R. Porta, F. Cardenal, C. Camps, M. Majem, G. Lopez-Vivanco, D. Isla, M. Provencio, A. Insa, B. Massuti, J. L. Gonzalez-Larriba, L. Paz-Ares, I. Bover, R. Garcia-Campelo, M. A. Moreno, S. Catot, C. Rolfo, N. Reguart, R. Palmero, J. M. S+ínchez, R. Bastus, C. Mayo, J. Bertran-Alamillo, M. A. Molina, J. J. Sanchez and M. Taron, Screening for Epidermal Growth Factor Receptor Mutations in Lung Cancer, *New England Journal of Medicine*, 2009, 361, 10, 958-967

84. Gefitinib: Assessment Report, European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001016/WC500036361.pdf, Accessed: 08.10.2012.

85. E. S. Kim, V. Hirsh, T. Mok, M. A. Socinski, R. Gervais, Y. L. Wu, L. Y. Li, C. L. Watkins, M. V. Sellers, E. S. Lowe, Y. Sun, M. L. Liao, K. +ÿsterlind, M. Reck, A. A. Armour, F. A. Shepherd, S. M. Lippman and J. Y. Douillard, Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial, *The Lancet*, 1922, 372, 9652, 1809-1818

86. T. S. Mok, Y. L. Wu, S. Thongprasert, C. H. Yang, D. T. Chu, N. Saijo, P. Sunpaweravong, B. Han, B. Margono, Y. Ichinose, Y. Nishiwaki, Y. Ohe, J. J. Yang, B. Chewaskulyong, H.

Jiang, E. L. Duffield, C. L. Watkins, A. A. Armour and M. Fukuoka, Gefitinib or Carboplatin & Paclitaxel in Pulmonary Adenocarcinoma, *New England Journal of Medicine*, 2009, 361, 10, 947-957

87. N. Thatcher, A. Chang, P. Parikh, P. J. Rodrigues, T. Ciuleanu, P. J. von, S. Thongprasert, E. H. Tan, K. Pemberton, V. Archer and K. Carroll, Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer), *Lancet*, 2005, 366, 9496, 1527-1537

88. F. R. Hirsch, M. Varella-Garcia, P. A. Bunn, W. A. Franklin, R. Dziadziuszko, N. Thatcher, A. Chang, P. Parikh, J. R. Pereira, T. Ciuleanu, J. von Pawel, C. Watkins, A. Flannery, G. Ellison, E. Donald, L. Knight, D. Parums, N. Botwood and B. Holloway, Molecular Predictors of Outcome With Gefitinib in a Phase III Placebo-Controlled Study in Advanced Non-Small-Cell Lung Cancer, *Journal of Clinical Oncology*, 2006, 24, 31, 5034-5042

89. R. Maruyama, Y. Nishiwaki, T. Tamura, N. Yamamoto, M. Tsuboi, K. Nakagawa, T. Shinkai, S. Negoro, F. Imamura, K. Eguchi, K. Takeda, A. Inoue, K. Tomii, M. Harada, N. Masuda, H. Jiang, Y. Itoh, Y. Ichinose, N. Saijo and M. Fukuoka, Phase III Study, V-15-32, of Gefitinib Versus Docetaxel in Previously Treated Japanese Patients With Non-Small-Cell Lung Cancer, *Journal of Clinical Oncology*, 2008, 26, 26, 4244-4252

90. Catumaxomab: Assessment Report, European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000972/WC500051808.pdf, Accessed: 02.05.2012.

91. M. M. Heiss, P. Murawa, P. Koralewski, E. Kutarska, O. O. Kolesnik, V. V. Ivanchenko, A. S. Dudnichenko, B. Aleknaviciene, A. Razbadauskas, M. Gore, E. Ganea-Motan, T. Ciuleanu, P. Wimberger, A. Schmittel, B. Schmalfeldt, A. Burges, C. Bokemeyer, H. Lindhofer, A. Lahr and S. L. Parsons, The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial, *International Journal of Cancer*, 2010, 127, 9, 2209-2221

92. A. Burges, P. Wimberger, C. K++mper, V. Gorbounova, H. Sommer, B. Schmalfeldt, J. Pfisterer, M. Lichinitser, A. Makhson, V. Moiseyenko, A. Lahr, E. Schulze, M. J+ñger, M. A. Str+¦hlein, M. M. Heiss, T. Gottwald, H. Lindhofer and R. Kimmig, Effective Relief of Malignant Ascites in Patients with Advanced Ovarian Cancer by a Trifunctional Anti-EpCAM +ù Anti-CD3 Antibody: A Phase I/II Study, *Clinical Cancer Research*, 2007, 13, 13, 3899-3905

93. P. Ruf, M. Kluge, M. J+ñger, A. Burges, C. Volovat, M. M. Heiss, J. Hess, P. Wimberger, B. Brandt and H. Lindhofer, Pharmacokinetics, immunogenicity and bioactivity of the therapeutic antibody catumaxomab intraperitoneally administered to cancer patients, *British Journal of Clinical Pharmacology*, 2010, 69, 6, 617-625

94. Fulvestrant: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000540/WC500021171.pdf, Accessed: 09.05.2012.

95. L. Mauriac, J. E. Pippen, J. Quaresma Albano, S. Z. Gertler and C. K. Osborne, Fulvestrant (Faslodex) versus anastrozole for the second-line treatment of advanced breast cancer in subgroups of postmenopausal women with visceral and non-visceral

metastases: combined results from two multicentre trials, *European Journal of Cancer*, 2003, 39, 9, 1228-1233

96. J. F. R. Robertson, C. K. Osborne, A. Howell, S. E. Jones, L. Mauriac, M. Ellis, U. R. Kleeberg, S. E. Come, I. Vergote, S. Gertler, A. Buzdar, A. Webster and C. Morris, Fulvestrant versus anastrozole for the treatment of advanced breast carcinoma in postmenopausal women, *Cancer*, 2003, 98, 2, 229-238

97. Study 0004, AstraZeneca, 2005, http://www.astrazenecaclinicaltrials.com/_mshost800325/content/clinical-trials/resources/pdf/9256854, Accessed: 24.08.2012.

98. L. Perey, R. Paridaens, H. Hawle, K. Zaman, F. Nol+¬, H. Wildiers, M. Fiche, D. Dietrich, P. Cl+¬ment, D. K+¦berle, A. Goldhirsch and B. Th++rlimann, Clinical benefit of fulvestrant in postmenopausal women with advanced breast cancer and primary or acquired resistance to aromatase inhibitors: final results of phase II Swiss Group for Clinical Cancer Research Trial (SAKK 21/00), *Annals of Oncology*, 2007, 18, 1, 64-69

99. Toremifene: Scientific Discussion, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000091/WC500020684.pdf, Accessed: 09.05.2012.

100. M. Gershanovich, A. Garin, D. Baltina, A. Kurvet, L. Kangas, J. Ellm+¬n and E. E. S. G. Eastern European Study Group, A phase III comparison of two toremifene doses to tamoxifen in postmenopausal women with advanced breast cancer, *Breast Cancer Research and Treatment*, 1997, 45, 3, 251-262

101. D. F. Hayes, J. A. Van Zyl, A. Hacking, L. Goedhals, W. R. Bezwoda, J. A. Mailliard, S. E. Jones, C. L. Vogel, R. F. Berris and I. Shemano, Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer, *Journal of Clinical Oncology*, 1995, 13, 10, 2556-2566

102. S. Pyrhonen, R. Valavaara, H. Modig, M. Pawlicki, T. Pienkowski, S. Gundersen, J. Bauer, G. Westman, S. Lundgren, G. Blanco, O. Mella, I. Nilsson, T. Hietanen, I. Hindy, J. Vuorinen and A. Hajba, Comparison of toremifene and tamoxifen in post-menopausal patients with advanced breast cancer: a randomized double-blind, the 'nordic' phase III study, *Br J Cancer*, 1997, 76, 2, 270-277

103. Imatinib: Scientific Discussion (HES/CEL), European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion_-_Variation/human/000406/WC500022211.pdf, Accessed: 11.05.2012.

104. G. Cervetti, S. Galimberti, G. Carulli and M. Petrini, Imatinib therapy in Hypereosinophilic Syndrome: a case of molecular remission, *Leuk.Res*, 2005, 29, 9, 1097-1098

105. K. F. Chung, M. Hew, J. Score, A. V. Jones, A. Reiter, N. C. Cross and B. J. Bain, Cough and hypereosinophilia due to FIP1L1-PDGFRA fusion gene with tyrosine kinase activity, *Eur Respir.J*, 2006, 27, 1, 230-232

106. N. Frickhofen, E. Marker-Hermann, A. Reiter, C. Walz, B. Jung, H. Bauer and A. Hochhaus, Complete molecular remission of chronic eosinophilic leukemia complicated by CNS disease after targeted therapy with imatinib, *Ann Hematol.*, 2004, 83, 7, 477-480

107. S. Imashuku, N. Kakazu, I. Ueda, A. Morimoto, H. Harada, T. Teramura, S. Tamura, Y. Fukushima-Nakase and H. Kuroda, Response to imatinib mesylate in a patient with idiopathic hypereosinophilic syndrome associated with cyclic eosinophil oscillations, *Int J Hematol.*, 2005, 81, 4, 310-314

108. A. D. Klion, J. Robyn, C. Akin, P. Noel, M. Brown, M. Law, D. D. Metcalfe, C. Dunbar and T. B. Nutman, Molecular remission and reversal of myelofibrosis in response to imatinib mesylate treatment in patients with the myeloproliferative variant of hypereosinophilic syndrome, *Blood*, 2004, 103, 2, 473-478

109. S. R. La, G. Specchia, A. Cuneo, D. Beacci, C. Nozzoli, L. Luciano, A. Aventin, C. Sambani, N. Testoni, M. Foppoli, R. Invernizzi, P. Marynen, M. F. Martelli and C. Mecucci, The hypereosinophilic syndrome: fluorescence in situ hybridization detects the del(4)(q12)-FIP1L1/PDGFRA but not genomic rearrangements of other tyrosine kinases, *Haematologica*, 2005, 90, 5, 596-601

110. M. Malagola, G. Martinelli, M. Rondoni, E. Ottaviani, P. P. Piccaluga, P. Ricci, G. Visani and M. Baccarani, Soft tissue and skeletal involvement in FIP1L1-PDGFR-alpha positive chronic eosinophilic leukemia: imatinib mesylate may induce complete molecular and imaging remission, *Haematologica*, 2004, 89, 8, ECR25

111. G. Martinelli, M. Malagola, E. Ottaviani, G. Rosti, E. Trabacchi and M. Baccarani, Imatinib mesylate can induce complete molecular remission in FIP1L1-PDGFR-a positive idiopathic hypereosinophilic syndrome, *Haematologica*, 2004, 89, 2, 236-237

112. G. Martinelli, D. Cilloni, M. Rondoni, F. Messa, E. Ottaviani, E. Gottardi, P. P. Piccaluga, M. Malagola, S. Soverini, S. Merante, G. Alimena, C. Bosi, A. Devivo, G. Rosti, G. Emilia, F. Pane, B. Izzo, N. Testoni, S. Paolini, F. Rancati, R. Bassan, C. Mecucci, M. Baccarani and G. Saglio, Imatinib mesylate can induce molecular complete remission in idiopathic hypereosinophilic syndrome (HES): A phase II multicentric Italian clinical trial, *AACR Meeting Abstracts*, 2006, 2006, 1, 274-227d

113. A. M. Muller, U. M. Martens, S. C. Hofmann, L. Bruckner-Tuderman, R. Mertelsmann and M. Lubbert, Imatinib mesylate as a novel treatment option for hypereosinophilic syndrome: two case reports and a comprehensive review of the literature, *Ann Hematol.*, 2006, 85, 1, 1-16

114. J. Musial, B. Brzezinska-Kolarz, M. Sanak and A. Szczeklik, Improved cardiac function in a patient with hypereosinophilic syndrome treated with imatinib, *Eur J Haematol.*, 2005, 75, 1, 87-88

115. C. Roche-Lestienne, S. Lepers, V. Soenen-Cornu, J. E. Kahn, J. L. Lai, E. Hachulla, F. Drupt, A. L. Demarty, A. S. Roumier, M. Gardembas, M. Dib, N. Philippe, N. Cambier, S. Barete, C. Libersa, O. Bletry, P. Y. Hatron, B. Quesnel, C. Rose, K. Maloum, O. Blanchet, P. Fenaux, L. Prin and C. Preudhomme, Molecular characterization of the idiopathic hypereosinophilic syndrome (HES) in 35 French patients with normal conventional cytogenetics, *Leukemia*, 2005, 19, 5, 792-798

116. K. J. Smith, E. Jacobson, S. Hamza and H. Skelton, Unexplained hypereosinophilia and the need for cytogenetic and molecular genetic analyses, *Arch Dermatol*, 2004, 140, 5, 584-588

117. P. Vandenberghe, I. Wlodarska, L. Michaux, P. Zachee, M. Boogaerts, D. Vanstraelen, M. C. Herregods, H. A. Van, D. Selleslag, F. Roufosse, M. Maerevoet, G. Verhoef, J. Cools, D. G. Gilliland, A. Hagemeijer and P. Marynen, Clinical and molecular features of FIP1L1-PDFGRA (+) chronic eosinophilic leukemias, *Leukemia*, 2004, 18, 4, 734-742

118. Victrelis: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002332/WC500109789.pdf, Accessed: 04.05.2012.

119. B. R. Bacon, S. C. Gordon, E. Lawitz, P. Marcellin, J. M. Vierling, S. Zeuzem, F. Poordad, Z. D. Goodman, H. L. Sings, N. Boparai, M. Burroughs, C. A. Brass, J. K. Albrecht and R. Esteban, Boceprevir for Previously Treated Chronic HCV Genotype 1 Infection, *New England Journal of Medicine*, 2011, 364, 13, 1207-1217

120. F. Poordad, J. McCone, B. R. Bacon, S. Bruno, M. P. Manns, M. S. Sulkowski, I. M. Jacobson, K. R. Reddy, Z. D. Goodman, N. Boparai, M. J. DiNubile, V. Sniukiene, C. A. Brass, J. K. Albrecht and J. P. Bronowicki, Boceprevir for Untreated Chronic HCV Genotype 1 Infection, *New England Journal of Medicine*, 2011, 364, 13, 1195-1206

121. Boceprevir in Combination With Peginterferon Alfa-2a and Ribavirin in Participants With Chronic Hepatitis C Genotype 1 Who Failed Prior Treatment With Peginterferon/Ribavirin (Study P05685AM2)(COMPLETED), ClinicalTrials.gov, 2011, http://www.clinicaltrials.gov/ct2/show/study/NCT00845065?term=p05685&rank=1&sect=X0125, Accessed: 05.05.2012.

122. Telaprevir: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002313/WC500115532.pdf, Accessed: 21.03.2012.

123. I. M. Jacobson, J. G. McHutchison, G. Dusheiko, A. M. Di Bisceglie, K. R. Reddy, N. H. Bzowej, P. Marcellin, A. J. Muir, P. Ferenci, R. Flisiak, J. George, M. Rizzetto, D. Shouval, R. Sola, R. A. Terg, E. M. Yoshida, N. Adda, L. Bengtsson, A. J. Sankoh, T. L. Kieffer, S. George, R. S. Kauffman and S. Zeuzem, Telaprevir for previously untreated chronic hepatitis C virus infection, *N Engl.J Med*, 2011, 364, 25, 2405-2416

124. K. E. Sherman, S. L. Flamm, N. H. Afdhal, D. R. Nelson, M. S. Sulkowski, G. T. Everson, M. W. Fried, M. Adler, H. W. Reesink, M. Martin, A. J. Sankoh, N. Adda, R. S. Kauffman, S. George, C. I. Wright and F. Poordad, Response-guided telaprevir combination treatment for hepatitis C virus infection, *N Engl.J Med*, 2011, 365, 11, 1014-1024

125. S. Zeuzem, P. Andreone, S. Pol, E. Lawitz, M. Diago, S. Roberts, R. Focaccia, Z. Younossi, G. R. Foster, A. Horban, P. Ferenci, F. Nevens, B. Mullhaupt, P. Pockros, R. Terg, D. Shouval, H. B. van, O. Weiland, H. R. Van, M. S. De, D. Luo, G. Boogaerts, R. Polo, G. Picchio and M. Beumont, Telaprevir for retreatment of HCV infection, *N Engl.J Med*, 2011, 364, 25, 2417-2428

126. Virology Follow up Study in Subjects Previously Treated With Telaprevir (Study 112), ClinicalTrials.gov, 2011, http://clinicaltrials.gov/ct2/show/NCT00916474, Accessed: 21.03.2012.

127. Ivacaftor: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002494/WC500130766.pdf, Accessed: 23.04.2013.

128. B. W. Ramsey, J. Davies, N. G. McElvaney, E. Tullis, S. C. Bell, P. D+Öev+¡nek, M. Griese, E. F. McKone, C. E. Wainwright, M. W. Konstan, R. Moss, F. Ratjen, I. Sermet-Gaudelus, S. M. Rowe, Q. Dong, S. Rodriguez, K. Yen, C. Ordo+¦ez and J. S. Elborn, A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation, *New England Journal of Medicine*, 2011, 365, 18, 1663-1672

129. Degarelix: Scientific Discussion, European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000986/WC500023256.pdf, Accessed: 29.04.2013.

130. L. Klotz, L. Boccon-Gibod, N. D. Shore, C. Andreou, B. E. Persson, P. Cantor, J. K. Jensen, T. K. Olesen and F. H. Schr+¦der, The efficacy and safety of degarelix: a 12-month, comparative, randomized, open-label, parallel-group phase III study in patients with prostate cancer, *BJU International*, 2008, 102, 11, 1531-1538

131. Everolimus: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/001038/WC500130788.pdf, Accessed: 28.05.2013.

132. J. Baselga, M. Campone, M. Piccart, H. A. Burris, H. S. Rugo, T. Sahmoud, S. Noguchi, M. Gnant, K. I. Pritchard, F. Lebrun, J. T. Beck, Y. Ito, D. Yardley, I. Deleu, A. Perez, T. Bachelot, L. Vittori, Z. Xu, P. Mukhopadhyay, D. Lebwohl and G. N. Hortobagyi, Everolimus in Postmenopausal Hormone-ReceptorGÇôPositive Advanced Breast Cancer, *New England Journal of Medicine*, 2011, 366, 6, 520-529

133. J. Baselga, V. Semiglazov, P. van Dam, A. Manikhas, M. Bellet, J. Mayordomo, M. Campone, E. Kubista, R. Greil, G. Bianchi, J. Steinseifer, B. Molloy, E. Tokaji, H. Gardner, P. Phillips, M. Stumm, H. A. Lane, J. M. Dixon, W. Jonat and H. S. Rugo, Phase II Randomized Study of Neoadjuvant Everolimus Plus Letrozole Compared With Placebo Plus Letrozole in Patients With Estrogen ReceptorGÇôPositive Breast Cancer, *Journal of Clinical Oncology*, 2009, 27, 16, 2630-2637

134. Lapatinib: Assessment Report, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000795/WC500044960.pdf, Accessed: 24.05.2013.

135. D. Cameron, M. Casey, C. Oliva, B. Newstat, B. Imwalle and C. E. Geyer, Lapatinib Plus Capecitabine in Women with HER-2 Positive Advanced Breast Cancer: Final Survival Analysis of a Phase III Randomized Trial, *The Oncologist*, 2010, 15, 9, 924-934

136. C. E. Geyer, J. Forster, D. Lindquist, S. Chan, C. G. Romieu, T. Pienkowski, A. Jagiello-Gruszfeld, J. Crown, A. Chan, B. Kaufman, D. Skarlos, M. Campone, N. Davidson, M. Berger, C. Oliva, S. D. Rubin, S. Stein and D. Cameron, Lapatinib plus Capecitabine for

HER2-Positive Advanced Breast Cancer, *New England Journal of Medicine*, 2006, 355, 26, 2733-2743

137. Protocol Summary for EGF103659, GlaxoSmithKleine, 2013, Accessed: 24.05.2013.

138. N. U. Lin, V. Di+¬ras, D. Paul, D. Lossignol, C. Christodoulou, H. J. Stemmler, H. Roch+¬, M. C. Liu, R. Greil, E. Ciruelos, S. Loibl, S. Gori, A. Wardley, D. Yardley, A. Brufsky, J. L. Blum, S. D. Rubin, B. Dharan, K. Steplewski, D. Zembryki, C. Oliva, D. Roychowdhury, P. Paoletti and E. P. Winer, Multicenter Phase II Study of Lapatinib in Patients with Brain Metastases from HER2-Positive Breast Cancer, *Clinical Cancer Research*, 2009, 15, 4, 1452-1459

139. Pertuzumab: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002547/WC500141004.pdf, Accessed: 28.05.2013.

140. J. Baselga, J. Cort+¬s, S. B. Kim, S. A. Im, R. Hegg, Y. H. Im, L. Roman, J. L. Pedrini, T. Pienkowski, A. Knott, E. Clark, M. C. Benyunes, G. Ross and S. M. Swain, Pertuzumab plus Trastuzumab plus Docetaxel for Metastatic Breast Cancer, *New England Journal of Medicine*, 2011, 366, 2, 109-119

141. J. Baselga, K. A. Gelmon, S. Verma, A. Wardley, P. Conte, D. Miles, G. Bianchi, J. Cortes, V. A. McNally, G. A. Ross, P. Fumoleau and L. Gianni, Phase II Trial of Pertuzumab and Trastuzumab in Patients With Human Epidermal Growth Factor Receptor Positive Metastatic Breast Cancer That Progressed During Prior Trastuzumab Therapy, *Journal of Clinical Oncology*, 2010, 28, 7, 1138-1144

142. L. Gianni, T. Pienkowski, Y. H. Im, L. Roman, L. M. Tseng, M. C. Liu, A. Lluch, E. b. Staroslawska, J. de la Haba-Rodriguez, S. A. Im, J. L. Pedrini, B. Poirier, P. Morandi, V. Semiglazov, V. Srimuninnimit, G. Bianchi, T. Szado, J. Ratnayake, G. Ross and P. Valagussa, Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial, *The Lancet Oncology*, 2012, 13, 1, 25-32

143. Trastuzumab (gastric cancer): Scientific discussion, European Medicines Agency, 2010, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000278/WC500074921.pdf, Accessed: 19.12.2012.

144. Y. J. Bang, E. Van Cutsem, A. Feyereislova, H. C. Chung, L. Shen, A. Sawaki, F. Lordick, A. Ohtsu, Y. Omuro, T. Satoh, G. Aprile, E. Kulikov, J. Hill, M. Lehle, J. R++schoff and Y. K. Kang, Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial, *The Lancet*, 2010, 376, 9742, 687-697

145. Ziagen: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000252/WC500050343.pdf, Accessed: 30.04.2012.

146. Kivexa: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000581/WC500043730.pdf, Accessed: 30.04.2012.

147. Trizivir: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000338/WC500043448.pdf, Accessed: 28.07.2011.

148. S. Mallal, E. Phillips, G. Carosi, J. M. Molina, C. Workman, J. Toma++i-ì, E. J+ñgel-Guedes, S. Rugina, O. Kozyrev, J. F. Cid, P. Hay, D. Nolan, S. Hughes, A. Hughes, S. Ryan, N. Fitch, D. Thorborn and A. Benbow, HLA-B*5701 Screening for Hypersensitivity to Abacavir, *New England Journal of Medicine*, 2008, 358, 6, 568-579

149. Zoledronic acid: Withdrawal Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2011/03/WC500102910.pdf, Accessed: 28.05.2013.

150. M. Gnant, B. Mlineritsch, H. Stoeger, G. Luschin-Ebengreuth, D. Heck, C. Menzel, R. Jakesz, M. Seifert, M. Hubalek, G. Pristauz, T. Bauernhofer, H. Eidtmann, W. Eiermann, G. Steger, W. Kwasny, P. Dubsky, G. Hochreiner, E. P. Forsthuber, C. Fesl and R. Greil, Adjuvant endocrine therapy plus zoledronic acid in premenopausal women with early-stage breast cancer: 62-month follow-up from the ABCSG-12 randomised trial, *The Lancet Oncology*, 2011, 12, 7, 631-641

151. A. Llombart, A. Frassoldati, O. Paija, H. P. Sleeboom, G. Jerusalem, J. Mebis, I. Deleu, J. Miller, N. Schenk and P. Neven, Immediate Administration of Zoledronic Acid Reduces Aromatase Inhibitor Associated Bone Loss in Postmenopausal Women With Early Breast Cancer: 12-Month Analysis of the E-ZO-FAST Trial, *Clinical Breast Cancer*, 2012, 12, 1, 40-48

152. A. M. Brufsky, L. D. Bosserman, R. R. Caradonna, B. B. Haley, C. M. Jones, H. C. F. Moore, L. Jin, G. M. Warsi, S. G. Ericson and E. A. Perez, Zoledronic Acid Effectively Prevents Aromatase Inhibitor Associated Bone Loss in Postmenopausal Women with Early Breast Cancer Receiving Adjuvant Letrozole: Z-FAST Study 36-Month Follow-up Results, *Clinical Breast Cancer*, 2009, 9, 2, 77-85

153. N. J. Bundred, I. D. Campbell, N. Davidson, R. H. DeBoer, H. Eidtmann, A. Monnier, P. Neven, G. von Minckwitz, J. C. Miller, N. L. Schenk and R. E. Coleman, Effective inhibition of aromatase inhibitor-associated bone loss by zoledronic acid in postmenopausal women with early breast cancer receiving adjuvant letrozole, *Cancer*, 2008, 112, 5, 1001-1010

154. Imatinib: Scientific Discussion (GIST), European Medicines Agency, 2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000406/WC500022212.pdf, Accessed: 18.07.2012.

155. C. D. Blanke, C. Rankin, G. D. Demetri, C. W. Ryan, M. M. von, R. S. Benjamin, A. K. Raymond, V. H. Bramwell, L. H. Baker, R. G. Maki, M. Tanaka, J. R. Hecht, M. C. Heinrich, C. D. Fletcher, J. J. Crowley and E. C. Borden, Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033, *J Clin Oncol*, 2008, 26, 4, 626-632

156. C. D. Blanke, G. D. Demetri, M. von Mehren, M. C. Heinrich, B. Eisenberg, J. A. Fletcher, C. L. Corless, C. D. M. Fletcher, P. J. Roberts, D. Heinz, E. Wehre, Z. Nikolova and H. Joensuu, Long-Term Results From a Randomized Phase II Trial of Standard- Versus Higher-Dose Imatinib Mesylate for Patients With Unresectable or Metastatic Gastrointestinal Stromal Tumors Expressing KIT, *Journal of Clinical Oncology*, 2008, 26, 4, 620-625

157. Erbitux: Assessment Report, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000558/WC500029117.pdf, Accessed: 26.03.2012.

158. C. S. Karapetis, S. Khambata-Ford, D. J. Jonker, C. J. O'Callaghan, D. Tu, N. C. Tebbutt, R. J. Simes, H. Chalchal, J. D. Shapiro, S. Robitaille, T. J. Price, L. Shepherd, H. J. Au, C. Langer, M. J. Moore and J. R. Zalcberg, K-ras mutations and benefit from cetuximab in advanced colorectal cancer, *N Engl.J Med*, 2008, 359, 17, 1757-1765

159. D. J. Jonker, C. J. O'Callaghan, C. S. Karapetis, J. R. Zalcberg, D. Tu, H. J. Au, S. R. Berry, M. Krahn, T. Price, R. J. Simes, N. C. Tebbutt, H. G. van, R. Wierzbicki, C. Langer and M. J. Moore, Cetuximab for the treatment of colorectal cancer, *N Engl J Med*, 2007, 357, 20, 2040-2048

160. C. E. Van, C. H. Kohne, I. Lang, G. Folprecht, M. P. Nowacki, S. Cascinu, I. Shchepotin, J. Maurel, D. Cunningham, S. Tejpar, M. Schlichting, A. Zubel, I. Celik, P. Rougier and F. Ciardiello, Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status, *J Clin Oncol*, 2011, 29, 15, 2011-2019

161. C. E. Van, C. H. Kohne, E. Hitre, J. Zaluski, C. R. Chang Chien, A. Makhson, G. D'Haens, T. Pinter, R. Lim, G. Bodoky, J. K. Roh, G. Folprecht, P. Ruff, C. Stroh, S. Tejpar, M. Schlichting, J. Nippgen and P. Rougier, Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer, *N Engl.J Med*, 2009, 360, 14, 1408-1417

162. A. F. Sobrero, J. Maurel, L. Fehrenbacher, W. Scheithauer, Y. A. Abubakr, M. P. Lutz, M. E. Vega-Villegas, C. Eng, E. U. Steinhauer, J. Prausova, H. J. Lenz, C. Borg, G. Middleton, H. Kroning, G. Luppi, O. Kisker, A. Zubel, C. Langer, J. Kopit and H. A. Burris, III, EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer, *J Clin Oncol*, 2008, 26, 14, 2311-2319

163. C. Bokemeyer, I. Bondarenko, J. T. Hartmann, B. F. de, G. Schuch, A. Zubel, I. Celik, M. Schlichting and P. Koralewski, Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study, *Ann Oncol*, 2011, 22, 7, 1535-1546

164. K. M. Tveit, T. Guren, B. Glimelius, P. Pfeiffer, H. Sorbye, S. Pyrhonen, F. Sigurdsson, E. Kure, T. Ikdahl, E. Skovlund, T. Fokstuen, F. Hansen, E. Hofsli, E. Birkemeyer, A. Johnsson, H. Starkhammar, M. K. Yilmaz, N. Keldsen, A. B. Erdal, O. Dajani, O. Dahl and T. Christoffersen, Phase III Trial of Cetuximab With Continuous or Intermittent Fluorouracil, Leucovorin, and Oxaliplatin (Nordic FLOX) Versus FLOX Alone in First-Line Treatment of

Metastatic Colorectal Cancer: The NORDIC-VII Study, *Journal of Clinical Oncology*, 2012,

165. Panitumumab: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000741/WC500047707.pdf, Accessed: 22.02.2013.

166. E. Van Cutsem, M. Peeters, S. Siena, Y. Humblet, A. Hendlisz, B. Neyns, J. L. Canon, J. L. Van Laethem, J. Maurel, G. Richardson, M. Wolf and R. G. Amado, Open-Label Phase III Trial of Panitumumab Plus Best Supportive Care Compared With Best Supportive Care Alone in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer, *Journal of Clinical Oncology*, 2007, 25, 13, 1658-1664

167. R. G. Amado, M. Wolf, M. Peeters, E. Van Cutsem, S. Siena, D. J. Freeman, T. Juan, R. Sikorski, S. Suggs, R. Radinsky, S. D. Patterson and D. D. Chang, Wild-Type KRAS Is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer, *Journal of Clinical Oncology*, 2008, 26, 10, 1626-1634

168. Alipogene tiparvovec: Assessment Report, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002145/WC500135476.pdf, Accessed: 25.04.2013.

169. Methylthioninium chloride: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002108/WC500107131.pdf, Accessed: 09.05.2012.

170. Imatinib for MDS/MPD: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion_-_Variation/human/000406/WC500022210.pdf, Accessed: 08.03.2012.

171. J. F. Apperley, M. Gardembas, J. V. Melo, R. Russell-Jones, B. J. Bain, E. J. Baxter, A. Chase, J. M. Chessells, M. Colombat, C. E. Dearden, S. Dimitrijevic, F. X. Mahon, D. Marin, Z. Nikolova, E. Olavarria, S. Silberman, B. Schultheis, N. C. Cross and J. M. Goldman, Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta, *N Engl.J Med*, 2002, 347, 7, 481-487

172. J. Cortes, F. Giles, S. O'Brien, D. Thomas, M. Albitar, M. B. Rios, M. Talpaz, G. Garcia-Manero, S. Faderl, L. Letvak, A. Salvado and H. Kantarjian, Results of imatinib mesylate therapy in patients with refractory or recurrent acute myeloid leukemia, high-risk myelodysplastic syndrome, and myeloproliferative disorders, *Cancer*, 2003, 97, 11, 2760-2766

173. J. L. Garcia, M. J. Font de, J. M. Hernandez, J. A. Queizan, N. C. Gutierrez, J. M. Hernandez and J. F. San Miguel, Imatinib mesylate elicits positive clinical response in atypical chronic myeloid leukemia involving the platelet-derived growth factor receptor beta, *Blood*, 2003, 102, 7, 2699-2700

174. F. H. Grand, S. Burgstaller, T. Kuhr, E. J. Baxter, G. Webersinke, J. Thaler, A. J. Chase and N. C. Cross, p53-Binding protein 1 is fused to the platelet-derived growth factor

receptor beta in a patient with a t(5;15)(q33;q22) and an imatinib-responsive eosinophilic myeloproliferative disorder, *Cancer Res*, 2004, 64, 20, 7216-7219

175. R. L. Levine, M. Wadleigh, D. W. Sternberg, I. Wlodarska, I. Galinsky, R. M. Stone, D. J. DeAngelo, D. G. Gilliland and J. Cools, KIAA1509 is a novel PDGFRB fusion partner in imatinib-responsive myeloproliferative disease associated with a t(5;14)(q33;q32), *Leukemia*, 2005, 19, 1, 27-30

176. M. K. Magnusson, K. E. Meade, R. Nakamura, J. Barrett and C. E. Dunbar, Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene, *Blood*, 2002, 100, 3, 1088-1091

177. A. Pardanani, T. Reeder, L. F. Porrata, C. Y. Li, H. D. Tazelaar, E. J. Baxter, T. E. Witzig, N. C. Cross and A. Tefferi, Imatinib therapy for hypereosinophilic syndrome and other eosinophilic disorders, *Blood*, 2003, 101, 9, 3391-3397

178. V. Pitini, C. Arrigo, D. Teti, G. Barresi, M. Righi and G. Alo, Response to STI571 in chronic myelomonocytic leukemia with platelet derived growth factor beta receptor involvement: a new case report, *Haematologica*, 2003, 88, 5, ECR18

179. A. M. Safley, S. Sebastian, T. S. Collins, C. A. Tirado, T. T. Stenzel, J. Z. Gong and B. K. Goodman, Molecular and cytogenetic characterization of a novel translocation t(4;22) involving the breakpoint cluster region and platelet-derived growth factor receptor-alpha genes in a patient with atypical chronic myeloid leukemia, *Genes Chromosomes Cancer*, 2004, 40, 1, 44-50

180. P. Trempat, C. Villalva, G. Laurent, F. Armstrong, G. Delsol, N. Dastugue and P. Brousset, Chronic myeloproliferative disorders with rearrangement of the platelet-derived growth factor alpha receptor: a new clinical target for STI571/Glivec, *Oncogene*, 2003, 22, 36, 5702-5706

181. J. L. Vizmanos, F. J. Novo, J. P. Roman, E. J. Baxter, I. Lahortiga, M. J. Larrayoz, M. D. Odero, P. Giraldo, M. J. Calasanz and N. C. Cross, NIN, a gene encoding a CEP110-like centrosomal protein, is fused to PDGFRB in a patient with a t(5;14)(q33;q24) and an imatinib-responsive myeloproliferative disorder, *Cancer Res*, 2004, 64, 8, 2673-2676

182. K. Wilkinson, E. R. Velloso, L. F. Lopes, C. Lee, J. C. Aster, M. A. Shipp and R. C. Aguiar, Cloning of the t(1;5)(q23;q33) in a myeloproliferative disorder associated with eosinophilia: involvement of PDGFRB and response to imatinib, *Blood*, 2003, 102, 12, 4187-4190

183. B. Wittman, J. Horan, J. Baxter, J. Goldberg, R. Felgar, E. Baylor, B. Cromwell, N. Cross and J. M. Bennett, A 2-year-old with atypical CML with a t(5;12)(q33;p13) treated successfully with imatinib mesylate, *Leuk.Res*, 2004, 28 Suppl 1, S65-S69

184. Dasatinib: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000709/WC500056995.pdf, Accessed: 30.05.2013.

185. J. Cortes, P. Rousselot, D. W. Kim, E. Ritchie, N. Hamerschlak, S. Coutre, A. Hochhaus, F. Guilhot, G. Saglio, J. Apperley, O. Ottmann, N. Shah, P. Erben, S. Branford, P. Agarwal, A. Gollerkeri and M. Baccarani, Dasatinib induces complete hematologic and

cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis, *Blood*, 2007, 109, 8, 3207-3213

186. Final Clinical Study Report for CA180015: Lymphoid Blast Phase CML, Bristol-Myers Squibb, 2008, http://ctr.bms.com/pdf/CA180015ST-1.pdf, Accessed: 30.05.2013.

187. Final Clinical Study Report for CA180015: Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia, Bristol-Myers Squibb, 2008, http://ctr.bms.com/pdf//CA180015ST-2.pdf, Accessed: 30.05.2013.

188. Final Clinical Study Report for Study CA180002, Bristol-Myers Squibb, 2006, http://ctr.bms.com/pdf//CA180002.pdf, Accessed: 30.05.2013.

189. M. Talpaz, N. P. Shah, H. Kantarjian, N. Donato, J. Nicoll, R. Paquette, J. Cortes, S. O'Brien, C. Nicaise, E. Bleickardt, M. A. Blackwood-Chirchir, V. Iyer, T. T. Chen, F. Huang, A. P. Decillis and C. L. Sawyers, Dasatinib in Imatinib-Resistant Philadelphia Chromosome Positive Leukemias, *New England Journal of Medicine*, 2006, 354, 24, 2531-2541

190. Imatinib: Scientific Discussion (ALL), European Medicines Agency, 2007, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000406/human_med_000808.jsp&mid=WC0b01ac058001d124, Accessed: 29.05.2013.

191. O. G. Ottmann, B. J. Druker, C. L. Sawyers, J. M. Goldman, J. Reiffers, R. T. Silver, S. Tura, T. Fischer, M. W. Deininger, C. A. Schiffer, M. Baccarani, A. Gratwohl, A. Hochhaus, D. Hoelzer, S. Fernandes-Reese, I. Gathmann, R. Capdeville and S. G. O'Brien, A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome positive acute lymphoid leukemias, *Blood*, 2002, 100, 6, 1965-1971

192. R. Capdeville, T. Krahnke, A. Hatfield, J. M. Ford, I. Van Hoomissen and I. Gathmann, Report of an international expanded access program of imatinib in adults with Philadelphia chromosome positive leukemias, *Annals of Oncology*, 2008, 19, 7, 1320-1326

193. B. J. Druker, C. L. Sawyers, H. Kantarjian, D. J. Resta, S. F. Reese, J. M. Ford, R. Capdeville and M. Talpaz, Activity of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in the Blast Crisis of Chronic Myeloid Leukemia and Acute Lymphoblastic Leukemia with the Philadelphia Chromosome, *New England Journal of Medicine*, 2001, 344, 14, 1038-1042

194. B. Wassmann, H. Pfeifer, N. Goekbuget, D. W. Beelen, J. Beck, M. Stelljes, M. Bornh+ñuser, A. Reichle, J. Perz, R. Haas, A. Ganser, M. Schmid, L. Kanz, G. Lenz, M. Kaufmann, A. Binckebanck, P. Br++ck, R. Reutzel, H. Gschaidmeier, S. Schwartz, D. Hoelzer and O. G. Ottmann, Alternating versus concurrent schedules of imatinib and chemotherapy as front-line therapy for Philadelphia-positive acute lymphoblastic leukemia (Ph+ALL), *Blood*, 2006, 108, 5, 1469-1477

195. O. G. Ottmann, B. Wassmann, H. Pfeifer, A. Giagounidis, M. Stelljes, U. D++hrsen, M. Schmalzing, L. Wunderle, A. Binckebanck and D. Hoelzer, Imatinib compared with chemotherapy as front-line treatment of elderly patients with Philadelphia

chromosome-positive acute lymphoblastic leukemia (Ph+ALL), *Cancer*, 2007, 109, 10, 2068-2076

196. A. Delannoy, E. Delabesse, V. Lheritier, S. Castaigne, F. Rigal-Huguet, E. Raffoux, F. Garban, O. Legrand, S. Bologna, V. Dubruille, P. Turlure, O. Reman, M. Delain, F. Isnard, D. Coso, P. Raby, A. Buzyn, S. Cailleres, S. Darre, C. Fohrer, A. Sonet, C. Bilhou-Nabera, M. C. Bene, H. Dombret, P. Berthaud and X. Thomas, Imatinib and methylprednisolone alternated with chemotherapy improve the outcome of elderly patients with Philadelphia-positive acute lymphoblastic leukemia: results of the GRAALL AFR09 study, *Leukemia*, 2006, 20, 9, 1526-1532

197. M. Yanada, J. Takeuchi, I. Sugiura, H. Akiyama, N. Usui, F. Yagasaki, K. Nishii, Y. Ueda, M. Takeuchi, S. Miyawaki, A. Maruta, H. Narimatsu, Y. Miyazaki, S. Ohtake, I. Jinnai, K. Matsuo, T. Naoe and R. Ohno, Karyotype at diagnosis is the major prognostic factor predicting relapse-free survival for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia treated with imatinib-combined chemotherapy, *Haematologica*, 2008, 93, 2, 287-290

198. Arsenic Trioxide: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000388/WC500042839.pdf, Accessed: 14.05.2013.

199. S. L. Soignet, P. Maslak, Z. G. Wang, S. Jhanwar, E. Calleja, L. J. Dardashti, D. Corso, A. DeBlasio, J. Gabrilove, D. A. Scheinberg, P. P. Pandolfi and R. P. Warrell, Complete Remission after Treatment of Acute Promyelocytic Leukemia with Arsenic Trioxide, *New England Journal of Medicine*, 1998, 339, 19, 1341-1348

200. S. L. Soignet, S. R. Frankel, D. Douer, M. S. Tallman, H. Kantarjian, E. Calleja, R. M. Stone, M. Kalaycio, D. A. Scheinberg, P. Steinherz, E. L. Sievers, S. Coutr+¬, S. Dahlberg, R. Ellison and R. P. Warrell, United States Multicenter Study of Arsenic Trioxide in Relapsed Acute Promyelocytic Leukemia, *Journal of Clinical Oncology*, 2001, 19, 18, 3852-3860

201. Amprenavir: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000264/WC500022925.pdf, Accessed: 20.06.2012.

202. PROAB3004 - results summary, GlaxoSmithKline, 2005, http://download.gsk-clinicalstudyregister.com/files/675.pdf, Accessed: 09.09.2013.

203. M. Maguire, D. Shortino, A. Klein, W. Harris, V. Manohitharajah, M. Tisdale, R. Elston, J. Yeo, S. Randall, F. Xu, H. Parker, J. May and W. Snowden, Emergence of resistance to protease inhibitor amprenavir in human immunodeficiency virus type 1-infected patients: selection of four alternative viral protease genotypes and influence of viral susceptibility to coadministered reverse transcriptase nucleoside inhibitors, *Antimicrob.Agents Chemother.*, 2002, 46, 3, 731-738

204. Atazanavir: Scientific Discussion, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000494/WC500056384.pdf, Accessed: 11.01.2013.

205. E. Siwak, T. Mikula, W. Sta+äczak and A. Wiercinska-Drapalo, Efficacy and safety of boosted atazanavir in HIV-infected, ARV-naive patients GÇö results from 48/96 weeks Castle study, *HIV & AIDS Review*, 2009, 8, 1, 5-8

206. Darunavir: Scientific Discussion, European Medicines Agency, 2008, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000707/WC500041754.pdf, Accessed: 08.03.2012.

207. Atripla: Scientific Discussion, European Medicines Agency, 2007, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000797/WC500028105.pdf, Accessed: 23.07.2012.

208. N. A. Margot, J. Enejosa, A. K. Cheng, M. D. Miller, D. J. McColl and a. t. Study, Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared With Lamivudine/Zidovudine and Efavirenz in Study GS-01-934, *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 2009, 52, 2,

209. J. E. Gallant, E. DeJesus, J. R. Arribas, A. L. Pozniak, B. Gazzard, R. E. Campo, B. Lu, D. McColl, S. Chuck, J. Enejosa, J. J. Toole and A. K. Cheng, Tenofovir DF, Emtricitabine, and Efavirenz vs. Zidovudine, Lamivudine, and Efavirenz for HIV, *New England Journal of Medicine*, 2006, 354, 3, 251-260

210. Emtricitabine: Scientific Discussion, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000533/WC500055657.pdf, Accessed: 20.08.2012.

211. C. A. Benson, C. van der Horst, A. LaMarca, D. W. Haas, C. K. McDonald, C. R. Steinhart, J. Rublein, J. B. Quinn, E. Mondou, F. Rousseau and a. t. FTC-, A randomized study of emtricitabine and lamivudine in stably suppressed patients with HIV, *AIDS*, 2004, 18, 17,

212. Enfuvirtide: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000514/WC500024739.pdf, Accessed: 21.09.2012.

213. J. P. Lalezari, K. Henry, M. O'Hearn, J. S. G. Montaner, P. J. Piliero, B. i. Trottier, S. Walmsley, C. Cohen, D. R. Kuritzkes, J. J. Eron, J. Chung, R. DeMasi, L. Donatacci, C. Drobnes, J. Delehanty and M. Salgo, Enfuvirtide, an HIV-1 Fusion Inhibitor, for Drug-Resistant HIV Infection in North and South America, *New England Journal of Medicine*, 2003, 348, 22, 2175-2185

214. A. Lazzarin, B. Clotet, D. Cooper, J. Reynes, K. Arast+¬h, M. Nelson, C. Katlama, H. J. r. Stellbrink, J. F. o. Delfraissy, J. Lange, L. Huson, R. DeMasi, C. Wat, J. Delehanty, C. Drobnes and M. Salgo, Efficacy of Enfuvirtide in Patients Infected with Drug-Resistant HIV-1 in Europe and Australia, *New England Journal of Medicine*, 2003, 348, 22, 2186-2195

215. Eviplera: Assessment Report, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002312/WC500118803.pdf, Accessed: 31.07.2012.

216. J. M. Molina, P. Cahn, B. Grinsztejn, A. Lazzarin, A. Mills, M. Saag, K. Supparatpinyo, S. Walmsley, H. Crauwels, L. T. Rimsky, S. Vanveggel and K. Boven, Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial, *The Lancet*, 1916, 378, 9787, 238-246

217. C. J. Cohen, J. ndrade-Villanueva, B. Clotet, J. Fourie, M. A. Johnson, K. Ruxrungtham, H. Wu, C. Zorrilla, H. Crauwels, L. T. Rimsky, S. Vanveggel and K. Boven, Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial, *The Lancet*, 1916, 378, 9787, 229-237

218. Fosamprenavir: Scientific Discussion, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000534/WC500035343.pdf, Accessed: 30.04.2013.

219. J. C. J. Gathe, P. Ive, R. Wood, D. Sch++rmann, N. C. Bellos, E. DeJesus, A. Gladysz, C. Garris and J. Yeo, SOLO: 48-week efficacy and safety comparison of once-daily fosamprenavir /ritonavir versus twice-daily nelfinavir in naive HIV-1-infected patients, *AIDS*, 2004, 18, 11,

220. A. Rodriguez-French, J. Boghossian, G. E. Gray, J. P. Nadler, A. R. Quinones, G. E. Sepulveda, J. M. Millard and P. G. Wannamaker, The NEAT Study: A 48-Week Open-Label Study to Compare the Antiviral Efficacy and Safety of GW433908 Versus Nelfinavir in Antiretroviral Therapy-Naive HIV-1-Infected Patients, *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 2004, 35, 1,

221. Lopinavir/ ritonavir: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion_-_Variation/human/000368/WC500039047.pdf, Accessed: 16.01.2013.

222. Nelfinavir: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000164/WC500050625.pdf, Accessed: 16.05.2013.

223. M. S. Saag, P. Tebas, M. Sension, M. Conant, R. Myers, S. K. Chapman, R. Anderson and N. Clendeninn, Randomized, double-blind comparison of two nelfinavir doses plus nucleosides in HIV-infected patients (Agouron study 511), *AIDS*, 2001, 15, 15, 1971-1978

224. Tenofovir: Scientific Discussion, European Medicines Agency, 2006, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000419/WC500051732.pdf, Accessed: 23.05.2013.

225. R. T. Schooley, P. Ruane, R. A. Myers, G. Beall, H. Lampiris, D. Berger, S. S. Chen, M. D. Miller, E. Isaacson and A. K. Cheng, Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study, *AIDS*, 2002, 16, 9, 1257-1263

226. K. Squires, A. L. Pozniak, G. Pierone, Jr., C. R. Steinhart, D. Berger, N. C. Bellos, S. L. Becker, M. Wulfsohn, M. D. Miller, J. J. Toole, D. F. Coakley and A. Cheng, Tenofovir

disoproxil fumarate in nucleoside-resistant HIV-1 infection: a randomized trial, *Ann Intern.Med*, 2003, 139, 5 Pt 1, 313-320

227. J. E. Gallant, S. Staszewski, A. L. Pozniak, E. Dejesus, J. M. Suleiman, M. D. Miller, D. F. Coakley, B. Lu, J. J. Toole and A. K. Cheng, Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial, *JAMA*, 2004, 292, 2, 191-201

228. Lapatinib: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000795/WC500044957.pdf, Accessed: 24.05.2013.

229. Tipranavir: Scientific Discussion, European Medicines Agency, 2005, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000631/WC500025932.pdf, Accessed: 18.07.2012.

230. C. B. Hicks, P. Cahn, D. A. Cooper, S. L. Walmsley, C. Katlama, B. Clotet, A. Lazzarin, M. A. Johnson, D. Neubacher, D. Mayers and H. Valdez, Durable efficacy of tipranavir-ritonavir in combination with an optimised background regimen of antiretroviral drugs for treatment-experienced HIV-1-infected patients at 48 weeks in the Randomized Evaluation of Strategic Intervention in multi-drug reSistant patients with Tipranavir (RESIST) studies: an analysis of combined data from two randomised open-label trials, *Lancet*, 2006, 368, 9534, 466-475

231. J. Gathe, D. A. Cooper, C. Farthing, D. Jayaweera, D. Norris, G. Pierone, C. R. Steinhart, B. Trottier, S. L. Walmsley, C. Workman, G. Mukwaya, V. Kohlbrenner, C. Dohnanyi, S. McCallister, D. Mayers and f. t. RESIST-, Efficacy of the Protease Inhibitors Tipranavir plus Ritonavir in Treatment-Experienced Patients: 24-Week Analysis from the RESIST-1 Trial, *Clinical Infectious Diseases*, 2006, 43, 10, 1337-1346

232. P. Cahn, J. Villacian, A. Lazzarin, C. Katlama, B. Grinsztejn, K. Arasteh, P. L+¦pez, N. Clumeck, J. Gerstoft, N. Stavrianeas, S. Moreno, F. Antunes, D. Neubacher and D. Mayers, Ritonavir-Boosted Tipranavir Demonstrates Superior Efficacy to Ritonavir-Boosted Protease Inhibitors in Treatment-Experienced HIV-Infected Patients: 24-Week Results of the RESIST-2 Trial, *Clinical Infectious Diseases*, 2006, 43, 10, 1347-1356

233. J. D. Baxter, J. M. Schapiro, C. A. Boucher, V. M. Kohlbrenner, D. B. Hall, J. R. Scherer and D. L. Mayers, Genotypic changes in human immunodeficiency virus type 1 protease associated with reduced susceptibility and virologic response to the protease inhibitor tipranavir, *J Virol*, 2006, 80, 21, 10794-10801

234. Trial Synopsis 1182.51, Boehringer Ingelheim, 2004, http://trials.boehringer-ingelheim.com/content/dam/internet/opu/clinicaltrial/com_EN/results/1182/1182.51_U04-1726.pdf, Accessed: 20.07.2012.

235. Trial Synopsis 1182.52, Boehringer Ingelheim, 2003, http://trials.boehringer-ingelheim.com/content/dam/internet/opu/clinicaltrial/com_EN/results/1182/1182.52_U03-3236.pdf, Accessed: 20.07.2012.

236. M. Markowitz, L. N. Slater, R. Schwartz, P. H. Kazanjian, B. Hathaway, D. Wheeler, M. Goldman, D. Neubacher, D. Mayers, H. Valdez and S. McCallister, Long-term efficacy and safety of tipranavir boosted with ritonavir in HIV-1-infected patients failing

multiple protease inhibitor regimens: 80-week data from a phase 2 study, *J Acquir.Immune Defic.Syndr.*, 2007, 45, 4, 401-410

237. P. Tajik, A. H. Zwinderman, B. W. Mol and P. M. Bossuyt, Trial designs for personalizing cancer care: a systematic review and classification, *Clin Cancer Res*, 2013, 19, 17, 4578-4588

238. N. A. Alymani, M. D. Smith, D. J. Williams and R. D. Petty, Predictive biomarkers for personalised anti-cancer drug use: discovery to clinical implementation, *Eur J Cancer*, 2010, 46, 5, 869-879

239. S. Matsui, R. Simon, P. Qu, J. D. Shaughnessy, Jr., B. Barlogie and J. Crowley, Developing and validating continuous genomic signatures in randomized clinical trials for predictive medicine, *Clin Cancer Res*, 2012, 18, 21, 6065-6073

240. D. Cunningham, Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, M. Mueser, A. Harstrick, C. Verslype, I. Chau and E. Van Cutsem, Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer, *New England Journal of Medicine*, 2004, 351, 4, 337-345

241. F. Cappuzzo, T. Ciuleanu, L. Stelmakh, S. Cicenas, A. Szcz+¬sna, E. b. Juh+ísz, E. Esteban, O. Molinier, W. Brugger, I. Melez+¡nek, G. l. Klingelschmitt, B. Klughammer and G. Giaccone, Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study, *The Lancet Oncology*, 2010, 11, 6, 521-529

242. Gefitinib: Summary of Product Characteristics, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001016/WC500036358.pdf, Accessed: 08.10.2012.

243. L. Paz-Ares, D. Soulieres, I. Melezinek, J. Moecks, L. Keil, T. Mok, R. Rosell and B. Klughammer, Clinical outcomes in non-small-cell lung cancer patients with EGFR mutations: pooled analysis, *J Cell Mol Med*, 2010, 14, 1-2, 51-69

244. D. H. Lee, K. Park, J. H. Kim, J. S. Lee, S. W. Shin, J. H. Kang, M. J. Ahn, J. S. Ahn, C. Suh and S. W. Kim, Randomized Phase III Trial of Gefitinib versus Docetaxel in Non-Small Cell Lung Cancer Patients Who Have Previously Received Platinum-Based Chemotherapy, *Clinical Cancer Research*, 2010, 16, 4, 1307-1314

245. Eviplera: Summary of Product Characteristics, European Medicines Agency, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002312/WC500118802.pdf, Accessed: 31.07.2012.

246. J. Cools, D. J. DeAngelo, J. Gotlib, E. H. Stover, R. D. Legare, J. Cortes, J. Kutok, J. Clark, I. Galinsky, J. D. Griffin, N. C. Cross, A. Tefferi, J. Malone, R. Alam, S. L. Schrier, J. Schmid, M. Rose, P. Vandenberghe, G. Verhoef, M. Boogaerts, I. Wlodarska, H. Kantarjian, P. Marynen, S. E. Coutre, R. Stone and D. G. Gilliland, A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome, *N Engl J Med*, 2003, 348, 13, 1201-1214

247. M. Baccarani, D. Cilloni, M. Rondoni, E. Ottaviani, F. Messa, S. Merante, M. Tiribelli, F. Buccisano, N. Testoni, E. Gottardi, V. A. de, E. Giugliano, I. Iacobucci, S. Paolini, S.

Soverini, G. Rosti, F. Rancati, C. Astolfi, F. Pane, G. Saglio and G. Martinelli, The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRalpha-positive hypereosinophilic syndrome. Results of a multicenter prospective study, *Haematologica*, 2007, 92, 9, 1173-1179

248. C. Rose, S. Dupire, C. Roche-Lestienne, N. Grardel, E. Bourgeois, N. Cambier and C. Preudhomme, Sustained molecular response with imatinib in a leukemic form of idiopathic hypereosinophilic syndrome in relapse after allograft, *Leukemia*, 2004, 18, 2, 354-355

249. Alipogene tiparvovec: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002145/WC500135472.pdf, Accessed: 25.04.2013.

250. Pertuzumab: Summary of Product Characteristics, European Medicines Agency, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002547/WC500140980.pdf, Accessed: 28.05.2013.

251. J. Baselga, I. Bradbury, H. Eidtmann, S. Di Cosimo, E. de Azambuja, C. Aura, H. G+¦mez, P. Dinh, K. Fauria, V. Van Dooren, G. Aktan, A. Goldhirsch, T. W. Chang, Z. Horv+íth, M. Coccia-Portugal, J. Domont, L. M. Tseng, G. Kunz, J. H. Sohn, V. Semiglazov, G. Lerzo, M. Palacova, V. Probachai, L. Pusztai, M. Untch, R. D. Gelber and M. Piccart-Gebhart, Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial, *The Lancet*, 2012, 379, 9816, 633-640

252. M. J. Piccart-Gebhart, M. Procter, B. Leyland-Jones, A. Goldhirsch, M. Untch, I. Smith, L. Gianni, J. Baselga, R. Bell, C. Jackisch, D. Cameron, M. Dowsett, C. H. Barrios, G. Steger, C. S. Huang, M. Andersson, M. Inbar, M. Lichinitser, I. í. L+íng, U. Nitz, H. Iwata, C. Thomssen, C. Lohrisch, T. M. Suter, J. R++schoff, T. í. S++t+æ, V. Greatorex, C. Ward, C. Straehle, E. McFadden, M. S. Dolci and R. D. Gelber, Trastuzumab after Adjuvant Chemotherapy in HER2-Positive Breast Cancer, *New England Journal of Medicine*, 2005, 353, 16, 1659-1672

253. Atazanavir sulphate: SUmmary of Product Characteristics, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000494/WC500056380.pdf, Accessed: 11.01.2013.

254. K. W. Lyles, C. S. Col+¦n-Emeric, J. S. Magaziner, J. D. Adachi, C. F. Pieper, C. Mautalen, L. Hyldstrup, C. Recknor, L. Nordsletten, K. A. Moore, C. Lavecchia, J. Zhang, P. Mesenbrink, P. K. Hodgson, K. Abrams, J. J. Orloff, Z. Horowitz, E. F. Eriksen and S. Boonen, Zoledronic Acid and Clinical Fractures and Mortality after Hip Fracture, *New England Journal of Medicine*, 2007, 357, 18, 1799-1809

255. A. Brufsky, W. G. Harker, J. T. Beck, R. Carroll, E. Tan-Chiu, C. Seidler, J. Hohneker, L. Lacerna, S. Petrone and E. A. Perez, Zoledronic Acid Inhibits Adjuvant Letrozole Induced Bone Loss in Postmenopausal Women With Early Breast Cancer, *Journal of Clinical Oncology*, 2007, 25, 7, 829-836

256. G. D. Demetri, M. von Mehren, C. D. Blanke, A. D. Van den Abbeele, B. Eisenberg, P. J. Roberts, M. C. Heinrich, D. A. Tuveson, S. Singer, M. Janicek, J. A. Fletcher, S. G. Silverman, S. L. Silberman, R. Capdeville, B. Kiese, B. Peng, S. Dimitrijevic, B. J. Druker, C. Corless, C. D. M. Fletcher and H. Joensuu, Efficacy and Safety of Imatinib Mesylate

in Advanced Gastrointestinal Stromal Tumors, *New England Journal of Medicine*, 2002, 347, 7, 472-480

257. Crizotinib: Assessment Report, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002489/WC500134761.pdf, Accessed: 27.03.2013.

258. Dasatinib: Summary of Product Characteristics, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000709/WC500056998.pdf, Accessed: 30.05.2013.

259. Nelfinavir: Summary of Product Characteristics, European Medicines Agency, 2012, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000164/WC500050679.pdf, Accessed: 16.05.2013.

260. C. Bokemeyer, I. Bondarenko, A. Makhson, J. T. Hartmann, J. Aparicio, F. de Braud, S. Donea, H. Ludwig, G. Schuch, C. Stroh, A. H. Loos, A. Zubel and P. Koralewski, Fluorouracil, Leucovorin, and Oxaliplatin With and Without Cetuximab in the First-Line Treatment of Metastatic Colorectal Cancer, *Journal of Clinical Oncology*, 2009, 27, 5, 663-671

261. J. Eron Jr, P. Yeni, J. Gathe Jr, V. Estrada, E. DeJesus, S. Staszewski, P. Lackey, C. Katlama, B. Young, L. Yau, D. Sutherland-Phillips, P. Wannamaker, C. Vavro, L. Patel, J. Yeo and M. Shaefer, The KLEAN study of fosamprenavir-ritonavir versus lopinavir-ritonavir, each in combination with abacavir-lamivudine, for initial treatment of HIV infection over 48 weeks: a randomised non-inferiority trial, *The Lancet*, 2005, 368, 9534, 476-482

262. D. Allingham-Hawkins, A. Lea and S. Levine, ERCC1 Expression Analysis to Guide Therapy in Non-Small Cell Lung Cancer, *PLoS Curr*, 2010, 2, RRN1202

263. R. A. Walker, Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment I, *Histopathology*, 2006, 49, 4, 406-410

264. C. R. Taylor and R. M. Levenson, Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II, *Histopathology*, 2006, 49, 4, 411-424

265. T. Seidal, A. J. Balaton and H. Battifora, Interpretation and quantification of immunostains, *Am J Surg Pathol*, 2001, 25, 9, 1204-1207

266. A. Vilmar, J. Garcia-Foncillas, M. Huarriz, E. Santoni-Rugiu and J. B. Sorensen, RT-PCR versus immunohistochemistry for correlation and quantification of ERCC1, BRCA1, TUBB3 and RRM1 in NSCLC, *Lung Cancer*, 2012, 75, 3, 306-312

267. K. A. Olaussen, A. Dunant, P. Fouret, E. Brambilla, F. Andre, V. Haddad, E. Taranchon, M. Filipits, R. Pirker, H. H. Popper, R. Stahel, L. Sabatier, J. P. Pignon, T. Tursz, T. Le Chevalier, J. C. Soria and I. B. Investigators, DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy, *N Engl J Med*, 2006, 355, 10, 983-991

268. S. Detre, G. Saclani Jotti and M. Dowsett, A "quickscore" method for immunohistochemical semiquantitation: validation for oestrogen receptor in breast carcinomas, *J Clin Pathol*, 1995, 48, 9, 876-878

269. C. R. Taylor, Standardization in immunohistochemistry: the role of antigen retrieval in molecular morphology, *Biotech Histochem*, 2006, 81, 1, 3-12

270. T. Strachan, Read, A., Human Molecular Genetics, 2010, Garland Science, New York,USA; Abingdon, UK

271. J. M. Berg, Tymoczko, J. L., Stryer, L., Biochemistry, 2010, W.H. Freeman & Company, New York, USA

272. M. Kubista, J. M. Andrade, M. Bengtsson, A. Forootan, J. Jonak, K. Lind, R. Sindelka, R. Sjoback, B. Sjogreen, L. Strombom, A. Stahlberg and N. Zoric, The real-time polymerase chain reaction, *Mol Aspects Med*, 2006, 27, 2-3, 95-125

273. J. Wilhelm and A. Pingoud, Real-time polymerase chain reaction, *Chembiochem*, 2003, 4, 11, 1120-1128

274. S. A. Bustin, Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays, *J Mol Endocrinol*, 2000, 25, 2, 169-193

275. K. Dheda, J. F. Huggett, S. A. Bustin, M. A. Johnson, G. Rook and A. Zumla, Validation of housekeeping genes for normalizing RNA expression in real-time PCR, *Biotechniques*, 2004, 37, 1, 112-114, 116, 118-119

276. A. Radonic, S. Thulke, I. M. Mackay, O. Landt, W. Siegert and A. Nitsche, Guideline to reference gene selection for quantitative real-time PCR, *Biochem Biophys Res Commun*, 2004, 313, 4, 856-862

277. K. J. Livak and T. D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods*, 2001, 25, 4, 402-408

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