Cancer Treatment Reviews 39 (2013) 592–601

Contents lists available at SciVerse ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevierhealth.com/journals/ctrv

Anti-Tumour Treatment

Targeted therapy in metastatic colorectal cancer – An example of personalised medicine in action

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ARTICLE INFO

Article history:
Received 18 September 2012
Received in revised form 3 December 2012
Accepted 9 December 2012

Keywords:
Biomarker
Cetuximab
Colorectal cancer
Companion diagnostic
Epidermal growth factor receptor
Genomics
KRAS testing
Panitumumab
Personalised medicine
Targeted therapy

ABSTRACT

In metastatic colorectal cancer (mCRC), an improved understanding of the underlying pathology and molecular biology has successfully merged with advances in diagnostic techniques and local/systemic therapies as well as improvements in the functioning of multidisciplinary teams, to enable tailored treatment regimens and optimized outcomes. Indeed, as a result of these advancements, median survival for patients with mCRC is now in the range of 20–24 months, having approximately tripled in the last 20 years. The identification of *KRAS* as a negative predictive marker for activity of epidermal growth factor receptor (EGFR)-targeted monoclonal antibodies (mAbs), such as panitumumab (Amgen, Thousand Oaks, USA) and cetuximab (ImClone, Branchburg, USA), has perhaps had the greatest impact on patient management. This meant that, for the first time, mCRC patients unlikely to respond to a targeted therapy could be defined ahead of treatment. Ongoing controversies such as whether patients with *KRAS* G13D- (or BRAF V600-) mutated tumours can still respond to EGFR-targeted mAbs and the potential impact of inter- and intra-tumour heterogeneity on tumour sampling show that the usefulness of *KRAS* as a biomarker has not yet been exhausted, and that other downstream biomarkers should be considered. Conversely, a predictive biomarker for anti-angiogenic agents such as bevacizumab (Genentech, San Francisco, USA) in the mCRC setting is still lacking. In this review we will discuss the discovery and ongoing investigation into predictive biomarkers for mCRC as well as how recent advances have impacted on clinical practice and ultimately the overall cost of treatment for these patients.

Introduction

Until relatively recently we believed that cancer could essentially be treated using the same combinations and sequences of locoregional (surgery and/or radiotherapy) and systemic (chemotherapy) treatments in all patients. However, we are now in a transitional period where we are embracing a more personalised approach to cancer management. The heterogeneous nature of cancer means that personalised medicine (i.e. tailoring therapy to an individual patient) is a promising approach for maximising efficacy and minimising the toxicity of treatment. It also facilitates efficient healthcare delivery and generates cost savings because treatment is only given to those likely to benefit and so costs associated with drug wastage, hospital resource utilisation and side-effect management are reduced. To successfully deliver personalised medicine, it is necessary to have a clear understanding of the pathology and molecular underpinnings of a disease, as well as the associated clinical characteristics that define different patient sub-populations with different outcomes in relation to a given treatment. Identifying the optimum treatment strategy also involves an understanding of a patient's medical history, disease status, and sometimes, their socio-economic situation, and consideration of the wider healthcare framework, such as the availability of hospital resources and reimbursement.

The ultimate goal of personalised medicine is to define a disease sufficiently to enable identification and treatment of only those

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0305-7372
http://dx.doi.org/10.1016/j.ctrv.2012.12.011

patients most likely to respond. Although personalised medicine is almost exclusively discussed in the context of targeted therapies, chemotherapy also has the potential to be tailored to individual patients. Advances in genomic and proteomic technologies and the implementation of major collaborative studies such as the human genome project and genome-wide association studies (GWAS), have already generated much data and are leading to the identification of many biomarkers – a characteristic that can be objectively measured and evaluated as an indicator of pathogenic processes or treatment responses. Biomarkers have been identified for: early detection/risk stratification (diagnostic markers); the likely course of a given disease (prognostic markers); and prediction of treatment safety/efficacy outcome (predictive markers).

The principle of targeted therapy was first proposed by Paul Ehrlich more than 100 years ago, when he coined the term 'magic bullet'.[1] Immunohistochemistry (IHC) provided one of the first opportunities to personalise medicine and was effectively used in breast cancer to identify patients with tumours expressing oestrogen and/or progesterone receptors, who were candidates for 'targeted' hormonal therapies like tamoxifen (AstraZeneca, Delaware, USA). Furthermore, since its discovery more than 30 years ago,[2]hybridoma technology has enabled production of large amounts of monoclonal antibodies (mAbs) targeted to specific tumour antigens, and has led to a vast array of new diagnostic and therapeutic options. These advances are already revolutionising cancer screening, drug development and treatment selection, and are major factors in personalising medicine in the 21st century. This concept has gained momentum in recent years with the development of other successful therapies such as imatinib mesylate (Novartis, New Jersey, USA)[3] for chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours (GIST) and trastuzumab (Genentech, San Francisco, USA)[4] for breast and gastric cancers. These agents target specific molecular alterations (abnormal protein tyrosine kinase activity for imatinib, overexpression of human epidermal growth factor receptor-2 [HER-2] for trastuzumab), which are now used as predictive biomarkers of response, thereby allowing these drugs to be targeted to individuals with the appropriate tumour characteristics.

Colorectal cancer (CRC) is perhaps one of the best examples of how an increased understanding of disease molecular biology has successfully merged with improved diagnostic techniques, advances in local/systemic therapy, and improvements in the functioning of multidisciplinary teams, to enable tailored treatment regimens and optimized outcomes.

Evolution of personalised therapy in metastatic CRC (mCRC)

Globally, CRC is the third most commonly diagnosed cancer in males and the second in females[5] and is the second leading cause of cancer mortality in the United States, accounting for 9% of all cancer deaths.[6] Approximately one-quarter of CRC patients have metastases at diagnosis and a further 33–50% develop metastases over their disease course.[5,7] Surgical resection offers the possibility of cure for a small minority of patients with mCRC and isolated metastases.[8] Management by a multidisciplinary team including, for example, surgeons, oncologists, interventional radiologists, radiotherapists, and nurses, increases the number of patients able to undergo potentially curative treatment and has consequently improved patient survival.[7,9] Together, advances in local and systemic therapy have led to improvements in survival[10] with median survival in mCRC increasing from approximately 8–24 months[9,11] over the last 20 years. The improvements in survival times in mCRC patients diagnosed between 1990 and 2006 at two large specialised institutes are exemplified in Fig. 1.[12] The availability of new cytotoxic and targeted therapies and the implementation of personalised medicine have been instrumental in this process.[13]

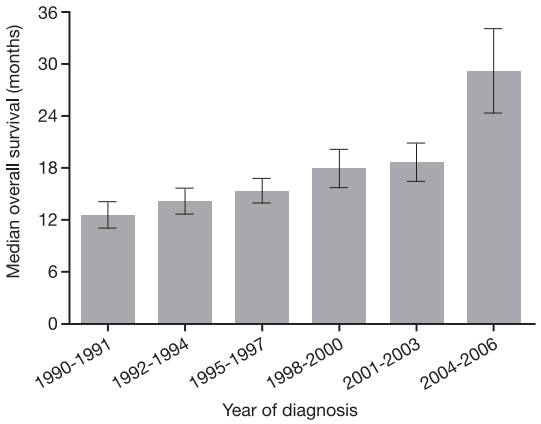

Fig. 1. Median overall survival for patients with metastatic colorectal cancer treated at the M.D. Anderson Cancer Center and the Mayo Clinic by year of diagnosis (error bars are 95% confidence intervals).[12] Reprinted with permission © 2009 American Society of Clinical Oncology: Kopetz S, et al. J Clin Oncol 2009; 27(22):3677–3683. All rights reserved.

Evolution of systemic therapy for mCRC

Chemotherapy has been standard care for mCRC patients for many years, and is based mainly on the use of three agents: 5-fluorouracil (5-FU; APP Pharmaceuticals, Schaumburg, USA), irinotecan (Pfizer, New York, USA)[14,15] and oxaliplatin (Sanofi-Aventis, Bridgewater, USA).[16,17] Infusional 5-FU regimens-such as FOLFIRI[18] or FOLFOX[19] have better efficacy than earlier bolus 5-FU regimens and currently provide the backbone of therapy.[20] Capecitabine (Genentech, San Francisco, USA),[21] an oral formulation of 5-FU, is also available.

Whilst the vast majority of biomarker research has focussed on targeted therapies, efforts are continuing to identify predictive markers of response or resistance to chemotherapy. Up to now, however, there are only a few noteworthy examples. Although results are somewhat conflicting, high thymidylate synthase (TS) expression has generally been linked with poorer outcomes during 5-FU-based therapy,[22,23] and 5-FU adjuvant treatment may also be ineffective in tumours with microsatellite instability.[13] Irinotecan was one of the first chemotherapy agents to be dosed based on the recipient's pharmacogenomics; reduced irinotecan doses should be considered in patients homozygous for the *28 variant form of UGT1A1 as they are unable to clear irinotecan as quickly as normal and, therefore, suffer more severe haematological side effects.[24] Furthermore, homozygosity for the *28 variant form of UGT1A1 has been linked with improved efficacy of FOLFIRI.[25]

The most promising predictive marker of resistance to oxaliplatin is excision repair cross-complementing C1 (ERCC1) expression,[26] and although there is currently no standard test available, it is possible that ERCC1 testing may become routine in mCRC patients in the future. Genetic differences in the glutathione transferase pathway have also been suggested to lead to higher rates of neurotoxicity during oxaliplatin therapy,[27] however, this is yet to be confirmed and has not yet impacted on clinical practice. In addition, a FOLFOX response predictor has recently been constructed based on gene expression profiles of responding and non-responding patients.[28] Initial results suggest that the overall accuracy of this predictor is high (92.5%) and therefore it may offer the possibility of selecting patients who would benefit from FOLFOX.

The emergence of targeted therapies

Targeting angiogenesis – bevacizumab

The first biological targeted therapy to be used in mCRC was the vascular endothelial growth factor-A (VEGF-A)-targeted agent, bevacizumab (Genentech, San Francisco, USA). When used in combination, bevacizumab improves progression-free survival (PFS) compared with chemotherapy alone, while the effects on objective response rate (ORR) and overall survival (OS) are less consistent in the 1st-[29–31] and 2nd-line[32] mCRC settings. Generally the overall magnitude of benefit appears to vary depending on the choice of chemotherapy backbone, and is seemingly greater with irinotecan-based regimens.[33,34]

As more effective therapies became available, the need for predictive biomarkers to enable optimum treatment selection for each patient increased. Such markers are particularly important in mCRC because of the heterogeneity of response among colon tumours and the toxicities and costs associated with the available therapies.[13] Despite much research[35–40] and the broad application of bevacizumab in many patients, it is still poorly understood which patients/tumour characteristics are best treated with anti-angiogenics, and no validated predictive markers of response/resistance are currently available.[41] Recently, VEGF-A, TS, and tissue inhibitor of metalloproteinase 3 were identified as factors that were differentially expressed in responding or non-responding patients.[39] A model utilising these three genes appeared to accurately predict response to bevacizumab[39] but needs further evaluation in larger numbers of patients. In addition, recent data from the AGITG MAX trial suggest that high VEGF-Dexpression could be predictive of resistance to bevacizumab.[40] Results of a second study suggested that high levels of an anti-angiogenic splice variant of VEGF-A ($VEGF_{165}b$) could have similar effects.[42] Further investigation of the impact of these potential markers of bevacizumab activity is warranted. Some tumours show intrinsic resistance to bevacizumab and, when they do occur, responses are often transitory with patients showing restoration of tumour growth due to evasive resistance.[43] Furthermore, in some preclinical studies, anti-angiogenic treatment has been shown to elicit malignant progression of tumors and to increase local invasion and distant metastasis.[44] Various mechanisms appear to be involved in different tumour contexts[43] such as upregulation of alternative proangiogenic signalling,[45] recruitment of vascular progenitor cells and pro-angiogenic monocytes[46] and increased tight pericyte coverage in the tumour vasculature.[47] However, these have not yet been shown to occur in patients with mCRC undergoing anti-angiogenic treatment. Nonetheless, these preclinical observations should motivate additional studies and should they be validated, could lead to combinatorial treatment strategies integrating anti-angiogenics with drugs targeting the appropriate resistance mechanisms. Based on results of a recent preclinical study,[44] it is of interest to clinically evaluate strategies combining anti-invasive and anti-metastatic drugs with anti-angiogenics, with the aim of producing a more enduring efficacy.

Targeting epidermal growth factor signalling – panitumumab and cetuximab

Advances in the understanding of mCRC have also led to the development of mAbs targeting the epidermal growth factor receptor (EGFR), such as panitumumab (Amgen, Thousand Oaks, USA) or cetuximab (ImClone, Branchburg, USA). Initial monotherapy studies included patients whose tumours expressed EGFR, but produced low ORRs,[48] suggesting that other factors were important for response to these agents.[49] Nonetheless, combining chemotherapy with EGFR-targeted mAbs improved efficacy in the 1st-[50–52] and 2nd-line[53,54] mCRC settings. Such regimens were generally associated with higher ORRs compared with the equivalent bevacizumab-containing regimens,[55] which may impact on treatment choice in patients with resectable/potentially resectable disease. Furthermore, unlike bevacizumab, EGFR-targeted mAbs are active as monotherapy in later lines of treatment.[48,56] Choice of chemotherapy backbone may also impact on the effectiveness of EGFR-targeted mAbs, although such observations have only been noted with cetuximab to date. The COIN[57] and NORDIC VII[58] trials reported no efficacy benefits on adding cetuximab to oxaliplatin-based regimens (capecitabine/oxaliplatin and 5-FU/oxaliplatin/folinic acid, respectively), raising concern about using these agents in combination. The use of oral/bolus fluoropyrimidines in both studies (rather than an infusional regimen) may explain these results. This hypothesis is substantiated by the FUTURE study, which reported inferior results (not statistically significant) for cetuximab/UFT (oral) vs. cetuximab/FOLFOX4 (infusional).[59] In line with this, efficacy benefits were noted on addition of EGFR-targeted mAbs to FOLFOX4 in the PRIME[52] and OPUS[60] trials. Interestingly, in the AIO 0104[61] and CELIM[62] trials, which used oral and infusional fluoropyrimidines, respectively, cetuximab had similar activity when combined with oxaliplatin- or irinotecan-based regimens.

The KRAS gene: a game changer for mCRC

Biomarker development for EGFR-targeted mAbs has focussed on the impact of alterations in EGFR and its downstream effectors (Fig. 2). The most important development in mCRC management in recent years was the discovery that mutated tumour *KRAS* status predicted for lack of response to EGFR-targeted mAbs. Approximately 27-43% of tumours in mCRC patients harbour *KRAS* gene mutations, leading to constitutive activation of downstream

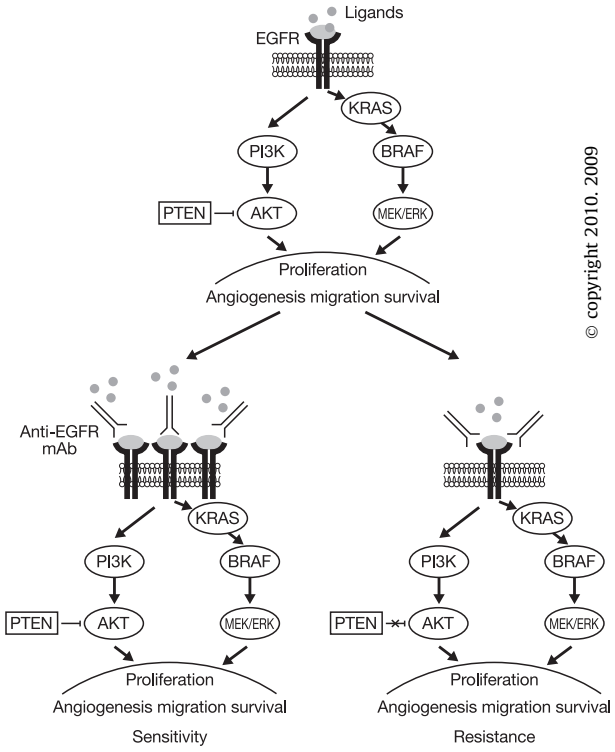

Fig. 2. An overview of the epidermal growth factor receptor (EGFR) pathway and its main downstream effectors (top). Expected outcomes of anti-EGFR monoclonal antibody (mAb) therapy (bottom): sensitivity (tumour response) when EGFR is activated (increased copy number, ligand overexpression, other unknown mechanisms) and downstream effectors are wild type (left); Resistance (tumour growth and metastasis) when downstream effectors such as KRAS, BRAF or PI3K are activated or PTEN is inactivated (right).[106] Reprinted by permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: Di Fiore F, et al. Br J Cancer 2010; 103(12):1765–1772.

signalling and conferring lack of response to these agents.[63] This discovery meant that for the first time in mCRC, patients unlikely to benefit from a targeted therapy could be identified ahead of treatment. Indeed, tumour *KRAS* testing is now mandatory in potential candidates for EGFR-targeted mAb therapy. The presence of *KRAS* mutations in CRC tumours may also be an adverse prognostic indicator,[64] particularly if the glycine to valine alteration at codon 12 (G12V) is present.[65] Furthermore, there is a negative interaction in patients with *KRAS*-mutant tumours receiving an EGFR-targeted mAb combined with oxaliplatin-based therapy; these patients have worse efficacy outcomes than similar patients receiving oxaliplatin-based therapy alone.[52,57] Interestingly, however, when treated with oxaliplatin-based regimens such as FOLFOX6 alone, recent retrospective data suggest that patients with *KRAS*-mutant disease respond better than patients with *KRAS*-wild-type mCRC.[66]

Cost implications of KRAS testing

Although the implementation of *KRAS* testing is associated with additional upfront costs, as might be expected, overall, it is associated with cost savings.[67–71] For example, under most scenarios tested, using *KRAS* testing to select mCRC patients for EGFR-targeted mAb therapy saved $7500-$12,400 per patient in the United States and €3900–9600 per patient in Germany (Table 1).[69] Based on cost savings for monotherapy of $7456-$8040, using *KRAS* testing should save ~$377–402 million in the United States each year. In another European cost-effectiveness analysis, using *KRAS* testing to limit treatment to patients with *KRAS*-wild-type tumours led to savings of €779.42 per patient per cycle.[71] Whereas, in Japan, implementation of *KRAS* testing before deciding whether to use EGFR-targeted mAbs was reported to save £32 million per year.[70]

The G13D controversy

Within the *KRAS* gene, most mutations occur in codons 12 and 13 and seven common mutations in these regions account for ~98% of all observed *KRAS* mutations in CRC.[72] The original analyses of response by tumour *KRAS* status during EGFR-targeted mAb therapy grouped *KRAS* codon 12 and 13 mutations together, and did not look at the impact of individual mutations. Recently, however, reports have suggested that different *KRAS* mutations may have different biological characteristics with respect to treatment sensitivity. Tumours harbouring mutations of the glycine to aspartate at *KRAS* codon 13 (G13D) have been suggested to retain cetuximab sensitivity and several small retrospective studies reported improved outcomes in some patients harbouring these mutations during cetuximab therapy.[73–77] In contrast, in a pooled, post-hoc analysis of data from patients receiving panitumumab treatment in three phase III trials, no single *KRAS* mutation consistently predicted PFS or OS outcome.[78] Indeed, in one of these studies, the G12V mutation was favourably associated with OS (but not PFS) while G13D was unfavourably associated with both OS and PFS in the panitumumab arm. Nonetheless, a trend towards benefit was observed on adding panitumumab to FOLFIRI in patients with G13D-mutated tumours, which taken together with data for cetuximab[73] suggests a potential benefit when EGFR-targeted mAbs are used alongside irinotecan-based therapy in such patients. However, until any association between specific *KRAS* mutations and response are confirmed in prospective studies including predefined patient populations (e.g. the AGITG ICE CREAM trial[79]), it seems prudent to limit use of EGFR-targeted mAbs to the licensed population (i.e. those with *KRAS*-wild-type tumours). Interestingly, a recent retrospective study suggested that *KRAS* codon 12 and 13-mutated mCRC may also have differential impact on prognosis, with codon 13-mutated mCRC presenting as a more aggressive disease frequently associated with local and distant metastases at diagnosis.[80]

Ultimately, these data highlight the need to correctly validate a biomarker in homogenous and, therefore, more clinically meaningful populations before it is put into clinical practice. However, the process of validating such markers can be complex, especially for uncommon mutations where it can be difficult to get sufficient patient numbers for an accurate picture to emerge of their impact. There is also a need for consistent testing across centres and for quality assurance measures to be in place to ensure any potential associations between mutation status and outcome are robust. The need for external quality assurance for *KRAS* testing has recently been demonstrated;[81] only 70% of laboratories included in a recent study correctly identified the *KRAS* mutational status of all test samples.[81] Notably, of the mutations found, 30% were false positives and false negatives, both of which would likely negatively impact patient care through their influence on treatment choice.

Table 1
Cost and effectiveness data associated with *KRAS* testing in the United States and Germany.[69] Reprinted with permission of John Wiley and Sons: Vijayaraghavan A, et al. Int J Cancer 2012; 131(2):438-445,copyright 2012

Strategy	Cost/pt (US)	Cost saving/ pt (US)	Cost/pt (Germany)	Cost saving/pt (Germany)	Effectiveness (weeks)	ICER ($ or € per LYS)
Panitumumab monotherapy with *KRAS* testing	$19,656	$7546	€13,787	€4612	18.26	–
Panitumumab monotherapy without *KRAS* testing	$27,202	–	€18,399	–	18.26	Higher cost, same effectiveness compared with *KRAS* testing
Cetuximab monotherapy with *KRAS* testing	$22,893	$8040	€13,588	€3856	19.78	–
Cetuximab monotherapy without *KRAS* testing	$30,933	–	€17,444	–	19.78	Higher cost, same effectiveness compared with *KRAS* testing
Combination therapy[a] with *KRAS* testing (combination therapy for *KRAS* WT & no chemotherapy for *KRAS* MT)	$35,075	$13,501	€26,292	€9560	24.26	Less expensive, less effective than combination therapy without *KRAS* testing
Combination therapy[a] with *KRAS* testing (combination therapy[a] for *KRAS* WT & irinotecan/FOLFIRI only for *KRAS* MT)	$36,148	$12,428	–	–	25.83	$35,539 per LYS compared with combination therapy with *KRAS* testing assuming *KRAS* MT patients will not receive chemotherapy
Combination therapy[a] without *KRAS* testing	$48,576	–	€35,852	–	25.83	Higher cost and same effectiveness compared with previous *KRAS* testing strategy

ICER, incremental cost-effectiveness ratio; LYS, life-year saved; MT, mutant; pt, patient; US, United States; WT, wild-type.
[a] Combination therapy is cetuximab + irinotecan in the United States and cetuximab + FOLFIRI in Germany. The FOLFIRI regimen consists of irinotecan, 5-fluorouoracil and leucovorin.

Future of personalised medicine in mCRC

Although mutant *KRAS* is undoubtedly an excellent predictor for lack of activity of EGFR-targeted mAbs, with its implementation response rates only rose from ~15% in an unselected population to ~30% in those with *KRAS*-wild-type tumours,[13] therefore, a prognostic impact of mutant *KRAS* is likely and additional factors influencing the course of disease must be important.[82,83] The ongoing need to identify and validate other biomarkers of response and resistance has led to much research in this area, with the aim of further improving our ability to specify likely responders and subsequently improve treatment outcomes and decrease costs.

Potential new predictive biomarkers for EGFR-targeted mAbs

Tumour *KRAS* testing for codon 12/13 mutations is now a prerequisite ahead of undergoing EGFR-targeted mAb treatment,[84–86] but should we consider testing the status of a panel of other biomarkers in mCRC patients at this point in time? Although there has been much research into other potential biomarkers, many results have been inconsistent and there is also a lack of validated tests, and so at present most are not routinely used in clinical practice. For example, there is some evidence suggesting that mutations at *KRAS* codons 61 and 146 (present in ~2% of colorectal tumours) have similar impact to mutations in codons 12 and 13[87] as do mutations in *NRAS*.[88] However, additional research in larger groups of patients is needed if we are to incorporate these mutations into routine clinical practice.

Emerging biomarkers for activity of EGFR-targeted mAbs and their stage of development are briefly reviewed below and the relationships between such biomarkers and treatment response are summarised in Fig. 3.

BRAF V600E mutations

After *KRAS* mutations, *BRAF* V600E mutations currently have the strongest evidence to support their use as a predictive biomarker for EGFR-targeted mAb activity. Overall, *BRAF* V600E activating mutations occur in approximately 10–15% of CRC tumours and are generally mutually exclusive to *KRAS* mutations.[13] Most but not all of the available evidence links *BRAF* V600E mutations with resistance to EGFR-targeted mAb therapy,[89–94]however, the impact of tumour *BRAF* status on efficacy of these treatments has not yet definitively been addressed due to the relatively small number of patients with *BRAF* mutations. Indeed, a retrospective analysis of data from the CRYSTAL trial showed numeric improvements in median PFS and OS (not statistically significant) on addition of cetuximab to FOLFIRI in patients with *KRAS*-wild-type/*BRAF*-mutant disease.[51] Mutated *BRAF* was also shown to be a negative prognostic marker for outcome irrespective of treatment received and the authors speculated that this strong prognostic effect could explain in part why previous single-arm analyses were interpreted as indicating that EGFR-targeted mAbs were ineffective in patients with *BRAF*-mutant mCRC. BRAF inhibitors also show limited single-agent activity in tumours bearing these mutations.[95] A recent study suggested that resistance to BRAF inhibition in *BRAF V600E*-mutant CRC may be caused by feedback activation of EGFR,[93] therefore, it could be of interest to evaluate treatment strategies targeting both BRAF and EGFR inhibition in a controlled clinical trial in patients with tumours of this genotype.

In addition to worse prognosis, mutated *BRAF* is associated with a characteristic pattern of gene expression.[96,97] Furthermore, in one study, some *BRAF*-wild-type/*KRAS*-mutated tumours and double wild-type tumours showed a *BRAF*-mutated-like gene expression profile and similarly poor prognosis,[97] suggesting a common biology that would not be detected by *BRAF* testing alone. Interestingly, the prevalence of *BRAF* V600E mutations appears considerably higher in older females with *KRAS*-wild-type right-sided colon cancers (50%) compared to unselected patients (10%).[96] This suggests that certain clinicopathological and molecular features may be useful to identify mCRC patients with a higher prevalence of *BRAF* V600E mutation or worse prognosis. A validated test has recently been launched for the *BRAF* V600E mutation,[98] but validated tests for other *BRAF* mutations are as yet unavailable. Testing *BRAF*V600E status in patients with *KRAS*-wild-type disease is associated with additional upfront costs. Nonetheless, a recent European study using this approach to define which patients should receive cetuximab treatment found it to be the most cost-effective strategy compared with various alternative scenarios including where only tumour *KRAS* status was determined.[99] *BRAF* V600E testing is now starting to be used in clinical practice, but isn't yet considered a fully validated marker for EGFR-targeted mAb activity and isn't routinely used in treatment decision-making in most centres.

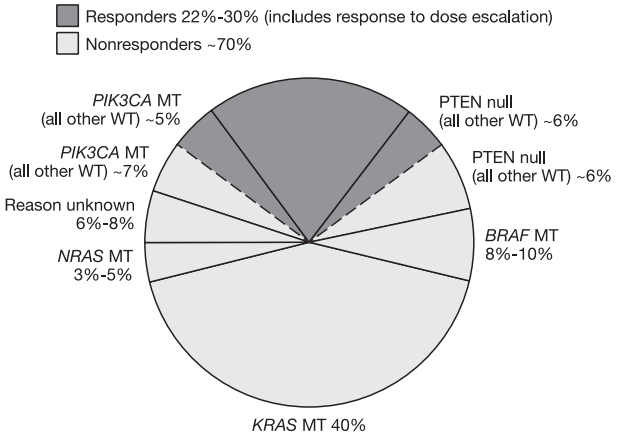

Fig. 3. Relationship between biomarkers and response to epidermal growth factor receptor (EGFR) inhibitors in chemorefractory colorectal cancer. Approximately 70% of responders may have an increased EGFR copy number. WT, wild-type; MT, mutant.[83] Reprinted with permission© 2010 American Society of Oncology: Hawkes E, et al. J Clin Oncol 2010; 28(28):e529-e531. All rights reserved.

Alterations to PI3K signalling

Changes in PI3K signalling, such as *PIK3CA* mutations[94,100,101] and loss of *PTEN* expression/activity[94,101–103] have generally been linked with lack of response to EGFR-targeted mAbs, although these data have been somewhat inconsistent and come from relatively small studies. The PI3K pathway is in part modulated by *KRAS* activation during EGFR signalling and so it is plausible that alterations could predict for activity of EGFR-targeted mAbs. Furthermore, data suggests that combining *KRAS* and *BRAF* mutational analysis with evaluation of *PIK3CA* mutations and *PTEN* expression status may permit identification ~70% of patients unlikely to respond to EGFR-targeted mAbs.[104] However, there are no standardized approaches for assessing these changes, particularly for PTEN, with mutational status, IHC and gene copy number all being used, making consolidation of the available data difficult. This emphasizes the importance of standardization of approach and the need for validated tests if *PIK3CA* and PTEN status are to become routinely used in clinical practice.

Overexpression/amplification of EGFR and its ligands

EGFR overexpression/amplification has potential as a prognostic marker in *KRAS*-wild-type patients[105] but investigations into its

use as a predictive marker of response have yielded inconsistent results,[105–108] likely due in part to differences in assay methodology. Therefore, EGFR overexpression/amplification is not currently used as a predictive marker for EGFR-targeted mAbs. Initial studies have indicated that overexpression of EGFR ligands such as amphiregulin and epiregulin may, however, predict response to cetuximab.[109–111] For example, in one study, amphiregulin and epiregulin expression were each significantly associated with ORR, PFS and OS outcomes in patients with *KRAS*-wild-type tumours undergoing cetuximab plus irinotecan treatment.[109] However, further studies are required to determine if these molecules could become useful biomarkers in clinical practice and to determine if reproducible assays can be defined.

Mutation testing – is one tumour sample sufficient?

It is debatable whether evaluating biomarker status of a single biopsy from a single tumour is sufficient to direct choice of targeted therapy in mCRC because deregulation of EGFR signalling can differ between primary and metastatic sites,[112] potentially impacting response to EGFR-targeted mAbs. For example, a recent study suggested that while *KRAS*, *BRAF* and *PIK3CA* status could be considered adequate markers in metastatic disease (concordant in 91%, 100% and 94% of cases, respectively), a much higher degree of discordance was found between tumour sites in expression of EGFR (concordant in 61%), PTEN (66%) and pAKT (54%).[113]

Mutation analysis of a single tumour biopsy sample may also underestimate the mutational burden of heterogeneous tumours,[114,115] making it difficult to accurately determine likely resistance and also to validate new biomarkers of response. Phylogenetic reconstruction of tumour clonal architecture reveals branched evolutionary growth, but reassuringly, common mutations at the trunk of the phylogenetic tree are consistently expressed and so may provide robust markers and therapeutic targets.[115] Intratumoural heterogeneity of *KRAS* and *BRAF* status has been reported in multiple blocks taken from the same primary tumour. In one study, the authors suggested that testing DNA from only a single tumour block could lead to tumour *KRAS* status being wrongly assigned in ~10% of patients.[114] However, preparing a 'DNA cocktail' from two or more blocks could improve detection at minimal additional cost.

Mutation status may also change over the course of therapy as resistance develops, begging the question whether serial mutation testing could be useful. While repeated tumour biopsies at disease progression may be a valuable approach to understand emerging resistance, this strategy certainly imposes a burden on patients. From an ethical standpoint, serial biopsies might find acceptance if it is clear that these could benefit the patient by guiding an effective course of action to overcome resistance. Two recent reports have suggested that the emergence of *KRAS* mutations or *KRAS* amplification during cetuximab or panitumumab treatment may be frequent drivers of resistance.[116,117]Interestingly, these mutations could be detected non-invasively in patient sera after 5–6 months of treatment, months before radiological progression. Mathematical modelling suggested that resistant clones bearing these mutations were highly likely to be present at very low levels in the patient's tumours before treatment commenced, and that these expanded rapidly following initiation of treatment.[116] Importantly, the *KRAS*-mutant tumours were found to be sensitive to combined EGFR-targeted mAb/MEK inhibitor treatment.[117] Monitoring for *KRAS* mutations in sera during EGFR-targeted therapy could, therefore, permit early initiation of combination treatment that could prevent or delay progression, without the need for more invasive tumour sampling. In addition, the emergence of EGFR mutations have been linked to the development of cetuximab resistance during treatment,[118] which if detected, could trigger a change of therapy to a more effective agent. Interestingly, tumours with acquired EGFR ectodomain mutations (S492R) that prevent cetuximab binding and, therefore, produce cetuximab resistance can retain sensitivity to panitumumab,[118,119] suggesting that each of these agents may interact with the EGFR slightly differently.

How can we sustain the progress?

The importance of sustaining development of specific targeted therapies and their associated predictive biomarkers was highlighted in a recent pooled analysis.[120] Here, hazard ratios for PFS and OS from published randomised controlled trials were pooled and compared for three groups of agents: those directed against a specific molecular target for which the target population was selected by biomarker (e.g. panitumumab/cetuximab in patients without *KRAS* mutations); less specific biologic targeted agents (e.g. bevacizumab); and chemotherapeutic agents.[120] The clinical benefit from targeted therapies was greater than for chemotherapies, with the highest relative benefit observed when the target population was selected by biomarker. It is, therefore, vital that we continue to identify druggable proteins driving cancer progression alongside suitable biomarkers that enable accurate selection of patients if more effective anticancer therapies are to be developed. In the first instance we must ensure that future clinical trials are designed according to established and validated biomarkers (e.g. *KRAS*) and then optimised biobanks from these studies can be used to explore the impact of potential next-generation biomarkers (e.g. alterations in PI3K signalling and expression levels of EGFR ligands, etc.).

Supporting basic and translational research is key to furthering the 'omics' revolution and will ensure the continuing identification of new drug targets and biomarkers. The integration of optimum technologies such as high-throughput, next-generation sequencing and protein and DNA microarrays is already revolutionising biomarker discovery programmes, however, moving such discoveries from bench to clinic remains a costly and time-consuming process involving many branches of science and medicine.[121] Encouraging open communication and collaboration between academia (basic and clinical research), industry, patients and regulators is, therefore, vital. It is also imperative that pharmaceutical companies embrace personalised medicine in their clinical development programmes, perhaps by implementing dedicated biomarker discovery programmes. With this in mind, the National Institute of Health Voluntary Genomic Data Submission Program aims to encourage companies to integrate genomics into their development programmes by permitting the discussion of genetic information with the Food and Drug Administration (FDA) in a forum separate from the product review process.[121] Such discussions may facilitate optimisation of trial designs, thereby helping to ensure biomarker studies are pre-specified in large clinical trials (most current data derive from retrospective analyses or small case series)[122,123] and that new targeted combination strategies are evaluated effectively. A phase III trial schema that has been proposed for use in evaluating potential new predictive markers is shown in Fig. 4.[123]

Although prospective biomarker studies will undoubtedly become more commonplace, tissue banks and blood samples from completed trials will continue to provide a valuable opportunity to retrospectively link tumour characteristics/blood-borne markers to clinical outcomes. Genetic epidemiology studies such as GWAS,[124] are also important in identifying potential new drug targets. As such studies yield results, it is important to ensure that all relevant genomic, transcriptomic and proteomic data are deposited into freely accessible databases, so that data can be accessed and processed globally.[125]

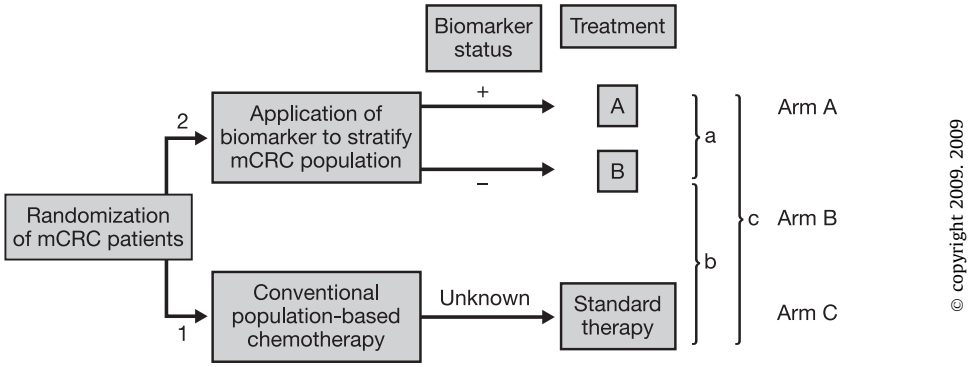

Fig. 4. Trial schema to evaluate a potential new predictive biomarker. Patients with metastatic colorectal cancer (mCRC) would be randomised (in a 2 to 1 ratio) to stratification by the biomarker or to receive unselected/population-based treatment. All three groups of patients would receive the same treatment, giving the potential to test three hypotheses and link the clinical outcome data to health and economic parameters (that treatment A equals treatment B equals standard therapy). On the basis of this several hypotheses can be tested. The first is that the biomarker-positive group has a superior clinical outcome compared with the biomarker-negative group following identical treatment. The second hypothesis is that the conventional population-based group has a superior clinical outcome compared with the biomarker-negative group following identical treatment. The third hypothesis is that the biomarker-positive group has a superior clinical outcome compared with the conventional population-based group following identical treatment. If sufficient evidence has been accumulated for a particular predictive marker, treatments A and B can differ to test the hypothesis that distinct treatments result in the same outcomes depending on marker status.[123] Reprinted by permission from Macmillan Publishers Ltd: Walter E, et al. Nat Rev Cancer 2009; 9(7):489–499.

After its initial identification, developing any new biomarker for clinical use is a time-consuming, multi-step process involving extensive testing, optimisation and validation. There is also a need to demonstrate that the new marker offers benefits over currently available methods i.e. that it has clinical utility. For any new biomarker, testing should be standardised and diagnostic tests should be validated with external quality assurance protocols. In some cases it may be deemed necessary for testing to be performed in specialised central laboratories to ensure quality. The National Comprehensive Cancer Network (NCCN) has published a timely report discussing the importance of the validation and clinical utility of tumour markers in oncology.[126] This report highlights the importance of both analytic validation (determining how accurately/reliably a test measures the characteristic of interest) and clinical validation (assessing the strength of association between assay result and outcome of interest in biomarker studies). Importantly, analytic validation also aims to standardise preanalytic specimen handling, preparation and storage. This report culminates in a series of recommendations that provide useful guidance for anyone involved in the tumour biomarker development process. While the NCCN report focusses on optimising the conduct of biomarker studies, guidance is now also available in the form of the REMARK guidelines[127,128] as to how biomarker data should be reported in the literature, but at the moment these focus only on the reporting studies of potential prognostic markers. Interestingly, a recent article by one of the authors of the REMARK guidelines highlighted that the literature on biomarkers continues to be plagued by issues of non-publication bias, selective reporting and incomplete reporting and suggested that the development of a tumour marker registry may help address some of these issues.[129]

Once a validated diagnostic test is available, it needs to be brought into clinical use as quickly as possible. For this reason, co-development programmes are becoming more commonplace, where diagnostic tests are developed alongside new targeted therapies. Such programmes have led to the development of trastuzumab and the Herceptest®(Genentech, San Francisco, USA) for HER-2-positive breast and gastric cancers and, more recently, vemurafenib (Genentech, San Francisco, USA) and the Cobas® 4800 *BRAF* V600 mutation test (Roche, Pleasanton, USA) for *BRAF* V600E-positive melanoma. Once validated, these 'companion' diagnostics can be licensed alongside the targeted agent, meaning that the treatment can be rapidly implemented into clinical practice as there is already a method available to identify the patients most likely to benefit. In line with this, in 2011 the FDA issued draft guidance clarifying that, in instances where the companion diagnostic is essential for the safe and effective use of a therapy, both products should be approved together.[130] A recent example of this is for crizotinib (Pfizer, New York, USA), a multi-targeted tyrosine kinase inhibitor, which was approved by the FDA for the treatment of anaplastic lymphoma kinase (*ALK*) rearranged non-small cell lung cancer (NSCLC) alongside its companion diagnostic – the Vysis® ALK Break Apart FISH Probe Kit (Abbott, Illinois, USA).[131] Notably, crizotinib was approved under the agencies accelerated approval program; no OS data were submitted and approval was based on ORRs achieved in two single-arm trials.[132] It is also an example of how co-development programmes can accelerate movement of discoveries from bench to bedside – it took an unprecedented 4 years from discovery of the *ALK* rearrangement in NSCLC to the approval of crizotinib.[132]

The cost of incorporating any new test into clinical practice is an important consideration. However, as was seen with *KRAS*[67–71] and also *BRAF*[99] testing, these costs are likely to be offset by savings in patient care, such as the cost-saving of avoiding unnecessary treatment and hospital stays in patients unlikely to respond.

Conclusions

Before the era of personalised medicine, cancer diagnosis, prognosis and treatment decisions were mainly based upon the histopathologic characteristics of the tumour. Nowadays, a more holistic approach is being taken where new molecular biomarkers and bioinformatic patient data are integrated to improve the accuracy of predicting prognosis and treatment efficacy. Huge advances have already been made, which can be exemplified by recent progress in the management of mCRC, particularly the discovery and implementation of *KRAS* as a predictive biomarker. Indeed, the implementation of new technologies is leading to the accumulation of huge amounts of genomic and proteomic data and the identification and validation of predictive biomarkers for existing and new targeted therapies, and will likely improve patient outcomes in the future. Taking discoveries from bench to clinic is a costly and time-consuming process. Ensuring all stakeholders across the healthcare spectrum are fully engaged and understand the importance of personalised medicine will help ensure that progress in science becomes progress in practice.

Although initial investment may be high, it should ultimately lead to huge long-term benefits and a cost-effective and rewarding future for cancer management. Ultimately, the first step will always be to incorporate biomarker discovery and validation into clinical trial design.

Conflicts of interest statement

VH has received honoraria for speaking at symposia and participating in advisory boards for Amgen, Roche, Merck Serono and Sanofi. He has also received research funding from Amgen, Roche, Merck Serono and Sanofi and travel support from Roche and Merck Serono. JYD has participated in advisory boards and/or spoken at symposia for Amgen, Merck Serono, Roche and Pfizer and has received research funding from Merck Serono. MD has acted as a consultant to Roche and Merck Serono and has participated in advisory boards and/or symposia for Amgen, Merck Serono, Roche, Novartis, Ipsen, Pfizer and Sanofi. MP has acted as a consultant and participated in advisory boards for Amgen and has also received honoraria and research funding from Amgen, Merck Serono, Ipsen, Novartis, Roche, and Sanofi.

Acknowledgements

Medical writing support (funded by Amgen [Europe] GmbH) was provided by Dawn Batty PhD from Bioscript Stirling Ltd. Amgen also reviewed the accuracy of the data regarding panitumumab and the overall context of the panitumumab data in line with other studies.

References

1. Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. *Nat Rev Cancer* 2008;**8**:473–80.
2. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;**256**:495–7.
3. Novartis Pharmaceuticals Corporation. Gleevec® (imatinib mesylate) prescribing information 2012. Available at: http://www.pharma.us.novartis.com/product/pi/pdf/gleevec_tabs.pdf.
4. Genentech Inc. Herceptin® [trastuzumab] prescribing information 2010. Available at: http://www.gene.com/gene/products/information/pdf/herceptin- =prescribing.pdf.
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011;**61**:69–90.
6. American Cancer Society. Cancer Facts and Figures 2010. Available at: http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-026238.pdf.
7. Garden OJ, Rees M, Poston CJ, et al. Guidelines for resection of colorectal cancer liver metastases. *Gut* 2006;**55**(Suppl. 4). iii1–8.
8. Chiappa A, Makuuchi M, Lygidakis NJ, et al. The management of colorectal liver metastases: expanding the role of hepatic resection in the age of multimodal therapy. *Crit Rev Oncol Hematol* 2009;**72**:65–75.
9. Gallagher DJ, Kemeny N. Metastatic colorectal cancer: from improved survival to potential cure. *Oncology* 2010;**78**:237–48.
10. Van Cutsem E, Nordlinger B, Cervantes A. Advanced colorectal cancer: ESMO clinical practice guidelines for treatment. *Ann Oncol* 2010;**21**(Suppl. 5): v93–97.
11. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012;**62**:10–29.
12. Kopetz S, Chang GJ, Overman MJ, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. *J Clin Oncol* 2009;**27**:3677–83.
13. Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. *Gut* 2011;**60**:116–29.
14. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000;**355**:1041–7.
15. Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000;**343**:905–14.
16. Capdevila J, Elez E, Peralta S, Macarulla T, Ramos FJ, Tabernero J. Oxaliplatin-based chemotherapy in the management of colorectal cancer. *Expert Rev Anticancer Ther* 2008;**8**:1223–36.
17. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000;**18**:2938–47.
18. Fuchs CS, Marshall J, Mitchell E, et al. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. *J Clin Oncol* 2007;**25**:4779–86.
19. Goldberg RM, Sargent DJ, Morton RF, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J Clin Oncol* 2004;**22**:23–30.
20. Pinedo HM, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol* 1988;**6**:1653–64.
21. Hirsch BR, Zafar SY. Capecitabine in the management of colorectal cancer. *Cancer Manag Res* 2011;**3**:79–89.
22. Lurje G, Manegold PC, Ning Y, Pohl A, Zhang W, Lenz HJ. Thymidylate synthase gene variations: predictive and prognostic markers. *Mol Cancer Ther* 2009;**8**:1000–7.
23. Afzal S, Gusella M, Jensen SA, et al. The association of polymorphisms in 5-fluorouracil metabolism genes with outcome in adjuvant treatment of colorectal cancer. *Pharmacogenomics* 2011;**12**:1257–67.
24. O'Dwyer PJ, Catalano RB. Uridine diphosphate glucuronosyltransferase (UGT) 1A1 and irinotecan: practical pharmacogenomics arrives in cancer therapy. *J Clin Oncol* 2006;**24**:4534–8.
25. Cecchin E, Innocenti F, D'Andrea M, et al. Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. *J Clin Oncol* 2009;**27**:2457–65.
26. Ross JS, Torres-Mora J, Wagle N, Jennings TA, Jones DM. Biomarker-based prediction of response to therapy for colorectal cancer: current perspective. *Am J Clin Pathol* 2010;**134**:478–90.
27. Weickhardt A, Wells K, Messersmith W. Oxaliplatin-induced neuropathy in colorectal cancer. *J Oncol* 2011;**2011**:201593.
28. Watanabe T, Kobunai T, Yamamoto Y, et al. Gene expression signature and response to the use of leucovorin, fluorouracil and oxaliplatin in colorectal cancer patients. *Clin Transl Oncol* 2011;**13**:419–25.
29. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**:2335–42.
30. Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008;**26**:2013–9.
31. Tebbutt NC, Wilson K, Gebski VJ, et al. Capecitabine, bevacizumab, and mitomycin in first-line treatment of metastatic colorectal cancer: results of the Australasian Gastrointestinal Trials Group Randomized Phase III MAX Study. *J Clin Oncol* 2010;**28**:3191–8.
32. Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 2007;**25**:1539–44.
33. Bevacizumab: new drug. Metastatic colorectal cancer: good in theory, not in practice. Prescrire Int 2006;15:94–7.
34. Welch S, Spithoff K, Rumble RB, Maroun J. Bevacizumab combined with chemotherapy for patients with advanced colorectal cancer: a systematic review. *Ann Oncol* 2010;**21**:1152–62.
35. Jubb AM, Hurwitz HI, Bai W, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. *J Clin Oncol* 2006;**24**:217–27.
36. Price TJ, Hardingham JE, Lee CK, et al. Impact of KRAS and BRAF gene mutation status on outcomes from the phase III AGITG MAX trial of capecitabine alone or in combination with bevacizumab and mitomycin in advanced colorectal cancer. *J Clin Oncol* 2011;**29**:2675–82.
37. Hurwitz HI, Yi J, Ince W, Novotny WF, Rosen O. The clinical benefit of bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase III study of bevacizumab with chemotherapy in previously untreated metastatic colorectal cancer. *Oncologist* 2009;**14**:22–8.
38. Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. *J Natl Cancer Inst* 2005;**97**:981–9.
39. Watanabe T, Kobunai T, Yamamoto Y, et al. Gene expression of vascular endothelial growth factor A, thymidylate synthase, and tissue inhibitor of metalloproteinase 3 in prediction of response to bevacizumab treatment in colorectal cancer patients. *Dis Colon Rectum* 2011;**54**:1026–35.
40. Weickhardt AJ, Williams D, Lee C, et al. Challenges for patient selection with VEGF inhibitors. *J Clin Oncol* 2011;**29**(Suppl.). abstract 3531.
41. Longo R, Gasparini G. Challenges for patient selection with VEGF inhibitors. *Cancer Chemother Pharmacol* 2007;**60**:151–70.
42. Bates DO, Catalano PJ, Symonds KE, et al. Association between VEGF splice isoforms and progression-free survival in metastatic colorectal cancer patients treated with bevacizumab. *Clin Cancer Res* 2012;**18**:6384–91.
43. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008;**8**:592–603.
44. Paez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* 2009;**15**:220–31.

45. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* 2005;**8**:299–309.
46. Shaked Y, Ciarrocchi A, Franco M, et al. Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. *Science* 2006;**313**:1785–7.
47. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 2003;**111**:1287–95.
48. Van Cutsem E, Peeters M, Siena S, et al. 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. *J Clin Oncol* 2007;**25**:1658–64.
49. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009;**101**:1308–24.
50. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;**360**:1408–17.
51. Van Cutsem E, Köhne CH, Lang I, et al. 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**:2011–9.
52. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010;**28**:4697–705.
53. Sobrero AF, Maurel J, Fehrenbacher L, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:2311–9.
54. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* 2010;**28**:4706–13.
55. Peeters M, Price T. Biologic therapies in the metastatic colorectal cancer treatment continuum: applying current evidence to clinical practice. *Cancer Treat Rev* 2012;**38**:397–406.
56. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;**357**:2040–8.
57. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;**377**:2103–14.
58. Tveit KM, Guren T, Glimelius B, et al. 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. *J Clin Oncol* 2012;**30**:1755–62.
59. Douillard JY, Zemelka T, Fountzilas G, et al. Randomized phase II study evaluating UFOX plus cetuximab versus FOLFOX4 plus cetuximab as first-line therapy in metastatic colorectal cancer: FUTURE. *Ann Oncol* 2012;**23**(Suppl. 4). abstract O-0017.
60. Bokemeyer C, Bondarenko I, Hartmann JT, et al. 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**:1535–46.
61. Moosmann N, von Weikersthal LF, Vehling-Kaiser U, et al. Cetuximab plus capecitabine and irinotecan compared with cetuximab plus capecitabine and oxaliplatin as first-line treatment for patients with metastatic colorectal cancer: AIO KRK-0104–a randomized trial of the German AIO CRC study group. *J Clin Oncol* 2011;**29**:1050–8.
62. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. *Lancet Oncol* 2010;**11**:38–47.
63. Siddiqui AD, Piperdi B. KRAS mutation in colon cancer: a marker of resistance to EGFR-I therapy. *Ann Surg Oncol* 2010;**17**:1168–76.
64. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter RASCAL study. *J Natl Cancer Inst* 1998;**90**:675–84.
65. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the RASCAL II study. *Br J Cancer* 2001;**85**:692–6.
66. Basso M, Strippoli A, Orlandi A, et al. KRAS mutational status affects oxaliplatin-based chemotherapy independently from basal mRNA ERCC-1 expression in metastatic colorectal cancer patients. *Br J Cancer* 2013;**108**:115–20.
67. Shankaran V, Bentrem DJ, Mulcahy MF, Bennett CL, Benson A, III. Economic implications of KRAS testing in metastatic colorectal cancer (mCRC). Gastrointestinal Cancers Symposium, San Francisco, CA, USA; 2009 [abstract 298].
68. Mancl EE, Kolesar JM, Vermeulen LC. Clinical and economic value of screening for Kras mutations as predictors of response to epidermal growth factor receptor inhibitors. *Am J Health Syst Pharm* 2009;**66**:2105–12.
69. Vijayaraghavan A, Efrusy MB, Goke B, Kirchner T, Santas CC, Goldberg RM. Cost-effectiveness of KRAS testing in metastatic colorectal cancer patients in the United States and Germany. *Int J Cancer* 2012;**131**:438–45.
70. Gallagher J, Cancer cost crisis warning from oncologists BBC News 26-9-2011. Available at: http://www.bbc.co.uk/news/health-15032862.
71. Königsberg R, Hulla W, Klimpfinger M, et al. Clinical and economic aspects of KRAS mutational status as predictor for epidermal growth factor receptor inhibitor therapy in metastatic colorectal cancer patients. *Oncology* 2011;**81**:359–64.
72. Smith G, Bounds R, Wolf H, Steele RJ, Carey FA, Wolf CR. Activating K-Ras mutations outwith 'hotspot' codons in sporadic colorectal tumours – implications for personalised cancer medicine. *Br J Cancer* 2010;**102**:693–703.
73. Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol* 2012;**30**:3570–7.
74. De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA* 2010;**304**:1812–20.
75. Bando H, Yoshino T, Shinozaki E, et al. Clinical outcome in patients with metastatic colorectal cancer harboring KRAS G13D mutation treated with cetuximab. *J Clin Oncol* 2011;**29**(Suppl. 4). abstract 448.
76. Bardelli A, Lamba S, Di Nicolantonio F, et al. Individual KRAS mutations modulate response to targeted agents in colorectal cancer. *J Clin Oncol* 2011;**29**(Suppl.). abstract e14123.
77. Modest DP, Reinacher-Schick A, Stintzing S, et al. Cetuximab-based or bevacizumab-based first-line treatment in patients with KRAS p.G13D-mutated metastatic colorectal cancer: a pooled analysis. *Anticancer Drugs* 2012;**23**:666–73.
78. Peeters M, Douillard JY, Van Cutsem E, et al. Evaluation of individual codon 12 and 13 mutant (MT) KRAS alleles as prognostic and predictive biomarkers of response to panitumumab (pmab) in patients with metastatic colorectal cancer (mCRC). European Multidisciplinary Cancer Congress, Stockholm, Sweden; 2011, abstract 33LBA.
79. Australasian Gastro-Intestinal Trials Group (AGITG). Randomised phase II study of cetuximab alone or in combination with irinotecan in patients with metastatic CRC with either KRAS WT or G13D mutation. ICE CREAM: The Irinotecan Cetuximab Evaluation and the Cetuximab Response Evaluation Among patients with G13D Mutation. Available at: http://agitg.org.au/clinical-trials/trials-open-to-recruitment/ice-cream/. Accessed 28 November 2012.
80. Modest DP, Stintzing S, Laubender RP, et al. Clinical characterization of patients with metastatic colorectal cancer depending on the KRAS status. *Anticancer Drugs* 2011;**22**:913–8.
81. Bellon E, Ligtenberg MJ, Tejpar S, et al. External quality assessment for KRAS testing is needed: setup of a European program and report of the first joined regional quality assessment rounds. *Oncologist* 2011;**16**:467–78.
82. Wong R, Cunningham D. Using predictive biomarkers to select patients with advanced colorectal cancer for treatment with epidermal growth factor receptor antibodies. *J Clin Oncol* 2008;**26**:5668–70.
83. Hawkes E, Cunningham D. Relationship between colorectal cancer biomarkers and response to epidermal growth factor receptor monoclonal antibodies. *J Clin Oncol* 2010;**28**:e529–531.
84. European Medicines Agency. Vectibix® (panitumumab) prescribing information 2011. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000741/WC500047710.pdf.
85. European Medicines Agency. Erbitux® (cetuximab) prescribing information 2011. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000558/WC500029119.pdf.
86. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Summary of opinion (post authorisation) – Vectibix (panitumumab) 2011. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion/human/000741/WC500107994.pdf.
87. Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 2009;**101**:715–21.
88. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. *Genes Chromosomes Cancer* 2011;**50**:307–12.
89. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:5705–12.
90. Fornaro L, Baldi GG, Masi G, et al. Cetuximab plus irinotecan after irinotecan failure in elderly metastatic colorectal cancer patients: clinical outcome according to KRAS and BRAF mutational status. *Crit Rev Oncol Hematol* 2011;**78**:243–51.
91. Seymour MT, Brown SR, Richman S, et al. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. *J Clin Oncol* 2011;**29**(Suppl.). abstract 3523.
92. Mao C, Liao RY, Qiu LX, Wang XW, Ding H, Chen Q. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. *Mol Biol Rep* 2011;**38**:2219–23.
93. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF (V600E) inhibition through feedback activation of EGFR. *Nature* 2012;**483**:100–3.
94. De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol* 2011;**12**:594–603.

95. Yang H, Higgins B, Kolinsky K, et al. Antitumor activity of BRAF inhibitor vemurafenib in preclinical models of BRAF-mutant colorectal cancer. *Cancer Res* 2012;**72**:779–89.
96. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. *Int J Cancer* 2011;**128**:2075–84.
97. Popovici V, Budinska E, Tejpar S, et al. Identification of a poor-prognosis BRAF-mutant-like population of patients with colon cancer. *J Clin Oncol* 2012;**30**:1288–95.
98. Reuters press release 2011. Molecular Response expands clinical offerings with BRAF mutation testing. Available at: http://www.reuters.com/article/2011/08/17/idUS220170+17-Aug-2011+BW20110817.
99. Blank PR, Moch H, Szucs TD, Schwenkglenks M. KRAS and BRAF mutation analysis in metastatic colorectal cancer: a cost-effectiveness analysis from a Swiss perspective. *Clin Cancer Res* 2011;**17**:6338–46.
100. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 2009;**69**:1851–7.
101. Sood A, McClain D, Maitra R, et al. PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer. *Clin Colorectal Cancer* 2012;**11**:143–50.
102. Mao C, Liao RY, Chen Q. Loss of PTEN expression predicts resistance to EGFR-targeted monoclonal antibodies in patients with metastatic colorectal cancer. *Br J Cancer* 2010;**102**:940.
103. Razis E, Briasoulis E, Vrettou E, et al. Potential value of PTEN in predicting cetuximab response in colorectal cancer: an exploratory study. *BMC Cancer* 2008;**8**:234.
104. Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. *PLoS One* 2009;**4**:e7287.
105. Adams RA, James MD, Smith CG, Sabourin JC. Epidermal growth factor receptor (EGFR) as a predictive and prognostic marker in patients with advanced colorectal cancer (aCRC): the MRC COIN trial experience. *J Clin Oncol* 2011;**29**(Suppl. 4). abstract 359.
106. Di Fiore F, Sesboue R, Michel P, Sabourin JC, Frebourg T. Molecular determinants of anti-EGFR sensitivity and resistance in metastatic colorectal cancer. *Br J Cancer* 2010;**103**:1765–72.
107. Heinemann V, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. *Cancer Treat Rev* 2009;**35**:262–71.
108. Tol J, Dijkstra JR, Klomp M, et al. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. *Eur J Cancer* 2010;**46**:1997–2009.
109. Jacobs B, De Roock W, Piessevaux H, et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. *J Clin Oncol* 2009;**27**:5068–74.
110. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol* 2007;**25**:3230–7.
111. Baker JB, Dutta D, Watson D, et al. Tumour gene expression predicts response to cetuximab in patients with KRAS wild-type metastatic colorectal cancer. *Br J Cancer* 2011;**104**:488–95.
112. Molinari F, Martin V, Saletti P, et al. Differing deregulation of EGFR and downstream proteins in primary colorectal cancer and related metastatic sites may be clinically relevant. *Br J Cancer* 2009;**100**:1087–94.
113. Cejas P, Lopez-Gomez M, Aguayo C, et al. Analysis of the concordance in the EGFR pathway status between primary tumors and related metastases of colorectal cancer patients: implications for cancer therapy. *Curr Cancer Drug Targets* 2012;**12**:124–31.
114. Richman SD, Chambers P, Seymour MT, et al. Intra-tumoral heterogeneity of KRAS and BRAF mutation status in patients with advanced colorectal cancer (aCRC) and cost-effectiveness of multiple sample testing. *Anal Cell Pathol (Amst)* 2011;**34**:61–6.
115. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012;**366**:883–92.
116. Diaz Jr LA, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 2012;**486**:537–40.
117. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012;**486**:532–6.
118. Montagut C, Dalmases A, Bellosillo B, et al. Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* 2012;**18**:221–3.
119. Metges J, Raoul J, Achour N, et al. PANERB study: panitumumab after cetuximab-based regimen failure. *J Clin Oncol* 2011;**28**(Suppl.). abstract e14000.
120. Amir E, Seruga B, Martinez-Lopez J, et al. Oncogenic targets, magnitude of benefit, and market pricing of antineoplastic drugs. *J Clin Oncol* 2011;**29**:2543–9.
121. Hamburg MA, Collins FS. The path to personalized medicine. *N Engl J Med* 2010;**363**:301–4.
122. Mandrekar SJ, Sargent DJ. Clinical trial designs for predictive biomarker validation: one size does not fit all. *J Biopharm Stat* 2009;**19**:530–42.
123. Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. *Nat Rev Cancer* 2009;**9**:489–99.
124. US Department of Health & Human Services 2012. Genome-Wide Association Studies (GWAS). Available at: http://gwas.nih.gov/.
125. Diamandis M, White NM, Yousef GM. Personalized medicine: marking a new epoch in cancer patient management. *Mol Cancer Res* 2010;**8**:1175–87.
126. Febbo PG, Ladanyi M, Aldape KD, et al. NCCN Task Force Report: evaluating the clinical utility of tumor markers in oncology. *J Natl Compr Canc Netw* 2011;**9**(Suppl. 5):S1–32.
127. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Cancer Inst* 2005;**97**:1180–4.
128. Altman DG, McShane LM, Sauerbrei W, Taube SE. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 2012;**9**:e1001216.
129. McShane LM, Hayes DF. Publication of Tumor Marker Research Results: The Necessity for Complete and Transparent Reporting. *J Clin Oncol* 2012;**108**:115–20.
130. US Food and Drug Administration 2012. Draft guidance for industry and Food and Drug Administration staff – in vitro companion diagnostic devices. Available at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm262292.htm.
131. US Food and Drug Administration 2011. FDA approves Xalkori with companion diagnostic for a type of late-stage lung cancer. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm269856.htm.
132. Ou SH. Crizotinib: a drug that crystallizes a unique molecular subset of non-small-cell lung cancer. *Expert Rev Anticancer Ther* 2012;**12**:151–62.