

Contents lists available at ScienceDirect

Seminars in Oncology

journal homepage: www.elsevier.com/locate/ysonc

Critical parameters in targeted drug development: the pharmacological audit trail

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ARTICLE INFO

Keywords:

Pharmacological audit trail
Biomarkers
Pharmacodynamics
Pharmacokinetics

ABSTRACT

The Pharmacological Audit Trail (PhAT) comprises a set of critical questions that need to be asked during discovery and development of an anticancer drug. Key aspects include: (1) defining a patient population; (2) establishing pharmacokinetic characteristics; (3) providing evidence of target engagement, pathway modulation, and biological effect with proof of concept pharmacodynamic biomarkers; (4) determining intermediate biomarkers of response; (5) assessing tumor response; and (6) determining how to overcome resistance by combination or sequential therapy and new target/drug discovery. The questions asked in the PhAT should be viewed as a continuum and not used in isolation. Different drug development programmes derive different types of benefit from these questions. The PhAT is critical in making go-no-go decisions in the development of currently studied drugs and will continue to be relevant to discovery and development of future generations of anticancer agents.

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1. Introduction

Molecularly targeted drugs have become an integral component of the treatment of cancer patients over the last three decades and their importance continues to increase. There have been successes where targeted agents have shown benefit in disease subtypes such as melanoma (vemurafenib [1]) and renal cancer (sorafenib [2]) where conventional chemotherapy had close to no efficacy. Molecular therapeutics have also added incremental patient benefit for diseases such as diffuse B-cell lymphoma (rituximab [3]) where chemotherapy was already of proven benefit. However, despite successes, there have been many failures and there is a distinct feeling in the oncology research community that the full potential of molecularly targeted approaches has not yet been realised. Many failures of novel anticancer drugs to meet endpoints in phase III studies have led to an economic model of drug discovery and development that is unsustainable to the pharmaceutical companies [4–6] and to pricing of drugs that will often be out of reach for healthcare systems and cancer patients [7].

The Pharmacological Audit Trail (PhAT) is based on addressing essential questions relating to biomarkers (Fig. 1A) at the appropriate

stages of drug development, aiming to maximize our chances of success [8–10]. It is designed to help researchers in evidence-based decision-making at various points in the life cycle of drug discovery and development (Fig. 1B).

2. Population identification for targeted drugs

Many drug discovery campaigns target protein products of specific genetic alterations linked to a tumor type or a subset of patients with poor prognosis within a cancer type. Thus, before the initiation of a first-in-human clinical trial there is often a biologically defined patient population. Clear examples include *BRAF* mutations in melanoma [11] or *HER-2* amplifications in breast cancer [12]. An extension to this is to include the drugs that target an aberrant pathway downstream of a pre-specified genetic alteration, such as MEK inhibitors used in the setting of melanoma driven by *BRAF* mutations [13]. However, this approach does not always lead to finding populations of patients with mutant oncogenes that are likely to respond to treatment; for example, *PIK3CA* mutations do not exclusively predict response to mammalian target of rapamycin (mTOR) inhibitors [14]. Nevertheless, mutation and amplification status of tumors are increasingly being seen as critical to regulatory approval (Table 1).

The availability of affordable hotspot mutation [15,16] and next-generation sequencing platforms [17] have made clinical testing of specific target-based hypotheses possible even in early-stage clinical

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A

Pharmacological Audit Trial (PhAT)**Defining target population**

- Clinically testable hypothesis

Pharmacokinetics

- ADME, PK-PD-toxicity relationships
- Population PK, food effect, drug interactions

Pharmacodynamics

- Proof of mechanism (POM)
- Proof of concept (POC)

Intermediate biomarkers of response

- Early prediction of response/resistance

Reassessment of tissue at resistance

- Understand mechanisms of acquired resistance

Overcome Resistance

- Combination/sequential therapy
- New targets/drugs

B

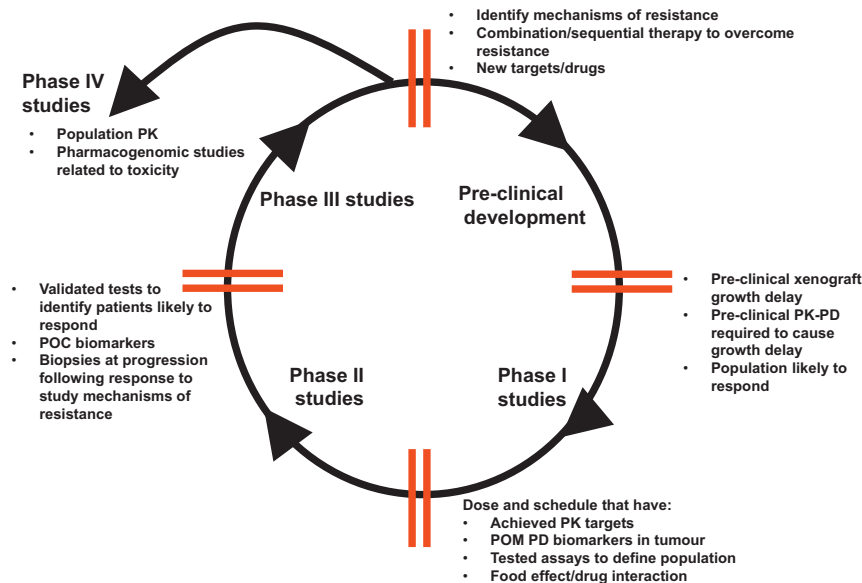
Anticancer drug development life cycle and PhAT

Fig. 1. The pharmacological audit trail (PhAT). (A) The six crucial aspects of the pharmacological audit trail. (B) The relationship of the PhAT to the various phases of the life cycle of an anticancer drug. The red lines indicate 'checkpoints' between different phases of drug development where go-no-go decisions are made.

trials. This has led to the possibility of conducting 'basket' clinical trials where subpopulations of patients with a specific mutation can be tested irrespective of their tumor type [18]. In addition to DNA mutations, protein expression can also be used to define tumor subtypes likely to respond to treatment. As previously noted, *HER-2* amplification has been used to define patients likely to respond to *HER-2*-targeting therapy and this can be detected by overexpression of the protein in cancer cells.

Immunotherapy has made huge advances in the last decade and more recently programmed death receptor ligand-1 (*PDL-1*) expression [19] is currently being used to stratify patient groups entering clinical trials of anti-*PDL-1* antibodies [20]. Of recent interest, the evaluation of unexpected responders in early phase clinical trials (sometimes called *n=1* studies) has led to the

retrospective study of determinants of sensitivity to targeted anticancer drugs [21].

To help define and validate a patient population biomarker before the start of a first-in-human clinical trial, various approaches have been tried. The use of large (> 500) cancer cell line panels is now feasible. This approach has been retrospectively validated by identifying patient populations with, for example, *BRAF* or *EGFR* mutations that are predictive for the activity of *BRAF* or *EGFR* inhibitors, respectively, and remains a promising approach. However, prospective validation of new subgroups of patients suggested by this approach is needed [22]. Other methodologies include use of mRNA gene expression signatures; examples include *RAF* or *RAS*-like signatures [23,24], which have been proposed and are currently being used in clinical trials [25]. The use of established cancer cell

Table 1
Licensed targeted anticancer drugs, their targets, and tests done to define sensitivity or resistance to these agents.

Drug	Target	Test
Imatinib	c-KIT	<i>KIT</i> mutations
Trastuzumab	HER-2	HER-2 IHC, HER-2 FISH
Lapatinib		HER-2 IHC, HER-2 FISH
T-DM1		HER-2 IHC, HER-2 FISH
Pertuzumab	HER-3	HER-2 IHC, HER-2 FISH
Erlotinib	EGFR	<i>EGFR</i> mutations
Afatinib		<i>EGFR</i> mutations
Dabrafenib	BRAF	<i>BRAF</i> mutations
Vemurafenib		<i>BRAF</i> mutations
Trametenib	MEK	<i>BRAF</i> mutation
Crizotinib	ALK	<i>ALK</i> rearrangement FISH
Cetuximab	EGFR	<i>KRAS</i> mutation
Tamoxifen	ER	Estrogen receptor IHC
Rituximab	CD20	CD-20 IHC

FISH = fluorescence in situ hybridization; IHC = immunohistochemistry.

line panels and tumor xenografts to study drug sensitivity has been questioned, and the use of patient-derived xenografts [26] and alternative models such as organoid cultures and patient 'avatar' models have been hypothesized to better reflect patients' tumors [27].

In rare instances, biomarkers have been introduced into prescribing guidelines after the drug has been licensed, as was done when *KRAS* mutation status was found to determine resistance to cetuximab in the setting of colorectal cancer [28]. However, there are clear examples of targeted drugs that have been licensed without a link to a specific biomarker for the activity of the drug, as in the case of histone deacetylase (HDAC) inhibitors used to treat cutaneous T-cell lymphoma [29], or the administration of vascular endothelial growth factor receptor (VEGFR) inhibitors in renal cancer [30].

While appreciating that many drug development programs will have different features, the definition of a patient subgroup likely to respond to treatment is a critical early step in all cases. Multiple responses may be demonstrated in specified populations, even early in phase I, as was the case for *BRAF* mutations predicting response to *BRAF* inhibitors [1], or germline *BRCA* mutations

predicting response to poly ADP ribose polymerase (PARP) inhibitors [31]. If predictive biomarker assays can be tested in phase I and refined in phase II studies, they are more likely to be used as companion diagnostics in phase III studies and the post-registration setting.

3. Pharmacokinetics

Pharmacokinetic (PK) studies are essential in drug development, predominantly adding value in early-stage studies. First, attaining a PK exposure in humans that causes anticancer activity in preclinical models plays an important part in go-no-go decisions during phase I studies. Bench-marking human PK to preclinical models is now routine practice, and we have used this approach to evaluate AKT, mTOR, HSP90, and HDAC inhibitors at our institution [32–35]. Second, PK studies can help clarify toxicity profiles in early phase development, especially by correlating PK profiles with dose-limiting toxicities. Some drugs have toxicities related to early C_{max} (the maximal concentration achieved in plasma), as in the case of serous retinopathy observed with MEK inhibitors [36,37], or hyperglycemia, as observed with AKT inhibitors [35,38]. Adjusting the dose of the drug such that the C_{max} falls below levels that cause unacceptable toxicity is a critical aspect of phase I studies. Third, PK characterization is essential for determining the schedule of oral anticancer agents. For example, the allosteric mTOR inhibitor everolimus has a half-life of approximately 26 hours and is administered once daily [39], while mTORC1/2 kinase inhibitors such as AZD2014 have a half-life of approximately 3 hours and are administered twice a day [40]. Fourth, PK studies are also critical to determine dosing recommendations for use of the drug in relation to the consumption of food and concomitant medications. For example, plasma concentrations of lapatinib can be significantly higher when taken with food, and this may have implications for interpreting toxicity and efficacy [41]. Fifth, because targeted drugs are often administered orally, PK drug interaction studies are important due to their metabolism by CYP3A4 [42].

Therapeutic drug monitoring (TDM) uses PK measurements to determine efficacy and toxicity in routine clinical practice in areas such as anti-epileptic or anticoagulation therapy; however, its use

Pharmacokinetics and PhAT

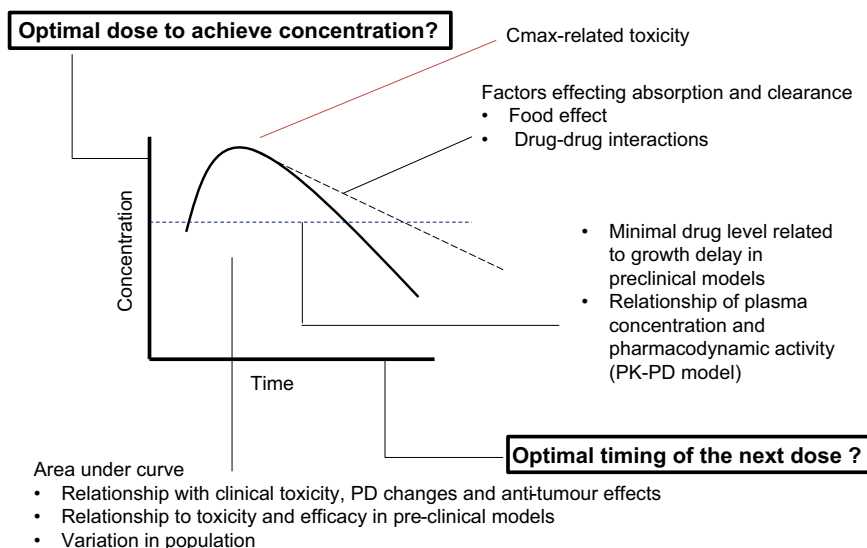


Fig. 2. Pharmacokinetics in the PhAT. The two main questions asked using PK analysis in early phase drug development are highlighted. Multiple parameters studied within the umbrella of pharmacokinetics and their clinical importance are also shown.

in cancer therapy is not widespread [43,44]. In those instances where cancer has become a disease that requires chronic treatment, such as imatinib for the treatment of chronic myeloid leukaemia [45], TDM will become increasingly relevant [46].

As the above examples demonstrate, PK assessment is essential to decision-making in phase I studies and also in preclinical discovery and development (Figs. 1B and 2). On rare occasions PK studies can play a role in drug development as late as the post-marketing phase.

4. Pharmacodynamic and proof of mechanism biomarkers

Use of pharmacodynamic (PD) biomarkers, including proof of mechanism (POM) endpoints, is a critical aspect of the PhAT in the development of targeted agents (Fig. 3). It is extremely important to demonstrate modulation of the target by the drug, and also to ensure that target engagement results in downstream perturbation of the intended biochemical pathway and the subsequent biological phenotype.

The first question to be asked is what is the best POM biomarker? An ideal biomarker is one that measures changes in the target itself or in proteins that are in close functional proximity to the target. An example of studying changes in the target itself is quantifying phospho-AKT (p-AKT) while evaluating an allosteric AKT inhibitor such as MK2206 where the POM biomarker is a phosphorylation site on the target [38]. An example of a proximal mechanistic biomarker is quantifying the accumulation of dehydrocorticosterone while evaluating the CYP17:C17,20 lyase inhibitor abiraterone [47]. A more commonly used strategy includes the measurement of protein biomarkers ‘downstream’ of the intended target such as quantification of p-ERK while studying MEK inhibitors including trametinib and selumetinib [36,48].

The second question to be asked in developing PD endpoints is what is the best tissue in which to study the biomarker? Clinical trials often use surrogate normal tissues at early dose levels in phase I studies because it may be considered unethical to take tumor biopsies from patients at dose levels that are unlikely to show PD effects. Normal tissue also has the advantage of being

able to be sampled repeatedly. Examples of normal tissue used in POM-PD studies include measuring p-AKT in platelet rich plasma while studying PI3K inhibitors [49,50], or using peripheral blood mononuclear cells to measure histone acetylation or p-ERK levels when evaluating HDAC [34,51] and MEK [36] inhibitors, respectively. Normal cells extracted from blood have the advantage of allowing PD endpoints and plasma drug levels to be determined simultaneously, thus making PK-PD modeling particularly relevant. Examples of PK-PD modelling using combined PK and PD determinations in blood to recommend a phase II dose include the use of p-S6 or protein acetylation in peripheral blood mononuclear cells in the case of mTOR or HDAC inhibitors, respectively [32,34].

However, blood-derived POM-PD can be criticised for showing positive PD effects even if a drug has limited penetration outside the vascular space. Assessing hair follicles circumvents this problem. Histone acetylation or p-PRAS40 quantification in hair follicles while evaluating HDAC [34,51] and AKT [38] inhibitors, respectively, and punch skin biopsies to quantify p-ERK when evaluating MEK inhibitors [37] have been used successfully in first-in-human studies.

The use of normal tissues as surrogates can be problematic because they may not represent rapidly proliferating tumor tissue and lack the activating mutations that drive cancer. Hence, pre- and post-treatment tumor biopsies are the gold standard for evaluating mechanistic biomarkers to examine PD changes in the target malignant tissue in question [52]. Pre- and post-treatment tumor biopsies are often done in the last few cohorts of a dose-escalation phase I study and have been successfully used to support recommendation of phase II doses of BRAF [1], MEK [48], PI3K [49], and AKT [35,38] inhibitors. Tumor biopsies serve to study cancer cells, but can also be used to study the stroma. Tumor infiltration by specific subsets of lymphocytes (CD8⁺) could be used as POM biomarkers while evaluating immunotherapeutic agents such as PDL-1 antibodies [20].

Pre- and post-treatment tumor biopsies are not without their own problems. Although considered safe in the literature [53], they are invasive procedures and have the potential for complications such as bleeding. Biopsies from multiple sites of resected tumors have shown considerable genetic heterogeneity [54], and

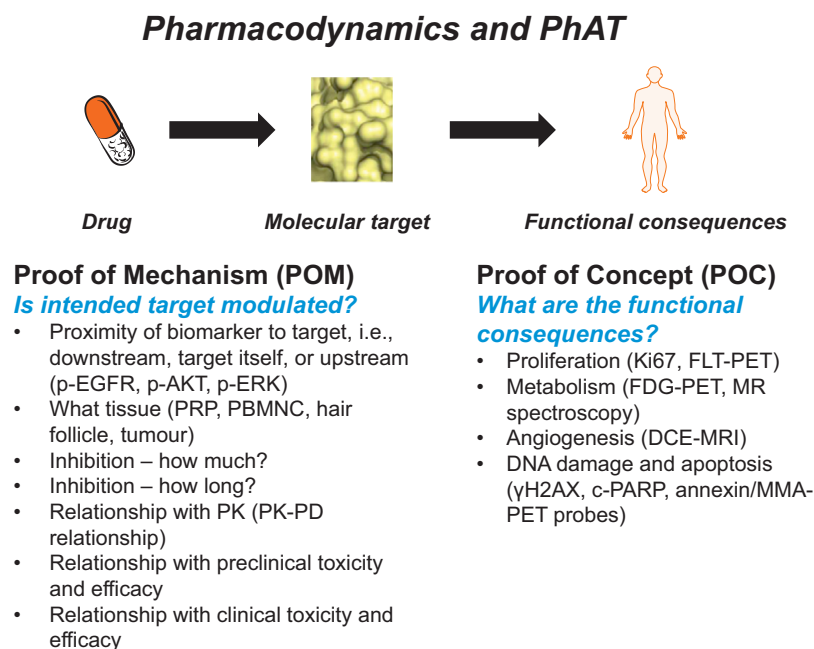


Fig. 3. Pharmacodynamics in the PhAT. Pharmacodynamic biomarkers can be conceptually divided into proof of concept or proof of mechanism biomarkers. The key questions, tissues studied, and platforms used to study them are shown.

this has the potential to influence interpretation of POM-PD effects. In some instances, it is possible to circumvent being misled by intra-tumoral heterogeneity by using imaging to assess POM-PD biomarkers. An example is the use of 89Zr-labeled trastuzumab positron emission tomography (PET) scans to detect degradation of HER-2 caused by the HSP90 inhibitor AUY922 or luminespib [55].

The third important question asked in relation to PD assays is how much target inhibition is enough? Because tumor biopsies are not done in sufficient numbers across all dose levels, it may not be possible to demonstrate a dose-response relationship in tumor tissue; instead, this is commonly done in normal tissue where it is sometimes possible to demonstrate a sigmoid shaped curve in which no additional significant biomarker modulation occurs upon increasing drug doses once a plateau is reached. Examples include HSP70 induction while evaluating HSP90 inhibitors [33] (where, in fact, depletion of protein clients is more important) and p-ERK inhibition while evaluating MEK inhibitors [36], but data from normal tissue identifying a plateau in POM-PD response should not be the sole criterion used to halt dose escalation, as PD changes in normal tissue may not mimic tumor tissue. In some early-phase trials where it has been possible to biopsy multiple patients (for example, while evaluating BRAF inhibitors such as vemurafenib in the setting of melanoma), correlation of the degree of biomarker modulation (in this case p-ERK inhibition) to tumor response has been achieved [56]. Quantitation of PD effects is important, especially where > 95% of the signaling output must be inhibited to have the desired effect, as with many kinase inhibitors.

It is important to discuss the possible downside of conducting PD biomarker studies. For drugs that target a relatively large number of kinases, the final activity of a drug might not be related to the POM-PD biomarker being studied. For example, phase I trials of sorafenib, initially developed as a RAF inhibitor, successfully demonstrated reduction in p-ERK levels [57]. However, it proved to be sorafenib's anti-angiogenic activity that led to approval for renal cell cancer [2], acting on VEGF and other targets; trials of sorafenib in *BRAF* mutation-driven tumors, such as melanoma, were negative [58]. As discussed in other articles in this issue of *Seminars in Oncology*, the use of POM-PD biomarkers does entail careful technical validation, which adds additional costs [59,60]. However, the cost of failure of late-phase development in clinical trials is very high, and we believe that the increase in cost resulting from use of PD biomarkers in early drug development is entirely justified as they could prevent drugs not modulating target from progressing to expensive phase II and III trials [61]. For example, the PARP inhibitor iniparib failed to meet its endpoint in a phase III study and, regrettably, it was only at this stage that its mechanism as a PARP inhibitor was questioned, which is something that should have been discovered in phase I [62].

5. PD proof of concept biomarkers

The ultimate effect of inhibiting a cancer target is usually related to modulating one of the hallmark traits of the cancer cells [63]. Proof of concept biomarkers thus predominantly focus on assessing functional biological consequences of inhibiting targets in tumor tissue (Fig. 3).

5.1. Proliferation

Examples of PD biomarkers for proof of concept have included assessing proliferation through use of Ki67 determined by immunohistochemistry [64]. As an example, use of this biomarker has accompanied POM-PD studies in a clinical trial of trametinib,

where tumor Ki67 effects were measured to show the functional consequence of inhibiting p-ERK [48]. In later stage phase II studies, changes in Ki67 can be used as a surrogate measure to determine clinical efficacy; for example, it has been employed in randomized phase II studies of fulvestrant to demonstrate the efficacy of two doses of the drug [65]. Other methods of determining proliferation include the use of imaging modalities like FLT-PET [66], where it has been possible to correlate immunohistological markers of proliferation such as Ki67 to SUVmax values. Post-treatment FLT-PET changes in rapidly proliferative malignancies like lymphoma not only confirm reduction in DNA synthesis and, hence, proliferation as a consequence of treatment, but could also be used as an early predictor of complete response [67].

5.2. Metabolism

There are multiple imaging platforms that detect changes in metabolism caused by targeted anticancer agents. FDG-PET has been extensively used to study glucose metabolism while evaluating anticancer drugs [68]. Changes in glucose metabolism are usually not directly linked to the mechanism of action of the drug, but rather a downstream consequence of inhibiting the intended target. Examples include the FDG-PET changes observed in gastrointestinal stromal tumors (GIST) following treatment with imatinib, which inhibits c-KIT [69], or the alterations within tumors in patients with melanoma following treatment with the BRAF or MEK inhibitors vemurafenib [70] and trametinib [48], respectively. Magnetic resonance spectroscopy has been used to observe changes in phosphocholine and, more recently, lactate levels that could be used to evaluate drugs targeting metabolism such as BRAF, PI3K, and HSP90 and HDAC inhibitors [71].

5.3. Angiogenesis

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) studies have demonstrated changes in angiogenesis during evaluation of anti-angiogenic agents such as bevacizumab [72,73] and sorafenib [74,75]. These are examples of studying downstream POC biomarkers of drugs that target VEGF or VEGFR.

5.4. DNA damage and apoptosis

DNA damage and apoptosis are important endpoints of many targeted agents. For example, DNA damage caused by PARP inhibitors has been studied using phosphorylation of γ H2AX in hair follicles [31]. Multiple 18F-labeled PET probes such as methylmalonic acid (MMA) [76], caspase-3 [77], and annexin [78,79] are under clinical evaluation to quantitate and determine the distribution of apoptosis within tumors before and after drug treatment.

The expectations for and interpretation of these biomarker endpoints depends on the setting in which they are used. In phase I studies, biomarkers for proof of concept are often used to accompany POM-PD biomarkers where it is critical to determine that target modulation has led to functional consequences in the tumor. Examples discussed above in this section include evaluating the degree of modulation of proliferation (Ki67, FLT-PET), apoptosis (cleaved caspase-3), angiogenesis (DC-MRI), or metabolism (FDG-PET). In later, phase II–III studies these biomarkers are often correlated to clinical outcomes such as response or time to progression. In this setting there has been some success, such as the use of FDG-PET predicting early response to treatment in specific subtypes of lymphoma [80,81]. Although widely used, such biomarkers should not be the sole criteria for decision-making [82].

6. Intermediate endpoints of clinical response

This is an area of emerging importance in the PhAT. Having an early insight into whether a patient will benefit from treatment or not is important as it empowers the treating clinician to change treatment early, improving patient outcomes, reducing unnecessary toxicity experienced by the patient, and improving efficiency in healthcare delivery.

6.1. Tumor-specific circulating biomarkers

Prostate-specific antigen (PSA) has been used to follow response in prostate cancer, as there is often 'bone only' disease, making imaging to delineate clinical outcome challenging. An early drop in PSA has been shown in some clinical trials to predict survival [83], though this is not universally accepted [84]. CA-125 has been conventionally used to follow treatment outcomes in ovarian cancer; however, tests such as CA-125 have been validated in patients receiving cytotoxic chemotherapy where rapid tumor regression is seen [85]. More sophisticated analysis is required to interpret data related to CA-125 when evaluating molecularly targeted agents such as tamoxifen where rapid tumor regression may not be seen [86].

6.2. Circulating tumor cell count and circulating free DNA

Circulating tumor cell (CTC) counts have been used to assess early response to treatment of prostate cancer [87]. CTCs have many advantages, including the ability to sample multiple time points, and use in a disease where the only site of metastasis is bone, where conventional imaging is difficult [88]. Quantification of circulating free DNA (cfDNA) has been linked to tumor burden and prognosis in patients with metastatic cancers entering phase I studies [15], and it is being studied further to determine whether changes in cfDNA levels can be used as an early biomarker of response or resistance in breast cancer [89].

6.3. Imaging techniques

FDG-PET has provided insights into early response to targeted treatment such as imatinib [90] for the treatment of GIST or in Hodgkin's lymphoma with chemotherapy [81]. Early changes in cell number within a tumor can be studied using diffusion-weighted MRI [91], and this is currently being validated to study response in tumors such as ovarian [92], prostate [93], and colon cancer [94].

Overall, intermediate endpoints of response or resistance to treatment are thus critical to drug development (Fig. 4). However, they need extensive validation, often in a large number of patients. Also of note, care should be taken to differentiate intermediate biomarkers of response from prognostic biomarkers.

7. Reassessment of molecular alteration at disease progression

The use of targeted anticancer agents in patients with tumors that have been genetically characterized has led to considerable success; however, it has increasingly become evident that acquired resistance is inevitable. For example, resistance occurs within 6–12 months of treatment with vemurafenib for the treatment of *BRAF*-mutant melanoma [95] or gefitinib for the treatment of *EGFR*-mutant lung cancer [96].

Obtaining tumor biopsies at progression following initial response has given researchers insights into mechanisms of resistance. For example, biopsies obtained after patients with melanoma became resistant to *BRAF* inhibitors were compared to pretreatment samples, and these revealed (among other changes) the presence of new *MEK1* and *MEK2* mutations [97].

Sampling circulating cells has the advantage that it can be repeated at many time points. CTCs have many applications [88]. In addition to quantifying them, it is possible to detect novel mutations that cause resistance to targeted treatment, for example, T790M *EGFR* mutations were found in CTCs from patients with *EGFR*-mutant lung cancer receiving *EGFR*-targeted treatment [98]. Serial blood samples to study cfDNA is an important approach, and can be used in early phase clinical trials [15] in a variety of tumor

Intermediate endpoints of response and reassessment of disease on progression

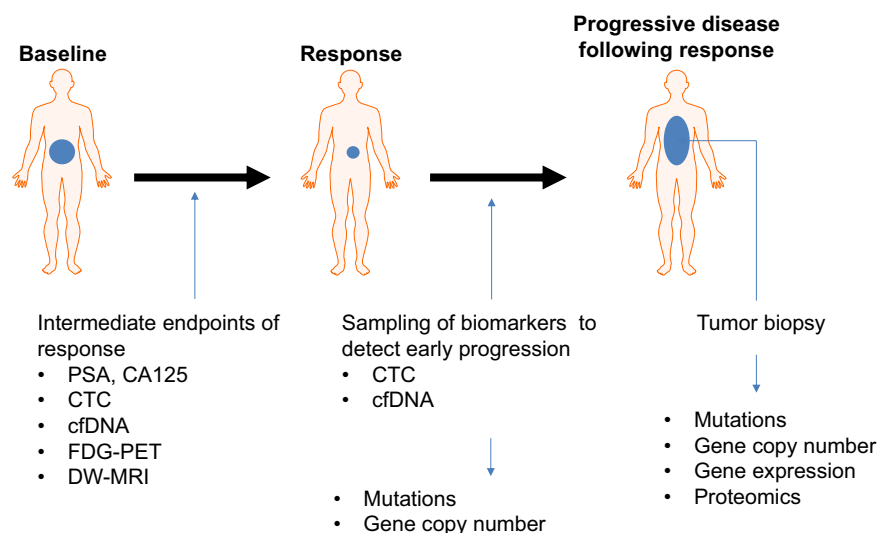


Fig. 4. Intermediate endpoints of response and reassessment of disease at progression. Sampling strategy of biomarkers related to PhAT in the patient journey. Examples of tissue used and tests done while evaluating these biomarkers are shown.

types for detecting new mutations after resistance has appeared following treatment with chemotherapy or targeted anticancer agents (Fig. 4) [89,99].

Growing tumor xenografts [100,101] from patient biopsies allows parallel treatment of the xenografts while the patient is undergoing treatment. These tumor xenograft models, variously called patient-derived xenografts (PDX) or avatar models, are being used to elucidate mechanisms of resistance in patients [27], and their use will be more widespread in the future.

Thus, reassessment of tissue, cells, and circulating cells and DNA from human tumors is critical to understand the mechanism of resistance and plan further treatment. Technological advances now allow us to sample cfDNA representing tumor in blood samples, which could, in the future, enable us to change treatment or propose combination therapy in real time.

8. Reversal of resistance by new drugs or combination therapy

Reassessment of tumors when a patient has become clinically resistant to the treatment had led to the identification of alterations within the original target. This information can be successfully exploited, as is exemplified in the case of detection of ‘gate keeper’ T790M *EGFR* mutations occurring following treatment with gefitinib [98]. These observations led to the development of irreversible *EGFR* inhibitors such as afatinib [102] and, more recently, the development of the T790M *EGFR*-specific inhibitor AZD9291 [103].

While analysis of mutations can help us understand mechanisms of resistance, cross talk and altered feedback loops in signal transduction networks that are not a consequence of mutation can also be a mechanism of resistance [104]. An example of the clinical importance of feedback loops is *EGFR* phosphorylation following *BRAF* inhibition in *BRAF*-mutant colon cancer. This was brought to light with elegant experiments using shRNA screens in the presence of a *BRAF* targeted drug as a means of identifying synthetic lethality [105]. This mechanism of resistance could be circumvented by combining *BRAF* and *EGFR* inhibitors, as currently being evaluated in hypothesis-testing clinical trials.

Also of importance is understanding transient, reversible changes in signalling following the exposure to a drug. For example, transient SOX-10-mediated transforming growth factor-beta (TGF- β) signaling

in melanomas can follow treatment with *BRAF* and *MEK* inhibitors; this could theoretically be circumvented by re-introducing the drug after a ‘drug holiday’ [106]. Interestingly, high throughput platforms now being used have correctly predicted resistance even before such events have occurred in the clinical development of the drug. For example, over-expression of a large panel genes in a V699E mutant melanoma cell line to look for genes conferring resistance to *BRAF* inhibitors led to the elucidation of the mechanism of COT-mediated *MEK* activation [107,108], subsequently overcome by combinations of *BRAF* and *MEK* inhibitors [109].

Combining anticancer agents is an attractive proposition to overcome resistance but there may be considerable challenges due to combinatorial toxicity [110]. Careful attention to detail of scheduling and dose, using all aspects of the PhAT, are necessary to fully realize the clinical promise of innovative combinations (Fig. 5) [111].

9. Future of the PhAT

The PhAT is a conceptual framework that we developed which codifies a series of biomarker-driven questions that are used to support pharmacologic understanding and evidence-based decision-making in drug discovery and development [8–10].

Over the last three decades, the focus of cancer drug discovery has changed substantially from the development of one-size-fits-all cytotoxic chemotherapy agents, to personalized or precision molecular targeted agents that modify oncogenic signal transduction and epigenetic control mechanisms, and more recently to immunotherapy and antibody drug conjugates. The development of all targeted treatments has benefited from asking the critical questions detailed in the PhAT. Each category of drugs and individual agents presents different unique challenges and rewards as well as specific technical differences when using the PhAT.

Newer drugs, however, like immune checkpoint modulator antibodies, notably anti-CTLA4, PD-1, and PDL-1, have resulted in clinical response in an increasingly wide range of tumor types without the use of specific patient-selection biomarkers for example. Further, clinical trials have not reported widely on PK and PD of these agents. Note that this is not a failure of the PhAT, but reflects the speed of development of these agents, which have been taken forward without answering multiple key questions

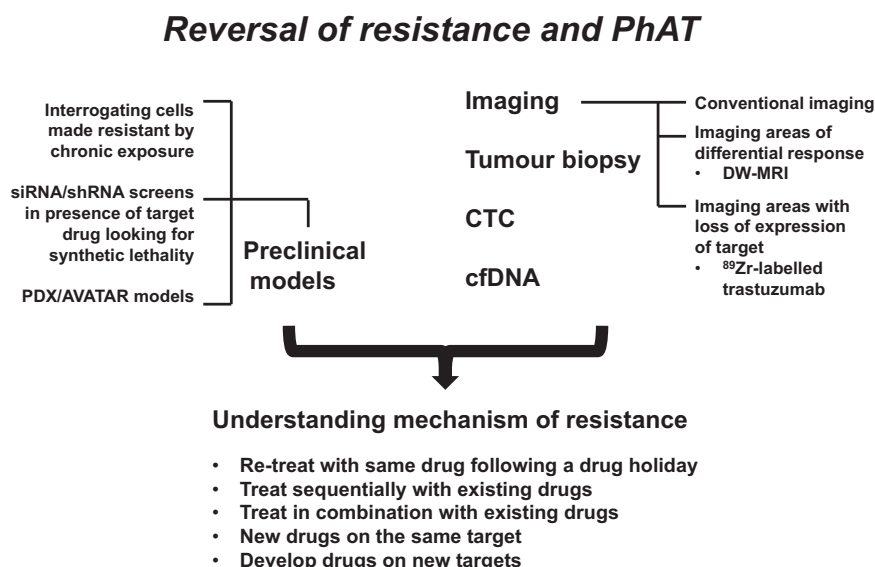


Fig. 5. Reversal of resistance and PhAT. Resistance to targeted anticancer drugs can be studied using clinical material and preclinical methodologies shown. Following analysis of the data and understanding the mechanisms of resistance, methods of clinically overcoming acquired drug resistance are discussed.

defined by the PhAT. Immune checkpoint agents do have significant toxicity; POM and POC biomarkers could be used to further refine dosing and schedules in the future. Biomarkers that predict response will rationalize giving such drugs to specific subgroups of patients who will gain the most benefit.

Drug discovery and development in oncology will continue to respond to the understanding of the biology of cancers. Hand-in-hand with developing drugs that block oncogene addiction and target very small populations of patients with specific genetic abnormalities such as ALK inhibitors, drug discovery efforts are re-discovering the virtues of targeting a range of mechanisms that are important in cancer such as cell cycle checkpoints (CDK4/6, CHK1), DNA repair (PARP, ATR), and epigenetic regulation. This group of targets and drugs will bring its own set of challenges of identifying patient populations and choosing the correct PD biomarkers to help define dosing and schedules.

It is very important to view the PhAT as a continuum of critical questions in the life cycle of an anticancer drug (Fig. 1B). Often specific aspects, such as cost, stimulate passionate arguments [59–61]. Further, while scientific rigor should not be compromised, the degree of validation needed for PD assays in early-phase clinical trials should be balanced with the important information acquired. Extent of validation should not be an obstacle for using these tests [112–115], when data can be further confirmed in increasing detail in future trials. Fit-for-purpose validation will be key in the future. The fact is that different aspects of the PhAT will offer more important information in some drug discovery projects than others, but that should not stop the oncology community from asking all the relevant questions. The relationship between different phases of clinical trials, ie, phase I/II/III, is also likely to evolve. There have been provisional licences granted to anticancer drugs based on phase II data, and the questions currently posed later in the PhAT are now being asked earlier than before. Reviewing the progress and development in the past three decades and taking stock of the current trends, we believe the PhAT is and will remain relevant and important irrespective of the direction cancer drug discovery and development takes in the future.

Conflicts of interest

Dr Banerji has received research funding from Astra Zeneca, Novartis, Chugai, and Onyx. He has also received honoraria for attending advisory boards for Astex, Novartis, and Debiopharm. Dr Workman is a former employee of AstraZeneca and declares commercial interactions with Yamanouchi (now Astellas), Piramed Pharma (acquired by Roche), Genentech, Vernalis, Novartis, Chroma Therapeutics, Astex Pharmaceuticals, AstraZeneca, Cyclacel, Onyx Pharmaceuticals, Merck Serono, Sareum, Janssen, Willex and Nextech Ventures. Dr Workman has received research funding from Vernalis for the discovery of HSP90 inhibitors, and intellectual property for this program was licensed to Vernalis Ltd., and Novartis. He has also been involved in a research collaboration with AstraZeneca in the area of stress and chaperone pathways, has been a consultant to Novartis, is a founder of Chroma Therapeutics, and is scientific advisory board member of Astex.

Drs Workman and Banerji are employees of The Institute of Cancer Research, which has a commercial interest in the discovery and development of anticancer drugs, including HSP90, PI3K, AKT, and HDAC inhibitors discussed in this paper.

Acknowledgments

The authors are grateful to patients and their families who have taken part in multiple phase I studies referenced in this article. The

authors acknowledge funding from Cancer Research UK (grants C309/A8274/A309/A11566 and C51/A6883) to The Institute of Cancer Research. The authors further acknowledge funding under the Experimental Cancer Medicine Centres program funded by Cancer Research UK and NIHR, CRUK to support our CRUK Centre and NIHR funding to our Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust.

Research performed at: The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust.

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