



Can molecular biomarker-based patient selection in Phase I trials accelerate anticancer drug development?

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Anticancer drug development remains slow, costly and inefficient. One way of addressing this might be the use of predictive biomarkers to select patients for Phase I/II trials. Such biomarkers, which predict response to molecular-targeted agents, have the potential to enrich these trials with patients more likely to benefit. Doing so could maximize the efficiency of anticancer drug development by facilitating earlier clinical qualification of predictive biomarkers and generating valuable information on cancer biology. In this review, we suggest a new model of early clinical trial design, which incorporates patient selection through predictive molecular biomarkers for selected targeted agents.

Problems with the anticancer drug discovery pipeline

Increased knowledge of the molecular pathways of oncogenesis has led to a new paradigm of targeted therapies against individual molecules in these pathways [1–5]. These new agents have the potential to result in greater efficacy and less toxicity than conventional cytotoxic agents through more specific targeting of cancer cells. Critical dependence of some tumours on the dysfunction of a single oncogene for their continued growth and proliferation is termed ‘oncogene addiction’ [6]. Therapeutic targeting of such critical oncogenes has found clinical application in the use of imatinib for BCR-ABL-driven chronic myeloid leukaemia and KIT-driven gastrointestinal stromal tumours and the use of trastuzumab for *HER2*-amplified breast cancer [7–9]. However, the majority of solid tumours are unlikely to have dysfunction of a single critical oncogene. Rather, a large number of oncogenic changes, which together with substantial molecular heterogeneity, abnormalities of metabolism and response to stress, are likely to contribute to key characteristics of the malignancy and its response to treatment [10–14].

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Nevertheless, the identification of the deregulated oncogenic processes that maintain the malignant phenotype in individual cancers remains critically important, as these are likely to provide the best targets for gaining clinical benefit from treatment with rationally designed novel agents [15]. A recent high-profile example supporting this approach has been the identification of the presence of wild-type, non-mutated *KRAS* in predicting clinical benefit to colorectal cancer patients treated with epidermal growth factor receptor (EGFR)-targeting agents, as compared with patients with *KRAS* mutations who are resistant [16–23].

Despite the elegance of the concept of molecular-targeted treatments, their development and incorporation into clinical practice remains slow and expensive. Although it has been suggested that targeted agents might have more successful development rates than conventional cytotoxic chemotherapies, attrition rates are still unacceptably high [24–26]. Moreover, the cost of developing an anticancer drug are typically US\$ 700–1700 million, figures that are strongly influenced by the high rate of failure of evaluated agents and the length of time the process typically takes (eight to ten years from discovery to registration) [27]. Only 1 in 20 cancer drugs entering clinical trials gains regulatory approval: of the agents tested at each stage, 70% fail at Phase II, 59% fail at Phase III and 30% fail at the registration stage. The major causes for failure are inadequate therapeutic activity (30%) and toxicity (30%) [24]. While the primary aims of Phase I trials are to define toxicity and maximum tolerated dose and to use pharmacodynamic (PD) and pharmacokinetic (PK) assessments to assess optimal dose and schedule, objective response rates within these trials remain low. Earlier analyses of tumour responses in unselected patients recruited to Phase I trials indicate a response rate of 3.8%, with a risk of toxic death of 0.54% [28]. More recent information from European Phase I units focusing on targeted agents seems to show little improvement in objective responses (5% [29] or 7.2% [30]), although potential clinical benefit, as assessed by disease stabilization for more than three months, is more common. In the context of an inefficient drug development process, there is a clear scientific, ethical and financial imperative to improve Phase I trial design.

Most Phase I trials do not select patients for targeted anticancer drug administration based on tumour molecular biomarkers. In our institution, and in most other drug development units, empirical clinical and practical factors have, in general, been the primary determinants in selecting patients for specific trials (Box 1). Evolving beyond this empirical approach to specific analyses of the molecular characteristics of each patient's individual tumour has much promise in improving patient selection for Phase I trials. Such an approach could potentially be crucial for accelerating drug development by enabling predictive biomarker studies to identify the cancer patient subpopulations most likely to respond to a therapy. For anticancer drug development to provide maximum value to the community, in terms of both benefit to patients and cost-effectiveness, a more rational and targeted deployment of agents (or combinations of agents) to molecularly defined subpopulations is warranted. In this article, we propose that optimizing biomarker development, validation and implementation

BOX 1

Factors used in the Drug Development Unit at the Royal Marsden Hospital to predict a possible response to targeted agent

- Known preclinical data on probable mutations for that particular cancer
- *In vitro* and *in vivo* preclinical antitumour activity data on the novel agent in question
- Preclinical data identifying and validating (potential) pharmacodynamic and predictive biomarkers
- Clinical data on responses in cancers from previous trials of agents with similar mechanisms of action
- Previous exposure to chemotherapy and targeted agents – scope for strategies targeting resistance to either
- Any other existing standard treatment options

is crucial throughout the clinical drug development process, from Phase I to Phase III studies and beyond. Moreover, deploying biomarkers to guide patient selection in information-rich, hypothesis-driven early-phase clinical trials might facilitate the use of these biomarkers in subsequent wider studies, providing exciting opportunities for their efficient and informative use.

Biomarkers and the regulatory pathway

In recognition of the pressing need for the more appropriate development of biomarkers for the development and approval of molecularly targeted agents, the US Federal Drug Administration (FDA) created the 'Critical Path Initiative to New Medical Products' [31] under the auspices of both the FDA and the American Association for Cancer Research (AACR). In addition, both the National Cancer Institute/FDA/AACR Cancer Biomarkers Collaborative in the United States and the Cancer Research UK Initiative on Biomarkers aim to develop strategies and guidance on endpoints in specific clinical situations [32].

Crucial to novel biomarker development is rigorous scientific and analytical validation, followed by clinical validation or qualification [33,34]. We have proposed a biomarker-based framework for rational decision making in clinical trials known as the 'pharmacologic audit trail' [33–38]. This links various biomarkers together and provides a logical framework for decision making in drug development. There are different types of biomarkers [33]. Risk biomarkers identify predisposition to a tumour; prognostic biomarkers indicate the probable course of disease and associate with outcome measures, such as overall survival; and intermediate endpoint or surrogate biomarkers attempt to replace clinical endpoints and would expedite regulatory drug approval. Phase I studies generally require pharmacological PK–PD biomarkers to measure the effects of a drug treatment on a specific target, pathway or biological feature of tumour biology. The use of these is increasing in Phase I trials [39]. Predictive biomarkers, which are under-utilized in Phase I trials, identify patient subpopulations that are most likely to respond to a therapy. Although PK–PD biomarkers remain crucial to the successful conduct of Phase I studies, the focus of this present article is on the emerging use of predictive biomarkers.

In an FDA guidance document, biomarkers have been described as ‘exploratory’, ‘probably valid’ and ‘known valid’ [31]. This document defines a ‘valid biomarker’ as ‘a biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiological, toxicological, pharmacological, or clinical significance of the test results’ [31]. To date, many of the biomarkers used in clinical trials of novel agents have been exploratory and not well validated, leading to inefficient or unsuccessful deployment. Furthermore, biomarkers are also often introduced too late to have an impact on early clinical trials.

An important lesson on predictive biomarker validation can be learnt from the development of *HER2* testing for trastuzumab use. Quality of assay performance was a major issue in later stage randomized clinical trials of trastuzumab using *HER2* testing to select patients for treatment. Importantly, there was poor concordance for *HER2* testing between reference and community-based laboratories [40]. In addition, immunohistochemistry (IHC) assays showed a poor concordance with fluorescence *in situ* hybridisation (FISH). This might have been related to technical procedures, tissue quality, type of antibody used and subjective biases between operators [41].

Validation and qualification maps for biomarkers now need to be developed by consortia involving the regulatory authorities, academic research groups and industry. These must facilitate biomarker approval for successful drug development and accelerate the implementation of novel assays [42,43].

Biomarker utilization: early or late in drug development?

There has been some debate about the role of biomarkers early in the drug development process after the publication of a meta-analysis on the use of biomarkers in 2458 Phase I trial abstracts in the period 1991–2002 [39,44,45]. This article concluded that biomarkers supported dose selection for Phase II studies in only 13% of the trials, with little evidence of these biomarkers making a substantial contribution towards establishing dose and schedule or understanding of antitumour effects [39]. Some investigators have raised concerns that the use of biomarkers in early clinical trials is subject to imprecise assays, excessive cost, ethical issues surrounding tumour biopsies and, most importantly, the potential to abandon effective drugs on the basis of incorrect patient selection. Sorafenib, for example, was initially developed as a RAF kinase inhibitor but was subsequently recognized to be a weak RAF inhibitor and a more potent vascular endothelial growth factor receptor 2 tyrosine kinase inhibitor (TKI), leading to its development and successful FDA approval for clear cell renal carcinoma [46]. In retrospect, a clearer understanding of the mechanisms of action of sorafenib preclinically should have impacted the clinical drug development process, particularly biomarker and patient selection. Such preclinical understanding, combined with the careful selection of PD and predictive biomarkers in early drug clinical trials, might be crucial to accelerating successful drug development. Such preclinical studies are also a key to the early clinical qualification of analytically validated predictive biomarker

assays. In addition, substantial insights into the mechanisms of clinical response and primary and acquired drug resistance can result from such early studies. Importantly, biomarkers also provide a valuable means of interrogating disease biology in the context of human patients.

There are multiple examples of biomarker development lagging behind the drug development process. The EGFR TKIs erlotinib and gefitinib have been in the clinic for several years for the treatment of non-small cell lung cancer (NSCLC). Optimal predictive biomarkers for these drugs, however, are still being evaluated. Importantly, with respect to overexpression of EGFR, there is no clear correlation with drug sensitivity, and there are important discrepancies between the results of several studies [47–49]. By contrast, EGFR mutations in NSCLC have been strongly associated with sensitivity to gefitinib and erlotinib; there is a higher rate of clinical benefit to treatment with EGFR TKIs seen in patients with EGFR mutations than in those without such mutations [50,51]. Furthermore, mechanisms of resistance have not been sufficiently explored in early studies of EGFR TKIs. A recent study has shown overexpression of c-MET as a mechanism of resistance to erlotinib in 22% of NSCLC cell lines studied, supporting the potential role of c-MET inhibitors in the treatment of erlotinib-resistant disease and the testing of c-MET expression before treatment with these agents, rather than unselected patient recruitment into such programmes [52].

Overall, it is important that Phase I trial designs increasingly consider incorporating the use of scientifically and analytically validated predictive biomarkers to question and answer key biological issues and to provide information about underlying disease biology, such as mechanisms of resistance (Box 2). Such studies, as demonstrated in the examples discussed below, might provide vital information that could decrease late and expensive drug attrition, accelerate drug development and reduce the overall cost of this process.

BOX 2

Steps in the successful development of a new PI3K inhibitor: questions that must be addressed by hypothesis-testing early-phase clinical trials

- Can the drug inhibit PI3K at safe and tolerable doses?
- What is the extent and duration of PI3K inhibition in tumour?
- What is the consequence of PI3K inhibition (e.g. apoptosis, reduction in cell proliferation or angiogenesis inhibition)?
- What is the variability in PI3K inhibition in the target population?
- What is the impact of PI3K inhibition on tumour cell growth in patients with a non-activated pathway versus those with molecular evidence for PI3K pathway addiction (e.g. p110 α mutation or PTEN loss, or evidence of upstream pathway activation such as HER2 amplification)?
- How does clinical experience correlate with preclinical validation of potential predictive biomarkers?
- What is the impact of crosstalk with other signalling pathways in leading to resistance of the PI3K inhibitor (e.g. consequence of activated RAS/RAF signalling and impact of dual inhibition with both a PI3K and MEK inhibitor)?

Biomarker use as key components of early-phase studies: some examples

PARP inhibition in BRCA mutation carrier cancer patients

We recently completed a Phase I study of the poly-ADP-ribose polymerase (PARP) inhibitor olaparib (KuDOS/AstraZeneca; KU-0059436, AZD2281), in which the concept of synthetic lethality was studied in patients with BRCA1 or BRCA2 mutations [53]. As indicated by preclinical data from PARP inhibitors from the same chemical series as olaparib [54], our *a priori* hypothesis was that olaparib would have therapeutic activity in tumours with homologous recombination (HR) repair defects. The dose-escalation phase of the study was initially enriched with BRCA mutation carriers, whereas the maximum tolerated dose expansion cohort only included patients with known BRCA mutations. Treatment induced PARP inhibition in mononuclear cells and tumour biopsies was demonstrated. The formation of γ H2AX foci, a marker of DNA double strand breaks, on plucked eyebrow hair follicles was also shown [53]. The trial demonstrated a clinical response rate of 46% in patients with BRCA-mutated ovarian cancer but little antitumour activity in patients with unknown BRCA status. This experience has shown that specific targeting of a selected patient subpopulation through the use of a defined predictive biomarker, such as BRCA mutation status, can provide a strong early signal of antitumour activity that might benefit specific patient cohorts and accelerate drug development.

Importantly, this PARP inhibitor 'proof of concept' trial has shed much light on disease biology and has now led us to rapidly address several further key issues, including: how do we best identify sporadic cancer patients with HR repair deficiency? What is the incidence of HR repair defects in patients with sporadic or familial cancers? Does resistance to PARP inhibitors occur as a result of a secondary mutation of BRCA, and how can this be overcome? Do different forms of BRCA mutations result in varying sensitivities to PARP inhibitors by causing differing degrees of HR repair defects?

CYP17 blockade in CRPC

Abiraterone acetate is a potent and highly selective irreversible inhibitor of CYP17 that blocks androgen and oestrogen synthesis [55]. Phase I and II clinical trials of abiraterone acetate conducted at our institution and other centres have reported disease regression in 50–70% of patients resistant to multiple hormonal, cytotoxic and experimental agents [56–58]. However, because up to 40% of castration-resistant prostate cancer (CRPC) patients do not demonstrate clinical benefit, a study of predictive biomarkers could prove important to the future successful development of this agent, increasing the likelihood of its approval in randomized trials. We have measured the levels of those steroid precursors that we predicted would be suppressed by abiraterone acetate and have confirmed that abiraterone acetate suppressed steroids downstream of CYP17, reporting an association between clinical benefit and pretreatment hormone levels [56].

We have also investigated assays that could stratify patients with CRPC based on underlying molecular biology and select those most suitable for hormone therapies. Approximately 40–70% of prostate cancers have an *ERG* gene rearrangement that

can be detected by FISH studies [59,60]. We used archival tumour biopsies and circulating tumour cells (CTCs) (see section 'Analysing predictive biomarkers') isolated from blood collected from patients in Phase I/II studies of abiraterone acetate to molecularly characterize patients by FISH [61], as demonstrated in Fig. 2. These studies reported that the presence of an *ERG* rearrangement was associated with magnitude of PSA decline on abiraterone acetate ($P = 0.007$). This association is undergoing further evaluation in an ongoing international randomized double-blind Phase III trial of abiraterone acetate and prednisone versus prednisone and placebo (NCT00638690) and could inform the design of future abiraterone acetate clinical studies. These studies have provided important insights into the molecular biology of CRPC and the mechanism of action of abiraterone acetate and give an example of how biomarker evaluation in early clinical studies can inform later trial design.

Inhibition of ALK in patients with ALK rearrangements

Chimeric fusion proteins of the ALK gene produce constitutively active tyrosine kinases and increased activation of downstream pathways, including the PI3-kinase and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways [62,63]. ALK fusion proteins have been found in NSCLC (4% of cases, predominantly those of adenocarcinoma histology), anaplastic large-cell lymphoma and other malignancies. A Phase I trial of PF02341066, an oral MET and ALK inhibitor, was designed to have both a dose-escalation phase and a 'molecularly selected cohort' at the recommended Phase II dose of patients with MET or ALK activation. Clinical activity in a patient with an inflammatory myoblastic tumour (a rare sarcoma known to be driven by ALK fusion proteins) led to enrolment in a molecularly selected cohort of 27 patients with NSCLC demonstrated by FISH break-apart assay to have an EML4-ALK fusion protein. Of 19 evaluable patients, 10 achieved partial response and a further 5 had stable disease for >8 weeks with a clinical benefit rate of 79%. Although patients with MET amplification or mutation will also be enrolled in a further expansion at the recommended Phase II dose in this Phase I trial, a Phase III trial is now planned in the *EML4-ALK* fusion gene associated NSCLC population based on these Phase I data. Using such an adaptive trial design and a robust predictive biomarker, a clear signal of antitumour activity has been demonstrated, with facilitation of rapid transition to subsequent phase trials, which is hoped will further validate the utility of this biomarker.

Inhibition of V600E BRAF in melanoma

Activating mutations of BRAF have been identified in up to 60% of cutaneous melanomas [64]. The majority of these are point mutations resulting in substitution of valine with glutamate at the 600 position (V600E). Proof of concept that inhibition of V600E BRAF was a valid target for drug development was provided by small interfering RNA experiments, which resulted in the inhibition of proliferation and induction of apoptosis [65]. PLX4032 is a rationally designed small-molecule inhibitor of BRAF V600E that has impressive selectivity over wild-type BRAF, CRAF and other kinases. PLX4032 has been evaluated in Phase I clinical trials [66] with promising antitumour activity reported

in patients with cancers that carry the BRAF V600E mutation [67]. In total, 16 patients with the mutation were treated at last reporting on this Phase I study; 9 of these patients have had a partial response, with a further 7 patients having stable disease. Five patients with BRAF wild-type disease all showed evidence of progressive disease, further supporting the concept that BRAF mutations are predictive of response to selective mutant BRAF inhibitors.

A range of PD biomarkers were conducted in this study: IHC showed a reduction in both Ki-67 and phosphorylated extracellular signal-regulated kinase (ERK) levels in tumour samples after drug administration, as well as a reduction in fludeoxyglucose (FDG) uptake in selected patients having positron emission tomography (PET) scans [68]. These observations have confirmed that V600E BRAF is a valid therapeutic target and suggest that the development of a BRAF-V600E-specific inhibitor might potentially lead to new treatment options for melanoma.

In contrast to this is clinical data acquired to date with mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibitors. Preclinical data reported that BRAF mutation predicts anti-tumour activity after inhibition of MEK signalling [69]. Despite this, clinical studies of several MEK inhibitors have had infrequent responses in Phase I trials employing no process of molecular selection, resulting in a difficult transition to latter stage trials. It is not yet clear whether this is due to inadequate MEK blockade by these agents or to signalling pathway crosstalk bypassing the MEK blockade.

Overall, the examples discussed illustrate how predictive biomarkers can be used successfully in Phase I clinical trials. Adaptive designs using such biomarkers can increase the likelihood of patient benefit, generate important information on disease biology and impact the drug development process. More sophisticated Bayesian designs warrant further evaluation in this setting to guide molecular patient selection [70].

Analysing predictive biomarkers

Technologies for tumour acquisition and molecular assessment are evolving in parallel with developments in drug discovery. Optimization of the incorporation of these developments into early-phase trials is a profound challenge and important opportunity. Acquisition of tissue at different stages of disease and treatment is being facilitated by these new technologies. Plasma, CTCs and whole blood are being interrogated by molecular technologies to provide potential minimally invasive readouts of tumour biology and response to treatment, for both predictive and PD biomarkers. CTCs have been isolated and studied in a range of common malignancies, including lung, breast, colorectal and prostate cancers [71–73]. Molecular characterization of CTCs by multicolour FISH, mutation analyses by sequencing (Fig. 2) and protein expression by immunofluorescence (Fig. 2) hold promise for selecting patients for targeted treatments, monitoring efficacy and studying mechanisms of resistance [74–76]. Small volumes of tumour tissue can now be used to provide increasingly complex information that can be analysed by high-throughput methods. Such technologies include DNA, RNA and protein analyses and functional assessments of tumour activity.

DNA-based predictive biomarkers

Comparative genomic hybridisation (CGH) detects loss, gain and amplification of gene copy number at the level of chromosomes and enables the analysis of the whole genome at increasingly high definition [77]. Mass-spectroscopy-based technology can also be used to identify such changes, as well as multiplex evaluation of gene mutations, and is now increasingly affordable. Comprehensive sequencing of the entire genome of an individual's cancer using Solexa technology is also becoming increasingly feasible [78,79]. These studies will become more affordable, which – along with the ability to sequence smaller volumes of tissue, including single-cell studies – will generate many important opportunities. It is envisioned that these analyses will be used for patient selection in early-phase trials, but they might be limited by the challenges of intratumoural cellular molecular heterogeneity [61].

RNA-based predictive biomarkers: gene expression microarrays

Gene expression microarrays have the ability to define tumour signatures with powerful prognostic capability so that patients can be classified into good and poor outcomes, and one such gene signature (MammaPrint, Agendia) has been cleared by the FDA for patients under 61 years of age who have node-negative stage I or II disease with a tumour size of 5 cm or less [80]. Such studies might provide predictive information related to activation of oncogenic pathways and, ultimately, define sensitivity to molecularly targeted agents. A gene expression signature for PTEN protein loss has been reported from human breast cancer biopsies [81]. This was then validated in independent datasets for other tumour types, and prediction by the gene expression signature of pathway activity correlated significantly with poor patient prognosis [81]. Studies of microRNA expression might also have clinical relevance in predictive biomarker analyses [82].

Protein-based predictive biomarkers

Although CGH, sequencing and transcriptional profiling provide data on DNA and RNA status and levels, they cannot assess the functional effects that these changes have on cancer cell biology [83]. Assessment of mRNA levels by expression arrays can generate a signature of functional activity but does not directly measure, for example, the level of expression, post-translational modification and functional activity of key proteins. Proteomic studies have the potential to assess relative activity of multiple pathways. Reverse-phase protein microarray can quantitate phosphorylated and total protein expression [83] and is promising in the identification of multiplex predictors of response to PI3-kinase and MEK inhibition in breast cancer [84,85].

A functional readout of a cancer's molecular activity might lead to a more accurate signature of cancer drivers and lead to the targeting of both oncogene and 'non-oncogene' addictions [14]. Phosphopeptide arrays can evaluate tyrosine and serine/threonine kinase peptide substrates covering a high proportion of the kinome. These can determine the functional activity of kinases from tumour lysates determining pathway activation status [86]. Such arrays can simultaneously analyse the reversible phosphor-

ylation of multiple protein residues central to various oncogenic signalling pathways.

Molecular imaging

Further functional information can be provided by modern molecular imaging techniques [87]. Positron emission tomography (PET) technology can detect HER2 positivity [88] and markers of hypoxia (18-fluoromisonidazole (FMISO)), [89] and angiogenesis (integrin $\alpha_v\beta_3$ and VEGF) [90,91]. Specific molecular pathways might also be analysed, for example, for abnormal PI3K/AKT activity, by measuring the accumulation of ^{11}C acetate by PET and that of the RAS/RAF/MEK/ERK pathway by changes in labelled choline [92]. Changes in PET parameters might provide earlier information about treatment response than conventional imaging does, potentially enabling earlier decisions about stopping an ineffective therapy, and might also delineate changes in metabolic activity of tumours in the absence of changes in their size [93]. Dynamic contrast-enhanced MRI uses transverse relaxation time (T2) captured images immediately after contrast injection (evaluating perfusion) and longitudinal relaxation time (T1) images over a few minutes to examine extravasation of contrast (evaluating blood volume within tumour and microvascular permeability) [94]. Measurable changes in tumour perfusion or vascular permeability provide PD evidence of antiangiogenic effect and information early in a treatment course about probable response to such therapies [95].

Pharmacogenomics

Identifying host somatic cell single-nucleotide polymorphisms that associate with treatment benefit and/or drug toxicity also has the potential to provide crucially important data on selecting treatment and its dosing [96]. These pharmacogenomic markers are now increasingly available but remain poorly utilized [96,97]. An important example is the identification of allelic variations in *CYP2D6* as predictors of response to tamoxifen. Tamoxifen is metabolised by *CYP2D6* to 4-hydroxy-tamoxifen and endoxifen, which have approximately 100× greater affinity for ER α [98]. Patients homozygous for the null allele *CYP2D6**4 have shorter disease-free survival than those heterozygous or homozygous for the wild-type allele [99]. The FDA has approved technology for the identification of these *CYP2D6* genotypes, although this has not been routinely incorporated into clinical practice to date.

Another important example is the hepatic glucuronidation by UGT1A1 of the major metabolite of irinotecan, SN-38, to the inactive SN-38-G. This is a major pathway for irinotecan metabolism. Genetic variants of UGT1A1 are found in 10–20% of the population and can lead to increased exposure to SN-38 and severe, late-onset irinotecan toxicity [100–103]. The most common polymorphism (UGT1A1*28) has seven TA repeats in the gene promoter, rather than the more common six repeats. A rapid testing kit for the homozygous seven-repeat genotype (Invader UGT1A1, Third Wave Technologies, Madison, WI) has been approved by the FDA, although this assay does not evaluate other UGT1A1 variants, and its predictive capacity is poor [104]. Consequently, to maximize the early recognition of such pharmacogenetic variants in the development of novel agents, we recommend that all patients treated on early clinical trials should have somatic cell DNA collected from a blood sample to support such studies.

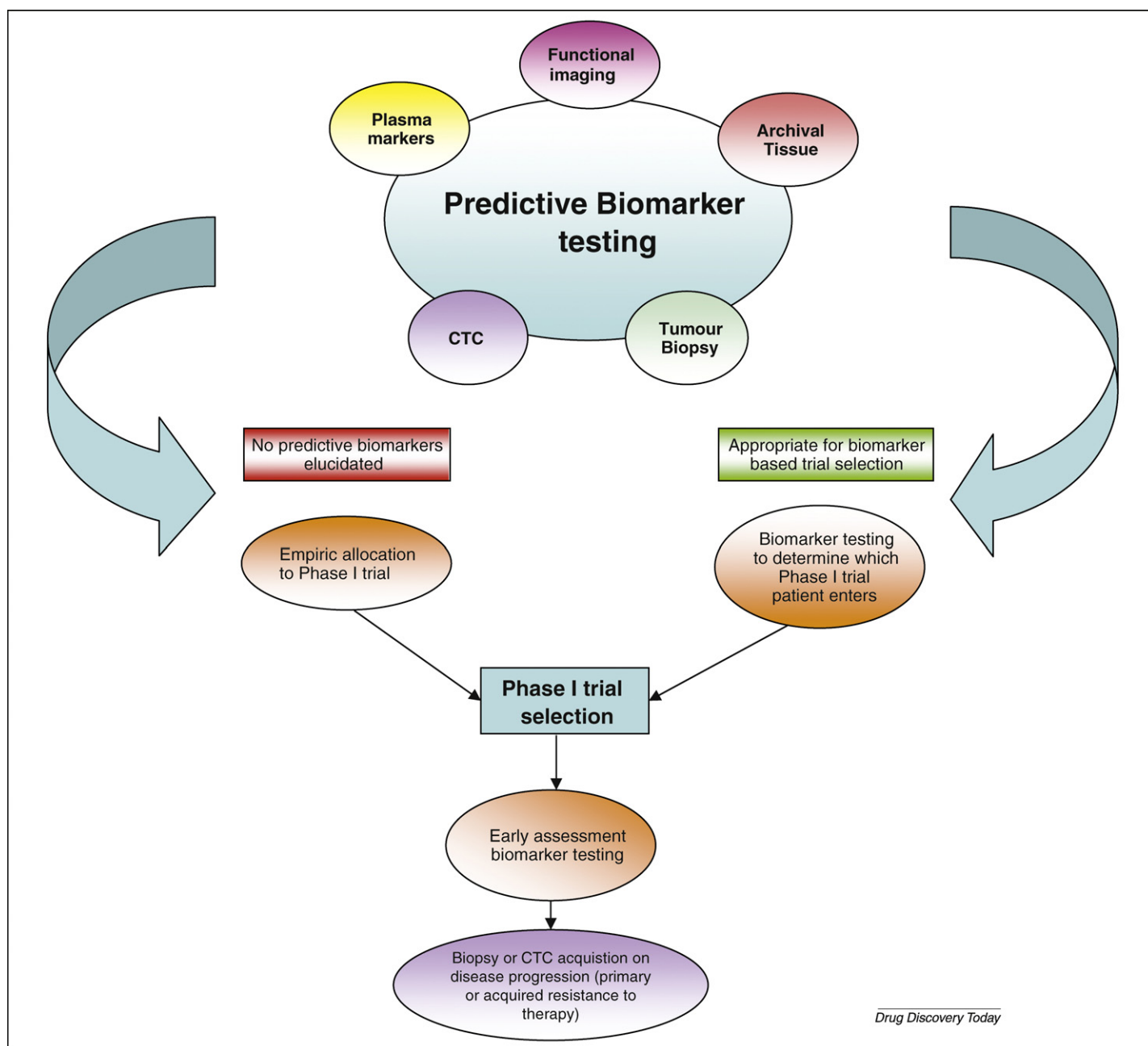
Concluding remarks: developing a new model of Phase I patient selection

Overall, new models of Phase I study design that incorporate patient selection based on predictive biomarkers have the potential to accelerate anticancer drug development for many molecular-targeted novel agents (Fig. 1). Indeed, it is probable that the early identification of such predictive biomarkers will improve the odds of eventual drug registration. This might not, however, be applicable for all novel agents. Compounds with unknown mechanisms of action, multiple targets or targets that are generic to all cancers (e.g. antiangiogenics) might not merit such predictive biomarker evaluation. Using such biomarker studies, however, can facilitate the development of information-rich, hypothesis-testing, adaptive trials directed by disease biology. Such trials might support the molecular subclassification of diseases through the use of robust and analytically validated predictive biomarkers. Most importantly, in doing so, they might improve the likelihood of benefit for patients accrued to these early trials.

Although much of the exploration and full validation of 'potential predictive' biomarkers will still occur in the context of subsequent Phase II and III trials, we believe that the use of molecular strategies to enrich Phase I trials with patient populations that have a higher chance of response should now be pursued. Such studies are conducted under the auspices of Clinical Laboratory Improvement Amendments (CLIA) in the USA, and Good Laboratory Practice (GLP) in the UK. Careful analytical validation of such biomarkers remain crucially important to increase the possibility of patient benefit, which can then lead to increasing confidence in the drug under evaluation and its target(s) and provide early clinical qualification of the predictive biomarker.

Conducting Phase I trials in this manner will require a significant infrastructure to support the acquisition of tumour material including archival tissue, fresh tumour biopsies and CTCs, as well as early radiology reviews to gauge the feasibility of safe tumour biopsies in suitable patients. Considerable education and provision of information for patients on the rationale for the process will be key, as will consent for histology review, tissue retrieval, pathological analyses of archival tissue and fresh tumour or CTC acquisition. These analyses must be streamlined to decrease the time required for this selection process, which must be expedited to reduce treatment delay. Such a biomarker-driven drug development process requires a detailed understanding of available technologies and drug mechanism of action and an accurate definition of the probable molecular oncogenic abnormalities for each cancer, so that the appropriate selection of the optimal modality of testing is made. The large data sets of predictive biomarker analysis will require bioinformatics expertise and the rapid evolution of hospital molecular pathology departments. Crucially, thorough preclinical validation of predictive biomarkers must be performed as early as possible before Phase I trials commence, so that these biomarkers can be incorporated into the early-phase trial programme of a new drug.

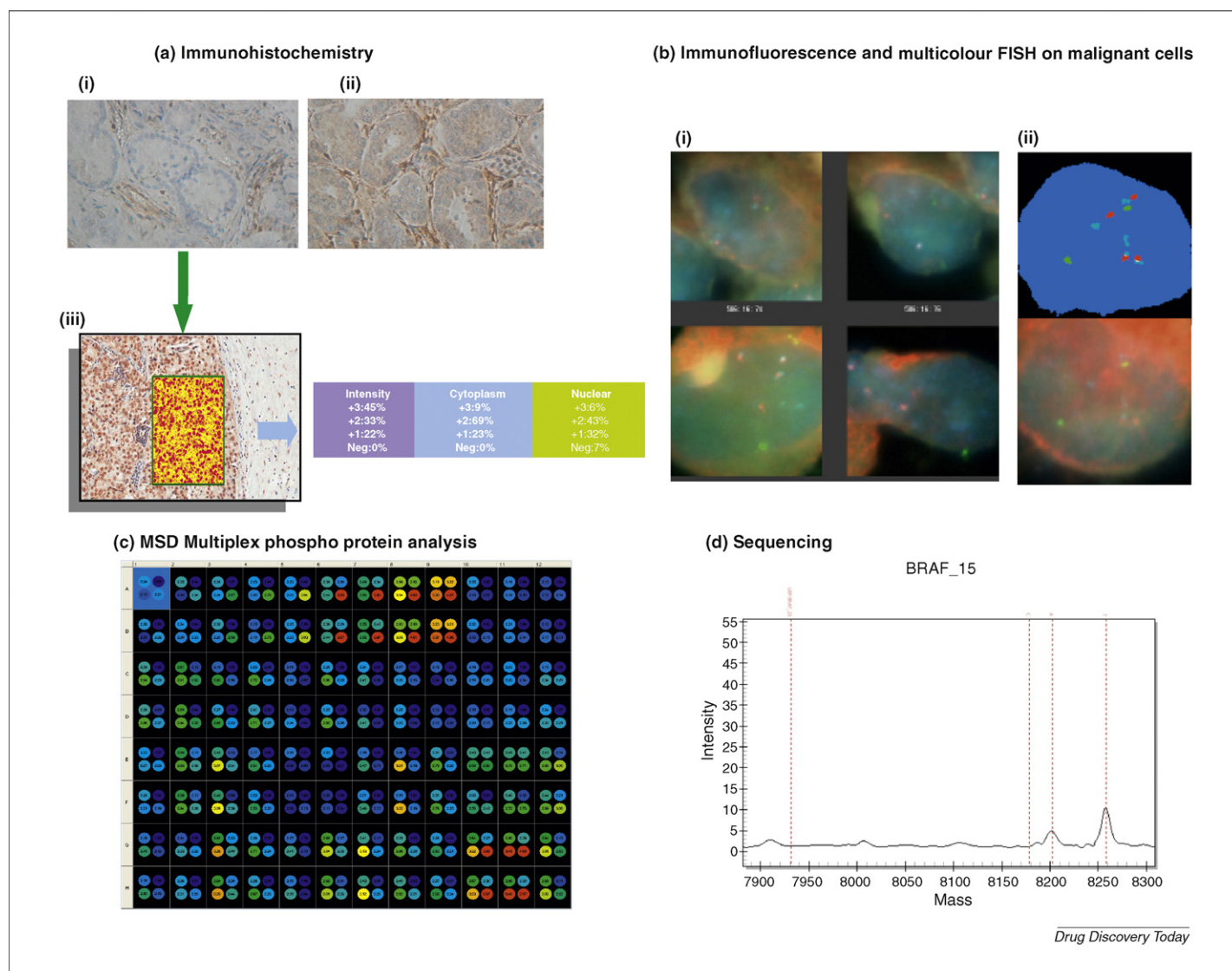
Finally, defining patient populations by molecular methods might enable drug application across the traditional boundaries of anatomical disease site and histological appearance typical of Phase II studies into defined populations across multiple histologies based primarily on molecular characterization. We envision patient selection for such studies based on tumour RAS/RAF

**FIGURE 1**

Potential Phase I trial selection process incorporating predictive and on-treatment biomarker assessment. Current Phase I trials select patients for particular trials based on the clinical factors listed in Box 1. We propose a restructuring of the patient selection process to incorporate predictive biomarker assessment as part of the initial workup of the patient before entry into the trial. This assessment might involve molecular evaluation of archival tissue, serum markers, fresh tumour biopsies, circulating tumour cells or functional imaging. Should a biomarker based decision not be feasible or not appropriate, the patient is allocated empirically to a Phase I trial in the usual way. If the patient and available trials warrant a biomarker-based approach, the relevant biomarker testing would be carried out, with entry onto a Phase I trial targeted towards the matched molecular abnormality. Early post-treatment biomarker testing would then be performed and repeated should the patient develop resistance to therapy. By evaluating tumour biology before trial entry, we propose that the patient will have a higher chance of receiving an effective treatment.

mutant status, PI3K pathway activation or *BRCA*, *PTEN* or *TP53* functional status for the appropriate molecular agents. Rather than unselected recruitment of patients into phase I trials of, for example, drugs targeting the PI3K/AKT pathway, these studies could recruit patients with PIK3CA mutation or amplification, PTEN loss, or receptor tyrosine kinase overexpression. This might

ultimately impact the design of later stage trials, shifting our focus from developing drugs for geographical tumour types to the treatment of diseases based on specific molecular drivers. This could decrease attrition of drugs in later phase trials, reduce cost, accelerate anticancer drug development and fix the broken anticancer drug pipeline.

**FIGURE 2**

Potential methods of predictive biomarker analyses. **(a)** Immunohistochemistry (IHC). PTEN IHC is demonstrated in panel (i), which is from a prostate cancer biopsy specimen that was PTEN negative on fluorescence *in situ* hybridisation (FISH), in contrast to the prostate cancer biopsy specimen in panel (ii), which was PTEN positive on FISH. Panel (iii) shows the quantitative image analysis (Aperio ScanScope imaging and software, CA, USA) of the corresponding PRAS40 IHC staining from the biopsy specimen used in panel (i), indicating strong positivity. **(b)** Immunofluorescence and FISH on malignant cells. Panel (i) shows four LNCaP cells spiked in blood and photographed using the Ikonisys system (Ikonisys, CT, USA). These cells were demonstrated to be DAPI positive (blue nuclear staining) and cytokeratin positive (orange cytoplasmic staining). FISH probes show the androgen receptor (green signal) and ERG genes (red and aqua nuclear probes). Panel (ii) shows a further example of malignant cells, illustrating the utility of the same FISH probes in demonstrating diploid androgen receptor copy number but ERG amplification. **(c)** Meso Scale Discovery (MSD, MD, USA) 96 well AKT multiplex plate. Each well shown in (c) provides a quantitative readout of the phosphorylated forms of AKT, p70S6K and GSK3β. Standard curves are shown in the top two rows and right three columns. **(d)** Sequenom DNA sequencing (Sequenom, CA, USA). This demonstrates a BRAF V600E mutation as analyzed in melanoma cell line SK-MEL-28. This mass-spectroscopy-based technology enables multiple common mutations to be analysed in small volumes of tissue. Images courtesy of Ruth Riisnaes, Joanna Moreira, Mateus Crespo, Jeremy Clark, Geraldine Perkins, Christophe Massard and Juliet Dukes.

Conflict of interest statement

All the authors are employees of The Institute of Cancer Research, which has a commercial interest in the development of inhibitors of HSP90, PI3-kinase, AKT, BRAF, PARP, CYP17, CDK and chromatin-modifying enzymes. The authors have potentially

relevant commercial interactions with Vernalis Ltd, Novartis, Piramed Pharma (recently acquired by Roche), Astex Therapeutics, AstraZeneca, GSK, Cougar Biotechnology Inc. (acquired by Johnson & Johnson) and Cyclacel Pharmaceuticals.

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