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Competing interests statement
The authors declare competing financial interests; see web version for details.

DATABASES
UniProtKB: http://www.uniprot.org
ANGPT1 | ANGPT2 | BV8 | DLL4 | FGF2 | HIF1α | MMP9 | Neuropilin 1 | PLGF | TGFβ1 | TIMP2 | TIMP3 | VEGFA | VEGFR2

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OPINION

Envisioning the future of early anticancer drug development

Timothy A. Yap, Shahneen K. Sandhu, Paul Workman and Johann S. de Bono

Abstract | The development of novel molecularly targeted cancer therapeutics remains slow and expensive with many late-stage failures. There is an urgent need to accelerate this process by improving early clinical anticancer drug evaluation through modern and rational trial designs that incorporate predictive, pharmacokinetic, pharmacodynamic, pharmacogenomic and intermediate end-point biomarkers. In this article, we discuss current approaches and propose strategies that will potentially maximize benefit to patients and expedite the regulatory approvals of new anticancer drugs.

Major advances in our understanding of the genetics and biology of cancer have revealed dependencies and synthetic lethalities that can be exploited with targeted molecular therapeutics that form the basis of personalized medicine[1–4]. Current evidence-based medicine requires large randomized multi-centre studies that aim to definitively prove the superior efficacy of new therapies compared with the gold standard, generally without molecular stratification of patients[5]. Although such trials have revolutionized medical practice, this 'one size fits all' approach does not take into account the now well-established patient-to-patient variation that exists in the molecular drivers of both cancer and drug sensitivity[6,7].

The new generation of molecularly targeted drugs underlines the potential for personalized medicine, which promises more efficacious and less toxic anti-tumour therapies in patients who have defined molecular aberrations[3,8]. Selecting patients based on molecular predictors could also accelerate the drug approval process, which remains slow and inefficient. There is a clear biological, ethical and financial imperative to increase the odds of the successful approval of new cancer therapies, especially as a high proportion of cancer drugs still fail late and expensively in Phase III trials[9–11]. To achieve this goal, a new paradigm is emerging that involves the use of customized, adaptive, hypothesis-testing early trial designs incorporating analytically validated and clinically qualified biomarkers (BOX 1) from the earliest possible stage.

Although traditional drug development has involved a 'compound-to-trial' process, there is increasing evidence that this should now change to a 'biology-to-trial' approach, starting with the unravelling of the

fundamental molecular mechanisms of cancer targets, which may then drive initial drug discovery and subsequent clinical studies (FIG. 1A). Key molecular targets or pathways to which certain cancers are addicted, or which present opportunities for synthetic lethality, should be actively pursued and dissected to improve our understanding of these pathways and to identify predictive biomarkers that could be integrated early in the drug discovery process. Such preclinical data could also support optimal clinical trial design. In this Opinion article, we focus on the tools and strategies currently in use and propose new approaches to enhance early-phase clinical trials and accelerate development of targeted anticancer agents.

The drug development toolkit

Studies of molecular biomarkers in trials that aim to correlate clinical data with pharmacological drug effects have arisen as a result of a greater understanding of cancer genetics and biology, the advent of molecularly targeted agents and advances in biotechnological tools[12]. Given that targeted therapeutics are optimal when applied in the appropriate molecular context[13], biomarkers can be used in clinical trials for multiple purposes. They can guide the selection of patients likely to respond to therapies, predict the probability of success or failure of a drug and provide meaningful correlations of target and pathway modulation in Phase I clinical trials. It is important for these molecular assays to be scientifically sound and analytically validated in the laboratory so that they are primed for clinical use (BOX 1). Biomarkers should ideally be clinically qualified as far as possible but, in first-in-class, first-in-human trials, the use of specific biomarkers could be the beginning of the journey towards qualification. These biomarkers could be broadly classified as pharmacodynamic (PD), pharmacokinetic (PK), pharmacogenetic, predictive, enrichment and intermediate end-point biomarkers (FIG. 1b).

Pharmacokinetics, pharmacodynamics and pharmacogenomics. PD biomarkers together with corresponding PK data should be used to confirm target and pathway modulation, to help identify the biologically active dose range and to make 'go' or 'no-go' drug development decisions[14–16]. We have generally moved away from using body surface area to determine drug doses and now use fixed doses of targeted therapies. Nonetheless, Phase I studies of targeted agents should evaluate the association between body surface area and weight and height with drug clearance to support fixed drug dosing[17]. PK–PD relationships should also be reported (BOX 2); this will allow the drug development process to continue with confidence to larger and more costly trials.

PK and PD can be affected by inter-patient variation, which can influence both treatment-related responses and toxicities owing in part to host pharmacogenomic factors[18]. To minimize such effects, prospective single nucleotide polymorphism (SNP)-based dose optimization Phase I studies should be considered; however, these are rarely conducted. SNPs that impact expression or function of proteins involved in drug metabolism or the target of the drug under evaluation can directly affect treatment efficacy and toxicity. Such studies might not be appropriate for all drugs but are potentially useful for agents that have clearly defined pharmacogenomic profiles. Alternatively, and more commonly, the possible influence of pharmacogenomic factors can be assessed retrospectively following completion of Phase II and even Phase III trials. In this approach, all patients are treated initially at a fixed, recommended, generic drug dose, which is then adjusted based on the presence or absence of toxicities. This approach should, however, take into consideration that a substantial proportion of such patients will be undertreated and could benefit from dose escalation. Dose escalation is less frequently pursued than dose reductions for patients who receive too high a dose, which results in toxicity. A detailed discussion on pharmacogenetics and pharmacogenomics is beyond the scope of this article, but the reader is directed to excellent publications on the subject[18,19].

The pharmacological audit trail. PD and PK data together allow the construction of a framework for rational decision making in clinical trials, known as the 'pharmacologic audit trail' (PhAT), which we first described in 2003 (REFS 14,20,21). This allows all key stages in drug development to be linked and interpreted in relation to measured parameters (such as PK and PD), and provides a stepwise 'audit' to assess the risk of failure during the development of a novel compound at any particular stage (FIG. 2). The application of the PhAT is illustrated by the preclinical and Phase I studies of the heat-shock protein 90 inhibitor tanespimycin[22–24] (17-allyamino-17-demethoxygeldanamycin (17AAG); Bristol–Myers Squibb/Kosan Biosciences), the CYP17 inhibitor abiraterone acetate[25–27] (Johnson and Johnson/Cougar Biotechnology) and the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib[28–30] (AstraZeneca/KuDOS Pharmaceuticals) (FIG. 2), which were conducted at our institution.

We now present an updated PhAT to reflect the evolving drug discovery and development landscape, implementing the evaluation of potential predictive assays earlier in the drug development process and strategies to reverse resistance mechanisms (FIG. 2).

Predictive biomarkers. The use of predictive markers is pivotal to accelerating the drug development process[6,31]. Predictive biomarkers have been successfully used in clinical

Box 1 | Preclinical validation and clinical qualification of biomarkers

Drug development and biomarker validation should ideally occur in parallel. Prior to their acceptance and use as a clinical trial end point, it is crucial that 'fit-for-purpose' biomarkers are scientifically and technically validated and clinically qualified with a suitable degree of rigour[94]. The scientific validation of biomarkers focuses on relating the marker to the molecular target and associated pathway or the mechanism of action of a drug, and understanding its association with therapeutic outcome. This should be followed by technical or methodological validation of the biomarker assay, assessing appropriate performance criteria, including reproducibility, variability, sensitivity and specificity[95]. Preclinical pharmacodynamic biomarker validation in animal models is illustrated by our work on heat-shock protein 90 (HSP90) inhibitors[24,96,97] and PI3K inhibitors[62,63]. The aim here is to produce a robust and reproducible biomarker assay that is progressively validated and qualified to a degree that is fit for purpose[14,94]. Such a biomarker can then be incorporated into an early-phase trial as an exploratory end point to allow early hypothesis-testing or hypothesis-generating clinical studies to be carried out[8,14]. Fit-for-purpose validation makes economic sense, as conducting a large amount of validation would be wasteful if the drug is terminated early. For predictive biomarkers, if they are proved robust and potentially useful in early clinical trials, these assays can then be subjected to further clinical qualification through prospective or retrospective evaluation in large randomized controlled trials before regulatory approval[31]. For biomarkers used in 'go' or 'no-go' drug development decision making, minimum standards set by Good Clinical Laboratory Practice (UK) or Clinical Laboratory Improvement Amendments (USA) should be adhered to in order to ensure technical standardization[98].

trials of trastuzumab[32,33] (Herceptin; Roche/Genentech), pertuzumab[34] (Omnitarg; Roche/Genentech), trastuzumab-DM1[35,106] (Roche/Genentech) and lapatinib[36] (Tykerb/Tyverb; GlaxoSmithKline), by evaluating ERBB2 overexpression in breast cancers and by BCR–ABL detection in predicting response to imatinib (Gleevec; Novartis) in chronic myelogenous leukaemia[37]. Other recent notable examples of the successful use of predictive biomarkers in Phase I trials include the detection of *BRCA1* and *BRCA2* mutations, which portend sensitivity to the PARP inhibitor olaparib[29] (BOX 2); of EML4– anaplastic lymphoma kinase (ALK) fusions that predict response to the ALK and MET inhibitor PF-02341066 (Pfizer) in non-small cell lung cancer (NSCLC)[38]; and of the V600E BRAF mutation that predicts response to the mutant BRAF-selective inhibitor PLX4032 (Plexxikon) in melanoma[39].

The upfront use and testing of putative predictive biomarkers in early clinical trials could minimize the need for retrospective subgroup dredging for predictive biomarkers in later phase trials carried out in unselected populations. Notable examples of clinical studies that used retrospective subgroup analyses include the randomized Phase III trial comparing the epidermal growth factor receptor (EGFR)-targeted antibody panitumumab (Vectabix; Amgen) with best supportive care in EGFR-positive metastatic colorectal cancer (CRC)[40], and the Iressa Survival Evaluation in Lung Cancer (ISEL) study, which investigated the EGFR small-molecule inhibitor gefitinib (Iressa; AstraZeneca) versus placebo in patients with advanced NSCLC[41]. In both trials, a retrospective analysis of tumour tissue led to the discovery that selected molecular subgroups attained greater benefit — for patients who had wild-type *KRAS* and were treated with panitumumab[40] and patients who had mutant *EGFR* and were treated with gefitinib[42]. A similar scenario was also encountered in the CRYSTAL trial, which assessed the combination of the EGFR-targeted antibody cetuximab (Erbitux; ImClone/Merck/Bristol–Myers Squibb) with 5-fluorouracil and irinotecan (Camptosar; Pfizer) (in the FOLFIRI regimen) in EGFR-positive metastatic colorectal cancer[43]. A retrospective subgroup analysis demonstrated that patients who had wild-type *KRAS* and were treated with the cetuximab–FOLFIRI regimen[44] had increased benefit compared with patients who had mutant *KRAS*. These examples emphasize the importance of retrospective studies, which might be essential when new data on predictive biomarkers become available after prospective trials are conducted. However, these examples also suggest the importance of *a priori* drug evaluation in hypothesized appropriate molecular contexts early on in the drug development process, for example in Phase I/II trials, to test and begin to clinically qualify predictive biomarkers in selected populations. When such hypothesis-testing studies are carried out upfront before large and costly clinical trials, they might also decrease the number of patients receiving ineffective treatments and late drug attrition.

In addition, it is important to note that the strategy of matching predictive biomarkers with molecularly targeted agents will not always be applicable to all novel therapies, for example, broad-spectrum inhibitors that block multiple signalling pathways. Other issues could also arise, including the lack of preclinically validated biomarkers, regulatory issues impacting clinical trial conduct and difficulties in recruiting suitable patients. A further matter to consider when using predictive biomarkers to select patients is that the potential beneficial effects of the targeted therapy in a more broadly defined patient population could be missed. Therefore, if the prevalence of a predictive biomarker is already known to be high in an unselected cohort and the new therapy has the potential to benefit the broader population, or if no clear differentiation between patients who benefit and those who do not seems achievable, then patient selection should be avoided. An often-cited example is sorafenib (Nexavar; Bayer/Onyx), which was initially developed as a CRAF inhibitor, only to later achieve regulatory approval as a multi-kinase inhibitor that has predominant effects on the vascular endothelial growth factor receptor (VEGFR) in advanced renal cell carcinomas[45,46]. Questions also remain as to whether trastuzumab therapy has clinical benefit in patients described as having ERBB2-negative disease. This could be a result of false-negative ERBB2 testing or intra-patient heterogeneity in which patients have tumour clones driven by ERBB2 and these clones are not present in the analysed tumour biopsies. These complexities might be difficult to dissect and support the case for initially evaluating new therapies in an unselected population and subsequently selecting for molecular aberrations that enrich for sensitive tumours (BOX 2).

Enrichment biomarkers. Clinical trial designs for targeted therapies are most effective when a biological hypothesis is evaluated using a validated predictive marker that has an established cut-off point for determining the status of the marker[47]. Although there is currently no formal consensus, we believe the term 'predictive biomarkers' should strictly be limited to those biomarkers that are scientifically sound and for which the

Glossary

Biologically active dose range
The range of drug doses required to result in the modulation of the cellular target of the drug to produce its expected effect.

Continual reassessment method
This tool uses statistical modelling and is employed in dose-finding clinical trials to estimate the dose at which the desired toxicity level can be expected to minimize risk of toxicity to patients.

Maximum tolerated dose
The highest dose of a drug or treatment that does not cause unacceptable side effects.

Pharmacodynamics
The relationship between drug concentration and its biological effects (what the drug does to the body).

Pharmacogenetics
This term was coined in 1959 and represents the study of genetic factors that influence response to drugs and chemicals[18].

Pharmacogenomics
Recent advances and improvements in large genome-scale sequencing and bioinformatic tools for processing data have led to the transition of pharmacogenetics to pharmacogenomics, which involves studies of the entire spectrum of genes in the human genome[18].

Pharmacokinetics
The concentration of drugs in the body over a period of time, including the processes by which drugs are absorbed, distributed in the body, localized in tissues, metabolized and excreted (what the body does to the drug).

Predictive biomarker
Any measurement associated with response to or lack of response to a particular therapy.

Response Evaluation Criteria In Solid Tumours
A set of published rules that define when cancer patients improve (respond), stay the same (stable) or worsen (progress) during treatments.

Single-arm Phase II trial
A trial that demonstrates the safety and activity of a drug in a selected group of patients. This is in contrast to randomized clinical trials, which involve the random allocation of different treatments (including placebo) to patients in different groups.

Surrogate threshold effect
The minimum treatment effect on the surrogate end point necessary to predict a non-zero effect on the true end point.

Synthetic lethality
In genetics, a phenomenon in which the combination of two otherwise non-lethal mutations results in a non-viable cell.

methodology has been validated preclinically, and clinically qualified in randomized clinical trials to robustly and reproducibly predict anti-tumour responses in the selected population (BOX 1). We therefore propose a new term — 'enrichment biomarkers' — to describe biomarkers that have strong scientific rationale and preclinical evidence for anti-tumour responses, but which are yet to be clinically qualified. Importantly, such enrichment biomarkers need to be scientifically and technically validated preclinically before entering Phase I clinical trials (BOX 1).

Some of these enrichment biomarkers could eventually evolve into predictive biomarkers following greater clinical qualification. For example, based on this definition, the mutation status of *BRCA1* and *BRCA2* in a patient would strictly be considered as an enrichment biomarker for PARP inhibitors until further definitive validation as a qualified predictive biomarker in appropriate clinical trials (BOX 2). Other potential enrichment biomarkers include PTEN loss or *PIK3CA*-activating mutations for PI3K–Akt–mTOR pathway inhibitors[48,49]. For such agents, in view of the complex network of feedback loops involved, it is probable that a biomarker signature of more than one marker will eventually be required to predict a response to inhibitors of this key signalling network. Also, although *MET* amplification or mutations have been shown in a range of cancers in preclinical studies, these have not yet been shown to strongly predict which patients will respond to MET inhibitors in the clinic[50,51].

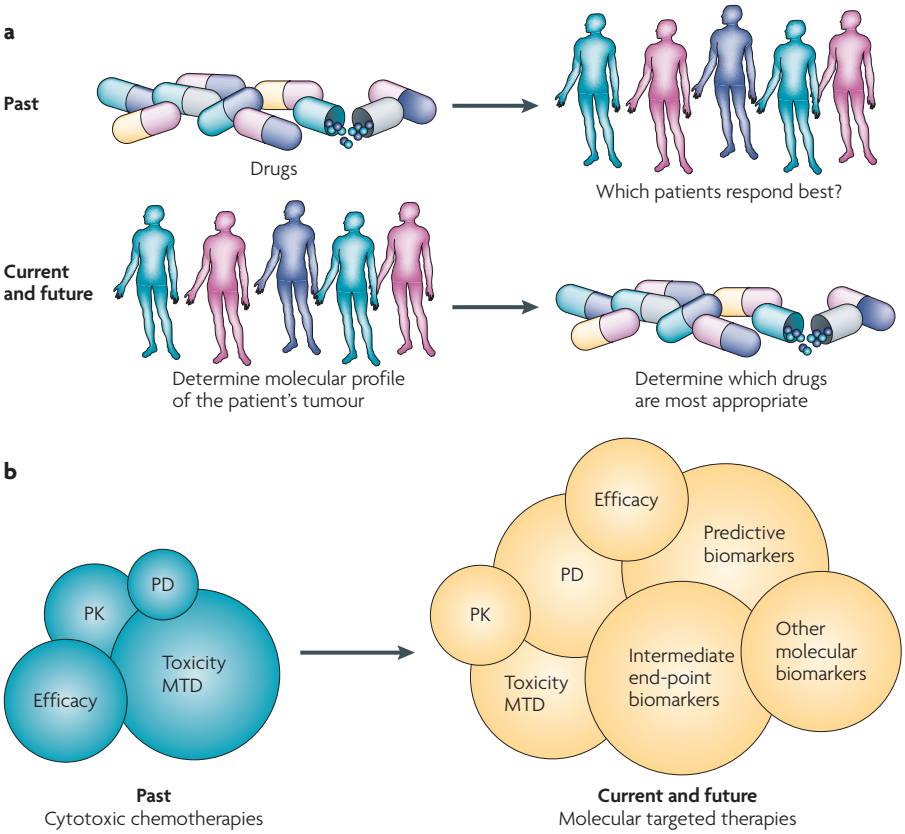

Figure 1 | **The shifting focus of old versus new Phase I clinical trial designs. a** | Preclinical and early clinical data have shown that using predictive biomarkers to match individual tumour genotypes with appropriate targeted agents will increase the odds of patient benefit. Therefore, we should consider shifting from Phase I trial designs in which all patients are treated regardless of their molecular status to strategies that include patient enrichment through biomarker analyses. **b** | Dose-related toxicities have traditionally been considered key end points of Phase I trials and the maximum tolerated dose (MTD) is regarded as the optimal dose that provides the best efficacy with manageable toxicity — the tried and tested model for cytotoxic chemotherapies[71]. Although important, pharmacokinetic (PK) and pharmacodynamic (PD) end points still take a backseat to toxicity in Phase I studies, despite a shift towards the development of molecularly targeted agents[68,102]. The development of targeted inhibitors has challenged the paradigms used in cytotoxic chemotherapy trial design on many levels[98]. Molecularly targeted agents do not necessarily maintain the same dose–toxicity relationship as cytotoxic agents and can produce minimal organ toxicity. Furthermore, molecular therapeutic agents may result in prolonged disease stabilization and provide clinical benefit without achieving the dramatic tumour shrinkage seen with cytotoxic agents, therefore necessitating alternative measures of anti-tumour efficacy[103]. This has prompted interest in functional mechanistic-based end points to delineate an active biological dose range[104]. These end points include biologically relevant drug exposures, PD biomarker measures of target inhibition, intermediate end-point biomarkers, such as circulating tumour cells and other molecular biomarkers, including functional imaging[53,79,83,102,105].

Intermediate end-point biomarkers. Intermediate end-point or surrogate biomarkers are those that accurately reflect treatment efficacy and clinical benefit at an earlier time point than would be required to attain the primary objective of the study. They are intended to substitute for the clinical primary end point and must therefore be modified by therapy and correlate robustly with response and survival end points[12].To establish the ability of intermediate end points to function as surrogates of overall survival, complex meta-analytical statistical designs involving multiple Phase III trials may be required to demonstrate a surrogate threshold effect[52]. If qualified intermediate end points of clinical benefit can be established, these could accelerate drug approval and facilitate earlier and accurate decisions about treatment efficacy, mitigating additional costs and treatment-related morbidity[53]. Promising biomarkers that could function as intermediate end points include circulating tumour DNA, the caspase-cleaved cytokeratin product M30, the concentration of the antigen KI67 in blood plasma and circulating tumour cell (CTC) counts[54–56].

CTC counts are an example of a promising intermediate end-point biomarker, and might even be considered as a multi-purpose marker, as changes in CTC counts from baseline during treatment are now recognized as a prognostic marker in patients with metastatic breast, prostate and colorectal cancer[53,57,58]. CTCs are currently being formally assessed as an intermediate end point for overall survival in ongoing prospective clinical trials, including a randomized Phase III trial of patients who have castration-resistant prostate cancer (CRPC) treated with the CYP17 inhibitor abiraterone acetate (NCT00638690; see Further information for a link to the ClinicalTrials.gov website), which is powered to address this question.

Another exciting development is the increase in information that the molecular characterization of CTCs brings to oncology drug development. The ability to longitudinally evaluate gene amplifications,

mutations, deletions or translocations that have crucial roles in underlying tumour pathogenesis provides unique insights into the underlying and evolving biology of the tumour, without the necessity for invasive biopsies. It also allows patient stratification according to the molecular profiles of risk, prognosis and likely response. These nascent molecular characterization studies are already yielding important results, including recent data showing that TMPRSS2–ETS rearrangements detected by fluorescence *in situ* hybridization in CTCs of patients with CRPC predicts an improved response to abiraterone[59]. It is also possible to identify *EGFR*-activating mutations from CTCs isolated from patients with NSCLC, including the gatekeeper T790M mutation, which confers drug resistance to first-generation EGFR tyrosine kinase inhibitors[60]. Therefore, studies of CTCs could function as a 'liquid biopsy' and be integrated with genomic and proteomic platforms to support patient selection, monitor treatment efficacy and identify mechanisms of acquired resistance.

Animal models. To improve clinical trials and address vital molecular and therapeutic questions accurately, we should also aim to maximize the potential of animal model systems to better reflect the disease being studied. For example, mice that are genetically engineered to recapitulate human cancer and xenografts of human malignancies that have detailed molecular profiling could be used to investigate aspects of tumour biology that might not be possible in the clinic and to demonstrate proof of concept for targeted agents and companion biomarkers[61].

Animal models might also be useful for defining the quantitative extent and duration of target inhibition required for biological and therapeutic effects. These data could then be used to establish preclinical PK–PD efficacy relationships by relating quantitative drug exposure and target modulation levels to efficacy and toxicity. Such PK–PD modelling can subsequently be used to inform a Phase I trial by providing target levels of PK and PD to aim for in the clinic. This PK–PD efficacy relationship is exemplified by our recent experience with the PI3K inhibitor GDC-0941, for which preclinical results showed that greater than 90% inhibition of AKT phosphorylation over several hours is necessary for 50% reduction in the number of proliferating cancer cells *in vitro* and a corresponding level of growth arrest in tumour xenografts[62,63]. GDC-0941 is currently being assessed in Phase I clinical trials, and the value of these preclinical PK–PD relationships is being evaluated[64,65].

Modern Phase I trial design

The clinical use of biomarkers in early drug development is a rapidly evolving and controversial area[66–69]. It is, however, clear that the trial framework for defining optimal dosing through establishing the maximum tolerated dose (MTD) and treatment efficacy according to Response Evaluation Criteria In Solid Tumours (RECIST) are less applicable to the development of molecularly targeted agents, in contrast to cytotoxic chemotherapies (FIG. 1B). Other functional determinants of the biological effects and clinical responses of a drug are now also required to prove the mechanism of action[70,71]. Analytically validated predictive, PD and intermediate end-point biomarkers can empower drug development — in the same way that PK and toxicity data have been the cornerstones for decision making in the past — as integrated components of a modern, comprehensive and biologically driven drug development process (FIG. 1B).

Modern, mechanistically based Phase I trial design should include a dose-escalation scheme that allows rapid and safe patient accrual with procurement of sufficient PK and PD data, clear and customized definitions of dose-limiting toxicities, a limited number (for example, three or fewer) of study sites to ensure familiarity with the drug[72,73] and, importantly, the implementation of an adaptive approach for analysing information accrued in 'real time' (FIG. 3). Such novel designs will facilitate the prospective modifications of dynamic study protocols to allow interrogation of the key clinical and scientific hypotheses being explored.

Crucial to the success of such trials is a thorough understanding of the target biology and of the drug pharmacology, based on detailed preclinical studies, as well as the expected molecular and biological effects. *A priori* provisions in trial design should also be made, if possible, to allow cohort enrichment with patients whose tumours have molecular aberrations that might increase the likelihood of responses (BOX 2). The ultimate goal of this model is personalized medicine, based on real-time molecular profiling of tumour and surrogate material and the identification of the key genetic events driving oncogenesis[74,75]. With several increasingly cost-effective genomic alteration-screening platforms now available, we can rapidly interrogate multiple mutations across numerous oncogenes simultaneously to prospectively guide rational therapeutic selection for each patient (FIG. 2).

For Phase I trials, we support the use of accelerated dose-escalation schemes with initial 100% dose increments in small cohorts of patients (typically three) to define the active dose range of molecular targeted agents[76]. A potential alternative to such a dose-escalation

Box 2 | **Predictive biomarkers in early-phase studies: an example**

Hypothesis-testing preclinical studies showed that *BRCA1*$^{-/-}$ and *BRCA2*$^{-/-}$ cells were 1000-fold more sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors than wild-type cells or cells heterozygous for *BRCA1* or *BRCA2*, demonstrating a clear therapeutic window for this synthetic lethal strategy[28,99]. The assay for detecting *BRCA1* or *BRCA2* mutations in patients with cancer was already well established clinically. These key elements were incorporated into the first-in-human proof-of-concept Phase I trial of olaparib (AstraZeneca/KuDOS Pharmaceuticals), a potent and selective PARP inhibitor[29]. Following preclinical data, *a priori* provisions in the study protocol allowed enrichment during dose escalation for patients with cancer who were *BRCA1* or *BRCA2* mutation carriers. This allowed testing of the hypothesis that patients with cancer who have *BRCA1* or *BRCA2* mutations would respond, as well as the continued rapid accrual of patients with these mutations and unselected patients. Dose escalation was guided by parallel pharmacokinetic and pharmacodynamic evaluation of normal tissue, including peripheral blood mononuclear cells from hair follicles, and tumour tissue. These data were used to establish the biologically active dose range of olaparib. The minimally invasive sampling of normal tissue allowed specimens to be obtained safely at multiple time points, minimizing the impact of intra-patient variability. Dose escalation continued to the maximum tolerated dose (MTD) to maximize drug delivery. Following this and preliminary efficacy data in patients who had *BRCA1* or *BRCA2* mutations, the MTD expansion cohort was limited to this population of patients[30]. Indeed, overall results showed clinical benefit in this patient population but no objective responses in unselected patients. The efficacy of olaparib in patients who had mutations in *BRCA1* or *BRCA2* with advanced breast and ovarian cancers was recently confirmed in two Phase II trials[86,100]. These trials also demonstrated that the MTD seemed to be more efficacious than a biologically active dose (as defined by pharmacodynamic biomarkers), emphasizing the importance of dosing to the MTD. This rapid translation from scientific rationale to robust preclinical data to clinical efficacy was enhanced by incorporating biomarkers into the early trial design.

scheme is the continual reassessment method (CRM), although the practical benefits of this remain controversial[77,78].

Although entering one patient per dose level is feasible, a major limitation of this approach is the limited PK and PD data generated from single-patient cohorts. Furthermore, in a multi-site study, single-patient cohorts are unlikely to save time compared with recruiting three patients.

Dose escalation should continue in 100% increments until Grade 2 drug-related toxicity is observed, when more conservative increases should be pursued. We advocate dose escalation in Phase I trials to the MTD and not just the biologically active dose range, which is usually lower than the MTD[79,80] (BOX 2). This is crucial because, for example, PD data obtained from normal tissue such as blood does not necessarily represent tumour target blockade. PD biomarkers might also not reflect the complexity of drug delivery and drug effects on the entire heterogeneous tumour, which can have poorly perfused, acidic and hypoxic regions with poor drug delivery[81]. In addition, drugs can have a limited ability to penetrate tumour tissue and do not reach all viable tumour cells in an equally effective concentration. Tumour biopsies performed for PD studies are usually obtained from the periphery of the lesion and, therefore, even if target blockade is shown, such findings should not always be taken to imply that central, hypoxic areas are inhibited to the same extent. Importantly, regions of lower drug exposure might rapidly become drug resistant through various mechanisms[82]. Therefore, maximizing drug delivery to all of the tumour by dosing to the MTD could minimize potential resistance to anticancer agents. Imaging studies that evaluate PK and PD might be helpful and should be included whenever possible, although cost is frequently a restriction[83].

This concept of selecting the highest possible dose below the toxic dose might not apply to all classes of agents. For example, it might not be possible with drugs that do not reach MTD in dose-escalation studies, as seen with certain monoclonal antibodies[84,85]. In such instances, a recommended Phase II dose (RP2D) has to be established based on other parameters such as PK and PD estimates in the PhAT in relation to preclinical data. Overall, the optimal dose selection for targeted agents should be based on consideration of all available data from the different stages of early drug development.

The use of PK determinations to ensure adequate drug exposure and validated PD measurements to confirm target and pathway inhibition, as well as other relevant exploratory biomarkers for on-target and off-target effects (including functional imaging), can allow the determination of a biologically active dose range to answer key questions related to the development of an experimental agent. Once this dose is established, we advocate expanding selected doses between the biologically active dose range and the MTD in a larger cohort of patients (for example, 10–20 patients) to assess safety and efficacy, preferably in selected subgroups of patients who have specific molecular aberrations[30]. As a biologically active dose range is likely to be reached while dose escalation to MTD is ongoing, patients could be accrued in parallel to an expanded cohort at a pharmacologically active dose. Patients in these expansion cohorts could also have paired pre- and post-treatment tumour biopsies to facilitate a better delineation of the PK–PD relationship at doses between the biologically active dose range and MTD[23]. Further expansions at the eventual RP2D should also be carried

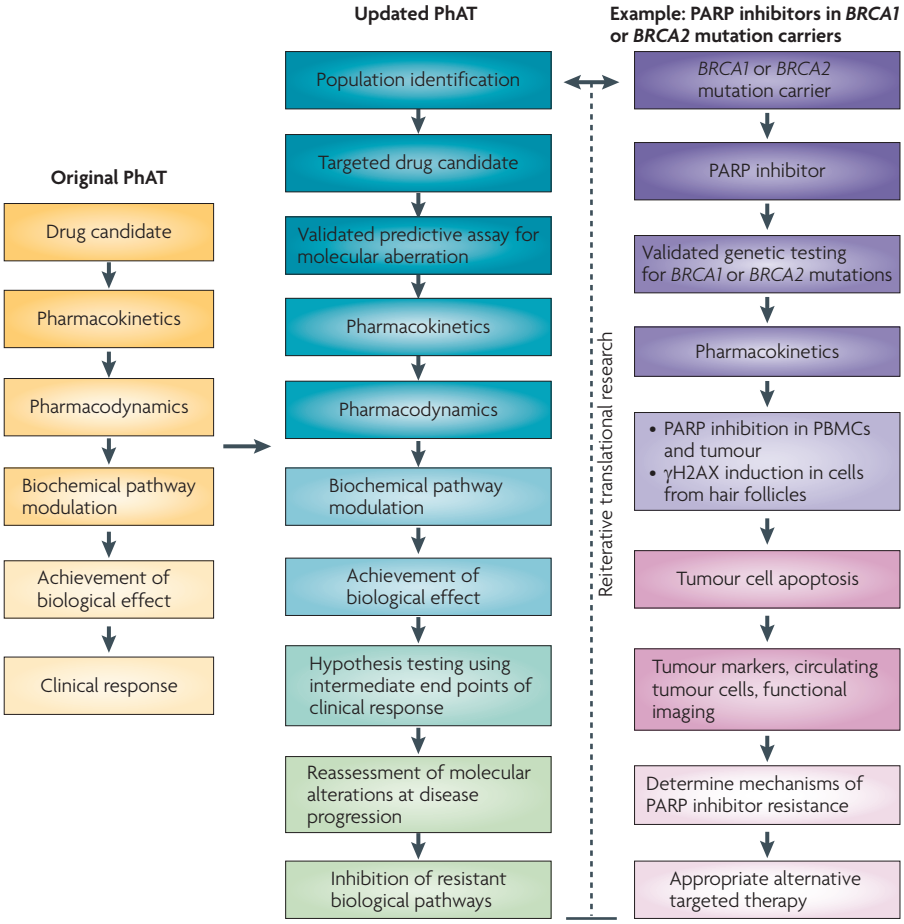

Figure 2 | **Updating the pharmacological audit trail.** The 'pharmacological audit trail' (PhAT) provides a rational framework for assessing the risk of failure of the development of a new agent at any particular stage, with the likelihood of drug attrition decreasing as the hierarchy of sequential questions are successfully answered. It also provides the basis for making key decisions, such as determining the optimal dose range and schedule of a new compound and whether to continue or terminate a drug development programme. First proposed by us in 2003 (REFS 14,20,21), the updated application of the PhAT is illustrated in this figure by the preclinical and clinical studies of the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib[22–24]. To reflect the surge in implementation of biomarkers into early-phase clinical trials, we have modified and enhanced the original PhAT model[20–22]. We postulate that it will become imperative to include the identification and initial clinical qualification of robust predictive biomarker assays for patient selection early in the drug development process, as recently shown with inhibitors of PARP, V600E BRAF and anaplastic lymphoma kinase (ALK)[29,38,39]. Intermediate end-point biomarkers should also be identified and studied in the audit trail as early predictors of anti-tumour activity. These could include circulating tumour cells, circulating endothelial cells and functional imaging modalities. Following the development of drug resistance to new agents[91], potential resistance mechanisms can also be investigated in these studies and alternative strategies pursued to overcome resistance. PBMC, peripheral blood mononuclear cell.

out in molecularly defined cohorts to potentially increase treatment responses, if feasible (BOX 2). We recommend, whenever possible, that randomized Phase II studies comparing the MTD and lower biologically active dose range be pursued for optimal dose (and schedule) selection. If this is not feasible, however, the highest possible dose should be used as the RP2D[86].

Accelerating the transition to Phase III

There is accumulating evidence that non-randomized Phase II trials have limited usefulness and that an increased focus should perhaps be placed on randomized Phase II studies[87–89]. By carrying out exploratory hypothesis-testing studies in expansion cohorts of Phase I trials at a selected dose or doses, early signals of activity could be detected without multiple single-arm Phase II trials. Such Phase I expansions could instead lead directly to randomized Phase II/III trials that incorporate early-stopping rules. For example, such a randomized Phase II/III trial could mandate an interim data analysis. The statistical cost for this is a limited amount of alpha spend; nonetheless, if such an interim analysis is defined prior to trial onset, this approach should be considered. If the efficacy data following an early data analysis suggests that the trial does not meet preset requirements, a no-go decision might be made based on these randomized data, resulting in early trial cessation. However, if the data are promising and meet preset criteria, a decision could be made to proceed seamlessly to Phase III evaluation using a larger cohort. Alternatively, randomized Phase II trials using selection designs[90] can be conducted with smaller patient numbers, using, for example, higher alpha and beta values and one-sided testing. We believe such a change is now warranted, as many Phase II non-randomized trials have taught us little. Overall, by adopting such a strategy following smart Phase I cohort expansions based on tumour biology-driven, hypothesis-testing studies, one may potentially accelerate development and increase the odds of approval of targeted therapies.

In addition, when novel targeted agents in selected patient populations show high and compelling levels of anti-tumour activity in patients with end-stage cancer, a change in the regulatory requirements for drug approval becomes an ethical imperative. We are, for example, now observing high response rates in early-phase clinical trials with molecular therapeutics when matched with patients who have the appropriate genetic aberrations. This is illustrated, as discussed, by Phase I studies of the ALK inhibitor PF-02341066 in patients with NSCLC who have the *EML4–ALK* gene fusion[38]; the BRAF inhibitor PLX4032 in patients with melanoma who have the V600E BRAF mutation[39]; and the PARP inhibitor olaparib in patients with cancer who have *BRCA1* or *BRCA2* mutations[29]. Such experiences raise the question of whether such highly efficacious agents, if well tolerated, should be given provisional rapid approval for use in specific patient subpopulations without the necessity for large randomized Phase III studies. It is envisioned that the development and use of better intermediate end points, involving analytically validated and clinically qualified biomarkers could support accelerated drug approval when compelling activity is seen in molecularly defined populations.

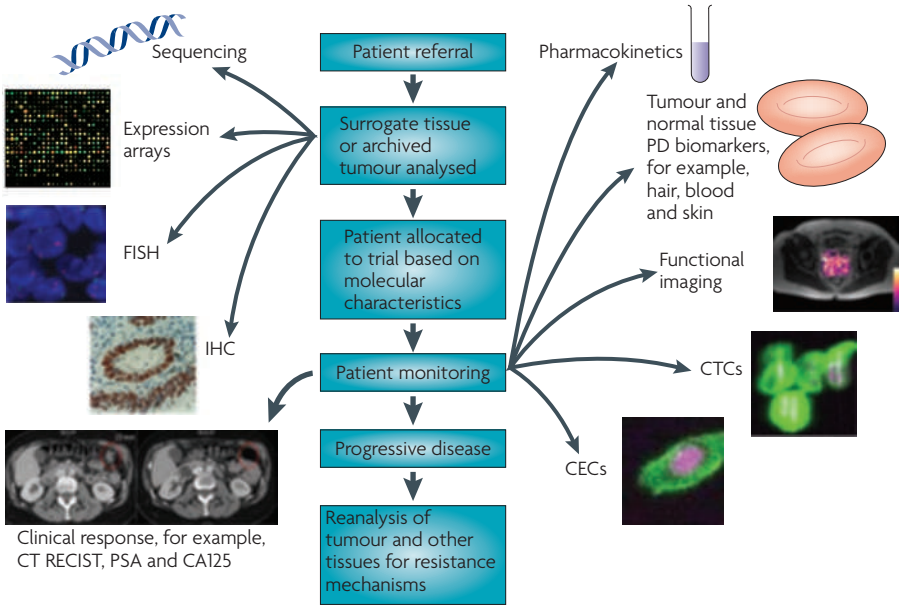

Figure 3 | **Future clinical track for early-phase clinical trials.** Currently, patient selection for early clinical trials of molecular targeted therapies is based on a 'best-guess' approach using available scientific data. It is envisioned that, in the future, predictive biomarkers will have a key role in guiding the individualization of such therapies for patients based on the genetic and molecular characteristics of their tumours. Patients will first have their tumour interrogated for genetic aberrations across many cancer genes in 'real time' and enter a Phase I trial of a drug targeted towards the relevant molecular abnormality. While on therapy, patients will be monitored with improved intermediate end-point biomarkers to gauge their response to treatment. Pharmacodynamic biomarkers will also be used to confirm an appropriate level and duration of target and pathway modulation, ideally defined in preclinical models. These 'on-trial' biomarkers will be analysed in normal tissues, such as blood (platelet-rich plasma or peripheral blood mononuclear cells) and hair follicles, and tumour tissue. Any known mechanism-based toxicities such as epidermal growth factor receptor inhibitor-induced skin rash could be monitored to increase confidence of target inhibition. Anti-tumour responses should be evaluated at regular intervals by conventional radiological and biochemical assessments, as well as exploratory functional imaging modalities. At disease progression, patients could be re-evaluated to identify resistance mechanisms and alternative suitable therapies. This approach has the potential to personalize treatment for patients and to maximize data generation from early-phase trials, and might reduce attrition rates and decrease late-phase trial costs.CA125, cancer antigen 125 (also known as MUC16); CECs, circulating endothelial cells; CT, computerized tomography; CTCs, circulating tumour cells; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; PSA, prostate-specific antigen; RECIST, Response Evaluation Criteria In Solid Tumours. The CTC and CEC images are courtesy of J. Dukes and D. Olmos, The Institute of Cancer Research, UK.

Conclusions

There is increasing evidence that the traditional route of drug development and registration should be adapted for the development of molecularly targeted drugs (BOX 3). We should therefore shift the focus of Phase I studies to include patient subpopulation identification by genomic and molecular analyses with analytically validated biomarkers. Early-phase clinical trials present the opportunity to test and begin to qualify key biomarkers before more rigorous confirmatory assessments in larger studies.

We should also continue to build on our current portfolio of well-characterized, targeted drugs to develop optimal combination therapies. This will allow the creation of cocktails of different regimens guided by

Box 3 | **A multidisciplinary translational strategy**

Multiple barriers to the implementation of new molecular approaches to improve early clinical trials exist, including scientific, technical, cultural and regulatory ones[69,101]. Greater investment by industry and funding bodies is required to help develop, in a timely fashion, biomarkers that can be used not only for decision making in early- and late-stage clinical trials, but also for hypothesis-generating clinical studies that could, for example, lead to the identification of new biomarkers, targets and resistance mechanisms. To sustain the intense requirements of the new trial paradigm discussed in this article, it is important to accept that translational medicine is no longer a one-way process from 'bench to bedside', but rather a continuous dynamic and iterative cycle between laboratory and clinic, involving a multidisciplinary approach to drug development. Furthermore, the timescales for iterative cycles between the laboratory and clinic are now much shorter. The current model at our large comprehensive cancer centre that includes the Royal Marsden Hospital and The Institute of Cancer Research — and one that is also being developed at other large centres — reflects the necessity for a highly integrated multidisciplinary and team-based approach to drug development. The physical housing of both preclinical and clinical teams under one roof fosters active and efficient interaction and transfer of knowledge between different specialist teams and facilitates translational studies, including biomarker-driven drug development. This is enhanced by collaborations with other academic centres, the pharmaceutical industry and regulatory bodies. These interactions facilitate a free flow of ideas and information, and foster partnerships between individual teams specializing in areas, such as cancer genetics, basic molecular oncology, molecular pathology, bioinformatics, systems biology, functional imaging and cancer therapeutics.

the unique molecular biomarker signatures of different malignancies, which might truly individualize cancer therapies. Iterative, real-time analysis will allow the study of predictive biomarkers for the targeted therapy, including target mutation, feedback loops and pathway switching, to be monitored and the appropriate therapeutic corrections made (FIG. 3). The repeated analysis of specific biomarkers will also allow the evolving biology of a tumour to be monitored during therapy and changes in response to treatment to be identified[91]. In the future, we envision that it will be possible to examine the dynamic structure of the human genetic, epigenetic and protein interaction network (interactome) as a means of predicting patient outcome[92]. This will bring rapidly emerging systems or network biology approaches into the clinic[93].

Finally, in this era of targeted treatments, it may soon be time for a paradigm shift away from the previously established management of cancer, which was based on anatomical sites and histological classifications, to one which is augmented by molecular and target-specific strategies. An example of this approach is the treatment of patients with cancer who have either *BRCA1* or *BRCA2* mutations with the PARP inhibitor olaparib, for which anti-tumour responses were observed in different malignancies, including ovarian, breast and prostate cancers[29] (BOX 2). This fundamental alteration in drug development strategy could allow us to further expedite the approval of targeted therapies for cancer patients and thereby accelerate our progression to personalized cancer medicine.

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Competing interests statement
The authors declare competing financial interests; see Web version for details.

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