OPINION

Compressing drug development timelines in oncology using phase '0' trials

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Abstract | The optimal evaluation of molecularly targeted anticancer agents requires the integration of pharmacodynamic assays into early clinical investigations. Phase '0' trials conducted under the new Exploratory Investigational New Drug Guidance from the US Food and Drug Administration can provide a platform to establish the feasibility of assays for target modulation in human samples, evaluate biomarkers for drug effects and provide pharmacokinetic data. Phase 0 trials could facilitate rational drug selection, identify therapeutic failures early, and might compress timelines for anticancer drug development. We expect that such trials will become a routine part of early-phase oncological drug development in the future.

About 10% of Investigational New Drug (IND) applications for new molecular entities submitted to the US Food and Drug Administration (FDA) progress beyond the investigational phase[1]. The success rate is even lower in oncology (~5%)[2]. The problematic issues that underlie the low rate of approval of new oncological drugs include the lack of preclinical systems (both *in vitro* assays and *in vivo* animal models) that can accurately predict the efficacy and toxicity of new agents[3,4,5], the prolonged timeline for drug development, the high costs involved (in terms of financial, patient and professional resources) and the increasing complexity of clinical trials that involve molecularly targeted agents and advanced technologies.

The shift away from the use of nonspecific cytotoxic chemotherapeutic agents in cancer therapy to more specific, molecularly targeted agents has necessitated a re-evaluation of the cancer-drug development process[6]. There is an increased need for therapeutic target development and assessment studies, an endeavor that requires the availability of reliable assays before clinical development, as well as the initiation of first-in-human, proof-of-concept trials that carefully evaluate target modulation. Unfortunately, current strategies for drug development are still predominantly based on the inherent assumption that the investigational agent under study has a dose–toxicity relationship, and that efficacy is somehow related to toxicity[7]. Therefore, the primary objective of phase 1 trials has traditionally been safety, in which the highest dose that is associated with tolerable toxicities, that is, the maximum tolerated dose (MTD), is the endpoint, and that dose is then used for further clinical testing in phase 2 efficacy studies. Objective tumour shrinkage is the marker that is usually used for clinical benefit in phase 2 trials, and forms the basis for deciding whether a drug will be evaluated further in a randomized phase 3 trial.

However, strategies used for the development of cytotoxic agents might not be applicable or optimal for the development of molecularly targeted agents[8–11]. Determining the 'biologically effective dose' instead of the MTD might be the most relevant objective of an early-phase trial evaluating these agents. However, despite the accumulation of an extensive array of preclinical data before IND submission in the United States, the development and integration of well-qualified pharmacodynamic tools and biomarkers for first-in-human cancer clinical trials has been uncommon over the past decade[12]. The development of rigorous assays to evaluate pharmacodynamic biomarkers requires extensive resources that are not readily available at most medical centres engaged in anticancer drug development[13,14]. Because of the increasing number of molecularly targeted agents, better preclinical models are urgently needed to facilitate the development of biomarkers that might provide the investigative capability to make predictive correlations with early clinical endpoints. The routine availability of predictive pharmacodynamic markers for early clinical trials would provide the basis for a new drug-development paradigm in oncology. This new approach would enable the systematic de-prioritization of investigational agents that clearly do not show expected biological effects early in drug development on the basis of human pharmacology data rather than that collected in animal models. For example, even though no effects were observed on the pharmacodynamic markers evaluated in the early-phase trials of matrix metalloproteinase inhibitors, clinical development proceeded to phase 3 trials that showed no evidence of clinical benefit[15,16].

The phase '0' clinical trial

We propose that some of the above concerns can be addressed and the timeline for anticancer drug development compressed by altering the traditional drug-development sequence (TABLE 1). At present, following preclinical investigations, phase 1 toxicity studies are followed by phase 2 efficacy trials, and then, if warranted, phase 3 randomized studies are performed to compare new agents to standard treatment (FIG. 1a). The incorporation of exploratory, phase 0 or target-development clinical trial designs that focus on extensive agent characterization

Table 1 | **Role of phase 0 clinical trials in cancer-drug development**

Current challenges in cancer-drug development	Phase 0 trials
Suboptimal use of target assessment and imaging techniques in early-phase clinical trials	Biomarker development and assay qualification in human tissues before the initiation of the trial
	The evaluation of imaging studies that provide functional and metabolic information about the effects of a drug on its target(s)
	The integration of such assays and/or imaging studies in phase 0 trials to establish the mechanism of action *in vivo* in actual patient samples
Establishment of the maximum tolerated dose as a primary endpoint in trials with molecularly targeted agents	Evaluation of target modulation is a primary endpoint
Late-stage failures with low rates of anticancer drug approvals	Allow for the systematic de-prioritization of investigational agents that do not show expected biological effects
Long timelines for the development of promising agents	The early initiation of first-in-human, proof-of-concept trials that provide data to better inform and expedite subsequent clinical development should shorten drug-development timelines
Increasing number of complex trials that require substantial resources	Investing resources in early-phase trials that involve a small number of patients should help prioritize resource allocation for subsequent larger trials

and target-assay development (including molecular imaging studies) in a limited number of patients could yield results that would optimally inform and expedite the subsequent development of molecularly targeted agents (FIG. 1b). Conducting phase 0 trials should not delay drug development, as they can be conducted earlier than the traditional dose escalation, safety and tolerance studies that ordinarily initiate a clinical drug-development programme (see below). Extensive preclinical development leading to the incorporation of real time pharmacokinetic and pharmacodynamic assays in these first-in-human trials could help to evaluate the effects of an agent at the molecular level, select the lead agent from a group of compounds, assist in optimizing the selection of the starting dose for subsequent studies and aid in developing rational dose-escalation schedules for the examination of target effects. Phase 0 clinical trials could also guide patient selection and response evaluation (for example, pharmacodynamic assays and imaging studies) in subsequent definitive studies that focus on the assessment of drug effects on the target ('target assessment' or combined phase 1 and 2 trials). This could expedite the process of drug development and reduce the likelihood of failure in late-phase studies, potentially reducing overall costs (FIG. 1b).

Because phase 0 trials will involve a relatively small number of patients, about 10–15, who will each be exposed to a limited number of doses of the study agent (for example, dosing for less than 7 days), the associated risk of toxicity will be lower than in traditional phase 1 trials (TABLE 2). Therefore, the toxicology testing required before initiating phase 0 clinical trials is reduced, enabling these trials to be initiated substantially sooner than the standard phase 1 study. Phase 0 trials will be conducted under the purview of an Exploratory Investigational New Drug (xIND) Application, a component of the FDA Critical Pathway Initiative, as outlined in an FDA guidance issued early in January 2006 (REF. 1) (BOX 1). Phase 0 trials have no therapeutic intent, and therefore will not have the potential to provide definitive conclusions regarding the safety or efficacy of a new anticancer agent. However, they can provide the data on which to base informed decisions about further clinical development of a specific agent, as well as the design and execution of phase 1–2 trials, potentially speeding the delivery of new anticancer agents to the bedside.

Phase 0 clinical trial settings

Some examples of possible phase 0 clinical trial settings are shown below.

Single-agent trials. These trials will be single dose first-in-human studies of a molecularly targeted agent, and will include an evaluation of the biological effects on the target in tumour biopsies obtained pre- and post-drug administration, and a determination of the pharmacokinetics of the agent in humans (FIG. 2). Inter-patient dose escalation, giving a single dose per patient, can be performed within the range established in preclinical models, with the objective of evaluating target modulation and not clinical efficacy or toxicity, therefore enabling dose selection for future studies. Such trials would provide the opportunity to use and refine a biomarker assay, developed with a preclinical model system, during a study in humans, and to optimize tissue handling and processing procedures that are required to obtain reliable, reproducible data. The assay could be used to study the effects of the drug in question in tumour samples and peripheral blood or other surrogate tissues, providing initial data on the possible association between drug effects on tumour cells and on surrogate cell populations. In order to limit the number of invasive biopsies, tumour biopsies could be obtained once a target level of systemic exposure is achieved in the plasma or some evidence of biological effect is observed in peripheral blood cells in the initial patients. A clear indication that the surrogate tissue reliably predicts drug effects in the tumour, potentially reducing or obviating the need for tumour biopsies in future trials, would be of particular interest and should be explored in preclinical studies and early clinical trials. In case the expected effect on target is not observed in the trial, more preclinical evaluation of the potential causes for lack of target inhibition and exploration of other targets (for example, a kinase inhibitor affecting other pathways) should be done before clinical development is continued or terminated.

The pharmacokinetic data obtained from such a trial could provide a closer approximation to a safe, but potentially effective starting dose, therefore permitting more aggressive dose escalation in future studies, thereby reducing the number of patients needed to draw definitive conclusions from phase 1–2 trials. Robust pharmacokinetic data from such phase 0 trials might also provide the basis for the use of more limited pharmacokinetic sampling strategies in subsequent trials.

Based on the conclusions from this type of trial, a multi-dose (with a limited number of doses) phase 0 trial could also be conducted to provide additional preliminary data to guide decisions about the optimal schedule to evaluate in definitive investigations. However, only substantial differences in results among dose levels would be detectable with the small sample sizes used in a phase 0 trial.

Combination trials. One of the crucial questions for combination therapy, either with two targeted agents or a targeted agent and a conventional cytotoxic agent, is the determination of the schedule and sequence to use in combining the agents to optimize efficacy. Therefore, a phase 0 trial could be performed in which a single dose or limited number of doses of each agent would be administered in various schedules with pharmacokinetic and pharmacodynamic evaluations. The pharmacodynamic evaluation could focus on assays that examine, for example, the level of DNA damage produced by the cytotoxic chemotherapy. The optimal schedule for further evaluation would be the one that maximized the level of DNA damage in tumours by the addition of the targeted agent. For drugs in combination, the modulatory effects of one drug on another might occur at doses well below the MTD. Without adequate pharmacodynamic investigation, an opportunity for improved trial design is lost. The pharmacokinetic and pharmacodynamic data for different schedules of administration would provide the basis for further trials. Therefore, many schedules could be evaluated, involving a limited number of patients and doses, over a relatively short period of time, informing future clinical development.

Selection of a lead agent for clinical development. During traditional drug discovery, many structurally-related analogues of a given class are generated, and decisions about lead agent selection for further clinical development are made on the basis of *in vitro* and animal model data. Owing to limited resources, full toxicology and manufacturing packages are usually developed for the lead agent only. This approach might result in promising compounds not being developed, as the preclinical data used to make development decisions might not reliably predict efficacy or toxicity in humans[3,5]. Phase 0 trials enable the evaluation of the agents in humans with a limited toxicology package, giving few doses of different analogues to provide essential human pharmacokinetic and pharmacodynamic data that will form the basis for selecting the lead agent for further development.

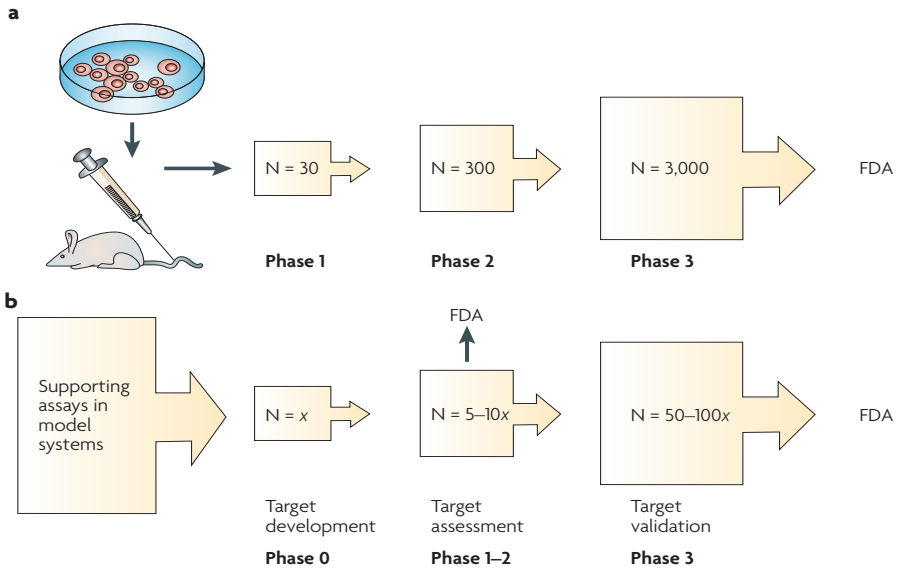


Figure 1 | **Stages of drug development. a** | Current stages of therapeutics development in oncology. N is the approximate number of patients required to complete the phase of the study. **b** | Proposed stages of targeted therapeutics development. N is the approximate number of patients required to complete the studies, *x* is the approximate number of patients needed to complete a phase 0 trial (~10–15). A phase 0, or 'target development' trial, is a first-in-human pre-phase 1 trial that involves a small number of patients and single doses or shortened courses of treatment instead of continuous treatment. The objectives might include the development of pharmacodynamic assays in human tumours or surrogate tissues, the estimation of pharmacokinetic parameters, evaluation of the drug effect on its target(s) or imaging the target of interest. This type of proof-of-concept trial involves early integration of real-time biomarker assays to establish the mechanism of action *in vivo* in patient samples with the intent to inform and improve the efficiency of subsequent drug development. Several phase 0 trials of different agents with the same molecular target might facilitate prioritization with respect to further development in phase 1 and 2 trials. A phase 1–2 or 'target assessment' trial evaluates the safety and efficacy of an agent in patients with various types of cancer, provides definitive pharmacokinetic assessments and uniformly collects sufficient tumour and surrogate tissues. Such a trial could provide a preliminary evaluation of whether target inhibition is associated with clinical endpoints (that might include tumour response), and with unique cellular or molecular characteristics of the patients' tumours. In rare cases, the results of the phase 1–2 trial might be so compelling with respect to both the clinical endpoint and its relation to the molecular endpoint that applying directly to the US Food and Drug Administration (FDA) for accelerated approval, in advance of a definitive phase 3 study, could be considered. Phase 3 or 'target validation' trials (usually randomized) are powered sufficiently to definitively evaluate both clinical endpoints following the administration of specific therapies, and predictive relationships between specified clinical endpoints and the molecular characteristics identified in phase 1 and 2 trials.

Early application of molecular-imaging studies during anticancer drug development. Another rapidly-evolving field in oncology is receptor or target imaging to provide a non-invasive whole-body image of the receptor or target status in various tissues including tumours, which might help to evaluate receptor or target heterogeneity between tumours at different sites within a patient, and between patients, for a given tumour type[17]. Radiopharmaceuticals need to be highly specific and have favourable metabolic and clearance characteristics to ensure optimal tumour to background ratios. Different radiopharmaceuticals can be effectively evaluated using microdoses in a limited number of patients in the context of a phase 0 trial to study biodistribution, as well as the presence and effect of an agent on a given target. Phase 0 trials also provide a platform to test the feasibility of target-specific imaging, ranging from assessing the presence of a target to evaluating whether a particular drug is adequately intracellularly transported. Such trials will help define patient populations for further studies[18]. Imaging modalities can also be used to evaluate downstream target effects, for example, the use of DCE-MRI (dynamic contrast-enhanced magnetic resonance imaging) in the evaluation of an anti-angiogenic agent can be defined in a phase 0 trial before conducting such resource-intense studies in larger trials[19,20].

Phase 0 infrastructure

The development and conduct of phase 0 trials requires a smoothly integrated infrastructure with a collaborative and highly motivated multi-disciplinary team of professionals. Phase 0 trials provide an opportunity to establish feasibility and qualify assay methodology in limited numbers of human samples before embarking on studies that involve larger numbers of patients receiving

Table 2 | **Differences between phase 0 and phase 1 trials**

Variable	Phase 1 trials	Phase 0 trials
Primary endpoint	Establish the maximum tolerated dose	Target modulation or ability to image the target of interest
Dose escalation	Determine safety and toxicities	Achieve desired systemic exposure or target modulation, enabling dose selection for future studies
Preclinical biomarker studies	Not consistently performed before the trial	Required to have plasma drug (pharmacokinetic) and preclinical biomarker (pharmacodynamic) assay development and assay qualification before the initiation of the clinical trial
Biomarker assays	Not performed consistently, most phase 1 trials do not emphasize pharmacodynamic markers	Biomarker assays and/or imaging studies are integrated to establish the mechanism of action in actual patient samples
Number of patients	Usually >20	10–15
Dosing	Multiple	Limited
Therapeutic benefit	None expected; however, tumour response is evaluated to enable continued dosing in case evidence of clinical benefit is found	None
Tumour biopsies	Optional	Serial tumour biopsies required to evaluate the effect of the drug on its target(s)
Pharmacokinetic/ pharmacodynamic analysis	Samples are usually batched and analysed at a later time	Real time

higher, potentially toxic, doses. Phase 0 trials require the rigorous development of assays for target modulation and pharmacokinetic analysis before the initiation of the clinical trial, and real-time analysis of patient samples during the conduct of the study. This raises certain unique logistical, ethical and statistical considerations, in addition to the intricacies of the required pharmacodynamic and pharmacokinetic studies, that need to be carefully planned and managed before and during the conduct of phase 0 trials.

Logistical considerations

Pharmacokinetic and pharmacodynamic assays. The development of reliable pharmacokinetic and pharmacodynamic assays requires close collaboration between laboratory, clinical and regulatory personnel dealing with issues such as optimal acquisition and tissue-handling procedures, assay development and validation, assay reproducibility across technicians and different laboratories, and access to animal models, human tissue samples and biological fluid samples for analysis (FIG. 3). Qualified tissue assays, that is, those that are performed on uniformly handled tissue samples after testing in preclinical systems and preferably replicated in a second laboratory, are required to draw reliable conclusions from a given trial. Therefore, before initiating a phase 0 trial, an essential component of preclinical assay development is to establish standard operating procedures (SOPs) for sample collection and processing that mirror the clinical procedure, enabling meaningful analysis of clinical data. This requires starting to qualify the assay much earlier in the drug-development process than usual. Such SOPs and assays can then be incorporated early in target development and assessment trials to provide a more effective and rigorous scientific basis on which to make clinical development decisions. In support of this resource-intensive effort, the United States National Cancer Institute (NCI) is leading a nationwide initiative to standardize the collection of high-quality human biospecimens through its Office of Biorepositories and Biospecimen Research in order to optimize their use for investigational purposes.

Box 1 | **Exploratory IND guidance**

The development of the US Food and Drug Administration's (FDA's) Exploratory Investigational New Drug (IND) Guidance began in earnest early in 2003 as a part of the FDA's Critical Pathway Initiative. This process involved extensive discussions between the FDA, pharmaceutical industry representatives and the US National Cancer Institute (NCI) about the nature of the preclinical data required for first-in-human, limited dosing, pilot studies of therapeutic agents and for initial investigations of new imaging agents. The entry of the first patient on a phase 0 clinical trial at the NCI under an exploratory IND occurred in June 2006, 5 months after the formal issuance of the FDA guidance. This trial is a first-in-human study of an inhibitor of the DNA repair enzyme poly-ADP-ribose polymerase, evaluating the effect of the compound on its molecular target in both tumour and surrogate tissues in real-time.

Historically, in phase 1 trials, pharmacokinetic and pharmacodynamic assays are often performed at the end of the trial, with all the patient samples being analysed in a single batch. However, in phase 0 trials where dose escalation is performed, and because toxicity is not anticipated, near real-time pharmacokinetic and pharmacodynamic data will have to be analysed promptly, as the rationale for dose escalation is based on either a pre-determined pharmacokinetic parameter or drug effect on its target.

The development of qualified assays and the performance of real-time pharmacokinetic and pharmacodynamic assays in trials is resource intense. In addition, given the non-therapeutic nature of such trials, financial reimbursement from third-party payers is not feasible. Therefore, the NCI is devoting new resources to help investigators conduct such studies.

Preclinical efficacy data. The essential components of types of efficacy studies that should be performed to support phase 0 trials include the determination of the IC_{50} of the agent for the molecular target in cell-free and tumour-cell assays, as well as a determination of time–concentration and area-under-the-curve (AUC) profiles *in vitro* and in animal tumour model(s). Optimally effective dosing schedules, biomarker assay(s), and preclinical imaging evaluations should also be fully developed during preclinical animal model efficacy studies so that the appropriate investigations can be planned for first-in-human phase 0 trials. The goal is to establish a direct link between the physical events that occur

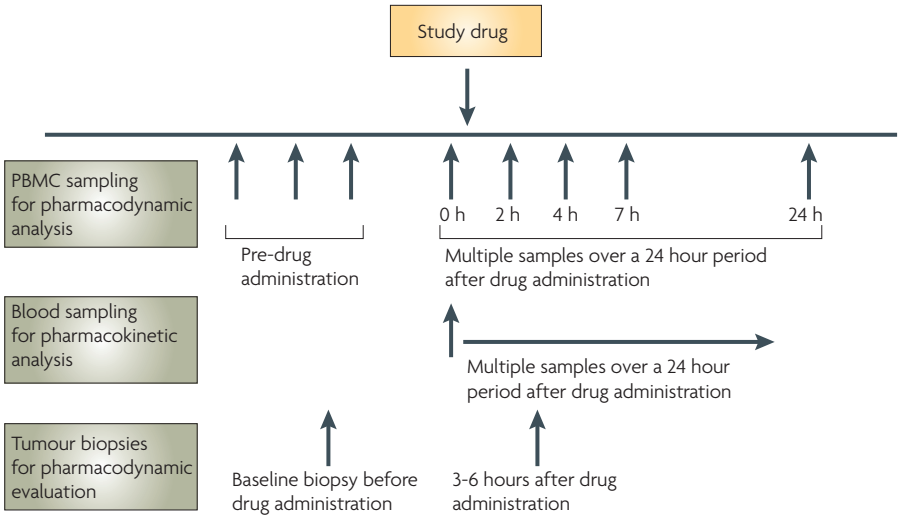


Figure 2 | **Single-dose phase 0 trial of a drug with serial sampling of blood and tumour for pharmacokinetic and pharmacodynamic analysis.** Serial baseline blood samples can be obtained before the administration of the drug to establish the baseline for the measurement of post-drug effects. Tumour biopsies could be obtained once a target level of drug is achieved in the plasma, or some evidence of biological effect is observed in peripheral blood mononuclear cells (PBMCs), to avoid unnecessary biopsies.

in the tumour (that is, growth inhibition or regression) with the molecular events that are defined by the biomarker(s), first in animal models and then in patients.

Ethical considerations

Informed consent for study participants in phase 0 trials must clearly document that the dose of the investigative agent administered will be lower than that which would be expected to cause appreciable toxicity or therapeutic benefit. The non-therapeutic nature of the phase 0 trial, and the need for several tests such as serial tumour biopsies, could significantly impair patient enrollment. However, there are patients who are highly motivated to contribute to research and who will participate knowing there is no therapeutic benefit. A frank discussion with prospective patients on the purpose of conducting such trials and their potential effect on the development of the anticancer drug being tested is essential during initial patient evaluation. At present, tumour biopsies for research purposes are often obtained during standard phase 1 trials. The administration of repeated cycles of the study agent and evaluation for tumour response by serial scans could result in a perception of therapeutic benefit, influencing patient acceptance of invasive procedures in phase 1 studies. As there is no associated therapeutic benefit to participation in phase 0 trials, the intent of the biopsy procedure is clear to the patients. Therefore, the consent for participation in the study and for invasive procedures is given with a clear understanding of the purpose of the trial. Careful documentation must be provided in the medical record that these considerations have been discussed in depth with the patient contemplating participation in phase 0 studies, and that true informed consent has been obtained.

An additional ethical concern surrounding participation in a trial with no therapeutic intent is that it might result in delay or even possible future exclusion from participation in certain clinical trials. In general, owing to their limited dosing schedules (often a single dose), the duration of participation in a phase 0 trial would be short, for example, 2 weeks. Therefore, participation should not result in a significant delay in proceeding to other trials. However, this issue should be discussed with the patient, and phase 0 trials should be considered only for those patients with advanced disease who do not have symptoms that require immediate therapy. In addition, we strongly recommend that future phase 1 and 2 studies specifically refrain from excluding patients who have received an investigational agent not given for therapeutic intent, such as during a phase 0 trial. Finally, because of the range of potential concerns about the lack of therapeutic intent of phase 0 studies, consultation with institutional bioethics and human research committees is strongly advised early in the process of protocol development. Conceptual discussion of the nature and design of phase 0 trials with these committees can help to provide a well-defined understanding of the intent and risk involved in these trials. Such consultations are likely to significantly improve the development process for subsequent, drug-specific protocol documents.

Pharmacokinetic considerations

The design and conduct of phase 0 trials will be directed in part by the pharmacokinetic properties of the agent evaluated. Conducting extensive preclinical testing will establish a starting dose and schedule and help make predictions about the circulating levels of the agent required to produce target modulation. Many of the new targeted agents are oral and are projected to be given as chronic daily therapy. The margin of safety in determining the starting dose can be achieved by either lowering the individual dose or by giving fewer doses, for example, a single dose. Therefore, giving a single dose of the projected chronic daily dose will generally be safe (and will have been addressed specifically in preclinical models), might have modulatory effects on the target, will present no unique challenges for analytical methods and should require no pharmacokinetic extrapolations from microdosing or tracer doses.

Pharmacokinetic monitoring during the trial will address questions such as oral bioavailability, time to maximum concentration in plasma, exposure and drug clearance. Dose escalation and timing of tumour biopsies in a phase 0 trial could be based on achieving a projected level of systemic exposure. To accomplish this objective, intensive, real-time pharmacokinetic monitoring is required. In trials that require tumour biopsies to measure the effect of a drug on its target, biopsies can be deferred until a desired level of systemic exposure is achieved. Such a strategy will minimize the number of invasive procedures performed and increase the likelihood of observing drug effects in the samples collected. Real-time pharmacokinetic data obtained in phase 0 trials in the pharmacological range should inform the starting dose and schedule, and, in future phase 1 and 2 trials, potentially fewer dose levels would need to be investigated and rapid dose escalation could be planned on the basis of such data.

Pharmacodynamic considerations

To optimally evaluate a particular molecularly targeted agent, it is essential to have a fundamental grasp of the molecular pathways that are believed to be altered by the drug, and to possess pharmacodynamic assays that accurately and reproducibly

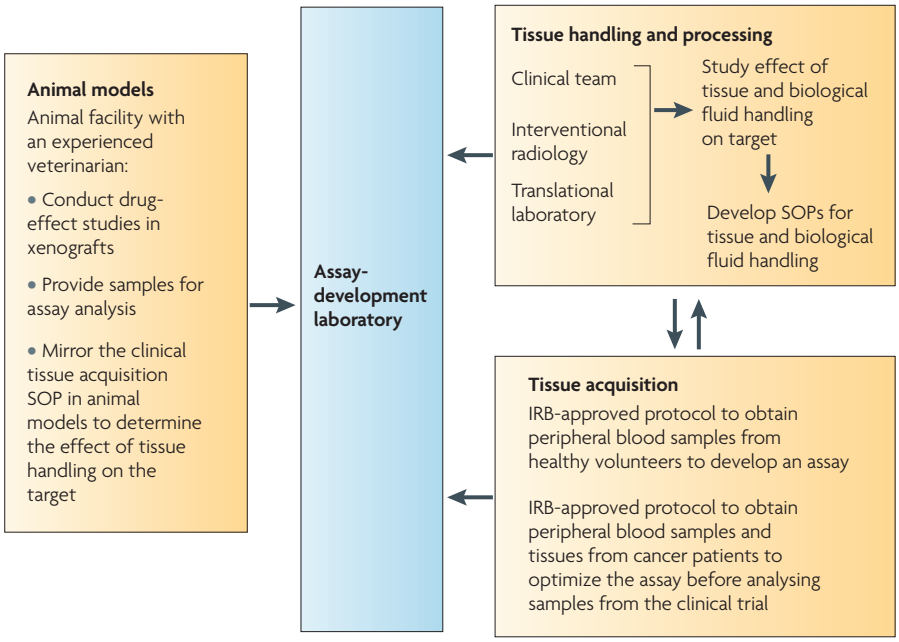


Figure 3 | **Pharmacodynamic assay development before the initiation of phase 0 clinical trials.** Rigorous assay development, including an emphasis on the development of standard operating procedures (SOPs) for tissue acquisition and handling, is essential before the initiation of a phase 0 trial. IRB, institutional review board

enable the evaluation of drug effect[11]. Once potential targets or pathways for the therapeutic agent of interest are identified, it is important to develop an understanding, first in animal models and then in humans, of the effects of the agent on the target pathway in tumour cells and normal cells *in vivo*, the effects of inhibiting the target and whether inhibition results in anti-tumour activity. It is also important to determine the level and duration of inhibition required to produce therapeutic activity ('optimal target inhibition'), and to evaluate any so-called 'off target' biological effects that might be important for a complete pharmacological understanding of the clinical outcome of the trial.

Many targeted therapeutics are designed to inhibit the enzymatic activity of their molecular targets, and development programmes frequently focus on inhibitors of dynamic cellular processes, such as phosphorylation, acetylation or farnesylation. Regardless of the exact mechanism of enzyme inhibition, the goal is to inhibit specific enzyme activities and reduce the intracellular levels of their specific reaction products to levels and for time periods that are required to achieve biological consequences that manifest as clinical efficacy. Assays for pharmacodynamic biomarkers are, in effect, attempting to measure predictors of clinical outcomes. As such, they will be applied across many patients, often more than one tumour and tissue type, and be required to detect changes in analyte levels associated with changing doses of the test drug. Early-phase clinical trials are often initiated without the rigorous development of pharmacodynamic assay methodology, including a lack of assay qualification across a spectrum of patient samples in several laboratories.

By contrast, the concept of phase 0 trials requires that the results of the pharmacodynamic assay be used to make decisions about the next step in clinical testing, whether that be dose escalation, the addition of patients at a particular dose level, or the achievement of the specified trial endpoint of target inhibition. Phase 0 trials will measure the specific activity of target function as a strategy to quantify drug effect as a possible primary endpoint. Therefore, the analytical performance of the biomarker assay is crucial and needs to be addressed before initiating early-phase trials in much the same manner as occurs with the development of pharmacokinetic assays. There are several considerations regarding assay development and qualification that must be examined during the development of a phase 0 study, in addition to the fact that the level of the enzyme product used as a pharmacodynamic marker is a dynamic endpoint that can be affected by many factors.

Requirements and limitations of pharmacodynamic assays. For the pharmacodynamic assay to be a reliable measure of the effect of a drug on its target, the test must be demonstrably accurate, precise, reproducible and have a dynamic range that enables the measurement of baseline and post-treatment modifications of the target (BOX 2), so that a reported result from one patient can be reliably related to the result from another patient in the same or a different treatment cohort. These requirements become even more important if a test will be run in more than one laboratory.

How reliably the assay standards correlate to the actual analyte (the molecule being quantified or analysed) in test specimens is a limiting factor in assay development. The availability of a matrix (the sample material in which the analyte is dissolved or dispersed, for example, serum, plasma, urine or cytosol) that mimics patient specimens, and in which the standards can be stabilized, is an important consideration. Selection of the matrix will depend on the type of specimen to be assayed, and if more than one specimen type will be used in the clinical trial (for example, plasma, peripheral blood mononuclear cells (PBMCs) or solid tumour biopsies) the selection becomes even more complex. In addition, stabilizing the standards in a cell matrix is often difficult owing to intrinsic enzyme activities and the binding of cellular components to the standards. An example would be the specification of an amount of protein in a cell or tumour extract to be loaded per assay well. This approach, in turn, requires a quantitative assay to be used in measuring the protein or nucleic acid load level, which would have to be performed before using the biomarker assay. Use of plasma as a specimen simplifies the issue, as total protein levels in plasma do not vary greatly from individual to individual except in specific diseases. For PBMCs, a viable cell count can be useful.

Biomarkers are dynamic endpoints. It is important to recognize that many of the enzyme activities targeted for therapeutic intervention will be biochemically balanced by a second enzyme, so that the product from the first is degraded by the activity of the second. This biochemical principle is crucial in making biological systems capable of rapidly adapting to signals and environmental conditions, and it provides for rapid and multiple control points for regulating the levels of specific biochemicals through the net activities of the producing and degrading enzymes. Therefore, the level of a particular

biochemical product is the net result of the dynamic processes of its production and its degradation, and the biological stresses of a cell might alter one of these processes but not the other. Because levels of biochemical indicators are determined by the net rate between production and reversion back to the original substrate, the level of an enzyme product used as a pharmacodynamic marker is a dynamic endpoint determined by two molecular targets, not one (BOX 3). For example, kinases and phosphatases, and acetylases and deacetylases catalyse reversible reactions. However, not all reactions are reversible, such as methylation and deamination. In addition, the activity of the therapeutic target over time might decline and then recover at the same time as drug levels rise and fall, whereas the activity of the balancing enzyme could fluctuate as a result of cellular response to target inhibition or natural biological processes like circadian rhythms. For example, the levels of small-molecule metabolites used as pharmacodynamic endpoints are determined by at least three dynamic systems: producing, degrading and utilization enzymes.

Interpreting changes in the levels of these substances as pharmacodynamic indicators is complicated, necessitating an investment in preclinical assay development and the conduct of phase 0 target-development trials. The development of functional pharmacodynamic assays using tissues obtained under 'clinical conditions' will, in fact, be a crucial goal of phase 0 trials, and provide the venue for the development of an understanding of assay characteristics and complexities that are unlikely to be confronted *in vitro* or through the use of animal-model systems. Phase 0 trials are not designed to provide a definitive assessment of the effect of the agent under study on its putative target. Only larger phase 1 and 2 target-assessment studies can address the molecular effects of targeted cancer therapeutics with precision (FIG. 1b). However, the timely completion of such target assessment trials that combine efficacy and biomarker endpoints will be markedly increased by the availability of well-qualified pharmacodynamic assays that emerge from phase 0 evaluations.

Box 2 | **Pharmacodynamic assay requirements**

Accuracy
The ratio of the observed assay readout to the actual quantity of *bona fide* analyte present at any point within the dynamic range of the assay. Traditional methods of establishing accuracy include the recovered fraction of a known mass of the analyte added to a clinical sample (spike recovery), and the measurement of interferences from materials likely to be found in a typical specimen.

Dynamic range
The range of concentrations in which the assay is capable of accurately measuring an analyte, ideally concentrations present in both treated and untreated specimens without additional specimen dilutions or processing steps.

Precision
A measure of the variability of results for a specimen around the determined value, performed in the range in which measured values approximate the true value. Usually accomplished by repeated assays of a set of specimens by several technicians on different days.

Reproducibility
The closeness of agreement between independent results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different laboratories and/or after different intervals of time). It is measured as the total imprecision of the assay from all sources, measured at several points within the dynamic range of the assay. This imprecision must be much less than the clinical endpoint selected: that is, if an undeveloped assay had a total imprecision of about 40%, this would make measurement of a 50% effect impossible.

Robustness
The assay must be transferable to other laboratories. The results obtained from the assay must be stable over time. As the patients for Phase 0 trials are being monitored in real time, there should be a method to determine that results obtained for the first specimen from the first patient and the last specimen from the last patient are comparable in precision and accuracy.

Sensitivity
Analytically, this is the slope of the standard curve. The assay should be sensitive enough to enable repeat determinations of the same specimen.

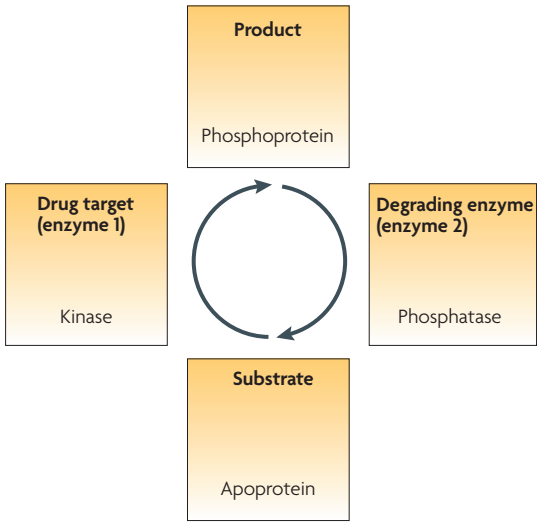
Toxicity considerations

The underlying rationale for the development of the exploratory IND is to enable the initiation of clinical trials at an earlier stage of development by reducing, in part, the toxicology requirements to support those studies. Toxicity studies performed to support phase 0 clinical trials must now focus on the safety of single doses or shortened courses of treatment instead of continuous treatment, as well as on the drug concentrations and systemic exposures that will probably be encountered in such trials. It is also important that these preclinical studies determine the safety and/or toxicity of effective doses and/or drug concentrations in animal models, including the effect of efficacious drug concentrations on pharmacodynamic markers in surrogate compartments[6]. These last studies are very important because they enable the determination of appropriate safe starting doses for the phase 0 study, the establishment of a therapeutic index and the determination of drug plasma levels that might be associated with toxicity. As single or a limited number of doses will be given as part of phase 0 trials, the risk of toxicity associated with participation in a phase 0 trial is less than in a standard phase 1 trial, and is likely to be related more to the procedures associated with tissue acquisition or the mechanics of conducting the trial (such as intravenous line placement or receiving imaging contrast material) than to the actual administration of the experimental agent. However, it remains clear that all patients enrolled in a first-in-human study must be carefully monitored for side effects given the potentially toxic nature of all anticancer therapies.

Statistical considerations

New statistical designs are needed for phase 0 trials owing to the limited number of patients to be enrolled and the complexities of analysing the pharmacodynamic and pharmacokinetic results, particularly as the pharmacodynamic and pharmacokinetic data can serve as primary endpoints for these trials. This section provides rough guidelines and suggestions to be followed for phase 0 statistical designs. In general, the effect of the agent will be evaluated on the basis of a biological endpoint(s), and it would be desirable to have the endpoint defined as a dichotomous outcome, such as the achievement of substantial biological effect (or not). For a participant, the threshold for declaring a biological effect should satisfy both biological and statistical criteria. For a dose level, the statistical criteria for declaring an effect should be such that the overall significance level (across all dose levels taken together) should not exceed 0.10 in a one-sided test of significance. A target effect across all participants should be given, and the power to detect it should be estimated. Ideally, there will be pre-agent administration endpoint measurements per participant to facilitate comparison with post-agent administration endpoints.

Box 3 | **Accurate assessment of drug action might require two assays**



The measured levels of many biochemicals used as markers of drug action are determined by a balance between production and degradation, and are therefore highly dynamic. For example, if a drug targets a kinase and the level of a specific phosphorylated protein reaction product is used to quantify the drug effect, the counterbalancing action of the phosphatase(s) that reverses the reaction by removing the phosphate group from the product should also be taken into account. Therefore, the measured level of the phosphorylated protein is the net rate of its production by the drug target (kinase) and its degradation by the phosphatase. It also means that an identical degree of drug-induced target inhibition in different patients could manifest as different pharmacodynamic assay results depending on individual rates of product degradation relative to elapsed time between drug administration and tumour biopsy.

The very real and important ramification of this line of reasoning is that all pharmacodynamic studies using enzyme products to quantify drug action might in fact require the development and validation of two assays to reach accurate conclusions about drug activity or inactivity. For example, if one patient has high levels of kinase activity but low levels of phosphatase activity, the harmacodynamic assay for a kinase inhibitor might indicate that the patient is not a responder to the inhibitor, compared with a patient who has both high kinase and high phosphatase activity, when in fact both patients are responders.

Two examples follow. The threshold for declaring an effect for a particular participant could be defined so that the probability of falsely declaring an effect in the case of no true biological effect is no more than 0.05.

First, if 5 participants are assigned to each of 2 dose levels, a significant biological effect for a particular dose level could be defined as the observation of an effect in at least 2 of the 5 participants. This design gives an overall type I error rate of slightly less than 0.05 (the probability of observing an apparent effect for at least one dose level when neither has any true biological effect) and 91% power to detect a 60% likelihood of participant effect across all participants for either dose level. A variant of this design would be to stop at 3 participants, for either dose level, if at least 2 patients have shown an effect (declare early success) or if no patient has shown an effect (declare early failure). This variant gives an overall type I error rate of 0.04 and 89% power to detect a 60% likelihood of participant effect across all participants for either dose level.

Second, if 10 participants are accrued to a single dose level, an effect for that dose level could be defined as the observation of an effect for at least 2 of the 10 participants. This design gives a type I error rate of 0.09 (the probability of observing an apparent effect when the dose level has no true biological effect), and has 91% power to detect a 35% likelihood of participant effect across all participants for the dose level. A variant of this design would be to stop at 5 participants if no patient has shown an effect (declare early failure) or if at least 2 patients have shown an effect (declare early success). This variant gives a type I error rate of 0.07 and 85% power to detect a 35% likelihood of participant effect across all participants for the dose level.

Targeting likelihoods of participant effect of 35%–60%, as in the above examples, might seem optimistic in first-in-human studies, where the dose levels are necessarily kept low to avoid toxicity. However, part of the rationale for doing phase 0 studies is the realization that new agents are being developed that promise significant biological effects in substantial percentages of participants at doses well below the levels expected to result in toxicity, based on preclinical results. Agents that do not seem to satisfy these criteria might not be suitable candidates for phase 0 trials. As is the case for other types of clinical trials, because there can be various circumstances that would create a need to modify the designs above, it is imperative that a statistician be consulted about the study design for phase 0 trials.

Conclusion

Phase 0 trials might provide a mechanism to compress the timeline for anticancer drug development at the same time as ensuring patient safety. Such trials could provide a platform to qualify and show the feasibility of assays for target modulation in human samples, to establish SOPs for tissue acquisition and handling, to obtain preliminary pharmacokinetic data, including bioavailability, and to evaluate biomarkers for determining drug effects in human tissues. Crucial data obtained from phase 0 trials can inform and expedite subsequent trials with the goal of eliminating therapeutic failures early in the cancer-drug development process.

Conducting phase 0 trials requires significant resources, and involves specific logistical, ethical and scientific considerations that need to be carefully addressed before study initiation. It is fair to state that there are limitations in preclinical animal models, and some of these will restrict the value of phase 0 trials. Nonetheless, as preclinical models improve, the value of phase 0 and other early clinical trials should improve as a consequence. The non-therapeutic nature of such trials might make reimbursement from third-party payers difficult to anticipate. Phase 0 trials should be considered for drugs that are relatively non-toxic in preclinical models and for which reliable pharmacokinetic and pharmacodynamic assays can be developed. Phase 0 trials should not be considered for drugs for which there is a lack of understanding of the mechanism of action and/or no reliable pharmacodynamic assays; the establishment of the MTD should remain the objective in early-phase trials of such agents. Therefore, phase 0 trials, in particular settings, provide an opportunity to substantively expedite and better inform the development of an oncological therapeutic, but are not obligatory before the initiation of phase 1 and 2 studies.

The complexity of optimally evaluating molecularly targeted agents requires that we re-assess our current drug-development paradigm to integrate pharmacodynamic assays, including molecular imaging, into early clinical investigations. This might require investing more resources in the early drug-development process, but could ultimately enable more rational drug selection and a more expeditious evaluation of promising anticancer therapies. As the concept of the

phase 0 trial is widely evaluated, we expect that pharmacodynamic-driven early therapeutic studies, as was the case for pharmacokinetic monitoring nearly 20 years ago[21], will become a routine part of future early-phase oncological drug development.

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Competing interests statement

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TUMOUR MICROENVIRONMENT — OPINION

The tumour microenvironment as a target for chemoprevention

Adriana Albini and Michael B. Sporn

Abstract | New data indicate that primary dysfunction in the tumour microenvironment, in addition to epithelial dysfunction, can be crucial for carcinogenesis. These recent findings make a compelling case for targeting the microenvironment for cancer chemoprevention. We review new insights into the pathophysiology of the microenvironment and new approaches to control it with chemopreventive agents. The microenvironment of a cancer is an integral part of its anatomy and physiology, and functionally, one cannot totally dissociate this microenvironment from what have traditionally been called 'cancer cells'. Finally, we make suggestions for more effective clinical implementation of this knowledge in preventive strategies.

The continuing pandemic of cancer deaths requires a reassessment of our basic assumptions about the nature of cancer and how to control it. Directly bearing on this, there has been an explosion of new information about the tumour microenvironment, which is a complex system of many cell types, including endothelial cells and their precursors, pericytes, smooth-muscle cells, fibroblasts of various phenotypes, myofibroblasts, neutrophils and other granulocytes (eosinophils and basophils), mast cells, T, B and natural killer lymphocytes, and antigen-presenting cells such as macrophages and dendritic cells. All these cells can participate in tumour progression. If the process of carcinogenesis and its end result, invasive and metastatic cancer, are viewed as the maladaptive response of an entire tissue or organ to both genetic and epigenetic stress[1–3], then knowledge and control of the immediate microenvironment within a developing tumour become as important as the corresponding knowledge and control of the dysfunctional epithelial cells within that tumour.

New data from studies on the tumour microenvironment suggest that we might need to revise the very definition of the term 'carcinoma' (currently defined in classical terms as a malignancy derived from epithelial cells), and that to control cancer in the future, we need to regard carcinogenesis and carcinomas as phenomena that occur in tissues, not in individual cancer cells. From this perspective, the microenvironment becomes an integral, essential part of the cancer.