CLINICAL INVESTIGATION

Pharmacodynamic end points in early-phase oncology trials

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Recent insights into cancer biology and the advent of molecularly targeted agents have presented exciting opportunities for the development of cancer therapeutics. However, excessive timelines for drug development, high costs that cannot be sustained and lack of translation of efficacy from preclinical models to humans, underscore the difficulties inherent in this effort. Optimal development of molecularly targeted agents requires an understanding of a specific drug–target interaction, which depends on many factors, such as the structural features of the target, its relationship to a broad array of signaling networks, as well as what the drug does to the target itself. Proof-of-mechanism clinical trials, with incorporation of validated biomarker assays early in the development process, can provide essential human tumor data to make go or no-go decisions early in the drug-development process. Pharmacodynamic data generated in early clinical trials process could potentially expedite development of promising candidates.

Keywords: biomarker assay • circulating tumor cells • molecularly targeted agent • pharmacodynamic • Phase I trial

Recent investments in cancer research have coincided with marketing approvals for seven new cancer therapies during the fiscal year 2011, a hallmark year for oncology drugs [101]. In 2012, vismodegib, an inhibitor of the Hedgehog pathway, was also approved by the US FDA for the treatment of metastatic basal cell carcinoma [102]. Unfortunately such successes are not typical; excessive timelines for drug development, high costs that cannot be sustained and a lack of translation of efficacy from preclinical models to humans, underscore the difficulties inherent in this effort.

Traditionally, Phase I clinical trials have served to evaluate the safety of new therapeutic agents. While this should remain a central tenet, the paucity of drugs gaining regulatory approval compared to the number entering clinical testing, and the high attrition rates in Phase II and III trials [1,2], suggest that the current process is inefficient. Increased understanding of the molecular features of cancer, along with development of new agents that target these features, has ushered in an era of molecularly targeted agents (MTA), which has necessitated the rethinking of conventional methods of drug development. Phase I clinical trials include the first introduction of a new drug or combination of drugs in humans and, as such, are pivotal to translating discoveries from laboratory to clinic. Thus, to improve the efficiency of oncologic drug-development today, a more thoughtful, rigorous approach incorporating suitable pharmacological end points early in clinical drug investigation is necessary to better select patients who may benefit from treatment [3–5].

R&D costs to bring new molecular entities to market reflect accounting procedures in which the costs of drugs that gain regulatory approval absorb

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the costs to develop those that are not successful [6,7]. Estimates using publically available data from a large number of pharmaceutical firms placed drug-development costs at US$1.214 billion in 2010 [8]. Such high costs may be difficult to sustain in the coming years. Furthermore, despite increased spending on R&D related activities compared to previous decades, there has been a decline in the actual number of new drug applications submitted to the US FDA [9,10].

Drug development from time of synthesis to marketing approval is a very lengthy process; with an estimated average of 7.4–7.8 years spent in clinical testing for drugs approved between 2000 and 2009 [11], a long time when one considers the very low 5-year survival rates for many cancers. Moreover, a greater percentage of antineoplastic drugs moved from Phase I to II testing and to much more expensive Phase III testing versus multiple other therapeutic categories, yet had a relatively low estimated probability (55%) of having an application for marketing approval submitted to the FDA. In contrast, anti-infective, musculoskeletal and respiratory drug categories have had relatively high estimated probabilities of reaching regulatory review after they had entered Phase III (79% or higher) [12]. Major reasons for poor success rates in 2000 were lack of efficacy and lack of safety [1], indicating that a major hurdle in oncologic clinical drug testing is our inability to predict the ultimate success of a novel candidate drug. Although efforts are underway to develop more predictive preclinical models for oncologic disease, there is a lack of early clinical end points that correlate with clinical benefit and there remains a need to carry out proof-of-concept (mechanism) clinical trials early in the development process to provide human data to guide further clinical development.

Rethinking the drug-development plan

■ Development of MTA

Cancer drug development focuses on agents that specifically interfere with molecular characteristics that define aberrant growth, such as oncogene-based proliferation, invasion and metastasis, angiogenesis and dysregulated apoptosis [13]. At least 37% of Phase I oncology trials reported in the literature between 1997 and 2009 involved a MTA, either as a single agent or in a combination [14], and this number is likely have increased since then.

Cytotoxic agents are drugs that result in cell death, eventual tumor shrinkage and are generally not specific, affecting all rapidly dividing cells. Early trial designs of cytotoxic drugs focus on toxicity and determining the dose recommended for Phase II trials based on the maximum tolerated dose determined in Phase I. The inherent assumption is that toxicity and therapeutic efficacy occur by a common mechanism of action and that higher doses result in greater efficacy [15]. In addition, it is common practice to use objective response as measured by static imaging criteria (e.g., Response Evaluation Criteria in Solid Tumors) in Phase II trials of cytotoxics as a signal of activity warranting further clinical evaluation. El-Maraghi and Eisenhauer found that objective response correlated with successful marketing approval for targeted drugs, supporting the use of objective-response rate to assess the antitumor activity of MTAs in early-phase trials [16]. However, some MTAs may have a predominantly growth-inhibiting effect with minimal tumor shrinkage, as assessed by time-to-progression or progression-free-survival end points [17]. Thus, in early-phase trials, functional imaging strategies, rather than anatomic strategies, may provide early evidence of an antitumor effect [18].

The traditional Phase I objective of establishing the maximum tolerated dose is less frequently achieved in trials of MTAs alone as compared with trials using cytotoxic agents [14]. Establishing the optimal biologic dose based on pharmacodynamic (PD) effects of the drug on the target in the tumor has been proposed as an alternate primary end point for early-phase trials of MTAs [2,19].

Since the efficacy of many MTAs hinges on their ability to inhibit a specific biochemical pathway, incorporation of mechanism-based biomarker assays in early-phase trials to determine if a drug 'hits' its target may expedite drug development. Such information, derived from early-phase clinical trials, could help select a lead agent from a group of compounds, determine optimal dose and schedule, and guide patient selection [20,21]. Despite the potential benefits of assessing target engagement in early drug development, the use of biomarkers in early-phase trials has not been widespread [19,22].

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or a pharmacologic response to a therapeutic intervention [23]. In cancer, several types of biomarkers are of importance and have been further defined [24]. Prognostic biomarkers provide evidence that may be important for the patient's overall disease outcome, independent of any specific intervention. Predictive biomarkers can reflect the probability of benefit or toxicity from a specific intervention. Surrogate biomarkers are a subset of biomarkers that are intended to serve as a substitute for a clinically meaningful end point.

Patient selection for treatment with a MTA based on the presence of a specific predictive biomarker has the advantage of reducing the risk of missing a beneficial therapeutic effect by treating a larger unselected population. Successful development of agents likely to benefit specific patient subsets depends on discovering the right patient population to treat in early trials of such agents. For example, the efficacy of crizotinib therapy in patients with advanced non-small-cell lung cancer (NSCLC) whose tumors harbor a specific type of alteration in the *ALK* gene [25,26] and vemurafenib for patients with melanoma who have the *V600E BRAF* gene mutation [27], might have been overlooked in trials of those drugs in larger, unselected groups of patients with NSCLC or melanoma. In particular, crizotinib was initially developed to target c-MET, but fortuitously was also known to inhibit ALK, which prompted rapid development of a diagnostic assay to screen NSCLC tumors for *ALK* rearrangement, permitting the study of crizotinib in clinically enriched populations. Both crizotinib and vemurafenib were approved by the FDA concurrently with their respective companion diagnostic genetic tests [101]. Thus, identifying patients who are likely to benefit from therapies by incorporating molecular and genomic information into early-stage clinical trials is one approach that could speed the clinical development of effective therapies.

PD biomarkers establish a direct pharmacological effect of a drug on the body and can be used to measure target modulation by drugs and assess for both the drug-related antitumor effects and potential for toxicity. It is important to emphasize that PD markers may not always correlate with clinical benefit, depending on the markers evaluated. For the purposes of this article, the discussion will be focused on the incorporation of PD biomarkers for assessment of drug effects on target into early-phase trials. Establishing drug effects on target (PD) is essential for the optimal evaluation of MTAs and should form the basis for strategies pursued for further development of such agents. The potential applications of PD biomarkers include: to provide proof-of-mechanism of action of a drug; to select the optimal dose and schedule of administration of the drug, when combined with pharmacokinetics (PK) and toxicity evaluations; to increase understanding of response and resistance mechanisms; to design rational combination therapies; and to make clinical development decisions (go, no-go). In order to make the best informed decision for the latter, extensive evaluation in preclinical models is required, demonstrating an association between desired target modulation and antitumor activity at achievable drug plasma levels, using validated PK and PD assays [28]. Thus, if in an early-phase clinical trial the desired PD effect is observed, further clinical development of the agent is warranted. However, if in spite of achieving adequate plasma exposures, the PD effect is not observed, further clinical development should be stopped with re-evaluation of the preclinical and clinical data. A decision could be made to still pursue clinical development of the agent with a broader assessment of drug effect on target (primary and downstream effects) or to switch to another analogue for clinical development. In all instances, for the PD biomarker result to inform and expedite drug-development decisions, the assay must be validated in preclinical models and PK–PD relationships along with associations with antitumor effects need to be established in preclinical models prior to initiating clinical trials. Standard operating procedures for sample handling, storage and processing of preclinical samples developed using methods that replicate the procedures used in the clinical setting to ensure clinically relevant results are essential [29]. Furthermore, as many biomarker-driven studies may involve invasive procedures such as biopsies, or multiple procedures in addition to drug administration, it is important to verify and assure patient understanding prior to the start of the clinical trial, to ensure that the study experience is commensurate with patient expectations [30].

Pharmacodynamic end points in early-phase trials

Pharmacodynamic end points in Phase I clinical trials should elucidate whether a new drug affects its intended target and the eventual consequences of target engagement. Assessing target inhibition in tumor tissue, rather than a surrogate tissue, is the standard since tumor is the ultimate tissue of interest. However, even tumor tissue can pose significant challenges in data interpretation. For example, considerable heterogeneity in gene expression exists between different regions of a single tumor [31], necessitating caution in interpretation of drug activity if studying drug effect on a focused group of biomarkers, as biomarker response will not be uniform across all sample sites [32]. Nonetheless, pre- and post-treatment biopsies can provide information about target inhibition and can be used to evaluate mechanisms of resistance. However, obtaining multiple tumor biopsies presents numerous considerations, such as patient risk, the ethics of research biopsies, the limited amount of tissue obtained, the limited number of time points possible for sampling and tumor heterogeneity [33,34]. Less invasive methods, such as functional imaging, or use of circulating tumor cells (CTCs), are being explored as alternates to tumor biopsy.

However, before an alternate tissue or imaging can be established as a true surrogate and is used to guide drug-development decisions, initial trials should establish the correlation between effects on the target in the tumor as compared with the surrogate target. If a molecular effect is observed in a surrogate tissue but not the tumor, the effect may have been missed in the tumor due to the kinetics of target-engagement versus tumor-sampling time points; it may represent a difference in drug exposure (for instance, CTCs and peripheral blood mononuclear cells are exposed to plasma concentrations of an agent while drug delivery may be an issue for large tumor lesions); or the effect of a drug on its target in tumor tissue may differ with that in non-malignant tissues. A differential effect of drug on tumor versus potential surrogate tissue was observed during a Phase II study of gefitinib in breast cancer, where PD end points were examined in tumor and skin [35]. Although inhibition of EGFR phosphorylation in skin with anti-EGFR agents had been reported [36], a correlation between EGFR inhibition in skin and tumor tissues, or effects downstream of EGFR inhibition in tumor tissues, had not been established. In the Phase II study, immunohistochemistry analyses of serial biopsies demonstrated good target inhibition in both tissues, including inhibition of EGFR and MAPK phosphorylation levels; however, effects downstream of MAPK differed. In skin, induction of p27 and decrease in Ki-67 was observed following treatment with gefitinib, while this was not observed in tumor biopsies. Thus, lack of gefitinib therapeutic activity in breast cancer may not have been due to lack of receptor inhibition, but rather, the differential downstream effects of inhibiting EGFR in tumor tissue. This trial highlights the importance of understanding the biology of the target and the consequences of target inhibition in tumor tissue before basing decisions on data generated from analysis of surrogate tissues.

The complexity of cellular signaling processes makes it difficult to define the optimum degree and duration of target inhibition that might predict an antitumor effect. Since it is essential that PD data are obtained early in the clinical trials process to inform and expedite the subsequent development of a promising agent (or prevent pursuit of an ineffective agent), considerable thought should be given to the operational characteristics of any PD marker prior to the initiation of clinical development. Developing a detailed understanding of the meaning of a specific drug–target interaction will depend on many factors, such as the structural features of the target, its relationship to a broad array of signaling networks, as well as what the drug does to the target itself. For example, ATP competitive [37,38] and allosteric inhibitors of AKT [39] both act to inhibit the PI3K/AKT pathway; however, their different mechanisms of target inhibition should be reflected in the PD analyses used to assess target inhibition. Since allosteric AKT inhibitors block the recruitment of AKT to the membrane where it is normally phosphorylated and activated, loss of AKT phosphorylation serves as a pharmacodynamic measure of target inhibition [40]. By contrast, AKT catalytic site inhibitors may actually increase AKT phosphorylation (although nonfunctionally) through loss of negative-feedback regulation of PI3K [37]. Therefore, for catalytic-site inhibitors, one would need to assess the loss of phosphorylation of downstream AKT substrates such as PRAS40, GSK3 or forkhead box transcription factors [41].

One target for drug development that has been the focus of much recent attention is the poly(ADP–ribose) polymerase (PARP) family of enzymes [42]. PARP enzymes are involved in the recognition and repair of DNA damage, and efforts to develop PARP inhibitors in combination with cytotoxic agents [43–46] are underway, based on the hypothesis that PARP inhibition could enhance the antitumor activity of DNA-damaging agents. To help determine early in the course of treatment whether an investigational PARP inhibitor was inhibiting its target, the US National Cancer Institute (NCI) developed two PD biomarkers using clinically relevant animal models. Poly(ADP-ribose) (PAR), is the product of PARP enzymatic activity and can be measured in clinical samples to determine the effect of PARP inhibitors. In addition, phosphorylation of histone H2AX (γH2AX) induced by DNA double-strand breaks can be detected by an antibody to measure DNA damage before and after patient treatment with DNA-damaging drugs [47]. Using procedures relevant to human tissue collection and processing in mouse xenograft models, a validated, quantitative chemiluminescent assay to measure levels of PAR [48] and immunofluorescent assay to measure γH2AX [29] were developed. These assays were successfully used to support a clinical trial of the PARP inhibitor veliparib in combination with topotecan [46], which demonstrated a reduction of >75% in PAR levels in post-treatment tumor biopsies. The expected downstream consequence of increased γH2AX was not detected in the two paired-tumor biopsies studied; however, an increase in γH2AX levels was observed in CTCs, obtained by Veridex's CellSearch™, following treatment. Thus, the target was effectively inhibited with the expected consequence shown as enhanced DNA damage. Since serial post-treatment biopsies at multiple time points in patients is not feasible, the use of CTCs offers a relatively noninvasive method of repeated sampling over time, allowing assessment of biomarkers that may fluctuate over time.

Where possible, the optimal extent and duration of target inhibition associated with antitumor activity should be quantified in preclinical studies to aid in determining the dose and schedule of an agent, since the levels and patterns of inhibition may be different between targets and diseases. For example, in patients with melanoma treated with vemurafenib who had tumor regressions, pathway analysis typically showed >80% inhibition of cytoplasmic ERK phosphorylation [49]. Patients treated with an earlier drug formulation that led to lower peak-drug levels achieved <80% inhibition of pERK, and did not experience objective remissions. Coupled with the agent's long serum half-life (57 h), this observation suggests that near-complete inhibition of ERK signaling may be needed for significant tumor response [49]. On the other hand, the clinical success of once-daily dasatinib, coupled with its short serum half-life (3–5 h), suggests that intermittent inhibition of BCR–ABL kinase activity is sufficient for clinical response [50]. In patients treated on early-phase clinical trials, an effort should be made to obtain mandatory pre- and post-treatment tumor biopsies using preclinically validated assays and standardized tissue handling and storage procedures.

A related consideration for optimal PD-marker development is baseline variation in the outcome measure under study, which needs to be appropriately characterized if the outcome is to be interpreted with confidence. For example, intra- and inter-individual variation in baseline PAR levels in peripheral blood mononuclear cells was observed when measured over time [51]. This suggested that decreases in PAR following administration of a PARP inhibitor must be interpreted with respect to the natural variation of the analyte. Therefore, in a Phase 0 trial of veliparib, peripheral blood mononuclear cells were sampled at three time points during the week prior to veliparib administration to establish a baseline for each patient and at least 50% inhibition of PAR levels was required to demonstrate a significant PD response [52,53].

Changes in markers of interest caused by sampling, handling and storage procedures are another set of challenges inherent in PD-marker development. Standards for these procedures must be established in preclinical models prior to the use of the markers in clinical trials and the availability of high-quality human biospecimens must be assured [103]. For example, HIF1α is an important marker of hypoxia in human tumors and could be of great interest to study responses in tumors to certain anticancer therapeutics, such as antiangiogenic agents. However, an important limitation is the lability of HIF1α protein in the presence of oxygen. Standard procedures for sample collection and storage utilizing degassed buffers has been developed by the NCI to optimize the yield of HIF1α from tumor samples, allowing assessment of this important marker [54].

■ Minimally invasive functional & molecular imaging

Given the variability between primary tumors and metastases, and the heterogeneity within a tumor, the ability to noninvasively image a specific molecular characteristic in multiple tumor deposits at the same time would be highly desirable. Efforts aimed at developing targeted molecular imaging methods have resulted in valuable, noninvasive tools that may accelerate development of cancer therapies [55] and should be incorporated into early-phase trials when available. Evidence of target engagement and pharmacodynamic effect are central to successful proof-of-mechanism testing in the clinic. As such, molecular imaging can confirm whether the tumor expresses the target, if the drug reaches the target, if the function of the target protein is modulated and if this results in the expected biological effect. For example, digital contrast-enhanced MRI can be used to assess antiangiogenic therapies [56], such as anti-VEGF antibodies or VEGF receptor tyrosine kinase inhibitors, VEGF-Trap, and vascular disrupting agents [57,58]. VEGF increases vascular permeability and perfusion, therefore, changes in the parameters measured by digital contrast-enhanced MRI, such as Ktrans, can serve as biomarkers of response of the tumor vasculature to antiangiogenic agents [56,57,59]. However, it must be noted that such changes do not necessarily correlate with clinical benefit, as observed in the development of vatalanib (PTK787), an oral inhibitor of the VEGF receptor, in colorectal cancer [60,61].

Another promising area in molecular imaging is the use of positron emission tomography (PET) with functional imaging probes to visualize molecular targets and processes [62]. Most commonly, PET is used for assessment of proliferation with 1F-18 fluorodeoxyglucose PET; early response determination and prediction of outcome for signal transduction inhibitors, such as for patients with gastrointestinal stromal tumors treated with imatinib [18]. PET scanning can also be used with a range of radiolabeled tracers and provide proof-of-principle for the proposed mechanism of action of novel therapeutics under investigation. For instance,

16-α-^{18}F-fluoro-17-β-estradiol (^{18}FES) PET has been used to identify estrogen receptor expression [63,64] and baseline tumor ^{18}FES uptake has been shown to be predictive of responsiveness to endocrine therapy in estrogen receptor-positive breast cancer [65,66]. Linden and colleagues studied 47 heavily pretreated patients with recurrent breast cancer whose primary tumors were all initially estrogen receptor-positive [66]. Initial ^{18}FES uptake was measured and correlated with subsequent tumor response following 6 months of hormonal treatment. Objective response was documented in 23% of patients, but specifically, in none of the patients lacking ^{18}FES uptake. Thus, quantitative ^{18}FES uptake can be used to identify patients with estrogen receptor expression at the time of treatment and is predictive of response to antihormonal treatment. ^{18}FES PET is being evaluated at the NCI in an ongoing Phase I trial of endoxifen (the active metabolite of tamoxifen), to establish proof-of-mechanism by demonstrating a decrease in ^{18}FES uptake following target engagement by endoxifen [104].

Imaging of biological changes in response to target inhibition serves as a PD biomarker for the interaction between the drug and the target. HSP90 is a molecular chaperone, which is upregulated in cancer; its inhibition downregulates the expression of many oncogenic proteins, including HER2. Thus, imaging of HER2 downregulation would serve as a potential biomarker for early response to HSP90-targeted therapies. In HER2-expressing tumor xenografts, radiolabeled trastuzumab PET scans, ^{68}Ga-DOTA-F(ab′)(2)-herceptin [67] and ^{89}Zr-trastuzumab [68] injected before and after treatment with an HSP90 inhibitor were utilized to detect HER2 downregulation. In both cases, a significant reduction was noted 24 h after treatment. In comparison, ^{18}F-FDG PET uptake was unchanged when a significant decrease in HER2 was measured by ^{68}Ga-DOTA-F(ab′)(2)-herceptin PET; marked tumor growth inhibition was noted 11 days post-treatment [67], making HER2 PET scan an attractive functional imaging modality to explore for both noninvasive evaluation of the PD effect of anti-HER2 therapies as well as an early predictor of response to anti-HSP90 therapies. Optical imaging technologies based on the detection of light (bioluminescence, fluorescence, near-infrared imaging and multispectral imaging) can also be used in preclinical studies [69], to study various aspects of cancer biology including response to therapeutics, such as the vascular response to bevacizumab [70]. Despite the attractiveness of noninvasive imaging modalities, limitations to their wider application including the resource intense and costly nature of such studies, varying expertise in different institutions, lack of standardization between imaging hardware and lack of defined response criteria for a given imaging biomarker, makes comparison difficult between institutions and widespread adoption extremely challenging.

■ CTCs

CTCs are rare cancer cells shed from either a primary tumor or metastases, which can be identified in the peripheral blood. Due to the relatively noninvasive nature of blood sampling, multiple time points can be explored for longitudinal target assessments using CTCs and sample handling can be standardized across institutions.

The number of CTCs and changes due to treatment is prognostic for progression-free and overall survival in several types of cancer, including breast, colorectal and prostate [71–73]. The Veridex's CellSearch Epithelial Cell Kit/CellSpotter™ Analyzer is approved by the FDA for monitoring or predicting cancer disease progression, response to therapy and for the detection of recurrent disease when used to enumerate CTCs of epithelial origin [105].

In the development of novel cancer therapeutics, CTCs can be examined for genetic characterization or assessment of the presence of a target at the time of treatment, which could improve patient selection for early-phase trials. Currently, archival tissue is most often used to determine whether a target needed for patient selection is present, despite well-described differences in target expression between primary and metastatic sites. In one study, which evaluated concordance in HER2-gene amplification status by FISH between primary tissue and CTCs in 42 CTC-positive breast cancer patients, it was found that in 9 patients (21%) the primary tumor was HER2-negative, but the CTCs had acquired *HER2* gene amplification [74]. This resulted in treatment with trastuzumab in four patients, with clinical benefit in three. PD effects can also be observed in CTCs in early-phase trials. However, the successful utilization of CTCs to determine PD effects has certain limitations. Cell yields are generally low (usually <10 cells/7.5 ml of blood [46]) and the number of CTCs varies widely between refractory cancer patients. Recent data also suggest that the CTCs of interest may be the ones that have associated stromal cells, providing an advantage to establish and grow new metastases [75]. In addition, EpCAM-based capture techniques for CTCs may not yield cells with more basal-like features. In a study reported by Punnoose *et al.*, CTC counts were higher in estrogen receptor-positive breast cancer patients compared to HER2-positive and triple-negative patients, with the latter having low EpCAM expression and a more mesenchymal phenotype [76]. Thus, utilization of antibodies for mesenchymal markers could further improve CTC capture efficiency for routine biomarker analysis [64]. CTCs have been examined for markers such as HER2 status, *KRAS* mutation detection, and EGFR staining by immunofluorescence. CTCs have also been utilized to evaluate drug effects, such as drug-induced DNA damage, through detection of γH2AX in individual CTCs following treatment with veliparib and topotecan [77].

Executive summary

Introduction

- Developing an anticancer drug is, on average, one of the more expensive drug development projects (~US$1.04 billion), takes the longest to complete amongst therapeutic categories and is frequently hindered by a lack of predictive preclinical models.

Rethinking the drug-development plan

- A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or a pharmacologic response to a therapeutic intervention.
- Establishing drug exposure (pharmacokinetics) and effects on target (pharmacodynamics) in patients is essential for the optimal evaluation of molecularly targeted agents.
- Assessing target inhibition in tumor tissue is the standard; however, obtaining multiple tumor biopsies presents numerous obstacles, such as patient risk, the ethics of research biopsies, the limited amount of tissue obtained, the limited number of time points possible for sampling and tumor heterogeneity. Less invasive methods, such as functional imaging or use of circulating tumor cells, are being explored as alternates to tumor biopsy.

Pharmacodynamic end points in early-phase trials

- Challenges in assessing pharmacodynamic effects include determining the degree and duration of target inhibition required for antitumor activity, quantifying the baseline variation in the outcome measure and establishing standardized sample handling and processing procedure; all of which should be established prior to initiation of clinical trials.

Future perspective

The ultimate goal of cancer drug development is to discover treatments that will benefit a large proportion of a specific patient population. Discerning a link between drug treatment and an observed biological response in tumor tissue should provide critical data required to make informed decisions in the future. A critical step in the process is the development of reliable assays to accurately measure PD effects in humans. This effort requires allocation of resources to earlier phases of drug development and a multidisplinary team with close collaboration between laboratory and clinical scientists at academic institutions and pharmaceutical companies.

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