Pharmacodynamic Biomarkers: Falling Short of the Mark?

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Abstract

In recent years, the clinical development of targeted therapies has been advanced by a greater understanding of tumor biology and genomics. Nonetheless, drug development remains a slow and costly process. An additional challenge is that targeted therapies may benefit only a subset of patients treated—typically those patients whose tumors are dependent on the target of interest. Thus, there is a growing need for the incorporation of both predictive and pharmacodynamic (PD) biomarkers in drug development. Predictive biomarkers are important to help guide patient selection, while PD biomarkers can provide information on the pharmacologic effects of a drug on its target. PD studies may provide insights into proof of mechanism (i.e., Does the agent hit its intended target?) and proof of concept (i.e., Does hitting the drug target result in the desired biologic effect?). PD studies may also provide information on the optimal biologic dosing or scheduling of a targeted agent. Herein, we review PD endpoints in the context of targeted drug development in non–small cell lung cancer, highlighting some of the key challenges encountered to date. In doing so, we discuss recent experiences with repeat tumor biopsies, surrogate tissue analysis, alternative clinical trial designs (e.g., window-of-opportunity trials), circulating biomarkers, and mechanism-based toxicity assessments. The application of such technologies and biomarkers in early clinical trials may facilitate rational drug development, while enhancing our understanding of why certain targeted therapies succeed or fail.

See all articles in this *CCR Focus* section, "Progress in Pharmacodynamic Endpoints."

Clin Cancer Res; 20(10); 2587–94. ©2014 AACR.

Introduction

The field of oncology has been transformed by new insights into the molecular biology of cancer. This improved understanding of the genomic and cell biology landscape driving cancer has placed greater emphasis on exploiting such characteristics through the use of targeted therapies. To date, targeted therapies have reshaped the management of chronic myeloid leukemia, BRAF-mutant melanoma, HER2-amplified breast cancer, certain thyroid cancers, and non–small cell lung cancers (NSCLC) harboring EGF receptor (*EGFR*) mutations and anaplastic lymphoma kinase (*ALK*) rearrangements. Despite these well-profiled successes, barriers remain for targeted drug development. In particular, it has become increasingly clear that targeted therapies may benefit only specific subgroups of patients. Moreover, among initial responders, resistance can develop over time. Thus, as the number of targeted agents in clinical development continues to grow, biomarkers have become increasingly important to guide patient selection, ensure target inhibition, and understand mechanisms of resistance.

The use of biomarkers in phase I trials has increased over time (1). A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (2)." Prognostic biomarkers provide information about disease course independent of treatment, whereas predictive biomarkers indicate the likelihood of response to a given therapy. Pharmacodynamic (PD) biomarkers provide information about the pharmacologic effects of a drug on its target. In the targeted therapy era, PD endpoints often assess whether a given agent is engaging its molecular target in the expected manner. PD markers can provide information about: (i) proof of mechanism (i.e., Does the drug hit its intended target?), (ii) proof of concept (i.e., Does hitting the drug target alter the biology of the tumor?), (iii) selection of optimal biologic dosing, and (iv) understanding response/resistance mechanisms.

This review focuses on PD biomarkers in the development of targeted therapies. We use NSCLC as a paradigm to examine these issues. Despite widespread use of targeted therapies and predictive biomarkers in NSCLC, PD biomarker studies have generally been lacking. We therefore aim to illustrate the challenges and potential alternative approaches for the incorporation of PD endpoints into clinical trials. Specifically, we review repeat tumor biopsies, surrogate tissue sampling, window-of-opportunity trials, circulating biomarkers, and on-target toxicity-based

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doi: 10.1158/1078-0432.CCR-13-3132

assessments. Other areas, such as genome-wide association studies and imaging as PD tools, will be discussed elsewhere in this *CCR Focus* section (3–6).

Predictive Biomarkers in NSCLC: An Overview

Historically, systemic chemotherapy has been the mainstay of treatment for advanced NSCLC. In 2004, however, activating *EGFR* mutations were found in a subset of NSCLC patients, ushering in a new era of molecularly targeted therapies (7). Subsequently, large-scale genotyping efforts have revealed that ~50% of lung adenocarcinomas possess genetic alterations in oncogenic drivers—genes that are critical for cell growth and survival (Fig. 1; ref 8). Several of these genetic alterations, such as *EGFR* mutations and *ALK* rearrangements, are predictive markers for sensitivity to treatment with genotype-specific tyrosine kinase inhibitors (TKI; refs. 7 and 9). Still other genetic alterations may serve as negative predictive biomarkers, such as *KRAS* mutations or the *EGFR* T790M gatekeeper mutation, which are associated with lack of responsiveness to EGFR inhibitors (10, 11).

EGFR mutations are identified in 10% to 30% of patients with NSCLC. These mutations are more commonly found among female patients and those with no or minimal smoking history, East Asian ethnicity, and adenocarcinoma histology (12). *EGFR* mutations confer sensitivity to treatment with EGFR TKIs. In randomized trials, EGFR-mutant patients treated with EGFR TKIs experienced high objective response rates (ORR) and significant improvements in progression-free survival (PFS) compared with the first-line chemotherapy (13).

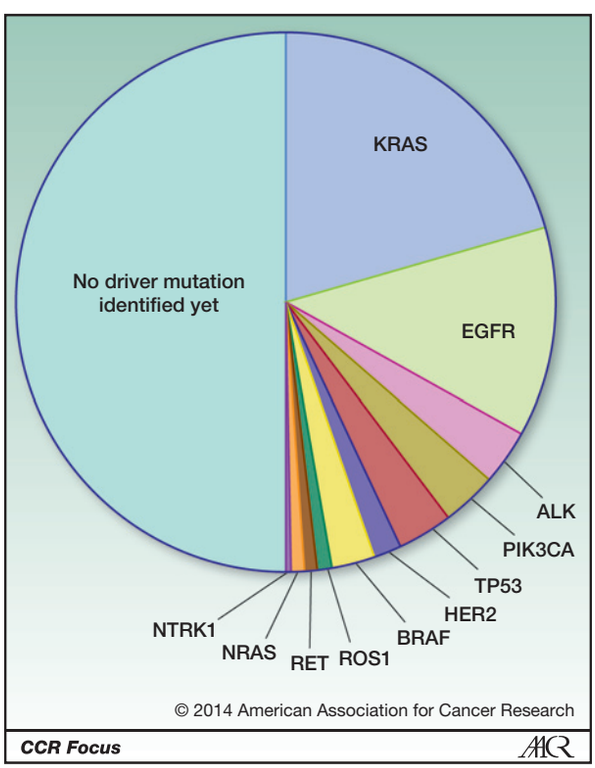

Figure 1. Distribution of oncogenic driver mutations in lung adenocarcinomas. Frequencies have been adapted from prior reports (8, 15).

Like *EGFR* mutations, *ALK* rearrangements have emerged as powerful predictive biomarkers in NSCLC (14). *ALK* rearrangements are identified in 4% to 6% of patients with NSCLC and are associated with sensitivity to treatment with the ALK TKI crizotinib (9, 14, 15). In single-arm studies, crizotinib was associated with high ORRs in ALK-positive patients (9). Crizotinib has also been found to significantly prolong PFS compared with standard single-agent chemotherapy administered in the second-line setting (16). As a result, clinical testing for *EGFR* mutations and *ALK* rearrangements is now recommended for all patients with advanced lung adenocarcinomas.

Pharmacodynamic Biomarkers

As clinical trials are designed for increasingly smaller, molecularly defined patient populations, PD biomarkers are likely to grow in importance. In particular, PD studies may provide insights into optimal biologic dosing, toxicity, and proof of mechanism for targeted therapies.

Serial tumor biopsies

Ideally, PD studies are performed directly on tumor specimens, allowing investigators to evaluate the functional and molecular effects of targeted agents within the tissue of interest. As illustrated in Table 1, PD endpoints may include assessments of protein phosphorylation markers, measures of cellular proliferation/apoptosis, cell-cycle regulation biomarkers, and epigenetic changes (17–26).

One illustrative example of the potential usefulness of PD evaluations can be found outside of NSCLC—in early trials of vemurafenib in BRAF-mutant melanoma (24, 27). In these studies, subsets of patients underwent serial tumor biopsies to assess for changes in mitogen-activated protein kinase (MAPK) signaling. Vemurafenib-treated patients exhibited reductions in tumor levels of phosphorylated extracellular signal-related kinase (p-ERK) as well as decreased tumor cell proliferation, as assessed by reductions in cyclin D1 and Ki67. Taken together, these studies helped establish that the predominant PD effect of vemurafenib is through on-target inhibition of MAPK signaling. These studies did not pursue dose-related questions such as what is the optimal biologic dose or the minimal effective dose of the drug.

In NSCLC, clinical trials with serial biopsies to assess PD endpoints have been limited. In one small phase II study, unselected patients with advanced NSCLC were treated with erlotinib after failure of first-line chemotherapy (28). In 14 patients, paired tumor biopsies were obtained before treatment and after 6 weeks of therapy. Significant reductions in p-EGFR and p-MAPK were observed following treatment; however, no objective responses were described. The authors concluded that treatment with erlotinib resulted in EGFR pathway inhibition (i.e., proof of mechanism), but

Table 1. Selected pharmacodynamic markers in tumor and surrogate tissues

		Examples in phase I/II trials		
Pharmacodynamic markers	**Description**	**Drug target**	**PD measurement(s)**	**Reference**
Protein phosphorylation markers	Evaluate changes in target protein phosphorylation or the activation status of downstream signaling/adapter molecules.	EGFR	p-EGFR, p-ERK	18
		MET	p-MET, p-FAK	17
		PI3K	p-AKT, p-PRAS40, p-4EBP1, p-S6	19
		MEK	p-ERK	20
		BRAF	p-ERK	27
		BCR–ABL	p-CRKL	21
Proliferation/apoptosis markers	Assess downstream events, such as changes in cellular proliferation (e.g., Ki67) or induction of apoptosis (e.g., TUNEL assay). Such assessments are generally not target specific.	—	Ki67	22
		—	TUNEL assay	17
			Nuclear caspase-3 cleavage	23
Cell-cycle regulation markers	Evaluate progression through the cell cycle. Such assessments are generally not target specific.	—	Cyclin D1	24
		—	p27	24
Epigenetic markers	Assess epigenetic modifications in response to therapy, such as DNA acetylation or demethylation.	HDAC	Acetylated histone 3	25
		DNA methyltransferase	*MAGE1A* CpG island methylation, 5-methyl-2′-deoxycytidine levels, HbF expression	26

Abbreviations: FAK, focal adhesion kinase; HbF, fetal hemoglobin; HDAC, histone deacetylase; MEK, mitogen-activated protein/extracellular signal-regulated kinase; p, phospho; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling.

that such inhibition was not sufficient to produce antitumor effects (i.e., lack of proof of concept). Importantly, only one of these patients harbored an activating *EGFR* mutation.

More commonly, sequential tumor biopsies have been performed in NSCLC to determine molecular markers of resistance to targeted therapies (Fig. 2). Repeat biopsies obtained at the time of disease progression on targeted therapies have been important tools for understanding acquired resistance (11, 29–31). In the case of *EGFR* mutations, for example, repeat biopsies have revealed that ~50% of patients with resistance to EGFR inhibitors harbor a secondary mutation, T790M, in the *EGFR* gatekeeper residue (11, 29). Based upon analyses of patient-derived biopsy specimens, additional mechanisms of EGFR TKI resistance have been identified, including *EGFR* amplification, *MET* amplification, *PIK3CA* mutations, *BRAF* mutations, *HER2* amplification, increased AXL expression, epithelial-to-mesenchymal transition, and transition to small-cell lung cancer (32).

Similar evaluations of repeat biopsy specimens have been performed in crizotinib-resistant, ALK-positive NSCLC. In these studies, resistance mutations in the *ALK* kinase domain (e.g., the gatekeeper mutation L1196M) have been identified in approximately 20% to 30% of patients (30, 31, 33). In addition to *ALK* resistance mutations, analysis of repeat biopsy specimens has revealed *ALK* gene amplification and upregulation of bypass signaling pathways as alternative mechanisms of resistance (30, 31). Bypass signaling pathways have included ligand-dependent activation of EGFR and *c-KIT* amplification (30).

Although tumor tissue is considered the gold standard for PD assessments, these studies may be limited by differences in drug exposure among patients and the potential for inter- and intratumoral heterogeneity. Moreover, serial biopsies may be difficult to obtain because of the invasiveness of these procedures as well as patient preferences, logistical constraints, and costs. Although studies have shown that clinical trial participants often consider rates of potential biopsy-related complications to be acceptable, nearly 50% believe that research-related biopsies may have treatment implications (34). This highlights the importance of ensuring appropriate informed consent and minimizing the risks of research biopsies. Moreover, there is a greater need for noninvasive biomarkers and improved technological platforms to allow comprehensive molecular analyses on smaller specimens, such as fine-needle aspirates.

Surrogate tissue biopsies

Certain malignancies (e.g., leukemia) are accessible to repeated samplings during therapy. In solid tumors, however, it is more difficult to collect serial biopsies. Investigators have therefore explored using normal tissues, such as skin, peripheral blood mononuclear cells, and plucked hair follicles, as potential surrogate biomarkers (Fig. 3). For targeted inhibitors, this requires the therapeutic target of interest to be highly expressed in normal tissue. Such PD studies may provide insights into target inhibition (i.e., proof of mechanism) and the time course/duration of such effects, but not necessarily proof of concept. Additional limitations of normal tissue as a surrogate biomarker include (i) differences in drug penetration between normal and tumor tissues; (ii) possible differences in gene expression between tissues (e.g., ALK is aberrantly expressed in ALK-rearranged NSCLC, but it is not expressed in normal skin); (iii) normal tissues lack somatic mutations in the oncogenic target; (iv) mutant enzymes in tumor tissue may lead to significant differences in drug sensitivity as compared with wild-type enzymes in normal tissue (e.g., BRAF, EGFR); and (v) there may be differences in signal transduction pathway regulation in tumors (e.g., oncogene addiction) as compared with normal cells.

Early clinical studies of EGFR TKIs relied on skin biopsies as surrogate markers for PD assessments (18). In these studies, skin biopsies revealed decreases in EGFR phosphorylation with treatment, indicative of target modulation. Despite this proof of mechanism, however, EGFR inhibitors were ultimately found to have only limited antitumor activity in unselected NSCLC populations (35). It is now recognized that wild-type EGFR and the mutant enzyme

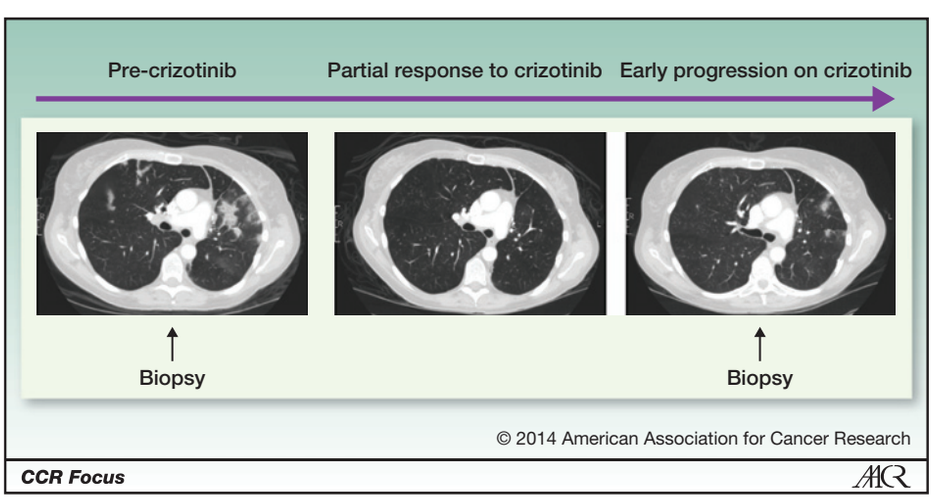

Figure 2. Sequential computed tomography images of a patient with ALK-positive lung cancer at baseline and following treatment with crizotinib, demonstrating an initial response to therapy followed by the development of acquired resistance and progression. A biopsy was performed at baseline for tumor genotyping, as well as at the time of tumor progression on crizotinib.

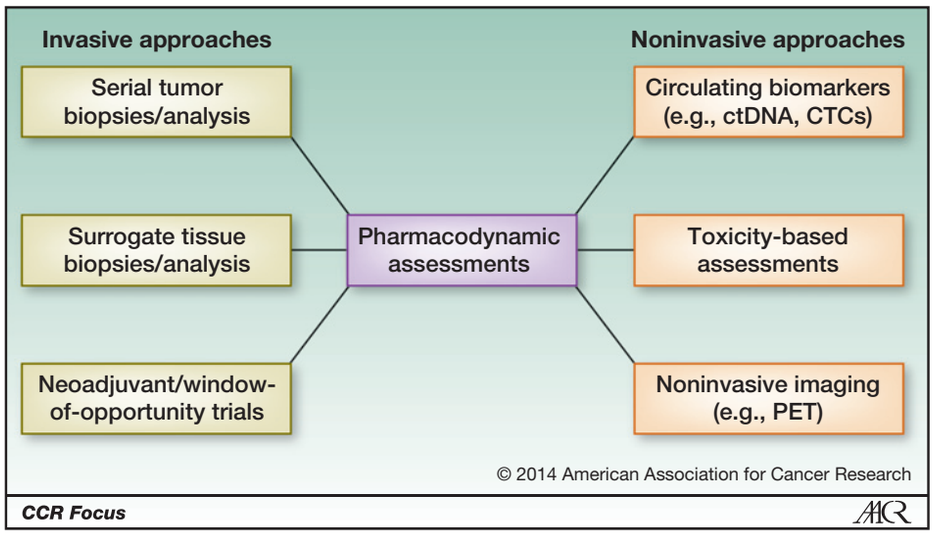

Figure 3. Depiction of various pharmacodynamic biomarker assessments, including tissue-based (invasive) approaches and noninvasive tools. Use of particular pharmacodynamic assessments may depend on the target of interest as well as the study disease. Assessments may also be complementary to one another.

differ in their sensitivity to EGFR inhibition. Moreover, tumors harboring *EGFR* mutations are more "addicted" to EGFR signaling. Thus, when targeted agents have greater potency against mutated forms of the target, PD studies conducted on surrogate tissues may provide misleading signals. Nevertheless, depending on the target of interest, surrogate tissue studies may allow investigators to confirm that an agent can produce target/pathway inhibition. These biopsies may also assess the time course of such inhibition, a first step toward effective antitumor use.

Window-of-opportunity studies

One alternative strategy to obtain tumor tissue for PD assessments is to pursue neoadjuvant or "window-of-opportunity" trials. In such studies, patients with early-stage disease are treated with a molecularly targeted therapy during a brief "window" period, after which surgical resection is performed. The resection specimen can then be examined for evidence of antitumor activity. Obviously, the disadvantage to such trials is that patients with early-stage disease may experience a delay in surgical resection—a potentially curative therapy. Also, to be of value, a significant response after a brief period of experimental therapy is needed to provide proof of concept. Some authors have therefore proposed requirements for window-of-opportunity trials (36), such as (i) the targeted therapy should have an adequate safety profile, (ii) the agent should have demonstrated preliminary activity in advanced disease, and (iii) there should be a strong rationale for use of the agent in the intended disease setting.

Although neoadjuvant studies have been pursued extensively in breast cancer as a path for drug development and approval, they have not been routinely used in NSCLC, perhaps because of a lack of validated surrogate endpoints (e.g., pathologic complete response) and the rarity of major responses in the brief period of treatment. Several studies evaluating the use of neoadjuvant EGFR TKIs have been conducted in early-stage NSCLC; however, such studies were generally performed in unselected patient populations (37, 38) and/or lacked PD assessments of target inhibition (37–39). Recently, a window-of-opportunity study was performed in patients with NSCLC with stage I/II disease who were treated with neoadjuvant pazopanib (36). This study included gene-expression profiling on pre- and posttreatment specimens, which revealed transcriptional changes in pazopanib target genes and other angiogenic factors (e.g., VEGFR-1, PDGFR-α) following treatment suggestive of on-target effects.

Circulating biomarkers

Given the challenges of obtaining serial tumor biopsies in patients with advanced cancer, efforts are ongoing to identify noninvasive biomarkers, such as circulating tumor cells (CTC) and circulating tumor DNA (ctDNA). Specifically, there is interest in using these "liquid biopsies" to provide information on prognosis, genetic determinants of response, dynamic tumor assessments, and monitoring of resistance (40, 41).

Studies in colorectal, prostate, and breast cancer have demonstrated that CTC levels are prognostic biomarkers (40). Preliminary analyses have also suggested that CTCs may be prognostic biomarkers in NSCLC (42), but data on CTCs as PD endpoints in lung cancer are limited. Krebs and colleagues reported on 101 patients with advanced NSCLC who had CTC levels measured at baseline and following one cycle of cytotoxic chemotherapy (42). Patients were divided into favorable and unfavorable groups based upon a cutoff of 5 CTCs per 7.5 mL of blood. The median PFS and overall survival (OS) durations for the favorable group (<5 CTCs) were 6.8 and 8.1 months, respectively. In contrast, the median PFS and OS for the unfavorable group (≥5 CTCs) were 2.4 and 4.3 months, respectively. The authors also assessed changes in CTC number following one cycle of chemotherapy in a subset of patients ($n = 18$), finding that a reduction in CTC number after therapy was predictive for improved survival. In a separate phase II study of erlotinib

and pertuzumab in advanced NSCLC (43), on-treatment decreases in CTC counts were found to correlate with radiographic response and PFS. It should be noted that in both studies, measurements of the absolute numbers of CTCs were used as the PD biomarker, rather than specific targets within CTCs. Such findings will require prospective validation in larger cohorts.

Despite the promise of CTCs as biomarkers, a number of challenges have emerged. Several different CTC detection technologies have been developed. This has led to a lack of standardization for CTC detection, cutoffs, and data analysis, making reproducibility and generalizations across studies difficult (44). To date, the only commercially available system approved by the U.S. Food and Drug Administration is the CellSearch CTC platform (Veridex); however, this system is currently not approved for use in NSCLC. Nonetheless, several reports in NSCLC have highlighted the potential of CTCs. For example, Maheswaran and colleagues used a microfluidic-based platform to isolate CTCs, identifying activating *EGFR* mutations and the appearance of an *EGFR* T790M resistance mutation in patients (45). More recently, *ALK* rearrangements in CTCs were also detected (46). Moving forward, it is hoped that such technology may be used for dynamic tumor assessments.

In addition to CTCs, extracellular microRNAs (miRNA) and ctDNA have been proposed as noninvasive biomarkers (41, 47). miRNAs are a class of small, non–protein-coding RNAs that serve as negative gene regulators. miRNAs can exist stably in circulation in tumor-derived exosomes or microvesicles (47). To date, early studies in NSCLC have largely focused on using miRNA as biomarkers for early disease detection and prognosis, but PD studies have been lacking (48, 49).

ctDNA is thought to be released into the bloodstream from cancer cells undergoing apoptosis or necrosis. Although it represents only a small proportion of the total cell-free DNA in circulation (<1%; ref 41), ctDNA can be identified and distinguished from normal cell-free DNA by testing for genetic mutations that are tumor specific (e.g., point mutations, chromosomal rearrangements). ctDNA levels have correlated with changes in tumor burden in a number of different malignancies (41, 50). Thus, ctDNA has potential to serve as a noninvasive tool for PD assessments.

In studies of NSCLC to date, ctDNA has been predominantly used to identify predictive biomarkers, such as *EGFR* and *KRAS* mutations (51, 52). ctDNA has also been used to track the development of resistance, such as the *EGFR* T790M resistance mutation (53). More recently, preliminary studies have also described serial assessments of ctDNA to monitor response, suggesting that ctDNA may be useful as a PD endpoint. In one report, Oxnard and colleagues performed droplet digital PCR of ctDNA to quantify plasma levels of mutant *EGFR* in patients with EGFR-mutant NSCLC receiving erlotinib (54). Plasma levels of mutant *EGFR* became undetectable in 8 of 9 (89%) patients following initiation of therapy, and the ninth patient also experienced a reduction in plasma levels of mutant *EGFR*. Interestingly, plasma levels of mutant *EGFR* eventually rose to detectable levels in 6 (67%) of these patients, preceding objective radiographic progression by up to 4 months. Although the sample size from this study is small, it provides early proof of principle that such PD assessments are feasible using ctDNA.

On-target toxicity assessments

Clinical parameters can also serve as PD markers. Specifically, assessments of mechanism-based, or "on-target" toxicities, have been used as PD biomarkers in clinical trials of various targeted therapies. These adverse events can arise when the molecular drug target is also expressed on normal cells. Importantly, such on-target toxicities do not necessarily correlate with disease response, but rather they may provide support for the proposed mechanism of action of an agent. Illustrative examples include EGFR inhibitors, which are associated with rash; vascular endothelial growth factor inhibitors, which are associated with arterial hypertension; and phosphatidylinositide-3-kinase (PI3K) inhibitors, which affect insulin and glucose (55).

Conclusions

In summary, the number of targeted agents in clinical development continues to grow. Historically, the development of such agents has been costly and slow, but with the use of predictive biomarkers for patient selection, increasingly rapid approval is possible. The experience in NSCLC highlights that targeted therapies are likely to benefit only specific subgroups of patients. Therefore, in addition to the continued development of predictive biomarkers, there is a greater need for the incorporation of PD endpoints in clinical trials.

Thus far, the use of PD studies in oncology in general and in NSCLC in particular has been somewhat limited. This may be because of a number of issues, including technical factors (e.g., insufficient dynamic range of assays), practical considerations (e.g., cost, patient preferences), inter- and intratumoral heterogeneity, and the invasive nature of biopsies. Nonetheless, PD studies may provide valuable insights into optimal biological dosing, toxicity, and, most importantly, proof of mechanism and proof of concept. PD studies are of greatest importance in phase I/II studies evaluating investigational agents with novel mechanisms of action or compounds directed at new targets. Ideally, such assessments should be performed directly on tumor tissue and focus on mechanism-based PD endpoints (e.g., changes in target protein phosphorylation status or the activation status of downstream signaling molecules in response to a TKI). Because objective tumor responses are relatively uncommon in phase I studies, such assessments may provide early insights into proof of mechanism.

At present, PD studies have not been incorporated into routine clinical practice in NSCLC because of the invasive nature of repeat tumor biopsies and the lack of validated

PD endpoints. Moving forward, invasive biomarker studies will likely need to be combined with less invasive strategies, such as imaging-based assessments and circulating biomarkers (e.g., CTCs and ctDNA). Together, these assessments may allow investigators to gain deeper insights into the dynamics of tumor response and the emergence of resistance. This may in turn inform early phases of future drug development and clinical practice.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.F. Gainor, D.L. Longo
Development of methodology: J.F. Gainor
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.F. Gainor
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.F. Gainor, D.L. Longo
Writing, review, and/or revision of the manuscript: J.F. Gainor, D.L. Longo, B.A. Chabner
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.F. Gainor

Received January 31, 2014; revised March 7, 2014; accepted March 21, 2014; published online May 15, 2014.

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