Rationale for Biomarkers and Surrogate End Points in Mechanism-Driven Oncology Drug Development

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INTRODUCTION

Recent progress in understanding the molecular basis of cancer has redefined the landscape for cancer drug discovery and development. Among the most exciting and promising benefits are molecular targeted cancer therapies, which can be seen as progress toward personalized medicine for cancer patients. However, it is already clear that the translation of molecular insights into useful therapeutic approaches is highly complex, and the success of any particular approach is by no means guaranteed. Traditional paradigms of drug development are not well suited to these new challenges and may not fully exploit the potential of molecular advances.

Instead, an integrated, collaborative effort is needed among pharmaceutical, biotechnology, government, academic, and patient advocacy groups to translate laboratory insights into rationally designed agents. A key goal of this proposed enterprise is the integration of complementary efforts (e.g., molecular diagnostics and therapeutics technologies). To this end, the enterprise will provide access to key common resources (e.g., development and implementation of a national tissue bank) and information (e.g., data pertinent to target discovery or validation). These efforts will be complemented by the development of consensus standards regarding processes and validation. In addition, the enterprise will establish rational, science-based strategies and frameworks for clinical testing and approval of molecular therapies and imaging modalities. This enterprise can be guided by and build on the success of other collaborative scientific efforts, such as the landmark mapping of the human genome.

Integral to this effort is the discovery and validation of mechanism-based biomarkers to facilitate the efficient development of new cancer medicines. Biomarkers (i.e., testable end points indicative of a relevant biological aspect of cancer) may be discovered using molecular, cellular, and/or imaging methodologies focused on drug and disease mechanisms, thus providing critical feedback about the interaction of novel therapies with their intended target and about the disease itself. Ideally, the development of targeted therapies can be closely coupled to the identification and validation of cognate biomarkers, thus maximizing the potential for rational development of such agents based on rigorous mechanistic assessments of their effects. This information can be used at critical decision points throughout drug discovery, screening, preclinical testing, and all phases of clinical testing, as well as during the United States Food and Drug Administration (FDA) accelerated and full approval processes to increase efficiency (e.g., more successful candidates) and accelerate progress (e.g., more rapid feedback).

In this way, mechanism-based biomarkers can serve as true intermediate or surrogate end points during the drug development process. For example, these biomarkers can validate a new target or pathway, allow optimization of lead agents, predict response and hence select patients for therapy, determine dose/schedule regimens, reflect potential drug resistance mechanisms, and provide rationale for optimal combination therapies.

This article discusses the uses of biomarkers as intermediate/surrogate end points in mechanism-driven oncology drug development. The use of biomarkers to facilitate the development of novel anticancer agents now has clear precedents. As examples, we review the close connection between HER2 gene and protein assays and the development of the anti-HER2 monoclonal antibody (MAb) trastuzumab (Herceptin) and between studies of the BCR-ABL tyrosine fusion protein kinase and the development of the BCR-ABL kinase inhibitor imatinib mesylate (Gleevec). In both cases, biomarkers indicative of disease mechanisms became targets for therapy and further served as tools for design of preclinical studies and patient selection in clinical trials and as predictive factors for treatment response in the clinic. However, recent results with other novel targeted agents have highlighted the difficulties and complexities of successful development of these new approaches. Many agents, despite promising scientific rationale, have shown disappointing efficacy in clinical trials, or clinical studies have revealed benefit in some trials but not others, for reasons that are not yet fully understood. In most cases, it remains uncertain why the agents failed. Did the targeted therapy hit the target? If so, is the target or pathway not as important as postulated? If not, can the agent be used differently, against certain molecularly defined tumors, combined with other agents, or further modified and optimized for better effect?

These examples highlight the prospects and challenges in developing biomarkers and surrogate end points for mechanism-based development of molecularly based therapies. Despite the obstacles, biomarkers and surrogate end points have the poten-
tial to make drug development more rational, thereby optimizing the clinical trial process and delivering timely benefits to patients. Without stifling originality and creative paradigms, effective collaborative interactions and a consensus approach are needed to realize the potential of these opportunities.

NEW TECHNOLOGIES FOR BIOMARKER DEVELOPMENT

Recent technological advances now provide an unprecedented opportunity for the development of biomarkers relevant to cancer mechanisms. Prominent among these are molecular profiling approaches based on genomics or proteomics to define the molecular signature of cancer, patterns of gene and protein expression and DNA alterations (e.g., gene hypermethylation, single nucleotide polymorphisms, microsatellite instability, and gross chromosomal aberrations) associated with different cancers. For example, mass spectrometry in conjunction with bioinformatics algorithms has identified serum protein profiles that can be used to diagnose ovarian cancer, even though many of the proteins are unknown (1). Similar algorithms are in development to discern protein patterns that may have prognostic significance or may predict treatment responsiveness. Similarly, expression microarrays are now being widely used to characterize the transcriptional profiles associated with various tumor types, stages, and/or phenotypes, for example in colon carcinogenesis (2). At the genomic level, techniques such as comparative genomic hybridization and spectral karyotyping can detect chromosomal alterations associated with certain cancers, such as hematological malignancies (3). Methodologies to detect and characterize patterns of mutated or aberrantly methylated DNA in body fluids such as serum, sputum, or breast ductal lavage have been applied in patients with lung (4, 5), liver (6), or breast (7) cancer. Cell-based technologies relevant to biomarker development include those permitting detection and quantification of circulating tumor or endothelial and endothelial progenitor cells. Methods to sensitively detect and enumerate rare circulating tumor cells have shown promise as a marker of disease status or treatment response, including studies in breast (8) and prostate (9) cancers. Isolation of such cells offers the further possibility of applying molecular profiling techniques to cells that may be surrogates of minimal residual disease (“micrometastasis”) or metastatic cancer. Assays of circulating endothelial cells or endothelial progenitors may also be informative about cancer progression and/or tumor angiogenesis (10–12).

In addition to these molecular and cellular techniques, advances in novel imaging technologies also have profound implications for biomarker development. These molecular and functional imaging approaches include technologies to assess cellular metabolism (e.g., 18F-fluorodeoxyglucose positron emission tomography), cell proliferation and apoptosis (e.g., 18F-fluoro-L-thymidine and 99mTc-annexin imaging), resistance to chemotherapy [99mTc-sestamibi imaging (13–15)], and angiogenesis and vascular dynamics (e.g., dynamic contrast-enhanced computed tomography and magnetic resonance imaging). Other technical advances include simultaneous combined modality imaging (e.g., positron emission tomography/computed tomography or positron emission tomography/magnetic resonance imaging), which offers great promise in integrating functional and anatomical information. Optical technologies under development using fluorescence and bioluminescence may also prove useful in early drug development.

FACILITATING DRUG DEVELOPMENT WITH BIOMARKERS

Uses of Biomarkers during Drug Development

Biomarkers have many potential uses in all phases of the drug development process, from target discovery and validation through pivotal clinical trials for drug registration. Feasible uses of biomarkers in each phase are highlighted in the following sections.

Target Discovery and Validation. Biomarkers have been used to identify and justify targets for therapy. Prominent examples of targets for drug development include receptor and signaling molecules, e.g., human epidermal growth factor [EGF (HER/ErbB)] receptor-tyrosine kinases (TKs), BCR-ABL, platelet-derived growth factor receptor, vascular endothelial growth factor (VEGF), VEGF receptor, ras, and so forth. As discussed in detail below, the HER2/c-erbB2 proto-oncogene is frequently amplified in breast cancer and, when amplified, is associated with poor prognosis. This correlation between the biomarker and clinical outcome was a key factor in establishing the rationale for anti-HER2 therapeutic strategies that ultimately led to the development of trastuzumab (see “Case Studies: Trastuzumab, Imatinib, EGFR Inhibitors and Angiogenesis Inhibitors”). As molecular profiling efforts yield increasingly massive amounts of new information about potential targets, it will be important to characterize the biological and clinical relevance of these potential targets to evaluate their status as biomarkers and to prioritize the most promising targets for therapy.

Lead Discovery and Optimization. Most oncology drug development efforts use target-associated assays to identify leads and evaluate the effects of molecular targeted drugs in preclinical development. These data are critical in developing and optimizing drug candidates, including those based on small molecules, MAbs, or other therapeutic platforms. Indeed, evidence that the molecular target is significantly impacted by the “targeted” agent, as indicated by an effect on an appropriate biomarker in a relevant model, is almost a sine qua non for selecting drugs for further research. Of course, a drug may show various effects in experimental systems in addition to its intended effect on the proposed target. Nevertheless, the increasing trend toward precise molecular targeting means that biomarkers that indicate target effects provide crucial validation for a given therapeutic approach. Lead agents developed against a given target can be further optimized based on biomarker end points in model systems or clinical studies.

Preclinical Studies. Critical proof of concept studies typically involve appropriate animal models of cancer. The complexities of modeling human cancer in experimental systems are well known and have impeded cancer drug development over the years. Newer, genetically engineered cancer models (e.g., using the conditional or cell-specific expression of transgenes or the restricted introduction of somatic mutations using viral vectors) have addressed some of these limitations (16); advances are also being made in refining and improving
human tumor xenograft models, such as by using genetically marked or fluorescence-labeled tumor cells to track and measure primary or metastatic disease in noninvasive, quantitative ways (17, 18). However, current models still do not accurately mimic most disease settings, especially in advanced stages. As a result, most current models have limited capability for predicting clinical effects. Models that feature biomarker properties comparable with those seen in patient populations will enhance their utility as predictive models; biomarkers can play an essential role in the validation of new disease models. Specific effects on biomarkers in such models can, in turn, provide proof of concept for therapeutic approaches. For example, to facilitate the development of appropriate models for cancer, the National Cancer Institute established the Mouse Models of Human Cancer Consortium in 1999. This collaborative effort has made strides toward delineation of appropriate models for preclinical evaluation of different classes of anticancer agents. These include the RIP1-Tag2 mouse, which contains a transgene composed of the upstream region of the rat insulin II gene linked to simian virus large-T antigen (19). This mouse model, in which islet cell pancreatic tumors develop by 12–14 weeks, has been used to test the preclinical efficacy of novel angiogenesis inhibitors (20). Transgenic mouse models of breast cancer include those expressing HER2/neu [e.g., driven by the mouse mammary tumor virus promoter (21)]. EGF receptor (EGFR) TK inhibitors have been found effective in this model of estrogen receptor-negative mammary tumorigenesis (22).

**Phase I/II Clinical Trials.** Appropriate biomarkers can provide critical feedback on the clinical testing. To date, however, the lack of validated biomarkers for clinical application has been deeply problematic, particularly with regard to end points relevant to targeted therapies (23). In principle, optimization of dose and schedule can be based on pharmacological effects on biomarker-based end points rather than on maximum tolerated dose. Similarly, mechanistic information about new agents can complement standard safety evaluations. Perhaps most importantly, biomarker-based studies may provide early evaluations of the key question of mechanistic success or failure (“hitting the target”). Lack of mechanistic activity in early clinical trials can help to curtail further costly clinical testing and redirect efforts toward additional preclinical studies. Effects on biomarkers that indicate the mechanistic impact of a novel agent can be correlated with observed clinical effects. Biomarkers indicative of mechanism can also help guide rational selection of regimens involving combinations of agents. Although there is considerable interest in combinations of novel targeted agents with either standard anticancer drugs or other novel agents, information to guide the selection of effective combinations has usually been scanty or nonexistent. Judicious use of biomarkers may illuminate useful or detrimental mechanistic interactions among drugs, thereby facilitating the identification of additive or synergistic combinations.

**Phase III Clinical Trials.** The development of biomarkers as surrogate end points for accelerated or full drug approval and registration is a unique and important case. Key examples are discussed in the target organ-specific papers accompanying this article [e.g., the colorectal adenoma in colon cancer (24), CA-125 in ovarian cancer (25), and PSA in prostate cancer (26)]. It should be noted that the development and validation of mechanism-based biomarkers that reflect fundamental disease activity and/or essential interactions between disease targets and targeted therapy may lead to new surrogate end points of clinical benefit. If sufficiently linked to disease outcomes, these markers could in principle be used to assess clinical benefit in Phase III trials.

**Collaborations for the Development of Biomarkers**

Successful development of biomarkers in conjunction with novel agents requires multidisciplinary research, and a collaborative enterprise will be necessary to translate biomarker research into truly useful clinical tests and therapies. This enterprise may share some features with the current drug development process, although a new philosophy that stresses the integration of disciplines, synergy, and the collaboration between the public and private sectors will be required. In this section, we survey several current collaborative efforts that may serve as models for these future endeavors.

**Industry Investment.** One of the barriers to better integration of biomarker development and drug development has been the limited interactions between the industry groups involved in these respective areas. Traditionally, biomarker development has been pursued most avidly by diagnostics, imaging, and testing companies, whereas therapeutics have been the domain of biotechnology and pharmaceutical companies. Coordination of effort between these two groups has not been routine. This difference in perceived mission and culture has even occurred within large companies or organizations comprising both activities. Recently, some companies have begun to bridge the gap between their internal diagnostics and therapeutic efforts. For example, the Integrated Cancer Care Unit at Roche unites diagnostics with pharmaceuticals through an integrated approach to drug development (27). Similarly, Johnson & Johnson’s Molecular Diagnostics Unit in the Advanced Diagnostics Systems division of Ortho Clinical Diagnostics collaborates with their Pharmaceutical Research & Development Company in preclinical and clinical development of cancer therapeutics. Despite these promising developments, an industry-wide approach is needed. For example, this might include consortium-building involving diagnostics and imaging companies with drug developers.

**Collaborations between the Public and Private Sectors.** Effective collaborative interactions among industry, federal agencies (e.g., FDA and NIH), and academia are essential to the development and acceptance of biological markers as surrogate end points. The discovery of new markers for diagnosis and targets for drug development often results from basic research in academic laboratories. Whereas the NIH facilitates such research and encourages industry-academia collaborations, the FDA must be involved in the validation of such markers as useful mechanistic end points in clinical trials and/or as surrogate end points for clinical outcomes.

A number of examples of collaborations among industry, the NIH, and academia can be cited as precedents for a new collaborative enterprise. For example, the Single Nucleotide Polymorphism Consortium, a collaborative that represents the collected efforts of 13 companies and 4 academic institutions, has discovered and characterized nearly 1.8 million single nu-
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cleotide polymorphisms in the human genome. Other examples include the Alliance for Cellular Signaling, a consortium supported by two pharmaceutical companies and the National Institute of General Medical Sciences that was established to define cellular signal transduction networks, and the International Genomics Consortium, a research organization focused on standardizing sample collection and genomic array analyses for cancer. The field of biomarker research is an excellent venue for such an alliance because it spans many disciplines and has myriad applications for disease states.

CASE STUDIES: TRASTUZUMAB, IMATINIB, EGFR INHIBITORS, AND ANGIGENESIS INHIBITORS

In this section, we detail four prominent case studies illustrating the role of biomarkers in the development of novel targeted agents in oncology. All of these cases involved biomarkers that served as the basis for rational drug design. However, implementation of biomarker testing and translation to the clinic posed different challenges in each case.

The cases of trastuzumab and imatinib demonstrate how biomarkers with strong clinical validation provided compelling targets for therapy, followed by clinical development that centrally involved biomarker-based patient selection or intermediate efficacy assessments.

Other highly promising targets for cancer drug development, including HER-1 (also called EGFR and ErbB1) and mediators implicated in angiogenesis (e.g., matrix metalloproteinases and VEGF), have proven challenging to fully exploit for therapy, due in no small part to incomplete mechanistic information about the effects of the targeted agents. Recent clinical results with EGFR kinase inhibition and VEGF inhibition have finally provided encouraging evidence of benefit, culminating in recent FDA approvals for targeted agents. However, a number of other studies with the same agents were less encouraging. The case discussions of EGFR inhibitors and angiogenesis inhibitors highlight the need for and possible approaches to the development of more useful biomarkers for these therapeutic strategies.

Trastuzumab

HER2 (also called ErbB2 and Neu) is a cell surface glycoprotein with intrinsic TK activity that is involved in cell growth and development (28). Activation of HER2 initiates signaling cascades leading ultimately to increased cell proliferation, survival, angiogenesis, adhesion, and resistance to certain anticancer agents (28, 29). HER2 became a potential biomarker with the initial observation that the HER2 gene was amplified in 25% of axillary lymph node-positive breast cancers and, when present, correlated with worse prognosis (30). Additional studies confirmed that HER2 protein overexpression was also a prognostic biomarker in breast cancer, correlating with decreased relapse-free and overall survival (30–33). Moreover, additional clinical data have shown that HER2 amplification/overexpression is a predictive biomarker for greater or lesser response to certain chemotherapies or hormonal therapies in breast cancer (34–41).

The role of HER2 as an oncogenic protein and clinically relevant biomarker led directly to the development of a specific targeted therapy: trastuzumab (Herceptin; Genentech, South San Francisco, CA), a recombinant MAb directed against the extracellular domain of HER2. In advanced breast cancers with HER2 overexpression, trastuzumab was shown to be active as a single agent in second-/third-line therapy (42, 43) and subsequently as first-line therapy (44). Trastuzumab is particularly effective in combination with chemotherapy; the addition of trastuzumab to chemotherapy (paclitaxel or anthracycline/cyclophosphamide) was associated with a longer time to disease progression, a higher rate of objective response, and longer overall survival in a Phase III trial (45). Based on these results, trastuzumab was approved in 1998 by the FDA as second-/third-line monotherapy or first-line therapy in combination with paclitaxel for the treatment of HER2-overexpressing metastatic breast cancer.

HER2 as a biomarker contributed to this successful result in several ways. First, the initial finding that HER2 is a negative prognostic factor suggested that it might be an attractive target for therapy. That is, because HER2-overexpression was associated with more aggressive breast cancer, this implied a pathogenic role for the protein and also indicated that this subpopulation might particularly benefit from an appropriately targeted therapy. Second, the in vivo efficacy of various anti-HER2 MAbs, including trastuzumab, correlated strongly with the degree of HER2 overexpression in preclinical models (46, 47). This observation led to the design of clinical trials requiring HER2 overexpression as a strict criterion for study entry. An immunohistochemistry (IHC) assay with a semiquantitative scoring system (0–3+) was developed by the company sponsor and used throughout Phase I–III trials. This was important because routine HER2 testing was not in place at most centers and, when performed, was not standardized. In this way, trastuzumab clinical trials directly facilitated refinement of HER2 testing methods. The ultimate effect was to focus clinical testing of trastuzumab on those patients with the molecular abnormality being targeted and thus most likely to benefit. Rigorous HER2 testing in clinical trials also led to additional useful observations. In the pivotal Phase II trial of trastuzumab monotherapy, HER2 overexpression at the 3+ level was associated with a higher objective response rate than HER2 overexpression at the 2+ level (17% versus 4%), as well as a longer time to disease progression (3.3 versus 1.9 months; P = 0.0034; Ref. 43). A number of clinical trials have confirmed that patients with high levels of HER2 receptor overexpression (IHC 3+) are most likely to receive clinical benefit from trastuzumab and further indicated that HER2 gene amplification as detected by fluorescence in situ hybridization is most predictive (48).

In retrospect, the clinical benefits of trastuzumab would almost certainly have been obscured if the agent had been tested solely in unselected patient populations and without data indicating HER2 status. All existing evidence indicates that patients whose breast cancers lack HER2 overexpression are highly unlikely to respond to trastuzumab alone. This evidence includes in vitro and in vivo preclinical studies in model systems, the clear “antigen dose”–response relationship between HER2 gene/protein levels and trastuzumab response in breast cancer, and the lesser activity observed to date for trastuzumab in other tumor types with lower HER2 overexpression. Hence, in the
initial Phase II study in metastatic breast cancer patients (42), the observed response rate to trastuzumab of 12% in HER2-overexpressing patients would have been much lower in unselected patients, most likely <5%. Such a result would almost certainly have entailed discontinuation of the clinical program by the company sponsor.

The successful development of trastuzumab demonstrates that biomarker-based patient selection at an early stage in the clinical trial process can be critical to the evaluation of a targeted agent. Furthermore, active investigation into other biomarkers indicative of signaling of the HER2 pathway and related pathways may provide support for new ways to use trastuzumab, such as in combination with other signal transduction inhibitors.

**Imatinib**

One of the most venerable biomarkers in oncology is the Philadelphia chromosome, a t(9;22) translocation found in 95% of cases of chronic myeloid leukemia (CML) and in some cases of acute lymphoblastic leukemia. The presence of the Philadelphia chromosome can aid in the diagnosis of these diseases and has prognostic significance in acute lymphoblastic leukemia. More importantly, the translocation led to discovery of the BCR-ABL fusion as the critical initiating event in CML, which in turn enabled the development of molecular tests for this biomarker that can sensitively detect minimal residual disease, even when standard hematological parameters appear normal. The development of imatinib mesylate (STI571, Gleevec; Novartis, Basel, Switzerland), a small molecule inhibitor of the BCR-ABL TK, has transformed treatment paradigms in patients with CML (49). CML progresses through three distinct phases (chronic or stable, accelerated, and blast) within an average time span of 4–6 years (50). Before the development of imatinib mesylate, stem cell transplantation, chemotherapy, and IFN-α-based regimens were the only modes of treatment for CML. Of these, stem cell transplantation is the only proven curative therapy for CML (51), although this approach is impractical in the majority of CML patients (50, 52).

Imatinib mesylate is a rationally designed, molecular targeted small molecule that exemplifies the successful use of biomarkers in cancer drug development. Drug design was based on the characterization of BCR-ABL, which acts as a transforming oncogene in hematopoietic cells via its dysregulated TK activity (53). Imatinib is a phenylaminopyrimidine-based compound selected for its potent inhibition of the ABL and platelet-derived growth factor receptor TKs. This inhibition is relatively selective but also extends to the c-KIT kinase (54). In a Phase I trial in CML patients who were resistant or intolerant to IFN-α, treatment with >300 mg/day imatinib mesylate produced complete hematological responses in 98% of patients in chronic phase and in 55% of those in blast phase (55). Further clinical testing has confirmed these striking results (56–59). The high rate of hematological responses and marked reduction in patients who progress to accelerated or blast phases suggest a possible survival benefit from the use of imatinib (49). Determination of clinical benefit was greatly facilitated by concomitant testing for the molecular target in these trials, including conventional cytogenetics, fluorescence in situ hybridization-based assays of the BCR-ABL translocation, and reverse transcription-PCR-based detection of BCR-ABL transcripts. Responses in these biomarkers provided important early evidence for drug efficacy. Furthermore, in individual patients, these biomarker results are now commonly used to assist in treatment decisions (60).

The case of imatinib illustrates how biomarkers derived from BCR-ABL not only stimulated initial drug discovery efforts but also served as a useful end point of treatment effect. The success of imatinib has promoted the search for other agents with activity against BCR-ABL-expressing cell lines, many of which will ultimately be tested in combination with imatinib in clinical trials (61). Elucidation of the effects of imatinib on the progression of CML has also identified other downstream biomarkers, including phosphatidylinositol 3′-kinase, AKT, and ras, as possible therapeutic targets. Moreover, knowledge gained about mechanisms of resistance to imatinib has stimulated the testing of new and rational drug combinations in CML therapy.

The ability of imatinib to inhibit the receptor TK encoded by the oncogene c-KIT (CD117) led to clinical testing of the drug in another disease, gastrointestinal stromal tumor. Treatment with imatinib in these studies showed a high degree of antitumor efficacy in what was previously a refractory tumor with few good treatment options (62, 63). Preliminary data further suggest that the presence of particular activating mutations of c-KIT correlate with response to the drug, indicating that these mutations may serve as additional biomarkers for patient selection in future trials (64).

**EGFR Inhibitors**

The overexpression of EGFR in a variety of human cancers, including non-small cell lung cancer (NSCLC) and colorectal, head and neck, bladder, brain, pancreatic, ovarian, breast, prostate, gastric, and brain tumors, suggested the potential for a prognostic biomarker and a target for drug development (65, 66). A variety of EGFR TK inhibitors, including the quinazoline-based small molecules gefitinib (ZD1839, Iressa; AstraZeneca, London, United Kingdom) and erlotinib (OSI-774, Tarceva; OSI Pharmaceuticals, Uniondale, NY) and the MAb cetuximab (IMC-C225, Erbitux; ImClone Systems, New York, NY) have now been studied in thousands of patients (67). Other EGFR and ErbB inhibitors in clinical development include CI-1033, a small molecule inhibitor of all of the active ErbB kinases (EGFR, HER2, and ErbB4); GSK572016, a small molecule inhibitor of EGFR and HER2; and ABX-EGF, a recombinant human MAb against EGFR derived from transgenic mice containing human antibody genes (29, 68–71).

**The Case of Gefitinib.** Recent Phase II studies of gefitinib monotherapy in second or third line after chemotherapy in advanced NSCLC have clearly shown antitumor responses with acceptable toxicities (72, 73). Based on these results, gefitinib received accelerated FDA approval in 2003 for the treatment of advanced NSCLC after disease progression on chemotherapy. Although very encouraging, these results contrast with Phase III data in which gefitinib failed to show any significant clinical benefit in combination with first-line chemotherapy in NSCLC. Recently announced Phase III results with erlotinib in advanced NSCLC also indicated no significant additional benefit for that agent in combination with chemotherapy; at least some preclinical models predicted additive or
synergistic effects of EGFR inhibition and standard chemotherapy (74, 75). Furthermore, results with EGFR kinase inhibitors in other solid tumors have thus far shown only modest single-agent activity. Thus, despite meaningful responses in some patients with NSCLC, the utility of EGFR inhibition in other settings and in other diseases remains to be seen, despite already extensive clinical testing.

It is clear that a more sophisticated mechanistic understanding about the role of the EGFR pathway in individual tumors and the corresponding effects of EGFR inhibitors on these tumors is needed. Examples of the types of biomarker data that could answer key mechanistic questions and contribute to further development of EGFR inhibitors are cited in the following sections.

Which Patients/Tumors Are Truly Dependent on EGFR TK Activity? Initial attempts at identifying predictive biomarkers for EGFR inhibitors have been disappointing. Unlike the case of HER2, EGFR overexpression in tumor tissue as assessed by IHC does not appear to predict response to these agents; for example, in a Phase II trial of patients with platinum-refractory NSCLC, erlotinib responses did not correlate with percentage or intensity of EGFR staining (76). In retrospect, this result is not surprising in light of the known mechanisms for EGFR activation. Unlike the HER2 proto-oncogene, amplification of EGFR does not occur frequently in solid tumors, with the notable exception of gliomas. Similarly, EGFR is probably not constitutively active in most cancers but instead is subject to direct ligand activation by transforming growth factor-α, EGF, and related growth factors. Hence, EGFR overexpression is not required for EGFR-dependent tumorigenesis and tumor progression. Current efforts are attempting to identify biomarkers that will better indicate EGFR dependence, including those that are upstream of the target, such as expression of EGFR ligands or other ErbB receptors, as well as those that are downstream of the target, such as the signaling elements extracellular signal-regulated kinase 1/2, phosphatidylinositol 3'-kinase, or Akt. In addition, the absence of one or more survival pathways may influence the efficacy of EGFR inhibitors. Expression array studies have been undertaken to identify potential genes associated with sensitivity and resistance to EGFR inhibitors (77–79). Studies using surrogate tissues, including skin biopsies, are also being pursued to correlate phosphorylation of EGFR and downstream components with treatment outcomes (80, 81).

Do Different Anti-EGFR Strategies Produce Different Results? Several different therapeutic strategies have been invoked to achieve EGFR inhibition (5, 82, 83). The MAbs cetuximab and ABX-EGF recognize epitopes near the ligand binding site of the EGFR extracellular domain and thereby competitively inhibit receptor activation by the EGF family of ligands. As chimeric (cetuximab) and human (ABX-EGF) MAbs, these agents may also elicit host immune mechanisms. In contrast, small molecule quinazolines gefitinib and erlotinib occupy the intracellular ATP binding site of EGFR and thus reversibly inhibit EGFR phosphorylation on receptor dimerization. Other small molecule inhibitors such as CI-1033 or EKB569 bind irreversibly to the ATP site of EGFR as well as that of other ErbB receptors. Dissection of the mechanistic effects of these drugs in human studies may help to sort out whether different therapeutic platforms have different clinical effects on the same target.

Is EGFR Inhibition Useful in Earlier Stages of Cancer (e.g., Minimal Residual Disease/Adjuvant Therapy, Pre-malignant Disease, and Cancer Prevention)? A widely stated explanation for the relative paucity of profound antitumor responses observed with EGFR inhibitors (and many other targeted agents) is that these therapies are cytostatic and hence not likely to have major impact on established metastatic disease. Whether or not this is true, it is reasonable to hypothesize that these agents may have greater utility in earlier stages of cancer. Certainly, their favorable toxicity profile makes this an attractive and feasible strategy. If predictive biomarkers are validated in advanced disease, these markers can facilitate studies in early cancers. Furthermore, valid biomarkers are particularly important as surrogate end points in the prevention setting because conventional end points such as disease incidence and, in particular, survival can require immense numbers of subjects in randomized studies.

Which Combinations of Agents Are Most Rational? As noted, Phase III trials with both gefitinib and erlotinib have thus far failed to show any enhancement of platinum-based chemotherapy in advanced NSCLC. On the other hand, cetuximab combined with irinotecan has shown anticancer activity in advanced colorectal cancer, even in patients previously resistant to irinotecan alone (84). Recently, cetuximab received accelerated FDA approval for treatment of metastatic colorectal cancer; it is to be used in combination with irinotecan or alone (if irinotecan cannot be tolerated). It remains unclear how EGFR inhibitors should be combined with chemotherapy, including which cytotoxic agent(s) should be used and whether combination or sequential administration makes a difference. Studies incorporating biomarkers that can illuminate interactions between pathways may help resolve such questions more than purely empirical “trial and error” clinical trial designs. “Combinatorial therapy” including two or more agents that target intersecting or interdependent pathways has been posited as a key strategy to increase the utility of targeted therapies, and this too will require further mechanistic understanding to focus clinical testing on the most compelling combinations. For example, it is likely that EGFR inhibition is producing effects on or is affected by other signaling pathways that are also important in various cancers, including steroid hormone signaling in hormone-sensitive cancers. Furthermore, multiple interventions targeting different points along the same pathway, such as inhibition of EGFR phosphorylation and phosphatidylinositol 3’-kinase or ras activation, may act synergistically to shut down signaling activity.

Angiogenesis Inhibitors

Angiogenesis, the growth of new blood vessels from an existing vasculature, appears to be a key component in tumor pathogenesis. New endothelial cells can come from division of pre-existing, differentiated endothelial cells in mature vessels (“local” or “sprouting” angiogenesis); alternatively, they can arise via mobilization of bone marrow-derived endothelial progenitor cells into the peripheral circulation, followed by differentiation and incorporation into sites of angiogenesis (“systemic vasculogenesis”) (11, 85). The ability to manipulate tumor vasculature through inhibition of these two processes represents a promising avenue for cancer therapy, and a diverse group of...
drugs is currently being developed to target components and pathways associated with these processes. To date, more than 300 potential angiogenesis inhibitors have been identified, and approximately 80 antiangiogenic drugs are currently being tested in clinical trials [12 are in Phase III trials (86)]. These agents vary considerably in both structure and mechanism of action. Many, including standard chemotherapeutic agents as well as biological agents such as thalidomide, are “accidental” antiangiogenic drugs, developed originally for a variety of reasons other than inhibition of tumor angiogenesis (87). The numerous antiangiogenic agents currently in clinical trials, however, have often failed to meet the promise suggested by preclinical studies. In retrospect, these disappointments may reflect incorrect identification of targets, insufficient potency of the agent(s) tested, redundancy of targets, or a lack of robust understanding of the mechanistic pathways of angiogenesis and vasculogenesis. Angiogenesis is regulated through numerous complex pathways, and most tumors express multiple angiogenic pathways (88). As new angiogenic pathways are discovered, the relative importance of each to tumorigenesis must be evaluated early in the clinical trial process.

The Case of Bevacizumab. Most of the problems and challenges already discussed can be illustrated by two recent Phase III clinical trials of bevacizumab (Avastin), a recombinant humanized MAB to VEGF, in combination with standard chemotherapy regimens. The trial in advanced breast cancer was assessed as a failure (89), whereas the trial in advanced colorectal cancer was an unequivocal success (90) that led to FDA approval. The “negative” trial involved treatment with bevacizumab in combination with capecitabine (Xeloda) in advanced breast cancer, whereas the “positive” trial involved testing first-line chemotherapy [irinotecan, 5-fluorouracil, and folinic acid/leucovorin (IFL)] in combination with bevacizumab as a first-line therapy in metastatic colorectal carcinoma. When these trials were designed, the implicit assumption was that tumor angiogenesis in advanced breast or colon cancers would be driven predominantly by VEGF. However, recent results cast considerable doubt on this assumption. Based on results from a number of laboratories, it is likely that elevated levels of VEGF expression occur in approximately 40–50% of metastatic breast cancers (91, 92). Therefore, it may have been the case that at least half the patients in the breast cancer trial who received bevacizumab could not, in retrospect, have benefited from receiving the drug. It is possible that the proportion of patients whose metastatic tumors express high levels of VEGF is higher in colorectal cancer, which could be one of the factors accounting for the discrepant results between the two Phase III trials. These considerations may also help explain the encouraging results of bevacizumab monotherapy in metastatic renal cell cancer in a randomized, placebo controlled Phase II clinical trial (93) because VEGF expression is especially prominent in this disease due in large part to inactivation of the von Hippel-Lindau gene (94).

The molecular picture in the Phase III bevacizumab trial in breast cancer may have been even more daunting than stated above. For example, a considerable proportion of VEGF detected in tissue sections is not necessarily bound to VEGF receptors on tumor-associated endothelial cells but is instead sequestered and hence biologically inactive as a result of binding to heparin sulfate proteoglycans present in the extracellular matrix (95). In addition, there are reports showing that as breast cancer progresses to more advanced stages of disease, the number of different proangiogenic growth factors expressed increases, i.e., there is greater redundancy of potential proangiogenic mechanisms (96). This raises the possibility that even high levels of biologically active VEGF may not always be functionally significant for more than brief periods of time, if at all, which further implies that specific anti-VEGF therapy may only produce a transient response in such situations. Indeed, in the bevacizumab/capecitabine trial, there was a significant improvement in the response rate in patients who received the combination as opposed to those who received capecitabine alone; however, the responses were not durable enough to translate into significant prolongations of time to progression or survival.

It is also not clear whether the optimal dose of bevacizumab was used in this or other clinical trials of the drug. In a preceding randomized Phase II clinical trial in colorectal cancer (97), two doses of bevacizumab were tested in combination with chemotherapy; the lower dose (5 mg/kg) but not the higher dose (10 mg/kg) showed enhanced activity compared with chemotherapy alone. In contrast, the higher dose, but not the lower dose, was effective in a randomized Phase II clinical trial of single agent bevacizumab. These differences might have contributed to the failure of the Phase III randomized breast cancer trial: was the optimal dose of bevacizumab used?

All of these considerations highlight the difficulties in establishing that VEGF is a valid and important target and that drugs that target the VEGF/VEGFR receptor axis are potentially effective drugs. Some of the critical outstanding mechanistic questions and biomarker-based strategies to address them are discussed in the following sections.

Which Patients/Tumors Are Truly Dependent on VEGF Activity? Failure to identify patients who express VEGF in a functionally significant manner could obscure an otherwise useful drug (98). For example, even if only 20–30% of breast cancers are driven by VEGF, this still represents a sizeable patient population. But clearly, strategies are needed to screen for patients whose tumors are most amenable to specific targeted agents, as are strategies for monitoring the activity of these agents in patients on trials.

A number of these problems may be solved by the intelligent use of biomarkers, some of which are currently in development. First, it is notable that some biomarkers have not yet proven useful in this setting, such as serum VEGF or primary tumor VEGF expression by IHC, and may not be informative based on the complexity of the pathophysiology involved. On the other hand, newer approaches include antibodies that specifically recognize VEGF only when bound to VEGF receptor 2, also known as Flik-1/KDR (99). Such a reagent may be of more value in identifying tumors with VEGF-dependent signaling. In addition, VEGF expression on tumor-associated endothelial cells may prove a useful biomarker for selecting patients for therapy. There is also considerable interest in enumerating the levels and viability of circulating endothelial precursor/progenitor cells; these cells, which originate in the bone marrow, can be detected in the peripheral circulation, from which they can incorporate into sites of ongoing angiogenesis and differentiate into fully mature endothelial cells (100, 101). A number of
laboratories have reported declines in the levels and viability of such cells in tumor-bearing mice during and after treatment with various antiangiogenic drugs (102). This technology is now being standardized and applied to patients receiving antiangiogenic therapies. If validated, it could represent a means to monitor the activity of some or most antiangiogenic drugs. Also under intensive investigation by many groups are functional imaging approaches, which may also eventually prove to be informative biomarkers of antiangiogenic drug activity.

**Which Combinations of Agents Are Most Rational?**

As discussed, one of the pitfalls in evaluating novel targeted therapies such as the angiogenesis inhibitors is the complexity of combining such agents with chemotherapy. It is assumed that addition of a new targeted agent to standard chemotherapy will improve the benefits of the latter. Whereas this has indeed been shown for trastuzumab and has now been shown for bevacizumab in colorectal cancer, this assumption has usually not been based on mechanistic grounds. This raises the important issue of preclinical models to test new targeted therapies in vivo. For example, there were no preclinical studies testing the effects of capecitabine in combination with bevacizumab before undertaking the Phase III randomized trial in breast cancer. If such preclinical studies had been undertaken, models involving metastatic disease, which are becoming available, might have provided a more accurate picture of the potential of a particular therapy in the clinic (103). Advances in preclinical models have ranged from the use of transgenic/knockout mice, which recapitulate many of the genetic alterations in the particular human disease counterpart, to the use of novel imaging technologies to visualize and quantify metastatic disease in mice injected with human tumor xenografts. These new animal models can now provide rigorous preclinical evaluations of putative surrogate markers for targeted anticancer therapy (103); conversely, evaluation of biomarker properties of these models can help to establish their use as mimics of specific disease settings.

Also, it may be necessary to optimize the dose/schedule of the chemotherapy drug(s) for administration with a new targeted agent, rather than simply adopting a standard chemotherapy regimen. For example, a number of laboratories have shown that frequent low-dose “metronomic” chemotherapy regimens give better antitumor efficacy in a variety of preclinical models, at least partly via antiangiogenic mechanisms (104–107). Metronomic administration of chemotherapy may also facilitate combination with a targeted antiangiogenic agent. However, although promising, some significant issues remain with regard to the use of metronomic chemotherapy regimens. For example, it is not clear how the optimum dose/schedule of a particular chemotherapeutic drug should be defined; as with targeted antiangiogenic agents, this question can be addressed by the development of appropriate biomarkers to guide dosing and activity.

**SUMMARY AND RECOMMENDATIONS**

This article highlights the potential, as well as the associated challenges, of using mechanism-based biomarkers to facilitate the development of molecular targeted therapies. Targeting drugs to molecularly defined populations is difficult to implement and has not been a traditional approach in the development of new drugs. By providing insight into disease mechanisms and interactions with therapy, the successful implementation of biomarkers can significantly advance the effort to rationally develop targeted agents. This use of biomarkers and surrogate end points has already proven invaluable in some oncology drug development efforts and offers the promise of increasing the efficiency and accelerating the progress of oncology drug development. However, to fulfill this potential, the development of novel mechanism-based and clinically relevant biomarkers will require its own investment in technology and translation. Specifically, recommendations for accomplishing better integration of novel biomarkers into drug development include the following items.

**Biomarkers Can and Should Be Applied Throughout the Drug Development Process for Novel Agents**

Biomarkers and surrogate end points have the potential to make drug development more rational, thereby optimizing preclinical and clinical efforts and ultimately delivering timely benefits to patients. Applications include the following: (a) to identify and validate therapeutic targets; (b) to screen and optimize candidate targeted agents; (c) to provide proof of concept for agents and models; (d) to enhance mechanistic understanding of drug or drug combination effects (e.g., as clear indicators of target engagement, cell death, and changes in tumor biology); (e) to identify optimal target populations; (f) to predict response, resistance, and toxicity; and (g) to rapidly distinguish responders from nonresponders to therapeutic intervention.

**New Biomarkers Emerging from Promising Molecular, Cellular, and Imaging-Based Technologies Should Be Codeveloped with Novel Agents**

Close integration of biomarker development with drug development is necessary to expedite the evaluation and validation of novel biomarkers, thereby providing robust, relevant predictive factors and mechanistic end points for clinical testing of novel agents.


Because of the increasingly complex, multidisciplinary nature of the research required, traditional paradigms of drug development are not well suited to translate molecular advances into clinically useful interventions. Effective collaborative interactions and a consensus approach are essential to the development, validation, and implementation of biomarkers as intermediate and surrogate end points. The effort can be modeled after other precompetitive consortia (e.g., the Human Genome Project, Mouse Models of Human Cancer Consortium, Cancer Genome Anatomy Project, Single Nucleotide Polymorphism Consortium, and the Avon Foundation).

Key goals of the enterprise include the following: (a) to advance research in methodology and validation; (b) to facilitate information and technology flow among different sectors and partners; (c) to encourage integration of complementary efforts such as diagnostics and therapeutics; (d) to establish consensus...
regarding standards, processes, and validation; and (e) to develop frameworks for using biomarkers in clinical trials.

Ongoing Clinical Research Offers Critical Opportunities for the Development and Validation of Biomarkers

This essential activity should be encouraged by funding directed specifically to the incorporation of correlative biomarker evaluation studies in Phase I, II, and III oncology drug trials. Funding should be drawn from pharmaceutical companies as well as government agencies such as the NIH. New mechanisms of funding should be established to foster interdisciplinary research in this area.

Biomarker-Based Drug Development Is a Proven, Successful Strategy in the Development of Novel Anticancer Drugs

There are now multiple examples of biomarkers providing key rationale and end points in the development of successful molecular targeted agents; also, there are many examples in which further mechanistic insight is required to fully exploit important new therapeutic approaches, including the following examples.

Imatinib. This molecular targeted agent is highly efficacious in two malignancies (CML and gastrointestinal stromal tumor) that are difficult to treat with conventional modalities. The well-established BCR-ABL translocation in CML provided a biomarker and a therapeutic target for this rationally designed small molecule. In clinical trials of imatinib, assessment of biomarker-based responses (i.e., molecular as well as hematological remissions) facilitated proof of clinical benefit in CML and is now used in clinical decision-making. c-KIT expression provided a rationale for use of imatinib in gastrointestinal stromal tumors, and clinical trials showed substantial anticancer effect. Additional studies indicating differential activity of imatinib based on particular Kit mutations suggest that biomarker-based patient selection may be useful for imatinib therapy.

Trastuzumab. This humanized MAb was derived based on the oncogenic role of HER2, including studies showing frequent amplification and overexpression of HER2 in breast cancer and the role of HER2 as negative prognostic factor. Clinical development of trastuzumab initially focused on HER2-overexpressing breast cancer patients because this phenotype was closely linked to treatment response in preclinical studies. Patient selection based on HER2 overexpression was critical to demonstration of efficacy. Biomarker-based patient selection by IHC has since been supplemented by HER2 gene amplification as detected by fluorescence in situ hybridization.

EGFR Inhibitors. EGFR, which is commonly overexpressed in many major cancers, has served as a target for a number of antibody- and small molecule-based inhibitors. To date, extensive clinical testing has yielded evidence of clinical benefit in certain settings, but not in others. Gefitinib received accelerated approval based on efficacy in second- or third-line monotherapy of advanced NSCLC but failed to show significant benefit in combination with first-line chemotherapy in the same disease. Efforts to develop useful biomarkers predictive of response to EGFR inhibitors include factors both upstream (e.g., EGFR ligands) and downstream (e.g., extracellular signal-regulated kinase 1/2, phosphatidylinositol 3'-kinase, and Akt) of EGFR. Efficacy may also be influenced by the mechanism of inhibition, other agents used in combination, and stage of cancer; these questions underscore the need for early indicators of response.

Angiogenesis Inhibitors. Antiangiogenesis agents such as the anti-VEGF MAb bevacizumab have shown variable efficacy in different tumor types. To identify those patients most likely to benefit from this and related agents, efforts to develop biomarkers to assess and monitor tumor angiogenesis are in progress; these include assays of ligand activation and signaling, detection of circulating endothelial cells and precursors, and dynamic imaging strategies. As an example, Herbst et al. (108) have recently used molecular imaging of blood flow to monitor the clinical antiangiogenic activity of human recombinant endostatin.

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Mechanism-Driven Oncology Drug Development

Clinical Cancer Research

Rationale for Biomarkers and Surrogate End Points in Mechanism-Driven Oncology Drug Development

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