From Bench to Bedside: Lessons Learned in Translating Preclinical Studies in Cancer Drug Development

Christopher H. Lieu, Aik-Choon Tan, Stephen Leong, Jennifer R. Diamond, S. Gail Eckhardt

The development of targeted agents in oncology has rapidly expanded over the past 2 decades and has led to clinically significant improvements in the treatment of numerous cancers. Unfortunately, not all success at the bench in preclinical experiments has translated to success at the bedside. As preclinical studies shift toward defining proof of mechanism, patient selection, and rational drug combinations, it is critical to understand the lessons learned from prior translational studies to gain an understanding of prior drug development successes and failures. By learning from prior drug development, future translational studies will provide more clinically relevant data, and the underlying hope is that the clinical success rate will improve and the treatment of patients with ineffective targeted therapy will be limited.


Because standard chemotherapy demonstrates limited efficacy against a range of adult solid tumor malignancies, there has been an impetus toward the development of targeted agents in oncology. Likewise, there has been a shift of translational research away from simple screening studies of activity in preclinical models toward studies that define proof of mechanism, patient selection, and rational drug combinations. These strategies are substantially changing the preclinical rationale used to drive clinical development. Although these more robust preclinical studies have successfully guided the development of targeted agents in several tumor types, not all success at the “bench” has translated to success at the “bedside.” As preclinical models become more sophisticated, translational studies of targeted agents will have the potential to produce more clinically relevant data not only to guide “go/no-go” decisions but also to investigate resistance pathways and rational drug combinations. This review will provide examples of lessons learned from prior preclinical studies used in the development of targeted agents and addresses strategies moving forward.

Epidermal Growth Factor Receptor Targeted Agents

One of the most broadly active classes of targeted agents for solid malignancies has been the development of small molecule tyrosine kinase inhibitors (TKIs) and monoclonal antibodies against the epidermal growth factor receptor (EGFR). EGFR overexpression and activation is common in epithelial cancers (1,2), and the efficacy of targeting this pathway was initially demonstrated preclinically in vitro by blocking epidermal growth factor–stimulated phosphorylation of membrane receptors, leading to inhibition of tumor cell proliferation among a range of cancer types (3–6). These results were then recapitulated in a diverse array of xenograft models, leading some to speculate whether this would be the first example of “pathway targeting” versus “disease targeting” as a strategy for clinical development (7–12). Interestingly, early research suggested that the number of EGFRs was not an important determinant in the efficacy of antibody-mediated EGFR blockade because efficacy against T222 (non–small cell lung cancer [NSCLC], squamous) or A431 (vulvar squamous carcinoma) cells was comparable despite an approximately 100-fold higher number of EGFRs in the A431 cells (8). In colorectal cancer (CRC), preclinical studies indicated that antibodies directed against EGFR would be effective and that the addition of cetuximab to irinotecan-refractory CRC tumors could “resensitize” them to irinotecan, resulting in greater efficacy with the combination over cetuximab alone (13–15). These studies were largely reiterated clinically in CRC, where single-agent treatment with cetuximab or panitumumab resulted in improved overall and progression-free survival and a randomized phase III study of cetuximab in combination with irinotecan vs cetuximab monotherapy revealed improvements in these same measures in patients receiving the combination (Figure 1) (16–18). Interestingly, when cetuximab was initially approved for the treatment of CRC, it was only indicated for patients with tumors exhibiting overexpression of the EGFR. However, when investigators retrospectively analyzed the tumors of patients receiving cetuximab monotherapy or cetuximab in combination with irinotecan with EGFR-negative CRC, major objective responses were observed, suggesting that these patients had the potential to respond to EGFR-based antibody therapy (19). Similar results were observed with panitumumab, with no statistically significant difference seen in overall response rate,
progression-free survival, or overall survival between patients with low/negative EGFR and patients with high EGFR (20). The lack of correlation between EGFR overexpression and response to EGFR antibodies was supported by scant data in preclinical models but suggested the opposite of what was considered to be common sense, indicating that the application of patient-selection biomarkers should be more comprehensively studied in preclinical models and/or that clinical trials should incorporate biomarker-negative subsets (21–23). However, as discussed below, such rules may be less stringent when targeting pathways that appear to be critical drivers in disease subtypes.

Unfortunately, the erroneous application of EGFR immunohistochemistry was not the only mistake made in EGFR antibody development in CRC. After the publication of large randomized trials, several retrospective studies revealed that patients whose tumors had a mutation in KRAS derived no benefit from therapy with cetuximab or panitumumab, leading to subsequent revision of the marketing label for both agents (24–28). Although there was a strong scientific rationale for activation of the RAS/RAF/MEK pathway to mediate resistance to upstream EGFR blockade, the limited preclinical studies investigating this were suggestive but not conclusive before the launch of large trials in unselected patients (29–33). More recently, a preclinical phase II trial of patient-derived human tumor xenograft models treated with cetuximab confirmed the key role of KRAS mutation in cetuximab resistance, suggesting that more extensive evaluation in relevant preclinical models may have prevented (or perhaps limited) the treatment of patients unlikely to attain benefit from EGFR inhibitors (Figure 2) (34).

Similarly, the discovery that EGFR mutation predicts clinical benefit from EGFR TKIs in patients with NSCLC was again initially observed retrospectively from clinical samples obtained from patients with gefitinib-responsive lung cancer and then subsequently corroborated in preclinical models (35,36). Consequently, substantial advancements in EGFR targeting of NSCLC have been made by acquiring tissue from patients developing resistance, developing preclinical models that mimic clinical resistance mechanisms, and using these models to develop new inhibitors, a true “bedside-to-bench-and back” approach (37–41). For example, in the approximately 50% of patients with EGFR-mutant lung cancers who develop acquired resistance through mutation of the T790M gatekeeper threonine residue, second-generation irreversible EGFR inhibitors have been shown in preclinical models to be more potent in targeting the T790M mutant than either gefitinib or erlotinib, whereas further studies of one of these, BIBW-2992, in combination with cetuximab, demonstrated that only the combination induced dramatic shrinkage of erlotinib-resistant tumors harboring the T790M mutation.

**Figure 1.** Preclinical studies investigating epidermal growth factor receptor inhibition in colorectal xenografts correlated with improved patient outcomes. A) Growth inhibition of a preclinical study of CPT-11 refractory colorectal tumor xenografts in nude mice. Mice with established DLD-1 (a) or HT-29 (b) tumors were treated with two cycles of CPT-11 therapy (100 mg/kg) on days 0 and 7. Mice with tumors that did not respond to CPT-11 therapy (defined as >2x initial tumor volume at day 12; shown as dotted vertical line) were selected, randomized, and then treated with IMC-C225 at 1 mg/dose/every 3 days (•), continued CPT-11 at 100 mg/kg/week (□), or received combination therapy (▪). Bars represent standard error (14). B) Time to disease progression in two study groups on a phase III clinical trial investigating cetuximab as monotherapy or in combination with irinotecan, in patients refractory to irinotecan (18). Reprinted with permission from the American Association for Cancer Research and the New England Journal of Medicine.
through depletion of phosphorylated and total EGFR (42). Given the promising preclinical results, this work is now translating back to the bedside, with an ongoing clinical trial of the combination of BIBW-2992 and cetuximab in patients with unresectable NSCLC (Figure 3).

A very surprising clinical result that was not accurately predicted in preclinical models was the combination of EGFR and vascular endothelial growth factor (VEGF) inhibitors. Among preclinical studies suggesting promising activity were those in CRC and NSCLC, where the combination of cetuximab or gefitinib with the anti-VEGF receptor antibody DC101 led to striking supra-additive–to–synergistic tumor growth inhibition in CRC and NSCLC models, respectively (43, 44). Despite these and numerous other studies of similar combinations (45–48), the clinical results were disappointing, and in a randomized phase III study in advanced CRC of chemotherapy and bevacizumab vs the same with panitumumab, the combination of the two targeted agents resulted in increased toxicity and decreased progression-free survival, with similar results when cetuximab was added to bevacizumab and chemotherapy, despite a promising randomized phase II trial (27, 49). In NSCLC, vandetanib, a small molecule TKI of VEGFR2, EGFR, and RET induced sustained tumor regressions in an EGFR TKI-resistant mouse model in vivo, and the combination of bevacizumab and erlotinib demonstrated similar efficacy (50). However, a clinical study of vandetanib failed to meet its primary objective of demonstrating an overall survival benefit, and another phase III clinical trial of bevacizumab plus erlotinib vs erlotinib alone revealed no difference in overall survival (51, 52). The lack of concordance between the preclinical and clinical studies is likely multifactorial and due to issues such as tumor heterogeneity, inability to accurately predict clinical toxicity in murine models, pharmacokinetic interactions, and overestimation of angiogenesis dependence in preclinical models, among others.

Figure 2. Preclinical studies in patient-derived human tumor xenograft (PDTX) models correctly predict lack of response to epidermal growth factor receptor (EGFR) antibodies in KRAS-mutant colorectal cancer (CRC). Results from a retrospective analysis evaluating the effect of KRAS mutational status on overall survival in patients treated with cetuximab (A, B) and results from a PDTX trial evaluating the effect of KRAS mutational status on overall survival in PDTX patients treated with cetuximab (C, D) (24). KRAS-mutant PDTX patients treated with cetuximab had similar survival curves compared with control (CTRL) PDTX patients (C). KRAS wild-type PDTX patients treated with cetuximab had statistically significantly improved survival compared with control PDTX patients (D). The results from the xenopatient trial mirror the results seen clinically, suggesting that the lack of efficacy of EGFR monoclonal antibodies in KRAS-mutant CRC could have been predicted preclinically (34). Reprinted with permission from the New England Journal of Medicine and the American Association for Cancer Research.
In advanced pancreatic cancer, for which erlotinib is approved in conjunction with gemcitabine, data from preclinical studies were underwhelming with regard to preclinical study endpoints and the number of models tested. In one preclinical study, an EGFR TKI (PKI166) was studied in the L3.6pl pancreatic orthotopic cell line xenograft model, and the results demonstrated a 45% and 59% reduction in tumor volume by PKI166 or gemcitabine, respectively, but an 85% reduction with the combination (53). Perhaps more relevant to the clinical trials, in a study of erlotinib as monotherapy or in combination with gemcitabine and wortmannin against patient-derived pancreatic cancer xenografts, a twofold increase in apoptosis in tumors treated with gemcitabine, wortmannin, and erlotinib was observed relative to the vehicle control (54). It should be noted that only two patient-derived xenografts were evaluated, tumor growth inhibition was not measured, and the combination of gemcitabine and erlotinib in one of the xenografts did not increase apoptosis in the absence of wortmannin. Despite the lack of robust preclinical data, a phase III clinical trial of the combination of gemcitabine and erlotinib vs gemcitabine alone in patients with advanced pancreas cancer resulted in an increase in overall survival of just 0.33 months, or roughly 10 days (55). The scientific literature is replete with negative phase III trials of novel agents in combination with gemcitabine in pancreatic cancer, the majority of which were preceded by preclinical studies (56–60). Numerous reviews have been written proposing biological mechanisms for this discordance, including poor tumor penetration in patients, inability to adequately recapitulate stromal effects in preclinical models, and tumor heterogeneity (61–63). Future preclinical studies will need to address these issues to improve the preclinical rationale needed for future clinical trials in pancreatic cancer.

Finally, an important question that has been investigated preclinically and clinically is whether monoclonal antibodies and small molecule TKIs directed toward EGFR share similar mechanisms of action in preventing ligand-induced receptor activation and blockade of EGFR-dependent pathways that could make them interchangeable in multiple disease types. Preclinical studies have shown that some of the mechanisms of action and their antitumor effects are not completely overlapping. Anti-EGFR monoclonal antibodies, but not EGFR TKIs, have the capacity to form receptor-containing complexes that result in receptor internalization, an important mechanism for attenuating receptor signaling (64). In addition, anti-EGFR monoclonal antibodies can elicit antibody-dependent cellular cytotoxicity, which increases antitumor efficacy (65). In contrast with anti-EGFR monoclonal antibodies, low-molecular-weight EGFR TKIs can induce the formation of inactive EGFR homodimers and EGFR/HER2 heterodimers, which impair EGFR-mediated transactivation of the HER2 tyrosine kinase (66–68). Clinically, EGFR monoclonal antibodies and EGFR TKIs have had different activity profiles, particularly with the lack of efficacy seen with EGFR TKIs in CRC, which is most likely due to the low incidence of mutations in the ATP site of the EGFR tyrosine kinase domain (0.34%) (69–71). These data highlight the importance
of fully understanding mechanisms of action, even when drugs target similar pathways, because different classes of drugs may lead to clinical development in different tumor types.

**Lessons Learned From EGFR Inhibition**

Overall, preclinical studies of EGFR inhibitors have largely predicted clinical benefit in the malignancies for which they are approved—CRC, NSCLC, head and neck cancer, and pancreatic cancer—although patient selection strategies have been discovered retrospectively, thereby exposing thousands of patients to ineffective and potentially toxic treatments. Current studies suggest that patient-derived human tumor xenografts would have been able to accurately predict the lack of efficacy of EGFR monoclonal antibodies in KRAS-mutant CRC, and increasingly these models are being used to test molecular hypotheses of response, although clearly they have their own limitations (72–77). The lack of correlation between EGFR overexpression and response to EGFR inhibitors also highlights the importance of comprehensively evaluating patient selection biomarkers in the preclinical setting and not overinterpreting scant preclinical data before clinical implementation. The disparate results from preclinical and clinical studies of EGFR inhibition in pancreatic cancer have also confirmed the long-held observation that preclinical activity overestimates clinical benefit, which can be because of multiple factors, including tumor heterogeneity, microenvironmental factors, and unrealistic approximations of drug exposure (8,53,78,79). Lastly, two common sense assumptions have been refuted in preclinical and clinical models—that of the ability to select patients for EGFR pathway-based therapy independent of disease subtype and the notion that blockade by either small molecule EGFR TKI or antibody targeting is equivalent in diseases where one or the other has been clinically benchmarked.

**Targeted Therapies Against Drivers of Oncogenesis**

A highly successful strategy in targeted drug development in oncology has been with agents that target critical mediators of oncogenesis (80–83). One of the earliest examples was trastuzumab, a monoclonal antibody directed against the Her2/neu receptor that is currently used for the treatment of breast and gastric cancer. Early data in cell lines revealed that the Her2/neu pathway was important in tumorigenesis, and amplification of the Her2/neu oncogene was associated with neoplastic transformation and a high rate of tumor cell proliferation (84–86). Clinical data also supported the importance of amplification of Her2/neu because its presence was inversely correlated with the median overall survival of breast cancer patients (87). These observations led to the subsequent development of antibodies directed against the receptor, with in vitro and in vivo studies conducted more than a decade ago revealing activity both as a single agent and in combination with chemotherapy (86,88–91). Importantly, these early preclinical studies demonstrated a direct association between Her2/neu overexpression/amplification and reversal of the malignant phenotype by antibody blockade (91–93). Many years after the publication of these studies, a phase III trial of trastuzumab with chemotherapy vs chemotherapy alone in patients with metastatic breast cancer overexpressing Her2/neu revealed a longer time to disease progression and overall survival in patients receiving trastuzumab (94). Despite the ensuing controversies surrounding the optimal methodology for documenting Her2/neu overexpression (95–97), as pointed out in the analysis by Simon and colleagues, if this study had been performed in an unselected population, it is very likely that a survival benefit would not have been detected (98). In some respects, the development of trastuzumab is an exemplar of preclinical-to-clinical translation, although interestingly, its use in other diseases where Her2/neu is overexpressed has only been proven clinically effective in gastric cancer, indicating the disease-specific context of such targets (80).

In most gastrointestinal stromal tumors (GIST), the proto-oncogene c-kit has an activating mutation, and in preclinical studies in human GIST cell lines, the receptor TKI imatinib was shown to rapidly and completely eliminate GIST cells containing a c-kit mutation (99). These results were concordant with clinical activity in GIST, as early trials demonstrated promising efficacy in this chemotherapy-refractory disease and a phase III study confirmed a statistically significant survival benefit of imatinib in patients with unresectable CD117-positive GIST (81,100). Bedside-back-to-bench research used patient samples from a phase II clinical trial of imatinib to correlate mutations to clinical outcome and guide therapy, whereas preclinical studies conducted in GIST cell lines revealed that most mutations in the c-kit activation loop were resistant to clinically achievable doses of imatinib (101). Interestingly, it was in studies of chronic myeloid leukemia (CML) that analysis of the crystal structure of dasatinib-bound abelson leukemia kinase (ABL) kinase suggested that the increased binding affinity over imatinib was at least partially due to its ability to recognize multiple states of breakpoint-cluster gene, abelson leukemia gene (BCR-ABL), including BCR-ABL–activating loop mutants (102). Given this finding in CML, further preclinical studies of dasatinib in GIST cell lines were performed, revealing induction of apoptosis in the imatinib-resistant, c-kit-activating loop mutant cell lines (102). These results were translated into ongoing clinical trials evaluating the efficacy of dasatinib in imatinib-resistant unresectable GIST, reflecting, in this case, the relevance of transdisciplinary preclinical research into resistance mechanisms of specific classes of agents (103). Perhaps such basic resistance processes that alter receptor structural biology and drug binding affinity are more readily adaptable from one disease to another, indicating the importance of not only detailed characterization in vitro and in vivo but also multidisciplinary collaboration.

The rapid clinical development of vemurafenib, an inhibitor of BRAF in BRAF-mutated melanoma, although obviously an example of success, has illustrated the importance of preclinical models in assessing the disease-specific activity of targeting mutations as well as resistance mechanisms. Initially, vemurafenib was shown to potently inhibit melanoma cell lines bearing the BRAF V600E mutation but not in cells lacking oncogenic BRAF, in vitro and in vivo (104–106). These preclinical results were recapitulated clinically, as vemurafenib was found to statistically significantly increase overall survival in patients with BRAF V600E mutant metastatic melanoma when compared with standard chemotherapy (82). Although scant preclinical data existed on the activity of vemurafenib in BRAF-mutant CRC, single-agent vemurafenib was
administered in a phase I extension trial of patients with previously treated metastatic CRC with only one confirmed partial response among 19 evaluable patients, clearly indicating de novo resistance in this disease (107). Subsequently, preclinical studies investigating the activity of vemurafenib in BRAF-mutant CRC have revealed inherent resistance mechanisms involving the c-met, EGFR, and PI3K pathways, among others, suggesting that up-front rational combinations will be required in CRC (108–110).

Another powerful application of preclinical research has been the investigation into the acquired resistance mechanisms that occur fairly rapidly in patients with BRAF V600E–mutant metastatic melanoma receiving single-agent vemurafenib. Although dramatic pictures of a patient initially responding and then developing dramatic resistance to vemurafenib have been widely disseminated, importantly, this devastating outcome has been investigated comprehensively in preclinical models using patient tissue, cell lines, and next-generation sequencing technology (111–119). One of the most “actionable” resistance mechanisms has been the discovery of MAP kinase pathway reactivation, leading to the preclinical and clinical combination of RAF and MEK inhibitors for advanced melanoma (111,120). In preclinical models, rapid recovery of MAPK pathway signaling was associated with BRAF inhibitor resistance, and complete inhibition of the MAPK pathway induced cell death in BRAF V600E melanoma (111). When the combination of a BRAF inhibitor and a MEK inhibitor was studied in a clinical trial, progression-free survival was statistically significantly improved (121). An interesting dilemma that may be more easily tested in preclinical models is whether dual targeting up-front vs sequential targeting is more effective in combating resistance mechanisms because the clinical trials have focused on the combination vs monotherapy in untreated patients. The differential biological impact of targeting BRAF in melanoma and CRC is a clear example of an instance where more extensive and unbiased preclinical studies could have defined mechanisms of de novo and acquired resistance earlier and hastened the development of effective rational combinations. As it is, groups working in this area rapidly exploited clinical data to investigate resistance mechanisms in preclinical studies, setting the standard for comprehensive translational drug development strategies in oncology (109,112,121,122).

Inhibition of the anaplastic lymphoma kinase (ALK) in lung cancer, although clearly an example of the therapeutic benefit of targeting driver mutations, also illustrates the iterative and nonlinear nature of translational studies in drug development. During its initial preclinical development, crizotinib was identified as an ATP-competitive small-molecule inhibitor of the catalytic activity of c-met kinases that also inhibited ALK at pharmacologically relevant concentrations in lymphoma cell lines expressing the NPM-ALK oncogenic fusion protein (123). Concurrent with phase I trials of the agent, a transforming fusion gene comprising portions of the echoderm microtubule-associated protein-like 4 (EML4) gene and ALK gene was described in 6.7% of NSCLC tumors (124). This finding led investigators to screen and select patients in the latter stages of the phase I trial for the ALK fusions by fluorescence in situ hybridization (FISH), resulting in dramatic tumor regression and disease stabilization when these patients were treated with crizotinib, a result that was ultimately confirmed in a phase II registration trial and a recently published phase III trial (83,125,126). These clinical results were recapitulated mechanistically in preclinical models, whereas experiments in ALK knockout mice performed years earlier corroborated the relative lack of toxicity observed in the clinical trials (127,128).

Finally, the subsequent identification of resistance mechanisms to crizotinib has been an elegant example of the value of “bench-to-bedside-and-back” translational research. Repeat FISH analysis and DNA sequencing characterized the amplification of the ALK fusion gene and mutations in the kinase domain of ALK from patients progressing on crizotinib as the potential molecular mechanisms of resistance (129–131). In vitro NSCLC cell lines selected for resistance to crizotinib also show amplification of the ALK fusion gene as well as kinase domain mutations such as L1196M (130). Data from both resistant patient tumor samples and cell lines have led to rationales for novel second-generation ALK TKIs that inhibit these mechanisms of resistance to now be tested in the clinic.

Lessons Learned in Targeted Therapies Against Drivers of Oncogenesis

The last decade has witnessed an impressive growth of agents directed at drivers of the oncogenic process. By definition, these agents may be easier to study in preclinical models and to develop clinically, although clinical and scientific observations must be exchanged as seamlessly as possible between patients and preclinical models, respectively, to derive the greatest benefit. An important concept is that although acquired treatment-associated mutations may be effectively targeted across tumor types, such as use of dasatinib after imatinib failure in CML and GIST, the drivers of oncogenesis to be targeted may differ among disease sites because of inherent resistance mechanisms. This is clearly illustrated in the striking difference in efficacy of vemurafenib in BRAF V600E–mutant melanoma and BRAF V600E–mutant CRC, with preclinical studies highlighting the differences in resistance mechanisms. This provides an opportunity for investigators to interrogate disease-specific banks of cell lines and patient-derived tissues for the presence of drivers and the functional impact of targeting them and to assess resistance mechanisms and develop rational targeting strategies. In fact, this shift of preclinical research away from drug efficacy screening and toward molecular and functional studies of responsiveness or resistance is paving the way for the future of translational research. The ALK story with crizotinib also highlights the importance of early identification and rapid translation of novel targets, so that even in phase I trials patients can be screened for rare genetic drivers.

Targeting Angiogenesis

An important obstacle to the development of antiangiogenic agents has been the lack of suitable preclinical models for assessing their efficacy. In fact, although there have been clear clinical successes (132–135), these agents have been tested in numerous phase III trials with negative results, often based upon either nonexistent or misleading preclinical data (136–138). Clearly, in vitro assays, although perhaps useful for screening, do not recapitulate angiogenesis in vivo because of the absence of critical factors such as host- and tumor-derived microenvironmental factors (139). In vivo, one of the earliest metrics for assessing antiangiogenic effects preclinically
was the use of microvessel density in murine models (140–142). After about a decade of attempts at clinical translation with poor results (Figure 4), the assessment of microvessel density in cancer patients as a pharmacodynamic marker of angiogenesis inhibition was finally abandoned, and its demise was the subject of an elegant review by Dr Judah Folkman (143). More recently, functional imaging studies dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) have been assessed in murine models and in patients and appear to be a valid translatable pharmacodynamic endpoint for assessing the effects of VEGF blockade, although it is less clear for other angiogenic targets (144–146). Figure 5 depicts the results of a bench-to-bedside study of DCE-MRI after treatment of mice and patients with G6-31 (see below) or bevacizumab, respectively (147).

A limitation to the preclinical testing of antibodies that target angiogenic growth factors in murine models has been the presence of both murine and human tumor–associated angiogenic growth factors. Thus, bispecific murine/human antibodies must be developed, a process that has often lagged behind the clinical development of these agents. For example, Genentech developed their bispecific murine/human monoclonal antibody against VEGF A/B, G6-31, available to investigators in 2006 for preclinical studies, after bevacizumab had completed positive randomized phase II and phase III trials in CRC (132,148). Before that, most preclinical studies were conducted with either bevacizumab (ignoring the murine stromal VEGF) or DC101, an antibody against the murine VEGFR2 receptor. Nonetheless, the activity of antibodies targeting the VEGF pathway was quite robust in preclinical models of CRC and breast cancer, among others, despite the fact that single-agent activity is very limited in patients (149,150). This has prompted closer scrutiny of human xenograft models, leading to the conclusion that the exaggerated antitumor efficacy is most likely due to rapid tumor and vascular growth in these models that results in greater dependence on limited angiogenesis growth factors and thus responsiveness to antiangiogenic therapy (151–153).

An interesting transgenic model that has been clinically benchmarked recently is the rat insulin promoter (RIP)–T-antigen transgene (Tag) model developed by Hanahan and colleagues in which the RIP directs expression of the SV40 large Tag to beta cells of the pancreatic islets (154,155). This model was designed to avoid the confounding biases of neutralizing antibody responses by developing an immunodeficient variant, rendering it completely deficient in adaptive immunity. This allowed the investigators to demonstrate that B and T lymphocytes were not factors in pancreatic islet tumorigenesis. In this study, the inhibition of VEGFR2, but not VEGFR1, markedly disrupted the angiogenic switch, angiogenesis, and initial tumor growth (156). In late-stage tumors, phenotypic resistance to VEGFR2 blockade emerged, and potential resistant mechanisms were discovered, including induction of the proangiogenic fibroblast growth factor. Further investigation with sunitinib, a TKI of primarily VEGFR2, VEGFR3, c-kit, and PDGFR, was also shown to be effective in inhibiting growth in the RIP-Tag model, and a phase III clinical trial of sunitinib in patients with advanced, well-differentiated pancreatic neuroendocrine tumors demonstrated an improvement in overall response rate, progression-free survival, and overall survival (157,158). It is also important to note that efficacy of sunitinib was not observed across multiple tumor types, despite the elegant work performed in the RIP-Tag model. A phase II trial of sunitinib in patients with metastatic refractory CRC was completed despite a relative lack of preclinical evaluation, and the trial failed to demonstrate a meaningful single-agent objective response in patients (159).

Another limitation of preclinical evaluation is the inability to accurately predict the toxicity of a particular agent, especially in combination with other chemotherapeutic agents. Preclinical evaluation of the TKI vatalanib, an inhibitor of VEGFR-1/Fit-1, VEGFR-2/KDR, VEGFR-3/Fit-4, PDGF-β, c-Fms, and c-kit (but with greater potency for VEGFR-1 and VEGFR-2), revealed tumor growth and angiogenesis inhibition in cell line-derived xenografts, but phase III evaluation of the combination of FOLFOX4 and vatalanib resulted in no statistically significant improvement in response rate, progression-free survival, or overall survival (160,161). Of note, there were clinically significant adverse events

**Figure 4.** Preclinical microvessel density studies have not accurately predicted clinical efficacy with angiogenesis inhibitors. **A** A preclinical study of vatalanib (PTK787) in H1975 non–small cell lung cancer (NSCLC) xenografts exhibits statistically significant changes in tumor microvessel density index (MDI), tumor vessel size (VSI), and apparent diffusion coefficient (ADC). The **blue bars** represent vehicle-treated tumors, and the **red bars** represent vatalanib-treated tumors (224). **B** Kaplan–Meier plots for progression-free survival (PFS) in patients with NSCLC treated with vatalanib in a phase II clinical trial demonstrating a median PFS of 2.1 months (225). Reprinted with permission from PLoS One and Oxford University Press.
associated with this combination, reflected by an 18.1% study discontinuation rate because of toxicity (161).

Extensive reviews have described the transitory nature of the clinical benefit from angiogenesis inhibitors due to evasive resistance and adaptation to circumvent angiogenic blockade (151,152). Bedside-to-bench investigation is ongoing in an effort to target known resistance mechanisms, identify predictive markers of response, and develop combination regimens that synergize with VEGF inhibitors (136,162–164). Recently, evaluation of the role of placental-growth factor (PlGF) has led to a potential improvement in angiogenic therapy in CRC. PlGF has been shown to be upregulated in response to anti-VEGF therapy in both preclinical models and prospective evaluation in patients treated with anti-VEGF therapy (162,163,165). Results from a recent phase III trial of a dual-inhibitor of PI/GF and VEGF-A in patients with refractory metastatic CRC demonstrated that continued blockade of VEGF family members confers a modest clinical benefit in patients with acquired resistance to anti-VEGF therapy, but because of the study design it is currently unclear whether patients were benefitting from continued VEGF inhibition, inhibition of PI/GF, or both (166).

Lessons Learned From Angiogenesis

The clinical benchmarking of the first angiogenic agents followed decades of elegant preclinical studies that likely overstated their single-agent activity and led to numerous failed clinical trials. Preclinical studies investigating the combination of chemotherapy and antiangiogenic agents were also lacking before clinical trials. Since then, much has been learned about the process of angiogenesis and the inherent bias that resides in preclinical models.

Nonetheless, progress has been made with the development of more robust and translational pharmacodynamic markers of activity and with murine models that are designed to mimic specific disease subtypes. Going forward, there will continue to be challenges with effectively assessing the activity of these agents in humans using preclinical models, and this will continue to be complicated by the lack of predictive biomarkers for selecting patients. However, these obstacles should not hinder development of angiogenesis inhibitors but should stimulate more relevant model development and further research into predictive and pharmacodynamic biomarkers.

Targeting Basic Cellular Processes

Another focus in targeted cancer therapy research has been the inhibition of basic cellular processes that appear to be particularly critical for maintenance of the malignant phenotype, such as protein processing and epigenetic gene regulation (167–170). Many investigators have focused on the ubiquitin–proteasome pathway, where inhibition of the degradation of cell cycle regulatory proteins by proteasome inhibition can induce apoptosis (167,171,172). Bortezomib, the only proteasome inhibitor approved for therapy, demonstrated activity against preclinical models of multiple myeloma in vitro and in vivo and in patient-derived multiple myeloma cells (172–175). The mechanism of its activity in multiple myeloma is thought to be partially due to blocking of NF-κB–mediated transcription of interleukin 6 and insulin-like growth factor 1 by inhibition of the proteasomal degradation of IκB (172,173). The preclinical antitumor and mechanistic studies of bortezomib in multiple myeloma were successfully translated clinically, as reflected in the phase I trial of bortezomib in patients with
refractory hematologic malignancies, which demonstrated clinical activity in nine patients with multiple myeloma comprised of one complete response and eight patients with a reduction in paraprotein levels and/or marrow plasmacytosis, as well as in a phase III clinical trial of bortezomib vs dexamethasone (Figure 6) (176,177). Likewise, subsequent studies in pediatric tumors demonstrated preferential activity against pediatric acute lymphocytic leukemia models vs solid tumors, which was subsequently recapitulated clinically, where the combination of bortezomib and induction chemotherapy resulted in striking activity in pediatric patients with relapsed B-precursor acute lymphoblastic leukemia (178,179).

Surprisingly, despite the marked preclinical in vivo activity documented against an array of solid tumors, the clinical activity of bortezomib has been unremarkable both as a single agent and in combination (168,180–184). One major disappointment was in pancreatic cancer, for which, in preclinical studies, bortezomib demonstrated activity both in vitro and in vivo and was hypothesized to reverse NF-κB–mediated chemotherapy resistance (168,181,185,186). After the failure of bortezomib in a phase II trial in patients with metastatic pancreatic cancer, a repeat investigation of bortezomib and gemcitabine in an orthotopic pancreatic adenocarcinoma mouse model revealed tumor inhibition with gemcitabine but tumor promotion with bortezomib by induction of angiogenesis (187). Additionally, other studies have highlighted the difficulties of achieving adequate and consistent exposure with bortezomib in xenograft models, whereas in patients with solid tumors, there is concern that the agent does not achieve sustained exposure to modulate proteasomal targets (188–190). The challenges of bortezomib in solid tumors have illustrated the importance of pharmacology and accurate approximation of human exposure in preclinical models, particularly when transitioning from hematologic to solid malignancies, as well as the difficulty of precisely establishing target modulation with agents that exhibit variable context-dependent effects (187–189).

A somewhat similar scenario in nonhematological malignancies has been observed with the development of the histone deacetylase (HDAC) inhibitors, epigenetic modulators that have protean effects on cells but in cancer are thought to potentially de-repress cell cycle regulatory genes and nuclear receptors such as estrogen receptor α (191–193). The first HDAC inhibitor approved for clinical use, vorinostat, exhibited in preclinical models a broad spectrum of epigenetic activity as well as efficacy against a range of solid tumors in vitro and in vivo, particularly in combination with other agents (194–196). However, in this case, the activity in cutaneous T-cell lymphoma, for which both vorinostat and romidepsin are approved, was identified serendipitously in a phase I trial, with subsequent confirmation in single-arm phase II trials with 30% and 34% objective response rates, respectively (197,198). Although investigations into the mechanisms of actions are ongoing, preclinical investigations in cutaneous T-cell lymphoma cell lines have shown that vorinostat induces an accumulation of acetylated histones, an increase of p21<sup>WAF1</sup> and bax, a decrease of STAT6, and activation of caspase 3 (199). Similar findings have been seen with romidepsin (200). Similar to bortezomib, a large number of clinical studies of vorinostat with a variety of agents were launched, often with proposed mechanisms that were worked out both in vitro and in vivo (201–207). Additive or synergistic effects against tumor cell growth have been reported in numerous preclinical studies in multiple solid tumors where vorinostat was combined with chemotherapy or targeted agents, but several clinical studies have failed to successfully build upon the preclinical results (205,208,209).

Hypotheses for the lack of efficacy seen in certain tumor types include the difficulty of achieving intratumoral therapeutic effect of vorinostat in CRC (208), inability to exert synergistic cytotoxicity within the central nervous system in recurrent glioblastoma multiforme (205), and limited drug exposure because of poor tolerability (210). More recent studies have investigated improved patient selection and predictive markers using a bedside-back-to-bench approach, highlighted by a phase II study of vorinostat combined with tamoxifen that demonstrated that histone hyperacetylation and HDAC2 expression may be viable pharmacodynamic and predictive biomarkers, respectively (211). A recent randomized phase II study investigating the HDAC inhibitor entinostat in combination with the EGFR TKI erlotinib in NSCLC revealed no additional efficacy, whereas a preplanned biomarker analysis revealed that the subset of patients with EGFR wild-type tumors and high e-cadherin protein expression exhibited statistically significantly longer overall survival (12.2 months vs 5.4 months; <i>P = 0.03</i>) (212).

**Figure 6.** Preclinical studies of bortezomib in multiple myeloma cell lines accurately predicted benefit in patients with multiple myeloma. **A** Preclinical study of bortezomib vs dexamethasone against multiple myeloma cell lines. Dexamethasone in control media (●) and with bortezomib 0.0025 (△) or 0.005 (●) × 10<sup>−6</sup> M. The preclinical study demonstrates statistically significant activity of bortezomib compared with dexamethasone (172). **B** Progression-free survival in a phase III clinical trial of bortezomib vs dexamethasone in patients with multiple myeloma. A statistically significant increase in progression-free survival was seen in patients receiving bortezomib compared with patients receiving dexamethasone (177). Reprinted with permission from the American Association for Cancer Research and the New England Journal of Medicine.

jnci.oxfordjournals.org
Lessons Learned From Targeting Basic Cellular Processes

Somewhat surprising has been the clinical approval and robust activity of agents impacting a range of targets in normal and malignant cells in a rather nonselective manner, providing support for continued preclinical work to identify and characterize such challenging targets. The ability to clinically translate the success of these agents from hematologic malignancies and preclinical models has been hampered by pharmacologic limitations and difficulties in adequately predicting drug exposure and toxicity. Therefore for success in preclinical models to be translated, comprehensive pharmacokinetic/pharmacodynamic analyses indexed to human exposure need to be included, with the huge caveat that toxicity may be poorly recapitulated in these models. Future clinical trials with these agents will be dependent on bedside-back-to-bench studies that will improve patient selection and biomarker development that will be critical for successful clinical development.

Preclinical Models and Prediction of Pharmacokinetics and Toxicity

Lack of efficacy and clinically significant toxicity are major reasons for targeted therapy failure, and therapeutics with favorable pharmacokinetics are more likely to be efficacious and safe. Thorough preclinical pharmacokinetic evaluation may guide dose selection in the phase I clinical trial setting. Pharmacokinetic endpoints that are shown to correlate with biological activity in preclinical studies may enable safer dose selection of molecularly targeted agents in phase I clinical trials (213). However, the predictive value of preclinical models may be limited by individual human genetic sensitivities, immunologically mediated phenomena, and idiosyncratic reactions (214). Preclinical data may also help formulate clinical trial design.

If the preclinical data suggest a wide therapeutic window with little toxicity, an aggressive dose titration may be reasonable. Imatinib is an example of a targeted agent that demonstrated dramatic efficacy as well as a favorable pharmacokinetic profile before any dose-limiting toxicity was encountered (215). However, if preclinical data suggest a narrow therapeutic window, more conservative phase I clinical trial design would be warranted. There are several approaches by which human pharmacokinetic data can be predicted from preclinical data, and although a comprehensive review of the prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data is beyond the scope of this review, comprehensive reviews of this topic are available in the literature (216).

Limitations in Preclinical Models and Future Directions

As new molecular targets for cancer therapy are discovered and characterized, the ability to efficiently and effectively translate these findings to patients has become rate limiting. In fact, results from internationally collaborative tumor sequencing efforts are yielding an unprecedented array of aberrations, many of which appear to be tractable targets for drug development (217–219). The push toward personalized medicine has now added another facet to preclinical drug development, beyond the usual demonstration of efficacy. Thus, preclinical studies increasingly rely upon larger banks of disease-specific cell lines and tissues that have undergone extensive genetic annotation. The future of drug development likely will take on a bilateral approach, in which drug responsiveness signatures are developed in cell lines and patient-derived xenograft models, concurrent with the molecular categorization of patient tissues (Figure 7). Convergence will occur at the patient exhibiting a...
response profile that is indexed to a molecular bin within a specific disease context. Nonetheless, challenges will remain in categorizing models as responsive or nonresponsive to improve the accuracy of preclinical models in predicting clinical benefit. Rather than discarding the process of preclinical drug development as being hopelessly flawed, the drug development community should strive for establishing appropriate model scenarios, greater stringency, and more guidance for what criteria constitute a sufficient body of data for clinical testing. For agents where a particular disease subtype has been clinically benchmarked, consideration should be given to incorporating a positive control (the tumor type known to respond in a clinical setting) into screens where an agent is being tested for use against other malignancies as an internal standard to gauge relative activity. Additionally, real-time retrotranslation should be conducted using tolerable drug exposures achieved in early clinical trials to determine whether the exposures achieved in humans are sufficient for preclinical efficacy to help guide development decisions because drug exposures in preclinical studies have typically surpassed achievable levels in humans (220–223). Patient-derived tumor xenografts, although arguably a more relevant in vivo model for testing than cell line xenografts, are still subject to the same limitations in terms of the tumor microenvironment and therefore should not be regarded as positive when only modest tumor growth inhibition, not regression, is observed (76). Likewise, combination therapies, where much of drug development is focused, for good reason, will need to excel beyond the standard assessments of antiproliferative synergy toward assays that detect the potentiation of cell death with the combination. This will become paramount as resistance pathways are identified clinically, along with use of tools such as gene set enrichment analysis and synthetic lethality that increasingly are revealing strategies for rational combinations. Although not the subject of this review, another important component of preclinical translation is the ability to design early clinical trials where feasibility testing of efficacy and patient selection strategies can occur (22). This has been the subject of numerous reviews, but suffice it to say that we need to overhaul the design of phase I trials to routinely accommodate disease-specific expanded cohorts, tissue acquisition upon disease progression, and early testing of rational combinations. The lessons learned thus far in preclinical translation of targeted agents in oncology should be viewed as facilitating a roadmap that will avoid the obstacles of the past and provide a way forward in the future.

References

colorectal cancers to anti-epidermal growth factor receptor antibody therapies. 
Cancer Res. 2007;67(6):2643–2648.


panitumumab efficacy in patients with metastatic colorectal cancer. J Clin 


30. McCarthy SA, Samuels ML, Pritchard CA, et al. Rapid induction of hepa-
rin-binding epidermal growth factor/diphtheria toxin receptor expression by 


34. Julien S, Merino-Trigo A, Lacroix L, et al. Characterization of a large panel of 
patient-derived tumor xenografts representing the clinical heterogene-

35. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal 
growth factor receptor underlying responsiveness of non–small-cell lung 

36. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: 

37. Engelma JA, Zeijunlallu K, Mitsudomi T, et al. MET amplification leads to 

38. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance 


40. Gatto C, Rieppi M, Borsotti P, et al. BAY 12–9566, a novel inhibitor of 

versus the matrix metalloproteinase inhibitor BAY 12–9566 in patients with 
advanced or metastatic adenocarcinoma of the pancreas: a phase II trial. 

42. End DW, Smets G, Todd AV, et al. Characterization of the antitumor 
effect of the selective farnesyl protein transferase inhibitor R115777 in 

43. Van Cutsem E, van de Velde I, Karasek P, et al. Phase III trial of gefi-
tinib plus tipifarnib compared with gefitinib plus placebo in advanced 

combined with gemcitabine alone in patients with advanced pancreatic cancer: a phase II trial. 

45. Philip PA, Mooney M, Jaffe D, et al. Consensus report of the National 
Cancer Institute Clinical Trials Planning Meeting on pancreatic cancer 

46. Giuntoli RA, Devi SS, Corcoran E, et al. Synergistic antitumor effects of 
combined epidermal growth factor receptor and vascular endothelial 

47. Ray S, Aronson S, Hughes G, et al. GEF2/p190Arf counteracts the 
activity of the novel vascular-targeting agent ZD6126 in human colo-
rectal cancer and non–small cell lung cancer (NSCLC) xenograft models 

carcinomatosis by antibodies to vascular endothelial and epidermal growth 

49. Hecht JR, Mitchell E, Chidicq T, et al. A randomized phase IIIB trial of 
chemotherapy, bevacizumab, and panitumumab compared with chemofor-
therapy and bevacizumab alone for metastatic colorectal cancer. J Clin 

growth factor receptor and epidermal growth factor receptor (EGFR) 
blockade inhibits tumor growth in xenograft models of EGFR inhibitor 

advanced non–small-cell lung cancer after prior therapy with an epidermal 
growth factor receptor tyrosine kinase inhibitor: a randomized, double-

versus erlotinib alone in advanced non–small-cell lung cancer after failure 
of standard first-line chemotherapy (BeTa): a double-blind, placebo-con-

growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to 
apoptosis of endothelial cells and therapy of human pancreatic carci-

54. Ng SSW, Tsaor M-S, Nicklee T, et al. Effects of the epidermal growth 
factor receptor inhibitor OSI-774, tarceva, on downstream signaling 


56. Pao W, Riely GJ, Yang JC, et al. Epidermal growth factor receptor muta-

57. Pao W, Riely GJ, Yang JC, et al. Epidermal growth factor receptor muta-

58. Pao W, Riely GJ, Yang JC, et al. Epidermal growth factor receptor muta-

59. Pao W, Riely GJ, Yang JC, et al. Epidermal growth factor receptor muta-

60. Pao W, Riely GJ, Yang JC, et al. Epidermal growth factor receptor muta-

61. Pao W, Riely GJ, Yang JC, et al. Epidermal growth factor receptor muta-


Threats to Validity of Nonrandomized Studies of Postdiagnosis Exposures on Cancer Recurrence and Survival

Jessica Chubak, Denise M. Boudreau, Heidi S. Wirtz, Barbara McKnight, Noel S. Weiss

Manuscript received February 12, 2013; revised June 18, 2013; accepted June 19, 2013.

Correspondence to: Jessica Chubak, PhD, Group Health Research Institute, 1730 Minor Ave, Ste 1600, Seattle, WA 98101 (e-mail: chubak.j@ghc.org).

Studies of the effects of exposures after cancer diagnosis on cancer recurrence and survival can provide important information to the growing group of cancer survivors. Observational studies that address this issue generally fall into one of two categories:

1. Threats to Internal Validity
2. Threats to External Validity