Resistance to Targeted Therapies: Refining Anticancer Therapy in the Era of Molecular Oncology

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Abstract The advent of targeted therapy for treatment of human cancers has added significantly to our armamentarium as we strive to prolong patient survival while minimizing toxicity. In cancers driven by a dominant oncogene, targeted therapies have led to remarkable improvements in response and survival, whereas in others the outcome has been more modest. One key aspect toward realizing the potential of targeted therapies is a better understanding of the intrinsic or acquired resistance mechanisms that limit their efficacy. The articles in this *CCR Focus* provide insights into molecular mechanisms of resistance to targeted therapy. Recent discoveries of the molecular pathways that mediate intrinsic resistance to targeted therapy have led to the identification of predictive biomarkers that allow for better patient selection for front line treatment. Equally important, the identification of mechanisms of acquired resistance following front line therapy has led to the discovery of novel agents that overcome these resistance mechanisms. Improving the efficacy of targeted therapies in the future will require expanding our understanding of resistance mechanisms, the development of new generations of rationally designed targeted agents, and translating this information to the clinic to select patients for appropriate therapy. (Clin Cancer Res 2009;15(24):7471–8)

The focus of oncology drug discovery has markedly evolved over the past 10 years from empiric development to one of a rationale approach. Current drug development emphasizes the development of targeted therapeutic agents based on the discovery of genetic alterations in human cancer and the signaling pathways they alter. In 2010, there are numerous targeted therapies that have been approved by the U.S. Food and Drug Administration (FDA) in both solid and hematologic malignancies, but the clinical benefit obtained from targeted therapies varies greatly. In patients with malignancies in which a dominant mutation, gene amplification, or translocation drives tumor growth ("oncogene addiction"), single agent-targeted therapies are highly effective but rarely curative. Examples include cKit mutations in gastrointestinal stromal tumors (GIST), epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC), HER2 amplification in breast cancer, and the BCR-ABL translocation in chronic myelogenous leukemia (CML). However, in most tumors a multiplicity of genetic alterations contributes to malignant growth, and, therefore, the benefit of targeted therapies can be modest and transient. Key to the successful development and application of targeted cancer agents is a better understanding of resistance mechanisms. In most tumor types, patients' tumors, unfortunately, are refractory to targeted therapies (intrinsic resistance). Even if an initial response to targeted therapies is obtained, the vast majority of tumors subsequently become refractory (i.e., acquired resistance) and patients eventually succumb to disease progression. Although we are still very early in the era of targeted therapies and in our understanding of resistance mechanisms, recent basic and clinical research has led to the identification of genetic alterations in tumors, which has enabled selection of appropriate initial targeted therapy for patients, and/or modifications of therapy upon disease progression. More robust and efficient molecular techniques have allowed investigators to interrogate a larger number of genes and proteins with greater speed and accuracy; providing further insights into why some drugs work, but equally important, why some do not.

In this series of *CCR Focus* articles, our current understanding of resistance mechanisms to targeted therapies is summarized for a variety of malignancies and therapeutic approaches (Fig. 1; Table 1). These insightful reviews focus on molecular biomarkers that help investigators identify the molecular phenotype of tumors, which aim to maximize patient benefit for specific targeted therapies.

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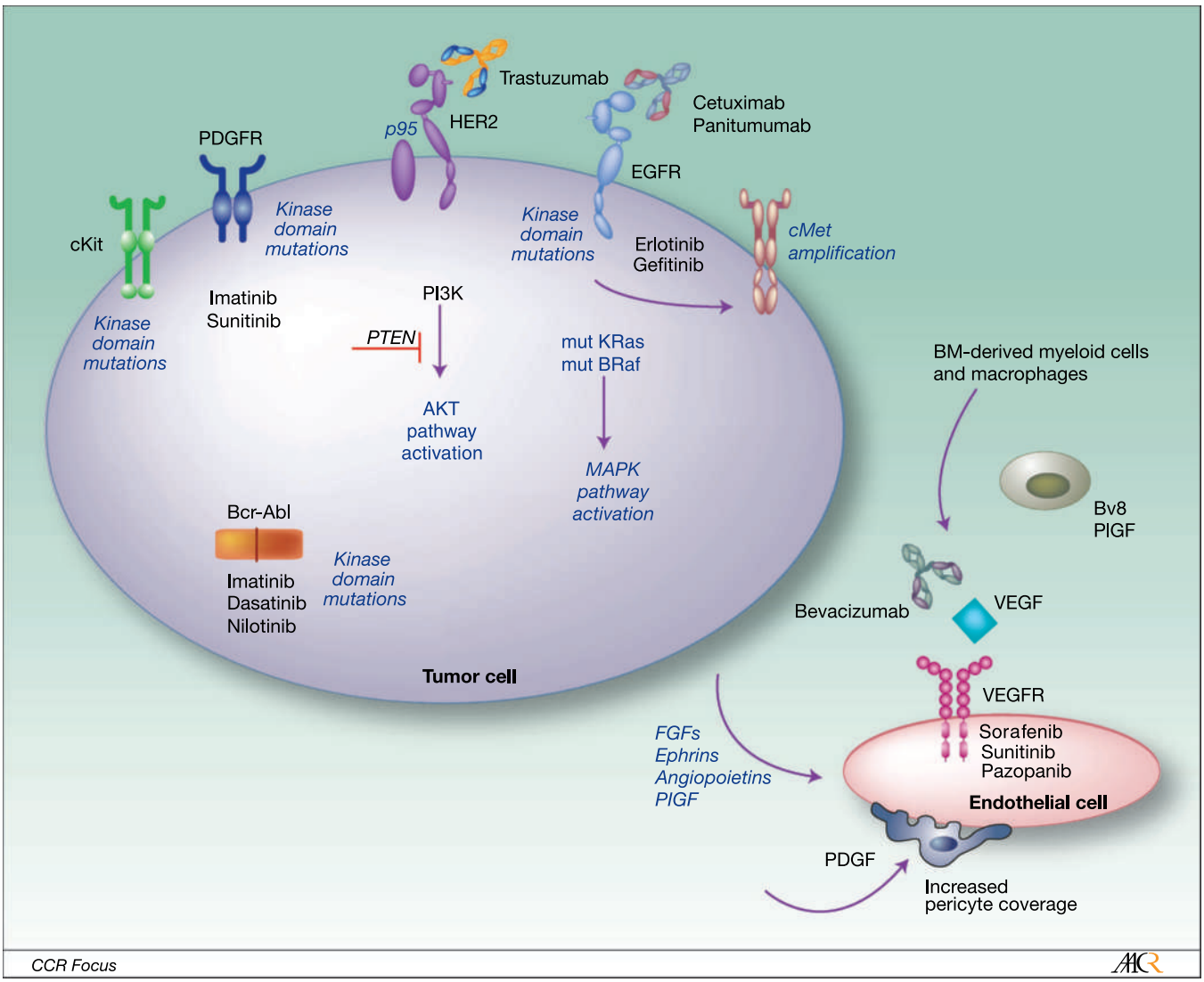

Fig. 1. Overview of resistance pathways to targeted therapies. Current targeted therapies are directed at inhibiting the kinase activity of dominant oncogenes and/or growth factor receptors with either TKIs or mAbs. Resistance in tumor cells may be inherent or acquired. Resistance is likely to be multifactorial. Common acquired resistance mechanisms include the selection of tumor cells with specific kinase mutations rendering drugs incapable of binding to the ATP pocket, and activation of redundant signaling pathways. Inherent resistance to targeted therapies may be due to activation and/or mutation of downstream signaling pathways or mutations in kinase domains, which do not allow access of the drugs to the ATP pocket. Resistance to angiogenic-VEGF-targeted therapies may be due to redundant angiogenic pathways, survival signals from perivascular cells, or myeloid-derived infiltrating cells that secrete pro-angiogenic factors.

Overview

Resistance to HER-2-targeted therapy. The introduction of trastuzumab for HER2-amplified breast cancer has been one of the most successful stories in targeted therapy (1). However, the majority of breast cancer patients who initially respond to trastuzumab eventually experience disease progression while still on therapy within 1 year of treatment initiation. Despite more than a decade of investigation into trastuzumab's mechanism of action, there is still considerable debate about how the drug works and a surprising paucity of clinical validation for potential mechanisms for resistance. Pohlmann and colleagues (2) have reviewed the mechanisms for trastuzumab's activity including down-regulation of HER2 signaling and stimulation of FcR-mediated immune effector mechanisms. Potential mechanisms of resistance to trastuzumab therapy include: (1) prevention of trastuzumab binding to HER2, (2) up-regulation of signaling pathways downstream of HER2, (3) up-regulation of alternative growth factor receptor-signaling pathways, and (4) inhibition of immune-mediated mechanisms. The majority of these resistance mechanisms was identified in preclinical studies and has yet to be validated clinically. However, two recent clinical-translational studies highlighted by Pohlmann and colleagues are worth noting. In one study Scaltriti and colleagues (3) found a significant inverse relationship between expression of p95HER2 (a truncated form of HER2 that trastuzumab does *not* bind) and trastuzumab clinical response. Importantly, p95HER2 is expressed at high frequency (~60%) in

HER2-overexpressing breast cancer. This result may provide one explanation why HER2 tyrosine kinase inhibitors (TKI) such as lapatinib are effective in HER2-overexpressing tumors that become resistant to trastuzumab therapy. In another study by Nagata and colleagues (4), PTEN loss was associated with resistance to trastuzumab therapy, highlighting the importance of resistance that may be mediated by activated downstream signaling pathways, common to several targeted therapies. Although these are retrospective studies with small numbers of patient samples, they provide the most compelling data to date for potential mechanisms of primary resistance to trastuzumab therapy.

Resistance to EGFR-targeted therapy. EGFR-targeted therapy has become an important treatment modality in both colorectal cancer (CRC) and NSCLC. The last few years have seen significant advances in our understanding of resistance mechanisms for these agents in both diseases. Banck and Grothey reviewed exciting recent clinical data demonstrating that *KRas* (and probably *BRaf*) mutations confer intrinsic resistance to EGFR monoclonal antibodies (mAb) in CRC (5). These landmark findings have led to recent changes in clinical practice (and FDA approval) for these agents, i.e., patient tumors are now routinely screened for *KRas* mutations and for those patients whose tumor harbor a *KRas* mutation, EGFR MAb therapy is not indicated. Although these findings are noteworthy, there is still considerable room to improve patient benefit with these agents in CRC. Banck and Grothey point out that not all patients' tumors with wild-type *KRas* tumors respond to EGFR MAb therapy and those who do respond eventually become resistant to therapy. We know very little about intrinsic or acquired resistance mechanisms for EGFR MoAbs in wild-type *KRas/BRaf* colorectal tumors, although recent data on *PI3K* mutations (6) and levels of the EGFR ligands epiregulin and amphiregulin have shown promise as predictive markers for efficacy (7). It will be of interest to define in future studies whether other known high frequency genetic alterations in CRC also play a role in resistance to these agents.

The observation that, in some cases, targeted therapies can actually increase the aggressiveness of CRC has been shown in several phase III trials in patients with metastatic CRC. In the recently reported PRIME trial, the addition of panitumumab to FOLFOX in patients with mutated *KRas* led to a significant decrease in progression free survival (PFS; ref. 8). In addition, in two phase III clinical trials, when EGFR mAbs were added to 5-fluorouracil, oxaliplatin, and bevacizumab-based regimens, PFS was significantly worse (9, 10). Determining the mechanisms by which EGFR mAbs interact negatively with underlying genetic alterations (mutant *KRas*) or specific chemotherapeutic agents (oxaliplatin-based chemotherapy) to lessen PFS is essential to keep from repeating the same outcome with newer regimens.

Early clinical studies with EGFR-targeted TKIs, including large randomized phase III studies in NSCLC, were disappointing (11, 12). However, retrospective genetic analyses of NSCLC tumors from a subset of patients with robust clinical response to these agents revealed unique mutations in the EGFR kinase domain, which conferred exquisite sensitivity to EGFR kinase inhibitors (13). Hammerman and colleagues discuss the important recent advances in our understanding of resistance mechanisms for EGFR TKI therapy (14). Remarkably, the

Table 1. Primary resistance mechanisms for FDA-approved targeted therapies discussed in the *CCR Focus* reviews

Drug	Drug class	Primary target	Diseases	Primary resistance mechanisms
Trastuzumab	mAb	HER-2	HER-2 amplified BrCa	p95HER2*[†], PTEN*[†]
Cetuximab	mAb	EGFR	CRC	*KRas* mutation*[‡], *BRaf*
Panitumumab	mAb	EGFR	CRC	*KRas* mutation*[‡], *BRaf*
Erlotinib	TKI	EGFR	NSCLC	*EGFR* or *HER2* exon 20 insertion*[‡], *KRas* mutation?*, EGFR TK mutation (T790M, D761Y, L747S, T854A)[‡§], cMet amplification[‡§], PI3K/AKT activation[†§]
Gefitinib	TKI	EGFR	NSCLC	*EGFR* or *HER2* exon 20 insertion*[‡], *KRas* mutation?*, EGFR TK mutation (T790M, D761Y, L747S, T854A)[‡§], *cMet* amplification[‡§], PI3K/AKT activation[†§]
Imatinib	TKI	Bcr-Abl, cKit, PDGFR	CML, GIST	*Bcr-Abl* mutation[‡§], *cKit, PDGFR* mutations
Nilotinib	Second generation TKI	Bcr-Abl, cKit, PDGFR	CML	TBD
Dasatinib	Second generation TKI	Bcr-Abl, cKit, PDGFR	CML	TBD
Sunitinib	TKI	VEGFR, cKit, PDGFR	RCC, GIST	Multiple, *cKit, PDGFR* mutations (for GIST)
Sorafenib	TKI	VEGFR, Braf, PDGFR	RCC, HCC	Multiple
Pazopanib	TKI	VEGFR, cKit, PDGFR	RCC	TBD
Bevacizumab	mAb	VEGF	CRC, NSCLC, BrCa, RCC, GBM	Multiple

Abbreviations: BrCa, breast cancer; SSCHN, squamous cell carcinoma of the head and neck; TBD, to be determined.
*Intrinsic resistance.
[†]Proposed on the basis of preclinical data.
[‡]Clinically validated.
[§]Acquired resistance.

response rates to EGFR TKI therapy have increased nearly an order of magnitude (<10% to 60-80%) when selecting patients with specific EGFR kinase mutations for EGFR TKI therapy. Of interest is that these mutations exist in NSCLC, but are absent or exist in very low frequency in other human cancers such as CRC. Although certain EGFR kinase mutations confer sensitivity to gefitinib or erlotinib, a very small subset of NSCLC patients with wild-type tumors also exhibit meaningful responses to these agents suggesting additional mechanisms of sensitivity and/or resistance to these agents in the remaining patients.

Moreover, patients selected for gefitinib or erlotinib therapy on the basis of their EGFR mutation status eventually develop acquired resistance to therapy within ~12 months. Hammerman and colleagues summarize the primary and acquired resistance mechanisms identified for EGFR TKI treatment (14). One well-characterized primary resistance mechanism is an insertion mutation in exon 20 of the *EGFR* or *HER2* gene. *KRas* mutation as a primary resistance mechanism for EGFR TKIs (or EGFR mAbs) in NSCLC has been proposed by preclinical studies, but clinical validation is still premature. Given the high frequency of KRas mutation in NSCLC, it will be important to conduct studies to answer this question in a prospective, conclusive manner. Emergence of resistance to EGFR TKI therapy is related to EGFR kinase domain mutations, the most common of which increases ATP binding affinity, and amplification of the *cMet* gene. Independent of these two mechanisms, preclinical studies have shown that tumor cells acquire resistance to EGFR TKIs by up-regulation of the PI3K/AKT pathway. The exact mechanism leading to this pathway up-regulation is unknown.

It is important to point out that EGFR molecular therapeutic approaches (i.e., mAbs versus TKIs) are not interchangeable. Although intended to inhibit the same target, TKI and MoAB inhibitors of EGFR mediate their effects by different mechanisms. These mechanisms may be dependent upon inherent molecular alterations in the EGFR kinase domain or downstream signaling. These mechanisms of activity extend beyond simple inhibition of kinase activity. Most mAbs are developed to inhibit ligand binding and receptor activation, but may also inhibit dimerization with other receptor tyrosine kinases (RTK), and subsequent downstream signaling. In addition, some mAbs can induce antibody-dependent cell-mediated toxicity (ADCC) *in vitro* (cetuximab, trastuzumab), but this mechanism of action and its contribution to efficacy are difficult to prove clinically. Importantly, at least some resistance mechanisms between TKIs and mAbs are likely to be distinct based on mechanisms of action. EGFR kinase domain mutations that constitutively activate the EGFR kinase (ligand-independent) may confer sensitivity to EGFR TKIs and resistance to EGFR mAbs. Other EGFR mutations may do the opposite. Whereas resistance to TKIs may develop with new mutations in the kinase domain, this does not seem to occur with mAbs, in which the mechanisms of action, such as blockade of ligand-receptor interaction, dimerization of receptors, or ADCC are likely to lead to entirely distinct mechanisms of resistance as discussed in the Grothey and Pohlmann reviews. Importantly, TKIs are not specific for a single kinase, but in reality inhibit a plethora of kinases owing to the conserved structure of the ATP binding pocket within the kinase domain. The promiscuity of TKIs can be advantageous; for example imatinib and sunitinib can inhibit both c-Kit in GIST as well as other kinases involved in GIST tumor growth, such as platelet derived growth factor receptor (PDGFR). "Vertical" target inhibition with mAbs and TKIs have recently been shown to overcome resistance due to the T790M EGFR receptor mutation in a preclinical model of lung cancer (15). Of course, additional preclinical studies are warranted to confirm this interesting observation prior to planning and initiating clinical trials.

Resistance in GIST. GISTs are driven by dominant oncogenes in which ~85% of tumors have oncogenic mutations in the c-KIT kinase domain, and another 5 to 7% of GISTs harbor mutations in the PDGFR-alpha kinase domain. Fortuitously, both of these RTK targets are blocked effectively by the FDA-approved kinase inhibitors imatinib and sunitinib, because of homology in the ATP binding sites of both RTKs. Nearly 90% of patients receive benefit from front-line TKI therapy, but acquired resistance primarily occurs owing to selection and/or acquisition of secondary c-Kit or PDGFR mutations. As reviewed by Gramza and colleagues (16), these mutations typically occur in the same receptor that harbored the primary mutations, i.e., Kit mutated tumors are resistant because of another Kit mutation, and the same holds true for the PDGFR. Whether resistance mechanisms are due to newly developed mutations or simply a selection of clones within the tumor mass remains a point of controversy. Of interest is the fact that different mutations may be present in different regions of the tumor, highlighting the principle of tumor heterogeneity (16). Fortunately, different kinase inhibitors are able to inhibit the activity of various mutations and conformations of c-Kit, and second-line therapy can lead to disease control in the majority of patients. The use of molecular and circulating biomarkers, and noninvasive imaging techniques are helping tailor therapy for patients with GIST, as discussed in a recent *CCR Translations* by Blanke (17). Although investigations into the molecular pathogenesis of GIST have provided a foundation for drug development and patient selection through multiple lines of therapy, few tumor types are driven by such well-defined mutations, and thus the lessons learned from GIST biology may be hard to directly translate to other tumor types driven by multiple molecular alterations, as shown by (18).

Resistance to Bcr-Abl-targeted therapy. The well-delineated oncogenic *Bcr-Abl* translocation in CML has allowed for the development of molecular therapies that have markedly improved survival in patients with this disease. The first generation Abl kinase inhibitor imatinib has dramatically changed the treatment of CML transforming it from a disease with poor prognosis to a more chronic disease. Apperley and Milojkovic (19) discuss in detail a number of diverse resistance pathways to imatinib. The most commonly observed resistance mechanism is the occurrence of point mutations in the Bcr-Abl kinase domain that affect the ability of imatinib to bind effectively in the ATP pocket. Although second generation TKIs may initially be efficacious, subsequent mutations emerge to these therapies, leading to subsequent resistance. Similar to GIST, it is unclear if new mutations develop, or if primary therapy selects for pre-existing resistant clones. Given that mutations are observed early in the disease process whereas patients are still maintained in response in chronic phase, it seems likely that at least a substantial portion of these mutations are pre-existing and selected only by therapy. Support from this also comes from *in vitro* data showing that these mutations often do not confer a significant

level of resistance (20). Insights into the molecular mechanisms of resistance may be easier to define in CML compared with solid malignancies owing to accessibility of tumor cells for study (phlebotomy). Although for both GIST and CML, we tend to focus on the identification of secondary mutations as mediators of resistance, we must keep in mind that other resistance pathways, such as activation of alternative signaling pathways, may also confer resistance and can be targets for therapy.

Perspective on Vascular Endothelial Growth Factor-Targeted Therapy

Agents that target the vascular endothelial growth factor (VEGF) pathway have become important treatment options for a number of human cancers. Although VEGF-targeted drugs have shown survival benefit in a number of indications, the benefit has been incremental rather than the robust benefit initially anticipated for these agents. Therefore, there has been particular interest in understanding the mechanism of resistance to anti-angiogenic drug treatment. We, and others, have recently published reviews on mechanisms of resistance to VEGF-targeted therapy (21–23). Proposed mechanisms of resistance to VEGF-targeted therapies fall into broad categories such as (1) induction of compensatory angiogenic cytokines (PlGF, B-FGF, PDGF, angiopoietins, Bv-8 etc); (2) infiltration of immune effector cells that may deliver angiogenic mediators to the microenvironment; and (3) other cellular components of the microenvironment (i.e., pericytes, fibroblasts). It should be emphasized that, to date, these mechanisms have been established only in preclinical models, in which often times, the therapy does not mimic that delivered in the clinic. For example, bevacizumab is administered in combination with chemotherapy in patients with NSCLC, CRC, and breast cancer (24–26), but preclinical studies on VEGF resistance in these, and other tumor types, are typically done in studies using single agent therapy, most of which include agents different than bevacizumab. Thus, it is possible that different or additional resistance mechanisms may be in place when therapy is delivered in combination with chemotherapy. Although some of these pathways have been recognized for years, clinical studies targeting these resistance pathways (specifically PDGFR inhibition) have yet to definitively show any benefit over VEGF-targeted therapies (i.e., bevacizumab) alone. Thus, as always, translating from the laboratory to the clinic is challenging, and, at times, even misleading. With the large number of trials and patients treated with VEGF-targeted therapies, we expect a greater emphasis in the near future to test hypotheses generated from preclinical studies and determine their validity in the clinic. Moreover, there are recent human studies that have provided the foundation for the discovery of potential biomarkers (such as VEGF single nucleotide polymorphisms), which require validation in clinical trials and patient populations (27).

Recent research and reports in this area have led to discussion and controversy in the field that are worth highlighting. In early 2009, publications by Paez-Ribos and colleagues and Ebos and colleagues reported on preclinical studies suggesting that resistance to VEGF-targeted therapy could lead to a more aggressive, invasive tumor phenotype and increase the rate of metastasis, despite controlling primary tumor growth (28, 29). These findings have potentially important implications for the field of VEGF-targeted therapy, especially because there are currently three VEGF-targeted agents approved by the FDA for 6 tumor types, and >500 clinical trials testing VEGF-targeted therapeutics in various indications. The critical question arises, "is VEGF-targeted therapy of detriment to patients with cancer?"

Why would VEGF-targeted therapy increase the rate of metastasis? Important biologic processes require more than one molecular mediator, and angiogenesis is no exception. Although there is no question about the key role of VEGF in angiogenesis, there are multiple additional angiogenic factors that have distinct or compensatory functions. In preclinical and clinical studies, inhibition of VEGF activity leads to an increase in the circulating levels of a number of cytokines including bFGF, PlGF, SDF-1, angiopoeitin-1, and others (30–33). Many of these factors are also considered important for controlling tumor cell invasion and metastasis. The hypoxic response to vessel destruction and/or vasoconstriction secondary to VEGF inhibition leads to up-regulation of a number of pro-angiogenic and pro-migratory factors (Fig. 2). Thus, one plausible explanation is that the hypoxic response overwhelms VEGF-targeted therapy and/or turns on additional pro-angiogenic mechanisms. However, not all of the compensatory response observed with VEGF-targeted therapy may be due to hypoxia. Ebos and colleagues have shown that blockade of VEGF signaling can lead to an increase in cytokine production in nontumor bearing animals (32). In subsequent studies, these investigators showed that pretreatment of mice with VEGF-targeted therapy prior to tumor cell injection preconditioned the mice for metastasis formation (28). As stated above, it is important to point out that these preclinical studies used single agent VEGF-targeted therapies, which may be relevant to certain tumor types such as renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), and glioblastoma, in which single agent VEGF-targeted therapies are FDA approved. However, single agent VEGF-targeted therapies in preclinical studies may not reflect the complex interactions of chemotherapy and VEGF-targeted therapies used in patients with metastatic breast cancer, NSCLC, and CRC. Thus, the alarming nature of these preclinical results needs to be carefully weighed against clinical evidence.

Does VEGF-targeted therapy accelerate metastasis in the clinic? We now have phase III data on the use of VEGF-targeted therapy in various indications, either alone, or in combination with chemotherapy in >10,000 patients. In addition, registries of patients are available who received VEGF-targeted therapy in the phase IV setting (34). It is unlikely that VEGF-targeted therapy accelerates metastasis in NSCLC and CRC, on the basis of the data showing improved overall survival when bevacizumab was added to chemotherapy for patients with NSCLC and CRC. In contrast, in the pivotal breast cancer trial, the addition of bevacizumab to paclitaxel significantly improved the response rate and PFS, but overall survival was not significantly improved (25). Some investigators hypothesized that this lack of improvement in overall survival is a result of increased aggressiveness due to VEGF-targeted therapies. However, a more commonly accepted interpretation is that the overall endpoint in this study was compromised by the dilutional effect of multiple subsequent lines of therapy over a long duration, which is

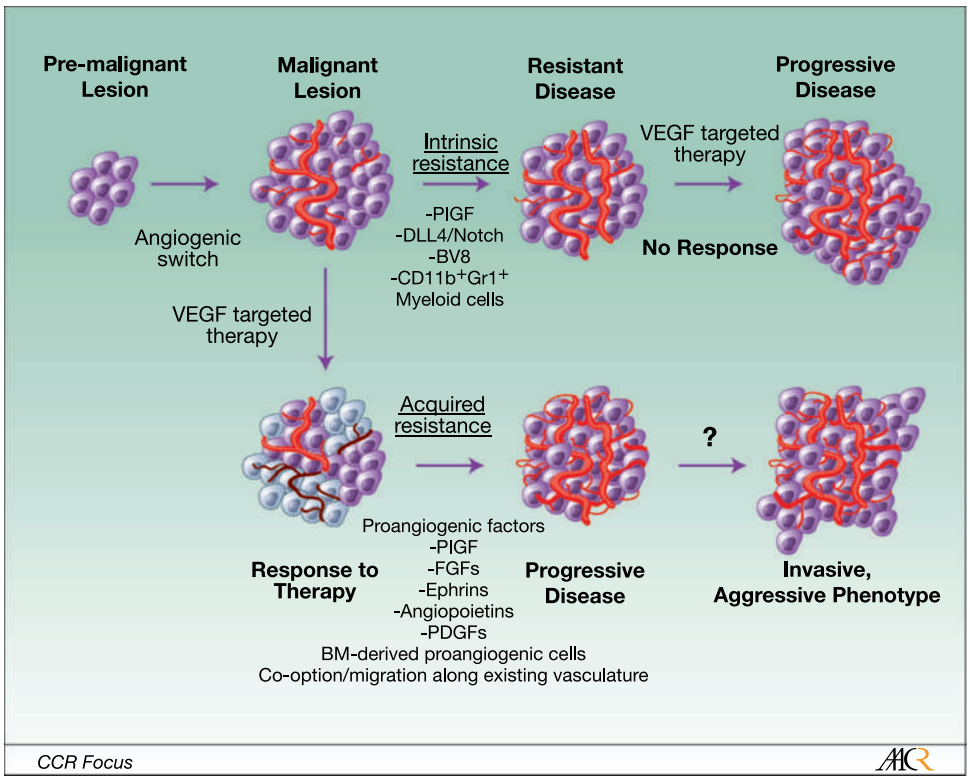

Fig. 2. Theoretical pathways by which VEGF-targeted therapies can increase tumor aggressiveness in preclinical models. Preclinical studies have identified four potential mechanisms of resistance to VEGF inhibitors: (1) activation and/or up-regulation of alternative angiogenic signaling pathways by tumor cells; (2) recruitment of bone marrow-derived cells that up-regulate angiogenic pathways; (3) increased pericyte coverage leading to VEGF-independent tumor vasculature; and (4) activation of invasion and/or metastasis mechanisms to supply tumor cells via established vasculature rather than stimulation of new blood vessels. During progression of malignant disease, these mechanisms may play a role in acquired (1) or intrinsic (2) resistance to VEGF-targeted therapy. In situations in which patients initially respond to VEGF-targeted agents, one or more of these resistance mechanisms are up-regulated leading to the eventual failure of therapy. Intrinsic resistance occurs when one or more resistance mechanisms are up-regulated by the natural course of the disease leading to initial failure of VEGF-targeted therapy. Recent studies propose that under some circumstances, VEGF inhibition can lead to an increase in levels of circulating proangiogenic and pro-invasive factors that could potentially lead to an increase in tumor aggressiveness after an initial response to therapy.

common in patients with metastatic breast cancer. In patients with metastatic CRC, survival has been shown to be increased with the addition of bevacizumab to chemotherapy (24). In the Brite Registry, an observational study of >1,900 patients who received chemotherapy and bevacizumab post-FDA approval, Grothey and colleagues studied the role of prolonged bevacizumab in patients who had experienced disease progression on first-line therapy; i.e., bevacizumab beyond progression. In this analysis, when bevacizumab was continued in subsequent lines of therapy while changing the chemotherapy backbone (35), patients achieved an overall survival of ~32 months, a duration that is far beyond the 20 months typically observed in the most recent phase III trials in patients with metastatic CRC. If bevacizumab accelerated tumor growth or metastasis in patients with metastatic CRC, this remarkable long-term survival would not have been observed. It must be emphasized that this study was an observational study with associated caveats. Two ongoing phase III clinical trials (SWOG 0600 in the US, AIO 0804 in Europe) should help to clarify the role of bevacizumab in long-term control of CRC growth.

More recently, Rini and colleagues reported their findings on a phase II trial in patients with RCC who had progressed on sorafenib therapy then were treated with axitinib, another VEGFR TKI (36). The response rate to axitinib in patients with sorafenib refractory disease was 23%, and PFS and overall survival were 7.4 months and 13.6 months, respectively. This disease control rate with continued VEGF-targeted therapy is significantly better than historical controls with placebo or mTOR inhibitors and shows that continued VEGF-targeted therapy may be of benefit to patients after progression on first-line therapy. Moreover, these clinical data do not support the hypothesis that VEGF-targeted therapy can increase tumor aggressiveness in RCC. An alternate possibility is that in some tumors there is an increase in tumor growth rate after discontinuation of VEGF-targeted therapy, as recently proposed in a kinetic analysis of tumor measurements obtained from patients enrolled in a randomized trial of bevacizumab alone in renal cancer (37). In that trial, an improvement in overall survival could not be discerned clinically after clear improvement in PFS was shown (38).

The results of an National Surgical Adjuvant Breast and Bowel Project (NSABP) adjuvant CRC trial were reported at the 2009 ASCO annual meeting (39). In this trial, patients with stage II and III resected CRC were randomized to receive chemotherapy for 6 months, or chemotherapy with bevacizumab followed by bevacizumab alone for 6 months. Thus, in this trial, if

single agent bevacizumab were thought to accelerate metastasis, we would expect to observe a shorter disease-free interval in the group treated with bevacizumab. However, the results of the trial demonstrate that bevacizumab may have provided an early benefit without any evidence of an increase in metastasis. Specifically, the investigators showed that multiple sites of metastases were exactly the same in the two study groups (18%), suggesting that in patients with CRC, bevacizumab does not accelerate metastatic outgrowth. This clinical study determined the effect of chemotherapy and bevacizumab on metastatic outgrowth of *presumed micro-metastasis* and differs in numerous respects from the preclinical studies by Ebos and Paez-Ribos (28, 29). Continued careful analysis of data from adjuvant clinical trials with VEGF-targeted therapies is essential to confirm the safety and overall patient benefit of this therapeutic approach.

Single agent VEGF-targeted therapy is now a primary therapeutic option in patients with RCC, HCC, and glioblastoma multiform (GBM). In patients with RCC and HCC, there is also little evidence, either from clinical trials or anecdotal reports that patients receiving single agent VEGF-targeted therapy lead to accelerated tumor growth or metastasis formation. However, anecdotally, several investigators have reported that patients with unresectable GBM who progress on bevacizumab demonstrate a more invasive tumor phenotype and/or numerous metastases within the central nervous system (CNS; refs. 40, 41). The presence of CNS metastasis from primary GBM is uncommon and many attribute this observation to the fact that this rapidly progressing primary disease leads to the demise of patients before metastases can develop. Thus, primary tumor control on bevacizumab could allow emergence of such metastases. However, an alternative hypothesis is that VEGF-targeted therapy could lead to increased tumor cell migration along vessels (21) and CNS metastasis in patients with GBM, after a relatively prolonged period of control of the primary tumor (40). In other words, the PFS is significantly prolonged by VEGF-targeted therapy, whereas the time from actual disease progression after bevacizumab administration until death may be slightly shortened. Importantly, the phase II studies suggest that the net effect of bevacizumab is an increase in overall survival in GBM (23, 42, 43).

Although there are no phase III clinical studies to suggest that VEGF-targeted therapies are detrimental to patient survival, clinical investigators should be mindful of the possibility that VEGF-targeted therapy can lead to conversion of a "moderately" aggressive cancer to an even more invasive or metastatic phenotype. The natural history of malignant disease is progressive growth, invasion, and metastasis, even if systemic therapy is transiently effective. Thus, with improvements in PFS with first-line therapy, micrometastatic disease that normally would *not* be clinically detectable at the time of a patient's death, may manifest itself as a new site of metastasis during the prolonged period of first-line therapy. Although some may view this as an increase in aggressiveness of the disease (which can occur with any oncologic therapy), most clinicians simply believe that these newly detected lesions are the outgrowth of micrometastases that had been present all along. There is no doubt that more effective systemic therapies are changing the *patterns of recurrence* in patients with advanced stage disease. Although there are instances in which phase III trials have documented that therapy can lead to a decrease in PFS in patients with metastatic CRC receiving dual mAb therapy (9, 10), there is little evidence to suggest that, in the clinic, VEGF-targeted therapies, in and of themselves, can induce a more aggressive tumor phenotype leading to a shorter overall survival.

Conclusions

Resistance to treatment remains the major challenge to targeted cancer therapy. Understanding resistance mechanisms will benefit patients in several ways. First, we will be able to select patients more likely to respond to a specific targeted therapy based on markers of response and resistance. Equally important, we can spare patients from an inactive therapy and allow oncologists to make earlier decisions for treatment with novel therapies that potentially may be more efficacious. Second, understanding resistance mechanisms will facilitate the development of novel agents for patients who have become refractory to target inhibition in earlier lines of therapy. However, we must consider whether it is best to switch therapies (i.e., discontinue current therapies), or add therapy that leads to reversal of resistance to the primary agent. Lastly, understanding resistance mechanisms will provide insight into novel mechanisms of tumor growth and potentially identify new targets and classes of drugs. As new resistance mechanisms are discovered we would expect new treatment paradigms to develop as well. Innovative biomarker studies are necessary to advance the field, but we must be cognizant of such issues as tumor heterogeneity, complications and costs of invasive biopsies, and patient comfort. Therefore, noninvasive biomarkers and new technologies, such as the use of circulating tumor cells and cytokines, should be explored to limit tumor biopsies and patient discomfort, costs, and inconvenience.

Disclosure of Potential Conflicts of Interest

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