Antiangiogenic Therapies: Going beyond Their Limits

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ABSTRACT

Tumor growth requires induction of an angiogenic program, and targeting of this program with antiangiogenic drugs shows an impact on tumor progression. However, although they are effective at reducing angiogenesis, these therapies have not produced widespread or enduring clinical benefit, which openly exposes their limitations. Here, we describe the current limitations of these therapies, including the known mechanisms and current controversies. Further, we present some of the recent approaches to predict these limitations and strategies to overcome them. With the development of meaningful predictive biomarkers and effective treatments that impede these limitations, longer and more robust efficacies will be achieved for a wider population of patients.

Significance: The clinical benefit of antiangiogenic drugs is restricted because of intrinsic and acquired limitations. Acknowledging and understanding these limitations will not only allow the development of effective predictive biomarkers but also help in devising new therapeutic strategies that achieve longer efficacies for a wider population of patients.

INTRODUCTION

Neoplastic growth requires increasing amounts of nutrients and oxygen to sustain expansive proliferation of tumor cells. Therefore, switching on an angiogenic program is a limiting step during neoplastic progression that provides the growing tumor with a supply of oxygen and nutrients. Although many cytokines and growth factors have been described to have proangiogenic properties, it is VEGF and its receptors (VEGFR) that are essential for tumor angiogenesis (1). Inhibitors of this signaling axis exhibit antiangiogenic properties with consequent antitumor effects; hence, these drugs have been robustly introduced into clinical protocols for the treatment of several types of tumors, either alone or in combination with traditional cytotoxic agents (2).

Although effective at reducing angiogenesis, these drugs have not produced widespread or enduring clinical benefits in many patients, thereby openly exposing their limitations. Here, we describe tumor responses and existing cellular and molecular knowledge on the limitations of antiangiogenic therapies, including the current controversies in this field (Fig. 1). Furthermore, we propose the identification of biomarkers of response and suggest several strategies or approaches to overcome these limitations to increase the efficacy and extend the benefits of current antiangiogenic therapies.

WHAT IS THE TARGET AND THE COMBINATION THERAPY TO USE

In the clinic, targeting VEGF ligand (i.e., with antibodies or receptor traps) or its receptors, VEGFRs [i.e., with small-molecule tyrosine kinase inhibitors (TKI)], shows effectiveness in different tumor types. Surprisingly, clinical benefits are achieved when ligand-blocking drugs are combined with chemotherapeutic agents or radiotherapy, but on the contrary, small-molecule inhibitors of the receptor are given as monotherapy and not combined (3, 4). This empirical (clinically validated) use of these kinds of drugs seems to be a limitation on its own, but has not yet been mechanistically explained. By ligand binding (i.e., bevacizumab) or trapping (i.e., aflibercept), or by inhibition of intracellular signaling pathways with small-molecule TKIs, these drugs achieve a significant inhibition of the VEGF–VEGFR signaling axis. But then, why is single-agent VEGF-targeted therapy efficacious in certain cancers, such as renal cell cancer (RCC) or hepatocellular carcinoma, but shows considerably less clinical benefit in others, for example in colorectal cancer, for which this therapy is administered in combination with chemotherapy?

TKIs Alone Only in a Subgroup of Tumors

Abnormalities of the tumor vasculature are dependent on endothelial cells as well as on other vascular cells such as perivascular cells or pericytes that are also affected by factors...
secreted by tumor cells. Tumor pericytes have a functionally relevant association with endothelial cells, which not only contributes to their barrier role but also can promote the direct survival of endothelial cells (5, 6). Indeed, these cells mediate the stabilization of vessels based on synthesis of new basement membrane and tight association of endothelial and pericyte junctions. Molecularly, a specific cross-talk between endothelial cells and pericytes that implicates VEGF and platelet-derived growth factor (PDGF) is key for vascular formation and maintenance and creates a crucial therapeutic opportunity that has been exploited (7). In preclinical studies, pericytes have been shown to be relevant targets for effective antiangiogenic therapies, and several preclinical studies have shown the increased efficacy of targeting both pericytes and endothelial cells (8–10). Molecularly, inhibition of the PDGF receptor (PDGFR) in pericytes with small-molecule TKIs enhances the benefit of anti-VEGFR antiangiogenics, as endothelial cells become more sensitive to apoptosis in the absence of pericyte coverage (7). Interestingly, a retrospective study compared the effects of sunitinib and bevacizumab (in combination with IFN-α) in advanced metastatic RCC and found sunitinib to be superior to bevacizumab plus IFN-α in terms of progression-free survival (PFS; ref. 11). As a consequence, PDGFR inhibition has been developed clinically in the context of combined inhibition of VEGFR and PDGFR with dual-specificity small-molecule inhibitors, such as sunitinib, sorafenib, pazopanib, and others, that have ultimately achieved significant and robust clinical efficacies in RCC and hepatocellular carcinoma, two typical VEGF-dependent tumor types (12, 13).

Although the preclinical and clinical development of this class of VEGFR TKI small molecules is expanding for the treatment of RCC and hepatocellular carcinoma, this class of drugs has not yet shown significant efficacy in other tumor types where combination with chemotherapy is necessary. What is the basis of this limitation? Several mechanistic studies have postulated that the deeper therapeutic impact that TKIs exert on vessels could be the cause of this incompatibility, which is not observed with anti-VEGFR drugs. Specifically, TKIs induce distinct vessel alterations via the inhibition of both intracellular VEGFR2 and pericytes through PDGFR, which is not achieved by anti-VEGFR ligand drugs (7). Nevertheless, several clinical studies testing various TKIs combined with chemotherapy have failed because of increased toxicity of these combination therapies. The mechanistic explanation could be based on off-target activity of small-molecule TKIs, which could add to the toxicities of chemotherapeutic drugs (14, 15). Indeed, many of the VEGFR2 TKIs show off-target inhibition of c-Kit, which plays an important role in bone marrow progenitor cell mobilization, and its inhibition could lead to myelosuppression and exacerbate the bone marrow toxicity of chemotherapy (3, 16, 17). The ultimate cause for this “incompatibility” is not yet fully understood and warrants further studies to overcome this limitation of VEGFR TKI drugs.

Anti-VEGF Ligand and Chemotherapy: Always Together?

Although it has been known for several years that anti-VEGF ligand drugs can enhance the antitumor effects of cytotoxic drugs, the underlying mechanism has been far from clear since the early positive results of drug combination trials (18). The most widespread explanation for such a mechanism is the “vascular normalization” theory, initially postulated by Jain (19). The theory proposes that anti-VEGF/VEGFR therapies induce a structural and functional normalization of tumor blood vessels and, as a result, blood flow is increased and cytotoxic drugs can more easily reach into the tumor (20). Indeed, with antiangiogenic treatment, immature blood vessels are pruned and vessel tortuosity and dilation decrease, producing a reduction of blood vessel leakage, vascular permeability, and interstitial fluid pressure (IFP), which alleviates edema in patients with cancer and provides an important clinical benefit (21–23). Several antiangiogenic drugs have been shown to induce morphologic normalization of the tumor vasculature in both preclinical and clinical studies (24–26), and in some cases the functionality of individual surviving blood vessels has improved (22, 27, 28).

Nevertheless, a more recent report has clinically shown that angiogenesis inhibitors can decrease the delivery of cytotoxic drugs to tumors in patients, and hence hinder their therapeutic benefits (29). By means of a very sensitive positron emission tomography (PET) imaging method, the authors evaluated uptake and retention of a cytotoxic drug (radionabeled docetaxel) in patients with advanced-stage nonsmall cell lung carcinoma (NSCLC) treated with bevacizumab. In these 10 patients, VEGF inhibition induced a fast and sustained decrease, rather than an increase, in the penetration of both water and docetaxel into the tumors. These results contrast with those of previous studies in patients with rectal cancer (21) and in patients with glioblastoma (24), showing...
that bevacizumab treatment induced vascular normalization and increased glucose uptake, which was used as a surrogate for cytotoxic drug uptake. The discrepancies between the recent observations and previous results could be due to differences in blood vessel networks and response to angiogenesis inhibitors between the cancer types studied, as it is known that these agents can affect blood vessels in different ways in various tissues (24, 30). However, the finding that, at least in patients with NSCLC, antiangiogenic therapy does not improve but rather decreases cytotoxic drug delivery to tumors is a limitation and could be the cause of the modest benefits of these combination therapies in NSCLC and other tumor types (31).

To circumvent this limitation (Fig. 1), several studies have aimed to pinpoint the time interval of the normalization effects of antiangiogenics (“window of normalization”) to optimize the benefits of vascular normalization-enhanced tumor drug delivery (32). However, there is high variation in preclinical studies and clinical data, and noninvasive imaging techniques that monitor tumor blood flow need to be developed to better define the duration of the “window of normalization” in every tumor type. Moreover, chronic treatment with VEGF pathway inhibitors eventually reduces tumor blood perfusion and increases tumor hypoxia in experimental animal studies (33, 34), suggesting that uninterrupted treatment may not be optimal for tumor vascular normalization-enhanced combination chemotherapy. Therefore, intermittent antiangiogenesis treatment schedules need to be investigated to ascertain whether, and under which conditions, repeated cycles of antiangiogenics to normalize the vasculature and increase drug uptake can be achieved. The potential of such cycles of renormalization of the tumor vasculature to facilitate vascular recovery and prolong synergy with chemotherapeutics needs to be carefully considered. Overall, studies are needed to verify whether sequencing of chemotherapy followed by antiangiogenic drugs or intermittent antiangiogenesis treatment could reveal important benefits to circumvent the limitations of this kind of drug.

**RESISTANCE AND HOW TO OVERCOME IT**

The initial hypothesis was that antiangiogenesis therapy would not induce resistance (“resistant to resistance”) because it targeted the genetically stable endothelial cells instead of the unstable tumor cells themselves (35). Nevertheless, clinical and experimental evidence indicates that resistance to antiangiogenic therapy does indeed occur (36, 37). Among tumor responses to therapy, it is essential to distinguish between refractoriness, sometimes called intrinsic resistance, and acquired resistance (ref. 38; Fig. 1). Intrinsic resistance is characterized by tumor indifference to antiangiogenic therapy and continued tumor growth despite treatment with antiangiogenics such as bevacizumab, sorafenib, or sunitinib (24, 39). On the other hand, acquired resistance to antiangiogenics seems to stem from tumor adaptations to therapy instead of mutations or gene amplifications that characterize acquired resistance to other therapeutic strategies. In this form of resistance, alternative mechanisms lead to activation of angiogenesis even when the target of the drug remains inhibited (36, 40–42). In fact, clinical evidence of
this plasticity has been described in metastatic RCC treated repeatedly with VEGFR inhibitors (37).

Several molecular mechanisms of intrinsic or acquired resistance have been described. The most prominent one stems from therapy-induced vascular trimming and hypoxia, where neoplastic cells respond to hypoxia by becoming tolerant and modifying their metabolic characteristics to resist low oxygenation, or by upregulating multiple proangiogenic molecules, including VEGFs, fibroblast growth factors (FGF), and angiopoietins, which promote revascularization and eventual resistance (41, 43). In this case, both tumor cells and the stroma can contribute to therapy resistance through recruitment of infiltrating cells, such as cancer-associated fibroblasts (CAF; ref. 44) and tumor-associated macrophages (TAM; ref. 45), that aid in the production of alternative proangiogenic factors (46, 47). Alternatively, tumors can be refractory to anti-VEGF therapies because they do not depend on sprouting angiogenesis driven by VEGF, as in the case of co-option of preexisting normal vessels of the tissue or vascular mimicry, in which neoplastic cells can directly form vessel walls (48).

Interestingly, there are several similarities among the mechanisms that lead to intrinsic or acquired resistance. The difference lies in the intrinsic characteristics of each tumor, as in the acquired type, tumors require some time to generate these molecular changes and become resistant to therapy, whereas in intrinsic resistance, tumors are immune to this therapy, as they have upfront expression of these alternative factors (49, 50).

Strategies to overcome the limitations of resistance to antiangiogenic therapies are currently an intense area of research in the field. Several studies have proposed cotargeting of VEGF and FGF signaling pathways to improve efficacy and overcome adaptive resistance to VEGF inhibition in the RIP-Tα2 model of pancreatic neuroendocrine tumors (43, 51). Specifically, the use of the dual FGF receptor (FGFR)/VEGFR TKI, brivanib, shows antiangiogenic activity due to inhibition of VEGFR1–3 and disruption of FGFR1–3, which overcomes resistance to VEGF-selective therapy and blocks FGF-dependent tumor proliferation (51, 52). Clinically, brivanib had shown promising activity as a single agent in hepatocellular carcinoma and in combination with cetuximab in colorectal cancer (53), but in a more recent phase III study in liver cancer, it did not show any benefit over sorafenib, a more restricted VEGFR inhibitor (BRISK-FL trial). Other studies have proposed to aim at the root of tumor adaptation with alternative combination strategies designed to overcome the emergence of hypoxia-induced resistance. Along these lines, dual-targeted strategies have been tested in xenografts with the combination of bevacizumab and hypoxia-inducible factor-1 (HIF-1) or Sp1 inhibitors, which enhanced the therapeutic efficacy of antiangiogenic treatments in these preclinical studies (47, 54).

Furthermore, as resistance reflects reversible adaptations to angiogenic blockade, it has been suggested that subsequent treatment with therapy that does not target angiogenesis might re sensitize patients to antiangiogenic strategies (55). Given this suggestion, sequential treatment with an antiangiogenic drug followed by a non-antiangiogenic drug (i.e., another targeted therapy or chemotherapy) could re sensitize patients to another antiangiogenic drug as a third line of treatment. Obviously, many studies are warranted to unravel the preclinical basis and clinical potential of this hypothetical sequential treatment and to determine its clinical benefit for patients.

**SELECTION FOR TUMOR-INITIATING (STEM) CELLS**

Tumor adaptation to antiangiogenic therapies may also involve alterations in signaling pathways in tumor cells and the accumulation of tumor-initiating cells or cancer stem cells (CSC), limiting treatment efficacy (ref. 56; Fig. 1). Studies in breast-cancer xenograft models treated with antiangiogenic agents (sunitinib and bevacizumab) showed accumulation of a tumor-initiating cell subpopulation that expressed the enzyme aldehyde dehydrogenase and initiated tumors when reimplanted in other mice. Similar cell populations have been described in tissue samples from patients with inflammatory breast cancer (57) and in glioblastoma treated with combination therapies in mice (58).

Several different mechanisms have been described to underlie the antiangiogenic-induced selection for cells with tumor-initiating (stemness) capacity. First and foremost, CSCs produce higher levels of VEGF in both normal and hypoxic conditions compared with their non-CSC counterparts (59), and they can recruit high amounts of endothelial cell precursors for revascularization and tumor growth or regrowth (60), which could make them less sensitive to anti-VEGF therapies. Another proposed mechanism involves antiangiogenic therapy–triggered vascular trimming and hypoxia. In this case, tumors treated with antiangiogenic agents activate not only a hypoxia-response program, but also the Akt/β-catenin signaling pathway, which regulates cell growth and adhesion between cells. This pathway has been previously implicated in the regulation of breast cancer progenitor cells (61). Furthermore, some intrinsic features of CSCs have been implicated in their resistance to chemotherapy and radiotherapy, and these same features could also be involved in their selection or insensitivity to antiangiogenic therapy. The most significant example is the low proliferative index that CSCs share with normal stem cells that could help them to survive under the conditions of nutrient and oxygen starvation generated by antiangiogenic therapies. The broad capacity of tumors to adapt to therapy and their intrinsic cellular plasticity initiates a rewiring of survival signals that triggers stemness and therapy resistance. Indeed, several reports have recently described the differential capacity of tumor-initiating cells to resist or survive after several treatment strategies, including not only chemo- and radiotherapy but also targeted therapies (62, 63).

How could this limitation of antiangiogenic therapies be overcome? One possibility would be to combine angiogenesis inhibitors with drugs that suppress the response of cancer cells to hypoxia and, as mentioned before in the case of acquired resistance, try to hit at the root or the cause of tumor adaptation. Another alternative could be the combination of antiangiogenics with inhibitors of the Akt/β-catenin pathway aimed at blocking the signaling pathways that mediate selection of stem-like cells and ultimately impeding tumor-initiating cell accumulation.
INCREASED AGGRESSIVENESS RESPONSE

Tumor angiogenesis is intimately associated with systemic dissemination of tumor cells and the consequent development of distant metastases. The typically disorganized vascular tumor network, characterized by a loose association of basement membrane with endothelial cells and pericytes and by leaky blood vessels, serves as a permissive escape route for tumor cells (64). Moreover, the consequent formation of edema due to blood extravasation, together with the pushing force generated by the expansion of growing tumor cells and the absence of draining lymphatic vessels, converts the tumor microenvironment into an area with high IFP (20, 65). Under these conditions, the IFP inside the tumor could equalize with the microvascular pressure and generate a fluid convection to the surrounding tissue that may contribute to tumor-cell dissemination via the lymphatic and blood vessels, especially when considering the venous contribution to tumor-cell metastasis (65, 66). Thus, the aberrant and hyperpermeable tumor vasculature provides the push (higher IFP) and the opportunity (endothelial gaps and openings) for “passive” tumor cell intravasation and systemic dissemination (20). Therefore, blocking tumor angiogenesis by inhibition of the VEGF pathway should not only reduce tumor growth but also impede the formation of metastases. However, angiogenesis inhibitors have been shown to make some tumors more aggressive in several animal models, with potent angiogenesis inhibition altering the natural history of tumors by triggering resistance to therapy and increasing invasion and lymphatic or hematogenous metastasis (refs. 67, 68; Fig. 1). Here again, the mechanism of this more malignant phenotype is associated with tumor adaptation to therapy-induced hypoxia. Indeed, evidence linking hypoxia to a more aggressive metastatic cell behavior is well established (69), with HIF–1α-dependent production of prometastatic proteins, the secretion of proteolytic enzymes, and alteration of adhesion molecules in tumor cells or in the extracellular matrix resulting in induction of epithelial-to-mesenchymal transition (70). Nevertheless, recent evidence suggests that other mechanisms, alone or in addition to hypoxia, could also be involved in the malignant response to these therapies, as in the case of activation of c-MET signaling leading to enhanced invasion and metastasis after anti-VEGF therapy (71–73).

Several studies have tried to address this issue in the clinical setting. Initially, a retrospective study analyzed tumor “rebound” after stopping antiangiogenic therapy in patients with metastatic cancer from five pooled phase III trials (74). The authors concluded that the disease developed with the same pattern of progression in bevacizumab- and placebo-treated patients, thus excluding an acceleration of tumor progression and an increase in mortality rates due to treatment. However, this analysis excluded a number of other clinical trials that reported somewhat discordant results (74, 75). More recent clinical studies in the adjuvant setting (the AVANT trial and the NSABP-C08 trial) reported that patients in the bevacizumab-containing arm who relapsed after adjuvant treatment seemed to have a higher rate of death than those treated with chemotherapy alone (76, 77). Therefore, it will be crucial to evaluate tumor aggressiveness in future trials with antiangiogenic therapies.

How can this detrimental limitation be overcome? Similar to the emergence of acquired resistance and the accumulation of tumor-initiating cells, the possibility of restoring tumor oxygenation represents an appealing option to prevent tumor invasiveness/aggressiveness, as hypoxia is one of the drivers of neoplastic metastases. Following Jain’s (20) “vascular normalization” theory, several studies showed that controlled normalization of tumor oxygenation can be obtained by modulating the endothelial cell response to hypoxia with reduced activity of the oxygen sensor prolyl hydroxylation domain protein 2 (PHD2; refs. 78, 79). In animals heterozygous for PHD2, normalization of the endothelial lining restored perfusion and oxygen supply, and tumors were less invasive and developed fewer metastases than tumors with wild-type vessels. Similarly, studies in a transgenic model of pancreatic neuroendocrine tumors showed that restoration of tumor oxygenation levels through the normalization of endothelial cells impaired neoplastic invasion and dissemination (80). In this study, recombinant semaphorin-3A (Sema3A) was used as a therapeutic agent to inhibit both angiogenesis and invasive/metastatic behavior (80, 81). Nevertheless, these approaches should be taken with caution, as chronic exposure of cells to moderate hypoxia levels represents a well-recognized experimental method to induce adaptation to growth in hypoxic conditions and subsequent selection of resistant clones. In addition, these same intermediate levels of oxygen could increase the metastatic capability of cells (82). Therefore, intensity and potency of normalization of the tumor vasculature and its associated tumor reoxygenation should be carefully evaluated to avoid undesirable side effects.

HETEROGENEOUS RESPONSES CALL FOR PREDICTIVE BIOMARKERS

Tumor responses to antiangiogenic therapies are everything but homogeneous. Although studies mostly report, and have to be based on, population (global) averages, the reality is that individual responses are very heterogeneous and variable. Indeed, results from preclinical models, case studies, and clinical trials report varying and often contradictory responses to antiangiogenic therapies, depending on the cancer type and the specific antiangiogenic therapy. The general agreement is that antiangiogenic treatments are more effective in terms of increasing PFS than prolonging overall survival, as in the case of VEGFR TKI for RCC (83, 84). In detail, anti-VEGF/R agents typically induce cavitary and loss of viable tumor mass, which exerts an impact on tumor growth, but this is not always associated with a significant alteration of tumor size and results in heterogeneity in individual Response Evaluation Criteria in Solid Tumors (RECIST) responses (38, 85). Indeed, the pattern of growth and the modifications induced at the site of tumor development are greatly influenced by the tumor type and in particular by its angiogenic features and proangiogenic capacity. A typical example is RCC, where angiogenesis is highly VEGF-dependent, in part due to frequent gene inactivation of the von Hippel-Lindau (VHL) tumor suppressor that drives dysregulated HIF-1 and VEGF overproduction (86). The same dependence on angiogenesis is thought to be
important for the efficacy of antiangiogenic therapy in hepatocellular carcinoma, where tumors are highly angiogenic and displace the normal parenchyma. In contrast, the metastatic foci of colorectal cancer that grow in the liver often replace rather than displace the liver parenchyma, leading to co-option of existing blood vessels instead of dependence on predicting angiogenesis (38, 87). This differential growth pattern, displacing or replacing the normal tissue, could be key in determining angiogenesis- and VEGF-dependency, and therefore response to antiangiogenic therapies. Furthermore, responses to antiangiogenic drugs also vary between primary tumors and their metastases (88). Interestingly, as the interplay between tumor cells and the tumor microenvironment is crucial for the development of neoplastic lesions, the same tumor–stromal cell collaboration is also involved in tumor responses to therapeutic inhibition of the VEGF pathway. Thus, together with tumor-cell characteristics, the stroma contributes to therapeutic inefficacy with a differential contribution in each cancer subtype, resulting in even more heterogeneity in responses to therapy.

With this broad spectrum of heterogeneity in tumor responses to antiangiogenics, it is logical that the different angiogenic features and vascular dependence of each type of tumor do indeed influence and limit the overall response to antiangiogenic therapy. But could this collection of limiting factors be managed and converted into predictors of sensitivity or response to therapy? Several years ago, hypertension was introduced as a putative biomarker for antiangiogenic treatments. Specifically, in a retrospective study in more than 500 patients with metastatic RCC, sunitinib treatment–induced hypertension was associated with an improved clinical outcome (89). These findings support the hypothesis that hypertension may be a viable “on-therapy biomarker” or a “monitoring biomarker” of antitumor efficacy in this patient population, although development of hypertension during sunitinib treatment was neither necessary nor sufficient for clinical benefit in all patients. Nevertheless, basal (pretreatment) hypertension in these patients did not associate with clinical benefit, and therefore blood pressure is not a useful predictive biomarker but rather an on-therapy monitor of treatment response. More recently, many studies have evaluated putative predictive factors of response to antiangiogenics, some stemming from tumor-cell adaptation to therapy (and its resulting hypoxia), and others initiating from the vascular structure and components (as it is the direct target of these therapies; Table 1). An example of the latter is a study where antiangiogenic-refractory tumors contained blood vessels with a prolific investment of pericytes expressing α-smooth muscle actin (α-SMA). Therefore, the occurrence of pericytes expressing α-SMA was postulated as a biomarker for tumors refractory to therapy (90). Obviously, further studies are warranted to validate this possibility. Another relevant example is the immune system and its intricate relationship with angiogenesis in cancer (91), as TAMs and myeloid-derived suppressor cells (MDSCs) produce proinflammatory cytokines, endothelial growth factors, and proteases that drive neoangiogenesis (92, 93). Along these lines, preliminary studies suggest that several immune markers such as intratumoral MDSCs and interleukin-8 (IL-8), or peripheral regulatory T cells, may predict clinical response to antiangiogenic therapy. Nevertheless, there is not yet a recognized and validated biomarker of response to antiangiogenics (94).

To be successful in the search for the elusive biomarkers of antiangiogenics, our view is that in the era of targeted/personalized therapy, patient populations have to be carefully studied to find the most appropriate marker or characteristic that defines dependence on tumor angiogenesis, and more specifically VEGF. Several morphologic, histologic, and molecular features in tumors are indeed known to be strongly associated with tumor vessel density, angiogenesis, and, therefore, VEGF signaling. However, not only VEGF is implicated in this process and, depending on the tumor, other proangiogenic factors overtake its function to become the main promoters of neovascularization (95). Therefore, due to their profound influence on therapeutic efficacy, we need to develop markers that quantify dependence on these factors to define subgroups of patients who will respond best to the specific blockade of the VEGF signaling pathway, angiogenesis, or other specific targets (36, 51).

Another important avenue with regard to biomarkers is the development of factors that predict the limitations of antiangiogenic therapies, because if we could predict the emergence of resistance or drawbacks of therapies, we would be able to develop strategies to overcome these limitations. The prediction of the limitations of antiangiogenics has to be based on the characteristics of each tumor at the tumor-cell and microenvironment levels. Indeed, tumor cells themselves can exhibit or switch on a repertoire of features that ensure their survival under therapeutic selection, such as the expression of prosurvival receptors like EGFR receptor (EGFR) and c-KIT or the activation of autophagic protection (96). Moreover, they can produce factors that modify the surrounding stroma, for example those related to HIF downstream genes. In addition, the stroma itself can also contribute to the failure of therapy if we consider the role of CAFs in vessel maintenance (97). With all these mechanisms and molecules described, we envision developing different biomarkers of response (or of failure) based on the detection of key molecules that critically contribute to each of the limitations to antiangiogenic therapies (Table 1).

On the other hand, the limitations to antiangiogenic therapies also exhibit very heterogeneous responses. In fact, preclinical data reveal that the same treatment elicits different and often contrasting responses depending on the tumor cell type and its microenvironment, varying from local adaptation to systemic dissemination, as mechanisms of escape from therapy (36, 98). A relevant example in the case of enhanced aggressiveness after antiangiogenics is the fact that sunitinib increases metastasis in orthotopic mouse models of breast and colon cancer, whereas it does not promote metastatic behavior in lung cancer, suggesting a role of intrinsic tumor-cell characteristics in this heterogeneous response to therapy (73). Could the different angiogenic features of each type of tumor also influence the escape behavior of tumor cells after antiangiogenic therapy? And does reduction of the oxygen and nutrient supplies due to inhibition of vessel growth more efficiently mobilize the angiogenesis-dependent tumor cells? In this case, and similar to the other limitations to antiangiogenic therapies previously described, the angiogenic...
### Table 1. Predictors of limitations to antiangiogenic therapies and avenues for intervention

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<td>Tumor oxygen tension MRI/CT imaging/ intratumoral needle</td>
<td>Promote vascular normalization (inhibition of PHD2, administration of Sema3A, etc.) and new schedules.</td>
<td>(20, 21, 47, 54, 65, 72, 79-81, 108, 110)</td>
</tr>
<tr>
<td>Resistance to glucose starvation</td>
<td>Glycolytic phenotype</td>
<td>Tumor cells</td>
<td>FDG uptake by PET imaging</td>
<td>Inhibition of AMPK</td>
<td>(111-113)</td>
</tr>
<tr>
<td></td>
<td>Expression of HIF target genes (GLUT1, LDHA, etc.)</td>
<td>Tumor cells</td>
<td>Expression of HIF target genes (GLUT1, LDHA, etc.); mutational profile (pVHL status)</td>
<td>Inhibition of HIF-1; specific inhibition of HIF downstream genes</td>
<td>(47, 54, 72, 86, 108)</td>
</tr>
<tr>
<td></td>
<td>Autophagy</td>
<td>Tumor cells</td>
<td>Autophagy markers (LC3, ATG16L, BNIP3, BNIP3L, p62)</td>
<td>Chemical inhibitors of autophagy; neutralization of p62</td>
<td>(114, 115)</td>
</tr>
</tbody>
</table>

(Continued)
features of the tumor and its angiogenesis dependence not only may be predictors of response to therapy but also may help to foresee the limitations that will limit the long-term benefits of antiangiogenic therapies.

CONCLUDING REMARKS

Overall, the current knowledge in the field emphasizes the need for a carefully balanced evaluation of the benefits and limitations of antiangiogenic therapies. In addition, the causes and mechanisms of these limitations have to be unraveled in order to therapeutically overcome them (Fig. 1). We now know that the molecular profile of tumor cells as well as the microenvironmental features of the tumor can deeply affect the efficacy of therapy. Thus, the combination of antiangiogenic therapy with treatments targeting the tumor-signaling pathways or specific stromal cells implicated in both intrinsic and acquired limitations could prevent or delay treatment failure (Table 1). Therefore, inhibitors of these limiting pathways should also be taken into consideration in the development of new antiangiogenic therapeutic approaches, where maximal benefits could be achieved with upfront combinatorial or multitargeted therapies that block tumor angiogenesis and overcome intrinsic limitations. Alternatively, these treatments could be improved by sequential scheduling of antiangiogenic drugs followed by additive combinations with other drugs that target acquired limitations. This is particularly the case with acquired resistance that accounts for tumor desensitization to treatment. Hence, targeting the alternative pathways triggered or selected by previous antiangiogenic drugs could result in a marked improvement of the duration of benefit.

Nevertheless, not all the mechanistic predictions result in effective and clinically beneficial combinations. In the clinic, even in the presence of well-documented molecular pathways and targets of resistance, combinatorial/multitargeted therapies still fail in some patients, as in the case of erlotinib and bevacizumab in patients with breast cancer (99). Altogether, predictive factors of response but also predictors of the limitations are urgently needed as they could ultimately allow for categorization of subgroups of patients or even individualization of combination treatments to extend the benefits of antiangiogenic therapies for a wider population of patients. It should be our aim as translational researchers to convert the limitations of antiangiogenic therapies into therapeutic benefits for the future.

### Table 1. Predictors of limitations to antiangiogenic therapies and avenues for intervention (Continued)

<table>
<thead>
<tr>
<th>Limitation to therapy</th>
<th>Biomarker</th>
<th>Tumor compartment contribution</th>
<th>Method of detection</th>
<th>Interventional therapy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant residual cells</td>
<td>EGFR/c-KIT, etc.</td>
<td>Tumor cells</td>
<td>Expression of specific receptor</td>
<td>Specific inhibition of the receptor</td>
<td>(96, 116)</td>
</tr>
<tr>
<td>CSC</td>
<td></td>
<td></td>
<td>Flow cytometry for specific markers (CD133, CD24, CD44, CD38, and CXCR4)</td>
<td>Neutralization of CSCs by inhibition of specific targets (Notch, CXCR4, AKT-β-catenin pathway, etc.); ATRA</td>
<td>(61–63)</td>
</tr>
<tr>
<td>Metastatic potential</td>
<td>CEC/CEP</td>
<td>Stroma</td>
<td>Flow cytometry in blood</td>
<td>Simultaneous inhibition of VEGFR1 and VEGFR2</td>
<td>(106, 107)</td>
</tr>
<tr>
<td>CTC</td>
<td></td>
<td>Tumor cells</td>
<td>Blood CellSearch Technology</td>
<td>Target molecules specifically expressed by CTCs</td>
<td>(117–119)</td>
</tr>
<tr>
<td>CSC</td>
<td></td>
<td>Tumor cells</td>
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<td>(61–63)</td>
</tr>
<tr>
<td>c-MET/EGFR</td>
<td></td>
<td>Tumor cells</td>
<td>Expression of specific receptor</td>
<td>Specific inhibition of the receptor</td>
<td>(72, 73)</td>
</tr>
<tr>
<td>Expression of HIF downstream pathways (CXCR4, SDF-1, MMP, etc.)</td>
<td></td>
<td>Tumor cells/stroma</td>
<td>Expression of HIF target genes (CXCR4, MMP, etc.); mutational profile (mTOR status)</td>
<td>Inhibition of PHD2/HIF-1; specific inhibition of HIF downstream genes; inhibition of mTOR</td>
<td>(47, 54, 72, 79, 108, 120)</td>
</tr>
</tbody>
</table>

NOTE: Basic mechanisms behind the limitations to antiangiogenic therapies with their predictive biomarkers and related methods of detection, together with opportunities for interventional therapy to overcome them.

Abbreviations: AMPK, AMP-activated protein kinase; ANG2, angiopoietin-2; ATRA, all-trans retinoic acid; CEC, circulating endothelial cell; CEP, circulating endothelial progenitor cell; CTC, circulating tumor cell; CT, computed tomographic scan; EC, endothelial cell; FDG, 2[18 F]fluoro-2-deoxy-d-glucose; GLUT1, glucose transporter-1; IHC, immunohistochemistry; LDHA, lactate dehydrogenase A; miRNA, microRNA; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; NRP1, neuropilin-1; PlGF, placental growth factor; pVHL, VHL protein; SDF-1, stromal cell–derived factor-1; SNP, single-nucleotide polymorphism; TEM, Tie2-expressing monocyte; vWF, von Willebrand factor.
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O. Casanovas has received honoraria from the Speakers Bureaus of Ipsen and Pfizer and is a consultant/advisory board member of Teva and Ipsen. No potential conflicts of interest were disclosed by the other authors.

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Writing, review, and/or revision of the manuscript: L. Moserle, G. Jiménez-Valero, O. Casanovas
Study supervision: O. Casanovas

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