

## Resistance and Escape From Antiangiogenesis Therapy: Clinical Implications and Future Strategies

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### ABSTRACT

Angiogenesis has long been considered an important target for cancer therapy. Initial efforts have primarily focused on targeting of endothelial and tumor-derived vascular endothelial growth factor signaling. As evidence emerges that angiogenesis has significant mechanistic complexity, therapeutic resistance and escape have become practical limitations to drug development. Here, we review the mechanisms by which dynamic changes occur in the tumor microenvironment in response to antiangiogenic therapy, leading to drug resistance. These mechanisms include direct selection of clonal cell populations with the capacity to rapidly upregulate alternative proangiogenic pathways, increased invasive capacity, and intrinsic resistance to hypoxia. The implications of normalization of vasculature with subsequently improved vascular function as a result of antiangiogenic therapy are explored, as are the implications of the ability to incorporate and co-opt otherwise normal vasculature. Finally, we consider the extent to which a better understanding of the biology of hypoxia and reoxygenation, as well as the depth and breadth of systems invested in angiogenesis, may offer putative biomarkers and novel therapeutic targets. Insights gained through this work may offer solutions for personalizing antiangiogenesis approaches and improving the outcome of patients with cancer.

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### INTRODUCTION

Angiogenesis plays a critical role in tumor growth and progression.<sup>1</sup> Tumors acquire their supply by a variety of means including vasculogenesis, co-option of previously established vasculature, and vascular mimicry.<sup>2,3</sup> Efforts to target tumor angiogenesis have focused on the vascular endothelial growth factor (VEGF) pathway.<sup>4</sup> VEGF targeting has shown promise for some cancers but has not proven as efficacious as hoped.<sup>5,6</sup> Evidence suggests mechanisms of escape mediated by tumor cells and by members of the microenvironment, leading to the hypothesis that simultaneous targeting of complementary and redundant pathways may hold promise in the treatment of solid tumors.<sup>7,8</sup>

### TARGETING VEGF

More than 30 years ago, on the basis of the recognition that tumor-associated endothelial cells play a fundamental role in tumor neovascularization, and given their presumed genetic stability, these cells were proposed as a therapeutic target. VEGF-A was identified as a central endothelial cell survival factor and angiogenesis promoter.<sup>9</sup> Bevacizumab (mono-

clonal antibody against VEGF-A165) was among the initial antiangiogenic agents developed.<sup>10</sup> This therapeutic strategy has provided clinical benefit in several solid tumor types, and bevacizumab remains approved for colorectal, renal, nonsquamous/non-small-cell lung cancer, and glioblastoma.<sup>11</sup> Although bevacizumab was approved for metastatic breast cancer in 2008, approval was withdrawn secondary to concerns about efficacy relative to toxicity.<sup>11</sup> In ovarian cancer, GOG 218 (Gynecologic Oncology Group 218; three-arm trial: paclitaxel/carboplatin chemotherapy (CT) v CT plus concurrent bevacizumab v CT plus concurrent and maintenance bevacizumab) and GIG ICON7 (Gynaecologic Cancer InterGroup International Collaboration on Ovarian Neoplasms 7; two-arm trial: CT ± concurrent and maintenance bevacizumab) were both conducted in the first-line adjuvant setting after tumor cytoreduction.<sup>12,13</sup> In both trials, modest improvements in progression-free survival (PFS) were noted in the groups receiving maintenance bevacizumab. Overall survival (OS) data are not mature but are not expected to be positive. Although interval to progression has improved, there seems to have been no improvement in the total number of patients who progressed. Small-molecule inhibitor data are less mature, but

similar observations have been made regarding sorafenib and sunitinib, prompting investigation into potential mechanisms of escape from anti-VEGF therapy.<sup>14,15</sup>

### ESCAPE/RESISTANCE MECHANISMS

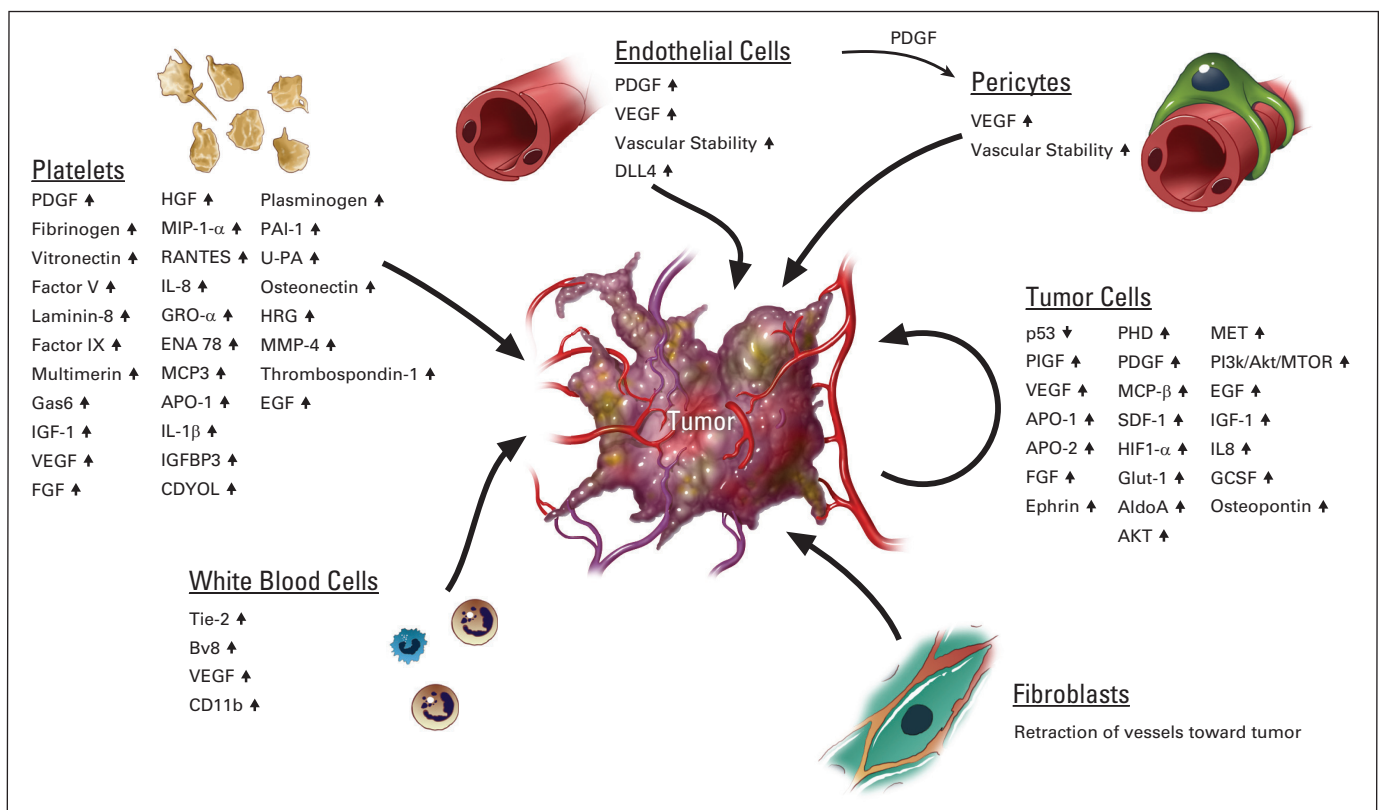
Evolutionary biology teaches that disruptions in an ecosystem by an imposed selection pressure produce reactionary dynamics and interactions, and natural selection will result in a resilient system. Cancer cells are characteristically heterogeneous and genetically unstable.<sup>16</sup> Furthermore, normal cells in the tumor microenvironment such as endothelial cells, pericytes, platelets, fibroblasts, and leukocytes are known to have normal functions that are co-opted to support tumor growth and progression. Potential mechanisms of resistance to antiangiogenic therapy, therefore, may result from the selection of directly and indirectly advantaged subpopulations of tumor and tumor-associated cells. If anti-VEGF therapy is considered as a selection pressure, and surviving cell populations are considered to be advantaged in the new environment, multiple plausible mechanisms of resistance and escape emerge for consideration (Fig 1).

#### Direct Selection Benefit to Tumor Cells

*Selection of clonal populations with upregulated alternative and compensatory pathways.* Various data raise the question of whether

anti-VEGF therapy selects for clonal populations that have upregulated alternative and compensatory proangiogenic signaling cascades. In pancreatic cancer models, treatment with a VEGF receptor 2 (VEGFR-2) –inhibiting antibody resulted in 10 to 14 days of tumor stasis followed by tumor regrowth, with apparent acceleration of tumor vascularity and increased expression of fibroblast growth factor 1 (FGF-1), FGF-2, Ephrin-A1 (Eph-A1), Eph-A2, and angiopoietin-1.<sup>7</sup> In tumors whose angiogenesis should otherwise be suppressed by ectopic expression of angiogenesis inhibitors, compensatory upregulation of VEGF, platelet-derived growth factor (PDGF), and FGF-2 is observed.<sup>17</sup> In human studies, predictable upregulation of FGF-2, VEGF, and placental growth factor (PIGF) has been detected in response to antiangiogenic therapy.<sup>18</sup> In sunitinib-treated mice, VEGF, granulocyte colony-stimulating factor, stromal cell–derived factor 1-alpha (SDF-1 $\alpha$ ), stem-cell factor, and osteopontin have all been found to be increased.<sup>18</sup>

FGFs are important promoters of angiogenic and mitogenic activity.<sup>19</sup> FGF-1 and FGF-2 induce angiogenesis, and preclinical models suggest that FGF binding protein releases FGF-2 from the extracellular matrix, allowing it access to receptors.<sup>20,21</sup> Cross talk between VEGF and FGF may stimulate angiogenesis synergistically, with variable effects on vessel size and function.<sup>19</sup> In a phase II trial of FOLFIRI + B (folinic acid, fluorouracil, irinotecan, and bevacizumab) in colorectal cancer, significant increases in FGF-2 as well as hepatocyte growth



**Fig 1.** Resistance and escape from antiangiogenesis therapy is multifactorial; it is driven by the intrinsic properties of cancer cell subpopulations and members of the tumor microenvironment, resulting in the evolution of an advantaged tumor ecosystem in response to the stimulus of antiangiogenic therapy. AldoA, Aldolase-A; APO, apolipoprotein; DLL, delta-like ligand; ENA, epithelial neutrophil-activating peptide; FGF, fibroblast growth factor; GCSF, granulocyte colony-stimulating factor; Glut-1, glucose transporter 1; GRO, growth-regulated oncogene; HGF, hepatocyte growth factor; HIF1- $\alpha$ , hypoxia-inducible factor-1 alpha; HRG, histidine-rich glycoprotein; IGF, insulin-like growth factor; IGFBP, IGF binding protein; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PIGF, placental growth factor; RANTES, regulated and normal T cell expressed and secreted; SDF, stromal cell–derived factor; U-PA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

factor, PlGF, SDF-1, and macrophage chemoattractant protein-3 were observed in plasma samples.<sup>22</sup> In patients with glioblastoma, treatment with cediranib, a pan-VEGFR inhibitor, resulted in decreased tumor edema by dynamic magnetic resonance imaging; subsequent increases in FGF-2 and SDF-1 levels correlated with progression.<sup>23</sup> Murine models of pancreatic and renal-cell carcinoma further support that upregulation of FGF-2 is associated with tumor progression in the face of VEGFR-2 blockade, and upregulation of FGF-2, angiopoietin-2, and PDGF-A has been implicated in bypass of antiangiogenic signaling.<sup>17</sup>

Recognizing the complexity of angiogenesis, other signaling systems are potentially implicated. In tumor models without hypoxia inducible factor 1-alpha (HIF-1 $\alpha$ ), interleukin-8 (IL-8) expression has been shown to support angiogenesis.<sup>24</sup> In a phase II trial of FOLFIRI+B in colorectal cancer, increased baseline IL-8 correlated with decreased PFS.<sup>22</sup> Increased insulin-like growth factor 1 has been associated with increased prostaglandin E2 expression, which correlates with increased VEGF expression and enhanced angiogenesis.<sup>25</sup> VEGF precursor mRNA undergoes alternative splicing that generates both pro- and antiangiogenic forms, and variant splicing has been hypothesized to explain both initial and acquired resistance to antiangiogenic therapy.<sup>26</sup> Studies of the murine double minute (*MDM2*) oncogene have demonstrated a p53-independent, hypoxia-driven translocation to the cytoplasm, with binding and stabilization of VEGF mRNA, increasing translation, VEGF protein production, cell survival, and angiogenesis.<sup>27</sup> Epidermal growth factor receptor (EGFR)–mediated activation of the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway can also upregulate HIF-1 $\alpha$  and VEGF, and there have been separate pathways described by which VEGF production via phosphoinositide 3-kinase is independent of HIF.<sup>28</sup> One report suggested that blockade with anti-EGFR antibody resulted in selection of tumor cell subpopulations with increased angiogenic potential.<sup>29</sup> These studies form the basis of dual targeting of EGFR and VEGFR with drugs such as vandetanib.<sup>30</sup>

*Selection of clones with greater invasive capacity in nonangiogenic environment.* Evidence suggests that when VEGFR/PDGF receptor (PDGFR) signaling is blocked with sunitinib (preclinical pancreatic and glioblastoma models) in addition to anticipated antitumor effects, a tumor phenotype is observed with heightened invasive and metastatic potential. In an orthotopic mouse model of glioblastoma multiforme, it was observed that blockade of VEGF with a neutralizing antibody resulted in a tumor phenotype that continued to grow, albeit more slowly, with persistent invasive capacity.<sup>31</sup> These models remain unsubstantiated and controversial, but they raise potential concerns regarding unintended consequences of blockade of these pathways, and mechanisms remain under exploration.<sup>32,33</sup>

Aside from the upregulated mitogenic pathways discussed previously, the hypoxic, acidic, high–interstitial fluid pressure features of the tumor microenvironment seem to favor tumor clones with more aggressive behavior.<sup>34</sup> It has been argued that this microenvironment fuels nonproductive angiogenesis that fails to relieve the hypoxic stress, perpetuating a self-reinforcing loop of pathologic angiogenesis.<sup>35</sup> Hypoxic conditions created by VEGF pathway inhibitors correlate with upregulation of the *MET* oncogene, promoting invasive behavior.<sup>36,37</sup> *MET* overexpression has been observed in variety of solid tumors including advanced ovarian cancer.<sup>36</sup> Cabozantinib (XL-184) is an oral, potent inhibitor of *MET* and VEGFR-2, and a phase II trial (100 mg once daily orally over 12 weeks) in advanced, progressive

epithelial ovarian cancer showed a promising clinical response rate (overall, 24%).<sup>38</sup>

*Selection of clones resistant to hypoxia.* Pancreatic ductal adenocarcinoma is characteristically hypovascular and in clinical trials has shown no treatment response to bevacizumab.<sup>37,39</sup> This tumor type seems to have underlying resistance to hypoxia, indicating a high probability that other cell populations could evolve this trait. Approximately 75% of these tumors carry inactivating mutations of the *p53* gene, which may contribute to survival in hypoxic conditions.<sup>40,41</sup> Additionally, 75% of pancreatic cancer cell lines in one study constitutively expressed HIF-1 $\alpha$ , correlating with resistance to apoptosis induced by hypoxia and/or glucose deprivation. Constitutive HIF-1 $\alpha$  expression correlates with increased expression of glucose transporter 1 and Aldolase-A, both of which are associated with anaerobic metabolism.<sup>42</sup> These data suggest that preferential utilization of particular metabolic pathways may favor certain cell populations when VEGF-targeted therapy imposes a hypoxic environment. The combination of p53 loss supporting cell survival and the utilization of metabolic pathways that additionally favor survival in a hypoxic environment would offer a simple mechanism by which clonal populations may thrive in the hypoxic environment.

Compensation for VEGF blockade and resistance to hypoxia can be mediated by the microenvironment. Hypoxic conditions promote recruitment of bone marrow–derived cells that include vascular progenitors (eg, endothelial and pericyte progenitors) and vascular modulators.<sup>43,44</sup> Vascular modulators are a class of cells including tumor-associated macrophages, immature monocytes, VEGFR-1 hemangiocytes, and CD11b–myeloid cells.<sup>45,46</sup> HIF-1 $\alpha$  has been implicated in the recruitment of CD45 myeloid cells with various subpopulations expressing Tie-2, VEGFR-1, CD11b, mature F4/80 tumor-associated macrophages, and endothelial and pericyte progenitors.<sup>46,47</sup> This environment favors selection of tumor clones protected by CD11b+Gr1+ myeloid cells that express proangiogenic factors such as Bv8, which has been shown to be partially responsible for angiogenesis promotion during VEGF blockade.<sup>48,49</sup> These cells facilitate progression to frank carcinoma and may represent an important alternative therapeutic target.<sup>50</sup>

### **Indirect Selection Benefit to Tumor Cells**

*Selection and support of normalized vasculature, supporting tumor cell growth and function.* Anti-VEGF therapy results in vascular normalization that remains of unclear therapeutic significance.<sup>35</sup> Abnormal tumor vasculature functions poorly, reflected by chaotic, stagnant, even reversed flow.<sup>34,51</sup> The hypoxic, acidic microenvironment promotes protease-mediated matrix remodeling, anchorage-independent growth, and resistance to apoptosis despite increasing genetic instability.<sup>52,53</sup> Inhibition of VEGF reduces vessel size and tortuosity; the remaining vessels have greater pericyte coverage, and the basement membrane is normalized.<sup>54</sup> Some studies have posited a therapeutic drug-delivery advantage (within a limited therapeutic window) gained by changes in vascular permeability.<sup>34</sup> Other studies have suggested that normalization and maturation may represent mechanisms of therapeutic escape. In one study, myeloid cell–driven angiogenesis was selectively ablated in a mouse model of breast cancer, resulting in reduced VEGF, reduced vascular density, increased maturation, and concomitant maturation/normalization; this normalization correlated with increased tumor growth and progression.<sup>55</sup>



The oxygen-sensing prolyl hydroxylase domain (PHD) proteins may also play a role in vascular normalization and maturation and contribute to more aggressive tumor behavior after antiangiogenic therapy. PHD-2 is an oxygen-sensing enzyme that hydroxylates HIF when sufficient oxygen is available, targeting the protein for degradation.<sup>56</sup> Under hypoxic conditions resulting from anti-VEGF treatment, a compensatory release of proangiogenic cytokines generates vessels characterized by irregular borders, hypermobile cells, loosely attached layers, and denuded areas.<sup>34,57</sup> In PHD-2 heterozygous mice, endothelial cells upregulate VEGFR-1 and VE-cadherin to stabilize vessels, reduce leakage, and improve vessel perfusion, allowing normalization of neovasculature; homozygous deficiency does not allow this function.<sup>57</sup> In some non-small-cell lung cancers, PHDs are present and upregulated, suggesting a possible vascular normalization function associated with overall tumor growth.<sup>58</sup>

The hyperactivated angiogenic state characteristic of some tumor microenvironments results in a dense, nonproductive vascular network that fails to support tumor growth. Recent data suggest that the Notch ligand Delta-like ligand-4 (DLL4), which is normally induced by VEGF, is actually a negative-feedback regulator of vascular sprouting and branching. In mice lacking DLL4, or in circumstances where it is inhibited, there is excessive and nonproductive angiogenesis (blind-end budding).<sup>59</sup> Despite the excess of angiogenesis, tumor growth is decreased, even in tumors resistant to anti-VEGF therapy. The current hypothesis is that in the absence of DLL4, there is inadequate maturation, and therefore inadequate function, of the vessels.<sup>59</sup> It has been shown that tumor resistance to bevacizumab can be induced by transfection of DLL4; blockade of Notch signaling reverses this resistance. Mechanisms implicated include increased stromal VEGFR-1, decreased vascular VEGFR-2, diffusely reduced VEGFR-3, and increased signaling through FGF-2/FGF receptor and Ephrin-B4/Ephrin-B2. Cell lines transfected with DLL4 were also resistant to a VEGFR-targeted multikinase inhibitor.<sup>60</sup>

Among vascular support structures, pericytes provide survival factors and temper the proliferation rate of endothelial cells.<sup>61-63</sup> Pericytes are positioned around endothelial cell junctions, provide physical support, and release low levels of endothelial survival factors such as VEGF; for new vascular branches to form, pericytes must detach.<sup>64</sup> Within tumors, pericyte coverage is variable, but it is less extensive than seen in normal tissue, and cells take on an abnormal shape and express markers characteristic of immature, less contractile mural cells.<sup>64-66</sup> Evidence suggests that tumor cell shedding into the circulation is inversely proportional to pericyte coverage.<sup>67</sup> Chronic VEGF expression in the tumor microenvironment seems to interfere with PDGF-B signaling, interfering with vascular smooth muscle cell function and smooth muscle recruitment.<sup>68</sup> VEGF blockade increases the signaling of angiopoietin-1, resulting in improved endothelial cell function and pericyte recruitment.<sup>69</sup>

PDGF/PDGFR signaling itself is implicated in rescue and escape from VEGF blockade.<sup>70</sup> The PDGF family provides mitogenic signaling necessary for pericyte recruitment and maturation. Immature vessels with poor pericyte investment seem vulnerable to anti-VEGF treatment, and post-treatment (surviving) vessels seem to have relatively high pericyte coverage.<sup>71,72</sup> In one study, PDGF-BB was expressed by endothelial and tumor cells, and PDGFR $\beta$  was expressed in pericyte-like cells; PDGF-BB increased the migration and VEGF production of these pericyte-like cells, and these noted functions could be blocked by PDGFR $\beta$  inhibitors.<sup>73</sup> AX102, a highly specific inhibitor of

PDGF-B signaling, was highly effective in combination with bevacizumab in ovarian cancer models.<sup>74</sup> Dual targeting of endothelial cells and pericytes has been considered to hold potential as an antivascular therapeutic approach in ovarian carcinoma, and agents such as pazopanib, sunitinib, sorafenib, and BIBF-1120 are being tested in clinical trials.

The role of PlGF in tumor angiogenesis remains controversial and poorly understood. PlGF overexpression in tumors in vivo has been shown to correlate with decreased tumor growth, and it has also been shown to correlate with normalization of tumor vasculature, possibly through heterodimerization with VEGF, reducing VEGF potency.<sup>75,76</sup> PlGF-null mice demonstrate reduced response to VEGF. However, PlGF is chemotactic for endothelial cells and monocytes in vitro; it may be involved with mobilization of bone marrow-derived cells, and it increases the response of cultured endothelial cells to VEGF-induced survival, proliferation, and migration.<sup>77,78</sup> Aflibercept (VEGF Trap) is a protein that contains the VEGF-binding regions of VEGFR-1 and VEGFR-2 fused to the Fc portion of human IgG1. It acts as a high-affinity soluble VEGFR decoy receptor and therefore inhibits the activity of both VEGF and PlGF.<sup>79</sup> Two randomized phase II studies showed that even in heavily pretreated patients, single-agent aflibercept could induce tumor response and delay progression.<sup>80,81</sup> A recent combined phase I/II trial of docetaxel plus aflibercept in patients with recurrent ovarian, primary peritoneal, or fallopian tube cancer resulted in a 54% response rate, and 24% of patients had stable disease.<sup>82</sup>

Ang1 and Ang2 exist in balance to promote organization and maturation of neovasculature. Overexpression of Ang1 compared with Ang2 results in dense hypervascularization with large vessels, and excess Ang2 binding to Tie-2 results in destabilized and leaky blood vessels.<sup>83</sup> Perivascular Tie-2-expressing monocytes are also proangiogenic.<sup>35</sup> Coordinated Ang1 and Ang2 signaling through Tie-2 receptors is thought to be an alternative pathway to VEGF through which neovascularization can occur, and coordinated upregulation may represent an additional mode of anti-VEGF-therapy bypass.

*Selection of subpopulations capable of co-opting normal/existing vasculature.* Grade 2 and 3 astrocytomas have been observed to develop neovasculature without an identified proliferative endothelial component.<sup>84,85</sup> Vascular acquisition in this case seems to be via perivascular tumor invasion with incorporation of normal vasculature into the tumor structure. As an apparent corollary, three human studies reported patients with multifocal recurrence while receiving bevacizumab therapy.<sup>37,86,87</sup> These findings, in conjunction with data suggesting that fibroblasts can draw existing vessels toward and into a fresh wound, point toward cell populations that have the phenotypic capacity to home toward existing vasculature and to incorporate it rather than relying on a cytokine-driven neovascular response. These clonal populations would be favored in an environment exposed to antiangiogenic therapy.

*Endothelial cell genetic instability and resistance to therapy.* Contrary to previous dogma stating that endothelial cells within tumors are genetically stable and therefore less likely to evolve resistance to therapy, recent work has identified cytogenetic abnormalities in human tumor-associated endothelial cells.<sup>88,89</sup> Although they have not been validated, these studies raise the possibility that high rates of endothelial cell mutation within tumors could contribute to therapeutic resistance to a greater degree than previously surmised.<sup>88,89</sup> What remains to be elucidated is why tumor-based endothelial cells have

significant cytogenetic instability and whether this high rate of mutation would confer the capacity to select for unique subpopulations of endothelial cells that would develop therapeutic resistance over (relatively) short periods of time.<sup>90</sup>

### ATTRACTIVENESS OF MULTIPLE-PATHWAY INHIBITORS

Given our increasing knowledge regarding the myriad alternative pathways for the development and maintenance of tumor vasculature, several new inhibitors have been engineered to block multiple proangiogenic signaling pathways. Sorafenib, axitinib, sunitinib, cediranib, and pazopanib variously target proangiogenic signaling cascades and are in clinical trials. The most promising of these is a phase III trial of pazopanib in advanced renal cell carcinoma. Compared with placebo, pazopanib improved PFS from 4.2 to 9.2 months. The difference in PFS was even more striking in patients who were treatment naive (11.1 v 2.8 months;  $P < .001$ ). Response rate was also improved (30% v 3%), and median duration of response was  $> 1$  year.<sup>91</sup>

Other drugs have been developed to target VEGF and FGF signaling. Brivanib (dual FGF/VEGF inhibitor) has shown activity in preclinical pancreatic cancer models that have developed resistance to VEGF inhibition. BIBF-1120 is a unique, triple-angiokinase inhibitor of VEGFR, PDGFR, and FGF receptor. In a randomized, phase II placebo-controlled trial, patients who had completed chemotherapy for relapsed ovarian cancer with evidence of response were treated with BIBF-1120. Three-year PFS rates were 16.3% and 5.0% in the BIBF 1120 and placebo groups, respectively (hazard ratio, 0.65; 95% CI, 0.42 to 1.02;  $P = .06$ ).<sup>92</sup> A current phase III (NCT01015118) trial is evaluating the addition of BIBF-1120 to carboplatin/paclitaxel in first-line chemotherapy in ovarian cancer.<sup>93</sup>

AMG386 is an investigational, angiopoietin antagonist peptide-Fc fusion protein that selectively binds Ang1 and Ang2. This binding prevents the interaction of Ang1 and Ang2 with Tie-2 and inhibits tumor endothelial cell proliferation and tumor growth.<sup>94</sup> In a randomized, double-blind, placebo-controlled phase II study to evaluate the safety and tolerability of AMG386 in combination with paclitaxel, the addition of AMG386 to paclitaxel demonstrated dose-responsive improvements in PFS with a manageable safety profile distinct from that of VEGF inhibition.<sup>95</sup> AMG386 has entered phase III investigation in the setting of recurrent ovarian cancer.

Attention has also shifted to nonreceptor kinases such as Src and Fak, which are implicated in multiple tumorigenic behaviors including angiogenesis.<sup>96</sup> The Src/Fak complex, in particular, has been the target of drug development.<sup>97-99</sup> In phase II investigation, the Src inhibitor dasatinib has been used as first-line therapy in advanced non-small-cell lung cancer. Of 34 patients, the overall disease control rate was 43%; however, only one patient had a partial response. Interestingly, 11 patients had a metabolic response, suggesting poor patient selection and/or dose selection.<sup>100</sup> An additional target of interest is EZH2. Increased EZH2 in endothelial cells results from paracrine VEGF stimulation, resulting in silencing of VASH1. Silencing of EZH2 in the endothelium results in decreased tumor angiogenesis and reduced ovarian cancer growth in an orthotopic model. EZH2 silencing in tumor cells has a similar effect.<sup>101</sup> EZH2 may also be subject to regulation by FGF-2 and miR101 signaling.<sup>102,103</sup>

**Table 1.** Selected Classes of Agents That Represent Novel Approaches to Targeting Biology of Angiogenesis Through Pathways Other Than VEGF Signaling

Mechanism of Action/Class	Representative Drugs	Current Clinical Phase
Vascular disrupting agents	ASA-404	II-III
	AVE-8062	II-III
	Ombrabulin	II-III
	CA4P	II-III
	Crolibulin	I-II
	DMXAA	I-II
	NPI-2358	I-II
	Plinabulin	I-II
	Soblidotin	I-II
	Denibulin	I
Oxi-4503	I	
ZD-6126	I	
FGFR targeting	Dovitinib	III
	BIBF-1120	II-III
	Brivanib	II-III
	Pazopanib	II-III
	BGJ-398	I
Angiopoietin targeting	AMG-386	II-III
	MEDI-3617	I-II
EphrinA2 targeting	Dasatinib	II-III
DLL4/Notch targeting	MEDI-0639	I
	REGN-421	I
PI3K/mTOR targeting	Demcizumab	I
	BKM-120	II-III
	Everolimus	II-III
	Temsirolimus	II-III
	BEZ-235	I-II
	BGT-226	I-II
	DS7-423	I-II
	GDC-0941	I-II
	GSK-2110183	I-II
	PF-04691502	I-II
	PX-866	I-II
	XL-147	I-II
	XL-765	I-II
	INK-1117	I
GSK-1059615	I	
GSK-2126458	I	
PKI-179	I	
SF-1126	I	
ZSTK-474	I	
EGFR targeting	Cetuximab	II-III
	Erlotinib	II-III
	Gefitinib	II-III
	Panitumumab	II-III
	Vandetanib	II-III
	Lapatanib	I-II
	MM-121	I-II
MET targeting	Cabozantinib	II-III
PDGFR targeting	Axitinib	II-III
	BIBF1120	II-III
	Cediranib	II-III
	Dovitinib	II-III
	Pazopanib	II-III
	Sunitinib	II-III
	Sorafenib	II-III
PIGF targeting	Aflibercept	II-III
Src/Fak complex targeting	Dasatinib	II-III
	Sunitinib	II-III
	KX2-391	I-II
IL-6 targeting	Siltuximab	II-III
AKT targeting	MK-2206	I-II
IGF1-R targeting	MK-0646	I-II

Abbreviations: DLL4, delta-like ligand-4; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; IGF1-R, insulin-like growth factor receptor 1; IL-6, interleukin-6; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; PIGF, placental growth factor.

## STRATEGIC CLINICAL INVESTIGATION

Given the exponential number of new drug combinations, as well as the cost of drug trials, careful consideration must be given to trial design and rational combination therapy. Conventional clinical trials rely on randomly assigning a heterogeneous array of eligible patients to a small number of therapeutic arms. These studies consistently fail to acknowledge the fundamental biologic variability of cancer and thus necessarily expose patients to potentially toxic medication in the absence of an expectation of benefit. Because of limited resources, validated, biomarker-driven designs are of great interest. These smart trials have the potential of enrolling fewer patients, anticipating larger treatment effects, and reducing unnecessary harms.

An alternative approach may be trial designs in which a pharmacodynamic or pharmacokinetic marker could be assessed early in therapy, and if the marker is undermodulated, the experimental agent could be withdrawn. In GOG 262 (randomized, phase III trial in primary ovarian cancer that includes a bevacizumab maintenance arm), a computed tomography–based perfusion scan is being evaluated to identify those who might benefit from long-term bevacizumab exposure. With regard to biologic tumor variation, circulating tumor cells and cell-free nucleic acids may offer a real-time assay for making more informed choices regarding dose and duration of therapy.

## NEW DIRECTIONS

Surgery and cytotoxic chemotherapy remain the mainstays of primary therapy. The use of targeted biologics combined with one another or with traditional therapy is intended to increase tumor response without significant increases in toxicity. Some have interpreted results of phase III studies of biologics as reflecting poor drug responsiveness. The true shortcoming of these trials lies in the failure of the trial design to account for tumor cell heterogeneity, both among individuals and within a given individual's tumor cell population; different underlying aberrant biology should be anticipated to be vulnerable to different drug targeting. As targeted biologic therapies push forward, it becomes increasingly important to identify susceptible tumor cell populations within a given patient to identify which cytotoxics and biologic inhibitors may provide that individual with clinical benefit.

Fresh targets are needed, and the microenvironment provides a rich array of necessary, targetable, angiogenic participants such as pericytes, endothelial cells, and bone marrow–derived precursors. Although multiple-kinase inhibitors have risk of increased toxicity, carefully considered combinatorial choices in well-designed trials hold real promise of clinical benefit. Additionally, vascular disrupting agents are a unique class of drugs that aim to collapse existing vascular structures.<sup>104</sup> Among these, combretastatin A-4 (modified to AC7700; now known as ombrabulin) was encouraging in preclinical sarcoma and lung cancer models.<sup>105</sup> CA4P, pinabulin, and ombrabulin have shown some promise in phase II clinical trials.<sup>106</sup> Combination with traditional antiangiogenic agents might further improve the overall antitumor effect.<sup>107</sup> Table 1 summarizes selected classes of agents in current development that represent novel and promising approaches

to targeting the biology of angiogenesis through pathways other than VEGF signaling.

A recent, compelling strategy involves metronomic dosing of chemotherapy, in which lower doses of cytotoxic agents at more frequent intervals is thought to target the tumor vasculature.<sup>108,109</sup> Clinically, semimetronomic, or dose-dense, regimens have shown intriguing superiority to traditional dosing regimens.<sup>110-113</sup>

MicroRNAs are a recent addition to the angiogenesis literature. More than 20 microRNAs have been identified that either target genes involved in angiogenesis or respond to angiogenic stimuli such as VEGF.<sup>114</sup> These microRNAs may represent transmissible genetic elements that can be dysregulated by tumors. The new finding of endothelial genetic instability may imply the presence of transposable genetic elements by which a genetically unstable cell can transmit unstable elements to surrounding cells.

Finally, the hematologic contribution to angiogenesis and tumorigenesis must be more fully explored and targeted. Transgenic mouse models of de novo skin carcinogenesis provide evidence that early hyperplasia and early increases in angiogenesis are correlated with mast cell recruitment and degranulation.<sup>115</sup> B lymphocytes initiate reactions resulting in mast cell recruitment, which correlates with increased angiogenesis and progression from hyperplasia to dysplasia to carcinoma.<sup>116</sup> Causality is supported by the fact that blocking B-lymphocyte responses, mast cell infiltration, and recruitment of immature myeloid cells decreases angiogenesis and tumor progression.<sup>115</sup> In a transgenic mouse model of breast cancer, the transition from adenoma to mammary intraepithelial neoplasia was accompanied by activation of angiogenesis that coincided with macrophage infiltration of the tumor. Depletion of these macrophages reduces angiogenesis and tumor progression; the provision of VEGF after macrophage depletion restores the progression of malignancy.<sup>117</sup> Platelets are targetable mediators of angiogenesis. Purinergic signaling from platelets influences cell migration and proliferation.<sup>118-120</sup> Alpha granule contents are known mediators of pro- and antiangiogenic effectors, supporting the notion that platelets may be sophisticated and integrated angiogenesis regulators.<sup>121</sup> IL-6 is implicated in paraneoplastic thrombocytosis, and targeting with siltuximab (a monoclonal antibody targeting IL-6) was shown in preclinical models to abrogate thrombocytosis and to have antitumor effects additive to conventional cytotoxic agents.

A better understanding of the biology of hypoxia and reoxygenation, including the biochemical, microenvironmental, and ecologic effects, could provide a model of solid tumor biology, facilitating wise choices of biomarkers and therapeutic interventions. Similarly, it may provide insights into natural history and prognosis that would aid in more holistic decision making for and by the patient as we aim to enter an age of individually focused care and improved outcomes.

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