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Review

Mechanisms of and strategies for overcoming resistance to anti-vascular endothelial growth factor therapy in non-small cell lung cancer

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ABSTRACT

Sustained angiogenesis is a hallmark of cancer. Because of the primary role of vascular endothelial growth factors (VEGFs) and their receptors in angiogenesis, VEGF-targeted agents have been developed to inhibit these signaling processes in non-small cell lung cancer (NSCLC). However, the clinical benefits are transient and resistance often rapidly develops. Insights into the molecular mechanisms of resistance would help to develop novel strategies to improve the efficacy of antiangiogenic therapies. This review discusses the mechanisms of resistance to anti-VEGF therapy and the postulated strategies to optimize antiangiogenic therapy. A number of multitargeted tyrosine kinase inhibitors currently in phase III clinical development for NSCLC are summarized. The emerging combination of antiangiogenic therapy with tumor immunotherapy is also discussed.

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1. Introduction

Non-small cell lung cancer (NSCLC) is the most common form of lung cancer, accounting for 84% of all lung cancers in the United States [1]. The 5-year survival rate for NSCLC is currently below 20% [1], highlighting the need for new treatment strategies.

Angiogenesis is an essential component of primary tumor growth and metastasis (Table 1) [2]. The key proteins involved in angiogenesis include members of the vascular endothelial growth factor (VEGF) family, which consists of 5 members in mammals: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF) [3]. Of these, VEGF-A is the primary growth factor associated with vessel formation [2,3]. VEGF binds to a family of receptor tyrosine kinases called VEGF receptors (VEGFRs), including VEGFR-1, VEGFR-2, and VEGFR-3, and causes dimerization of the tyrosine kinase domain. The dominant VEGFR in angiogenic signaling with VEGF-A is VEGFR-2 [3]. Neuropilin (NRP)-1 and NRP-2 are co-receptors for VEGF family members and play a role in VEGF-mediated angiogenesis [4,5].

Antiangiogenic therapies (Fig. 1) have been investigated (and some approved) in several solid tumors [2]. The first antiangiogenic agent approved for NSCLC is bevacizumab (approved in 2006; Avastin®, Genentech, South San Francisco, CA, USA), a monoclonal antibody to VEGF-A [6]. Bevacizumab in combination with carboplatin/paclitaxel improved both progression-free survival (PFS) and overall survival (OS) compared with chemotherapy alone in patients with advanced NSCLC [7]. However, similar to cancer cell targeted therapies, the clinical benefits from VEGF inhibitors are often on the orders of months and usually followed by the rapid emergence of resistance [8–10]. It is important to recognize that, unlike cancer cell targeted therapies that are only given to a subset of patients according to biomarkers, antiangiogenic agents are usually given to all patients for the approved indications. Therefore, informed selection of patients would likely significantly improve their clinical benefits. For example, recurrent glioblastoma patients with an increase in tumor blood perfusion after cediranib treatment survive about 6 months longer than those with stable perfusion [11]. Thus, the insights into the resistant mechanisms would improve the application of antiangiogenic therapy and achieve better clinical outcomes.

Both primary and acquired resistance can limit the efficacy of antiangiogenic therapy. Primary resistance occurs when the agent fails to have any effect on the tumor upon initial treatment, while acquired resistance to therapy describes tumor progression when treatment is ongoing following a previous response [12]. This article will provide an overview of proposed mechanisms of primary and acquired resistance to VEGF-targeted therapy, followed by a discussion of completed and ongoing clinical trials of multitargeted tyrosine kinase inhibitors (TKIs) in advanced NSCLC.

Table 1
Angiogenic factors involved in NSCLC.

Factor	Role in nontumor cells
ALK1 [28]	Type I transforming growth factor β subclass involved in vasculogenesis
Angiogenin [110]	Ribonuclease active in angiogenesis
Ang-1 [109]	Binds to Tie2 to control vessel stabilization
DLL4 [29]	Signaling in vascular development and angiogenesis
Ephrins [20]	VEGF-independent regulation of angiogenesis
FGFs (acidic and basic) [20]	VEGF-independent regulation of angiogenesis
HIF-1α [111]	Regulation of oxygen homeostasis
HGF [112]	Involved in embryonic angiogenesis
IL-8 [113]	Promotes angiogenesis in endothelial cells
NRP-1 and -2 [25]	Modulators of VEGF pathway
PD-ECGF [114]	Non-heparin-binding angiogenic factor, originally isolated from platelets
PDGF-β [109]	Involved in vessel wall development
PlGF [3]	Placental member of VEGF family
VEGF [3]	Primary signaling factors involved in angiogenesis

NSCLC, non-small cell lung cancer; ALK1, activin A receptor type II-like 1; Ang, angiopoietin; DLL4, delta-like ligand 4; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; HGF, hepatocyte growth factor; IL, interleukin; NRP, neuropilin; PD-ECGF, platelet-derived endothelial cell growth factor; PDGF, platelet-derived growth factor; PlGF, placental growth factor; VEGF, vascular endothelial growth factor.

2. Resistance to VEGF-targeted therapy

Primary resistance is likely attributed to a number of different mechanisms. These may include hypovascularity (eg, pancreatic cancer) [8], other modes of tumor vascularization (eg, vessel co-option and vasculogenic mimicry) [13], and pre-existing redundant proangiogenic pathways [8]. Even without primary resistance, eventual acquired resistance to antiangiogenic therapy usually occurs, also via multiple distinct mechanisms [12,14]. These may include mutations resulting from the chromosomal instability of endothelial cells [15], selection of hypoxia-resistant clones [16], recruitment of angio-promoting bone marrow-derived cells [8], and upregulated compensatory proangiogenic factors due to the plasticity and adaptability of cancer cells and stromal cells [8,17].

2.1. Redundant or compensatory proangiogenic factors

Compensatory proangiogenic factors (treatment-induced or intrinsic) may trigger VEGF-independent tumor neovascularization and cause resistance to VEGF-targeted therapy. For example, in mouse xenograft models of human lung adenocarcinoma, pericytes have been shown to adapt to anti-VEGF treatment and induce expression of epidermal growth factor (EGF), leading to vascular remodeling and resistance to antiangiogenic therapy [17,18]. Some tumors treated with anti-VEGF therapy can overcome inhibition through upregulation of platelet-derived growth factor C (PDGF-C) in tumor-associated fibroblasts [19]. Similarly, fibroblast growth factor 1 (FGF-1) and FGF-2 have been shown to be upregulated in pancreatic islet cell tumors that were unsuccessfully treated with an anti-VEGFR-2 antibody, with preclinical evidence that such increases may occur as part of a hypoxia-mediated phenomenon that ultimately leads to resistance to VEGFR blockade [20]. In a murine model, an anti-placental growth factor (anti-PlGF) antibody effectively inhibited growth of tumors resistant to treatment with an anti-VEGFR-2 antibody; these effects were attributed to its ability to prevent macrophage infiltration without causing severe hypoxia or triggering compensatory angiogenic activity [21]. However, a subsequent study showed that PlGF blockade did not inhibit tumor growth nor improve the effect of anti-VEGF antibody treatment in several murine tumor models [22]. In addition, aflibercept (VEGF Trap [ziv-aflibercept in the United States]; Zaltrap®, Sanofi, Paris, France; and Regeneron Pharmaceuticals, Tarrytown, NY, USA), which was designed to neutralize VEGF family ligands and PlGF simultaneously, did not improve OS when added to standard docetaxel therapy for advanced NSCLC [23]. The reasons for such discrepancy remain unclear, but may be related to tumor type and stage. Clinical observations also support that tumor progression on antiangiogenic therapy is preceded by an increase in angiogenic cytokines other than VEGF, such as basic FGF, hepatocyte growth factor, and interleukin (IL)-6 in advanced renal cell carcinoma [24]. Collectively, these results suggest that VEGF-independent signaling, such as FGF, PDGF, EGF, or PlGF, may be involved in escape from anti-VEGF therapy in some cancers [16].

In addition, some intrinsic angiogenic factors can modulate vessel formation and have been implicated in resistance to VEGF-targeted therapy. NRPs modulate the VEGF pathway and may compensate during VEGF blockade [25]. Using H1299 xenografts, a NSCLC model with high expression (vascular and stromal) of NRP-1, Pan and colleagues observed additive antitumor activity with the combination of anti-VEGF and anti-NRP-1 antibodies [25]. In addition, angiopoietins and their endothelial receptor Tie2 are involved in regulation of vessel stability [26] and have been implicated in resistance to VEGFR-targeted therapy in preclinical models [20,27]. The activin A receptor type II-like 1 (ALK1)

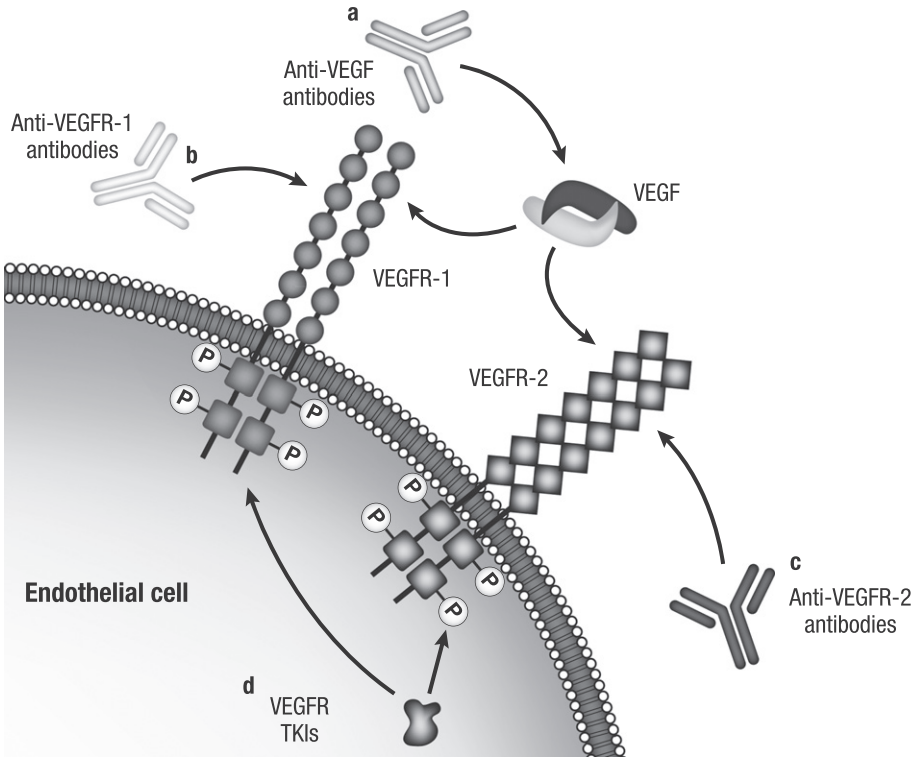

Fig. 1. Strategies to target angiogenesis. Diagram depicting the use of monoclonal antibodies that target a) VEGF, b) VEGFR-1, and c) VEGFR-2, as well as d) small molecule TKIs that target VEGF signaling. Adapted with permission from Macmillan Publishers Ltd: *Nature* [109], ©2005. Abbreviations: VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; TKI, tyrosine kinase inhibitor.

signaling pathway in proliferating endothelial cells has also emerged as a potential contributor of resistance to bevacizumab, possibly stemming from its ability to stabilize angiogenic vessels [28]. When analyzing human ALK1 expression in circulating endothelial cells, NSCLC was among the malignancies associated with the most pronounced increase relative to healthy controls [28]. Delta-like ligand 4 (DLL4), a member of the Delta/Jagged transmembrane ligand family, binds Notch receptors and is important in vascular development [29]. In a preclinical study, blockade of DLL4 induced a dysfunctional vascular network and delayed tumor growth in both anti-VEGF sensitive and resistant tumors [30].

2.2. Tumor stromal cells

The stromal compartment consists of heterogeneous cell populations, including immune cells, fibroblasts, and pericytes. Pericytes are a type of major vascular support stromal cell, which provide paracrine survival factors for endothelial cells, such as VEGF. Tumors with a high proportion of pericyte coverage are intrinsically resistant to VEGF blockade [20,31,32]. Cancer-associated fibroblasts (CAFs) are another type of proangiogenic stromal cell. CAFs are able to increase vessel pericyte coverage through the production of PDGF-C, which counteracts the vessel pruning effects of VEGF blockade. Neutralization of PDGF-C has exhibited additive anticancer effects with anti-VEGF antibody treatment [19,33]. Myeloid cells, such as myeloid-derived suppressor cells and tumor-associated macrophages, are a major source of various proangiogenic factors. Through this mechanism, myeloid cells mediate both primary and acquired resistance to anti-VEGF therapy in some tumor models, such as Lewis lung carcinoma [34]. In a series of studies to determine the role of myeloid cells in responsiveness to anti-VEGF therapy, Shojaei and colleagues showed that anti-VEGF refractoriness was associated with infiltration of the tumor tissue by $CD11b^{+}Gr1^{+}$ myeloid cells in some tumor models [34]. Upregulation of Bv8 in $CD11b^{+}Gr1^{+}$ myeloid cells enhances their infiltration into tumor tissue and promotes angiogenesis locally [35]. IL-17, a canonical cytokine of T helper type 17 (T_H17) cells, is also able to induce expression of VEGF and Bv8, and thus facilitate the proangiogenic capacity of $CD11b^{+}Gr1^{+}$ myeloid cells [36]. Blockade of VEGF and Bv8 or IL-17 simultaneously resulted in greater growth inhibition of refractory tumors than anti-VEGF therapy alone. In addition, tumor-infiltrating myeloid cells may exert unique proximate proangiogenic effects through direct incorporation into vessels or by establishing a local interaction with endothelial cells [37,38]. Therefore, specific strategies may be needed to overcome this type of resistance (eg, immune cell-specific depletion and polarization of macrophages away from a proangiogenic phenotype).

2.3. Other modes of tumor vascularization

Tumors can survive on other modes of vascularization, such as vasculogenic mimicry and vessel co-option. The role of vasculogenic mimicry in NSCLC is unclear; however, vessel co-option was first described in this setting. Vascular mimicry is a term used to describe the process by which an aggressive tumor generates its own network of vascular channels, providing an alternative source of blood supply for the tumor [39]. Vascular co-option is a process where malignant cells grow around pre-existing normal vessels and take their oxygen and other essential nutrients without evoking an angiogenic response [40]. Pezzella and colleagues identified a group of NSCLCs that did not have morphological evidence of neoangiogenesis, and grew by co-opting pre-existing pulmonary blood vessels; such nonangiogenic tumors were characterized as fast-growing and hypoxia tolerant [41,42]. It was also speculated that nonangiogenic tumors would be resistant to anti-VEGF therapy. Because angiopoietin-2 appears to play a key role in the regression of co-opted tumor vessels [40], targeting VEGF along with angiopoietins may more effectively control tumor growth than by targeting VEGF alone [13]. The outstanding question remains regarding whether vessel co-option contributes to resistance of NSCLC patients to bevacizumab, and thus may serve as a pathologic biomarker for selecting potentially nonresponsive patients.

2.4. Hypoxia

Antiangiogenic therapy often induces tumor hypoxia. Recently, the oxygen homeostasis regulator known as hypoxia-inducible factor (HIF)-1α was implicated in resistance to antiangiogenic therapy, with a combination of clinical and preclinical observations appearing to explain that at least some poor responses in a phase II trial of bevacizumab plus radiation therapy were associated with upregulated levels of HIF-1α [43]. Piao and colleagues reported the relative effects of bevacizumab versus the multitargeted TKI sunitinib (Sutent®, Pfizer, New London, CT, USA; targets VEGFR-1 through -3, platelet-derived growth factor receptor (PDGFR)-α/β, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog [c-kit], ret proto-oncogene [RET], fms-related tyrosine kinase 3 [Flt-3], and colony-stimulating factor 1 receptor [c-fms] [44]) in an orthotopic glioma model [45]. They found that bevacizumab significantly prolonged survival relative to untreated controls or sunitinib alone. This effect could be explained by the prolonged decrease in tumor vascularity observed with bevacizumab, which was associated with a delay in the development of hypoxia and myeloid cell infiltration. These results provide an interesting perspective on different types of antiangiogenic therapies, suggesting that the degree of hypoxia induced by a given agent may be a more important determinant of outcomes than the number of targets per se.

3. Strategies to delay or overcome resistance

3.1. Biomarkers of response to antiangiogenic therapy

Given that resistance to antiangiogenic therapies is clinically common, reliable biomarkers could help to predict efficacy and may also serve as a guide for developing more efficient regimens to delay or overcome resistance [46]. However, results to date have been inconsistent with antiangiogenic therapy. Across several trials evaluating bevacizumab in patients with various malignancies, higher plasma VEGF levels were associated with shortened PFS and OS [47], and specific VEGF isoforms were significantly correlated with poor survival by univariate analysis ($P = 0.001$) in patients with previously untreated NSCLC [48]. Interestingly, the simultaneous expression of multiple VEGF family members was recently proposed as a contributor of resistance to bevacizumab in the ovarian cancer population, with 38% of samples having positivity for VEGF-A plus VEGF-C, and 54% for VEGF-A plus VEGF-D [49]. Conversely, other studies have suggested that circulating VEGF levels are not significant predictors of survival [50,51]. While a review of other proposed biomarkers is beyond the scope of this discussion, Table 2 provides an overview of key findings from clinical trials that have evaluated the predictive or prognostic value of circulating, tissue, and clinical (eg, hypertension) biomarkers in patients receiving antiangiogenic therapy for NSCLC.

3.2. Means to tame hypoxia

3.2.1. Vascular normalization strategy

Among the mechanisms of resistance to VEGF-targeted therapy, hypoxia may be the hub. Hypoxia not only induces expression of various proangiogenic factors, but also regulates genes involved in processes such as proliferation, recruitment and polarization of myeloid cells, and cell "stemness" [52,53]. Judicious blockade of VEGF signaling has been shown to normalize the vasculature by pruning some abnormal vessels and remodeling the remaining vessels, resulting in a reduction in tumor hypoxia and improved efficacy of concurrent cytotoxic therapies [9,53]. Therefore, vascular normalization may provide a strategy to optimize the dosage and schedule of antiangiogenic agents and to delay resistance.

3.2.2. Oxygen sensor inhibitors

An alternative way to reduce hypoxia is to modulate oxygen sensors. Hypoxia mitigates expression of a variety of genes, including proangiogenic factors. Downregulation of HIF-1α inhibited tumor angiogenesis and improved concurrent chemotherapy in a preclinical model of hepatocellular carcinoma [54]. Prolyl hydroxylase domain 2 (PHD2) is an oxygen-sensing protein. A recent study suggested that haplodeficiency of PHD2 may pre-adapt endothelial cells to hypoxia while inducing vascular normalization and improving oxygenation [55]. Whether targeting oxygen sensors would alleviate hypoxia and improve antiangiogenic therapy needs further investigation.

3.2.3. Hypoxia-activated prodrugs

Hypoxia is a common feature in solid cancers that is often aggravated by antiangiogenic therapies. Hypoxia-activated prodrugs are designed to utilize hypoxia to convert its nontoxic prodrugs to a cytotoxic state. Thus, the rational integration of hypoxia-activated prodrugs into anti-angiogenic therapy may provide extra clinical benefits. Currently, there are 5 classes of hypoxia-activated prodrugs: nitro(hetero)cyclic compounds, aromatic N-oxides, aliphatic N-oxides, quinones, and metal complexes [56,57]. Evofosfamide (also known as TH-302) is a type of nitro compound and has shown encouraging antitumor activity in combination with different chemotherapeutic drugs in various human xenograft models, as well as in a phase I/II study in patients with cancer, including NSCLC [58,59]. In preclinical models, the combination of TH-302 and anti-VEGF therapy had greater antitumor activity than either treatment alone and provided the rationale for the clinical investigation of co-targeting tumor angiogenesis and tumor hypoxia [60]. Two phase I studies are currently investigating the combination of TH-302 with an antiangiogenic agent (pazopanib and sunitinib) in patients with solid tumors, and have shown promising preliminary activity in several treatment-refractory cancers [61,62]. TH-302 is also being studied in an ongoing phase I/II study in combination with bevacizumab in patients with recurrent glioblastoma following bevacizumab failure [63].

3.3. Multitargeted TKIs

As redundant angiogenic factors are likely among the key mechanisms underlying resistance to anti-VEGF therapy, multitargeted TKIs could be a reasonable solution because they are able to simultaneously inhibit multiple signaling pathways, including those mediated by VEGFRs, PDGFRs, FGF receptors (FGFRs), and c-kit [64]. Results are available for randomized, placebo-controlled trials that have evaluated the therapeutic potential of various multitargeted TKIs in combination with first-line chemotherapy for advanced NSCLC [65–68] or with the epidermal growth factor receptor (EGFR) TKI erlotinib (Tarceva®, Genentech, South San Francisco, CA, USA) in chemotherapy-pretreated disease [69]. These completed first-line phase III trials evaluated sorafenib (Nexavar®, Bayer, Leverkusen, Germany), which targets VEGFR-1 through -3, PDGFR-β, v-raf 1 murine leukemia viral oncogene homolog 1 (RAF), c-kit, Flt-3, and RET [65,67,70]; cediranib (Recentin™, AstraZeneca, Wilmington, DE, USA), which targets VEGFR-1 through -3, PDGFR-α/β, c-kit, and RAF [68,71,72]; motesanib (AMG 706, Amgen, Thousand Oaks, CA, USA), which targets VEGFR-1 through -3, PDGFR, c-kit, and RET [66,73,74]; or vandetanib (Caprelsa®, AstraZeneca, Wilmington, DE, USA), which targets VEGFR-2, VEGFR-3, EGFR, and RET [75–78].

In addition, sunitinib was combined with erlotinib in a population that was pretreated with 1 or 2 chemotherapy regimens [69]. All of these trials, for which results are described in Table 3, had OS as their primary endpoint but none demonstrated a significant improvement in this outcome among patients randomized to receive the multitargeted TKI. Two of the trials, the ESCAPE trial of sorafenib [65] and the BR29 trial of cediranib [68], had been closed early on the basis of preplanned interim analyses supporting futility for improved efficacy for targeted

Table 2
Selected predictive and prognostic biomarkers for response to antiangiogenic therapy.

Biomarker	Study description	Biomarker findings
Prognostic biomarker studies		
Plasma VEGF level	4 randomized phase III studies in metastatic colorectal cancer, lung cancer, and renal cell cancer of patients (N = 1816) treated with bevacizumab vs placebo [47]	Plasma VEGF levels associated with shortened PFS and OS for both treatment groups
	Study of surgically resected NSCLC patients (N = 57) [48]	Plasma VEGF189 isoform associated with decreased survival ($P = 0.001$)
Tumor VEGF level	Surgically treated NSCLC patients (N = 153) [115]	VEGF-A and VEGF-C expression have different prognostic value depending on tumor histology
	Multivariate analysis of VEGF variants using real-time quantitative RT-PCR on NSCLC specimens (N = 130) [50]	Only the VEGF189 isoform was prognostic of survival ($P = 0.03$); all other VEGFs were not prognostic
Predictive biomarker studies		
VEGF polymorphisms	ECOG 4599, a phase II/III trial of carboplatin/paclitaxel or carboplatin/paclitaxel + bevacizumab in patients (N = 878) with advanced NSCLC [51,116]	VEGF was predictive of response to bevacizumab, but not predictive of OS VEGF G634C was associated with significant improvement in OS ($P < 0.05$) [116]
VEGFRs	Heavily pretreated patients (N = 255) with NSCLC were screened for biomarkers and randomized to erlotinib, sorafenib, vandetanib, or erlotinib/bexarotene [117]	In the vandetanib arm, VEGFR-2 was significantly associated with improved DCR ($P = 0.05$)
ICAM	ECOG 4599, a phase II/III trial of carboplatin/paclitaxel or carboplatin/paclitaxel + bevacizumab in patients (N = 878) with advanced NSCLC [51,116]	Low ICAM levels were prognostic, but not predictive of response to bevacizumab + chemotherapy [51] ICAM T469C was associated with significant improvements in OS ($P < 0.05$) and PFS ($P < 0.05$) [116]
PlGF	MONET1, a phase III trial of carboplatin/paclitaxel + motesanib or placebo in patients (N = 1090) with advanced nonsquamous NSCLC [66]	PlGF increased from baseline to Week 4 in response to motesanib, but changes were not associated with OS, PFS, or response
IL-12	Phase II trial of patients (N = 35) with early-stage NSCLC treated with pazopanib [118]	Baseline levels of IL-12 correlate with response to pazopanib ($P = 0.0007$)
KRAS gene mutation	Heavily pretreated patients (N = 255) with NSCLC were screened for biomarkers and randomized to erlotinib, sorafenib, vandetanib, or erlotinib/bexarotene [117]	*KRAS* gene mutation is predictive of response to sorafenib
Combination of HGF and IL-12	Phase II trial of patients (N = 35) with early-stage NSCLC administered pazopanib [118]	A baseline signature of HGF and IL-12 led to 81% accuracy in prediction of response to pazopanib
Hypertension	ECOG 4599, a phase II/III trial of carboplatin/paclitaxel or carboplatin/paclitaxel + bevacizumab in patients (N = 878) with advanced NSCLC [51,119]	Patients who developed hypertension had numerically longer OS/PFS with bevacizumab
	Meta-analysis of 7 phase III trials of bevacizumab in metastatic cancers [120]	Hypertension was not predictive of outcome in 6 of the 7 studies

VEGF, vascular endothelial growth factor; PFS, progression-free survival; OS, overall survival; NSCLC, non-small cell lung cancer; RT-PCR, reverse transcriptase-polymerase chain reaction; ECOG, Eastern Cooperative Oncology Group; VEGFR, vascular endothelial growth factor receptor; DCR, disease control rate; ICAM; intercellular adhesion molecule; PlGF, placental growth factor; IL, interleukin; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; HGF, hepatocyte growth factor.

therapy plus chemotherapy versus chemotherapy alone. Of note, interim phase II results from an earlier phase II/III, placebo-controlled trial (BR24; N = 296) of first-line cediranib plus paclitaxel/carboplatin in patients with advanced NSCLC showed preliminary activity; however, the study was terminated and did not advance to phase III due to excessive toxicities with cediranib, prompting the initiation of the BR29 trial with a lower cediranib dose [79]. Overall, while results from phase III trials of a multitargeted TKI for advanced NSCLC reported to date have revealed significant benefits with respect to secondary efficacy outcomes of response rate (RR) and/or PFS (except for ESCAPE, in which there were no significant differences in response or PFS [65]), grade ≥3 toxicity incidences were likewise increased across all studies [65–69]. The earliest phase III results for use of a multitargeted TKI as a component of first-line chemotherapy for advanced NSCLC were from the ESCAPE trial of sorafenib [65], and the clinical development of this particular agent has since shifted to patients with relapsed or refractory nonsquamous disease after 2 or 3 prior therapies (NCT00863746).

There are 2 other multitargeted agents that have shown activity in phase II and phase III trials: pazopanib (Votrient®, GlaxoSmithKline, London, United Kingdom; which targets VEGFR-1 through -3, PDGFR-α/β, FGFR-1 and -3, c-kit, IL-2-inducible T-cell kinase, lymphocyte-specific protein tyrosine kinase, and c-fms [80]) and nintedanib (BIBF 1120, Boehringer Ingelheim, Ingelheim, Germany; which targets VEGFR-1 through -3, FGFR-1 through -3, and PDGFR-α/β, with additional activity against the v-src sarcoma [Schmidt-Ruppin A-2] viral oncogene homolog [Src] family of kinases and Flt-3 [81,82]). Pazopanib was evaluated for treatment of patients (N = 35) with operable, stage I/II NSCLC in a phase II trial, leading to tumor volume reduction in 86% of patients, including reductions ≥50% in 2 of 35 patients and partial response in 3 patients [83]. Increased alanine aminotransferase (ALT) elevation (6%) was the most commonly reported grade 3/4 adverse event (AE). A placebo-controlled phase II/III trial is evaluating pazopanib as adjuvant therapy in stage I NSCLC (NCT00775307), with disease-free survival as the primary endpoint for the phase III component. Another placebo-controlled trial, the European Organization for Research and Treatment of Cancer (EORTC) Lung Group MAPPING study (NCT01208064), will determine if maintenance pazopanib after first-line chemotherapy improves the primary endpoint of OS, with recruitment to meet the target of 600 patients ongoing as of June 2013. A phase II trial (N = 73) evaluating nintedanib in previously treated advanced NSCLC reported an overall median PFS of 6.9 weeks and median OS of 21.9 weeks; for patients with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (n = 56), median PFS was 11.6 weeks and median OS was 37.7 weeks [84]. The most common (>5%) grade 3/4 AEs were ALT increase (9.6%), diarrhea (8.2%), and nausea (6.8%). Results from the LUME-Lung 1 phase III trial showed significantly improved median PFS (primary endpoint) independent of histology with nintedanib plus docetaxel versus docetaxel plus placebo as second-line therapy (3.4 vs 2.7 mo, respectively; $P = 0.0019$) and prolonged OS in patients with adenocarcinoma (12.6 vs 10.3 mo; $P = 0.0359$); however, OS was not significantly different in the overall population (10.1 vs 9.1 mo; $P = 0.272$) [85]. The LUME-Lung 2 phase III trial of second-line nintedanib or placebo plus pemetrexed in patients with advanced, nonsquamous NSCLC was halted based on a planned PFS futility analysis after randomization of 713 patients [86]. Randomized patients were unblinded and continued per protocol, and follow-up showed an improvement in PFS with nintedanib plus pemetrexed (median 4.4 vs 3.6 mo with placebo; $P = 0.04$), but no difference in OS or RR.

Table 3
Phase III clinical trial results for multitargeted TKIs in advanced NSCLC.

Agent (trial)	Study design	Objective RR, investigational vs control	PFS, investigational vs control	OS, investigational vs control
Sorafenib (ESCAPE) [65]	N = 926 CT-naïve[a]; randomized to (1) sorafenib + carboplatin/paclitaxel or (2) placebo + carboplatin/paclitaxel	27.4% vs 24.0% ($P = 0.1015$)	4.6 vs 5.4 mo ($P = 0.433$)	10.7 vs 10.6 mo ($P = 0.915$)[b]
Sorafenib (NexUS) [67]	N = 904 CT-naïve, nonsquamous; randomized to (1) sorafenib + cisplatin/gemcitabine or (2) placebo + cisplatin/gemcitabine	28% vs 26% ($P = 0.27$)	6.0 vs 5.5 mo ($P = 0.008$)	12.4 vs 12.5 mo ($P = 0.401$)
Sunitinib [69]	N = 960 CT-pretreated (1–2 regimens)[a]; randomized to (1) sunitinib + erlotinib or (2) placebo + erlotinib	10.6% vs 6.9% ($P = 0.047$)	3.6 vs 2.0 mo ($P = 0.0023$)	9.0 vs 8.5 mo ($P = 0.1388$)
Cediranib (BR29) [68]	N = 306 CT-naïve[a]; randomized to (1) cediranib + carboplatin/paclitaxel or (2) placebo + carboplatin/paclitaxel	52% vs 34% ($P = 0.001$)	5.5 vs 5.5 mo ($P = 0.5$)[b]	12.2 vs 12.1 mo ($P = 0.74$)
Motesanib (MONET1) [66]	N = 1090 CT-naïve, nonsquamous[c]; randomized to (1) motesanib + carboplatin/paclitaxel or (2) placebo + carboplatin/paclitaxel	40% vs 26% ($P < 0.001$)	5.6 vs 5.4 mo ($P < 0.001$)	13.0 vs 11.0 mo ($P = 0.14$)
Nintedanib (LUME-Lung 1) [85]	N = 1314 CT-pretreated (1 regimen)[a]; randomized to (1) nintedanib + docetaxel or (2) placebo + docetaxel	NR	3.4 vs 2.7 mo ($P = 0.0019$)	10.1 vs 9.1 mo ($P = 0.272$) Adenocarcinoma histology only: 12.6 vs 10.3 mo ($P = 0.0359$)
Nintedanib (LUME-Lung 2) [86]	N = 713 CT-pretreated (1 regimen) nonsquamous; randomized to (1) nintedanib + pemetrexed or (2) placebo + pemetrexed	NR	4.4 vs 3.6 mo ($P = 0.04$)[b]	Not significant (HR, 1.03)
Vandetanib (ZODIAC) [77]	N = 1391 CT-pretreated (1 regimen); randomized to (1) vandetanib + docetaxel or (2) placebo + docetaxel	17% vs 10% ($P = 0.0001$)	4.0 vs 3.2 mo ($P < 0.0001$)	10.3 vs 9.9 mo ($P = 0.196$)
Vandetanib (ZEPHYR) [78]	N = 924 CT-pretreated (1–2 regimens), failure with prior EGFR TKI; randomized to (1) vandetanib or (2) placebo	2.6% vs 0.7% ($P = 0.028$)	1.9 vs 1.8 mo ($P < 0.001$)	8.5 vs 7.8 mo ($P = 0.527$)
Vandetanib (ZEST) [75]	N = 1240 CT-pretreated (1–2 regimens); randomized to (1) vandetanib or (2) erlotinib	12% vs 12% ($P = 0.98$)	2.6 vs 2.0 mo ($P = 0.721$)	6.9 vs 7.8 mo ($P = 0.83$)
Vandetanib (ZEAL) [76]	N = 534 CT-pretreated (1 regimen); randomized to (1) vandetanib + pemetrexed or (2) placebo + pemetrexed	19% vs 8% ($P < 0.001$)	17.6 vs 11.9 wks ($P = 0.108$)	10.5 vs 9.2 mo ($P = 0.219$)

TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression-free survival; OS, overall survival; CT, chemotherapy; NR, not reported; HR, hazard ratio.
[a] Eligibility was not restricted based on histology.
[b] Study was terminated early based on preplanned interim analyses of this endpoint.
[c] Study was amended to exclude patients with squamous tumors after a planned review showed increased mortality and gross hemoptysis rates among motesanib recipients with squamous disease; published results, as summarized here, are from the amended study in nonsquamous disease.

3.4. Antiangiogenic therapy and histologic subtype

Histologic subtype has become an important consideration in the treatment of NSCLC, particularly relating to the use of antiangiogenic therapy. While platinum-based doublets are standard treatment for advanced NSCLC, these combination regimens have shown comparable efficacy and toxicity overall and based on histology [87,88]. In contrast, better outcomes have been observed with pemetrexed in patients with nonsquamous NSCLC [89]. Moreover, some targeted drugs, such as antiangiogenic agents, appear to be safer and more effective in specific histologic subtypes [87,88].

For example, bevacizumab is the only antiangiogenic therapy currently approved for the treatment of NSCLC; however, its use is restricted to patients with nonsquamous histology. While early phase II and III studies demonstrated efficacy with bevacizumab in patients with NSCLC [7,90], clinically significant bleeding events, including major hemoptysis and pulmonary hemorrhage, were observed in some patients, particularly those with squamous histology [90]. Although it is currently unclear whether histology alone led to the increased risk of bleeding with bevacizumab, patients with squamous cell histology were excluded from later phase III trials with bevacizumab [88]. In the subsequent ECOG E4599 phase III pivotal trial of carboplatin/paclitaxel with or without bevacizumab in patients with recurrent or advanced nonsquamous NSCLC, results from a histologic subgroup analysis showed significantly longer survival with bevacizumab versus the control arm in patients with adenocarcinoma, while data for other histologies were inconclusive due to small patient sample sizes [91]. In a phase III randomized, placebo-controlled study of sorafenib in combination with carboplatin/paclitaxel in chemotherapy-naïve patients with advanced NSCLC, the addition of sorafenib did not improve PFS or OS in the overall population; however, PFS and OS were lower with the addition of sorafenib in patients with squamous cell histology [65]. In addition, a higher mortality rate was observed with sorafenib plus chemotherapy versus chemotherapy alone in patients with squamous cell NSCLC. Similar concerns regarding increased risk of serious bleeding events and other adverse safety signals have been observed with other antiangiogenic agents (eg, motesanib, sunitinib) in squamous cell NSCLC [66,92,93], thereby restricting further clinical development of these and other antiangiogenic agents based on NSCLC histology. Regardless of histology, antiangiogenic agents targeting VEGF signaling are associated with cardiovascular and noncardiovascular toxicities that must be considered, including hypertension, venous thromboembolism, myocardial ischemia, hemorrhage, proteinuria, gastrointestinal perforation, and fatigue [94]. More information related to AEs of antiangiogenic therapy can be found in http://www.uptodate.com/contents/toxicity-of-molecularly-targeted-antiangiogenic-agents.

4. Future perspectives

Compensatory proangiogenic factors play a central role in both primary and acquired resistance to VEGF-targeted therapy. Development of multitargeted TKIs may combat this resistance by simultaneously targeting several proangiogenic signaling cascades; however, this has not been convincingly demonstrated to date in NSCLC, with no phase III trials meeting their primary endpoint of survival benefit. It is likely that we do not yet fully understand the physiological coordination between different proangiogenic pathways and the molecular mechanisms underlying tumor resistance to single, dual, and multiple proangiogenic factor inhibitors. Therefore, rationally scheduled administration of a relatively specific and narrow spectrum of TKIs to target the evolving tumor evasion mechanisms may achieve better antiangiogenic efficacy. In addition, to reduce the profound systemic

toxicity of TKIs, novel technologies, such as nanotechnology, are awaited to develop new formulations of TKIs for targeted delivery and gradual drug release.

Biomarkers have long been considered a powerful tool to develop more efficient antiangiogenic treatment regimens. However, current biomarkers mainly focus on soluble factors and gene mutations and few are reliable. Cellular biomarkers, especially immune cells in peripheral blood and tumor parenchyma, are beginning to emerge as a new direction in oncology. For example, DeNardo and colleagues showed that the $CD68^{high}/CD4^{high}/CD8^{low}$ signature was a predictor of decreased OS and relapse-free survival in breast cancer patients [95]. In addition, a growing body of reports shows the interplay between angiogenesis and immunity [96–98]. Thus, immune cell components may be candidates as biomarkers for better clinical applications of antiangiogenic therapy.

Finally, current antiangiogenic therapies are predominantly combined with chemotherapy for the treatment of NSCLC in the clinic. The recent successes of tumor immunotherapies in multiple cancers have renewed the hope for incorporating this modality into NSCLC treatments [99–101]. A recent phase I trial reported a RR of 10% (5 of 49 patients) in advanced NSCLC patients receiving antiprogrammed death 1 monotherapy [102], suggesting that tumor immunotherapy may be a promising modality for NSCLC. Cancer genome sequencing studies also suggest that NSCLC and melanoma harbor many more mutations than other tumor types [103], indicating that a higher immunogenic potential may exist. Additionally, the dual roles of VEGF in promoting angiogenesis and immunosuppression provide good rationale to combine bevacizumab with tumor immunotherapy [104]. Elevated levels of VEGF have been shown to suppress dendritic cell maturation and function, inhibit T-cell immune response, and create an immunosuppressive tumor microenvironment [98,105,106]. Furthermore, preclinical data suggest that appropriate low-dose VEGF-targeted therapy could recondition the tumor immune microenvironment and enhance anticancer vaccine therapy [53]. A recent clinical study showed that pretreatment serum VEGF levels (≥43 pg/mL) were associated with decreased OS in melanoma patients treated with ipilimumab, indicating that serum VEGF could be a predictive biomarker for ipilimumab treatment [107]. The combination of bevacizumab and immune checkpoint blockers is also being tested in multiple cancer types, including NSCLC (NCT01454102) and melanoma (NCT01950390 and NCT00790010) [108]. Therefore, a rationally designed combination of antiangiogenic therapy and tumor immunotherapy could be a compelling option for investigation in NSCLC.

5. Conclusions

Antiangiogenic therapy with bevacizumab represents a significant advancement in the treatment of NSCLC, with a significant prolongation of OS in a phase III trial. However, clinical benefit remains modest and resistance represents a key challenge. Future success in antiangiogenic therapy may rely on insights into the molecular mechanisms of tumor evasion of various antiangiogenic therapies, the development of novel vascular modulation targets, and more effective treatment combinations.

Abbreviations

ALK1	activin A receptor type II-like 1
AE	adverse event
ALT	alanine aminotransferase
CAF	cancer-associated fibroblast
c-fms	colony-stimulating factor 1 receptor
c-kit	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
DLL4	delta-like ligand 4
ECOG	Eastern Cooperative Oncology Group
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EORTC	European Organization for Research and Treatment of Cancer
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
Flt-3	fms-related tyrosine kinase 3
HIF	hypoxia inducible factor
IL	Interleukin
NSCLC	non-small cell lung cancer
NRP	neuropilin
OS	overall survival
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PlGF	placental growth factor
PFS	progression-free survival
RAF	v-raf 1 murine leukemia viral oncogene homolog 1
RET	ret proto-oncogene
RR	response rate
Src	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
TKI	tyrosine kinase inhibitor
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

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Conflicts of interest

Dr. Huang has no potential conflicts of interest to disclose. Dr. Carbone has done occasional consultant/advisory board work for Genentech, GlaxoSmithKline, Merck, EMD Pharmaceuticals, Biodesix, Novartis, and Roche.

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