Targeting Angiogenesis in Non-Small Cell Lung Cancer (NSCLC)

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Abstract: The management of NSCLC has undergone a paradigm shift in the last 5 years with the survival advantage demonstrated by the addition of bevacizumab to standard frontline platinum-based doublet specifically, paclitaxel and carboplatin. The increased toxicity observed in patients with squamous histology and in elderly patients treated with angiogenesis-targeting agents has led to even greater scrutiny of other subpopulations of patients. Although recently, several anti- angiogenetic agents have been developed and tested in clinical trials, bevacizumab remains the only vascular-targeted agents to show overall survival benefit when combined with a cytotoxic agent in the frontline setting. The realization of the potential promise of personalized medicine requires that the appropriate treatment be given to the most appropriate group of patients. Given the modest benefit and the significant toxicity associated with the use of anti-angiogenesis agents in NSCLC patients, it is highly desirable that predictive markers of response and or toxicity be established to assist in the optimal selection of patients. Although a number of plasma-, tissue- and genomic-based markers have been explored, none of these has been robust or sensitive enough to reproducibly discriminate responding from non-responding patients. The presence of squamous differentiation on histologic evaluation remains the only established marker that predicts for increased risk of hemorrhagic complications. In this review, we discuss data establishing the role of angiogenesis-targeting agents and the clinical value of putative markers of angiogenesis.

Keywords: Angiogenesis, lung cancer, VEGF, chemotherapy, biomarker, bevacizumab.

INTRODUCTION

Lung cancer is the leading cause of cancer death in the United States with over 200,000 estimated new cases in the US in 2009 [1]. NSCLC accounts for approximately 85% of all lung cancer cases and is the leading cause of cancer death in both men and women worldwide [2]. While significant strides have been made in the treatment of NSCLC over the last decade, the prognosis for advanced stage disease remains poor with reported median survival rates following first-line treatment ranging between 10-13 months [3-5].

Platinum-based chemotherapy has been the cornerstone of treatment for NSCLC [6]. The incorporation of third generation chemotherapy agents (taxanes, vinorelbine, gemcitabine, pemetrexed) produced superior response rate (RR) and improvement of overall survival (OS) when compared to older, more toxic regimens [7]. Attempts at dose intensification using alternative schedules or triple combinations of traditional cytotoxic agents merely resulted in increased toxicity without survival benefit [8]. In recent years, an increased understanding of tumor biology and the availability of relatively well-tolerated agents that target key biological processes required for tumor growth provided an

opportunity to further improve the outcome of patients with advanced NSCLC.

Angiogenesis is a pathological adaptation of a normal biologic process by tumor cells to gain survival advantage. By altering the dynamic balance between proangiogenic and antiangiogenic factors, solid tumors attain the capacity to grow beyond a size that otherwise would be unsustainable with normal vasculature [9-13]. Preclinical and correlative studies in NSCLC showed that the degree of tumor-associated angiogenesis correlates with disease progression and serves as a marker of unfavorable survival outcome [14-16].

The realization of the potential promise of personalized medicine requires that the appropriate treatment be given to the most appropriate group of patients. Given the modest benefit and the significant toxicity associated with the use of anti-angiogenesis agents in NSCLC patients, it is highly desirable that predictive markers of response and or toxicity be established to assist in the optimal selection of patients. Although a number of plasma-, tissue- and genomic-based markers have been explored, none of these has been robust or sensitive enough to reproducibly discriminate responding from non-responding patients. The presence of significant squamous differentiation on histologic evaluation remains the only established marker that predicts for increased risk of bleeding complications. The development of hypertension early in the course of therapy may be an early signal to indicate optimal activity of the anti-angiogenic agent and has

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been shown to correlate with improved survival outcome. Although this is a useful indicator of therapeutic activity, there is insufficient clinical evidence to recommend discontinuation of anti-VEGF therapy in patients who fail to manifest blood pressure elevation while on therapy. Biomarker identification and validation remain a focus of intense research that may ultimately guide therapeutic decision making in this patient population.

Anti-angiogenesis therapy is one of the most active areas of clinical investigation in NSCLC and the only intervention in the first-line treatment of advanced NSCLC that has led to a major improvement in survival outcomes in the last 5 years [4]. This review is focused on the established role of anti-angiogenesis therapy in the clinic and discusses the various pharmacological approaches for targeting tumor-induced angiogenesis. We also review the clinical value of putative markers of angiogenesis, such as circulating serum levels of vascular endothelial growth factor (VEGF), VEGF receptor and adhesion molecule expression.

ANGIOGENESIS BASICS

There is a multitude of physiologic proangiogenic factors including acidic and basic fibroblast growth factor (aFGF, bFGF), epidermal growth factor (EGF), matrix metalloproteinases (MMPs), placental growth factor (PLGF), hepatocyte growth factor (HGF), angiogenin, interleukin-8, tumor necrosis factor (TNF) and platelet-derived growth factor (PDGF) (Fig. **1**). The most important proangiogenic factor is the vascular endothelial growth factor (VEGF), which drives the rate-limiting step in both physiologic and pathologic new vessel formation. VEGF has five isoforms, VEGFA, -B, -C, -D, and -E; when not further qualified, VEGF has generally been used to refer to the VEGFA isoform, which is the key driver of pathologic and physiologic angiogenesis [17-19]. The biological action of VEGF is mediated through its interaction with surface VEGF receptors (VEGFR) of which there are three members, VEGFR1, -2 and -3. VEGFR1 and 2 mediate vasculogenesis, while VEGFR3 mediates predominantly lymphangiogenesis. VEGF is a prosurvival factor for endothelial cells both *in vitro* and *in vivo* that stimulates endothelial cell proliferation, migration and increased vascular membrane permeability. High levels of VEGF have been correlated with poor prognosis in patients with NSCLC [20-24]. Angiopoietins (ANG-1 and ANG-2) are considered important growth factors for tumor development through the induction of angiogenesis and promotion of tumor metastasis by increased vascular extravasations [25]. The level of expression and activity of these growth factors as well as numerous other proangiogenic factors, such as bFGF, [26] EGF, [27] MMPs, [28] PLGF, [29] HGF [30] and PDGF [31] have been correlated with poor prognosis in NSCLC (reviewed by Ferrara *et al.*) [19].

A. Antiangiogenic Monoclonal Antibodies in NSCLC

Bevacizumab is a fully humanized monoclonal antibody which binds VEGF-A. It has proven efficacy either alone or in combination with cytotoxic agents in a number of solid tumors, including colorectal cancer, renal cancer, breast cancer, non-squamous NSCLC and glioblastomas. Although the precise details of the underlying biological mechanisms remain to be fully elucidated, the principal mechanism of action of bevacizumab appears to be through angiogenesis

inhibition. This results in a more mature vasculature that is thought to facilitate the delivery of chemotherapeutic agents by decreasing microvascular permeability and decreasing intra-tumoral pressure, [32, 33] which may explain why bevacizumab acts synergistically with cytotoxic or other targeted agents.

Bevacizumab in Combination with Chemotherapy

Bevacizumab was evaluated in combination with paclitaxel and carboplatin in patients with advanced NSCLC [34]. This trial enrolled 99 patients and evaluated 2 dose levels of bevacizumab (7.5mg/kg or 15 mg/kg) in combination with standard doses of paclitaxel (200 mg/m^2) and carboplatin (AUC of 6). The median time to progression (TTP) was similar between the control and the low dose bevacizumab arm (4.2 and 4.3 months, respectively) but significantly higher with the high dose arm (7.4 months; p=0.023). The incidence of mild (grade I or II) bleeding was increased from 6% in the control arm to 33% in the low-dose bevacizumab arm and to 44% in the high-dose bevacizumab arm. Six fatal pulmonary hemorrhages were observed in this study, four of which occurred in patients with squamous cell carcinoma. Nonetheless, the improved median TTP along with the response rates of 31.5% and 28.1% in high and low dose bevacizumab-containing regimen compared to 18.8% for standard chemotherapy alone prompted a subsequent phase III trial conducted by the Eastern Cooperative Oncology Group (ECOG) [34]. Due to safety consideration with respect to fatal pulmonary hemorrhage observed in the phase II trial, especially in squamous type NSCLC, the phase III trial was limited to patients at reduced risk of bleeding notably, patients without brain metastasis and those with non squamous histology. The ECOG phase III study (E4599) of paclitaxel, carboplatin with or without bevacizumab (15 mg/kg) was a pivotal study enrolling 878 patients with recurrent or advanced (stage IIIB and IV) non-squamous NSCLC. Patients were randomized to receive chemotherapy alone (n=444) or chemotherapy plus bevacizumab (n=434). This study met the specified improved survival endpoint with median overall survival (OS) of 12.3 months versus 10.3 months in favor of bevacizumab arm (hazard ratio [HR] for death, 0.79; 95% CI, 0.67 to 0.92; p=.003). The median progression-free survival (PFS) of 6.2 versus 4.5 months (HR=0.66; 95% CI, 0.57 to 0.77; p<0.001) and response rate of 35% versus 15% (p<0.001) were both in favor of the bevacizumab-containing arm. The incidence of adverse events such as bleeding, hypertension, proteinuria, neutropenia, febrile neutropenia, thrombocytopenia, hyponatremia, rash and headache were significantly higher among the patients treated with bevacizumab (p<0.05). Fifteen of the 17 treatment-related deaths were recorded in the bevacizumab arm of the trial. Nonetheless, the survival benefit in the overall patient population clearly outweighed the added toxicity risk which led to the approval of this regimen by both the United States Food and Drug Administration (FDA) in 2006 and the European Medicines Agency (EMEA) in 2007 [4].

The AVAiL ("Avastin in lung cancer") trial was a 3-arm phase III randomized trial conducted in Europe that evaluated 2 different doses of bevacizumab (7.5 mg/kg and 15 mg/kg) in combination with a different chemotherapy backbone, gemcitabine 1250 mg/m^2 and cisplatin 80 mg/m^2,
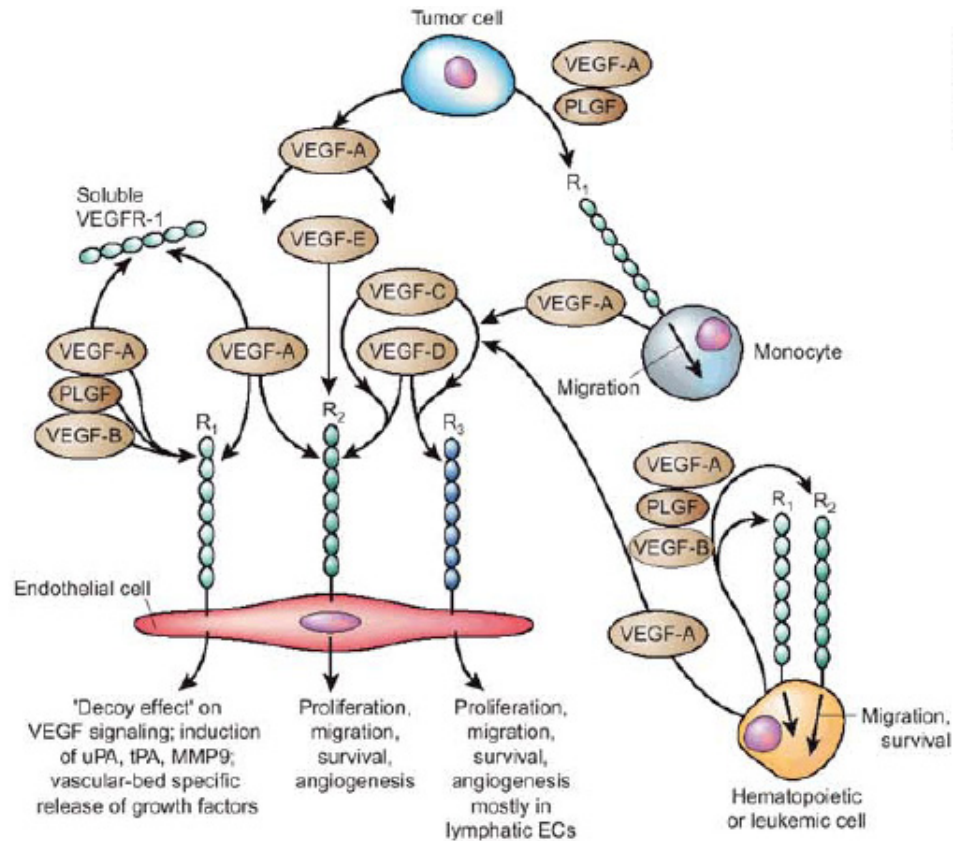
Fig. (1). Diagramatic representation of VEGF receptor signaling showing the interaction between different ligands and receptor subtypes. The biological consequence of receptor ligand interaction may result in endothelial cell proliferation, migration, improved survival and consequently, angiogenesis. Reprinted by permission from Macmillan Publishers Ltd: [Nature Medicine] (9, 669-676), copyright (2003).

as frontline therapy for advanced stage (wet IIIB and IV) nonsquamous NSCLC. The control arm was the same chemotherapy alone plus placebo. The original primary endpoint of this trial was OS. However, this was amended during the course of the study to PFS in order to expedite study completion and also to potentially avoid a confounding effect on the OS endpoint by the use of bevacizumab in the second-line setting. A total of 1,043 patients were randomized to placebo (n=347), low-dose bevacizumab (n=345) and high-dose bevacizumab (n=351) groups. The addition of bevacizumab to chemotherapy improved PFS with significant differences noted in both bevacizumab arms compared to the placebo arm. In the low dose bevacizumab arm the median PFS was 6.7 versus 6.1 months in the placebo arm with a HR of 0.75 (95% CI: 0.62 to 0.91); p=0.003, whereas in the high dose arm the median PFS was 6.5 months versus 6.1 months in the placebo arm with a HR of 0.82 (95% CI: 0.68 to 0.98); p=0.03. The RR was 34%, 30% and 20% respectively for low dose, high dose and placebo [35]. Unlike the E4599 trial, however, the improved RR and PFS did not translate into overall survival benefit. The reason for the failure of the AVAiL to demonstrate a survival advantage has been ascribed to different factors including the use of a potentially more effective cisplatin-containing chemotherapy regimen as well as the use of a 3-arm trial design that was relatively underpowered to detect a modest survival advantage conferred by bevacizumab. In this study, the rates of hypertension, bleeding, neutropenia and proteinuria were higher in the bevacizumab arms, especially

in the high dose bevacizumab arm where the incidence of serious adverse events (SAEs) was 44% compared to 35% in each of the other arms [35].

A review of contemporary management of an unselected patient population with NSCLC at a leading US academic cancer center revealed that greater than 70% of patients would be ineligible for frontline therapy with a bevacizumab-containing regimen based on the presence of one or more exclusion criteria employed in the E4599 trial, such as poor performance status (24%), brain metastasis (13%), therapeutic anticoagulation (26%) and squamous histology (11%) [36]. Several approaches have been explored for better patient selection and to demonstrate the safety of the regimen in some of the previously excluded patient population.

Multiple phase II trials have established the safety and improved efficacy of bevacizumab when combined with other platinum-based doublet. The docetaxel plus carboplatin regimen was tested in patients with metastatic NSCLC with encouraging results (ORR of 63% and median OS of 13.5 months) [37, 38]. Similarly, the combination of pemetrexed plus carboplatin and bevacizumab was evaluated in two separate studies. Skaff *et al.* presented the preliminary results of their study at the 2009 ASCO meeting. They reported a median TTP of 7.2 months, ORR of 36% and a very promising high disease control rate of 92% with acceptable toxicity in 27 patients treated with pemetrexed, carboplatin and bevacizumab followed by maintenance bevacizumab

[39]. Using a slightly different design of frontline pemetrexed, carboplatin and bevacizumab followed by maintenance pemetrexed plus bevacizumab, Patel *et al.* showed that the combination was well-tolerated with an ORR of 49%, TTP of 7.2 months and median OS of 14 months [40]. Finally, pemetrexed plus oxaliplatin was combined with bevacizumab in a phase II study in previously untreated patients with advanced non-squamous NSCLC. The combination was well tolerated with encouraging efficacy outcome: RR of 26%, PFS of 7.8 months and OS of 16.7 months [41]. Taken together therefore, these emerging data indicate that the findings of the two pivotal phase III trials E4599 and AVAil that used carboplatin/paclitaxel and cisplatin/gemcitabine, respectively, can be replicated successfully when bevacizumab is combined with other platinum doublets the choice of which may be warranted for reasons of toxicity or treating physician's preference and experience. Moreover, two observational phase III/IV studies are currently evaluating the safety of bevacizumab in combination with different chemotherapy regimens and enrolling patient groups who were underrepresented in the E4599 and AVAiL studies. The SAiL study is an international phase IV evaluation of bevacizumab (7.5mg/kg or 15mg/kg) in combination with investigators' choice of platinum-containing chemotherapy doublet. A total of 2116 patients were enrolled and treated for up to six cycles. Non-progressing patients continued with maintenance bevacizumab. This study confirmed the efficacy of bevacizumab and platinum-based chemotherapy with median OS of 14.6 months and no new significant safety signal. Vascular related adverse events including hypertension, proteinuria, thromboembolism and bleeding were observed in 65% of treated patients, majority (82%) of such adverse events resolved or improved without bevacizumab discontinuation [42]. Table **1** provides a the outcome summary of clinical trials that evaluated the efficacy of bevacizumab in combination with different chemotherapy backbone in NSCLC patient population.

Biomarkers

Biomarker analysis was performed using tissue and data from patients enrolled in the E4599 study with the expectation that the risk-benefit ratio of this regimen could be optimized if markers predictive of response to bevacizumab could be identified and employed for patient selection. Although bevacizumab targets VEGF, baseline serum VEGF levels surprisingly did not correlate with OS (p=0.15) [4]. Subset analysis of 146 patient samples for germline polymorphisms in angiogenesis pathway genes indicated that *ICAM* gene polymorphisms may predict RR. Furthermore, genetic mutations in the *ICAM, EGF and CXCR2* genes correlate with differences in PFS while mutations in *ICAM, WNK1* and *VEGF* genes correlate with OS [43]. Predictive analysis of serum-based biomarkers performed in 358 patients with available samples on the AVAiL trial showed that high (greater than median value) baseline serum levels of ICAM-1, VCAM, bFGF and VEGF were associated with a shorter OS irrespective of treatment received; low levels of ICAM-1 correlated with bigger

treatment effect on PFS with the 15 mg/kg dose bevacizumab, while high bFGF levels correlated with better OS outcome with the 7.5 mg/kg dose of bevacizumab [44]. While biomarker analysis represents a promising research tool, the high variability in biomarker selection, technique and cut-off set points make it difficult to establish any meaningful benchmarks for use in the clinical setting at the current time.

Bevacizumab in the Elderly

Subset analyses of outcome between elderly and younger patients have been performed for both the E4599 and the AVAiL trials. Ramalingam *et al.* published a comparative analysis of the outcome for elderly (>70 years) and younger patients (<70 years) enrolled in the E4599 trial. In the 224 elderly patients enrolled in the trial, treatment with bevacizumab was associated with a trend toward improved RR (29% versus 17%; p=0.067) and improvement in PFS (5.9 versus 4.9 months; p=0.063) but not in overall survival (11.3 versus 12.1 months; p=0.4). Compared to younger patients in the trial, the elderly suffered a higher incidence of neutropenia (34% versus 22%), proteinuria (7.9% versus 3.2%) and bleeding (7.9% versus 3.2%) with a resultant numerical increase in treatment-related deaths in elderly patients treated with bevacizumab compared to elderly patients treated with chemotherapy alone (6.3% versus 1.8%) [45]. A subset analysis of the AVAiL study did not show any major safety concern or survival difference based on age. As observed in the entire study patient population, the 304 bevacizumab-treated elderly patients (>65 years) derived an improvement in PFS versus placebo (HR 0.71, p 0.023 for 7.5mg/kg and HR 0.84, p= 0.25 for 15mg/kg bevacizumab). Furthermore, consistent with the result in the general patient population, there was no difference in overall survival between the treatment arms regardless of age. Perhaps due to the different age categorization for the elderly, the addition of bevacizumab also did not result in increased toxicity in the elderly contrary to the observation in the E4599 trial: grade ≥3 toxicities was recorded in 84%, 80% and 80% of older patients treated with 7.5 mg/kg, 15 mg/kg and placebo respectively. Treatment-related deaths were not significantly different between elderly patients and younger patients or within the elderly subgroup with respect to treatment with bevacizumab [46].

Bevacizumab Safety in Certain Clinical Settings

Similar in concept to the SAiL phase IV study, the ARIES observational phase IV study is enrolling patients treated in the community oncology practice setting in the US to establish the real world safety and efficacy profile of bevacizumab-containing regimens. Preliminary analysis after enrolling 1758 of the planned 2000 showed comparable safety profile to those reported in the pivotal phase III trials despite the inclusion of greater number of elderly patients and allowing patients with brain metastasis and those on anticoagulation. The median TTP and OS were 7.8 and 15.3 months, respectively, comparable to the outcome in the pivotal trials [47].

Table 1.   Selected Clinical Trials Evaluating the Combination of Cytotoxic Agents and Bevacizumab in NSCLC

Clinical Trial	Phase	Design	RR	PFS	OS	Remarks
E4599 [4]	III	CP *vs* CP + Bev	15% *vs* 35% (p<.001)	4.5 *vs* 6.2 months (p<.001)	10.2 *vs* 12.3 months (p=.003)	Increased incidence of AEs in bevacizumab arm.
Ramalingam *et al.* [45]	Post-hoc subset analysis	CP *vs* CP + Bev	29% *vs* 17% (p=0.067)	5.9 *vs* 4.9 months (p=0.063)	11.3 *vs* 12.1 months (p=0.4)	Increased toxicity in elderly patients; unplanned subset analysis comparing patients younger and older than 70years of age.
AVAiL Trial [35]	III	CisG *vs* CisG + Low dose Bev *vs* CisG + High dose Bev	20.1% *vs* 34.1% *vs* 30.4%	6.1 *vs* 6.7 *vs* 6.5 months (p=.003)	NR	No significant difference in OS; preliminary analysis showed > 13months OS in all arms.
PASSPORT [48]	Phase II	Chemotherapy + Bev or Erlotinib + Bev	NA	NA	NA	Enrolled both 1st and 2nd line patient population. Primary objective was to demonstrate safety of bevacizumab in the presence of brain metastasis.
ARIES [47]	IV	Observational Study	NR	NR	NR	Preliminary analysis after enrolment of 1750 patients out of 2000 planned.
SAiL [42]	IV	Bevacizumab+ chemotherapy	80.1	7.8	15.3	10.5% SAEs related to bevacizumab; RR – includes all patients with SD or better.

CP: Carboplatin/Paclitaxel; Bev: Bevacizumab; CisG: Cisplatin/Gemcitabine; NR: not reported; NA: not applicable.

Based on the initial observation in the phase II trial of bevacizumab plus carboplatin and paclitaxel that fatal pulmonary hemorrhage occurred predominantly in patients with centrally located tumors of squamous histology, such patients have been excluded from all prospective phase III trials evaluating anti angiogenesis therapy in NSCLC. The BRIDGE study was designed to establish the safety of bevacizumab in patients with squamous type NSCLC by giving two cycles of induction chemotherapy followed by four cycles of chemotherapy plus bevacizumab. It is anticipated that the initial 2 cycles will devascularize the tumor sufficiently enough to prevent any significant bleeding complications with subsequent therapy with bevacizumab. The final outcome of this trial is still awaited.

The safety of bevacizumab as part of first- or second-line treatment regimen in patients with treated brain metastasis was the primary objective of the PASSPORT trial. One hundred and fifteen patients were enrolled of which 76 received frontline therapy including bevacizumab after completing brain irradiation. The use of bevacizumab in this population appeared safe as there was no symptomatic grade 2 or greater CNS hemorrhage although there were 2 cases of fatal pulmonary hemorrhage [48]. This safety profile was replicated in the larger observational ARIES study discussed in the preceding section.

Ways to Enhance Bevacizumab-Based Therapy

There is significant cross-talk between VEGF signaling and epidermal growth factor receptor (EGFR) activation [49] whereby EGFR signaling induces VEGF expression and angiogenesis [50]. Conversely, EGFR inhibition results in the inhibition of VEGF secretion and reduced induction of angiogenesis by tumor cells [51-55]. Combined blockade of both EGFR and VEGF signaling is therefore expected to result in a synergistic interaction. Promising activity of this concept in a series of small phase II trials of bevacizumab and erlotinib combination, [56, 57] led to a phase III randomized study of bevacizumab plus erlotinib versus erlotinib alone in previously treated patients with NSCLC. While the combination resulted in increased PFS, there was no difference in OS (9.3 months for the combination arm versus 9.2 months for erlotinib alone; p=0.006) [58]. Similar comparison of bevacizumab and erlotinib combination against bevacizumab alone as maintenance strategy following frontline regimen of chemotherapy plus bevacizumab was evaluated in the ATLAS trial. The median PFS in the intent- to- treat population was 4.76 months for the combination arm and 3.75 months for the bevacizumab/placebo arm (HR=0.722, 95% CI: 0.59-0.88; p=.0012). The observed toxicity profile was characteristic and within the range expected based on single agent administration of both drugs [59]. The SWOG S0536 phase II study was designed to evaluate the safety and efficacy of combining standard frontline cytotoxic chemotherapy with agents targeting both the EGFR and the VEGFR signaling pathways. One hundred and ten treatment-naïve patients with advanced NSCLC were enrolled to receive a maximum of six 3-weekly cycles of paclitaxel (200mg/m2), carboplatin (AUC 6), cetuximab (400mg/m^2 loading followed by 250mg/m^2 weekly) and bevacizumab (15mg/kg) regimen. Non-progressing patients continued with bevacizumab and cetuximab as maintenance therapy [60]. The toxicity profile of this 4-drug regimen was comparable to that reported in the S0342 trial of carboplatin, paclitaxel and cetuximab [61] and the E4599 trial of carboplatin, paclitaxel and bevacizumab [4]. In 95 response evaluable patients, objective RR was 54%, and SD was 23%; PFS and OS were 7 months and 14 months respectively with median follow up of 15 months

[60]. A phase III trial comparison of paclitaxel, carboplatin plus bevacizumab with or without cetuximab is currently ongoing to confirm this promising outcome (NCT00946712).

B. Fusion Protein

Aflibercept

Aflibercept (VEGF Trap) binds all isoforms of VEGF-A and placental growth factor (PIGF) with a higher affinity than the natural receptors. It is, therefore, expected to be a potent inhibitor of angiogenesis. In a phase II trial in 33 patients with non-squamous NSCLC refractory to both platinum-containing regimen and erlotinib, aflibercept administered at 4.0 mg/kg once every 2 weeks was well-tolerated and was not associated with significant hemorrhagic complications; two patients attained PR confirming some activity but not enough activity to warrant single agent development [62]. This agent is currently being evaluated in combination with docetaxel in a phase III study (NCT00532155) and in combination with cisplatin and pemetrexed in a phase II trial (NCT00794417).

C. VEGF Receptor Tyrosine Kinase Inhibitors

Targeting the receptor tyrosine kinase activity of the VEGF receptor is an alternative approach for inhibiting angiogenesis. A number of orally administered agents have become available, many of which possess the additional advantage of simultaneously targeting multiple signaling pathways. Available evidence of the activity of this class of agents in NSCLC will be reviewed in this section.

Sorafenib

Sorafenib targets the serine threonine kinases c-Raf and b-Raf, the VEGFRs 1, 2 and 3, PDGFR, the proto-oncogene RET and c-KIT. In a phase II study of 54 patients with relapsed or refractory NSCLC previously treated with not more than two regimens, sorafenib 400 mg orally twice produced no objective responses; however, 59% of the 51 evaluable patients achieved disease stability; the median PFS was 2.7 months and median OS was 6.7 months. Sorafenib was well tolerated in this setting and the most frequent grade 3 treatment-related AEs were hand-foot syndrome (10%) and hypertension (4%) [63]. In a randomized discontinuation phase II trial, Schiller *et al.* enrolled 342 patients with refractory NSCLC and identified 97 patients with disease stability after 2 cycles of sorafenib. This cohort of 97 patients was subsequently randomized in a blinded fashion to either continue sorafenib or switch to a placebo. There was a near-doubling of the PFS (1.9 months versus 3.6 months) and maintenance of disease stability (5% versus 29%) in favor of patients randomized to continue sorafenib [64]. The modest single agent activity recorded in the refractory NSCLC patient population, however, could not be replicated in a window of opportunity trial in treatment naïve NSCLC patients. In this trial, newly diagnosed patients with stage IIIB/IV NSCLC received sorafenib on a continuous 4-week cycle schedule. The study recorded only 1 confirmed PR in the first cohort of 20 evaluable patients for an overall RR of 12% and median OS of 8.8 months. The study failed to meet the pre-specified RR required to proceed to second stage accrual and was, therefore, closed prematurely [65].

Sorafenib has also been evaluated as part of a combination regimen. A phase II trial of sorafenib plus erlotinib enrolled fifty chemo-naïve patients with stage IIIB/IV NSCLC. The median TTP was 4.6 months and the rate of non-progression at 6 weeks after 2 cycles of therapy was 76% (PR in 13 patients and SD in 25 patients) [66]. Intriguing clinical activity was also observed with this combination in a randomized phase II trial of erlotinib alone versus erlotinib plus sorafenib in 168 patients with stage IIIB/IV NSCLC previously treated with up to 2 prior chemotherapy regimens. The preliminary result of this study presented at the 2009 World Conference of the International Association for the Study of Lung Cancer (IASLC) in San Francisco showed improvement in PFS outcome with the combination regimen, which was also demonstrable in patients with squamous histology on subset analysis [67].

The ESCAPE trial was a phase III evaluation of the combination of sorafenib, carboplatin and paclitaxel versus carboplatin and paclitaxel alone in the frontline setting for all histologic types of NSCLC. The study was closed after a pre-planned interim analysis showed that the primary endpoint of a superior survival outcome for the sorafenib arm could not be met. More importantly, there was a strong indication of a deleterious effect of sorafenib in patients with squamous histology (214 patients or 24% of total accrual) who achieved a median OS of 8.9 months in comparison with 13.6 months for similar patients treated with chemotherapy alone. The participation in the study of the squamous cell carcinoma patients, the choice of the platinum-based regimen is among the proposed reason for treatment failure. Moreover, the carboplatin, paclitaxel regimen has been tested in metastatic melanoma patients with negative results, leading to the speculation that sorafenib may change the pharmacokinetics of the chemotherapeutic regimen [68]. The NEXUS trial is a similarly designed study using cisplatin and gemcitabine chemotherapy backbone. The study has completed the planned patient accrual and results are pending (NCT00449033).

Sunitinib

Sunitinib has a spectrum of activity similar to sorafenib by inhibiting VEGFR, PDGFR, c-KIT and RET. Similar to sorafenib it has limited single agent activity in patients with previously treated NSCLC. When given on a continuous schedule, sunitinib 37.5 mg daily achieved a confirmed PR of 2% and SD in 23.4% of patients [69]. A higher dose of 50 mg daily on an intermittent schedule, 4 weeks on and 2 weeks off, produced a higher response rate (PR-9.5%; SD-19%) but the rate of bleeding-related complications also increased [70]. The safety of this agent in patients with treated brain metastasis was demonstrated by a phase II study of single agent sunitinib in patients with NSCLC and irradiated brain metastases presented at the 2009 ASCO meeting in Orlando. No intracranial hemorrhage was reported and a modest clinical benefit was observed with median PFS of 10.9 weeks and best response of SD in 19% of the patients [71]. There is enough signal to warrant further evaluation of this compound in NSCLC and trials assessing the combination of sunitinib and cytotoxic chemotherapy are currently underway [72, 73].

Table 2. **Selected Clinical Trials of VEGF Receptor Tyrosine Kinase Inhibitors in NSCLC**

Clinical Trial	Phase	Design	RR	PFS	OS (Month)	Remarks
ZEST [77]	III	Vandetanib *vs* Erlotinib 2nd line	12% *vs* 12%	11.3 *vs* 8.9 weeks (HR=0.98; p=0.721)	6.9 *vs* 8.9 (HR=1.01; p=0.830)	Higher incidence of AEs in the vandetanib group
ZEAL [79]	III	Pemetrexed *vs* Pemetrexed + Vandetanib 2nd line	19.1% *vs* 7.9% (P<0.001)	11.9 *vs* 17.6 weeks (HR=0.86; p=0.108)	9.2 *vs* 10.5 (HR=0.86; p=0.219)	Did not meet the primary endpoint of superior PFS
ZODIAC [80]	III	Doc *vs* Doc + Vandetanib 2nd line	17% *vs* 10%	3.2 *vs* 4 months (HR=0.79; p<0.001)	10 *vs* 10.6 (HR=0.91; p=0.196)	Docetaxel plus vandetanib superior to docetaxel by RR and PFS but no OS benefit
Adjei *et al.* [65]	II	Sorafenib 1st line	12%	2.9 months	8.8	Failed to meet the primary endpoint
Scagliotti *et al.* [68]	III	CP + Sorafenib *vs* CP + Placebo 1st line	27.4% *vs* 24.0% (P=0.1015)	4.6 *vs* 5.4 months (HR=0.99; P=0 .433).	10.7 *vs* 10.6 (HR=1.15; P = 0.915)	Study terminated prematurely when interim analysis showed low likelihood of meeting the primary end point. Higher mortality in patients with squamous histology treated with sorafenib
Schiller *et al.* [64]	IIR	Sorafenib *vs* placebo ≥2nd line	49% *vs* 19% (p=0.01)	3.8 *vs* 2.0 months HR=2.18; 1.21-3.88; P<0.0001	11.9 *vs* 9.0; HR=1.5 (0.82- 2.72; P=0.18)	No difference between squamous and non squamous NSCLC. Adopted a randomized discontinuation trial in non progressing patients after 2 cycles of sorafenib therapy
Goss *et al.* [76]	II	CP + Cediranib *vs* CP + Placebo 1st line	38% *vs* 16%	5.6 *vs* 5.0 months HR=0.77; (0.56 to 1.08)	10.5 *vs* 10.1; HR=0.78; (0.57 to 1.06; P = .11)	Premature closure of trial due to increased toxicity with cediranib
Novello *et al.* [69]	II	Sunitinib ≥2nd line	2.1	3 months	9 months	Low dose (37.5mg) continuous
Socinski *et al.* [70]	II	Sunitinib ≥2nd line	9.5%	NR	NR	High dose (50mg) intermittent schedule

Doc: Docetaxel; Pem: Pemetrexed; CP: Carboplatin/Paclitaxel; NR: not reported.

Cediranib

Cediranib (AZD2171) is an orally administered VEGFR-selective TKI. The drug was safely combined with standard cytotoxic chemotherapy regimens for NSCLC in phase I trials [74, 75]. However, a phase II/III trial of cediranib plus paclitaxel and carboplatin was prematurely closed to accrual due to increased treatment-related toxicity despite meeting its primary efficacy endpoint at the end of phase II accrual; ORR (38% versus 16%, p<0.0001) and median PFS (5.6 versus 5.0 months, HR=0.77) were both in favor of the cediranib containing regimen [76]. Given the encouraging ORR observed in patients treated with cediranib, a randomized trial looking at a reduced dose of cediranib of 20mg instead of 30 mg versus placebo in combination with carboplatin and paclitaxel has been initiated in order to establish the efficacy of this agent while minimizing its toxicity (NCT00795340).

Vandetanib

Vandetanib (Zactima) is a multi-targeted TKI with potent activity against VEGFR-2, VEGFR-3, EGFR and RET. Given its inhibitory activity against both EGFR and VEGF signaling, this drug is one of the most tested TKI in lung cancer patients. After a series of early phase clinical trials, the drug has now been evaluated in the phase III settings for different indications. The ZEST trial compared vandetanib (300 mg, daily) to erlotinib (150 mg, daily) as salvage therapy in NSCLC patients who have progressed on frontline chemotherapy. The primary endpoint was to establish the superiority of vandetanib over erlotinib in terms of PFS.

With a total of 1240 patients enrolled, the study showed no difference in terms of RR (12% in both arms); PFS (HR=0.98; p=0.721) or OS (HR=1.01; p=0.830). The adverse event profile was however in favor of erlotinib with incidence of grade 3 or 4 toxicities of 50% versus 40% [77].

It has been suggested that the more potent activity of vandetanib against the kinase activity of VEGFR2 (IC$_{50}$ − 40 nM) over EGFR kinase activity (IC$_{50}$ − 500 nM) would lead to a predominant anti-VEGF effect when the drug is administered at a lower dose of 100 mg while a potent anti-EGFR effect is expected at the higher 300 mg per day dose [78]. To take advantage of this differential activity, lower doses of vandetanib were evaluated in combination with cytotoxic chemotherapy agents. The ZEAL study was a randomized, double-blind phase III trial, which enrolled 534 patients to compare standard dose pemetrexed versus vandetanib (100 mg once daily) plus pemetrexed. The 2-drug combination showed superior response rates (19.1% *vs* 7.9%, *P*<0.001). This however, failed to translate into survival advantage whether by the primary endpoint of PFS (HR=0.86; p=0.106) or by the secondary endpoint of OS (HR=0.86; p=0.219) [79].

In the ZODIAC trial, vandetanib (100mg) combined with docetaxel was compared to docetaxel alone. A total of 1391 patients were enrolled including 25% with squamous histology and 10% with treated brain metastases. The study met its primary endpoint of improved PFS with the combination arm (HR=0.79; p<0.001). Similar to the result in the ZEAL study, the ORR was also higher in vandetanib group (17% versus 10%; p<0.001) but there was no significant difference in OS [80]. Finally, the ZEPHYR trial is comparing vandetanib to best supportive care in patients who have progressed on chemotherapy and EGFR inhibitor therapy. The results of this trial are still awaited. While the "Z series" trials have shown some promise with vandetanib in the phase III setting, the combination with docetaxel appears to be the best strategy so far for incorporating this drug into combination regimens against NSCLC.

A number of other multitargeted kinase inhibitors with significant anti-VEGFR activity are at various stages of evaluation in NSCLC. These include motesanib which is currently being evaluated in combination with cytotoxic agents; [81, 82] axitinib, which showed promising single agent activity in a phase II trial and is now being evaluated in combination with cytotoxic chemotherapy; [83, 84] as well as pazopanib, which has been tested in the neoadjuvant setting [85]. Table **2** contains a summary of outcomes for clinical trials that evaluated VEGF receptor tyrosine kinase inhibitors in NSCLC patients.

D. Vascular Disrupting Agents

Vascular disrupting agents (VDA) selectively target newly formed tumor-associated blood vessels. They induce tumor-specific ischemia and necrosis and are therefore expected to induce vascular-related complications at a lower rate than observed with non-selective antiangiogenesis agents. 5,6 dimethylxanthenone-4-acetic acid (DMXAA) is a flavinoid VDA which has been shown to enhance the activity of radiation therapy (RT) and chemotherapy [86-88]. In a randomized phase II study enrolling newly diagnosed NSCLC patients including those with squamous histology, DMXAA (ASA404) in combination with paclitaxel and carboplatin was associated with comparable toxicity profile

to chemotherapy alone while conferring improved clinical outcome in terms of RR, disease progression and overall survival [89]. A phase III study of docetaxel combined with ASA404 versus docetaxel plus placebo is currently enrolling patients who have progressed on first line therapy.

CONCLUSIONS AND FUTURE PERSPECTIVES

The role of antiangiogenesis therapy has been established in the frontline treatment of advanced NSCLC using monoclonal antibody, bevacizumab, but its efficacy in the salvage therapy setting remains to be established. Ongoing evaluation of tyrosine kinase inhibitors targeting the VEGF signaling pathway look somewhat promising and may eventually fill this void. Similarly, since the safety and efficacy of anti-angiogenic agents has become well established in the frontline treatment for most subgroups of patients with advanced metastatic NSCLC, establishing their role in the treatment of microscopic residual and micrometastatic disease following curative surgery seems fitting. An ECOG phase III adjuvant trial (E1505) is currently enrolling NSCLC patients with stage IB to IIIA who have had adequate surgical resection of the primary tumor as well appropriate lymph node dissection. Patients are randomized to receive standard platinum-based adjuvant chemotherapy with or without bevacizumab (NCT00324805). This study may establish a role for the strategy of antiangiogenesis therapy in early stage disease. Nonetheless, several questions remain to be answered. Patient selection is important for the optimal use of these agents but no clear predictive marker of benefit has been validated well enough for clinical utility. Given the limited efficacy of all classes of antiangiogenesis agents when used as single agent for the treatment of NSCLC, the future development of these agents will require combinatorial strategies. As the number of agents under study increases, screening of potentially useful agents may require the development of innovative surrogate markers. Response assessment using current criteria (RECIST) has been shown to have significant limitations in assessing the efficacy of newer molecularly-targeted therapeutic agents [90, 91]. The increased availability and use of metabolic imaging (dynamic contrast MRI and PET) may provide an opportunity for a more optimal assessment of the efficacy of the increasing number of antiangiogenesis agents. The proposal to adopt a functional response criteria based on temporal changes in PET activity, so called PERCIST criteria, is an intriguing concept that may assist in the optimal evaluation of a number of novel agents that target angiogenesis but remains to be validated. Finally, the toxicity profile of novel antiangiogenesis agents as monotherapy and in combination with established chemotherapeutic agents require careful attention and further evaluation.

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