

## ANGIOGENESIS AND NON SMALL CELL LUNG CANCER

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### ABSTRACT

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Non small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers. Despite improvements in treatment protocols the survival rate in NSCLC remains poor. An improved understanding of cancer biology has led to the investigation of angiogenesis in NSCLC development and progression by intratumoral microvessel count or expression of angiogenic markers. The prognostic significance of several frequently assessed angiogenic factors and current antiangiogenic strategies which proved to be particularly promising in combination with chemotherapeutic agents for clinical management of NSCLC are here reviewed.

**Key words:** Angiogenesis; Non small cell lung cancer (NSCLC); Vascular endothelial growth factor (VEGF); Antiangiogenic agents

### INTRODUCTION

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Lung cancer is the leading cause of cancer-related mortality with 1.2 million new cases being diagnosed every year and one million deaths recorded worldwide in 2001 [1]. Histologically, lung cancer is divided into two major types: small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC) consisting of squamous cell carcinoma, adenocarcinoma, large cell carcinoma and other rare subtypes. Non small cell lung cancer accounts for approxi-

mately 80% of all lung cancers. Five-year survival rates reported for stage I NSCLC range from 50 to 80%, but overall survival rate is 10 to 15% [2]. Surgical removal is the mainstay treatment in patients with early-stage disease (stage I and II disease) [3] but a large portion of these patients eventually experience metastatic recurrence [1]. Moreover, 70% to 80% [3] of NSCLC patients present with locally advanced (stage III) or metastatic (stage IV) disease at diagnosis [1], and the primary treatment option for these patients is chemotherapy including cisplatin or carboplatin regimen. Despite improvement in survival rates due to chemotherapy overall (8-11 months) and 1-year survival (27-47%) rates for chemotherapy-treated patients with advanced disease remain low [4,5]. The improved understanding of cancer biology has led to the investigation of therapies directed against key biological processes in NSCLC development and progression. Antiangiogenic agents, including monoclonal antibodies and small-molecule tyrosine kinase inhibitors (TKI), have shown potential for increased selectivity and thereby reduced toxicity compared with standard chemotherapies [3].

**Angiogenesis and Tumor Development.** When tumors are small (<1 mm in diameter), they rely on diffusion to provide oxygen and nutrients for growth of cancerous cells [6]. When a tumor reaches 2-3 mm in diameter, further growth needs vascular supply [7]. Angiogenesis is the development of new capillary beds from the endothelium of pre-existing blood vessels [6]. Studies in animal tumor models have suggested that tumor endothelial cells may

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also be derived from circulating endothelial precursor cells originating from the bone marrow [8]. Angiogenesis is a multi-step process that involves activation of endothelial cells and production of matrix metalloproteinases (MMPs), which break down the surrounding extracellular matrix [9], proliferation of activated endothelial cells, migration of these cells to remote regions and then assembly into new capillary tubes and the synthesis of a new basement membrane and maturation of vessels with formation of a vascular lumen [10]. Initially, malignant tumors exploit the host's pre-existing vessels and become enriched with new blood capillaries only when they are on the verge of invading adjacent tissues. Normal tissues act as a barrier for tumor invasion and angiogenesis occurs simultaneously with the formation of new stroma (stromatogenesis). The loose and oedematous stroma is easily amenable to penetration by endothelial and tumor cells and allows endothelial cell migration and tumor cell invasion [8]. Thus, angiogenesis permits growth of the primary tumor and provides a pathway for migrating tumor cells to gain access to the systemic circulation and to establish distant metastases. Without a functional vascular supply tumors remain dormant and are unable to metastasize [1]. However, some NSCLCs have a non angiogenic vascular phenotype which is the same as that of normal alveolar vessels [11]. These tumors are invasive, exploiting the pre-existing alveolar vessels [12].

**Markers for Angiogenesis.** In NSCLC, angiogenesis has been evaluated by intratumoral microvessel count or expression of angiogenic markers [13]. The microvessel count is frequently assessed by using antibodies to clotting factor VIII, CD31 or CD34 antigens, expressed on the surface of endothelial cells [6]. High microvessel density has been used as a predictor of metastasis, poor survival [1] and recurrence in patients with stage I NSCLC [2]. In particular, high vascularity at the tumor periphery has been correlated with tumor progression [14]. However this method has several drawbacks. First, it assumes that the region with the most angiogenic activity reflects the activity of the tumor as a whole [6]. However, new blood vessel formation occurs mainly at the tumor periphery, while inner regions that form the great bulk of the tumor often lack vessels. Also in tumor periphery and inner parts of the tumor regions of extreme microvessel density ("hot

spots") alternate with others of vascular sparsity [8]. Secondly, the method does not distinguish between new and old vessels, or whether there is actual blood flow through the new vessels [6].

The expression of angiogenic substances by tumors may indicate the formation of new blood vessels. Initiation of angiogenesis is believed to rely on an angiogenic "switch" [14] that includes the secretion of angiogenic factors by tumors and their precursor cells [15]. The "switch" is activated during tumor progression by environmental (*e.g.*, hypoxia) and genetic changes that involve upregulation of oncogenes such as Src [10] and Ras [15], and downregulation by tumor suppressor genes such as p53 [10]. Angiogenesis is controlled by stimulatory factors, *e.g.*, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) [1], platelet-derived endothelial cell growth factor (PDEC-GF)/thymidine phosphorylase (TP) [10] and inhibitory factors (*e.g.*, angiostatin, endostatin [1]) whose balance determines the degree of angiogenesis [1]. Tumors can up- or downregulate these factors to produce an environment in which angiogenesis occurs [9]. Since most of the stimulatory factors are soluble and diffusible peptides, blood levels of these cytokines could reflect overall angiogenic tumor activity and provide an easier method to assay angiogenesis or proangiogenic factors in NSCLC [13].

The VEGF family consists of six growth factors [VEGF-A, -B, -C, -D and -E, and placental growth factor (PlGF)] and three receptors [1,9,16], of which VEGFR-2 is thought to be mainly responsible for the biological effects of VEGF. The binding of VEGF to VEGFR-2 promotes receptor dimerization and receptor tyrosine kinase phosphorylation and lead to endothelial cell proliferation, migration, and survival [3]. The VEGF/VEGFR signaling pathway is frequently upregulated in lung cancer, and VEGF overexpression is associated with tumor progression [16]. High VEGF levels have been identified as an independent prognostic factor correlated with poor prognosis in patients with lung cancer [14].

High expression of bFGF is also an independent indicator of poor outcome in NSCLC patients [14]. Basic fibroblast growth factor is released by proteolytic enzymes from the extracellular matrix and it increases the expression of other proteolytic molecules. It is proangiogenic and increases tumor

growth [6]. It has been shown that NSCLCs expressing bFGF did not express ELRpositive CXC chemokines, heparin-binding proteins that contain Glu-Leu-Arg (“ELR” motif) and play a role in the regulation and promotion of angiogenesis [17] or VEGF which supports the hypothesis that tumors develop unique “angiogenic signatures” characterized by the expression of one dominant angiogenic factor [18].

Platelet-derived endothelial cell growth factor is a growth factor with TP activity. In NSCLC, increased PDEC-GF/TP expression correlates with a higher vascular grade and poorer prognosis in patients with node-negative disease [9,14].

Platelet-derived growth factor is secreted from platelets and increases DNA synthesis [19], endothelial cell migration [20] and tumor growth [21]. The PDGF receptors are expressed on tumor neovascularity and upregulated during tumor progression [9]. The PDGF expression in stromal cells may be more important than tumor cell expression in its effect on angiogenesis and outcome in NSCLC [6].

The hypoxia-inducible factors HIF-1 $\alpha$  and HIF-2 $\alpha$  regulate a number of gene products relevant to angiogenesis [22]. Expression of both factors was found in NSCLC tissue samples, with HIF-2 $\alpha$  considered to be an independent prognostic indicator of poor outcome [14].

The MMPs are a family of more than 20 zinc-dependent neutral endopeptidases that are important for remodelling of the extracellular matrix during angiogenesis [23]. They are synthesized by connective tissue cells and are capable of degrading all of the components of the extracellular matrix [7]; MMP-12 expression significantly correlates with unfavorable outcome and metastasis in NSCLC [23].

Interleukin-8 (IL-8) is angiogenic for NSCLC *in vitro* and *in vivo* [24]. High IL-8 expression correlates with a higher microvessel density and is a significant prognostic marker [25]. An association was found between the presence of tumor-infiltrating macrophages and tumor IL-8 expression, suggesting a mechanism for how macrophages may adversely affect outcome in NSCLC [6].

**Antiangiogenic Strategies.** Since tumor growth and metastasis are angiogenesis-dependent, therapeutic strategies aimed at inhibiting angiogenesis are theoretically attractive. Potential targets for neo-angiogenesis include VEGF and the receptor

tyrosine kinases, as well as the MMPs [9].

A widely studied approach in the treatment of NSCLC is inhibition of the VEGF-VEGFR pathway which plays a central role in tumor angiogenesis. Bevacizumab is an anti-VEGF recombinant humanized monoclonal antibody which blocks the binding of VEGF to its receptors, thereby inhibiting its biological activities. In 2004, the Food and Drug Administration (FDA) in America approved bevacizumab as a first-line treatment for patients with colorectal cancer that has spread to other parts of the body. Bevacizumab, combined with 5-fluorouracil-based chemotherapy, is currently approved in Europe and the United States for first-line treatment of metastatic colorectal cancer, and in the United States for first-line treatment of advanced non squamous NSCLC [3]. In a phase II trial, Johnson *et al.* [26] showed that bevacizumab with carboplatin-paclitaxel increased the response rate (31.4 *versus* 18.8%) and median time to progression (7.4 *versus* 4.2 months) in patients with NSCLC compared with chemotherapy alone. Bleeding was the primary safety concern, especially for patients with squamous tumors which tend to be more centrally located in the chest, near large blood vessels [27]. A phase II/III trial was conducted by Sandler *et al.* [28] with the main differences from the phase II trial mentioned above being the exclusion of patients with squamous cell histology and the exclusion of a thrombotic or hemorrhagic history including hemoptysis disorders. Patients with lung cancer were randomly assigned to standard treatment or standard treatment plus bevacizumab. Median survival was significantly longer in the bevacizumab group than in the standard group (12.3 *versus* 10.3 months); response rate (35 *versus* 15%) and progression-free survival (6.2 *versus* 4.5 months), were also better. In 2006, based on the results obtained from the study of Sandler *et al.*, the FDA approved use of bevacizumab in combination with carboplatin/paclitaxel as treatment for patients with advanced non squamous NSCLC. Extension of the benefit of bevacizumab into early-stage NSCLC is being explored in the E1505 North American intergroup trial. The study will include 1,500 patients with stage IB-III A resected NSCLC, stratified by stage, histology, gender, and chemotherapy regimen [29].

Many antiangiogenic therapies, which target a single angiogenic factor or pathway, may be effec-

tive for only a limited proportion of patients [18]. Most smoke-related bronchogenic neoplasms possess multiple molecular alterations and show resistance to selective molecular-targeted agents [30]. Non small cell lung cancer is frequently associated with overexpression [31] or activation [32] of epidermal growth factor receptor (EGFR), which involves receptor dimerization and autophosphorylation of intracellular receptor tyrosine kinase [31]. The EGFR gene copy number and the absence of K-ras mutations have been identified as potential predictive biomarkers for the efficacy of anti-EGFR TKIs [33]. The combination of bevacizumab and EGFR TKI erlotinib demonstrated partial responses (20%), stable disease (65%) or progressive disease (15%) in a phase I/II study on patients with non squamous stage IIIB-IV NSCLC pre-treated with chemotherapy [34]. In a phase II study, the erlotinib plus bevacizumab regimen was well tolerated, with a low rate of adverse events and toxicities, and offered a promising rate of non progression at 6 weeks in the treatment of advanced non squamous NSCLC who had received no prior chemotherapy [35]. Some clinical trials on the combination of bevacizumab and erlotinib are ongoing. The study BO20571 is a phase II trial that compares, in terms of progression-free survival and safety, erlotinib plus bevacizumab with platinum-based chemotherapy plus bevacizumab in the first-line treatment of non squamous stage IIIB/IV NSCLC patients. The ATLAS trial is a phase III trial comparing bevacizumab plus erlotinib with bevacizumab as maintenance treatment after four cycles of chemotherapy plus bevacizumab in the first-line treatment of non squamous stage IIIB/IV NSCLC. The trial endpoints are progression-free survival after the end of first-line chemotherapy and safety and overall survival. The BeTA Lung trial is a phase III trial comparing erlotinib plus bevacizumab with erlotinib alone in terms of efficacy (survival) and safety in the second-line treatment of non squamous stage IIIB/IV NSCLC [1]. In the BeTA Lung trial, the median survival is similar but there is an increase in progression-free survival when bevacizumab and erlotinib are combined compared with erlotinib alone.

Targeting of multiple pathways in tumor growth may offer the benefits of combined therapy within a single agent. The small-molecule TKIs

prevent activation of VEGF receptors [14] through binding to the ATP pocket of their TK residues in the intracellular domain [16]. Tyrosine kinase inhibitors also frequently target other receptor TKs, such as EGFR and c-KIT [16]. Multi-TKIs such as ZD6474 (Zactima™), SU11248 (Sunitinib™) and BAY 43-9006 (Sorafenib™), are also under clinical evaluation in NSCLC. Zactima™ (ZD6474) selectively inhibits VEGF-dependent tumor angiogenesis and EGF-dependent tumor cell proliferation and survival. It acts as a potent and reversible inhibitor of ATP binding to the TK domain of VEGFR-2 and to EGFR [36]. Studies in a broad population of patients with advanced NSCLC show that ZD6474 is well tolerated alone and in combination with chemotherapy, with promising results in the treatment of recurrent disease [37]. Preliminary results of two phase II randomized trials indicate that ZD6474 plus docetaxel in patients with locally advanced or metastatic NSCLC, or ZD6474 plus carboplatin/paclitaxel in treatment-naive patients with locally advanced or metastatic NSCLC, are generally well tolerated [3,38]. A phase III study investigating ZD6474 plus docetaxel *versus* docetaxel alone has started accruing patients with advanced NSCLC who failed first-line chemotherapy [3].

Sunitinib (SU11248) also has anti-tumor and antiangiogenic activities. It has been identified as a potent inhibitor of VEGFR-1, VEGFR-2, fetal liver tyrosine kinase receptor 3 (FLT3), c-KIT [stem-cell factor (SCF) receptor], PDGFR (platelet-derived growth factor receptor) –a and –b [37]. In 2006, the FDA approved sunitinib malate for the treatment of gastrointestinal stromal tumor (GIST) after disease progression on, or intolerance to, imatinib mesylate (Gleevec) and for the treatment of advanced (metastatic) renal cell carcinoma. Sunitinib is under clinical evaluation in lung cancer in second-line treatment, both as single agent and in combination with erlotinib [37]. Two ongoing phase II studies are evaluating sunitinib malate monotherapy in advanced NSCLC: the first in previously treated patients with metastatic disease, and the second as consolidation therapy following first-line treatment with carboplatin/paclitaxel in locally advanced/metastatic disease. The agent is also under investigation in combination with erlotinib in previously treated advanced NSCLC [3].

Sorafenib (BAY 43-9006) has shown activity against RAF-kinase and several other kinases, including VEGFR-2, PDGFR-b, c-KIT and FLT3. It has the potential to prevent tumor growth by inhibition of tumor angiogenesis and of tumor cell proliferation [37]. In 2005, the FDA approved the use of sorafenib for the treatment of patients with advanced renal cell carcinoma (RCC). The potential activity of sorafenib in patients with advanced NSCLC is being evaluated in ongoing phase II/III trials, as single agent or in combination with gefitinib or chemotherapy [37]. Preliminary results from a phase I study of sorafenib plus gefitinib in patients with unresectable or recurrent advanced NSCLC, showed that the combination was well tolerated with no dose-limiting toxicities [39]. In a recent phase II trial, sorafenib monotherapy showed promising efficacy in patients with advanced NSCLC, with 59% of patients achieving disease stabilization [40]. A large randomized study presently in progress, is comparing chemotherapy (carboplatin-paclitaxel) alone with chemotherapy plus sorafenib in patients with advanced NSCLC [3].

Vatalanib (PTK787) is an oral inhibitor of VEGFR-1, -2, and -3 TKs and related kinases such as PDGFR- $\beta$  and c-Kit [41]. In a human lung adenocarcinoma model, PTK787 significantly suppressed vascular hyper permeability [42]. Its role in patients with lung cancer is being evaluated in a phase II study (the GOAL Study) designed to evaluate the efficacy of PTK/ZK in patients with stage IIIB/IV NSCLC [14]. In relapsed or progressive NSCLC (including squamous cell carcinomas) after first-line treatment with cisplatin-based chemotherapy, vatalanib given once or twice daily was associated with pulmonary hemorrhages, hypertension or thrombosis in some patients. In the first cohort (once a day), PTK787 induced a 2% response rate and a 33% stabilization rate at 12 weeks [7].

Clinical trials assessing the tolerability of several other kinase inhibitors, either in combination with chemotherapy or in chemo-naive patients, are ongoing. A phase I/II study is evaluating the efficacy of AMG 706 (an oral multi-kinase inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, c-Kit, and c-Ret) with paclitaxel/carboplatin and the anti-EGFR monoclonal antibody panitumumab in patients with advanced NSCLC [3]. CP-547,632, a selective VEGFR-2 TKI [43] showed good tolerabil-

ity in preliminary results from a phase I trial in combination with carboplatin/ paclitaxel for advanced NSCLC [44] and a phase I/II trial in chemo-naive NSCLC patients is ongoing [3]. AZD2171 is a potent inhibitor of VEGFR-2 TK activity [45] and is being assessed in a phase II study in combination with carboplatin-paclitaxel in patients with advanced NSCLC [3].

Alternatively, some inhibitors of MMPs have also shown potential for the treatment of lung cancer. Neovastat (AE-941) is an oral, naturally-occurring inhibitor of MMPs, derived from shark cartilage extract [9]. Patients with advanced cancer refractory to treatment or for which no standard treatments were available were enrolled in a phase I/II dose-escalation study of AE-941 (30, 60, 120, or 240 mL/day) received as monotherapy. No dose-limiting toxicity was reported with most frequent adverse events affecting the gastrointestinal system (nausea, vomiting) and a significant survival advantage was observed for patients receiving doses  $>2.6$  mL/kg/day compared to patients receiving lower doses (median, 6.1 *versus* 4.6 months). On the other hand, 26% of the patients in the high-dose group had stable disease compared to 14% in the low-dose group [46].

In conclusion, antiangiogenic agents currently in development and the available data indicate the significant potential of this therapeutic approach in the treatment of solid tumors. Given that lung cancer is a heterogeneous disease with many potential therapeutic targets, combinations of different biological agents to target multiple pathways simultaneously may lead to novel treatment for NSCLC.

## REFERENCES

1. Gridelli C, Maione P, Rossi A, De Marinis F. The role of bevacizumab in the treatment of non-small cell lung cancer: current indications and future developments. *Oncologist* 2007; 12(10): 1183-1193.
2. Dunder E, Oner U, Peker BC, Metintas M, Isiksoy S, Ak G. The significance and relationship between mast cells and tumour angiogenesis in non-small cell lung carcinoma. *J Int Med Res* 2008; 36(1): 88-95.
3. Giaccone G. The potential of antiangiogenic therapy in non-small cell lung cancer. *Clin Cancer Res* 2007; 13(7): 1961-1970.

4. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH, Eastern Cooperative Oncology Group. Comparison of four chemotherapy regimens for advanced non-small cell lung cancer. *N Engl J Med* 2002; 346(2): 92-98.
5. Socinski MA. The role of chemotherapy in the treatment of unresectable stage III and IV nonsmall cell lung cancer. *Respir Care Clin N Am* 2003; 9(2): 207-236.
6. Singhal S, Vachani A, Antin-Ozerkis D, Kaiser LR, Albelda SM. Prognostic implications of cell cycle, apoptosis, and angiogenesis biomarkers in non-small cell lung cancer: a review. *Clin Cancer Res* 2005; 11(11): 3974-3986.
7. Besse B, Ropert S, Soria JC. Targeted therapies in lung cancer. *Ann Oncol* 2007; 18 (Suppl 9): ix135-142.
8. Giatromanolaki A, Sivridis E, Koukourakis MI. Tumour angiogenesis: vascular growth and survival. *APMIS* 2004; 112(7-8): 431-440.
9. Brock CS, Lee SM. Anti-angiogenic strategies and vascular targeting in the treatment of lung cancer. *Eur Respir J* 2002; 19(3): 557-570.
10. Pang RWC, Poon RTP. Clinical implications of angiogenesis in cancers. *Vasc Health Risk Manag* 2006; 2(2): 97-108.
11. Hu J, Bianchi F, Ferguson M, Cesario A, Margaritora S, Granone P, Goldstraw P, Tetlow M, Ratcliffe C, Nicholson AG, Harris A, Gatter K, Pezzella F. Gene expression signature for angiogenic and nonangiogenic non-small-cell lung cancer. *Oncogene* 2005; 24(7): 1212-1219.
12. Offersen BV, Pfeiffer P, Hamilton-Dutoit S, Overgaard J. Patterns of angiogenesis in non-small cell lung carcinoma. *Cancer* 2001; 91(8): 1500-1509.
13. Dudek AZ, Mahaseth H. Circulating angiogenic cytokines in patients with advanced non-small cell lung cancer: correlation with treatment response and survival. *Cancer Invest* 2005; 23(3): 193-200.
14. Herbst RS, Onn A, Sandler A. Angiogenesis and lung cancer: prognostic and therapeutic implications. *J Clin Oncol* 2005; 23(14): 3243-3256.
15. Gazdar AF, Minna JD. Angiogenesis and the multistage development of lung cancers. *Clin Cancer Res* 2000; 6(5): 1611-1612.
16. Sun S, Schiller JH, Spinola M, Minna JD. New molecularly targeted therapies for lung cancer. *J Clin Invest* 2007; 117(10): 2740-2750.
17. Strieter RM, Belperio JA, Burdick MD, Sharma S, Dubinett SM, Keane MP. CXC chemokines: angiogenesis, immunoangiostasis, and metastases in lung cancer. *Ann NY Acad Sci* 2004; 1028: 351-360.
18. McClelland MR, Carskadon SL, Zhao L, White ES, Beer DG, Orringer MB, Pickens A, Chang AC, Arenberg DA. Diversity of the angiogenic phenotype in non-small cell lung cancer. *Am J Respir Cell Mol Biol* 2007; 36(3): 343-350.
19. Lynn JS, Gallagher KL, Clunn GF, Fexby SE, Patel MK, Hughes AD. PDGF stimulates DNA synthesis in human vascular smooth muscle cells via a novel wortmannin-insensitive phosphatidylinositol 3-kinase. *FEBS Lett.* 2003; 555(3): 591-596.
20. Battegay EJ, Rupp J, Iruela-Arispe L, Sage EH, Pech M. PDGF-BB modulates endothelial proliferation and angiogenesis in vitro via PDGF  $\beta$ -receptors. *J Cell Biol* 1994; 125(4): 917-928.
21. Ostman A. PDGF receptors-mediators of autocrine tumor growth and regulators of tumor vasculature and stroma. *Cytokine Growth Factor Rev* 2004; 15(4): 275-286.
22. Ben-Shoshan J, Schwartz S, Luboshits G, Maysel-Auslender S, Barzelay A, Polak-Charcon S, Tzahor E, Barshack I, Barak A, Levkovitch-Verbin H, Keren G, George J. Constitutive expression of HIF-1 $\alpha$  and HIF-2 $\alpha$  in bone marrow stromal cells differentially promotes their proangiogenic properties. *Stem Cells* 2008; 26(10): 2634-2643.
23. Hofmann HS, Hansen G, Richter G, Taege C, Simm A, Silber RE, Burdach S. Matrix metalloproteinase-12 expression correlates with local recurrence and metastatic disease in non-small cell lung cancer patients. *Clin Cancer Res* 2005; 11(3): 1086-1092.
24. Arenberg DA, Polverini PJ, Kunkel SL, Shanafelt A, Hesselgesser J, Horuk R, Strieter RM. The role of CXC chemokines in the regulation of angiogenesis in non-small cell lung cancer. *J Leukoc Biol* 1997; 62(5): 554-562.
25. Yuan A, Yang PC, Yu CJ, Chen WJ, Lin FY, Kuo SH, Luh KT. Interleukin-8 messenger ribonucleic acid expression correlates with tumor progression, tumor angiogenesis, patient survival, and timing of relapse in non-small-cell lung cancer. *Am J Respir Crit Care Med* 2000; 162(5): 1957-1963.
26. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, Langer CJ,

- DeVore RF 3rd, Gaudreault J, Damico LA, Holmgren E, Kabbinavar F. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer *J Clin Oncol* 2004; 22(11): 2184-2191.
27. Vokes E, Herbst R, Sandler A. Angiogenesis inhibition in the treatment of lung cancer. *Clin Adv Hematol Oncol* 2006; 4(11 Suppl 23):1-10.
28. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006; 355(24): 2542-2550.
29. Wakelee H, Dubey S, Gandara D. Optimal adjuvant therapy for non-small cell lung cancer—how to handle stage I disease. *Oncologist* 2007; 12(3): 331-337.
30. Ardizzoni A, Tiseo M. Combination of target agents: challenges and opportunities. *J Thorac Oncol* 2007; 2(Suppl 5): S4-6.
31. Klapper LN, Kirschbaum MH, Sela M, Yarden Y. Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. *Adv Cancer Res* 2000; 77: 25-79.
32. Kanematsu T, Yano S, Uehara H, Bando Y, Sone S. Phosphorylation, but not overexpression, of epidermal growth factor receptor is associated with poor prognosis of non-small cell lung cancer patients. *Oncol Res* 2003; 13(5): 289-298.
33. Le Tourneau C, Vidal L, Siu LL. Progress and challenges in the identification of biomarkers for EGFR and VEGFR targeting anticancer agents. *Drug Resist Updat* 2008; 11(3): 99-109.
34. Herbst RS, Johnson DH, Mininberg E, Carbone DP, Henderson T, Kim ES, Blumenschein G Jr, Lee JJ, Liu DD, Truong MT, Hong WK, Tran H, Tsao A, Xie D, Ramies DA, Mass R, Seshagiri S, Eberhard DA, Kelley SK, Sandler A. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005; 23(11): 2544-2555.
35. Groen HJ, Smit EF, Dingemans A. A phase II study of erlotinib (E) and bevacizumab (B) in patients (pts) with previously untreated stage IIIB/IV non-small cell lung cancer (NSCLC). *J Clin Oncol* 2007; 25(Suppl 18): Abstract 7625.
36. Ryan AJ, Wedge SR. ZD6474—a novel inhibitor of VEGFR and EGFR tyrosine kinase activity. *Br J Cancer* 2005; 92(Suppl 1): S6-13.
37. Tiseo M, Franciosi V, Ardizzoni A. Multi-target inhibitors in non-small cell lung cancer (NSCLC). *Ann Oncol* 2006; 17(Suppl 2): ii55-ii57.
38. Heymach JV, Johnson BE, Rowbottom JA, Fidias P, Lu C, Prager D, Roubec J, Csada E, Dimery I, Herbst RS. A randomized, placebo-controlled phase II trial of ZD6474 plus docetaxel, in patients with NSCLC. *J Clin Oncol* 2005; 23(Suppl 16): Abstract 3023.
39. Adjei AA, Mandrekar S, Marks RS, Hanson LJ, Aranguren D, Jett JR, Simantov R, B. Schwartz B, Croghan GA. A phase I study of BAY 43-9006 and gefitinib in patients with refractory or recurrent non-small-cell lung cancer (NSCLC). *J Clin Oncol* 2005; 23(Suppl 16): Abstract 3067.
40. Gatzemeier U, Blumenschein G, Fosella F, Simantov R, Elting J, Bigwood D, Cihon F, Reck M. Phase II trial of single-agent sorafenib in patients with advanced non-small cell lung carcinoma. *J Clin Oncol* 2006; 24(Suppl 18): Abstract 7002.
41. Wood JM, Bold G, Buchdunger E, Cozens R, Ferrari S, Frei J, Hofmann F, Mestan J, Mett H, O'Reilly T, Persohn E, Rösel J, Schnell C, Stover D, Theuer A, Towbin H, Wenger F, Woods-Cook K, Menrad A, Siemeister G, Schirmer M, Thierauch KH, Schneider MR, Dreves J, Martiny-Baron G, Totzke F. PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res* 2000; 60(8): 2178-2189.
42. Yano S, Herbst RS, Shinohara H, Knighton B, Bucana CD, Killion JJ, Wood J, Fidler IJ. Treatment for malignant pleural effusion of human lung adenocarcinoma by inhibition of vascular endothelial growth factor receptor tyrosine kinase phosphorylation. *Clin Cancer Res* 2000; 6(3): 957-965.
43. Beebe JS, Jani JP, Knauth E, Goodwin P, Higdon C, Rossi AM, Emerson E, Finkelstein M, Floyd E, Harriman S, Atherton J, Hillerman S, Soderstrom C, Kou K, Gant T, Noe MC, Foster B, Rastinejad F, Marx MA, Schaeffer T, Whalen PM, Roberts WG. Pharmacological characterization of

CP-547,632, a novel vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for cancer therapy. *Cancer Res* 2003; 63(21): 7301-7309.

44. Cohen RB, Simon G, Langer CJ, Schol JR, McHale J, Eisenberg P, Hainsworth JD, Liau KF, Healey D. Phase I trial of oral CP-547,632 (VEGFR2) in combination with paclitaxel (P) and carboplatin (C) in advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2004; 22(Suppl 14): Abstract 3014.

45. Wedge SR, Kendrew J, Hennequin LF, Valentine PJ, Barry ST, Brave SR, Smith NR, James NH, Dukes M, Curwen JO, Chester R, Jackson JA,

Boffey SJ, Kilburn LL, Barnett S, Richmond GH, Wadsworth PF, Walker M, Bigley AL, Taylor ST, Cooper L, Beck S, Jürgensmeier JM, Ogilvie DJ. AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. *Cancer Res* 2005; 65(10): 4389-4400.

46. Latreille J, Batist G, Laberge F, Champagne P, Croteau D, Falardeau P, Levinton C, Hariton C, Evans WK, Dupont E. Phase I/II trial of the safety and efficacy of AE-941 (Neovastat) in the treatment of non-small-cell lung cancer. *Clin Lung Cancer* 2003; 4(4): 231-236.