Insights into Angiogenesis in Non-Small Cell Lung Cancer: Molecular Mechanisms, Polymorphic Genes, and Targeted Therapies

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Abstract: Lung cancer is a highly prevalent disease worldwide. Currently, there are more than 150 million patients with lung cancer in the world, with more than 1 million new cases diagnosed per year. Tumoral angiogenesis is an important hallmark of this disease, but despite being extensively studied, the complete angiogenic mechanisms are not fully elucidated. Recent studies have reported a correlation between pharmacological inhibition of these angiogenic mechanisms and improvement of overall survival in lung cancer patients, mainly for those in advanced stages. The family of vascular endothelial growth factor (VEGF) proteins has critical roles in tumoral angiogenesis. An interaction between VEGF-A and VEGF receptor 2 (VEGFR-2) is the main pathway of activation and maintenance of angiogenesis. In tumors, this process is intimately correlative with progression and metastasis. Some studies suggested that serum levels of VEGF are higher in patients with lung cancer, especially in some types of non-small cell lung cancer (NSCLC). Other studies revealed that genetic polymorphisms of VEGF correlate with susceptibility, prognosis, and therapeutic response of some patients with NSCLC. This paper aims to review the impact of angiogenesis, especially on VEGF pathways, in NSCLC, and highlights the relevance of known and new patents disclosed of anti-angiogenic therapies in these patients.

Keywords: Angiogenesis, antineoplastic drug combination, biological markers, neovascularization, non-small cell lung cancer, pathology, polymorphism, vascular endothelial growth factor.

1. REVIEW CRITERIA

A systematic search was made in four important data bases: PubMed (www.pubmed.com), Scopus (www.scopus.com), Freepatentsonline (www.freepatentsonline.com) and Directory of Open Access Journals (www.doaj.org), with the following terms: "VEGF polymorphisms and lung cancer"; "antiangiogenic chemotherapy"; "angiogenesis mechanisms and lung cancer"; "angiogenesis and VEGF polymorphisms"; "VEGF drugs"; "VEGF polymorphisms and lung cancer prognosis". Studies dated between 1971 and 2011 were selected and evaluated. Case reports were not considered.

2. INTRODUCTION

Lung cancer is a disease with high mortality and morbidity, currently affecting more than 1 million people worldwide [1]. In 2006, approximately 12% of all cancer cases were diagnosed as lung cancer (LC) [2]. Non-small cell lung cancer (NSCLC) is the most common of LC types, accounting for more than 80% of all new cases in North America [1, 2]. It is an extremely lethal malignancy: all histological types have a 5-year survival rate of only ~15% [3, 4]. Histologically, the major subtypes are: adenocarcinoma (the most frequent), squamous cell carcinoma, and large cell carcinoma [5]. Cigarette smoking is related with almost 87% of lung cancer-related deaths [1, 6]. Evaluations of population patterns in smoking prevalence indicate that, in coming years, LC rates will probably decrease in Western countries due to lifestyle changes (mostly due to anti-smoking campaigns) [6]. Nevertheless, other factors account for cancer susceptibility, such as gender, ethnicity, radon exposure, clinical antecedents of lung tuberculosis, and occupational lung disease [2, 7]. Lung carcinogenesis is influenced by the cell's ability to repair DNA lesions induced by external agents, such as tobacco and nitrosamines that initiate the carcinogenic process [4]. These genetic lesions will affect genes that regulate cancer hallmark mechanisms: uncontrolled cellular proliferation; escape from programmed cell death; unlimited replicative potential; tissue invasion and metastization; and genesis of new blood vessels from pre-existent vasculature (angiogenesis) [8]. Therefore, the angiogenic process is a

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paramount feature of NSCLC and the introduction of targeted therapies directed at key molecules of this process, like bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF), have shown great benefits in NSCLC patient treatment, especially when the malignancy is in advanced stages [8-11]. This paper aims to review the impact of angiogenesis, especially of VEGF pathways, in NSCLC, the relevance of polymorphisms in angiogenesis-related genes, and discuss the impact of known and new patents of anti-angiogenic therapies on these patients.

3. ANGIOGENIC MECHANISMS AND VEGF FAMILY

Mechanisms of Tumor Vessel Creation Through VEGF Pathway

The creation of new tumor vessels influences not only the progression but also LC clinical behavior [12]. It is therefore fundamental to understand such mechanisms in order to acquire a better insight of the physiopathology and potential targeting treatments of this disease [12]. Since 1971, when Judah Folkman published his innovative hypothesis on tumoral vascularization [13], angiogenesis has been progressively considered responsible for the evolution of many cancer types [12, 14]. Folkman suggested that tumors above 2 mm would need new blood vessels in order to develop and grow, and predicted the existence of a tumor angiogenic factor responsible for inducing genesis of such vessels [15]. Currently, we know that the VEGF family is the main driver of this process, comprising six members: VEGF-A, -B, -C, -D, and -E and placental growth factor (PlGF) [16]. VEGF-A is the most important member and it is responsible for physiologic and pathologic mechanisms of angiogenesis [12]. Normally, it acts through binding with VEGF receptor 2 (VEGFR-2) generating a cascade of intracellular signaling, leading to activation of transcription factors in the nucleus that will ultimately lead to new vessel formation [10] as shown in Fig. (**1**). VEGF-A is a glycoprotein transcribed by a gene located on chromosome 6 (6p21.3). VEGF-A, or simply VEGF, exists in 6 isoforms categorized by the length of amino acid chains, $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$. *In vivo*, only three isoforms have been related to angiogenesis: $VEGF_{121}$, $VEGF_{145}$, and $VEGF_{165}$. The latter, with 165 amino acids, has been demonstrated to be a predominant isoform secreted by malignant and benign cells [17]. One of the major inducers of VEGF is hypoxia, which activates the hypoxia-inducible factor (HIF) 1α and 2α. HIF-1α function is to regulate body oxygen ho-

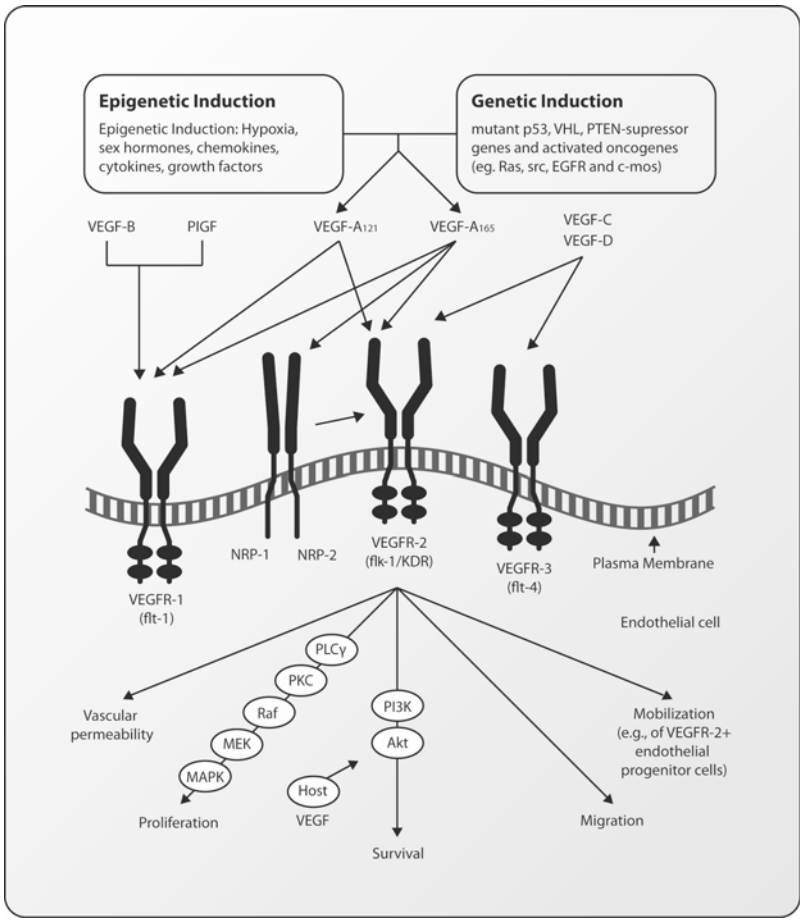

Fig. (1). Angiogenesis pathway. Adapted with permission from reference [12]. The major mediator of angiogenesis is VEGF, which circulates basically in two isoforms, VEGF-A_{121} and VEGF-A_{165}. Many factors, both environmental and genetic, may influence VEGF expression. The binding of VEGF-A to VEGFR-2, followed by the dimerization of the receptor, leads to a cascade of signaling pathways responsible for the many steps of tumor progression: vascular proliferation, cell proliferation, cell survival and migration.

meostasis and it is produced through a *cis*-acting sequence required to increase erythropoietin gene transcription in response to hypoxia [18]. HIF-1α is responsible for activating the transcription of genes encoding important signaling proteins shown in Table **1**, such as angiogenic factors, cellular surface receptors, and extracellular matrix proteins, thus promoting the first steps towards new vessel genesis [12, 18]. Paracrine mechanisms generated through VEGF production in tumor cells may also influence angiogenesis pathways, but those cells cannot adequately respond to the stimulus if they do not have enough cell membrane receptors for that purpose. Paradoxically, endothelial cells recruited during angiogenesis produce great amount of receptors, but produce little or no VEGF ligand. In this context, the amount of VEGF necessary to propel angiogenesis comes from several host cells in human body, like platelets, smooth muscle cells, and stromal cells, which, together, produce the necessary amounts of VEGF for angiogenesis to begin [19-21]. These vessels are significantly different from normal vasculature and do not have the same function. The structural heterogeneity of the new vasculature contributes to diversified behavior in each cancer regarding growth and tumor development [22] Table **2**.

Table 1. Categories of HIF-1α Target Genes and Corresponding Proteins.

Gene Category	Transcribed Protein
Angiogenic growth factors	VEGF
	PDGF
	Prokineticin
	Placental growth factor
	Erythropoietin
	Adrenomedulin
	Endothelin
	Angiopoietin 1 and 2
Cellular surface receptors and enzymes	$\alpha_{1\beta}$-Adrenergic receptor
	Chemokine receptor
	Transferring receptor
	VEGFR-2
Extracellular matrix proteins	Matrix metalloproteinae 2
	Collagen V α1-subunit Fibronectin 1
	Cathepsin D
Cytoskeletal protein	Ketarin 14, 19 and 18
	Vimentin

Abbreviations: VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; VEGFR, VEGF receptor.

VEGF Family

The other VEGFs family members are important for many diverse mechanisms of new vessel creation. The VEGF-B and PlGF act through VEGFR-1 in angiogenesis and in vasculogenesis. Although this receptor has higher affinity to VEGF than VEGFR-2, the interaction between VEGF-B and VEGFR-1 does not have good expression in angiogenesis pathways and its role remains unclear at this time [12]. The VEGF-C [3] and its close homolog, VEGF-D [20], influence lymphangiogenesis, through VEGFR-2 and VEGFR-3, and are also important in tumor metastization [3, 20]. The VEGF-E concurrent to VEGF-A, VEGF-B and PlGF acts by binding to neutrophilin 1, a nonkinase receptor expressed in vascular endothelium, neurons and tumor cells, potentiating VEGF-A´s binding to VEGFR-2, thus promoting the first steps of angiogenesis [16].

Notch-Delta-Like Ligand 4 (Dll4) Pathways

Recently, another pathway has proven to be very important for angiogenesis, the notch-delta-like ligand 4 (Dll4) pathway [23, 24]. The Notch membrane receptors are necessary in order to generate stimuli that signal important intracellular mechanisms [23]. The Dll4/Notch pathway acts by controlling the stimulus of angiogenesis [12, 23, 24]. Some studies suggest that tumors that depend on VEGF are able to induce the expression of Dll4 in endothelial cells and negatively regulate the vascular growth as a mechanism to control the angiogenic rate [23]. These signaling pathways act as brake mechanisms for the production of excessive amounts of blood vessels, promoting therefore the stabilization of an angiogenic process [23]. Therefore, if there is a blockade in the Notch/Dll4 pathway, this negative feedback will be destroyed and in consequence an excessive and unorganized production of blood vessels will take place favoring tumor progression [12]. Even so, these vessels are structurally abnormal and do not possess a good tissue perfusion, leading to tumoral hypoxia [12, 23].

VEGF Prognostic Value

The high expression and activity of VEGF has been observed in 30-40% of NSCLC [10]. Over the last decade, several studies have reported that VEGF influences NSCLC tumor behavior [3, 9, 10, 14, 25-28]. An immunohistochemical study of 102 NSCLC patients reported a higher expression of VEGF-A in adenocarcinomas when compared to squamous cell carcinomas [25]. However, VEGF-A expression was not associated with NSCLC prognosis [25]. Recently, another study [9] enrolling 134 patients diagnosed with NSCLC, evaluated the plasma levels of total VEGF during the treatment with platinum-based chemotherapy. The patient plasma VEGF baseline cut off value considered in this study was 275.2pg/mL, but it could not be related with overall survival (OS). Nevertheless, this study showed that patients in remission had a significant reduction of plasma VEGF levels in subsequent chemotherapy cycles, which suggested the utility of VEGF as a predictive biomarker in this type of patient [9]. Another study with 118 NSCLC patients showed that patients who present serum VEGF levels ≤630pg/mL had a significant OS increase as compared to those with higher levels (p = 0.04) [29]. It is important to

Table 2. Differences between Normal and Tumor Vasculature.

Normal Vasculature	Tumor Vasculature
Organized	Disorganized
Evenly distributed	Unevenly distributed
Uniform	Shaped twisted
Nonpermeable	Leaky
Vascular pressure is greater than interstitial pressure	Vascular pressure is similar to tumor interstitial pressure
Properly matured	Immature
Supporting cells present (e.g., pericytes)	Absence of supporting cells
Appropriate membrane protein expression	Inappropriate membrane protein expression
Independent of cell survival factors	Dependent on cell survival factors (e.g., VEGF)
Homogenous oxygenation of tissue within O_2 diffusion limit	Focal hypoxic and anoxic regions

Abbreviations: VEGF, vascular endothelial growth factor.

note that many factors may influence the results of the previously discussed studies, namely the isoform of VEGF that was quantified, or whether the total VEGF is measured, or even the collecting environment, storage and tubes used for venipuncture. The use of serum levels (and not plasma levels obtained from EDTA tubes) is recommended, processed until 1 hour after venipuncture and stored after centrifugation at -80ºC [10]. Overall, several studies have shown that serum VEGF can be an important and practical biomarker for the follow up of patients with lung cancer, allowing the possibility to individualize chemotherapy and perhaps predict the aggressiveness of several histological types [9, 10, 14, 24]. In 2010, Heymach *et al.* [30] studied 31 cytokines in 33 early stage NSCLC patients treated with pazopanib. They reported the important role of interleukine 4 and soluble VEGFR2 in tumor shrinkage, thus suggesting the central role of angiogenic biomarkers in tumor response [30]. In the E4599 phase II and phase III trials, many other biomarkers were evaluated, and good prognostic value of intracellular adhesion molecule 1 (ICAM-1) was found [31]. In the same study, serum VEGF was found to be a good predictive biomarker for patients treated with bevacizumab, but did not significantly correlate with OS [31]. Also, they suggested ICAM-1 as a strong prognostic marker for overall survival, and the circulating endothelial progenitor cells as a promising marker due to their responsiveness to anti-angiogenic agents.

4. ANTI-ANGIOGENIC TARGETED THERAPIES AND NEW PATENTS

For a long time, conventional chemotherapy, based mainly on platinum, has not shown satisfactory results in the treatment of lung cancer, especially in advanced stages, with an average OS under 10 months after diagnosis [1, 28]. Since then, targeted therapies, especially those associated with anti-angiogenic mechanisms such as tyrosine kinase inhibitors (TKI) and monoclonal antibodies, have shown some improvement in OS for several cancer types, including advanced stages of NSCLC Table **3** [11, 22, 28, 32-36]. TKIs are the compounds that selectively target the intracellular tyrosine kinase domain of receptors and act by competing with the ATP-binding site, thereby inhibiting the tyrosine phosphorylation and blocking the signaling pathways. The monoclonal antibodies usually bind to ligands and decrease the angiogenic activity by blocking its interaction with membrane receptor cell signaling.

VEGFR, EGFR and RET Multi-Target Tyrosine Kinase Inhibitors

The increased knowledge of the molecular mechanisms underlying cancer development and progression leads to the development of specific targeted therapies against key cellular players, with outstanding results in some tumor types [37-39]. Some drugs, mainly of the TKIs category, targeting the three VEGF receptors, VEGFR-1, VEGFR-2 and VEGFR-3, have been evaluated in phase II and phase III studies for their anti-angiogenic and clinical action. One example is the sorafenib, an oral anti-kinase inhibitor with anti-angiogenic and anti-proliferative activity; but it has not demonstrated clinical benefit in addition to standard chemotherapy in phase III studies [40, 41]. Another example is vandetanib, a multitarget TKI, which targets VEGFR2, epidermal growth factor receptor (EGFR) and RET, and has an improvement in progression-free survival (PFS) in phase II studies of NSCLC patients, as monotherapy or in combination therapy [36], and also when compared with gefitinib [42]. One study presented at the American Society of Clinical Oncology (ASCO) congress in 2010 confirmed vandetanib superiority PFS versus placebo in NSCLC patients, but did not demonstrate an OS benefit [43]. Currently the OS study results are not very consistent regarding this molecule, despite its benefit on PFS [44].

VEGFR, EGFR, PDGF, FGF and PlGF receptors tyrosine kinase inhibitors

Many other TKIs target multiple receptors, as is the case with sunitinib, montesanib, and the axitinib, which block the

activity of VEGFR-1, VEGFR-2, VEGFR-3, EGFR, and PDGF receptors. Their LC anti-tumor properties are currently being studied in phase I and phase II studies [34, 35, 45-50], but until this moment no significant data has been disclosed [51]. Nevertheless, some studies raised high expectations, like axitinib, which has proved to be a very powerful drug in a phase II study with an OS of 14.8 months [52]. The inhibition of endothelial proliferation *via* endostatin pathway was also recently showed to be promising. A novel invention in this field has been demonstrated to inhibit angiogenesis in mice lung cancer cells. This patent is a recombinant endostatin inhibitor (US7867975) with molecular weight between 18 to 20KDa and is capable of inhibiting endothelial cell proliferation *in vitro* [53]. Recently, patents such as BIBF 1120, have emerged in phase I and II trials as potent TKIs that simultaneously inhibit VEGFR 1-3, fibroblast growth factor receptors 1-3, and placental-derived growth factor α and β [54]. In fact, the results were not enough to conclude on OS and PFS, but the drug was well tolerated when used in a continuous treatment regimen. Another invention (US7875603) showed some benefit by inhibiting VEGFR in lung cancer treatment, as such as breast, colorectal, prostate and ovarian [39]. This invention decreases angiogenic activity by blocking the VEGF receptors.

EGFR Tyrosine Kinase Inhibitors

At the end of the of 1990´s, EGFR was shown to play an important role in tumor biology and behavior [55]. EGFR overexpression is present in approximately 43 to 83% of NSCLC, being more common in squamous cell carcinoma (70%), followed by adenocarcinoma (50%), and to a lesser extent in large cell carcinoma. This phenomenon is very rare in SCLC [55-58]. Its stimulation by external factors activates the intracellular signaling and cascades, through the downstream signaling pathway phosphoinositide 3-kinase (PIK3) regulation, Akt and mammalian target of rapamycin (mTOR), or through Ras-Raf-Mek pathway (MAPK), induces the expression of VEGF, and influences cellular proliferation, mobilization and angiogenesis [4, 59]. In tumor cells, it was found that the activation of cell proliferation mediated by EGFR would no longer need the external stimulus, but act independently and autonomously [55]. In the particular case of NSCLC, it was shown that the EGFR overexpression, as well as specific somatic mutations occurred in their intracellular domain with tyrosine kinase activity (between exons 18 and 21), were the factors of poor prognosis, being significantly related with stage, survival and chemotherapy response [55, 60-63]. This data led to the development and study of various substances, among which are monoclonal antibodies (e.g. cetuximab (Erbitux®), already approved for the treatment of colorectal carcinoma, but still under study in NSCLC) and TKIs, gefitinib (Iressa®) and erlotinib (Tarceva®) [55, 60, 64-67]. After, some papers [68-70] started to evaluate which was the best method to assess EGFR role in the response, PFS and OS of patients with NSCLC treated with gefitinib: EGFR mutation, copy number or quantitative of EGFR assessed by Fluorescence *in situ* hybridization (FISH) or real-time polymerase chain reaction (PCR) or EGFR detected by immunohistochemistry. The EGFR mutation (deletion in exon 19 or L858R in exon 21) has shown high sensitive (92%) and moderately specific (76%) for predicting response to EGFR TKI chemotherapy. Nowadays, this test is the preferred method for selecting patients with advanced, chemorefractory lung adenocarcinoma who are most likely to respond to erlotinib or gefitinib treatment [62]. The other methods, such as copy number by FISH or immunohistochemical positivity, may have a role for PFS predicting within an *EGFR*-mutated subset of patients, but they are not independently informative tests [62, 63].

Two TKI´s of EGFR, gefitinib and erlotinib, are currently used for the treatment of adenocarcinoma and NSCLC. Erlotinib was approved for refractory locally advanced/metastatic NSCLC [71]; and, since May 2010, gefitinib was approved by European Medicine Agency (EMEA) for use in first line treatment of metastatic advanced NSCLC, EGFR mutation positive based on its PFS and OS benefits when compared with the carboplatin-paclitaxel-treated group [72, 73] and the cisplatin-docetaxel-treated group [74]. Erlotinib in April 2010 was also approved for maintenance treatment in advanced NSCLC patients with stable tumor disease based on SATURN study [63, 72, 75]. Therefore, these therapies cannot only have an anti-neoplastic, but also an anti-angiogenic activity. Currently, the therapeutically resistance mechanisms for EGFR TKI are the subjects of discussion. There are several documented resistance point mutations to gefitinib and erlotinib, such as T790M, L747S and D761Y [56, 63]. The T790M is the most common secondary resistance mutation reported, accounting for about 50% of tumor relapse from prior TKI treatment [76].

The Role MET Inhibitors Drugs

In the last three decades, MET proto-oncogene was discovered that encodes the high affinity cell surface receptor (c-MET) for hepatocyte growth factor (HGF) and also controls carcinogenesis steps, including cell growth, proliferation, invasion and protection from apoptosis [77]. Recently, MET inhibitor patents are emerging as a new class of target therapies that promise good results in NSCLC patients [78]. The dual EGFR-MET inhibition [79] or VEGF/KDR receptor-HGF/c-MET dual inhibitor (US7790729) [78] are good strategies to improve anti-angiogenic approach by circumventing the ability of tumor cells overcome VEGF inhibition alone or overcoming MET-mediated resistance to EGFR inhibitors [78, 79]. In 2010, at the annual ASCO meeting, Wakelee *et al*. reported a new patent, XL184, that is an oral potent inhibitor of MET, VEGFR2 and RET in association with erlotinib in NSCLC patients. Its use is encouraged mainly in patients with erlotinib resistant and EGFRT790M and MET amplification [80]. Another recent patent, ARQ197, is a selective non ATP competitive inhibitor of c-MET that, when combined with erlotinib in treatment of second/third line EGFR inhibition, a *naïve* NSCLC increase of PFS was shown, mainly among patients with non-squamous histology, K-RAS mutations, and EGFR wild-type status [79]. In 2011, a new patent (US787211) provided a good weapon to suppress tumor growth toward c-MET pathways. This invention acts using RNA interference (RNAi) technology and adenovirus carrying siRNA (Ad met siRNA) target sequences that substantially reduces MET expression in human tumors cells. Ad met siRNA kills cancer cells by inducing apoptosis. *In vivo*, intra-tumoral infection with c-met

siRNA adenovirus vectors produces significant reduction in tumor growth [81]. Thus, this might be another choice in future to use associated with EGFR TKIs chemotherapies.

Anti-Angiogenic Monoclonal Antibody: Bevacizumab

Currently, drugs like bevacizumab act basically by reducing the interactions between VEGF and its receptors, diminishing the intracellular signaling that triggers the growth of new vessels, therefore improving the OS and also the PFS [14, 36]. Recently, some clinical trials have been developed aiming at demonstrating the effectiveness of new specific anti-angiogenic drugs for the improvement of the NSCLC approach as reported in Table **3** [11, 32-36, 41, 42, 46-50, 52, 71, 82-87]. In 2006, the Eastern Cooperative Oncology Group (ECOG) in a phase III trial (ECOG 4599) involving 878 patients diagnosed with advanced stage NSCLC showed [33] that adding bevacizumab, a monoclonal anti-body against VEGF, to the conventional chemotherapy protocols based on platinum, significantly increases the OS of 10.3 months in a group treated only with chemotherapy for 12.3 months in a group that underwent chemotherapy and bevacizumab (hazard ratio = 0.79, $p = 0.003$). There was also an increase in PFS from 4.5 months to 6.2 months in the group with bevacizumab (hazard ratio = 0.66, $p < 0.001$), and the therapeutic response also increased [33]. This monoclonal antibody was later approved by the FDA in 2006 for advanced NSCLC treatment [1]. Nevertheless, the same study [33] demonstrated that this treatment must be carefully considered in order to avoid the risk of thromboembolic events and even fatal hemorrhagic events more frequently associated to central tumors with squamous histology [11, 33]. The AVAiL phase III trial showed an arguable benefit of adding bevacizumab to conventional therapy in advanced NSCLC patients when the PFS improvement was not clinically significant, but the design could not compare with the best dosage (7.5mg/kg or 15mg/kg each 3 weeks) [11]. Recently, the final analysis of AVAiL resulted in still minor clinical PFS improvement when bevacizumab is combined with gemcitabine and carboplatinum, and without improvement in OS [88]. The ATLAS and PASSPORT studies demonstrated that it could be safely used in patients with brain metastasis [89] since they are first treated with brain radiotherapy and without progression evidence by imaging studies after four weeks since beginning treatment [90, 91]. Currently, bevacizumab is the only anti-angiogenic target therapy approved by FDA for NSCLC patients, especially in those aged less than 70 years old, stage IV and performance status zero to one [1, 90].

New Patents in Anti-Angiogenic Monoclonal Antibody Field

In the sequence, new patents such as VC300 (US7740844) [92], US7691977 [93], AMG102 [94], MetMab [95], $VEGF_{165}b$ (US7820178) [96] and US7875704 [97] emerged as promising inventions in the angiogenesis of monoclonal antibodies field as also shown in Table **4** and Fig. (**2**). VC300 is a recent monoclonal antibody that binds to amino acid residues Asn_{100} and Lys_{107} in human VEGF neutralizing its activity. This invention can be prepared by recombinant technology and it can be incorporated into pharmaceutical compositions suitable for an administration to a subject [92]. Another interesting new US patent (US7691977) of the last year was novel antibodies polypeptide sequence capable of binding the rodent and human VEGF with Kd values within 10 fold of each value wherein they inhibit the binding of VEGF to VEGFR. The antibody comprises a complementarity-determining region H1 (CDR-H1) comprising the amino acid sequence ASWIH, a CDR-H2 comprising the amino acid sequence AIYPYSGYTNY-ADSVKG, a CDR-H3 comprising the amino acid sequence WGHSTSPWAMDY, a CDR-L1 comprising the amino acid sequence RASQDVSTAVA, a CDR-L2 comprising the amino acid sequence SASFLYS, and a CDR-L3 comprising the amino acid sequence QQSYTTPPT. Further evaluations are warranted in this field in order to assess its feasibility in solid tumor, especially NSCLC [93]. In a phase Ib study, the AMG102, a monoclonal antibody against HGF, showed a possible benefit in solid tumor dimension reduction when combined with bevacizumab, but further evaluation with this patent is warranted [94]. Also, the monoclonal antibody acting directly against MET showed good clinical results. MetMab (Genentech) is a human recombinant antagonist of HGF-MET signaling pathway, demonstrated to be well tolerated in association with bevacizumab and effective in a phase Ib trial [95] and also with minor PFS improvement [98, 99].

Furthermore, as result of differentially splicing into a previously undescribed exon, exon 9, a new VEGF isoform in kidney cells was discovered with anti-angiogenic activity (called $VEGF_{165}b$, with 165 polypeptides sequence). This novel isoform may have an importance in future cancer treatment protocols [96]. The PlGF is another molecule that modulates angiogenesis. In 2011, a new patent provided a monoclonal antibody that inhibit PlGF binding with its receptor that can be used to significantly reduce the tumorigenicity steps with a decrease of side effects caused by an inhibition of physiological angiogenesis [97]. Thus, as molecular mechanisms of tumor biology are elucidated, new therapeutic possibilities are developed in order to improve the treatments for the NSCLC patients, as monotherapy or in association with the existent chemotherapy protocols [100].

5. GENETIC POLYMORPHISMS RELATED TO THE ANGIOGENESIS PATHWAYS

The human *VEGF* gene has more than 15 single nucleotide polymorphisms (SNPs) identified to date [101-103]. Due to the central roles of VEGF in angiogenesis, some of these gene polymorphisms have been recently studied in the context of different tumor types, including breast cancer [104], malignant melanoma [105], prostate cancer [106], and lung cancer [101, 103, 107-112]. Importantly, particular polymorphic variants have been shown to be functional [113-115] (i.e. they correlate with VEGF production), and have already been associated with tumor risk, angiogenesis, vascular density, and prognosis [101, 103-112]. Table **5** summarizes the major conclusions of studies addressing the relevance of individual VEGF polymorphisms in the context of lung cancer, particularly as putative risk factors for developing lung cancer and mediators of VEGF levels, tumor behavior, and patient prognosis. Table **6** summarizes the studies addressing

Table 3. Summary of Clinical Trials Evaluating Anti-Angiogenic Therapy in NSCLC Patients.

Reference	Phase	Treatment	n	OS(month)	RR(%)
Bevacizumab					
33	III	CBDCA + Pac*	427	10.3	35
		CBDCA + Pac* + bevacizumab 15mg/kg	440	12.3	15
11	III	CDDP + gem ** + placebo	347	NR	20.1
		CDDP + gem **+ bevacizumab 7.5mg/kg	345		34.1
		CDDP + gem **+ bevacizumab 15mg/kg	351		30.4
Sorafenib					
46	II	Sorafenib 400mg	15	NR	13
82	II	Sorafenib 400mg	54	7.0	29
48	II	Sorafenib 400mg	51	11.9	2
		Placebo	32	9.0	3
41	III	CBDCA + Pac*	462	10.7	24
		CBDCA + Pac* + Sorafenib 400mg	464	10.6	30
Vandetanib					
83	II	Vandetanib 100mg/200mg/300mg	53	NR	13
42	II	Vandetanib 300mg daily	83	6.1	8
		Gefitinib 250mg daily	85	7.4	1
84	II	Docetaxel	41	13.4	12
		Docetaxel + vandetanib 100mg	44	13.1	26
		Docetaxel + vandetanib 300mg	42	7.9	18
85	II	CBDCA+Pac*	52	NR	25
		CBDCA+Pac*+ vandetanib 300mg	56		32
		Vandetanib	73		7
Sunitinib					
35	II	Sunitinib 50 mg daily#	63	5.4	11.1
50	II	Sunitinib 37.5mg daily##	47	8.6	2
49	I	CDDP+gem** + sunitinib (37.5 or 50mg)	13	NR	23
Motesanib					
34	Ib	CBDCA + Pac*+motesanib 50, 125, 75 2id		NR	17
		Pan*** + motesanib 50, 125, 75 2id		NR	0
		CBDCA + Pac*+ Pan***+ motesanib 125 d		NR	17
Axitinib					
52	II	Axitinib 5 mg BID oral	32	14.8	32

Abbreviations: n, number of patients; OS = overall survival; RR = response rate; NR = not reported; *CBDCA + Pac: Carboplatin + paclitaxel; **CDDP + gem: Cisplantin + gemcitabin; *** Panitumumab each every 21 days cycle; #sunitinib administered daily for 4 weeks of 6 weeks cycle; ##Sunitinib administered daily continuously for 4 weeks cycles.

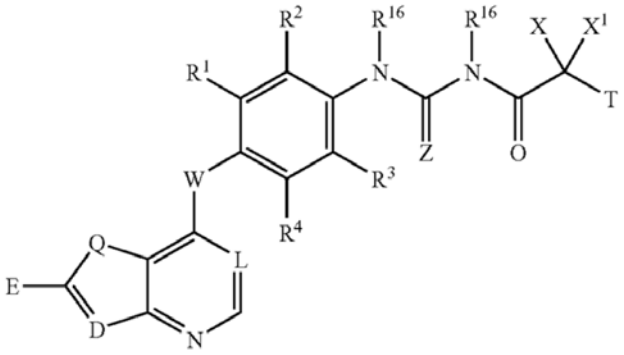

Fig. (2). Formula of the US7790729 patent. It is a compound that inhibits VEGFR and c-MET signaling.

Table 4. Summary of New Relevant Patents on Anti-Angiogenic Drugs.

#	Invention	Claim	Publication date	Patent reference (company)	Reference
1	Tirosine Kinase inhibitor	Compounds and methods for inhibition of VEGFR and HGF receptor signaling. The invention also provides compositions and methods for treating cell proliferative diseases and conditions	Sep 07, 2010	US 7790729 (Methylgene, Inc.)	[78]
2	Tirosine kinase inhibitor	Inhibit VEGFR and treat cancer with VEGF over expression	Jan 25, 2011	US 7875603 (Nova Southeastern University, FL, USA)	[39]
3	Monoclonal antibody	Anti-VEGF that inhibit with high affinity the binding of VEGF to VEGFR	Apr 6, 2010	US 7691977 (Genentech, Inc.)	[93]
4	Monoclonal antibody	Reduce angiogenesis by inhibiting PlGF pathways	Jan 25, 2011	US 7875704 (Natarajan, Meera)	[97]
5	Monoclonal antibody (VC300)	VC300 specifically binds with high affinity to amino acid residues Asn 100 to Lys 107 in human VEGF	Jun 22, 2010	US 7740844 (Taiwan Liposome Co. Ltd)	[92]
6	Polypeptide sequence	Potent specific inhibitor of endothelial proliferation and angiogenesis	Jan 11, 2011	US 7867975 (The Children's Medical Center Corporation, Boston, USA)	[53]
7	Polypeptide sequence ($VEGF_{165}b$)	An isolated VEGF polypeptide having anti-angiogenic activity	Oct 26, 2010	US 7820178 (University of Bristol, UK)	[96]
8	Adenovirus vector (Human si-hMet-$Ad5^{16}$)	Produce significant reduction in tumor growth by inducing apoptosis	Jan 18, 2011	US 7872117 (Van Andel Research Institute, USA)	[81]

Abbreviations: VEGF , vascular endothelial growth factor; VEGFR, VEGF receptor; HGF, hepatocyte growth factor; CDR, complementarity determining region; USA, United States of America; UK, United Kingdom; PlGF, placental growth factor .

the contribution of *VEGF* haplotypes (combinations of specific allelic variants in different polymorphism) in LC. This body of data emphasizes that some *VEGF* SNP variants may be LC biomarkers; however, some conflicting results have been reported in different studies, as some studies report statistically significant associations between *VEGF* polymorphic variants and tumor risk/behavior, while others fail to show this- see Table **5**. This may be due to differences in study design and analysis, small sample sizes, different ethnic backgrounds across studies, and experimental and procedural variations to assess, for example, *VEGF* gene variants, VEGF levels, and patient outcome. It is also clear that, despite the unquestionable relevance of VEGF in tumors, particularly highly angiogenic neoplasms like LC, and the use of anti-angiogenic therapies in some of these tumors, there is still a very limited number of studies addressing the relevance of *VEGF* polymorphisms in LC. Future well-designed, large, prospective studies are warranted to clearly establish

Table 5. Summary of *VEGF* Polymorphisms in Lung Cancer.

Polymorphism	Tumor Type	No. Cases / Controls	Ethnicity	Effects on Tumor Risk*	Functional and Clinical Effects (VEGF levels, Tumor Behavior, Survival)*	Reference
rs699947 (-2578 C/A)	NSCLC	36 / 0	N/A	N.Det.	CA genotype increased tumor VEGF expression and vascular density	[107]
	NSCLC	126 / 0	N/A	N.Det.	AA genotype associated with worse survival	[112]
	NSCLC	566 / 0	Asian	N.Det.	N.S.	[110]
rs1005230 (-2489 C/T)	NSCLC	566 / 0	Asian	N.Det.	N.S.	[110]
rs1570360 (-1154 G/A)	NSCLC	36 / 0	N/A	N.Det.	AA and GA genotypes increased tumor VEGF expression	[107]
	NSCLC	126 / 0	N/A	N.Det.	AA and GA genotypes associated with worse survival	[112]
rs833061 (-460 C/T)	Lung cancer	432 / 432	Asian	N.S.	N.Det.	[111]
	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
	NSCLC	462 / 0	N/A	N.Det.	N.S.	[108]
	NSCLC	126 / 0	N/A	N.Det.	CC genotype associated with worse survival	[112]
rs25648 (-7 C/T)	NSCLC	568 / 0	Asian	N.Det.	N.S.	[110]
rs2010963 (405 G/C)	NSCLC	36 / 0	N/A	N.Det.	GC genotype associated with increased tumor VEGF expression and vascular density	[107]
	Lung cancer	432 / 432	Asian	GG genotype reduces risk of SCC	N.Det.	[110]
	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
	NSCLC	462 / 0	N/A	N.Det.	C allele associated with improved survival	[108]
	NSCLC	462 / 0	N/A	N.Det.	GC and combined GC+CC genotypes associated with improved survival	[108]
	Lung cancer	88 / 0	N/A	N. Det.	N.S.	[109]
	NSCLC	126 / 0	N/A	N.Det.	N.S.	[112]
	NSCLC	566 / 0	Asian	N.Det.	N.S.	[110]
rs3025039 (936 C/T)	Lung cancer	432 / 432	Asian	CT or CT+TT genotypes reduce risk of SCC	N.Det.	[110]
	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
	NSCLC	462 / 0	N/A	N.Det.	N.S.	[108]
	Lung cancer	88 / 0	N/A	N. Det.	T allele increased *VEGF* gene expression and VEGF serum levels	[109]
	NSCLC	126 / 0	N/A	N.Det.	N.S.	[112]
	NSCLC	568 / 0	Asian	N.Det.	N.S.	[110]
rs10434 (1612 G/A)	NSCLC	560 / 0	Asian	N.Det.	N.S.	[110]

(Table 5) Contd…..

Polymorphism	Tumor Type	No. Cases / Controls	Ethnicity	Effects on Tumor Risk*	Functional and Clinical Effects (VEGF levels, Tumor Behavior, Survival)*	Reference
rs833069 (intron 2 G/A)	NSCLC	560 / 0	Asian	N.Det.	N.S.	[110]
rs833070 (intron 2 G/A)	NSCLC	560 / 0	Asian	N.Det.	N.S.	[110]
rs3024994 (intron 2 C/T)	NSCLC	568 / 0	Asian	N.Det.	N.S.	[110]
rs3025010 (intron 5 C/T)	NSCLC	567 / 0	Asian	N.Det.	N.S.	[110]
rs3025035 (intron 7 C/T)	NSCLC	568 / 0	Asian	N.Det.	N.S.	[110]
rs3025040 (3'-UTR T/C)	NSCLC	568 / 0	Asian	N.Det.	N.S.	[110]
rs3025053 (3'-UTR G/A)	NSCLC	568 / 0	Asian	N.Det.	N.S.	[110]

***** - Significant differences were considered when P<0.05.

Abbreviations: NSCLC, non-small cell lung cancer; SCC, small cell carcinoma; N/A, not available; N.Det., not determined; N.S., not significant.

Table 6. Summary of *VEGF* Haplotypes in Lung Cancer.

Haplotypes	Comparisons	Tumor Type	No. Cases/ Controls	Ethnicity	Effects on Tumor Risk*	Functional and Clinical Effects (VEGF levels, Tumor Behavior, Survival)*	Reference
-460,405,936	TCT vs all other haplotypes	Lung cancer	432 / 432	Asian	TCT reduces risk of lung cancer	N.Det.	[111]
	TGT vs all other haplotypes	Lung cancer	432 / 432	Asian	TGT increases risk of lung cancer	N.Det.	[111]
	Each haplotype vs all other haplotypes	AC	432 / 432	Asian	N.S.	N.Det.	[111]
	CGT vs all other haplotypes	SCC	432 / 432	Asian	CGT reduces risk of SCC	N.Det.	[111]
	TCC vs all other haplotypes	SCC	432 / 432	Asian	TCC increases risk of SCC	N.Det.	[111]
	TCC vs CGC (reference)	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
	TGC vs CGC (reference)	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
	CGT vs CGC (reference)	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
	TCT vs CGC (reference)	NSCLC	1900 / 1458	Caucasian	N.S.	N.Det.	[103]
405,936	All variant alleles vs GC (reference)	NSCLC	462 / 0	N/A	N.Det.	higher number of variant alleles increased overall survival	[108]

***** - Significant differences were considered when P < 0.05.

Abbreviations: NSCLC, non-small cell lung cancer; AC, adenocarcinoma; SCC, small cell carcinoma; N/A, not available; N.Det., not determined; N.S., not significant.

the biological and clinical implications of these polymorphisms in different types of LC, particularly how they affect tumor risk, progression, and response to therapies, especially those targeting angiogenesis-related molecules.

6. CURRENT & FUTURE DEVELOPMENTS

Currently, many efforts are underway in order to develop new therapeutic strategies against LC based on its molecular

and clinical features. The treatment of NSCLC patients with anti-angiogenic agents, like bevacizumab, together with other agents [116], like gefitinib and erlotinib for EGFR-positive tumors, has demonstrated how these fundamental new targeted therapies are in order to improve survival and the quality of life of these patients.

Combined chemotherapy composed by molecular therapies as well as conventional chemotherapy have shown better results in patients with NSCLC because they concurrently and synergistically act in different molecular pathways [32, 116]. Many clinical trials [11, 32-35, 41, 42, 46-50, 52, 71, 82-85, 87] have reported the beneficial impact of these combined therapies as demonstrated in Table **3**, commonly assessed at the levels of OS and PFS [32, 117]. The VEGF, through either its serum levels [9] or its tumoral levels, has shown evidence of being a putative prognostic marker in NSCLC [28]. Several VEGF genetic polymorphisms related directly or indirectly to angiogenesis like the +936 C/T [109], the +405 G/C [108], and the - 460 T/C [111] polymorphism extend a certain impact to the prognosis and/or the risk level for NSCLC patients. However, currently there are still no satisfactory biomarkers capable of correlating to risk, to therapeutic response, or to prognosis of NSCLC patients, which is currently a challenge in the field of oncology. It is extremely important to better understand anti-cancer pharmacogenomics in order to improve clinical practice. For this reason, more studies are necessary for a better understanding of the illness mechanisms and for the improvement of future therapeutic protocols, making it possible to evaluate, with reasonable accuracy and low expense, what type of treatment is best for each patient, as well as the potential NSCLC risk development.

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8. CONFLICT OF INTEREST

The authors declare that they not have any competing interest.

ABBREVIATIONS USED

VEGF	=	Vascular endothelial growth factor
LC	=	Lung cancer
NSCLC	=	Non small cell lung cancer
PlGF	=	Placental growth factor
VEGFR	=	VEGF receptor
HIF	=	Hypoxia inducible factor
Dll4	=	Delta-like ligand 4
OS	=	Overall survival
PFS	=	Progression free survival
EDTA	=	Ethylenediamine tetraacetic acid
ICAM-1	=	Intracellular cell adhesion molecule 1
TKI	=	Tyrosine kinase inhibitor
EGFR	=	Epigermal growth factor receptor
FISH	=	Fluorescence *in situ* hybridization
ASCO	=	American society of clinical oncology
PIK3	=	Phosphoinositide 3-kinase
mTOR	=	Mammalian target of rapamycin
EMEA	=	European Medicine Agency
US	=	United States
FDA	=	Food and drugs administration
HGF	=	Hepatocyte growth factor
KDR receptor	=	Kinase insert domain receptor
CDR	=	Complementarity-determining region
V_h	=	Variable region
SNP	=	Single nucleotide polymorphism

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