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Angiogenesis and Lung Cancer

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1. Introduction

Angiogenesis is the formation of new blood vessels from the existing vasculature, and neo-vascularization is a prerequisite for the growth of solid tumors beyond 1-2 mm in diameter [1]. Because of this, during tumorigenesis, tumor growth reaches a growth-limiting step where oxygen and nutrient levels are insufficient to continue proliferation.

Tumors acquire blood vessels by co-option of neighboring vessels from sprouting or intus-suscepted microvascular growth and by vasculogenesis from endothelial precursor cells [2]. In most solid tumors the newly formed vessels are plagued by structural and functional ab-normalities due to the sustained and excessive exposure to angiogenic factors produced by the tumor [3]. As a result of this, the new tumor-associated vasculature is abnormal and in-efficient, but it is essential for tumor growth and metastasis. Despite being abnormal, these new vessels allow tumor growth at early stages of carcinogenesis and progression from in situ lesions to locally invasive, and eventually to metastatic tumors.

As a result, tumors tend to become hypoxic. The normal cellular response to hypoxia is to produce growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor alpha (TGF-α), and platelet derived growth factor (PDGF), by neoplastic, stro-mal cells or inflammatory cells [4], and may trigger an angiogenic switch to allow the tumor to induce the formation of microvessels from the surrounding host vasculature [5], that stimulate neoangiogenesis [6].

VEGF is the most potent and specific growth factor for endothelial cells, and is associat-ed with tumor vessel density, cancer metastasis, and prognosis [7-10]: high levels of cir-culating VEGF have been reported in patients with non-small cell lung cancer (NSCLC)

[7,10-18]. VEGF is continuously expressed throughout the development of many tumor types, and is the only angiogenic factor known to be present throughout the entire tumor life cycle [19]. The clinical significance of circulating levels of VEGF in patients with NSCLC is controversial.

Since tumor growth and metastasis are angiogenesis-dependent, relying upon the generation of new blood vessels to sustain proliferation, survival and spread of the malignant cells, therapeutic strategies aimed at inhibiting angiogenesis area theoretically attractive. Targeting and damaging blood vessels can potentially kill thousands of tumor cells. The antiangiogenesis and vascular targeting strategies, therefore, may no result in whole tumor cell kill, but may maintain stable disease: this has given rise to the concept *cytostatic paradigm* [20].

The investigation and development of different anti-angiogenesis and vascular targeting strategies are of interest with respect to lung cancer.

2. Hypoxia and lung cancer, HIF-1α, carbonic anhydrase IX and glucose transporter glut

Hypoxia is one of the most important challenges for tumor growth and survival. The angiogenesis is a fundamental to avoid tumor necrosis (TN); every cell in a tissue is forced to be within 100µm capillary blood vessel [5].

Hypoxia inducible factor-1 (HIF-1) is a regulator of VEGF under hypoxia conditions [21].

HIF-1 is a heterodimer consisting of 2 subunits, HIF-1α and HIF-1β (otherwise known as the aryl hydrocarbon receptor nuclear translocator), which is stabilized by hypoxia. The expression of these subunits is different; HIF-1β is constitutively expressed, unlike HIF-1α, which is rapidly degraded under normoxic conditions [22]. In the presence of oxygen, HIF-1α is hydroxylated on conserved prolyl residues within the oxygen-dependent degradation domain by prolylhydroxylases and binds to von Hippel-Lindau protein (pVHL), which in turn targets it for degradation through the ubiquitin-proteasome pathway [23-26]. Hypoxia inhibits hydroxylation of prolyl residues 402 and 564 in the oxygen-dependent degradation domain that avoid binding of the pVHL. Similar hypoxia-dependent inhibition of hydroxylation of asparagines residues within the C-terminal activation domain increases HIF-1α transcriptional activity. Oxygen-dependent degradation of HIF-1α is inhibited by *src* and *ras* oncogenes [22-25].

The HIF-1 complex recognizes hypoxia response elements on the promoter of several genes, including VEGF, PDGF, and TGF-α [26].

Growth factors, cytokines and oncogenes, which stimulate p42/p44 mitogen-associated protein kinase (MAPK) and/or phosphoinositidyl-3 kinase (PI-3K) pathways, may enhance HIF-1 activity. HIF-1 binds to a conserved sequence (5-CGTG-3) known as the hypoxic response element in the promoter region of its target genes. These target genes are involved in processes that promote cellular survival, angiogenesis, blood vessel vasodilatation, erythro-

poiesis, anaerobic metabolism, buffering of the intracellular compartment and induction of growth factors. HIF-1 activity *in vivo* promotes tumor growth in the most of the studies and resistance to several chemotherapy agents, as platinum compounds [22]. Carbonic anhydrase (CA) IX and glucose transporter-1 are other transcriptional targets of HIF-1 and, along with HIF-1, have been identified as novel markers of hypoxia in different tumor types [27-31]. Up-regulation of CA IX in vivo in a perinecrotic pattern suggests this may be an important pathway in hypoxia, possibly regulating pH to allow survival of cells under hypoxic conditions [28].

Other study showed that HIF-1 is commonly expressed in NSCLC and is involved in the pathogenesis of NSCLC. HIF-1 expression seems associated with a poor prognosis and this was found to be as an independent factor. A similar observation has been made for the prognostic impact of the extent of TN, another marker for hypoxia in NSCLC, where although extensive TN predicts outcome in earlier stages of the disease, no such effect is seen in locally advanced disease. Thus, a number of other studies have included patients with locally advanced disease in different cancer types and reported an association between HIF-1 expression and prognosis [22]. Although some other studies have reported different results [32].

The associations between HIF-1, CA IX, TN and squamous NSCLC are coherent with the known pathways that regulate and are regulated by HIF-1. CA IX is regulated by HIF-1. TN and CA IX have been associated with a poor prognosis in NSCLC [22,31].

By other hand, glucose transporter GLUT-1 is a potential intrinsic marker of hypoxia in cancer [29]. VEGF and GLUT-1 are similarly regulated in response to hypoxia [33]. They may functionally help each other to endure hypoxia. Therefore, an upregulated expression of GLUT-1 allows the cell to better use an inadequate source of glucose, while an upregulated expression of VEGF will improve the reserve of glucose and oxygen through the recruitment of additional blood vessels [33].

3. Pathophysiology and clinical implications of VEGF

The role of angiogenesis in cancer biology was defended by Folkman in 1971, who first postulated that solid tumors remained latent at a specific size due to the absence of neovascularization, that was conditioned by the diffusion of oxygen and nutrients [34].

Subsequent studies have shown that angiogenesis is involved in tumor development from the initial stages to the most advanced stages of the disease [35]. Angiogenesis plays therefore, an important role in tumor growth and metastasis development.

Since then, one of the most important questions has been the identification of proangiogenic factors and the mechanisms in order to block its action. One of the most studied has been the VEGF.

VEGF is a potent mediator of angiogenesis. It is a growth factor that stimulates the proliferation and migration, promotes survival, inhibits apoptosis and regulates the permeability of

vascular endothelial cells. It belongs to the growth factors family, which includes four homologues VEGF-A (commonly referred to as VEGF)-B, -C, -D, -E and placental growth factor (PIGF). The biological activity of VEGF is mediated by binding to receptors with tyrosine kinase activity VEGFR-1 (also known as fms-like tyrosine kinase 1, ftl-1), VEGFR-2 (also known as kinase-insert domain receptor, KDR) and VEGFR-3 (ftl4).

When VEGF binds to its receptors it causes receptor dimerization, autophosphorylation, and downstream signaling of different pathways, as v-src sarcoma viral oncogene homolog (Src), phosphoinositol (PI)-3 kinase (PI3K) and phospholipase-C γ (PLCγ) which activate proliferation and angiogenesis.

In animal tumor models, VEGF is produced both by tumor cells and also by stromal tissues [4].

VEGF and its receptor are expressed in tumor cells in both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [36,37]. It is involved in tumor growth by neoangiogenesis, lymphangiogenesis and lymph nodal dissemination [38]. High levels of VEGF have been correlated with poor prognosis [39]. But there are several questions about the role of VEGF levels and its various isoforms plays as a potential biomarker, which may be useful in the use and selection of therapies against it. VEGF levels are elevated in lung cancer patients when compared to controls [40]. There is also a correlation between VEGF levels and the clinical stage in NSCLC patients [7,10,13,15] and an inverse correlation between the VEGF serum levels and survival [41]. Low levels of VEGF have shown to be correlated with a good response to chemotherapy [12]. Moreover, a study showed that low levels of VEGF were correlated with a good response to anti-EGFR. Furthermore, levels of VEGF in responders were not significantly different from volunteers, but were different from non-responders [42]. However, it remains unclear whether the clinical effects of anti-EGFR in patients with NSCLC are correlated with reductions in the levels of angiogenic growth factors. Furthermore, it is unclear whether these factors are correlated with response to anti-EGFR treatment, blocking EGFR autophosphorylation [43] and the subsequent signal transduction pathways implicated in proliferation, metastasis and inhibition of apoptosis, as well as angiogenesis [44,45]. The inhibition of EGFR has been shown to reduce production of angiogenic growth factors in various types of cancer cells [45,46].

Antiangiogenic drugs have demonstrated efficacy in the treatment of NSCLC in the last years. The more tested antiangiogenic drug in lung cancer is bevacizumab, a monoclonal antibody directed against VEGF, which is the first antiangiogenic approved for treatment of metastatic NSCLC in combination with chemotherapy. Two phase III studies have assessed the efficacy of chemotherapy combinations associated with bevacizumab. The AVAiL study [47] analyzed the combination of cisplatin and gemcitabine with or without bevacizumab in first line treatment for NSCLC. The primary endpoint was reached, showing a benefit in progression-free survival in the bevacizumab arm. The second study [48] compared the addition of bevacizumab with carboplatin and paclitaxel regimen, aiming differences in overall survival, progression-free survival and response rate.

These detailed studies further in subsequent chapters, show that bevacizumab is an effective and safe drug in the treatment of advanced NSCLC.

4. Pathophysiology and clinical implications of EGF/PDGF/VEG

It is known that other several growth factors regulate developmental processes, among which are the Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), growth factor Insulin-like type I (IGF-I) and Platelet Derived Growth Factor platelet (PDGF).

4.1. EGF

Members of the EGF family of peptide growth factors serve as agonists for ErbB family receptors. They include EGF, TGFα, amphiregulin (AR), betacellulin (BTC), heparin-binding EGF-like growth factor (HB-EGF), epiregulin (EPR), epigen (EPG), and the neuregulins (NRGs).

EGF is a polypeptide of 53 amino acids (6 Kda) that appears as a product of proteolytic processing of a large protein integral membrane (1207aa). This precursor protein is consisting of 8 domains called EGF-like, of which only one is active. The gene corresponding to this growth factor is located on chromosome 4q25 and stimulates epithelial cell proliferation, oncogenesis and is involved in wound healing. Its three-dimensional structure is characterized by the presence of common domain to other family ligands. This protein shows a strong sequential and functional homology with TGFα, which is a competitor for EGF receptor sites.

Collectively, these agonists regulate the activity of the four ErbB (Erythroblastic Leukemia Viral Oncogene Homolog) family receptors, each of which appears to make a unique set of contributions to a complicated signaling network.

EGF binds to a specific receptor on the surface of responsive cells known as EGFR (Epidermal growth factor receptor). EGFR is a member of the ErbB family receptors, a subfamily of four closely related to tyrosine kinase receptors: EGFR (ErbB1), Her2/c-neu (ErbB2), Her3 (ErbB3) and Her4 (ErbB4) (Fig.1). The EGF family ligands exhibits a complex pattern of interactions with the four ErbB family receptors; for example, EGFR can bind eight different EGF family members and Neuregulin 2beta (NRG2β) binds EGFR, ErbB3 and ErbB4. Given that ErbB2 lacks an EGF family ligand, ErbB3 lacks kinase activity, and the four ErbB receptor display distinct coupling patterns to different signaling effectors in the affinity of a given EGF family member as a key determinant of specificity for the ligand [49].

In response to toxic environmental stimuli, such as ultraviolet irradiation, or to receptor occupation by EGF, the EGFR forms Homo- or Heterodimers with other family members. Binding of EGF to the extracellular domain of EGFR leads to receptor dimerization, activation of the intrinsic PTK (Protein Tyrosine Kinase), tyrosine autophosphorylation, and recruitment of various signaling proteins to these autophosphorylation sites located primarily in the C-terminal tail of the receptor. Tyrosine phosphorylation of the EGFR leads to the recruitment of diverse signaling proteins, including the Adaptor proteins GRB2 (Growth Factor Receptor-Bound Protein-2) and Nck (Nck Adaptor Protein), PLC-&γ; (Phospholipase-C-γ), SHC (Src Homology-2 Domain Containing Transforming Protein), STATs (Signal Transducer and Activator of Transcription), and several other proteins and molecules (Fig 2).
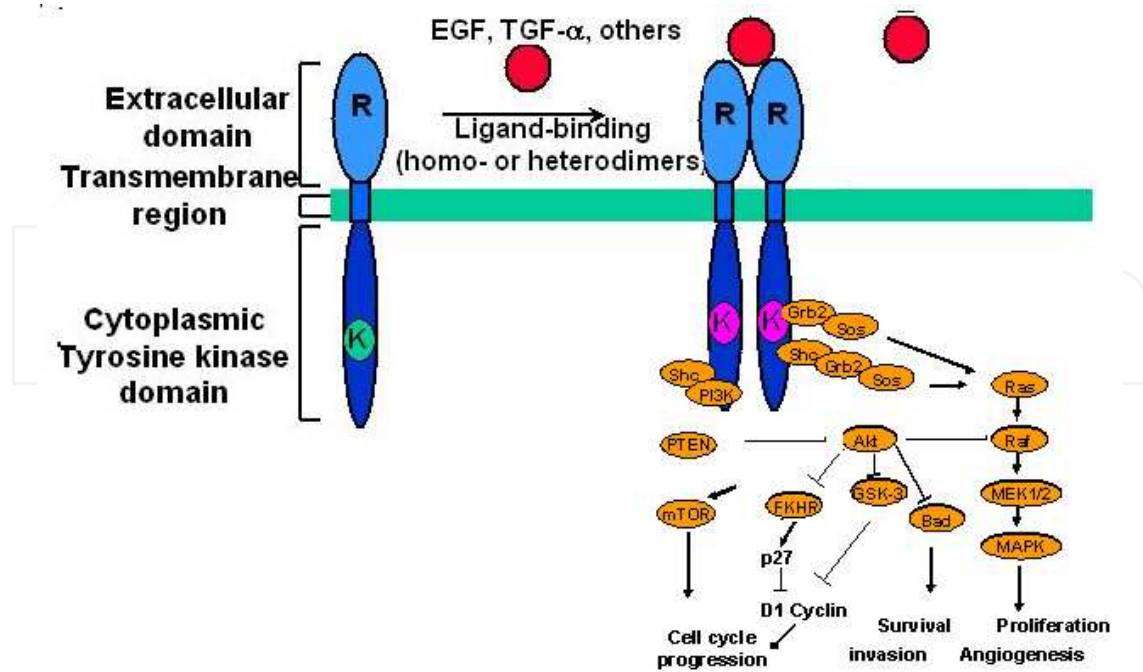
Figure 1. The binding of specific ligands to the receptor activates EGFR and generates a signal transduction cascade through its 2-way main PI3K/Akt and Ras / Raf / MAPK eventually stimulate proliferation, cell cycle progression, repair, angiogenesis and invasion.
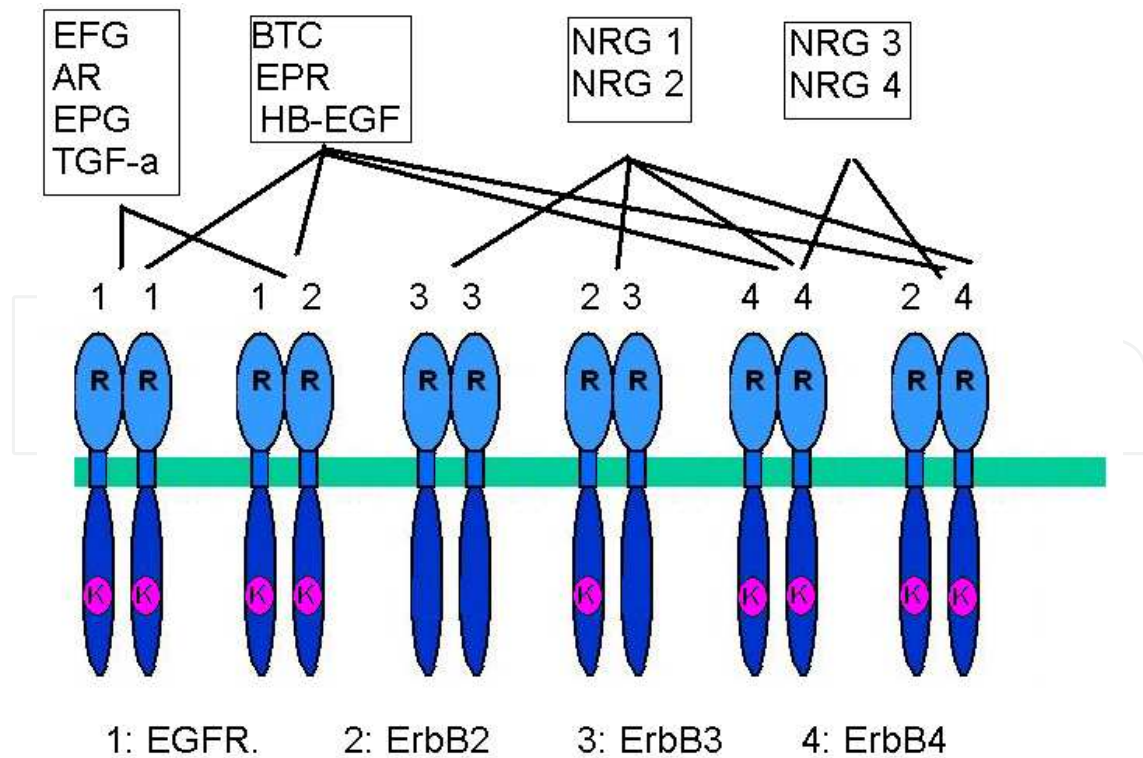

Figure 2. Binding specificities of EGF-related peptide growth factors

Although EGFR plays an important role in maintaining normal cell function, deregulation of EGFR pathway contributes to the development of malignancy progression, inhibition of apoptosis, induction of angiogenesis, promotion of tumor-cell motility and metastasis. Aberrant regulation of the activity or action of EGFR and other members of the RTK family have been involved in multiple cancers, including of brain, lung, breast and ovary. Furthermore, in many tumors EGF-related growth factors are produced either by the tumor cells themselves or are available from surrounding stromal cells, leading to constitutive EGFR activation. In gliomas, EGFR amplification is often accompanied by structural rearrangements that cause in-frame deletions in the extracellular domain of the receptor, the most frequent is the EGFRvIII variant. Somatic mutations in the tyrosine-kinase domain of EGFR were also identified in NSCLC.

When mutated, EGFR tyrosine kinase is constitutively activated, resulting in uncontrolled proliferation, invasion and metastasis. Expression of EGFR and their ligands, especially TGFα, by lung cancer cells, indicates the presence of an autocrine (self-stimulatory) growth factor loop. Activating EGFR mutations are observed in approximately 10% of North American and European populations and 30% to 50% of Asian populations [50] and are significantly more common in never-smokers (100 or less cigarettes per lifetime) or light former smokers (quit 1 year or more ago and less than ten-pack per year smoking history). The leucine to arginine substitution at position 858 (L858R) in exon 21 and short in-frame deletions in exon 19 are the most common mutations seen in adenocarcinomas of the lung. These mutations result in prolonged activation of the receptor and downstream signaling through phosphorylated Akt, in the absence of ligand stimulation of the extracellular domain. EGFR mutations are both prognostic for response rate to chemotherapy and survival irrespective of therapy and are predictive of response to specific inhibitors of the EGFR tyrosine kinase.

4.2. PDGF

Platelet-derived growth factor (PDGF) is a major mitogen for fibroblasts, smooth muscle cells (SMCs), and glia cells. Originally, was identified as a constituent of whole blood serum that was absent in cell-free plasma-derived serum, and was subsequently purified from human platelets [51]. Although the α-granules of platelets are a major storage site for PDGF, can be synthesized by a number of different cell types including fibroblasts, muscle, bone / cartilage, and connective tissue cells.

The synthesis is often increased in response to external stimuli, such as exposure to low oxygen tension, thrombin, or stimulation with various growth factors and cytokines [52].

PDGF is a family of cationic homo- and heterodimers of disulphide-bonded polypeptide chains. In mammals, a total of four different genes encode four PDGF chains (PDGF-A, PDGF-B, PDGF-C, and PDGF-D), which are assembled in five different isoforms known as: AA, AB, BB, CC and DD [53]. All members carry a growth factor core domain containing a conserved set of cysteine residues. The core domain is necessary and sufficient for receptor binding and activation. Classification into PDGFs is based on receptor binding. It has been generally assumed that PDGF is selective for their owns receptors.

PDGF isoforms exert their effects on target cells by activating two structurally related pro-tein tyrosine kinase receptors. The α and β receptors have molecular sizes of 170 and 180 kda, respectively, after maturation of their carbohydrates. Extracellularly, each receptor con-tains five immunoglobulin-like domains, and intracellularly there is a tyrosine kinase do-main that contains a characteristic inserted sequence without homology to kinases.

The human α-receptor gene is localized on chromosome 4q12, close to the genes for the SCF (stem cell factor) receptor and VEGF receptor-2, and the β-receptor gene is on chromosome 5 close to the *CSF*-1 (colony stimulating factor-1) receptor gene [54].

Because PDGF isoforms are dimeric molecules, they bind two receptors simultaneously and dimerize receptors upon binding. The α receptor binds both the A and B chains of PDGF with high affinity, whereas the β receptor binds only the B chain with high affinity. There-fore, PDGF-AA induces $\alpha\alpha$ receptor homodimers, PDGF-AB $\alpha\alpha$ receptor homodimers or $\alpha\beta$ receptor heterodimers, and PDGF-BB all three dimeric combinations of α and β receptors (Fig 3). General mesenchymal expression of PDGFRs is low in vivo, but increases dramati-cally during inflammation and in culture. Several factors induce PDGFR expression, includ-ing TGF-β, estrogen (probably linked to hypertrophic smooth muscle responses in the pregnant uterus), interleukin-1α (IL-1α), basic fibroblast growth factor-2 (FGF-2), tumor ne-crosis factor-β, and lipopolysaccharide [55].
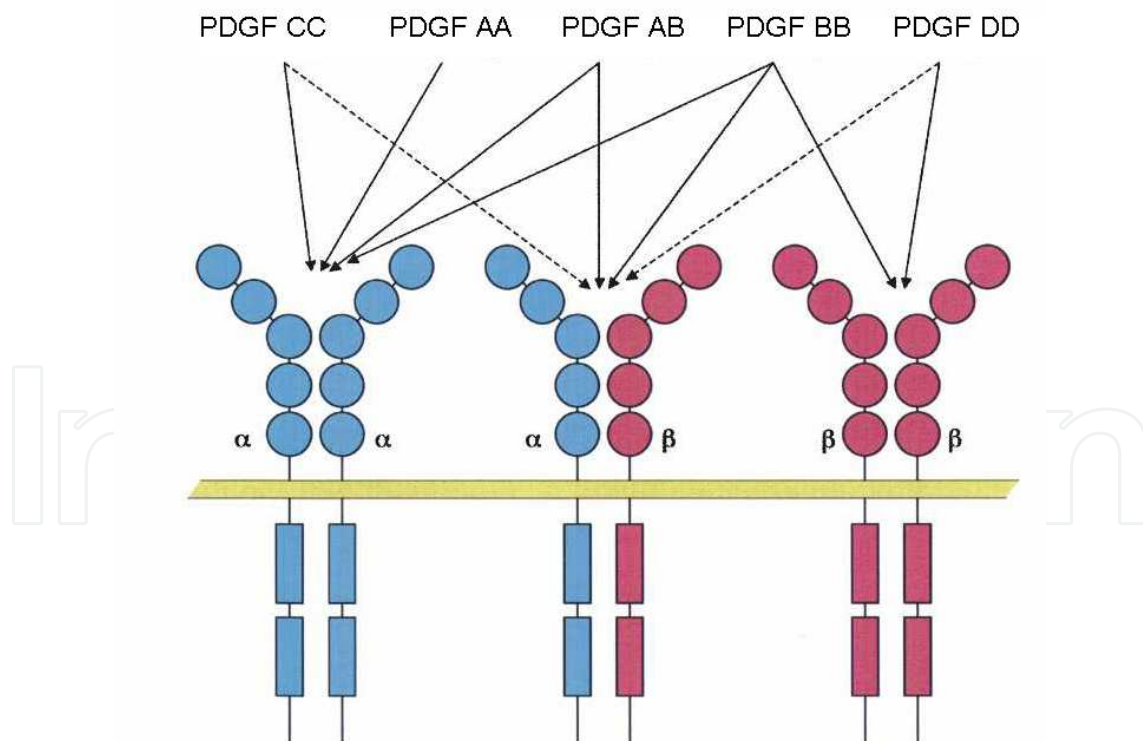

Figure 3. adapted from J Andrae 2008): PDGF–PDGFR interactions. Each chain of the PDGF dimer interacts with one receptor subunit. The active receptor configuration is therefore determined by the ligand dimer configuration. The top panel shows the interactions that have been demonstrated in cell culture. Hatched arrows indicate weak interac-tions or conflicting results.

The detailed expression patterns of the individual PDGF ligands and receptors are complex. There are some general patterns, however: PDGF-B is mainly expressed in vascular endothelial cells, megakaryocytes, and neurons. PDGF-A and PDGF-C are expressed in epithelial cells, muscle, and neuronal progenitors. PDGF-D expression is less well characterized, but it has been observed in fibroblasts and SMCs at certain locations (possibly suggesting autocrine functions via PDGFR-β). PDGFR-α is expressed in mesenchymal cells. Particularly strong expression of PDGFR-α has been noticed in subtypes of mesenchymal progenitors in lung, skin, and intestine and in oligodendrocyte progenitors (OPs). PDGFR-β is expressed in mesenchyme, particularly in vascular SMCs (vSMCs) and pericytes.

PDGF biosynthesis and processing are controlled at multiple levels and differ for the different PDGFs. PDGF-A and PDGF-B become disulphide-linked into dimers already as propeptides. PDGF-C and PDGF-D have been less studied on this regard. PDGF-A and PDGF-B contain N-terminal pro-domains that are removed intracellularly by furin or related proprotein convertases. Likely, PDGF-B also requires N-terminal propeptide removal to become active. In contrast, PDGF-C and PDGF-D are not processed intracellularly but are instead secreted as latent (conditionally inactive) ligands. Activation in the extracellular space requires dissociation of the growth factor domain.

Dimerization is the key event in PDGF receptor activation as it allows for receptor autophosphorylation on tyrosine residues in the intracellular domain. Autophosphorylation activates the receptor kinase and provides docking sites for downstream signaling molecules and further signal propagation involves protein–protein interactions through specific domains; e.g., Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains recognizing phosphorylated tyrosines, SH3 domains recognizing proline-rich regions, pleckstrin homology (PH) domains recognizing membrane phospholipids, and PDZ domains recognizing C terminal specific sequences. Most of the PDGFR effectors bind to specific sites on the phosphorylated receptors through their SH2 domains. Both PDGFR-α and PDGFR-β engage several well-characterized signaling pathways, e.g. Ras-MAPK, PI3K and PLC-γ, which are known to be involved in multiple cellular and developmental responses [56].

The PDGFR is expressed on capillary endothelial cells and PDGF has been shown to have an angiogenic effect. The effect is, however, weaker than that of fibroblast growth factors or VEGF, and PDGF does not appear to be of importance for the initial formation of blood vessels. PDGF B-chain produced by capillaries may have an important role to recruit pericytes that is likely to be required to promote the structural integrity of the vessels. PDGF has also been implicated in the regulation of the tonus of blood vessels [57].

PDGF functions have been implicated in a broad range of diseases. For a few of them, i.e., some cancers, there is a strong evidence for a causative role of PDGF signaling in this human disease process. In these cases, genetic aberrations cause uncontrolled PDGF signaling in the tumor cells.

4.3. VEG/PF

Vascular endothelial growth/permeability factor (VEG/PF) is a 40 kda disulphide-linked dimeric glycoprotein that is active in increasing blood vessel permeability, endothelial cell growth and angiogenesis. These properties suggest that the expression of VEG/PF by tumor cells could contribute to the increased neovascularization and vessel permeability that are associated with tumor vasculature. The cDNA sequence of VEG/PF from human U937 cells was shown to code for a 189-amino acid polypeptide that is similar in structure to the B chain of PDGF-B and other PDGF-B-related proteins. The overall identity with PDGF-B is 18%. However, all eight of the cysteines in PDGF-B were conserved in human VEG/PF, an indication that the folding of the two proteins is probably similar. Clusters of basic amino acids in the COOH-terminal halves of human VEG/PF and PDGF-B are also prevalent. Thus, VEG/PF appears to be related to the PDGF/v-sis family of proteins [58].

5. Angiogenesis and radiological assessment techniques

Neoangiogenesis, the formation of new blood vessels from a pre-existing vascular network, is essential for tumor growth, tumor proliferation and metastasis. The angiogenesis process is regulated by different proangiogenic and antiangiogenic factors, being the primary stimulus of new vessel formation the hypoxia induced by expansion of the growing tumor mass [59].

Tumor angiogenesis is an attractive target for anticancer therapy, and a wide range of novel therapies directed against tumor vascularity has been developed. Because many antiangiogenic agents are not cytotoxic but instead produce disease stabilization, measurement of tumor size alone may be not informative regarding therapeutic effects. For that reason, there has been great interest in the use of physiologic, rather than solely anatomic, imaging techniques [60]. Tumor vascularity has different features that are characteristic of malignancy, such as spatial heterogeneity, chaotic structure, fragility and high permeability to macromolecules. These structural abnormalities of new tumor vessels lead to pathophysiologic changes within the neoplastic tissue, including an increase in capillary permeability, volume of extravascular-extracellular space, and tumor perfusion, that permit distinction of malignant from benign vascularity with functional imaging techniques.

Several commonly available imaging modalities, including magnetic resonance (MR), computed tomography (CT), ultrasound and positron emission tomography (PET), have been used to indirectly assess the angiogenic status of human tumors [61]. But perfusion imaging with MR, and specially CT, are the most useful in clinical practice. They have the advantage of good spatial resolution, minimal invasiveness and rapid acquisition of data. Both techniques sequentially demonstrate passage of a bolus of contrast medium through a region of interest and allow quantification of the profile of tissue enhancement.

6. Perfusion CT

The fundamental principle of perfusion CT is based on the temporal changes in tissue at-tenuation after intravenous administration of iodinated contrast material (CM). This enhancement depends on the tissue iodine concentration, existing a direct linear relationship between contrast concentration and CT enhancement [62].

Recent progress in multidetector CT technology has enabled the rapid scanning of large anatomic volumes with high resolution. In perfusion CT, repeated series of images of the volume analyzed are performed in quick succession before, during and after intravenous administration of CM. The ensuing tissue enhancement can be divided into two phases based on CM distribution: a initial phase where the enhancement is attributable to the distribution of contrast within the intravascular space ("first pass", lasting 40-60 secs. from the contrast arrival), and a second phase as contrast diffuses from the intravascular to the extravascular compartment across the capillary basement membrane (2-5 minutes duration). To objectively quantify the "real" perfusion parameters of tissues from the density difference produced by the contribution of contrast material, a mathematic model is applied to the dynamic CT data. The quantitative parameters generated include blood volume (BV), blood flow (BF), mean transit time and capillary permeability surface.

Perfusion CT is a biomarker for angiogenesis that have been validated with other surrogate markers, such as VEGF levels, tumor perfusion and microvascular density (Fig 4) [63]. There has been a gradual increase of its use in oncology, ranging the wide spectrum of clinical applications of this technique, from lesion characterization, (differentiation between benign and malignant lesions), to prognostic information based on tumor vascularity and monitoring therapeutic effects of chemoradiation and antiangiogenic drugs. In a recent study using a 320-detector row CT, Ohno et al. concluded that perfusion CT has the potential to be more specific and accurate than PET/CT for differentiating malignant from benign pulmonary nodules [64]. Another study have also shown that in patients with NSCLC treated with sorafenib and erlotinib, early changes in tumor blood flow were predictive of objective response and tended to indicate a longer progression-free survival [65].
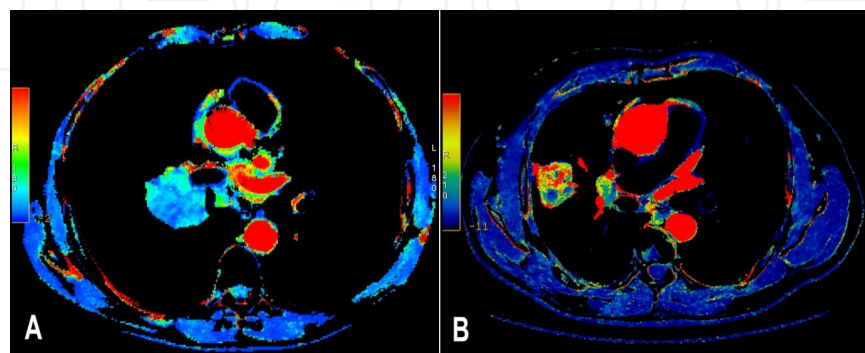

Figure 4. Parametric maps of perfusion CT studies representing blood flow in two different patients with NSCLC. (A) Tumor with very low perfusion depicted in blue and (B) a highly vascularized neoplasm showing yellow and red zones (scale at left).

Radiation exposure, the requirement of long breath holding during chest imaging acquisition and lack of standardized protocols, remain potential drawbacks of this technique. However, implementation of low-dose scanning strategies may allow a more widespread use in the future.

7. Dynamic MR

Quantification of tumor vascularity by dynamic MR (DCE MR) is technically more challenging than perfusion CT because there is a lack of a direct relationship between MR signal intensity and contrast agent concentration. This is due to the fact that tissue signal intensity on MR is related to the effect of CM on water in the microenvironment, which changes tissue relaxivity in complex and unpredictable ways [66].

While perfusion CT yield information is based predominantly on the first pass of CM (BV, BF), the MR imaging technique may sample a volume of interest over a longer time and yields parameters that reflect microvessel perfusion, permeability and extracellular leakage of space. In addition, by applying pharmacokinetic models to the MR imaging acquisitions, it is possible to calculate quantitative parameters, such as the transfer constant (K^{trans}) that describes the transendothelial transport of the CM.

A central flaw of dynamic MR is that acquisition and pharmacokinetic models vary widely. Thus, comparing studies from different institutions is difficult. This technique, on the other hand, is of limited value in organs with physiological movement such as the lungs.

Few studies have applied dynamic MR in the assessment of lung cancer. Ohno et al [67] evaluated the role of DCE MR as a prognostic indicator in NSCLC patients treated with chemotherapy using cisplatin and vincristine. In their study, the mean survival period of patients with lower slope of enhancement was significantly longer than that seen in the group with higher slope of enhancement. This study provides promising data for the application of dynamic MR in response assessment to chemotherapy and targeted therapy.

8. Current state of antiangiogenic therapy for NSCLC: VEGF as target treatment

In this section, we analyze the activity of a monoclonal antibody (bevacizumab) and other new antiangiogenic therapies.

8.1. Bevacizumab

Bevacizumab is a monoclonal antibody directed against VEGF and was the first antiangiogenic drug approved for the treatment of advanced NSCLC. Currently it's the only approved in this setting in Europe and the USA.

After proving the improvement in the response rate (RR) and progression free survival (PFS) of bevacizumab together with chemotherapy in first line in a randomized phase II study in which 99 patients with advanced or metastatic NSCLC were included [68], the ECOG group undertook a phase III trial (ECOG 4599) in first line, in which patients with brain metastasis, hemoptysis, and squamous histology were excluded, due to the risk of hemoptysis observed in the previous study with this histology [69]. The studied randomized 878 patients with recurrent or advanced NSCLC to receive carboplatin/paclitaxel with or without bevacizumab on a dose of 15 mg/kg every 21 days and crossover was not allowed. The main objective, overall survival (OS), was improved in the trial arm: 12.3 months vs 10.3 months, with a hazard ratio (HR): 0.79 (95% CI: 0.67-0.92; p=0.003). In addition, the RR was also improved (35 vs 15% (p<0.001)) and the PFS went from 4.5 to 6.2 months (HR: 0.66; 95% CI: 0.57-0-77, p<0.001). However, adding bevacizumab to the chemotherapy also increased toxicity; there were 15 toxic deaths (2 in the arm of chemotherapy alone) due to pulmonary hemorrhage, digestive bleeding, febrile neutropenia, ictus and lung embolism. A subgroup analysis found that patients over 70 had a higher incidence of grade 3-5 toxicities (87 vs 61%).

The AVAiL study [70] randomized 1043 patients to receive cisplatin and gemcitabine with or without bevacizumab in a dose of 7.5 or 15 mg/kg each 21 days. In this study the main goal was PFS, which was higher in patients which received the drug than those who took placebo, both in small dose arm (6.7 months vs 6.1 months; HR: 0.75, p=0.003) as well as in higher one (6.5 months vs 6.1 months, HR: 0.82, p=0.03). Nevertheless, OS didn't improve, which could be explained by the high percentage of patients who received treatment afterwards (more than 60%). Regarding toxicity, 7 patients died due to lung hemorrhage in the trial arm (2 in the control trial), although it was observed that in patients who were under anticoagulant treatment there was no lung hemorrhage.

The SAiL safety study, which included more than 2000 patients, showed the effectiveness of combining other doublets of chemotherapy; in terms of safety it displayed a grade 3 or higher lung hemorrhage incidence only in 1% of the patients [71].

An efficiency meta-analysis published in 2011 confirms effectiveness in terms of PFS, presenting uncertainty in terms of improvement of OS [72].

A meta-analysis published recently with 2210 patients evaluated the bevacizumab toxicity profile with high dose of bevacizumab (15 mg/kg), and stated that bevacizumab is related to a higher risk of toxicity deaths (HR: 2.04; 95% CI: 1.18-3.52), but it was not the case in lower doses of 7.5 mg/kg (HR: 1.20; 95% CI: 0.60-2.41). In addition, bevacizumab was associated to a greater incidence of grade 3-4 toxicities, especially in the group of high doses [73].

More studies have been conducted in sub-populations, for example, the PASSPORT study in 109 patients with brain metastasis, subgroup that had not been included in previous studies, and which proved that bevacizumab can be administrated in patients with controlled brain metastasis [74]. Another review on the incidence of bleeding in patients with brain metastasis treated with antiangiogenic drugs proved to be safe when it is administered to treated patients as well as patients with metastasis that appears during treatment [75].

The combination of bevacizumab with some of the new agents has been studied as well. In the ATLAS study, after having received four cycles of cisplatin-based chemotherapy and bevacizumab, patients were randomized to receive treatment with bevacizumab (15 mg/kg) and erlotinib (150 mg daily) or only bevacizumab. The main objective of this study was reached (PFS), with 4.8 months vs 3.7 months (HR: 0.72, p=0.0012); nevertheless no improvement was made in OS, a secondary goal of the study (14.4 months vs 13.3 months; p=0.56) [76].

The phase III BeTa trial compared the activity of the combination of bevacizumab and erlotinib vs erlotinib in second line in 636 patients. An improvement in PFS was found (3.4 vs 1.7 months; HR: 0.62, p<0.0001), but again, no significant differences were found in OS (9.3 vs 9.2 months; p=0.75)

Hypertension has been found to be a marker of clinical benefit from bevacizumab in various malignancies [77], although no single biomarker have proven to be ready for clinical use. Cytokines and angiogenic factors profiling may help identify drug-specific markers of activity.

8.2. Aflibercept

Aflibercept (VEGF-Trap) is a recombining fusion protein, which is added to VEGFR-1, VEGFR-2 and to the placental growth factor (PlGF).

In a phase II trial in patients with lung adenocarcinoma treated after several treatment lines, aflibercept in a dose of 4 mg/kg was administered intravenously every 14 days, reaching a RR of 2%, with a PFS of 2.7 months and an OS of 6.2 months [78]. A phase III trial in second line after failure to cisplatin-based chemotherapy compared aflibercept vs docetaxel (VITAL trial). This trial showed an improved RR (23.3& vs 8.9%) and PFS (HR: 0.82), but the primary endpoint, OS, was not reached (HR: 1.01).

9. Vascular disrupting agents

Vadimezan, fosbretabulin and plinabulin are vascular disrupting agents (VDA); fosbretabulin selectively disrupts VE-cadherin and plinabulin acts on cytoskeleton. A phase II trial of carboplatin, paclitaxel, bevacizumab and fosbretabulin was well tolerated and with a trend to improve OS and PFS [79], also a phase II trial with docetaxel with or without plinabulin showed a higher response rate with the combination (55% vs 5%) [80]; however, a randomized phase III study with vadimezan in first line failed to show an improvement in OS [81].

10. Multi-targeted tyrosine kinase inhibitors

Several anti-angiogenic small-molecule tyrosine kinase inhibitors (TKIs) are in current clinical development. An advantage of TKIs includes the fact that they inhibit multiple receptors

simultaneously, with anti-angiogenic and anti-proliferative activity against NSCLC, thereby potentially providing a higher likelihood of single-agent activity. Another benefit is that these agents are often available orally, offering patients greater convenience. However, toxicity remains a concern given the multi-targeted kinase inhibition and the additive adverse effects that may be of particular concern when the agents are combined with chemotherapy.

11. Sorafenib

Sorafenib is an oral multi-kinase inhibitor of VEGFR-2 and -3, PDGFR-β, RAF-kinase, c-Kit, RET, and Ftl-3.

In the phase II ECOG 2501 trial, 342 patients with NSCLC who has failed at least two prior chemotherapy regimens received sorafenib for two cycles. Those patients who were noted to have stable disease after two cycles (n = 97) were randomized to receive sorafenib or placebo. Sorafenib prolonged PFS compared with placebo (3.6 versus 1.9 months) [82]. In another phase II trial, of 52 patients with relapsed or refractory advanced NSCLC, 59% achieved SD, and in these patients, median PFS was 5.5 months [83].

The results of two phase III trials in the first-line treatment of NSCLC, ESCAPE (sorafenib plus paclitaxel/carboplatin) and NEXUS (sorafenib plus gemcitabine/cisplatin), were unsatisfactory. Because of the safety findings from the ESCAPE trial, patients with squamous cell histology were withdrawn from the NEXUS trial in February 2008 and excluded from analysis. Median OS, the primary endpoint of both trials, was similar in the sorafenib and placebo groups [84,85].

The Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) study randomized pretreated lung cancer patients to erlotinib, vandetanib, erlotinib plus bexarotene or sorafenib based upon biomarker results obtained from individual patients. K-ras-mutant patients treated with sorafenib had a non-statistically significant trend toward improved disease control rate (DCR) (61 versus 32%, p = 0.11), suggesting a preferential benefit of sorafenib in k-ras-mutant patients [86].

Phase III MISSION trial of sorafenib in patients with advanced relapsed or refractory non-squamous NSCLC whose disease progressed after two or three previous treatments, did not meet its primary endpoint of improving OS. An improvement in the secondary endpoint of PFS was observed [87].

These findings have led to suspend the development of sorafenib in NSCLC.

12. Vandetanib

Vandetanib is an oral TKI that inhibits VEGFR-2 and -3, RET and EGFR.

Vandetanib in combination with carboplatin/paclitaxel resulted in prolonged PFS (56 weeks; HR= 0.76, p= 0.098) compared with carboplatin/paclitaxel alone (52 weeks) in previously un-

treated patients with advanced NSCLC. The secondary endpoint of OS was not significantly different between the two arms [88]. Another phase II trial showed that vandetanib in combination with docetaxel was superior to docetaxel alone in pretreated NSCLC patients with regard to PFS (18.7 weeks versus 12 weeks; HR = 0.64, p = 0.037) [89].

The phase III ZODIAC trial randomized patients with advanced NSCLC to receive either docetaxel/vandetanib or docetaxel/placebo as second-line treatment. Although vandetanib improved ORR (17 versus 10%, p= 0.0001) and PFS (HR: 0.79, p< 0.0001), OS was not significantly improved (HR: 0.91, p= 0.196) [90]. In the ZEAL trial, vandetanib was investigated in combination with pemetrexed also in the second-line setting. Despite an improvement in ORR (19 versus 8%, p < 0.001), this study did not meet its primary endpoint of PFS (HR: 0.86, p = 0.108) [91]. In another phase III trial (ZEPHYR), patients who had progressed after chemotherapy and erlotinib were randomized to vandetanib versus placebo. PFS was improved (HR: 0.63, p < 0.0001), but not OS (HR: 0.95, p = 0.527) [92]. The above phase III trials did not carry out stratified analysis on the EGFR gene status and therefore were not able to further identify the potential populations that may benefit from vandetanib.

These results led to withdrawal of the application for approval of vandetanib in NSCLC.

13. Sunitinib

Sunitinib is an oral TKI of VEGFR-1, -2, -3, PDGFR-α/β, c-kit, Flt-3 and RET.

It has been studied in advanced NSCLC in two phase II trials. In the first one, 63 pretreated patients received sunitinib as single agent, achieving an ORR of 11.1% (95%CI: 4.6–21.6), median PFS of 12 weeks (95%CI: 10.0-16.1) and median OS of 23.4 weeks (95%CI: 17.0-28.3) [93]. In the other phase II trial, 47 pretreated patients received sunitinib on a continuous-dosing schedule (37.5 mg/day). The ORR was only 2.1%, but median PFS and OS were 11.9 weeks (95%CI: 8.6-14.1) and 37.1 weeks (95%CI: 31.1-69.7), respectively [94].

There are ongoing studies investigating sunitinib in patients with NSCLC, including the phase II CALGB 30704 trial evaluating sunitinib as second-line therapy and the phase III CALGB 30607 study of sunitinib as maintenance therapy.

14. Other multi-targeted TKIs

Axitinib, with VEGFR, PDGFR-β and c-Kit as its main targets, is currently the most potent TKI in inhibiting VEGFR signal pathways. In a phase II study in advanced NSCLC, in which 28% of patients had received no prior chemotherapy, ORR was 9.4%, with PFS and OS of 4.9 and 14.8 months, respectively [95]. Currently, three ongoing phase II studies are exploring the effectiveness and safety of axitinib-based combination therapies in non-squamous (AGILE1030: with paclitaxel/carboplatin; AGILE1039: with pemetrexed/cisplatin) and squamous NSCLC (AGILE1038: with cisplatin/gemcitabine).

Motesanib mainly inhibits targets including VEGFR, PDGFR, c-Kit and RET. In a phase II study of motesanib or bevacizumab in combination with carboplatin/paclitaxel as frontline treatment for advanced non-squamous NSCLC, the efficacy was similar, with a median PFS of 7.7 months (versus 8.3 months with bevacizumab) and a median OS of 14.0 months in both arms [96]. However, the phase III study of motesanib plus carboplatin/paclitaxel in patients with non-squamous advanced NSCLC (MONET1) did not meet its primary endpoint of improved OS (HR: 0.89, p = 0.137) [97].

BIBF 1120 inhibits VEGFR-1, -2 and -3, in addition to PDGFR-α/β and FGFR-1-3. In a phase II trial of 73 patients with relapsed or advanced NSCLC, the median PFS and OS were 11.6 and 37.7 weeks, respectively, with a disease control rate (DCR) of 46% [98]. BIBF 1120 is being studied, in the second-line NSCLC setting, in two phase III trials, in combination with docetaxel (LUME-Lung 1) and with pemetrexed (LUME-Lung 2).

A phase Ib/II study of cabozantinib, a TKI with potent activity against MET, VEGFR-2, RET, c-Kit and Ftl-3, with or without erlotinib in pretreated advanced NSCLC patients showed that the combination was well tolerated with evidence of clinical activity in a largely erlotinib pretreated cohort, including patients with EGFR T790M mutation and MET amplification [99].

Another multikinase inhibitors like pazopanib are in an earlier stage of development.

Although multitargeted TKIs have made certain advances in treating NSCLC, the outcomes remain unsatisfactory if they were applied non-selectively among NSCLC patients. Among the non-selective populations, TKI monotherapies showed no significant differences when compared with mono-targeted agent therapies (erlotinib, gefitinib) in treating NSCLC in terms of ORR, PFS and OS. Therefore, it is extremely important to identify populations that are suitable for TKIs. The future of multi-targeted drugs is highly depended on the capability of delivering these molecule-targeted therapies to patients most likely to benefit.

15. Conclusions

In recent years, we have acquired a lot of information regarding the role of angiogenesis and its pathophysiological relationship with some types of neoplasias, engaging in processes such as tumour growth and dissemination capacity as loco-regional as distant. In lung cancer, we know that neoangiogenesis is the result of the action of several growth factors (mainly VEGF, TGF-alpha, EGF, VEG/PF and PDGF) whose output is controlled by transcription factors hypoxia-induced such as HIF-1, whose expression has been associated as an independent factor of poor prognosis. Acquired knowledge has allowed designing therapeutic strategies aimed at blocking the action of various pro-angiogenic factors and thereby altering the disease natural course. Some drugs acting against VEGF, as bevacizumab, have demonstrated clinical efficacy improving OS and PFS although with treatment-related toxicities expected with blocking this pathway, as showed some trials, particularly in patients subsets with a known clinical profile that when is present

makes it more susceptible to those complications. Another line of research has been that of small-molecule tyrosine kinase inhibitors (sorafenib, vandetanib, sunitinib, axitinib, pazopanib and motesanib), showing some benefits in PFS but without a positive impact in OS when were applied non-selectively among NSCLC patients, so in the future probably we will need identify populations with a right profile that allows us to predict who have more chance to benefit from this therapy. Structural abnormalities in tumor neovascularization lead to pathophysiological changes within the neoplastic tissue. The study of these functional changes have allowed to develop imaging techniques that, not only differentiate a benign lesion from other malignant, but also provide prognostic information and monitor the therapeutic effects of drugs used. Thus, techniques such as perfusion CT and dynamic MR allow anatomical and functional assessment of neoplasia, based on the characteristics and changes of intratumoral capillary network.

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