Targeting angiogenesis in lung cancer - Pitfalls in drug development

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Abstract: In non-small-cell lung cancer, anti-angiogenic strategies like bevacizumab have developed into standard treatment options. New anti-angiogenic drugs like tyrosine kinase inhibitors generated optimistic results in phase II trials, but failed to translate into positive results in phase III trials. In this overview some critical aspects of the biology of tumor angiogenesis and potential pitfalls of anti-angiogenic drug development are discussed. These include the design of clinical trials, dosage of investigational drugs or the choice of combinational drugs, the lack of validated biomarkers and the complexity of the patho-biology of tumor angiogenesis. Future trials should also direct attention to the role of cigarette smoke and the stage of the disease, which is investigated.

Key Words: VEGF; VEGFR; anti-antiangiogenic therapy; tyrosine kinase inhibitor (TKI); bevacizumab

Introduction

In 1971, Judah Folkman hypothesized that tumor growth is dependent on angiogenesis and suggested that disrupting tumor angiogenesis would inhibit tumor growth, thus providing a method of controlling tumors (1). Thereby, tumor hypoxia is the key trigger to induce tumor angiogenesis and, in a simplified model, hypoxia and factors like epidermal growth factor (EGF), insulin-like growth factor (IGF) or platelet-derived growth factor (PDGF) lead to vascular-endothelial growth factor (VEGF)-secretion. Subsequently, VEGF activates VEGF receptors (VEGFR) which by means of dimerization induces the downstream signaling cascade of endothelial cells. Finally, proliferation, migration and permeability of endothelial cells are induced facilitating tumor growth and metastasis. During the following decades tumor angiogenesis has been the subject of extensive research and it is well known that angiogenesis is involved in early as well as in late carcinogenic processes and finally contributes to metastases (2).

Based on theoretical considerations, anti-angiogenic therapies could target either the VEGF itself by neutralization [Bevacizumab, VEGF-Trap (3)] or inhibition of the external epitope of the VEGF receptor with monoclonal antibodies. Further VEGF signaling can be blocked by VEGFR tyrosine kinase inhibitors (TKI) like sunitinib, stableorafenib, pazopanib, cediranib, axitinib, motesanib and so on (4). In contrast to monoclonal antibodies, these TKIs are not specific for VEGFR 1-3 but also inhibit a plethora of tyrosine kinases and signaling pathways (5,6).

The anti-VEGF monoclonal antibody bevacizumab was the first successfully applied antiangiogenic drug in humans. By testing bevacizumab in advanced non-small-cell lung cancer (NSCLC), the well known Sandler trial proved efficacy in all evaluated end-points including overall survival (OS), progression-free survival (PFS) and doubling of response rates (RR) (7). This study was the basis for the approval of bevacizumab in NSCLC, a new "standard of care". Impressive effects seen in daily routine supported the enthusiasm for this new anticancer strategy (*Figure 1*).

Nevertheless, the challenges, experienced during the clinical development in lung cancer, have to be kept in mind. When bevacizumab was evaluated in a randomized phase II trial in an unselected NSCLC cohort, 4 out of 66 treated patients experienced fatal bleedings (8). By
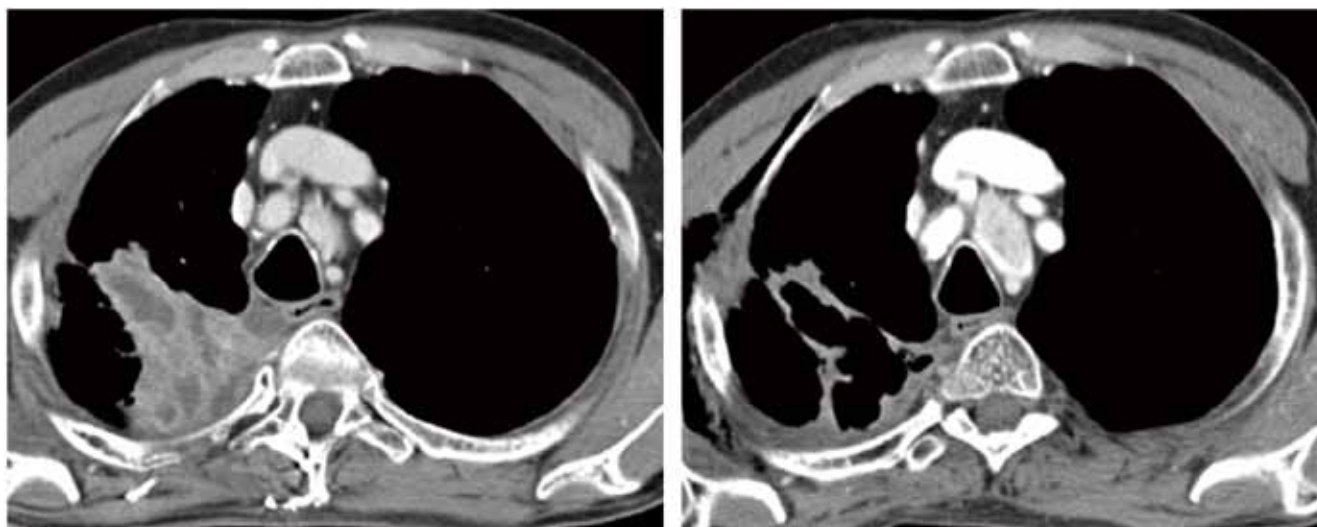
Figure 1 A 72-years old male patient, suffering from advanced NSCLC (adeno-carcinoma). After a pretreatment period of 23 months with erlotinib as monotherapy, bevacizumab was added at the time of progression in January 2009 resulting in a dramatic response within two months

defining a clear risk profile and by excluding "high-risk" patients, bevacizumab application proved to be tolerable in subsequent phase III trials (7,9).

Furthermore, first reports evaluating orally available kinase inhibitors also proved to be effective (10). These first positive results initiated a great engagement of the pharmaceutical industry to develop a number of similar products targeting VEGFR1, 2, 3 or other angiogenic cascades (11) (*Table 1*). However, these optimistic results generated in phase II trials did not translate into positive results in phase III trials. In the following, some critical aspects of the biology of tumor angiogenesis and potential pitfalls of anti-angiogenic drug development are discussed.

Tumor response but no prolongation of overall survival

Throughout the last years, most of the phase III studies evaluating anti-angiogenic drugs failed to prove a survival benefit despite improvement of PFS. A heavily discussed study, for example, is the AVAIL trial testing chemotherapy plus minus bevacizumab (9). By addition of bevacizumab in two different dosages, PFS improved significantly from 6.1 to 6.5 or 6.7 months, the median OS, however, failed to prove a beneficial effect of bevacizumab (13.1 *vs.* 13.6 or 13.4 months).

Afterwards, a series of phase III trials, evaluating kinase inhibitors, vascular disrupting agents or new molecules like the VEGF-trap, failed to improve overall survival despite optimistic results as far as PFS was concerned (21). Why does a drug, which proves efficacy in terms of tumor response or prolongation of PFS, not change patient's outcome? Possible answers for that question are manifold.

Firstly, the design of clinical trials could be an answer. Subsequent lines of treatment, including cross-over to similar products in later lines, might hide the absolute benefit of a drug (22). In that context the establishment of adequate criteria for response is warranted, since standard RECIST criteria measure tumor reduction in diameter but not the development of necrosis (23).

Secondly, the dosage of the investigational drug and the choice of combinational drugs might be other reasons. At the WCLC meeting in 2011, the presenting author of the ATTRACT study (13) therefore suggested that a suboptimal dosage of the investigational drug could be responsible for the negative outcome of the trial.

Thirdly, due to the absence of valuable biomarkers for antiangiogenic drugs (24), a proper selection of patients was impossible.

In the fourth place, another explanation supported by prominent preclinical data suggested that anti-angiogenic drugs might influence the biology of the disease. This

Table 1 Relevant phase III trials introducing anti-angiogenic agents in NSCLC

Drug	Class	Target	Clinical phase	Combination	PFS	OS	Ref (or clin. trials gov.)
Bevacizumab	MoAb	VEGF	III	Carboplatin/PXL	positive	positive	Sandler et al. (7)
			III	Cisplatin/Gemcitabine	positive	negative	Reck et al. (9)
Aflibercept	Soluble decoy receptor	VEGF	III	DXL	positive	negative	Novello et al. (12)
ASA4040	VDA	unknown	III	Carboplatin/PXL	negative	negative	Lara et al. (13)
BIBF1120	TKI	VEGFR-1, 2, 3, FGFR, PDGFR	III	DXL	pending	pending	NCT00805194
			III	Pemetrexed	pending	pending	NCT00806819
Cediranib	TKI	VEGFR-1, 2, 3, c-kit, Flt-3	III	Carboplatin/PXL	pending	pending	NCT00795340
			II/III	Carboplatin/PXL	negative	negative	Goss et al. (14)
Motesanib	TKI	VEGFR-1, 2, 3, PDGFR, RET, kit	III	Carboplatin/PXL	positive	negative	Scagliotti et al. (15)
Sorafenib	TKI	Raf, Kit, Flt-3, VEGFR-2 & 3, PDGFR-β	III	Carboplatin/PXL	negative	negative	Scagliotti et al. (16)
			III	Cisplatin/Gemcitabine	negative	negative	Gatzemeier et al. (17)
			III	Monotherapy	pending	pending	NCT00863746
Sunitinib	TKI	VEGFR-1, 2, 3, PDGFR-α, PDGFR-β, Flt-3, c-kit	III	Monotherapy	pending	pending	NCT00693992
Vandetanib	TKI	VEGFR-2 & 3, RET, EGFR	III	Docetaxel	positive	negative	Herbst et al. (18)
			III	Pemetrexed	negative	negative	De Boer et al. (19)
			III	Monotherapy	positive	negative	Lee et al. (20)

MoAb, Monoclonal antibody; VDA, Vascular disrupting agent; DXl, Docetaxel; PXL, Paclitaxel; TKI, Tyrosine kinase inhibitor

was pointed out by Ebos et Kerbel (21) stating that "Antiangiogenic therapies might initiate an array of stromal and microenvironmental defense mechanisms that contribute to eventual drug inefficacy and, more provocatively, may lead to a more aggressive and invasive tumor phenotype-one with an increased ability to metastasize". Their considerations were based on previous critical publications. In 2009, Ebos et al. (25) showed that sunitinib/SU11248 can accelerate metastatic tumor growth and decrease OS in mice receiving short-term therapy in various metastasis assays. Interestingly, mice, receiving sunitinib prior to intravenous implantation of tumor cells, also experienced an acceleration of metastases, suggesting possible "metastatic conditioning" by VEGFR inhibitors in various organs. At the same time, Paez-Ribes et al. (26) observed increased numbers of metastases in distant organs after VEGF-pathway inhibition in a mouse model. Despite an initial benefit, this mechanism could lead to limited OS benefits.

Complexity of the biology of tumor angiogenesis

Angiogenesis is dependent on a complex network of different cell compartments regulated by a balance of angiogenic and anti-angiogenic factors, which is much more complicated than described in the early days of the development of anti-angiogenic therapies (6). In pathologic angiogenesis the tumor cells themselves produce VEGF and other angiogenic factors such as beta-fibroblast growth factor (bFGF), angiopoietins, interleukin-8 and placental-derived growth factor (PlGF), which leads to an overweight of pro-angiogenic factors promoting the angiogenic switch. These factors stimulate resident endothelial cells to proliferate, loose cell interactions and migrate. An additional source of angiogenic factors is the adjacent tumor stroma, which is a heterogeneous compartment, comprising of fibroblastic, inflammatory and immune cells. Tumor-associated fibroblasts produce chemokines such as stromal cell-derived factor-1 (SDF-1), which may recruit bone-

marrow-derived angiogenic cells (BMC) (27). Tumor cells may also release stromal cell-recruitment factors, such as PDGF-A, PDGF-C or transforming growth factor (TGF). A well established function of tumor-associated fibroblasts is the production of growth factors such as EGFR ligands, hepatocyte growth factors and heregulin. Endothelial cells produce PDGF-B, which promotes recruitment of pericytes in the microvasculature after activation of PDGFR (28). A crucial paper, discussing resistance to a VEGF inhibitor, has been recently published and analyzes the influence of the tumor stroma in the development of resistance against anti-angiogenic therapies (29). They showed, that in a bevacizumab resistant mouse model, multiple genes (components of the EGFR and FGFR pathways) were up-regulated, and most of them occurred predominantly in stromal and not in tumor cells. Similarly, Solinas *et al.* (30) found that alterations of the endothelial microenvironment (e.g., by chemotherapy or radiation) leads to an induction of inflammatory mechanisms which increases the metastatic potential. Others stressed the key role of mast cells (31) which are involved in angiogenic switch, production of pro-angiogenic compounds and the induction of neo-vascularization. These data support the special role of the stromal tissue not only in promoting tumor angiogenesis but also in the development of evasive resistance mechanisms against therapies. From the clinical point it is well known that tumors exposed to antiangiogenic therapies will mostly become resistant thus leading to a re-growth of the tumor [for review see Jubb AM *et al.* (32) and Bergers G *et al.* (33)]. Various mechanisms are being discussed. On the one hand, other angiogenic factors like bFGF or PDGF could be up-regulated; on the other hand tumor vessels could be protected by an increased coverage of pericytes. A third option would be that tumor cells increase their invasiveness by an accumulation of mutations (34). Finally, endothelial progenitor cells, attracted from the bone marrow, could play a role in inducing resistance (35).

The most important trigger of the production of pro-angiogenic factors is the induction of tumor hypoxia. The role of hypoxia has already been elucidated by Carmeliet *et al*. (2). Hypoxic tumor cells switch to a pro-angiogenic phenotype. One key mediator in that regulation is the hypoxia-inducible factor 1 (HIF-1), which is a hetero-dimeric protein that activates the transcription of many genes that code for proteins involved in angiogenesis, glucose metabolism, cell proliferation/survival and invasion/metastasis (2,36). HIFs increase transcription of several angiogenic genes (for example, genes encoding VEGF,

PDGF and nitric-oxide species). HIFs also affect cellular survival/apoptosis pathways. In that particular setting, the role of anti-angiogenic therapies was investigated by M. Franco, showing that they increase the hypoxic tumor fraction (37). After a three-week treatment period using DC101 (VEGFR2 monoclonal antibody) the authors found a reduction in micro-vascular density, blood flow and perfusion, but also an increase in the hypoxic tumor fraction (measured with pimonidazole) and an elevation in HIF-1A expression. Tumors can cope with hypoxia by selection of hypoxia-tolerant clones and more malignant metastatic cells, which are less sensitive to antiangiogenic therapies (38,39). Furthermore, tumor cells might undergo an epithelial-mesenchymal transition to escape hypoxic conditions (39).

Reconsider the clinical development of anti-angiogenic drugs

Facing the complexity of tumor-angiogenesis together with the failure of phase III drug combination studies the traditional pharmaceutical development strategies have to be reconsidered. Planning of clinical trials evaluating anti-angiogenic drugs should consider the following points.

(I) Choice and dose of combinational drugs matter (34,40-42). Evidently, a combination of a platinum plus one of the third generation cytostatics with one of the anti-angiogenic kinase inhibitors does not seem to add any benefit. However, monotherapy with for example sorafenib revealed efficacy in single cases (10). We also learned that chemotherapies such as cyclophosphamide, administered at maximum tolerated doses, can mobilize circulating endothelial progenitor cells, which could contribute to re-growth of the tumor (34,35,40). On the other side metronomic therapy (closely spaced, less toxic doses of chemotherapy) can prevent mobilization of circulating endothelial progenitor cells (41,42).

(II) As a second point, the stage of the disease, in which the clinical trial is performed, could be an essential question. It is known that cancer can develop due to mechanisms evolved by tumors to escape from surveillance of immune cells (43) and that the immune defense mechanism are altered in late stage diseases (44,45). Still, the majority of preclinical studies with anti-VEGF inhibitors were performed in early tumor stages whereas the majority of clinical phase III trials were done in advanced metastatic disease. Preclinical evaluations are dominated by mouse models analyzing early tumor stages with tumor response

or progression as primary endpoints. In the clinical setting, patients are treated in an advanced stage of the disease and the primary end-point has to be overall survival (46). These discrepant stages of disease evaluating different end-points could be responsible for misleading interpretations of results. Therefore, there is an absolute need to develop appropriate cancer models for the development of anti-angiogenic drugs.

(III) Thirdly, every trial using anti-angiogenic drugs should include some kind of biomarker program. Since adequate in vivo models are missing, the biological role of these substances in humans has to be closely monitored (23,24,47). For example, the MD Anderson group around John V. Heymach (47) performed an extensive hypothesis generating biomarker program in 123 patients who were treated in a randomized phase II trial evaluating vandetanib (48). A large number of cytokines and angiogenic factors were evaluated at different days of the treatment and were correlated with progression risk. For example, plasma levels of VEGF increased and soluble VEGFR-2 decreased by day 43. Increase of VEGF was correlated with an increased risk of progression. However, validation of such biomarkers is warranted.

(IV) Finally, cigarette smoke induces oxidative/nitrosative stress, which increases the nitration of tyrosine residues on VEGFR2, rendering it inactive for downstream signaling. Active smoking could be responsible for an endothelial dysfunction (49). Therefore, a stratification of smoking behavior should be included.

In conclusion anti-angiogenic therapies are already used successfully in daily clinical practice. But there are still many questions to be answered about mode of action and optimal use of anti-angiogenic drugs. Further scientific efforts are necessary to analyze signal pathways and regulatory mechanisms which could possibly help to identify new targets and biomarkers. Special attention should be directed to the following points: Pivotal role of hypoxia, modes of resistance/microenvironment, need for optimal (mouse) models, role of cigarette smoke, choice of chemotherapy combination and the stage of the disease, which is evaluated.

Acknowledgements

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