Targeting Angiogenesis for Treatment of NSCLC Brain Metastases

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Abstract: Lung cancer is the leading cause of cancer-related mortality worldwide, and non-small cell lung cancer (NSCLC) accounts for about 85% of all new lung cancer diagnosis. The majority of people with NSCLC are unsuitable for surgery since most patients have metastatic disease at diagnosis. About 60% of brain metastases arise from lung cancer. Therapeutic approaches to brain metastases include surgery, whole brain radiotherapy (WBRT), stereotactic radiosurgery, chemotherapy and new biologic agents. Angiogenesis is essential for the development and progression of cancer, and vascular endothelial growth factor (VEGF) is a critical mediator of tumour angiogenesis. One of the targeted approaches most widely studied in the treatment of NSCLC is the inhibition of angiogenesis. Bevacizumab, an anti-VEGF recombinant humanized monoclonal antibody, is the first targeted agent which, when combined with chemotherapy, has shown superior efficacy versus chemotherapy alone as first-line treatment of advanced non-squamous NSCLC patients. Patients with central nervous system (CNS) metastases have initially been excluded from bevacizumab trials for the risk of cerebral haemorrhage as a result of the treatment. Nevertheless, the available data suggest an equal risk of intracranial bleeding in patients with CNS metastases treated with or without bevacizumab therapy. Several other anti-angiogenetic drugs are being investigated in the treatment of advanced NSCLC patients, but results of their activity specifically in CNS metastases are still lacking.

This review will focus on the potential role of bevacizumab and other anti-angiogenetic agents in the treatment of brain metastases from NSCLC.

Keywords: Angiogenesis, bevacizumab, brain metastases, intracranial bleeding, NSCLC, VEGF, small molecules.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the developed world in both men and women [1]. Non small cell lung cancer (NSCLC), including squamous carcinoma, adenocarcinoma and large cell carcinoma, accounts for more than 80% of all lung cancer types. The majority of patients diagnosed with NSCLC have advanced disease at diagnosis. In this setting of disease, the optimum management is still being determined, however, chemotherapy and/or radiotherapy represent the cornerstone of treatment.

The development of brain metastases is a common complication in lung cancer. Brain metastases arise in approximately 25-30% of patients with NSCLC and frequently are the first site of recurrence in early-stage NSCLC patients treated with definitive loco-regional therapies. Once metastasis to the brain is diagnosed, the median overall survival (OS) of untreated patients is 1-2 months.

Generally, whole-brain radiation therapy (WBRT) and steroids are the standard treatment for patients with multiple brain metastases, producing a symptomatic relief especially of headache and seizures in 75-80% of patients. The outcome after WBRT depends on different factors, like number and size of brain metastases and the performance status (PS). The commonly used radiation doses for WBRT are 30-40 Gy [2, 3].

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Radiosurgery or neurosurgical resection are indicated in solitary or oligometastatic disease. Stereotactic radiotherapy involves the delivery of a single high-dose fraction of external radiation to a targeted lesion in the brain using multiple cobalt sources (gamma knife), modified linear accelerator (LINAC) or cyber knife. It has a potential to achieve high local control and is essentially used as a substitute for surgical treatment in patients with lesions smaller than about 3 cm in diameter. This stereotactic radiotherapy is ideal to target, being small, spherical, and well defined with distinct margins on contrast enhancement. These characteristics help to achieve conformal dose distributions with minimal damage to surrounding tissues and target the areas where surgical resection is not possible.

The combinations of radiosurgery (stereotactic), neurosurgery, and/or WBRT can increase efficacy of treatment, however the OS can be extended only to 4-6 months [4, 5].

The prognosis for patients with brain metastases is poor, and it is due primarily to chemotherapy resistance considering that cytotoxic agents may not cross the blood brain barrier (BBB) efficiently. The recurrence at the site of brain metastasis resection as well as the development of metastases in other areas of the brain are frequently reported [6]. In fact, only a minority of patients have a long term control of intracranial disease after treatments.

Central nervous system (CNS) bleeding in patients with brain metastases is a serious complication that primarily occurs at sites of intracranial lesions. The rate of the bleeding is different across the types of tumour: a higher incidence was reported in thyroid cancer, melanoma (40-

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50%) and renal cancer (70%), a lower one in lung cancer (1-5%) [7].

Tumour angiogenesis is an important process in cancer development, invasion, progression and metastasis, and vascular endothelial growth factor (VEGF) is a critical mediator in physiological and pathological angiogenesis [8]. The inhibition of tumour-related angiogenesis has become an attractive target for anticancer therapy. The anti-VEGF therapy has demonstrated clinical benefit in several clinical trials involving different solid tumour types. However, an infrequent complication of this treatment is haemorrhage at tumour site or at distant sites [9]. Patients with brain metastases are frequently excluded from clinical studies of anti-VEGF therapy, due to the possibility of intracranial haemorrhage as a result of treatment.

To date, several antiangiogenetic agents have been investigated in advanced NSCLC treatment, however, to date, negative results are more commonly reported than positive ones.

This review will focus on the potential role of bevacizumab and other anti-angiogenetic drugs in the treatment of brain metastases NSCLC patients.

PATHOGENESIS OF BRAIN METASTASES

The outcome of the metastatic process depends on multiple and complex interaction of metastatic cells with host homeostatic mechanisms. Since 1889 the hypothesis (seed and soil) that the non random pattern of metastasis was not due to chance but, rather, that certain tumour cells (the "seed") had a specific affinity for the milieu of certain organs (the "soil") has been formulated [10]. Later, it has been proposed that spreading of metastatic cells may be depend on merely mechanical factors resulting from the anatomical arrangement of the vascular system [11].

To date, this hypothesis includes many principles: primary tumours and metastatic lesions are biologically heterogeneous and include multiple cell populations with different characteristics such as growth rate, cells surface receptors, sensitivity to cytotoxic agents, and angiogenic potential [12]; Metastatic spread is highly selective for cells that can complete all the step of metastatic process [13,14]; Metastases can have a clonal origin, and different metastases can originate from the proliferation of different single cells [15]; Finally, the outcome of metastasis is based on several interactions of metastatic cells with host homeostatic mechanisms that comprise the organ microenvironment which tumour cells use for their own growth [16].

The brain is a unique target organ because it is surrounded by the BBB and lacks lymphatic drainage. The microenvironment of the brain differs from that of other organs such as lung, liver, or bone. It is immersed in an interstitial fluid that has a high content of chloride, an environment that may not be conducive to many potential metastatic clones from cells of epithelial origin. It may be that the unique milieu of the brain attracts cells of neuroepithelial origin, such as small cell carcinoma of the lung or melanoma [16].

Experimental murine models were used to study the biology of lung, breast, melanoma and other cancer types of brain metastases [17]. The injection of metastatic cells into the internal carotid artery of mice simulates the hematogenous spread of tumour emboli to the brain and the release of tumour cells into the circulation. Tumour cells elongate their shape along the vessels, adhere to the vascular basement membrane *via* β1 integrins, and proliferate invading the vessel wall and on the top of the vascular basement membrane, they arrest in capillaries, penetrate and extravasate into the brain parenchyma with growing of tumour cells in the brain tissue [18]. Similar results were reported for a brain-tropic Lewis lung carcinoma line early after carotid injection, which was followed by a brain parenchymal growth pattern [19].

The microvasculature of the brain parenchyma is lined by BBB which is a peculiar anatomical structure that is mainly composed by tight junctions and adheres junctions between the brain endothelial cells, which regulate the flow of ions, nutrients, and cells into the brain [20].

The BBB, includes cerebral microvascular endothelium, which together with neurons, astrocytes, pericytes, and the extracellular matrix, constitute a "neurovascular unit". The endothelial cells of the BBB express a plethora of active transporters. Together, these transporters act as efflux pumps to send substances out of endothelial cells and back into circulation, away from the brain parenchyma. Most standard chemotherapeutics have been shown to be substrates of one or more of the active efflux transporters. The resistance of primary brain tumours and brain metastases to be treated by most chemotherapeutic drugs may be explained by two possible reasons: first, metastatic tumour cells in the brain are more resistant to chemotherapy than systemic metastases, implying that resistance may result from their late development after multiple rounds of prior chemotherapies, and could reflect accumulated mutations; Second, the remnants of the BBB prohibit adequate amounts of chemotherapy from reaching the metastases [21, 22]. The brain metastasis research field has debated the extent to which metastasis disrupts the BBB. Imaging studies, showing a greater uptake of contrast agents in brain metastases compared with surrounding brain tissue, have suggested that the barrier is open, whereas chemotherapeutic efficacy data suggest that, if the barrier is open, it is not open enough to permit sufficient drug accumulation. It is also not clear whether the pharmacokinetics of drug uptake into primary brain tumours are identical to those of brain metastases. Recent pharmacokinetic studies of two experimental brain metastasis models revealed that, although most metastases have some increased permeability compared with normal brain, heterogeneous uptake levels can occur, and only 10% had sufficient permeability to show a cytotoxic response to chemotherapy [23].

The integrity of the BBB is altered in primary brain tumours and in metastases and the BBB in metastases that are larger than 0.25 mm in diameter is leaky [24].

In the CNS, activated glial cells are involved in the innate immune response and generate different inflammatory mediators such as a chronic inflammatory reaction. A similar mechanism might support survival, growth, proliferation,

colonization, invasion and motility of metastatic tumour cells in the microenvironment of brain metastases [25]. Among the glial cells, astrocytes are the most copious cell population and play an essential role in maintaining homeostasis of the brain. In response to brain injury, astrocytes are activated and recruited to form a glial scar in the site of injury [26], and they can also protect neurons inducing apoptosis [27].

Histological analysis of resected human brain metastases revealed tumour cells interdigitated with activated microglia and astrocytes. Activation of astrocytes and microglia is widely evident around experimental brain metastases. Both *in vitro* and *ex vivo* studies support a functional interaction of cancer cells and the neural microenvironment. Astrocytes can enable the growth of brain-tropic tumour cell lines in co-culture experiments.

An interaction between astrocytes and tumour cells has been demonstrated. Lung tumour cells stimulate astrocytes by releasing interleukin 8 (IL-8), MIF, and PAI-1, then activated astrocytes stimulate the proliferation of tumour cells by releasing cytokines such as tumour necrosis factor-α (TNF-α), IL-1β, and IL-6. The reactive astrocytes can also provide neuroprotective properties on protecting tumour cells from cytotoxicity induced by chemotherapeutic drugs through direct physical contacts. The interaction between metastatic tumour cells and activated astrocytes are important to determine a favourable brain microenvironment for the tumour cells [28].

In order to identify pathways specific to the development of brain metastases and novel molecular targets/translational approaches, several groups have undertaken gene expression studies in animal models and human tissue cohorts.

During the metastatic processes to the brain, gene expression changes were observed comparing tissue specimens containing the primary tumour with a surgically resected brain metastasis from the same patient. Differences were reported in the expression of stem cell markers, receptor tyrosine kinases, hormone receptors, cyclooxygenase 2, proteins involved in apoptosis and DNA repair enzymes [29-31]. Many of these genes had previously been implicated in metastases to other organs, suggesting that brain colonization results from both general and site-specific metastatic pathways.

In lung cancer, the activation of the Wingless-type (WNT) pathway has been linked to bone and brain metastases. Binding of WNT ligands to their receptor stabilizes β-catenin (encoded by *CTNNB1*), which binds to the transcription factors of the lymphoid enhancer-binding factor (LEF) transcription factor (TCF) family. A TCF-related gene signature predicted lung cancer metastases-free survival but not breast cancer metastases-free survival. Expression of dominant-negative TCFs inhibited the brain and systemic metastases of lung cancer cell lines, and was mediated by alterations in LEF1 and homeobox protein HOXB9 [32].

Cytokines and their signalling pathways participate in metastatic colonization in the brain. Transforming growth factor-β (TGF-β) is a cytokine that has been widely reported to inhibit the initiation of tumorigenesis but to also stimulate tumour progression and metastases.

The STAT signalling pathway, which is downstream of many cytokines, was activated in brain metastases. Transfection of STAT3 into A375 brain-tropic tumour cells increased the incidence of brain metastases, as well as their blood vessel density, and decreased the survival of the injected animals [33].

The cadherins are a family of Ca2+ dependent cell-cell adhesion molecules and are involved in multiple processes inducing invasion and migration and promoting survival of cancer cells [34].

In a cohort of primary tumours from lung cancer patients who developed brain metastases CDH2 (N-cadherin) has been found to be significantly increased ($p = 0.009$) [35]. An N-cadherin antagonist, a polypeptide known as ADH-1 or Exherin, is currently being investigated in clinical trials.

VASCULAR SYSTEM IN BRAIN METASTASES

Irrespective of the origin of the tumour cells, the nearness to vascular supply, is an important determinant that governs its progression and survival. Data derived from examinations of human lung cancer brain metastases suggest that tumoral cell division takes place within 75 micron of the nearest blood vessel, while tumour cells residing beyond 150 micron from a vessel undergo programmed cell death. These evidences concord with the diffusion coefficient of oxygen in tumour tissue, which is approximately 120 micron [36].

Tumour growth is preceded by the development of new blood vessels, a process known as angiogenesis which provides a pathway for metastasis and nutrients essential for growth. The onset of angiogenesis within small clusters of tumour cells, known as the "angiogenic switch", is influenced by a complex interplay of proangiogenic molecules, such as VEGF and the angiopoietin family and antiangiogenic molecules, such as angiostatin [37, 38].

Normal tissues are exposed to a great number of inhibitor factors that maintain the vascular endothelium in a non proliferating state. The activation of the angiogenic switch may occur at any stage of tumour development; however, the beginning is usually correlated with increasing metabolic pressures, oncogenes activation, or mutation of tumour suppressor genes [39].

Angiogenesis can occur by either sprouting or nonsprouting processes. Sprouting angiogenesis occurs by branching (true sprouting) of new capillaries from pre-existing vessels. No sprouting angiogenesis results from the enlargement, splitting, and fusion of pre-existing vessels produced by the proliferation of endothelial cells within the wall of a vessel [40].

In experimental brain metastases and surgical specimens of human lung cancer, a density of blood vessels lower than in the adjacent tumour-free brain parenchyma has been observed. Blood vessels associated with brain metastases have dilated lumen and this vessel dilation did not occur merely by stretching of the blood vessel wall but rather as a consequence of endothelial cell division within the wall of the blood vessel [36].

VEGF AND VEGFR PATHWAY

The VEGF-related gene family comprises six angiogenic and lymphangiogenic secreted glycoproteins growth factors referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placenta growth factor (PlGF)-1 and PlGF-2. VEGF-A, commonly named as VEGF, is a 45-kDa homo-dimeric glycoprotein with a diverse range of angiogenic activities. VEGF signal is mediated by two identified vascular endothelial growth factor receptors (VEGFRs): fms-like tyrosine kinase-1 (Flt-1) and fetal liver kinase-1/kinase domain region (Flk-1/KDR). A tyrosine kinase receptor, VEGFR-3 also referred to as fms-like tyrosine kinase 4 (Flt-4), was activated by VEGF-C, VEGF-D and has been found to be primarily associated with lymphangiogenesis. These receptors are localized predominantly on vascular endothelial cells and contain seven immunoglobulin (Ig)-like domains in the extracellular region, a single transmembrane region, and a tyrosine kinase domain [41].

The binding of VEGF to VEGFR-2 leads to a cascade of different signalling pathways. This receptor mediates the biological action *via* the phospholipase C (PLC), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) pathway. The PLC directly binds to the autophos-phorylate VEGFR-2, then tyrosine-phosphorylated and is activated. The activated PLC stimulates the PKC activation and activated Raf-1 to MAPK cascade. This signalling transduction pathway results in up-regulation of genes involved in mediating the proliferation and migration of endothelial cells and promoting their survival and vascular permeability [41].

VEGF gene expression is regulated by several stimuli, including: hypoxia; growth factors as well as the platelet derived growth factor (PDGF), the basic fibroblast growth factor (bFGF), the epidermal growth factor (EGF), the tumour necrosis factor (TNF), TGF-β, and IL-1; tumour suppressor genes such as p53; oncogenes such as K-Ras, H-Ras, v-src; nitric oxide, HER-2, HER1/EGFR, and FOS. Moreover, tumour-derived VEGF regulates the function of tumour cells *via* autocrine signalling [41, 42].

Activation of the VEGF/VEGFR axis triggers several signalling pathways that result in a number of different effects on the vascular endothelium and endothelial cells. One of the most important properties of VEGF is to enhance microvascular permeability, especially with regards to the hyperpermeability of tumour vessels. VEGF is also an important mitogen for endothelial cells, the cells proli-feration appears to involve VEGFR-2 mediated activation of extracellular kinases Erk1/2 in addition to another member of the MAP kinase family, JNK/SAPK [43]. Other effects include changes in endothelial cell morphology, cytoskeleton alterations, and stimulation of endothelial cell migration and growth [44].

Several studies suggest that VEGF is also important for BBB functioning. VEGF upregulates ICAM-1 *via* phosphatidylinositol 3 OH-kinase/AKT/Nitric oxide pathway and modulates the migration of brain microvascular endothelial cells (BMECs), which are the major cellular constituent of the BBB [45]. VEGF secreted from cancer cells significantly increases the adhesion and penetration of tumour cells across the BMECs monolayer, *via* changes of VE-cadherin and also regulates focal adhesion assembly in BMECs through activation of FAK and RAFTK/Pyk2 [46]. Further, VEGF upregulates the expression of $\alpha 6$ integrin and increases the $\alpha 6\beta 1$ integrin expression in BMECs which is important for VEGF induced adhesion and migration, angiogenesis and tumour angiogenesis [47].

An important step in the initiation of angiogenesis is the degradation of the basement membrane which is necessary for endothelial cell migration and invasion. VEGF induces several enzymes and proteins involved in the degradation process, including matrix-degrading metalloproteinases, metalloproteinase interstitial collagenase, and serine proteases such as urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (TTPA) [48, 43]. The migration and sprouting of endothelial cells were facilitated by a prodegradative microenvironment due to the activation of these compounds.

Matrix metalloproteinases (MMP) are a family of glycoproteins that utilize zinc at the catalytic site. These proteins increase the invasive and metastatic potential of tumours through interaction with the extracellular matrix and angiogenesis. They are secreted as zymogens and are activated by proteinases within conserved and terminal region [49]. MMP play an important functional role in many normal physiological activities such as tissue remodelling, reproduction, and organogenesis and their activity has been found to correlate with invasiveness, metastasis, and poor prognosis in a variety of metastatic tumours including lung cancer [50-51].

MMPs and inhibitors of matrix metalloproteinases (TIMPs) also appear to be regulators of the angiogenic process, both by releasing proangiogenic peptides from the ECM and degrading the ECM to allow for the ingrowths of new blood vessels. The evidence that MMPs might have a role in angiogenesis emerged from observations that TIMPS, as well as synthetic MMP inhibitors, can greatly reduce the extent of endothelial cell tube formation *in vitro* [52]. MMPs have been shown to promote endothelial cell migration and may also function to trigger the angiogenic switch. MMP9 brings about the release of VEGF from the matrix necessary for the switch to an angiogenic phenotype [53]. Pre-clinical data suggest that tumours expressing MMP2 have more proliferating vasculature at the tumour-brain interface than MMP2-negative tumours, implicating a role for MMP2 in the stimulation of angiogenesis. As the tumour advances to invade the adjacent brain and elaborates new vasculature to sustain itself, it appears likely that MMP2 expression may be a key player in this process, enhancing both invasion and vascularisation [54].

In the last years, targeting the VEGF and its receptors has played a central role in advancing lung cancer research, treatment and patients outcome. Anti-VEGF strategies under investigation include monoclonal antibodies (mAbs) binding VEGF and small molecules which inhibit the corresponding receptor tyrosine-kinase activity (TKIs). Other antiangio-genic drugs, such as VEGF-Trap and cilengitide are being tested in ongoing clinical trials which will further define their role in the management of NSCLC.

Bevacizumab

Bevacizumab is a humanized mAb directed against the VEGF. Bevacizumab consists of 93% human and 7% murine components and it recognizes all isoforms of VEGF ligands with Kd of 8 $x10^{-10}$ M. It contains two identical light chains (214 amino acid residues) and two heavy chains (453 residues) with a total molecular weight of 149 kDa. The heavy chains demonstrate C-terminal heterogeneity (lysine variants) and also contain one N-linked glycosylation site at asparagines 303. The oligosaccharides consist of complex biantennary structures with a fucose core and with the two branches terminating mainly with zero (G0), one (G1) or two (G2) galactose residues. The G0 glycoform predominates with an 80% relative abundance. Each light chain is covalently coupled through a disulfide bond at cysteine 214 with a heavy chain at cysteine 226. The two heavy chains are covalently coupled with each other through two inter-chain disulfide bonds, which is consistent with the structure of a human IgG1. Bevacizumab exerts its antiangiogenic effects by binding to free circulating VEGF, thereby inhibiting the binding of VEGF to its receptors, preventing VEGF ligand receptor downstream signalling. The molecule has the same biochemical and pharmacologic properties as the natural antibody, but with reduced immunogenicity and a longer biological half-life [55].

Based on the results of two randomized phase III trials, bevacizumab is currently licensed for use in combination with carboplatin plus paclitaxel for the first line therapy at the dose of 15 mg/kg in United States, or in addition to platinum based chemotherapy in Europe at the dose of 7.5 or 15 mg/kg in patients with advanced non-squamous NSCLC. The first multicenter phase III clinical trial (ECOG 4599) evaluated bevacizumab plus carboplatin and paclitaxel (BCP, patients = 434) versus carboplatin and paclitaxel alone (CP, patients = 444) in advanced chemo-naive non squamous NSCLC patients. OS was significantly longer in patients receiving BCP compared to those treated with chemotherapy alone (12.3 versus 10.3 months, respectively; hazard ratio [HR] 0.80, $p = 0.003$); progression free survival (PFS) was 6.2 and 4.5 months (HR 0.66; $p < 0.001$) in the two treatment arms, with a corresponding response rate (RR) of 35% and 15%, respectively ($p < 0.001$). The addition of bevacizumab to chemotherapy resulted globally well tolerated, except for the rate of clinically significant bleeding which was 4.4% and 0.7% respectively ($p < 0.001$) [56].

The restriction of the patients population to non-squamous histology is based on life-threatening or fatal haemoptysis, which occurred in 4 of 13 patients with squamous histology who received the BCP regimen in a previous randomized phase II study [57].

Another phase III trial, AVAiL, evaluated the combination of bevacizumab (15 or 7.5 mg/kg every 3 weeks) to gemcitabine/ cisplatin chemotherapy versus the same chemotherapy regimen alone in previously untreated advanced non-squamous NSCLC patients. In this trial, not powered to compare the two doses directly, a significantly longer PFS, the primary study endpoint (6.1 in the control arm, 6.7 [$p = 0.002$], and 6.5 months [$p = 0.03$] in 7.5 mg/kg, and 15 mg/kg bevacizumab arms, respectively), and a higher RR (20%, 34%, and 30.4%, in the control, 7.5 mg/kg, and 15 mg/kg bevacizumab arms, respectively), were observed in patients randomized to receive bevacizumab therapy [58]. OS did not significantly increase in the two bevacizumab arms (7.5 mg/kg group: HR 0.93; $p = 0.420$ and 15 mg/kg group: HR 1.03; $p = 0.761$) compared to the placebo group, in fact OS was > 13 months in all treatment groups [59].

Patients with brain metastases have previously been excluded from clinical trials of bevacizumab following one case of a fatal cerebral haemorrhage from a previously undiagnosticated brain metastasis reported in hepatocellular carcinoma (HCC) patient treated with bevacizumab in a phase I trial [60]. However, CNS metastases from HCC have an intrinsic susceptibility to bleed because HCC patients are likely to have coagulopathy due to compromised liver function, resulting in a higher incidence of cerebral haemorrhage independent of the type of therapy received [61].

The ECOG 4599 trial excluded patients with a history of brain metastases, so there is a lack of documented experience in this subset of patients, and a lack of information about safety events in this increasing treatment setting. In this trial, three of 427 (0.70%) patients treated with bevacizumab experienced CNS haemorrhages compared to one of 441 (0.23%) patients treated with carboplatin/paclitaxel alone. Similarly, in the AVAiL trial which also excluded patients with brain metastases, three of 659 (0.45%) patients who received bevacizumab experienced CNS haemorrhage, compared with two of 327 (0.61%) patients who received placebo. The incidence of CNS haemorrhages among patients with NSCLC who received bevacizumab in these two phase III trials was similar to incidence of those reported in patients who did not receive bevacizumab. There was no association between CNS haemorrhage and on-study progression to symptomatic CNS disease.

In a multicenter, open-label, single-arm study, SaiL, the safety and efficacy of adding bevacizumab to first-line chemotherapy in a daily oncology practice population, has been assessed. This study showed that bevacizumb-based therapy resulted in a median OS of 14.6 months with a disease control rate (DCR) of over 88% and median time to disease progression (TTP) of 7.8 months. Efficacy was generally similar across chemotherapy regimens, with the exception of patients who received non-platinum doublets or monotherapy who had slightly lower median OS than patients treated with other regimens. The results of SAiL further document, in a real-world population, the safety and efficacy outcomes seen in phase III clinical trials of bevacizumab in this setting of disease. The presence of CNS metastases, even if previously treated, was an exclusion criteria, hence the specific safety of bevacizumab was assessed in patients who either developed CNS metastases during treatment or had occult CNS metastases at study entry. A low incidence of CNS bleeding was reported in the SAiL study. Of the 281 patients evaluated as having CNS metastases during the course of the study, five (2%) had CNS bleeding. Grade ≥ 3 CNS bleeding was reported in two (< 1%) of 2,212 patients enrolled in this trial [62].

A prospective phase IV clinical trial, ARIES, evaluated the efficacy and safety of first-line chemotherapy combined with bevacizumab in a real world setting of advanced non-

squamous NSCLC patients. This trial enrolled patients generally excluded from or under-represented in other clinical trials such as elderly, poor PS, or CNS metastatic patients. The median PFS and OS were 6.7 and 13.6 months, respectively, and were similar across all subgroups except for patients with PS ≥ 2. The rate of adverse events across subgroups were also similar to the overall cohort except for increased arterial thromboembolic events (ATE) in elderly (≥ 70 years old) and poor PS (PS ≥ 2) patients. The incidence of grade 3 to 5 bleeding events was 3.4% in all NSCLC patients. At baseline 150 patients (7.6%) had brain metastases and were generally treated with non platinum-containing chemotherapy and pemetrexed. In this subgroup the median PFS and OS were 6.0 and 11.7 months, respectively, and no grade 3 to 5 CNS haemorrhage were observed. These results suggest that patients with CNS metastases should not be necessarily excluded from bevacizumab treatment [63].

NSCLC patients with a history of treated CNS metastases were included in two prospective trials to evaluate specifically the safety of adding bevacizumab to chemothetapy in this population. The PASSPORT trial is a phase II single-arm study of bevacizumab in combination with first- or second-line therapy in non-squamous NSCLC patients with treated CNS metastases. Patients received as first-line bevacizumab (15 mg/kg) every 3 weeks with platinum-based doublet chemotherapy or erlotinib, and as second-line bevacizumab with single-agent chemotherapy or erlotinib, according to institutional standards. Patients with CNS metastases were allowed to enter the trial after previous treatment with WBRT and/or neurosurgery. After treatment of CNS metastases, the absence of progression or CNS haemorrhage had to be confirmed clinically and by magnetic resonance imaging or computed tomography. Concomitant treatment with low-dose aspirin, therapeutic heparin/ warfarin, and corticosteroids was permitted. The primary objective was to assess the rate of grade ≥ 2 symptomatic CNS haemorrhage during bevacizumab therapy. The majority of patients (80.0%) received WBRT for the treatment of brain metastases with or without radiosurgery and/or neurosurgery. Of the 115 enrolled patients as first line therapy 66/76 patients received carboplatin-based chemotherapy, while 3 were treated with erlotinib. As second line 22/39 patients received pemetrexed, and 9 erlotinib. Among 106 evaluable patients the median number of bevacizumab cycles received was five and about 24.5% of patients discontinued bevacizumab therapy because of any adverse event. No grade 1 to 5 CNS haemorrhage among patients who received bevacizumab-based therapy were reported. However, two grade 5 adverse events related to bevacizumab were reported; both were pulmonary haemorrhages, one occurring during treatment and the other occurring 6 weeks after the data cut off. The present study suggest that bevacizumab treatment can be administered with acceptable risks of intracerebral haemorrhages [64].

The phase III study, ATLAS, was designed to evaluate the combination of bevacizumab/erlotinib versus bevacizumab/placebo as maintenance therapy after 4 cycles of induction platinum-containing chemotherapy plus bevacizumab as first-line treatment in advanced NSCLC patients. A median PFS of 4.8 months for the combination treatment and 3.7 months for the bevacizumab/placebo group (HR 0.722; p = 0.0012) was reported. Because a statistically significant improvement in efficacy was found in the erlotinib group, the trial was stopped early [65]. About half of the patients in each arm went on to receive subsequent therapy. An equal percentage (39.7%) of patients in the bevacizumab/erlotinib arm and the bevacizumab/placebo arm received subsequent therapy with an EGFR-TKI. OS was not significantly different between patients who received bevacizumab/placebo and those treated with bevacizumab plus erlotinib [66]. In this trial patients with a history of CNS metastases were eligible for enrolment if they had received treatment with WBRT and/or neurosurgery while patients with an ongoing requirement at screening for dexamethasone treatment or cronic therapeutic warfarin were excluded. Among 25 CNS metastases evaluable patients, one grade 2 CNS bleeding was observed in a patient on post-progression therapy after 14 cycles of bevacizumab and patient's site of disease progression was CNS metastases [67].

A placebo-controlled, randomized, double-blind phase III study, BeTa Lung, evaluated the addition of bevacizumab to erlotinib for the second-line treatment of advanced NSCLC patients. A total of 636 patients were randomized to receive bevacizumab in combination with either erlotinib or erlotinib alone. The primary endpoint was not met, with median OS being similar in both arms of the study. However, the addition of bevacizumab to erlotinib increased PFS compared to erlotinib alone (3.4 versus 1.7 months, respectively; HR 0.62) and RR suggested some clinical activity of bevacizumab and erlotinib. The overall safety profile was consistent with known profiles of erlotinib and bevacizumab. This trial included patients with brain metastases previously treated with WBRT and neurosurgery/ stereotactic radiosurgery were permissible in addition to WBRT. Among 68 CNS metastases patients 37 received erlotinib plus bevacizumab and 31 erlotinib alone. No CNS haemorrhage or grade > 3 bleeding events while three grade 3-4 nervous system adverse events were reported in each arm. These data suggest an acceptable safety profile of this combination for the subset of brain metastases NSCLC patients [68].

However, the efficacy and safety of bevacizumab used with therapeutic intent for active brain metastases, as opposed to treated and inactive CNS disease, is unknown. A retrospective analysis of patients treated with chemotherapy regimens containing bevacizumab for active (treatment-naive or progressive) CNS metastases from NSCLC has been recently reported. The toxicity reported was consistent with expected events from bevacizumab, but there was no grade 2 CNS haemorrhage despite a prior history of spontaneous haemorrhage (2 patients) and therapeutic anticoagulation (3 patients). A grade 1 CNS asymptomatic intra-tumoral haemorrhage occurred in one of three patients receiving concurrent anticoagulation. RR was 33% with 2 CNS partial response, a stable disease (SD) occurred in three patients and the DCR was 83%. Bevacizumab should be used with caution in patients with active CNS metastases pending additional safety data [69]. Table **1** summarises the results concerning the use of bevacizumab in the treatment of CNS metastases from NSCLC.

Table 1. Bevacizumab in the treatment of CNS metastases from NSCLC.

Study	Type of analysis	Therapy	Line of therapy	N.pts	Characteristics of CNS metastatic pts	Comments
SAiL [62]	Retrospective	BV+CT	1st	281	Pts who either developed CNS metastases during treatment or had occult CNS metastases at study entry	5 pts had CNS bleeding
ARIES [63]	Prospective	BV+CT	1st	150	Brain metastases at baseline	Median PFS and OS were 6.0 and 11.7 months, respectively and no grade 3 to 5 CNS haemorrhage
PASSPORT [64]	Prospective	BV+CT or BV+ERL	1st 2nd	115	Pretreated CNS metastases with WBRT and/or neurosurgery	No grade 1 to 5 CNS haemorrhage
ATLAS [65, 66]	Retrospective	BV+ERL vs BV+Placebo	Maintenance	25	Pts with a history of CNS metastases pretreated with WBRT and/or neurosurgery	1 grade 2 CNS bleeding
BeTa Lung [68]	Retrospective	BV+ERL vs ERL	2nd	37	Brain metastases previously treated with WBRT and neurosurgery/stereotactic radiosurgery were permissible in addition to WBRT	No CNS haemorrhage
De Braganca [69]	Retrospective	BV+CT	1st 2nd	6	Active CNS metastases	A grade 1 CNS asimptomatic intra-tumoural haemorrhage occurred in one pts

CNS: central nervous metastases; NSCLC: non-small cell lung cancer; N.pts: number of patients; BV: bevacizumab; CT: chemotherapy; ERL; erlotinib; WBRT: whole brain radiotherapy.

An ongoing phase II trial, BRAIN, will assess the efficacy and safety of bevacizumab combined with first line paclitaxel-carboplatin (cohort 1) or second line erlotinib (cohort 2) in non-squamous NSCLC patients with asymptomatic untreated brain metastasis.

Additional safety data concerning the use of bevacizumab in this setting of patients result from a retrospective analysis of several clinical trials. This analysis, including more than 12,000 advanced/metastatic breast cancer, NSCLC, renal and colorectal cancer patients with treated CNS metastases, from 13 phase II/III randomized controlled trials, 2 open-label single-arm safety studies, and 2 prospective studies has been conducted to assess if patients with CNS metastases are at increased risk of cerebral haemorrhage when treated with bevacizumab. The safety results showed that the risk of cerebral haemorrhage is not increased in these patients, suggesting that the administration of bevacizumab should no longer be contraindicated based solely on the presence of CNS metastases. The rate of cerebral haemorrhage in the bevacizumab-treated group was 3.3% compared to 1.0% in the group no treated with bevacizumab, and the mortality rate was similar in both the bevacizumab and the control arm [70].

Further data supporting the use of bevacizumab in the treatment of high-grade gliomas, have called into question the exclusion of patients with CNS metastases from bevacizumab clinical trials. These tumours are highly vascularised compared to CNS metastases, and bevacizumab in this condition could precipitate cerebral haemorrhage; however the results of several clinical trials does not show an increased risk of cerebral haemorrhage (approximately 3%) in patients treated with bevacizumab [71, 72].

Based on these data, the European Medicines Agency (EMA), removed the contraindication concerning the use of bevacizumab in untreated CNS.

OTHER ANTIANGIOGENIC AGENTS IN PRECLINICAL AND CLINICAL DEVELOPMENT

Many different approaches targeting VEGF have been investigated as anticancer strategies, including neutralizing antibodies to the protein itself, antibodies that bind to VEGFR-2 thus blocking activation by the endogenous VEGF ligand, and orally active small molecules that block the kinase activity of the VEGFRs.

Several clinical trials have been launched to determine the safety and efficacy of various antiangiogenic agents in combination with either radiation therapy or single agent chemotherapy for the treatment of new or progressive brain metastases from solid tumours.

Sunitinib

Sunitinib is an oral TKI targeting a number of ligands involved in tumour proliferation and angiogenesis, including VEGFR-1, -2, and -3, PDGFR-α and -β, Flt3, c-kit. In preclinical study, sunitinib has demonstrated antitumor activity in several tumour cell lines, including NSCLC [73, 74]. Sunitinib has been tested in several clinical trials showing single agent antitumor activity in refractory NSCLC patients [75]. A phase II trial has recently evaluated the antitumor activity and safety, including risk of intracranial haemorrhage (ICH) associated with focal neurological deficit, of sunitinib in patients with pretreated NSCLC and irradiated brain metastases. The primary endpoint was PFS. A total of 64 patients received sunitinib 37.5 mg on a continuous daily dosing schedule. Median PFS and OS were 9.4 and 25.1 weeks. Serious neurologic adverse events occurred in six patients (9%), and none were treatment-related. No cases of ICH were reported [76].

The distribution of sunitinib and its active metabolite in brain and spinal cord tissue following oral or intravenous

administration in rodents and monkeys was demonstrated [77]. Sunitinib or its metabolite penetrated the CNS of monkeys with rapid clearance, but does not appear to accumulate. This result might suggest the potential antitumor activity of sunitinib in the brain.

Sunitinib is actually under investigation in combination with stereotactic radiosurgery for the treatment of brain metastases from several tumour types.

Cediranib

Cediranib is an orally active, highly potent inhibitor of the VEGFR-1, -2 and -3 tyrosine kinases. Using *in vivo* models, cediranib prevents both physiological and pathological angiogenesis and can constrain the growth of histologically diverse tumour xenografts when administered chronically [78]. Cediranib is currently under investigation in NSCLC within phase II/III clinical trials. The therapeutic potential of combining cediranib with radiotherapy has been explored in preclinical trials. Inhibition of VEGF signalling using cediranib enhances radiation response and causes substantial physiological changes in lung tumour xenografts. Tumour regression post radiotherapy was associated with high levels of apoptosis and necrosis, and pronounced antivascular effects in histological samples [79]. These data support the clinical investigation of cediranib in combination with radiotherapy considering that ablation of VEGFR-mediated signalling may have beneficial effects on the tumour response, in patients with brain metastases of several solid tumours.

Vatalanib

Valatinib is a VEGFR, PDGFR, and KIT inhibitor that is currently being studied in phase II/III trials. Data from a phase II trial examining vatalanib monotherapy administered once or twice daily in previously treated NSCLC patients have been reported. Single-agent treatment appeared active, with a trend toward greater efficacy with twice-daily treatment. Additionally, treatment was well tolerated, with no apparent differences between once- and twice-daily dosing [80]. The efficacy of vatalanib has been evaluated, in preclinical models, in mice injected with brain metastases selected variants, and showed reduced brain metastasis burden and decreased microvascular density. However, there was no significant increase in survival compared with vehicle treated controls [81]. Pre-clinical data supports the use of vatalanib in brain metastases, clinical trials are necessary to evaluate this agent in brain metastatic NSCLC patients.

CONCLUSIONS

Increasing understanding of tumour angiogenesis has allowed the development of several antiangiogenic agents targeting a variety of molecular mechanisms. Among targeting VEGF/VEGFR pathway biological agents, bevacizumab is the first to show a clear therapeutic potential in combination with chemotherapy, improving clinical outcomes including survival in advanced non-squamous NSCLC patients. A potential but infrequent side effect of bevacizumab administration is the haemorrhage at the tumour site or at distant sites. Since the occurrence of fatal intracranial bleeding in a patient with undiagnosed brain metastases from HCC, bevacizumab has been contraindicated in Europe for use in patients with CNS metastases. Consequently, the presence of CNS metastases was a stated exclusion criteria in several clinical trials investigating bevacizumab in advanced NSCLC patients. Ever since, the question has remained whether these exclusions are really necessary or are actually overly conservative. This restrictive factor has been evaluated over the last few years to try establish whether it may be feasible to treat patients with bevacizumab. Incidence of intracranial bleedings among patients enrolled in two randomized phase III trials of bevacizumab in NSCLC patients was similar to the incidence of CNS haemorrhages reported in patients who had not received this drug. Moreover, further experience treating primary or metastatic brain tumours including NSCLC revealed that intracranial haemorrhage was rare in patients receiving bevacizumab therapy. Subsequently, eligibility criteria for clinical trials of extracranial solid tumours were relaxed, allowing patients with previously treated, but inactive, brain metastases to participate. Data from prospective clinical trials enrolling such patients showed that the addition of bevacizumab to various chemotherapy agents or erlotinib seems to be safe and is associated with a low incidence of CNS haemorrhage.

Even though a randomized clinical trial with bevacizumab has not been specifically conducted, treated brain metastases NSCLC patients will likely achieve similar benefit from bevacizumab therapy as patients without brain metastases, thus producing significant benefit to a large number of NSCLC patients.

Other anti-angiogenetic agents have been investigated in the treatment of advanced NSCLC patients, but negative results are more frequent than positive ones. Several further small molecules are under investigation in this setting but, specific results concerning their activity in brain metastases are still lacking.

New therapies and strategies are increasingly being explored aiming to enhance drug delivery to the brain, complement the effects of radiation therapy, substitute the role of radiation therapy, or act as preventative agents against the development of new metastasis. Furthermore, with the development of new models and imaging techniques, preclinical treatment strategies can become more focused on established brain metastases, which is necessary for translational potential.

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Declared None.

CONFLICT OF INTEREST

Dr. Cesare Gridelli serves as a consultant and as speaker's bureau member for Roche. Dr. Antonio Rossi serves as a speakers bureau member for Roche. The other Authors declare no conflict of interest.

ABBREVIATIONS

NSCLC	=	non small cell lung cancer
OS	=	overall survival
WBRT	=	whole-brain radiation therapy
PS	=	performance status
LINAC	=	linear accelerator
BBB	=	blood brain barrier
CNS	=	central nervous system
VEGF	=	vascular endothelial growth factor
WNT	=	Wingless-type
LEF	=	lymphoid enhancer-binding factor
TCF	=	transcription factor
TGFβ	=	transforming growth factor-β
PIGF	=	placenta growth factor
VEGFRs	=	vascular endothelial growth factor receptors
Flt-1	=	fms-like tyrosine kinase-1
Flk-1/KDR	=	fetal liver kinase-1/kinase domain region
Flt-4	=	fms-like tyrosine kinase 4
PLC	=	phospholipase C
PKC	=	protein kinase C
MAPK	=	mitogen-activated protein kinase
PDGF	=	platelet derived growth factor
bFGF	=	basic fibroblast growth factor
EGF	=	epidermal growth factor
TNF	=	tumour necrosis factor
IL	=	interleukin
BMECs	=	brain microvascular endothelial cells
uPA	=	urokinase-type plasminogen activator
TTPA	=	tissue-type plasminogen activator
MMP	=	matrix metalloproteinases
TIMPs	=	inhibitors of matrix metalloproteinases
mAbs	=	monoclonal antibodies
TKIs	=	tyrosine-kinase inhibitors
BCP	=	bevacizumab plus carboplatin and paclitaxel
CP	=	carboplatin and paclitaxel
HR	=	hazard ratio
RR	=	response rate
HCC	=	hepatocellular carcinoma
DCR	=	disease control rate
TTP	=	time to disease progression
ATE	=	arterial thromboembolic events
SD	=	stable disease
EMA	=	European Medicines Agency
ICH	=	intracranial haemorrhage

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