Current status and future directions of anti-angiogenic therapy for gliomas

Wolfgang Wick, Michael Platten, Antje Wick, Anne Hertenstein, Alexander Radbruch, Martin Bendszus, and Frank Winkler

Neurology Clinic and National Center for Tumor Diseases, University of Heidelberg and German Consortium for Translational Cancer Research, German Cancer Research Center, Heidelberg, Germany (W.W., M.P., A.W., A.H., F.W.); Department of Neuroradiology, University of Heidelberg and German Cancer Research Center, Heidelberg, Germany (A.R., M.B.)

Corresponding Author: Wolfgang Wick, MD, Professor of Neurology, Department of Neurology, University Clinic Heidelberg, Im Neuenheimer Feld 400, D-69120 Heidelberg, Germany (wolfgang.wick@med.uni-heidelberg.de).

Molecular targets for the pathological vasculature are the vascular endothelial growth factor (VEGF)/VEGF receptor axis, integrins, angiopoietins, and platelet-derived growth factor receptor (PDGFR), as well as several intracellular or downstream effectors like protein kinase C beta and mammalian target of rapamycin (mTOR). Besides hypoxic damage or tumor cell starvation, preclinical models imply vessel independent tumor regression and suggest differential effects of anti-angiogenic treatments on tumorous and nontumorous precursor cells or the immune system. Despite compelling preclinical data and positive data in other cancers, the outcomes of clinical trials with anti-angiogenic agents in gliomas by and large have been disappointing and include VEGF blockage with bevacizumab, integrin inhibition with cilengitide, VEGF receptor inhibition with sunitinib or cediranib, PDGFR inhibition with imatinib or dasatinib, protein kinase C inhibition with enzastaurin, and mTOR inhibition with sirolimus, everolimus, or temsirolimus. Importantly, there is a lack of real understanding for this negative data. Anti-angiogenic therapies have stimulated the development of standardized imaging assessment and the integration of functional MRI sequences into daily practice. Here, we delineate directions in the identification of molecularly or image-based defined subgroups, anti-angiogenic cotreatment for immunotherapy, and the potential of ongoing trials or modified targets to change the game.

Keywords: angiogenesis, bevacizumab, cilengitide, evasive resistance, vascular normalization.

Glioblastoma is the most common primary malignant brain tumor in adults and among the most aggressive, making this disease a challenge to treat.1-12 Standard therapy for glioblastoma is maximal safe surgical resection1 and postoperative radiochemistry6 with temozolomide concomitantly and as maintenance.9,10 Fortunately, this a priori unspecific genotoxic treatment comes with a biomarker, that is, promoter methylation status of O6 methylguanine methyltransferase (MGMT).7-10

Hallmarks of the disease are heterogeneity, resistance to therapeutic approaches, diffuse infiltrative growth, immune evasion, and pathological angiogenesis. In the past 10 years an enormous amount of preclinical and clinical data have been generated for this last aspect.

Several mechanisms of tumor vessel formation are postulated: vasculogenesis, sprouting angiogenesis, vessel co-option, intussusception, vascular mimicry, and transdifferentiation of cancer cells into endothelial cells.11 The regulatory mechanisms are in part dependent on vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR), a pathway highly expressed and relevant for blood vessel formation in gliomas; but the mechanisms also depend on other hypoxia-inducible angiogenic factors; they are counterbalanced by endogenous inhibitors of angiogenesis such as soluble Fms-like tyrosine kinase 1 (sVEGFR1, a blocker of VEGF and placenta growth factor [PlGF]), angiotatin, endostatin, interferon, and thrombospondin 1 and 2.12 With those, a plethora of targets have evolved, of which the VEGF pathway is currently the one primarily tested in the clinic.

The VEGF antibody bevacizumab has increased the repertoire of medical treatment options for patients with recurrent glioblastoma. Two uncontrolled phase II studies13,14 were the basis for approval in patients with progressive glioblastoma in the US and Australia, whereas the European Medical Agency rejected approval in the EU in patients with progressive15 as well as newly diagnosed glioblastoma. The regulatory agencies in other countries, such as Japan, approved the treatment for
both primary and salvage therapy. In the United Kingdom, bevacizumab is funded for the treatment of neurofibromatosis type II–related vestibular schwannoma after a clinical trial in the US had demonstrated not only stabilization on imaging but also improvement in the hearing function.\textsuperscript{16,17}

Potential adverse effects of anti-angiogenic therapies are in part related to their mechanism of action. Anti-VEGF/VEGFR compounds,\textsuperscript{18} at least as part of their mode of action, induce normalization of the vasculature\textsuperscript{19} by inhibiting pathological proliferation of endothelial cells and immature vessel formation and reducing the pathological high permeability of existing tumor vessels. Only 1 or 2 days after initiation of therapy, this reduced permeability of the blood–brain barrier (BBB) results in decreased contrast enhancement and edema,\textsuperscript{18} potentially producing pseudoresponses, which are reductions of contrast enhancement as a consequence of mere BBB restoration rather than a tumor static or toxic effect. For these, an adaptation of the imaging criteria has been made by taking into account non–contrast enhancing tumor growth (Table 1).\textsuperscript{20} Despite a lack of clinical evidence, the debate continues about whether T2 progression is caused by anti-angiogenic therapy that permits, or even induces, a non-angiogenic invasive growth pattern of the disease (evasive resistance) (Fig. 1). On the other hand, it needs to be considered that neither the high response rates that VEGF/VEGFR-targeting agents produce\textsuperscript{21} nor the demonstrated improvement in progression-free survival (PFS) has translated into an overall survival (OS) benefit so far.\textsuperscript{22,23}

Decision algorithms have been proposed to account for the second challenging imaging phenomenon, which is pseudo-progression (Fig. 2A). Pseudoprogresion is an often clinically silent progression in the MRI, which is regarded as a consequence of (radio)chemo)therapy and not tumor growth and which may be reversible and best managed by continuation of present therapies and sometimes low-dose steroid usage.

The opposite, pseudoresponse, is an often clinically relevant reduction in the T1-contrast enhancing tumor parts, which are deemed a consequence of the restitution of the BBB and vessel normalization and not reduction of vital tumor mass. Objective responses need to be confirmed in a subsequent scan and are only possible for patients with a measurable\textsuperscript{21} lesion. Uncertainty remains, however, on the best evaluation of T2. Large series have proposed that T2 progress predicts subsequent T1 progress.\textsuperscript{24} Tumor progression including contrast-enhanced sequences of both T1 and T2 is more frequently diagnosed than considering only contrast enhanced T1 sequences.

Remarkably, other agents targeting glioblastoma angiogenesis at least as intuitive pathways produced similarly disappointing results not only for OS, but also in other aspects, including response rate and PFS.

### (Anti-)angiogenesis in Gliomas and Potential Mechanisms of Resistance

Integrins are a family of cell-cell and cell–extracellular matrix adhesion molecules involved in a variety of cellular processes, such as cell survival, proliferation, migration, invasion, angiogenesis, and thus can support tumor development.\textsuperscript{25} In particular α\textsubscript{v}β\textsubscript{3} and α\textsubscript{v}β\textsubscript{5} integrins are considered key mediators of crosstalk between tumor cells and the brain microenvironment in glioblastoma and are overexpressed on tumor cells and vasculature.\textsuperscript{26–28} Therefore, targeting integrins and the tumor microenvironment has been considered a promising therapeutic strategy in glioblastoma.\textsuperscript{29,30} Cilengitide is a selective inhibitor of α\textsubscript{v}β\textsubscript{3} and α\textsubscript{v}β\textsubscript{5} integrins.\textsuperscript{30}

Malignant gliomas show an activity of the VEGF-A pathway, which supports endothelial cell growth, permeability, and regrowth following endothelial cell injury after chemotherapeutic and radiotherapy.\textsuperscript{31–33}

The original concept of anti-angiogenic therapy regarded “tumor starvation” as the prime mechanism, by induction of endothelial cell apoptosis, limited growth of new blood vessels, obliteration of small vessels, decreased permeability, and decreased perfusion resulting in decreased delivery of oxygen and nutrients.\textsuperscript{34} However, more recent studies suggest that during the initial stages of treatment, anti-angiogenic agents transiently “normalize” abnormal tumor vasculature by reducing blood vessel diameter and permeability, which paradoxically improves vessel perfusion, reduces tumor interstitial pressure, and improves tumor oxygenation,\textsuperscript{18,19,35} leading to enhanced tumor response to radiotherapy\textsuperscript{19} and increased delivery of cytotoxic chemotherapy.

The role of PlGF in pathological angiogenesis is controversial, and clinical development has been aimed at a combination with bevacizumab.\textsuperscript{36} Preclinically, PlGF induces the formation of dilated and normalized vascular networks that are hypersensitive to

---

**Table 1. Summary of response assessment according to RANO criteria\textsuperscript{18}**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 gadolinium enhancing disease</td>
<td>None</td>
<td>&gt;50% ↓</td>
<td>&lt;50% but &lt;25% ↑</td>
<td>≥25% ↑*</td>
</tr>
<tr>
<td>T2/FLAIR</td>
<td>Stable or ↓</td>
<td>Stable or ↓</td>
<td>Stable or ↓</td>
<td>↑*</td>
</tr>
<tr>
<td>New lesions</td>
<td>None</td>
<td>None</td>
<td>NA*</td>
<td>Present*</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>None</td>
<td>Stable or ↓</td>
<td>Stable or ↓</td>
<td>↓*</td>
</tr>
<tr>
<td>Clinical status</td>
<td>Stable or ↑</td>
<td>Stable or ↑</td>
<td>Stable or ↑</td>
<td>↑*</td>
</tr>
<tr>
<td>Requirement for response</td>
<td>All</td>
<td>All</td>
<td>Any*</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; PD, progressive disease; PR, partial remission; SD, stable disease.

*Progression occurs when this criterion is present.

*Increase in corticosteroids alone will not be taken into account in determining progression in absence of persistent clinical deterioration.
anti-VEGF and anti-VEGFR2 therapy, leading to dormancy of a substantial number of avascular tumors.\textsuperscript{37}

The serine/threonine family of protein kinase (PK)C has an important role in tumor-derived VEGF-induced angiogenesis. Consequently, PKC inhibitors have been evaluated in preclinical tumor models for anti-angiogenic activity.\textsuperscript{38}

Serving as a central signaling hub integrating multiple intra- and extracellular cues, the serine/threonine kinase mammalian target of rapamycin (mTOR) is an attractive anticancer target. Mammalian TOR is involved in the formation of at least 2 multi-protein complexes, mTORC1 and mTORC2, which direct cell metabolism, growth, proliferation, survival, and angiogenesis. The rapamycin-sensitive mTORC1 essentially mediates phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt/PKB) signals.\textsuperscript{39}

Only recently, the impact of mTOR signals on tumor cell motility as well as the predominant role of mTORC2 as the main antitumor target has been deciphered.\textsuperscript{40}

All signaling cascades discussed share a profound preclinical rationale to be targeted in glioblastoma treatment. However, the level of preclinical preparation for the translation into the clinic shares some common weaknesses. Likewise, although unlikely to be a one-size-fits-all approach, all pathways were always addressed in all patients and not restricted to patients with a determined expression/relevance of the angiogenic cascade. Brain tumor–specific dose optimization is largely lacking. One preclinical study found a positive correlation between the dosing of bevacizumab monotherapy and its beneficial effects on glioma growth and survival, while vascular normalization

\textbf{Fig. 1.} Infiltrative progressive disease (FLAIR progression) in a patient with recurrent right temporal glioblastoma, prior to bevacizumab (A and B) and at 3 mo follow-up examination (C and D). The enhancement adjacent to the resection cavity on contrast enhanced T1-weighted images (A) no longer displays on contrast enhanced T1 follow-up (C). In contrast, FLAIR images (B and D) show an increase of the T2-signal intensity in the follow-up examination (arrows in D).

\textsuperscript{\textcopyright}Wick et al.: Update on glioma anti-angiogenesis

\textsuperscript{317}
was also achieved with subclinical doses. Clinically, no such positive correlation was found in retrospective studies. Furthermore, it is of crucial importance to better understand whether anti-angiogenic therapy has additive or even synergistic effects with chemo- and/or radiotherapy; or, to the contrary, whether it compromises one of them. While synergistic effects have been found in a preclinical model when radiotherapy was applied in a “normalization time window” under VEGFR2 blockade, inconsistent preclinical results have been reported for the combination of bevacizumab with alkylating chemotherapy. However, the Dutch BELOB trial (bevacizumab vs lomustine) implies that combination of anti-VEGF-A therapy with alkylating chemotherapy might be particularly effective in recurrent glioblastoma. All in all, we need a much better systematic understanding of how anti-angiogenic agents, with their complex mechanisms of action and profound changes of the tumor micromilieu, differentially influence the effectivity of radiotherapy and chemotherapy in malignant gliomas. This will help to identify clinical situations with the highest chances for net benefit, and perhaps also situations where they should be avoided.

Another challenge associated with anti-angiogenic therapies is the possible induction of evasive resistance: this is the activation of glioma cell motility and hence more diffuse T2/fluid attenuated inversion recovery (FLAIR) changes as a consequence of anti-angiogenic therapies. This may clinically result in the paradoxical reduction of contrast-enhancing tumor or even a relevant mass reduction with a parallel increase of diffusely growing tumor. Preclinical studies have indicated that anti-VEGF therapy may increase the tendency of tumor cells to invade by co-opting existing blood vessels. For the molecular mechanisms involved, 3 major hypotheses are discussed. Anti-angiogenic therapy increases hypoxia and acidosis, which activates survival signals, like the mTOR/PI3K

Fig. 2. (A) Pseudoprogression decision tree (after radiochemotherapy). (B) Pseudoresponse/pseudoprogression algorithm (at recurrence).
pathway, and promotes glycolytic energy metabolism and autophagy.50 Furthermore, VEGF-A inhibition may directly increase glioma cell motility and a mesenchymal transition via a MET/VEGFR2 complex.51 Alternatively or in addition, antiangiogenic therapy in combination with motility-enhancing radiotherapy52,53 further enhances this undesirable side effect. Adaptive but also primary resistance mechanisms can stem from redundancies of angiogenic pathways, including other VEGF isoforms,54 and the angiopoietin-2 (Ang-2) / Tie2 axis.55

Interestingly, despite being amongst the best-investigated phenomena in glioblastoma anti-angiogenesis, evasive resistance remains a mainly preclinical or casuistic consideration56 and does not hold in more systematic clinical assessments.57–59 As of today, we regard this mode of resistance as a relevant but not therapy-related complication of many tumors, mainly at late stages. This and the “pseudoresponse” phenomenon (Fig. 2B) of therapies restoring the integrity of the BBB rather than reducing the tumor mass induces major efforts in standardizing, harmonizing, and improving MRI evaluation criteria and examinations.

**Update on Trial Data**

Despite the overwhelming preclinical rationale starting in the early 1990s, glioblastoma was a late entry for the development of anti-angiogenic strategies. In addition to the general considerations in rare cancers, this was due to concern over potentially serious adverse events in the brain tumor population, such as intracranial hemorrhage and stroke. Casuistic, off-label experience and early evidence from clinical trials showed the rarity of vascular or coagulation complications. In the past 10 years, clinical studies of anti-angiogenic agents for glioblastoma have developed to a dominant research focus, catching up with the intensive clinical research with anti-angiogenic agents outside the brain. It is remarkable that most compounds, in part successfully developed outside the brain, failed not in late phase III development, but rather in uncontrolled or small controlled phase II trials. The general skepticism about anti-angiogenic therapies in glioblastoma is based on several disappointing phase II trials, but only a few phase III trials, indicating very early limitations for most of the compounds.

Data of trials for patients with glioblastoma in the newly diagnosed setting as well as at progression are summarized in Table 2.

In general, these trials are based on solid preclinical concepts. Most studies have focused on the VEGF/VEGFR axis. The trials are discussed together, although it is appreciated that blocking one receptor and one ligand might not lead to the same effects.

As a prototypical VEGFR inhibitor, cediranib had been promoted to phase III development based on a phase II study for patients with recurrent glioblastoma, in which 8/30 subjects (27%) achieved a partial radiographic response based on consensus-based response criteria.78 Cediranib is an orally available pan-VEGFR tyrosine kinase inhibitor with a half-life of 22 h, compatible with once-daily dosing. It has a sub-nanomolar half-maximal inhibitory concentration for VEGFR with additional activity against c-Kit and lower potency against platelet-derived growth factor receptor (PDGFR)β.79 Remarkably detailed imaging cediranib had early and in some patients maintained profound effects on tumor perfusion.18 Despite early clinical responses, there were too few durable stabilizations not leading to a PFS benefit. On the other hand, relevant clinical endpoints, like time to neurological deterioration and levels of steroid intake, have been improved. Why did the phase III trial in patients with recurrent glioblastoma fail to meet the prespecified target PFS advantage or even an OS effect? The doses used for the phase III trial had not been sufficiently determined in phase I glioblastoma trials. Whereas the trial used an optimal lomustine dose, the cediranib combination dose was taken from a metastatic lung carcinoma study (NCIC BR24).80 Patients had been included with first recurrences. This means that patients at second recurrence might have been treated with bevacizumab, which was used more frequently in the control groups and may or may not have had a more pronounced effect in patients from the control group, explaining at least the lack of an OS effect.

Early casuistic reports and the impressive response rates, PFS, and steroid-sparing effects in the BRAIN trial (NCT00345163)21 induced the development of bevacizumab in newly diagnosed disease. Although the 2 phase III trials had some relevant differences in design and outcome, they shared a lot of similarities as well. Major outcomes were a positive impact on PFS in both trials, which did not reach the prespecified significance level in the Radiation Therapy Oncology Group (RTOG) 0825 trial,23 but did so in the Avastin in Glioblastoma (AWGlia) trial22 and had a positive effect on survival time with low or no steroid cotreatment in both trials. Whereas the latter is a generally accepted benefit, the PFS data are challenged because the certainty of determining imaging results in times of pseudoprogression (with chemoradiotherapy), which may be alleviated by bevacizumab or pseudoresponse (with bevacizumab), is not uniformly considered robust.81 Even considering the PFS effects relevant, there is a lack of OS benefit in all trials that used bevacizumab in first line.22,23,63,64 The reasons for failure to translate PFS into OS benefit are unclear. Potential explanations are that the PFS benefit was mainly an imaging effect and no real gain in PFS, the escape mechanisms of the VEGF-inhibitory treatment made the recurrent tumor more aggressive, and (iii) there was a major impact of crossover, for which all practicing clinicians seem the most logical explanation, since the benefit from bevacizumab for many patients, especially when given as a salvage treatment, is relevant. Saying this, the outcome of the BELOB trial45 is easy to comprehend; the trial showed a fine translation of a PFS gain into an OS gain in a setting in which crossover was virtually excluded. However, this trial places a caveat on the monotherapy efficacy of anti-VEGF antibody, as the effects in monotherapy are as minimal as never reported in a bevacizumab trial before.45 A special focus has been put on trials, which restricted inclusion according to MGMT status allowing the study of temozolomide-free experimental arms (Fig. 3).

The development of cediradnige has some special aspects: the data from the phase II trial85 did not suggest a relative benefit over the historical data in the same patient population derived from the European Organisation for Research and Treatment of Cancer (EORTC) 26981 trial.5 The decision to rush into the phase III pivotal trial was therefore based mainly on theoretical considerations, which indicated that a potential
Table 2. Selection of published clinical data for anti-angiogenic therapies in glioblastoma

<table>
<thead>
<tr>
<th>Agents Trial Identifier</th>
<th>Phase</th>
<th>Primary/Progressive Disease</th>
<th>Patients, n</th>
<th>Primary [Secondary] Outcomes</th>
<th>Positive Prognostic Indicator(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflibercept NCT00369590</td>
<td>I/II</td>
<td>Progressive</td>
<td>42</td>
<td>PFS-6: 7.7% [ORR: 18%]</td>
<td>Circ. VEGF</td>
<td>60,61</td>
</tr>
<tr>
<td>Anti-PlGF + bevacizumab</td>
<td>I</td>
<td>Progressive</td>
<td>22</td>
<td>[ORR: 22.7%; PFS: 3.5 mo; OS: 8.5 mo]</td>
<td>NA</td>
<td>36</td>
</tr>
<tr>
<td>Bevacizumab + RT + TMZ</td>
<td>III</td>
<td>Primary</td>
<td>458</td>
<td>mPFS: 10.6 mo; mOS: 16.8 mo</td>
<td>Proneural subtype</td>
<td>22,62</td>
</tr>
<tr>
<td>Placebo + RT + TMZ (AAGlio) NCT00943826</td>
<td>III</td>
<td>Primary</td>
<td>463</td>
<td>mPFS: 6.2 mo; mOS: 16.7 mo</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Bevacizumab + RT + TMZ NCT00884741</td>
<td>III</td>
<td>Primary</td>
<td>320</td>
<td>mPFS: 10.7 mo; mOS: 15.7 mo</td>
<td>NA</td>
<td>23</td>
</tr>
<tr>
<td>Bevacizumab + CPT-11 + RT TMZ + RT (GLARIUS) NCT00967330</td>
<td>II</td>
<td>Primary (unmeth.)</td>
<td>116</td>
<td>mPFS: 9.7 mo [mOS: 18.2 mo]</td>
<td>NA</td>
<td>61</td>
</tr>
<tr>
<td>Bevacizumab + CPT-11 + RT/TMZ → Bevacizumab RT/TMZ→TMZ (TEMAVIR) NCT01022918</td>
<td>II</td>
<td>Primary (unresectable)</td>
<td>60</td>
<td>PFS-6: 50% [PFS: 7.1 mo; OS: 11.1 mo] [PFS: 5.2 mo; OS: 11.1 mo]</td>
<td>NA</td>
<td>64</td>
</tr>
<tr>
<td>Bevacizumab Bevacizumab + CPT-11 (BRAIN) NCT00345163</td>
<td>II</td>
<td>Progressive</td>
<td>85</td>
<td>PFS-6: 42.6%; ORR: 28.2%</td>
<td>6-mo PFS: 50.3%; ORR: 37.8%</td>
<td>NA 13</td>
</tr>
<tr>
<td>Bevacizumab + CCNU CCNU (BELOB) NCT00509821</td>
<td>I/IIa</td>
<td>Primary</td>
<td>52</td>
<td>OS-9: 63%</td>
<td>OS-9: 38%</td>
<td>NA 45</td>
</tr>
<tr>
<td>Cilengitide + TMZ + RT Cilengitide + TMZ + RT TMZ + RT (CENTRIC) NCT00689221</td>
<td>I/IIa</td>
<td>Primary</td>
<td>52</td>
<td>OS-9: 43%</td>
<td>OS-9: 43%</td>
<td>NA 45</td>
</tr>
<tr>
<td>Cediranib + CCNU (REGAL) NCT00777153</td>
<td>III</td>
<td>Progressive</td>
<td>300</td>
<td>mPFS: Cediranib 30 mg = 3 mo Cediranib 20 mg + CCNU = 4 mo CCNU = 2.7 mo</td>
<td>NA</td>
<td>66</td>
</tr>
<tr>
<td>Cilengitide NCT00948389</td>
<td>IIa</td>
<td>Progressive</td>
<td>81</td>
<td>mPFS: Cediranib 20 mg = 3 mo</td>
<td>6-mo PFS = 16%</td>
<td>NA 67</td>
</tr>
<tr>
<td>Dasatinib NCT00948389</td>
<td>I/II</td>
<td>Progressive</td>
<td>77</td>
<td>ORR: 0</td>
<td>mOS: 7.9 mo</td>
<td>NA 69</td>
</tr>
<tr>
<td>Enzastaurin + RT NCT00509821</td>
<td>I/II</td>
<td>Primary (MGMT unmeth.)</td>
<td>60</td>
<td>PFS-6 = 53.6 [39.8–65.6]</td>
<td>NA</td>
<td>70</td>
</tr>
</tbody>
</table>
vascular normalization with cilengitide would benefit mainly
the patient subgroup getting the biggest advantage from
temozolomide, without further optimizing the drug delivery or
schedule or considering testing for target expression, which
was performed only post hoc to understand the negative
data.\textsuperscript{66}

Therapeutical targeting of the pathways investigated so far
must, in principle, face potential consequences regarding gene-
ral effects on cellular behavior (eg, motility), homeostasis, and
repair processes of the vascular system, which might result in
major unwanted effects. However, in all trials evaluated for
this review, toxicity was not the major issue. Patients overall
did experience more arterial hypertension as soon as VEGF
pathway blockage occurred, and there is a potentially attribut-
ed increase in fatigue, although some patients do feel better,
especially when steroids can be reduced\textsuperscript{20} or there are objec-
tive responses; there is a slight increase in coagulation pathol-
ogies, including stroke, and there are drops in platelet counts.
However, a lack of antitumor effect observed in some ortho-
topic rodent xenograft models of glioblastoma\textsuperscript{82} suggests that
angiogenesis inhibitors have limited intrinsic antitumor activity,
at least in some tumors, and that their main benefit may be
limited to reductions in permeability and vasogenic cerebral
edema.\textsuperscript{13,18,67} Notwithstanding a better understanding of the
potential benefits of utilizing an optimal dose, schedule, and
drug combination,\textsuperscript{83} data from phase III clinical trials\textsuperscript{22,23} sug-
gest that some glioblastomas may be indeed intrinsically resis-
tant to anti-angiogenic therapy.

Given the improved laboratory data on the multitude of sig-
naling pathways relevant for glioma angiogenesis and the data
on escape mechanisms by switching gears to the utilization of
another system, combination approaches seem most logical.
These are done with toxic compounds or radiotherapy (see
above) or in the current early development of targeting both
the VEGF/VEGFR and the angiopoietin system—for instance,
by the application of an Ang-2/VEGF (cross monoclonal)
antibody.

<table>
<thead>
<tr>
<th>Drug Combination</th>
<th>Phase</th>
<th>Status</th>
<th>Primary and OS-12</th>
<th>MPPS</th>
<th>6-mo PFS</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzastaurin (STEERING)</td>
<td>III</td>
<td>Progressive</td>
<td>266</td>
<td>No significant effect on PFS (1.5 vs 1.6 mo), OS (6.6 vs 7.1 months), 6-mo PFS (P = .13), SD (38.5 vs 35.9%), or OR (2.9 vs 4.3%) respectively for enzastaurin vs lomustine</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Imatinib + vatalanib + hydroxyurea</td>
<td>I</td>
<td>Progressive</td>
<td>37</td>
<td>Vatalanib MTD = 1000 mg BID</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea ± imatinib</td>
<td>III</td>
<td>Progressive</td>
<td>240</td>
<td>Median PFS = 6 wk (both arms)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Temsirolimus + RT</td>
<td>II</td>
<td>Primary</td>
<td></td>
<td></td>
<td>mTOR phosphorylation</td>
<td></td>
</tr>
<tr>
<td>TMZ + RT (EORTC 26082/22081)</td>
<td>III</td>
<td>Progressive</td>
<td>65</td>
<td>6-mo PFS = 7.8%</td>
<td>p70S6 kinase</td>
<td></td>
</tr>
<tr>
<td>Temozolomide</td>
<td>II</td>
<td>Progressive</td>
<td>40</td>
<td>6-mo PFS = 12.5%</td>
<td>c-Kit</td>
<td></td>
</tr>
<tr>
<td>Vatalanib</td>
<td>I</td>
<td>Primary</td>
<td>19</td>
<td>[2 PR]</td>
<td>Potential: PlGF, sVEGFR1, sVEGFR2 and sTie2 in plasma</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BID, twice daily; CCNU, lomustine; maximum tolerated dose, MTD; MGMT, methylguanine methyltransferase; NA, not available; ORR, objective response rate; mOS, median OS; mPFS, median PFS; PR, partial response; RT, radiotherapy; TMZ, temozolomide.
Biomarker Data

There is no clinically validated biomarker today that tells us which patients will likely profit from anti-angiogenic therapy and which will not. This is true for glioma and all other tumor entities, despite more than a decade of research by the pharmaceutical industry and academia. Clearly, this is in sharp contrast to most other molecular therapies in oncology and might be one explanation for the failure of anti-angiogenic therapies to prolong OS in unselected patients with malignant glioma. However, it might also point to a possible solution for the future.

Early data suggest that early modification of circulating proteins, such as collagen IV, laminin, and VEGF, may be associated with response to anti-angiogenic therapies. Recently, a French case-control study provided evidence that high matrix metalloproteinase (MMP)-2 plasma levels in patients with recurrent high-grade glioma treated with bevacizumab, but not with a cytotoxic agent, are associated with prolonged tumor control and survival. These factors should be tested in randomized clinical trials that evaluate bevacizumab efficacy and reassess its biological role.

In the AVAglio trial, analysis of circulating biomarkers, mainly VEGF-A and sVEGFR, was not associated with outcome. However, expression analysis from a subset of the trial cohort revealed the proneural subtype according to the Phillips classification being predictive not only for PFS, but also for OS in bevacizumab-treated patients. The parallel RTOG-0825 trial also suggests an as yet not publicly available biomarker signature. Cross-validation of these signatures from the respective other trial cohorts would be a necessary next step to generate a biomarker or biomarker set. Whether the same subtype also mainly benefits from bevacizumab at recurrence will also be tested in the randomized EORTC 26101 phase II and III trial biomarker cohorts.

Patient-Related Endpoints

In parallel to the research on more effective treatment strategies as well as imaging and molecular biomarker surrogate endpoint, there is an increasing awareness that we need to focus on other endpoints than the mere measuring of time. Especially in the newly diagnosed situation, our patients often-times are remarkably little affected physically and cognitively by the disease. Several studies have found that tumor progression is the predominant cause of cognitive and functional decline in this patient population. It has to be our principle aim to maintain that positive situation for as long as possible and prevent a decline, be it due to progression of the disease or to unwanted effects of our therapies. Recent trials have included measures not only for the consumption of steroids during the disease period, but integrated measures on patient-reported quality of life and proxy-reported outcomes as well as scales to understand neurological functioning and neurocognition. Importantly, these data need to be obtained not only during a limited treatment phase, such as in a period of active trial participation, but also after progression, ideally until late stages of the disease. Further, we need to work on the most relevant scales from the many items of the health-related quality of life (HRQoL) assessments and scales for neurological functioning and cognition. As of now, the 2 phase III trials using bevacizumab in the newly diagnosed situation potentially are providing the most mature (though controversial and in part contradictory) data in that arena. In AVAglio, patients treated with bevacizumab or placebo in combination with radiotherapy + temozolomide maintained their relatively high baseline HRQoL until progression. Patients in the bevacizumab arm experienced extended PFS at these high levels. This is an important advantage in this patient population, where functioning and HRQoL are known to decline over time, particularly at progression and disease recurrence. On the contrary, the net clinical benefit substudies of RTOG-0825 revealed a greater deterioration over time in the bevacizumab group than in the placebo group in the EORTC QLQ-C30/BN20 scores for cognitive functioning (P = .008), motor dysfunction (P = .02), and communication deficit (P = .003).

This study also had a larger cohort being neurocognitively tested. Moreover, the composite scores on the neurocognitive-function test battery (P = .05), as well as the scores on the Controlled Oral Word Association Test (P = .003) and the Trail Making Test Part A (P = .04), compared negative for the bevacizumab arm. Importantly, the smaller neurocognitively tested subgroup of AVAglio did not show any negative trend for the bevacizumab-treated patients. Likewise the clinical impression is that patients specifically at recurrence tend to benefit in net clinical functions.

For these most part incompatible results have provoked an intensive debate in the community. First attempts to formally devaluate the RTOG data as not prespecified as a secondary objective or obtained from merely selected patients do not touch the core of the problem. Knowing that the patient cohorts are very similar in their baseline characteristics and that the same group of experts is actually consulting for both trials triggers the search for other factors. More relevant might be the difference in the assessment of progression in both trials. Considering that RTOG had done the analyses on patients in fact not stable anymore but clinically progressing would largely synchronize the datasets. The basis for this speculation is the difference in imaging evaluation that did not include T2 progression for the RTOG trial. On the other hand, this would further decrease the already relatively short PFS in the RTOG trial. There is consensus in the scientific community that data from both trials should be analyzed together to understand the differences and come to a unified conclusion. Further, there is preclinical research and speculation on potential adverse effects of bevacizumab on brain function as a consequence of treatment effects on the tumor tissue. Alternatively, bevacizumab might directly interfere with the function of the healthy brain. VEGF might not be essential for maintaining basal neurogenesis but relevant for maintaining homeostatic functions in the central nervous system. VEGF overexpression in the hippocampus of mice can augment learning and memory, whereas loss of function impairs this effect.

Future Aspects

Imaging

To ensure confidence in results of trials with anti-angiogenic agents, trials like the ongoing EORTC 26101 study in patients with recurrent glioblastoma chose OS as the primary endpoint.
The advantage is independence from subjectivity in the determination of progression. A disadvantage is the dilution of a potential effect by crossover; hence, the availability of the drug for outside trial treatments or inbuilt crossover challenges the endpoint. Current protocols, such as AVAglio, used T2-weighted or FLAIR MRI sequences to measure infiltrative/diffuse tumor growth in a methodology addressing the consensus reached in the Response Assessment in Neuro-Oncology (RANO) criteria. In the absence of widely available imaging technologies to differentiate pseudoprogression from true progression, steps were taken to ensure that patients with increased enhancement at the first post-radiotherapy scan (potential pseudoprogression) were not excluded from continuing study treatment (Fig. 2A). The post-hoc sensitivity analyses of PFS showed that the advantage of the bevacizumab-containing treatment was maintained after eliminating patients with potential pseudoprogression and pseudoresponse. In the prospective, controlled assessment, AVAglio demonstrated that the pattern of progression was similar between arms. When tumor pattern changed during treatment, survival was not affected in an exploratory analysis.

In essence, the provided algorithms greatly reduce the likelihood of inaccurate determination of the time point of progression. These algorithms (Fig. 2 and Table 1)—in addition to standardized protocols for MRI, central assessments, and the integration of multimodal MRI into daily clinical practice and trials—ensure the reliability of imaging 96 in the era of antiangiogenic compounds. Research activities are directed toward imaging being developed as a biomarker to select patients for anti-angiogenic treatments.

Several years ago, it was suggested that [18F]-fluorothymidine (FLT) PET would predict response of patients to bevacizumab and irinotecan therapy. In this study, a 25% reduction in tumor FLT uptake as measured by standardized uptake value was defined as a metabolic response. FLT-PET performed at 6 weeks was predictive for OS. A later study confirmed the predictive impact of FLT-PET, but not on OS, only on PFS. With another tracer, [18F]-FDOPA ([18F]-fluoro-DOPA) PET identified treatment responders to bevacizumab (plus irinotecan in most cases) as early as 2 weeks after treatment initiation. Despite the limitations of these uncontrolled series, the results are consistent. As commonly seen in PET research in neuro-oncology, prospective controlled data are missing and it remains to be determined whether the obtained sensitivity and specificity are sufficient to tailor treatments, especially if this means reducing or stopping therapy in a very delicate situation. Currently, there is a prospective trial with a new tracer, 2-fluoropropionyl-labeled pegylated dimeric arginine/glycine/aspartic acid peptide, to detect early responders in multiple cancers including glioblastoma treated with antiangiogenic agents on the basis of reduction of αvβ3-positive cells (NCT01806675).

Health-Related Quality of Life
As glioblastoma progresses, patients experience a decline in HRQoL. Delaying this decline is an important treatment goal, specifically in a disease like newly diagnosed glioblastoma, where patients considered eligible for a trial as well as patients in daily practice are not or are only minimally affected in the period following diagnosis. Furthermore, any treatment leading to more adverse effects may impact HRQoL. A secondary analysis of AVAglio showed that HRQoL was not negatively impacted despite earlier reports from the RTOG-0825 trial showing some decline in selected domains. Future trials, such as the ongoing EORTC 26101 trial, need to put an emphasis on these relevant patient-reported outcomes.

Biomarkers
Unlike most other indications for bevacizumab, tumor tissue or plasma biomarkers may help to decide which patients may derive the most (or least) benefit from anti-VEGF treatment. Interestingly, these might not be the initially proposed circulating VEGF-A, collagen IV, or laminin levels, but rather plasma MMP-2 and VEGFR2 expression on glioblastoma cells (or deficiency of phosphatase and tensin homolog) and pronuclear isocitrate dehydrogenase (IDH) wild-type glioblastoma defined by Phillips et al. Attention needs to be given to appropriate confirmation of all potential biomarkers in independent trial datasets, along with proper development of biomarker tests as a companion diagnostic. One interesting area of research is the development of MRI sequences that might predict response after a few days of therapy with an anti-angiogenic agent. Interestingly, in accordance with the vascular normalization hypothesis, an early increase in tumor perfusion and a change of tumor vascularity seem to be associated with better outcome in glioblastoma patients receiving an anti-angiogenic tyrosine kinase inhibitor.

Immune Modulation
Pathological tumor angiogenesis helps create an immunosuppressive microenvironment providing a significant barrier for effective antitumor immune responses. In addition to classic immunosuppressive cytokines, VEGF may contribute to glioma-induced immunosuppression by (i) inhibition of dendritic maturation and antigen presentation; (ii) induction of CD8+ T-cell apoptosis; (iii) promotion of regulatory T-cell activity; and (iv) restriction of T-cell migration across tumor vascular endothelium into tumors. In addition, agents with anti-angiogenic properties, such as enzastaurin, have been shown to limit the CNS influx of autoreactive T cells in preclinical animal models. Thus, current clinical trials focus on the combination of active vaccines targeting, for instance, HSPPC-96 (heat shock protein peptide complex 96; NCT01814813). The ReACT study (Clinicaltrials.gov: NCT01498328) demonstrated the safety and efficacy of the combination of a promising anti–epidermal growth factor receptor variant III peptide vaccine, rindopepimut, plus bevacizumab, with longer survival seen in patients with a higher antibody titer. Alternatively, the VEGF pathway may serve as a target for active immunotherapy. VEGFR2 contains CD8 epitopes suitable for vaccines. An oral DNA vaccine using a Salmonella vector targets VEGFR2 in patients with advanced pancreatic cancer.

Elderly Patients
Overall survival in elderly patients is short compared with OS in younger patients. Elderly patients receive less salvage therapy.
at recurrence than younger patients. In recurrent glioblastoma patients, bevacizumab has shown promising activity in particular in the elderly, and data from randomized trials of bevacizumab in patients with newly diagnosed glioblastoma (AVAglio, RTOG-0825) indicate a relevant increase in PFS. Only a minority of patients enrolled in AVAglio or RTOG-0825 qualified as elderly. In essence, elderly patients may be a subgroup in which any treatment that prolongs PFS will translate into an OS benefit and hence be particularly relevant. However, comorbidities in the elderly make it very likely that the triple combination of radiotherapy, temozolomide, and anti-angiogenic agents will be tolerated less well. As recent data justify a stratification based on MGMT promoter methylation status in elderly patients, a first-line trial of radiotherapy plus bevacizumab versus radiotherapy plus temozolomide would focus on elderly patients with newly diagnosed MGMT-unmethylated glioblastoma (Fig. 4).

Other activities will depend on the data from the phase III EORTC 26101 trial in recurrent glioblastoma and research performed on molecular subgroups as well as attractive combinations, especially with checkpoint-inhibiting compounds, or active immunotherapy.

### New Compounds

A search at Clinicaltrials.gov reveals trials with new compounds outside classical bevacizumab, cingletide, sorafenib, and VEGFR2 inhibition (Table 3). As one new target, Ang-1 and -2

![Table 3. Selection of ongoing clinical trials for anti-angiogenic therapies in glioblastoma](https://example.com/table3.png)


Abbreviations: BID, twice daily; CCNU, lomustine; c-Kit, type III receptor tyrosine kinases; FGFR, fibroblast growth factor receptor; FLT3, Fms-like tyrosine kinase receptor-3; MGMT, methylguanine methyltransferase; MTD, maximum tolerated dose; NA, not available; ORR, objective response rate; PD-L1, programmed death ligand 1; PR, partial response; RT, radiotherapy; TMZ, temozolomide.
potentially destabilizing vessels are discussed both as single targets and in combination with anti-VEGF strategies. Ang-2 and VEGF-A exhibit angiogenic synergy in a mutually compensatory fashion. Their inhibition (eg, with the novel Ang-2–VEGF-A cross monoclonal antibody) mediates antitumoral, antimetastatic, and anti-angiogenic efficacy in mouse models.

As outlined above, a different, though very attractive, strategy may apply an immunotherapeutic approach in conjunction with the angiogenic specifics of cancers. VXM01 is an orally available vaccine targeting VEGFR2, with a completed trial in pancreatic cancer (NCT01486329).

Funding
None declared.

Conflict of interest statement.
None declared.

Funding
None declared.

References
Wick et al.: Update on glioma anti-angiogenesis


