Targeting MET for glioma therapy

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Glioblastoma multiforme is the most common and most lethal of all primary brain tumors. Even with the standard therapy, life expectancy is still poor, with an average survival of approximately 14 months following initial diagnosis. Hence, there is an urgent need for novel treatment strategies that inhibit proliferation and angiogenesis in high-grade gliomas. One such strategy consists of inhibiting receptor tyrosine kinases, including MET and/or its ligand hepatocyte growth factor (HGF). Because of their widespread involvement in human cancer, HGF and MET have emerged as promising therapeutic targets, and some inhibitory agents that target them have already entered clinical trials. In this paper, the authors highlight recent evidence implicating HGF/MET pathway deregulation in glioblastoma multiforme, discuss therapeutic approaches to inhibit HGF/MET signaling, and summarize ongoing clinical trials targeting this pathway.

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KEY WORDS • MET • hepatocyte growth factor • glioblastoma multiforme

Glioblastoma multiforme (GBM; WHO Grade IV) is the most common and most lethal of all primary brain tumors. The incidence rate of GBM in the US is estimated to be 2.69 per 100,000 persons per year, comprising 12%–20% of all brain tumors.21,54 Even with the standard triad of surgery, chemotherapy, and radiation therapy, life expectancy is still poor, with an average survival of approximately 14 months following initial diagnosis.28 Hence, there is an urgent need for novel treatment strategies that inhibit proliferation and angiogenesis in high-grade gliomas. One such strategy consists of inhibiting receptor tyrosine kinases (RTKs).

Receptor tyrosine kinases are cell-surface receptors that regulate normal cellular processes through regulation by growth factors, cytokines, and hormones. There are 90 unique tyrosine kinase genes identified in the human genome, of which 58 are the transmembrane receptor type that can be classified into approximately 20 subfamilies.9 In addition to their physiological roles, RTKs are key determinants of malignancy in many human solid tumors, including non–small cell lung cancer, colorectal adenocarcinoma, prostate adenocarcinoma, squamous cell carcinoma of the head and neck, GBM, and gastric, pancreatic, breast, ovarian, and cervical cancers.62 According to The Cancer Genome Atlas genetic screening, 86% of human GBM samples harbor at least one genetic aberration in RTK pathways.15 These include frequent activating mutations in EGFR, ERBB2, PDGFRA, and MET. Additionally, overexpression of ligand and/or receptor and coexpression of both (autocrine loop formation) are frequent events in cancers, including GBM, and have been associated with increased malignancy and worse patient survival.35,45,61 Because of their critical roles in cancers, RTKs have become an attractive therapeutic target in cancers in general and for gliomas in particular.

MET is an RTK protein encoded by the MET proto-oncogene, and functions as a membrane receptor that is essential for embryonic development. MET and its ligand hepatocyte growth factor (HGF), also known as scatter factor (SF), are important mediators of malignancy in human cancers, including brain tumors. Aberrant MET activation in brain tumors enhances tumor growth by inducing cell proliferation, promoting tumor angiogenesis, inhibiting cell death, inducing tumor invasion, and promoting cancer stem cells.2,29,37,38,43,56 Activation of MET is associated with poor clinical outcomes.7,47 Inhibiting endogenous HGF and/or MET in experimental brain tumor models inhibits glioma growth and angiogenesis and promotes apoptosis.2,23 The HGF/MET oncogenic effects are mediated by a complex downstream signaling network: most importantly, the Ras/MAPK and PI3K/Akt pathways.55 Because of their widespread involvement in hu-
man cancer, HGF and MET have emerged as promising therapeutic targets, and some inhibitory agents that target them have already entered clinical trials. We highlight recent evidence implicating HGF/MET pathway deregulation in GBM, discuss therapeutic approaches to inhibit HGF/MET signaling, and discuss ongoing clinical trials targeting this pathway.

Structure and Signal Transduction of MET and HGF

Structure of HGF and MET

The human MET proto-oncogene is located in the 7q31 locus of chromosome 7.11 MET is initially synthesized as a 170-kD glycosylated single-chain precursor that is cleaved into a 50-kD extracellular α chain and a 140-kD transmembranous β chain that are linked together by a disulfide bridge.23 The β chain contains the cytoplasmic kinase domain and a protein docking site that contains 2 tyrosine residues essential for downstream signaling.8,22 The gene encoding the MET ligand HGF is located on chromosome 7q21.1.60 It produces a single-chain inactive precursor that is cleaved by serine proteases into a 69-kD α chain and a 34-kD β chain linked by a disulfide bond.48,53

MET-Dependent Signal Transduction

Binding of HGF to MET induces MET kinase catalytic activity, which triggers the phosphorylation of multiple residues in the receptor’s intracellular domain. When tyrosines Y1349 and Y1356 are phosphorylated, numerous substrates are recruited and bind to the MET docking site, including Gab1, Grb2, PI3K, Shc, Src, and others.44,55,64 This leads to the activation of downstream signal transduction pathways including the Ras/MAPK, PI3K/Akt, and STAT pathways. These interacting pathways mediate a diversity of cellular behaviors, including (Fig. 1) the following.

1) Activation of Ras/MAPK induces cell migration and proliferation.42,63 HGF triggers a cell survival signal through PI3K, Akt, Pak1, and NFκB that mediates resistance to apoptosis.12,19,20,41,67
2) The complex morphogenic phenomenon of branched tubules formation requires both Ras/MAPK and PI3K/Akt activation.8
3) The STAT pathway is necessary for endothelial tubule morphogenesis and endothelial cell proliferation, and has been implicated in MET-induced angiogenesis.10,32
4) HGF indirectly activates alternative RTKs such as EGFR by upregulating expression of EGFR ligands TGF-α and HB-EGF.57
5) β-catenin translocates into the nucleus following MET activation and participates in regulation of gene expression.49
6) Notch signaling is activated by the survival signal mediated through MET and Akt.28
7) MET enhances the so-called stemness of tumor-initiating cancer stem cells that contribute to tumor propagation and treatment resistance.20,65

HGF and MET Expression in Gliomas

MET is frequently overexpressed in GBM, and some gliomas show HGF autocrine activation of the MET signaling pathway.32 Several studies have found that HGF and MET are expressed at higher levels in human gliomas than in control brain tissue, and that expression levels correlate with tumor grade.32,58 The HGF content was measured in 74 clinical samples obtained from human low-grade and high-grade gliomas. The HGF expression in high-grade (WHO Grade III–IV) tumors was significantly higher than in low-grade (WHO I–II) tumors.38 Similarly, coexpression of HGF and MET is observed more frequently in Grade IV GBM than in low-grade glioma, consistent with the contribution of an HGF/MET autocrine loop to malignant progression in these tumors.32,51

HGF and MET are also overexpressed in glioma endothelial cells, consistent with the well-described role of HGF as a potent angiogenic factor. Several studies have shown that MET and HGF are expressed and functional...
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in neumicrovascular and brain tumor vascular cells. Moderate to high levels of immunoreactive and biologically active HGF were found in cultured brain-derived microvascular endothelial cells.\(^1\) Using double immunofluorescence staining and quantitative confocal laser scanning microscopy, it was also shown that the intensity of HGF and MET staining in human primary brain tumors is prevalent in both the infiltrating tumor cells and hyperplastic endothelium. Additionally, prominent HGF and MET immunostaining was observed in the vasculature of GBM, whereas less intense MET and HGF immunostaining was observed in areas of neovascularization within low-grade astrocytoma (WHO Grade I and II) and anaplastic astrocytoma (WHO Grade III).\(^2\) Using in situ hybridization, strong HGF mRNA expression was also detected in the majority of tumor cells and in vascular endothelial cells in GBM specimens, but was less prevalent in anaplastic astrocytoma, diffuse astrocytoma, pilocytic astrocytoma, and normal brain. MET immunoreactivity was also observed in GFAP-expressing astrocytic tumors and endothelial cells as well as in a subset of microglia/macrophages. Other experimental evidence points to autocrine and paracrine endothelium MET activation as a contributor to tumor angiogenesis.\(^3\)

### Oncogenic Effects of MET in Gliomas

#### Cell Proliferation

Activation of MET induces glioma tumor cell and endothelial cell proliferation. Treatment of glioma cells with exogenous recombinant HGF, or forced overexpression of HGF in glioma cells induce tumor cell and tumor endothelial cell DNA synthesis and proliferation.\(^4\) Conversely, inhibiting endogenous HGF or MET expression in glioma cells with U1snRNA/ribozymes, the HGF antagonist NK4, or an anti-HGF neutralizing monoclonal antibody (mAb) inhibits tumor cell proliferation.\(^5\) Moreover, inhibiting HGF and MET expression in human glioma xenografts reduces tumor cell proliferation.\(^6\) HGF/MET has been found to act at least in part through c-Myc activation of G1/S cell cycle progression.\(^7\)

#### Cell Survival

HGF is a potent inhibitor of apoptosis and chemotherapy- and radiotherapy-induced cell death in glioma tumor cells. Treating human glioblastoma cells with HGF partially inhibits the cytotoxic effects of gamma irradiation, cisplatin, camptothecin, Adriamycin, and Taxol.\(^8\) Similarly, forced expression of HGF in rat intracranial gliosarcomas reduces tumor cell sensitivity to in vivo gamma irradiation.\(^9\) Conversely, inhibiting endogenous HGF in either glioma cells or glioma xenografts leads to induction of apoptosis and cell death, with increased cleaved caspase-3 expression in cells and in xenografts.\(^10\) Inhibiting endogenous HGF and MET synergizes with radiation therapy in inhibiting the in vivo growth of GBM xenografts.\(^11\) This provides a rationale for using anti-HGF/MET therapies to sensitize brain tumors to radiation therapy.

### Cell Migration and Invasion

Hepatocyte growth factor stimulates cerebral microvascular endothelial cell motility and induces chemotactic migration of glioma cells.\(^11\) Conversely, MET inhibition via anti-MET U1snRNA/ribozymes attenuated glioblastoma cell migration in a 2D scratch assay.\(^12\) Additionally, treating glioma cells with HGF increases invasiveness in MET-positive malignant glioma tumor cells.\(^13\) Two studies have demonstrated that HGF increases invasiveness by inducing matrix metalloproteinase-2 (MMP-2) and urokinase-type plasminogen activator (uPA) expression and activation in glioblastoma cells.\(^14\)

### Cancer Stem Cell Regulation

Illustrative of its multiple roles, MET has been shown to contribute to glioma malignancy and resistance to antiproliferative therapies by regulating glioma stem cells (GSCs). Several preclinical studies showed that MET is highly expressed in GSCs.\(^15\) MET enhances GSCs via inducing the expression of reprogramming transcription factors and inducing differentiated cells to form multipotent stem cells.\(^16\) Additionally, MET pathway inhibition in GSCs inhibits tumor growth and invasiveness both in vivo and in vitro.\(^17\)

### Angiogenesis

The organization of endothelial cells into tubelike structures is an essential step in blood vessel formation. A study was conducted to analyze the capacity of angiogenic factors, including HGF, to induce endothelial tube formation in collagen gels.\(^18\) It showed that tissue levels of angiogenic factor extracts from high-grade gliomas were significantly more potent than extracts from low-grade tumors in stimulating endothelial cell tube formation.\(^19\) Additionally, HGF concentrations correlate with glioma grade and microvessel density, as determined by immunostaining for factor VIII–related antigen.\(^20\) Another study found that endothelial tubulogenesis increased in cocultures of endothelial cells and glioma tumor cells that secrete angiogenic factors, including HGF, but not with nontumorigenic cell types such as epithelial cells.\(^21\) A recent report showed that bevacizumab-resistant GBMs display increased hypoxia compared with pretreatment tumors in a manner correlating with their MET upregulation and activation. Moreover, silencing the MET gene in bevacizumab-resistant GBM–derived xenografts inhibited their invasion and survival in hypoxia, and converted them from bevacizumab resistant to bevacizumab responsive.\(^22\)

### HGF and MET as Targets for Glioma Therapy

Because of its widespread and profound involvement in human cancer, the MET pathway has emerged as an attractive target for cancer therapy. Among others, the following approaches have been developed to inhibit MET and/or HGF.

#### U1snRNA/Ribozymes and Antisense

Gene therapy approaches were the first used to in-
hibit HGF and MET expressions in experimental glioma animal models. Chimeric U1snRNA/ribozyme transgenes were the first agents used to inhibit HGF and MET expression in human cancer. Glioma xenografts were treated with U1snRNA/ribozymes delivered via liposome-DNA complexes and adenoviruses.2,3 There was marked tumor growth inhibition as well as tumor HGF and lower MET expression levels. Additionally, histological analysis showed a significant increase in brain tumor cell apoptosis and a significant decrease in tumor blood vessel density in glialoma tumor cells treated with U1snRNA/ribozymes. It was also shown that combining ionizing radiation therapy with U1snRNA/ribozyme achieves synergistic effects on angiogenesis inhibition.36 MET antisense oligonucleotides were also tested in glioma xenograft animal models. Antisense MET oligodeoxynucleotides enhanced the sensitivity of human glioma cells to paclitaxel; cell growth inhibition and apoptosis were more significantly affected than with either paclitaxel or the oligodeoxynucleotides alone.16

**NK4**

NK4 is a synthetic molecule that comprises the NH2-terminal hairpin domain and subsequent 4-kringle domains of HGF, but lacks the entire α chain. NK4 works by competitively inhibiting the specific binding of HGF to its receptor MET. In one study, mice bearing intracranial glioma xenografts received daily intratumoral injections of NK4 or buffer beginning 1–7 days after tumor cell implantation. Tumor volumes were reduced by 61% in the NK4-treated mice.13 The proliferative activity of the tumor cells and intratumoral microvessel densities were reduced by >30% regardless of when NK4 treatment was initiated. Additionally, the apoptotic fraction of tumor cells was increased up to 2-fold.13

**Small-Molecule Inhibitors**

Small-molecule kinase inhibitors are one of the most promising classes of targeted therapeutics for interfering with RTK pathway activation. These small molecules have good bioavailability following systemic delivery and have been clinically validated against many targets. There has been a recent surge in the development of small-molecule MET inhibitors, with at least 10 compounds in various phases of development.15,25 ARQ197 [ArQule] is one example of a non-ATP–competitive MET-specific inhibitor highly selective for the MET receptor. The half maximal inhibitory concentration values for MET inhibition and for multiple MET-driven cell responses are in the 50- to 100-nM range. ARQ197 is currently in Phase II and III trials for various systemic cancers. Other small-molecule inhibitors are somewhat less specific, but have the potential to simultaneously target multiple oncogenic kinases relevant to brain cancers. Exelixis has generated cabozantinib (XL-184), which inhibits both MET and VEGFR2. The cabozantinib Phase II study for relapsed GBM reported encouraging preliminary results. Of 26 patients assessed at 4 weeks, 10 patients (38%) achieved radiological response of ≥50% reduction from baseline, and 9 patients (35%) had tumor measurement reduction ranging from 24% to 49%.18

**Neutralizing mAbs**

Together with small-molecule inhibitors, mAbs presently constitute the most clinically applicable HGF/MET pathway inhibitors. Monoclonal antibodies are very specific and have a long biological half-life. Additionally, mAbs have the potential to chelate diffusible targets out of the brain and tumor parenchyma despite blood-brain barrier–associated limitations. Amgen, Inc. has generated and characterized several distinct, fully human mAbs, each of which binds to an epitope in the β chain of HGF, neutralizing it. One of these mAbs, rilotumumab (AMG 102), has already completed two Phase II clinical trials for recurrent GBM. The first trial studied rilotumumab alone,66 whereas the other studied the combination of rilotumumab with bevacizumab.6 In both trials, the overall survival was approximately 6 months. However, the first trial did not find significant antitumor activity, and the progression-free survival was similar among patients who had previously received bevacizumab compared with bevacizumab-naïve patients.66

It has not been possible to inhibit MET by using bivalent anti-MET antibodies because antibody-mediated receptor dimerization can induce receptor activation. MetMAb (OA-5D5) is a novel one-armed (monovalent) anti-MET antibody that has been developed to inhibit MET activation and GBM xenograft growth.67 Infusing the OA-5D5 anti-MET antibody directly into intracranial tumor xenografts inhibited the growth of HGF+/MET+ U87MG gliomas without affecting HGF+/MET+ G55 gliomas, thereby demonstrating mechanistic specificity. In OA-5D5–treated U87MG xenografts, cell proliferation and microvessel density were dramatically reduced, and apoptosis was significantly increased.67 This study demonstrated the feasibility and effectiveness of local intracranial delivery for brain tumor therapy. MetMAb has been humanized and affinity-matured to generate onartuzumab, which is under Phase II trial for recurrent GBM.

**Current Trials and Therapeutic Considerations**

Currently, there are 9 registered trials targeting HGF and MET in patients with gliomas. To date, these trials are in Phase I–II stages. Table 1 shows a summary of HGF and MET trials. Glioblastoma multiforme displays a considerable histopathological and genetic heterogeneity. Due to this complexity, it is most likely that only a subset of patients will benefit from HGF/MET pathway inhibition. It is therefore important to identify the factors that determine sensitivity to MET inhibition in GBM. Two recent studies found that HGF coexpression is the most important predictor of responsiveness to MET inhibition in animal models of GBM.68,69 Also, because of signal redundancy and compensatory mechanisms as well as the involvement of multiple molecular pathways in the growth, migration, invasion, and apoptotic resistance of tumor cells, it is unlikely that single molecular therapies would achieve substantial antitumor effects. Combination molecular therapies that simultaneously target different pathways are likely to achieve greater therapeutic success. Basic and translational research has provided a rationale for combining anti-HGF/MET therapies with
traditional cytotoxic therapies such as radiotherapy and chemotherapy. Other recent research has provided the rationale for combining anti-MET therapies with other molecular therapies. However, more research is required to uncover the interactions between MET and other oncogenic pathways in gliomas to devise new patient-tailored and more efficient MET combination therapies.

**Disclosure**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper. Author contributions to the study and manuscript preparation include the following: Conception and design: Awad. Acquisition of data: Awad. Analysis and interpretation of data: Awad. Drafting the article: Awad. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Abounader.

**References**


**TABLE 1: Summary of clinical trials targeting the MET/HGF signaling pathway**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Phase</th>
<th>Status</th>
<th>Patient Population</th>
<th>Combinations</th>
<th>Clinical Trial</th>
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<tbody>
<tr>
<td>Rilotumumab (AMG 102)</td>
<td>II</td>
<td>completed</td>
<td>advanced malignant glioma</td>
<td>bevacizumab</td>
<td>NCT00427440</td>
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<tr>
<td>Rilotumumab (AMG 102)</td>
<td>II</td>
<td>active</td>
<td>recurrent malignant glioma</td>
<td>bevacizumab vs bevacizumab (alone vs MetMAb mono-therapy, randomized)</td>
<td>NCT0113398</td>
</tr>
<tr>
<td>Onartuzumab (MetMAb)</td>
<td>II</td>
<td>active, not recruiting</td>
<td>recurrent GBM</td>
<td>lenvatinib</td>
<td>NCT0189513</td>
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<tr>
<td>Ficlatuzumab (AV-299)</td>
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<td>withdrawn</td>
<td>GBM</td>
<td>lenvatinib</td>
<td>NCT0143991</td>
</tr>
<tr>
<td>Golvatinib (E7050)</td>
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<td>active, not recruiting</td>
<td>advanced solid malignancies, GBM, unresectable Stage III/IV melanoma</td>
<td>lenvatinib</td>
<td>NCT01870726</td>
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<tr>
<td>INC28060 (INC280)</td>
<td>Ib/I</td>
<td>recruiting</td>
<td>recurrent GBM</td>
<td>buparlisib</td>
<td>NCT01644773</td>
</tr>
<tr>
<td>Crizotinib (PF02341066)</td>
<td>I</td>
<td>recruiting</td>
<td>diffuse intrinsic pontine glioma, high-grade glioma, pediatric</td>
<td>dasatinib</td>
<td>NCT02070488</td>
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<tr>
<td>Cabozantinib (XL-184)</td>
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<td>completed</td>
<td>GBM</td>
<td>temozolomide/radiation therapy</td>
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