Neuro-Oncology

Neuro-Oncology 16:viii7–viii13, 2014
doi:10.1093/neuonc/nou232

Targeted molecular therapies against epidermal growth factor receptor: Past experiences and challenges

David A. Reardon, Patrick Y. Wen, and Ingo K. Mellinghoff

Center for Neuro-Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

Corresponding Author: David A. Reardon, MD, Clinical Director, Center for Neuro-Oncology; Dana-Farber Cancer Institute; 450 Brookline Avenue, Dana 2134; Boston, MA 02215-5450 (david_reardon@dfci.harvard.edu).

Epidermal growth factor receptor (EGFR) has emerged as a highly attractive therapeutic target in glioblastoma (GBM) based on its high frequency of gene amplification and mutation and its identification as an upstream trigger of dysregulated cell signaling cascades that drive GBM pathophysiology. Extensive investment has been committed in an attempt to exploit EGFR therapeutically to improve outcome for GBM patients, including the development of a variety of EGFR-targeting therapeutics as well as the participation of hundreds of participants in multiple, carefully constructed clinical trials. In this review, we summarize the design and results of clinical trials evaluating EGFR tyrosine kinase inhibitors in recurrent and newly diagnosed GBM patients. While overall results thus far have been disappointing, it is premature to discount EGFR as a therapeutic target in GBM on the basis of these studies given the limitations in study design and the pharmacology of first-generation EGFR kinase inhibitors. Although important lessons have been learned, critical questions remain unanswered and warrant further study.

Keywords: EGFRvIII, epidermal growth factor receptor, glioblastoma, tyrosine kinase inhibitor.

The transmembrane receptor tyrosine kinase, human epidermal growth factor receptor (EGFR; HER-1), is an attractive therapeutic target for glioblastoma (GBM) based on substantive evidence supporting its role as an oncogenic driver. EGFR is amplified in ~40% of primary GBM tumors[1,2] Of note, EGFR overexpression is essentially absent in secondary GBMs[1] and is a mutually exclusive finding relative to IDH1/2 mutations.[3] In contrast to non–small cell lung cancer in which activating mutations localize to the intracellular kinase domain,[4] activation of EGFR in GBM is associated with gain-of-function missense mutations or in-frame deletions affecting the extracellular domain.[5,6] Approximately 50% of EGFR-amplified GBMs express EGFRvIII,[7] a constitutively active, ligand-independent mutant receptor with impaired downregulation.[8] In addition, EGFRc958, an EGFR mutant derived from a deletion of amino acids 521-603 resulting in enhanced ligand-dependent kinase activity, occurs in ~20% of EGFR-amplified GBM tumors.[9] EGFR activation occurs by receptor overexpression as well as multiple ligand-dependent and ligand independent mechanisms in glioblastoma tumors, and generates intracellular mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-OH kinase (PI3 K), and Src kinase pathway signalling as well as STAT transcription factor activation.[10] These mitogenic cascades direct gene transcription and cell activity to ultimately promote key phenotypic features of GBM cells including enhanced proliferation, survival, angiogenesis, and invasion. Preclinical data in orthotopic GBM models demonstrating anti-tumor benefit underscore the importance of EGFR activation and downstream mitogenic signaling.[11,12]

Enthusiasm for therapeutically targeting EGFR in GBM patients, initially ignited by proof-of-concept antitumor benefit observed with tyrosine kinase inhibitor (TKI) therapy against "oncogene-addicted" cancers such as chronic myelogenous leukemia and gastrointestinal stromal tumors, was further fueled by clinical benefit achieved by EGFR therapeutics for colorectal cancer,[13,14] head and neck carcinoma,[15,16] and non–small cell lung cancer.[17–20] A variety of EGFR inhibitors have been and continue to be evaluated for GBM patients including unarmed monoclonal antibodies (MAbs),[21–23] radiolabeled MAb injected intratumorally,[24] MAb/MAb fragment toxin conjugates administered either systemically or locally, and small molecule, ATP-competitive, EGFR TKIs (Table 1). EGFR TKIs are classified as first-generation reversible inhibitors that target EGFR and its coreceptor HER2 (gefitinib, erlotinib, and lapatinib); second-generation irreversible inhibitors (afatinib, dacomitinib, and neratinib); and third-generation inhibitors (AZD9291 and CLO-1686) targeting the T790M mutation associated with acquired resistance to first-generation EGFR TKIs in non–small cell lung cancer.[25,26] In addition, there are multitargeting TKIs with inhibitory capability against EGFR, as well as other growth factors, that have been evaluated for GBM such as vandetanib[27] and AAE788,[28] both of

Received 8 June 2014; accepted 4 August 2014, Advance Access publication 1 January 2014

Table 1. Targeted molecular inhibitors against epidermal growth factor receptor evaluated in clinical trials for glioblastoma

Mechanism of Action	Agent	Route of Administration	Ongoing GBM Clinical Trial	Citation
1st generation EGFR TKI	Erlotinib	Oral	NCT01110876 NCT00301418	34, 37, 38, 40, 43–45, 50, 61, 62, 64, 65, 67, 79–81
1st generation EGFR TKI	Gefitinib	Oral		33, 39, 42, 46, 60, 63, 66
1st generation EGFR/HER2 TKI	Lapatinib	Oral	NCT01591577 NCT0210905	
1st generation EGFR/VEGFR TKI	AEE788	Oral	None	28
1st generation EGFR/VEGFR TKI	Vandetanib	Oral	None	29
2nd generation EGFR/EGFRvIII TKI	Afatinib	Oral	None	68
2nd generation EGFR TKI	Dacomitinib	Oral	NCT01520870	
2nd generation EGFR TKI			NCT01112527	
2nd generation EGFR TKI	Neratinib	Oral	NCT01953926	
EGFR MAb	Cetuximab	Intravenous	NCT01238237	21, 23
EGFR MAb	Nimotuzumab	Intravenous	None	22
EGFR MAb	MAB 425	Intratumoral injection	None	24
EGFR MAb	Panitumimab	Intravenous	None	
EGFR/EGFRvIII MAb Toxin Conjugate	ABT 414	Intravenous	NCT01800695	
EGFR/EGFRvIII MAb toxin conjugate	AMG595	Intravenous	NCT01475006	
EGFRvIII MAb immunotoxin	MR1-1	Convection-enhanced delivery	None	

Abbreviations: EGFR, epidermal growth factor receptor; MAb, monoclonal antibody; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

which block EGFR and VEGFR2. This review will focus on the clinical experience with EGFR-specific TKIs for GBM.

Clinical Trials in Recurrent Glioblastoma Patients

Outcome for patients with recurrent GBM remains dismal. Meta-analyses of recently conducted clinical trials demonstrate overall radiographic response (ORR) and progression-free survival (PFS) rates at 6 months (PFS-6) between 0%–10% and 10%–15%, respectively, and provide a comparative benchmark to assess EGFR TKI trial results.[29–32] Importantly, all clinical trials for recurrent GBM patients evaluating first-generation EGFR TKIs have incorporated a continuous daily dosing schedule and have enrolled unenriched participants. None of the reported trials have required confirmation of enhanced EGFR expression or activation. A number of studies have evaluated these TKIs when administered as monotherapy, while others have evaluated combinatorial regimens.

The first study reported was a single-arm phase II trial of gefitinib in 57 GBM patients at first recurrence.[33] Gefitinib dosing was increased in patients concurrently receiving CYP3A enzyme-inducing antiepileptic drugs (EIAEDs). No radiographic responses were noted, progression-free survival at 6 months (PFS-6) was 13%, and median overall survival (OS) was 39.4 weeks. Of note, neither EGFR expression level nor presence of EGFRvIII mutation by immunohistochemistry was associated with outcome. Several subsequent studies evaluated single-agent erlotinib in recurrent GBM patients. No radiographic responses were observed, and the median PFS-6 was only 3% in 44 recurrent GBM participants with up to 2 prior episodes of progression treated with erlotinib on a single-arm phase II study,.[34] Of note, in 4 participants treated on this study with simultaneous pharmacokinetic analysis of plasma and resected tumor, the tissue-to-plasma ratios of erlotinib and its active metabolite OSI-420 were only 6%–8% and 5%–11%, suggesting inadequate intratumoral penetration of erlotinib.[35] A subsequent study supports poor CNS penetration of erlotinib due to interaction with efflux transporters P-gp and breast cancer resistance protein.[36] Accrual to a simultaneously performed single-arm phase II study of erlotinib in GBM patients at first recurrence was discontinued after the planned first-stage analysis due to poor outcome.[37] Forty-eight participants were treated on this study, and the ORR rate was 6%, PFS-6 was 20%, and median OS was 9.7 months. Of note, EGFR amplification did not correlate with outcome.

One randomized phase II study has been reported in recurrent GBM patients.[38] In this study, participants at first relapse were randomized to receive either chemotherapy ($n = 56$; temozolomide or carmustine) or erlotinib ($n = 54$). Median PFS-6 and OS for the erlotinib and chemotherapy arms were 11.4% versus 24.1% and 7.7 months versus 7.3 months, respectively. Correlation of molecular markers in erlotinib recipients was limited in this study due to small numbers, but immunohistochemical detection of EGFRvIII was associated with low PFS, while low pAKT predicted improved PFS. Pharmacokinetic analyses in this study also confirmed that concurrent EIAED administration enhances erlotinib metabolism and diminishes systemic exposures.

An important study evaluated 22 recurrent GBM patients who received gefitinib for at least 5 days prior to planned debulking surgery and then resumed gefitinib postoperatively until progression or unacceptable toxicity.[39] A control cohort of 12 participants with recurrent GBM, who underwent tumor resection without prior erlotinib therapy, was included. Median survival on this study was 8.8 months, and EGFR amplification status was not associated with outcome. Simultaneous tumor and plasma samples revealed a 20-fold increase in tumor gefitinib levels compared with plasma. In addition, EGFR was effectively dephosphorylated in gefitinib recipients compared with untreated control tumor samples. These findings suggest that gefitinib effectively penetrates GBM tumors and inhibits activation of EGFR. However, downstream-pathway analysis revealed no consistent difference in the phosphorylation status of canonical pathway effector molecules downstream of EGFR compared with untreated controls. Although gefitinib inhibited its intended target on the tumor cell surface, this finding suggested that it was ineffective at blocking downstream cell signaling. Inconsistent inhibition of EGFR or its downstream effectors was also observed in GBM patients treated with erlotinib[35] and the dual EGFR/HER2 inhibitor lapatinib.[5] While the quantitative analysis of signaling pathways in human GBM samples remains challenging, these data nonetheless suggest that first-generation EGFR TKIs do not sufficiently block the EGFR signaling network in GBM patients.

Following the limited antitumor benefit observed with single-agent EGFR TKI therapy, a number of studies were subsequently conducted that evaluated EGFR TKIs in combination with chemotherapeutics, inhibitors of cell signaling pathways, or antiangiogenic agents. Among chemotherapy combinatorial regimens, a phase I study determined the maximum tolerated dose (MTD) of erlotinib to be 450 mg/day and 200 mg/day for patients on and not on EIAEDs, respectively, when combined with temozolomide administered using the standard 5-day per 28-day cycle.[40] This phase I study enrolled a mixed population of stable and recurrent, grade III and IV malignant glioma patients and confirmed the detrimental impact of coadministered EIAEDs on systemic erlotinib exposures. A phase II study of erlotinib plus carboplatin (AUC 6 mgXml/min every 28 days) in 43 recurrent GBM patients with up to 2 prior recurrences yielded ORR and PFS-6 rates of 2.3% and 14%, respectively, with a median OS of 30 weeks. Interrogation of archival tumor failed to detect a correlation between EGFR, Akt, or phosphatase and tensin homolog (PTEN) expression and outcome.

Additional combinatorial regimens evaluated EGFR TKIs with inhibitors targeting intermediaries of dysregulated cell signaling pathways. The rationale for these studies included the possibility that compensatory activation of either downstream pathway components or alternative mitogenic/survival pathways may contribute to EGFR TKI resistance.[41] Several studies have evaluated the combination of an EGFR TKI with inhibitors of the mammalian target of rapamycin (mTOR), a key downstream mediator of PI3/Akt signaling. A phase I study of recurrent malignant glioma patients established the MTD of gefitinib and sirolimus, an oral mTOR inhibitor, and reported ORR and PFS-6 rates of 5.9% and 23.5%, respectively.[42] A follow-up, single-arm phase II study in 32 heavily pretreated, recurrent GBM patients treated with erlotinib plus sirolimus reported that no radiographic responses were achieved and that the PFS-6 rate was only 3.1%.[43] Presence of EGFRvIII, pEGFR, and EGFR amplification did not correlate with OS. A phase I/II study of erlotinib plus temsirolimus (another oral mTOR inhibitor) revealed significant toxicity associated with this combination requiring de-escalation of temsirolimus to a dose level ~1/3 that of single-agent therapy.[44] No radiographic responses were observed in the 42 participants treated on the phase II portion of this study, and PFS-6 was only 13%. A pilot study of 28 heavily pretreated malignant glioma patients treated with either gefitinib or erlotinib in combination with sirolimus reported an ORR rate of 15% with 25% achieving PFS-6.[45] Outcome of 22 recurrent GBM patients treated with gefitinib plus everolimus, an alternative mTOR inhibitor, revealed a 14% ORR rate, but only one participant (4.5%) remained progression-free on this combination for at least 6 months.[46]

An additional combinatorial regimen consisting of dual inhibition of EGFR and VEGFR2 signaling was advanced to the clinic based on data demonstrating compensatory activation of VEGF signaling as a mediator of EGFR resistance[47,48] as well as preclinical data in an orthotopic GBM model demonstrating that dual targeting of EGFR and VEGFR was superior to anti-EGFR therapy alone.[12] Based on these findings, a single-arm phase II study evaluating erlotinib plus bevacizumab, a humanized monoclonal antibody against VEGF that is FDA-approved for recurrent GBM,[49] was conducted in recurrent malignant glioma patients.[50] Erlotinib was administered at 200 mg/day and 500 mg/day for patients taking and not taking EIAEDs, respectively, and bevacizumab was administered at 10 mg/kg biweekly. In this study, PFS-6 and median OS were 28% and 42 weeks, respectively, and did not differ from outcome achieved with bevacizumab monotherapy.[51,52]

Clinical Trials in Newly Diagnosed Glioblastoma Patients

Median survival following multimodality therapy for newly diagnosed GBM patients, including maximum safe resection followed by radiation therapy with daily temozolomide and adjuvant cycles of temozolomide, is ~15 months[53] and has failed to improve despite recent efforts to dose-intensify temozolomide[54] and block angiogenesis with bevacizumab.[55,56] Enthusiasm for evaluating EGFR TKIs integrated into standard temozolomide chemoradiotherapy[53] for newly diagnosed GBM patients was based on preclinical data demonstrating that EGFR inhibition enhanced the therapeutic efficacy of radiation therapy and data suggesting that EGFR-triggered pathway activation conferred resistance to radiation and chemotherapy.[57–59]

Phase I studies confirmed that standard daily doses of EGFR TKIs can be safely coadministered with established temozolomide chemoradiotherapy.[60–63] Subsequent phase II studies have unfortunately failed to demonstrate an improvement in survival with the addition of EGFR TKIs to standard therapy for newly diagnosed GBM patients. Three phase II studies evaluated erlotinib for newly diagnosed GBM patients. Median OS was 15.3 months and did not correlate with the presence of EGFRvIII, EGFR amplification, or PTEN loss in the 97 patients treated on NCCTG NO177, a single-arm phase I/II study.[61] Two additional studies conducted in single-institution settings have reported somewhat discrepant results, the explanation for which is unclear. Median OS in one study of 27 patients was only 8.6 months.[64] In contrast, a second study of the same regimen in 65 newly

diagnosed GBM patients reported a median OS of 19.3 months.[65] Of note, outcome in the latter study was associated with PTEN expression by immunohistochemistry.

Two phase II studies have reported outcome with gefitinib for newly diagnosed GBM patients. Of note, gefitinib was added to external beam radiotherapy (XRT) alone, and participants treated in these 2 studies did not receive temozolomide. RTOG 0211 treated 147 newly diagnosed GBM patients with XRT and gefitinib, which was administered at 500 mg/day throughout XRT and for up to 18 months following XRT completion.[63] Median OS was only 11.5 months on this study and did not correlate with EGFR expression detected by immunohistochemistry. Ninety-eight participants were similarly treated on NCCTG N0074 and achieved a median OS of only 12 months.[66] In this study, neither EGFR expression nor amplification was associated with OS.

A recently reported single-arm, single institution, phase II study of standard temozolomide chemoradiotherapy plus daily erlotinib and bevacizumab noted a median OS of 19.8 months in 59 newly diagnosed GBM patients.[67] Although the regimen was adequately tolerated, outcome was not significantly different from that recently reported for 2 phase III, placebo-controlled studies evaluating the addition of bevacizumab to temozolomide chemoradiotherapy.[55,56]

Second-generation/Irreversible EGFR TKI Trials

Three studies evaluating second-generation/irreversible EGFR TKIs have been undertaken for GBM. These studies target recurrent GBM patients and utilize continuous daily dosing schedules. BI 1200.36 is a phase I/randomized phase II study of afatinib with and without protracted temozolomide in GBM participants at first or second recurrence after standard temozolomide chemoradiotherapy.[68] The rationale for incorporating protracted temozolomide in this study was based on encouraging outcome associated with daily protracted temozolomide in some recurrent GBM patients.[69] The phase I portion of this trial established the MTD of afatinib to be 40 mg/day when given with temozolomide administered at 75 mg/m2/day of each 28-day treatment cycle. On the phase II portion, 119 participants were randomized to receive either single agent afatinib ($n = 41$; 40 mg/day), protracted temozolomide ($n = 39$; 75 mg/m2/day of each 28 day treatment cycle), or afatinib plus protracted temozolomide ($n = 39$). The ORR rate with afatinib monotherapy was 2.4%, and PFS-6 for the afatinib-protracted temozolomide and combination therapy arms was 3%, 23%, and 10% respectively. None of the biomarkers evaluated in this study, including EGFR, EGFRvIII, PTEN, pAKT, and MGMT by immunohistochemistry as well as EGFR and PTEN copy number by fluorescence in situ hybridization, was associated with outcome, although a nonstatistically significant association between EGFRvIII expression and outcome was observed.

Two ongoing studies are evaluating dacomitinib in recurrent glioblastoma patients. Of note, these are the only EGFR TKI studies conducted to date in recurrent GBM patients with eligibility restricted to participants with archival tumor markers felt to predict a potentially beneficial response to EGFR blockade, as suggested by prior analyses.[70,71] NCT01520870, a phase II study of patients with either EGFR-amplified or EGFRvIII-positive tumors, is being conducted by the Grupo Español de Investigacion en Neurooncologia. This study will enroll 64 participants, and its primary study endpoint is PFS-6. NCO01112527 is a phase II study in patients with EGFR-amplified tumors and is enrolling 3 treatment cohorts. Participants in cohort A undergo surgical resection after daily dosing of dacomitinib. Results from this cohort will help determine the blood-brain barrier penetration of dacomitinib as well as its ability to inhibit intratumoral EGFR phosphorylation. Participants enrolling into cohorts B and C are bevacizumab naïve and refractory, respectively, and the primary endpoint for these cohorts is median PFS. The irreversible EGFR inhibitor neratinib is also under investigation in GBM in an open-label phase II study of patients with solid tumors demonstrating somatic EGFR mutation or EGFR gene amplification (NCT01953926).

Going Forward: Important Unanswered Questions

As summarized, aggregate clinical trial data to date evaluating EGFR TKIs appear to support a general conclusion that EGFR inhibition is not of value for GBM patients. Although this conclusion may ultimately prove to be correct, an argument can also be supported that acceptance of this conclusion is premature because compelling questions remain answered.

Nonetheless, given the large number of patients treated with these agents, the volume of studies conducted and the extent of resources committed, there are some conclusions that can be confidently drawn. First, first-generation EGFR TKIs administered on a continuous daily dosing schedule in unenriched GBM patients are inactive when administered as monotherapy in the recurrent setting or in combination with either standard radiotherapy or standard temozolomide radiotherapy in newly diagnosed patients. Second, first-generation EGFR TKIs are ineffective when combined with available mTOR inhibitors and do not improve outcome when combined with bevacizumab.

Several factors, as reviewed elsewhere in this supplement, may contribute to these disappointing results including inadequate tissue penetration,[35,36] inadequate target inhibition,[35] ineffective suppression of downstream signaling[39] due to redundant cell signaling pathways,[72] or compensatory activation of alternative signaling mediators[73,74] as well as cellular heterogeneity within and across GBM tumors.[75–77] It remains unclear whether alternative EGFR targeting reagents will sufficiently overcome these factors to generate improved therapeutic outcome.

Several critical questions also remain unanswered and warrant appropriate investigation before further conclusions can be accepted. First, are we using optimal inhibitors? We now know, based on recent identification of mutation patterns in the extracellular EGFR domain, that GBM tumors are predicted to respond poorly to first-generation EGFR inhibitors.[5] Perhaps an additional generation of inhibitors designed to interact effectively with EGFR mutations characteristic of GBM tumors will be required to realize therapeutic benefit.

Second, does the "holy grail" predictive biomarker of response to EGFR blockade exist? We know EGFR is differentially expressed among subclasses of GBM tumors and that all patients are not expected to respond equally.[78] Despite initial analyses suggesting that molecular determinants may be predictive of response,[70,71] relevant biomarkers, including expression of EGFR, EGFRvIII, pAkt, and PTEN as well as EGFR amplification, have not predicted outcome in prospectively conducted trials to date. Nonetheless,

these data warrant interpretation in the context of their generation in trials evaluating poorly active, first-generation EGFR TKIs. Further evaluation of relevant biomarkers should be incorporated into planned studies evaluating other, potentially more effective EGFR-targeting therapeutics. In addition, evaluation of delivery and suppression of downstream signaling, as elegantly demonstrated in the Hegi study,[39] should be incorporated into the development of future EGFR therapeutics in order to confidently determine if the drug is being effectively delivered and achieving its intended biologic effect.

Third, should we prioritize combinatorial therapy? Given the complexity and heterogeneity of GBM tumors as well as their unrelenting ability to adapt and acquire resistance, it is unlikely that monotherapy strategies will generate sufficiently durable benefit. Combinatorial regimens will likely be required. To date, the only combinatorial regimens evaluated with EGFR TKIs include either mTOR or VEGF inhibitors. Although the results of these studies, as described above, are disappointing, preclinical studies may guide the development of more effective combinatorial regimens.

Finally, are we dosing optimally? All of the studies to date have incorporated continuous daily dosing schedules. This dosing option is not based on data but rather dogma that continuous blockade is required to generate effective antitumor activity. Alternative dosing regimens, including pulsatile schedules, have proven feasible with first-generation EGFR TKIs in other cancer types[79–81] and are under evaluation in GBM (NCT01257594, NCT02101905). Similarly, rationally designed, sequential-dosing schedules for combinatorial regimens may generate greater antitumor effects and also warrant careful investigation.

In conclusion, future attempts to therapeutically target EGFR for GBM should incorporate determination of the concentration of drug delivered to both enhancing and nonenhancing components of the tumor as well as evaluation of the percent EGFR inhibition achieved using daily versus pulsatile dosing schedules. To do this, a carefully planned perioperative dosing study will be required that restricts enrollment to a genetically/biochemically defined, relatively uniform patient population with high-level EGFR phosphorylation. A quantitative bioassay to measure EGFR phosphorylation can then compare tumor material from treated patients with that of untreated control patients.

Conflict of interest statement. D.A.R. acknowledges the following: Advisory Boards for: Novartis, EMD Serono, Genentech/Roche, Merck/Schering, Midatech, Stemline Therapeutics, Amgen, and Momenta Pharmaceuticals. Speaker board activity for: Genentech/Roche and Merck.

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