Subject Review

Therapeutic Inhibition of the Epidermal Growth Factor Receptor in High-Grade Gliomas: Where Do We Stand?

Georg Karpel-Massler,1 Ursula Schmidt,2 Andreas Unterberg,1 and Marc-Eric Halatsch1

1Neurochirurgische Klinik und Poliklinik, Heidelberg, Germany and 2Klinik für Abdominal- und Transplantationschirurgie, Hannover, Germany

Abstract

High-grade gliomas account for the majority of intra-axial brain tumors. Despite abundant therapeutic efforts, clinical outcome is still poor. Thus, new therapeutic approaches are intensely being investigated. Overexpression of the epidermal growth factor receptor (HER1/EGFR) is found in various epithelial tumors and represents one of the most common molecular abnormalities seen in high-grade gliomas. Dysregulated HER1/EGFR is found in 40% to 50% of glioblastoma, the most malignant subtype of glioma. Several agents such as tyrosine kinase (TK) inhibitors, antibodies, radio-immuno conjugates, ligand-toxin conjugates, or RNA-based agents have been developed to target HER1/EGFR or its mutant form, EGFRvIII. To date, most agents are in various stages of clinical development. Clinical data are sparse but most advanced for TK inhibitors. Although data from experimental studies seem promising, proof of a significant clinical benefit is still missing.

Among the problems that have to be further addressed is the prediction of the individual patient's response to HER1/EGFR-targeted therapeutics based on molecular determinants. It is quite possible that blocking HER1/EGFR alone will not sufficiently translate into a clinical benefit. Therefore, a multiple target approach concomitantly aimed at different molecular sites might be a favorable concept. This review focuses on current HER1/EGFR-targeted therapeutics and their development for high-grade gliomas.

Introduction

Gliomas are the most common primary brain tumors (1). More than half of all gliomas are glioblastoma, the most aggressive grade. Unfortunately, refinements of available therapeutic modalities (i.e., microneurosurgery, radio-, and chemotherapy) have so far failed to substantially improve the poor prognosis of patients with high-grade gliomas. Median survival of patients with anaplastic astrocytoma is approximately 2 to 3 years, and in glioblastoma median survival is only 1 year (2, 3). Thus, new therapeutic approaches are urgently needed.

The epidermal growth factor receptor (HER1/EGFR) belongs to the HER family of receptors (4). All members of this receptor family consist of an extracellular ligand-binding site, a transmembrane part and an intracellular tyrosine kinase (TK) domain. The receptors of the HER family are expressed on many cell types and initiate signal transduction from the cell surface to the intracellular compartment by which diverse cellular functions, including proliferation and differentiation, are regulated (4). Once a ligand binds to the ligand-binding site of an HER family receptor, homodimerization or heterodimerization with a different HER receptor occurs and activates the intrinsic TK. Subsequently, autophosphorylation and initiation of cytoplasmic signaling cascades such as the ras-rafmitogen-activated protein kinase (MAPK) pathway or the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway occur (Fig. 1). Because of the different ligand-binding affinities of the HER family receptors and depending on how the homo- or heterodimer is composed, the downstream signaling characteristics vary. The most common ligands for HER1/EGFR are epidermal growth factor (EGF) and transforming growth factor-α (TGF-α; ref. 7). Noteworthy, HER2/neu does not bind to any known ligand.

In the 1980s, HER1/EGFR overexpression or activation was found in more than 50% of various epithelial tumors (8). Other members of the HER receptor family such as HER2/neu or HER3 were also shown to be overexpressed or activated in certain neoplasias such as breast cancer. The causative relationship between dysregulation of the HER1/EGFR and neoplastic disorder is explained by the affection of downstream signal transduction resulting in impaired apoptosis and or stimulation of proliferation, invasion, tumorigenesis, and angiogenesis (9).

There are several possible mechanisms leading to receptor dysregulation. One of these mechanisms is gene amplification causing HER1/EGFR overexpression. It was shown that in glioblastoma 40% to 50% of the tumors overexpress HER1/EGFR (10). Similar findings were reported for other tumor entities such as non-small cell lung cancer (NSCLC) (43-83%) or pancreatic carcinoma (21-50%; refs. 11-13). Another mechanism causing enhanced HER1/EGFR-mediated signaling is autocrine overproduction of HER1/EGFR ligands, leading to independent receptor activation and loss of external control of cell growth. A third way by which HER1/EGFR signaling might be pathologically altered is the intrinsic alteration of the receptor structure due to mutation. Many HER1/EGFR mutants have been described. One of the most interesting mutant forms that accounts for approximately 60% of all mutants is...
EGFRvIII resulting from an in-frame deletion of 801 base pairs in the DNA sequence encoding the extracellular ligand-binding domain (14). Lacking a ligand-binding site, EGFRvIII is constitutively activated and beyond external control (15). Besides its eminent expression in high-grade gliomas, EGFRvIII has been found in breast cancer and NSCLC (16). Expression of EGFRvIII is related to cellular transformation and enhanced tumorigenicity (17). Other HER1/EGFR mutant forms such as EGFRvII and EGFRvV that are found in high-grade gliomas appear less frequent and are of uncertain clinical relevance (14). The accumulation of evidence that HER1/EGFR is involved in the regulation of cellular proliferation and differentiation and promotes malignant transformation and tumor growth has generated substantial interest in the creation of therapeutic strategies targeting HER1/EGFR and or its mutant forms.

High-Grade Glioma and HER1/EGFR

Gliomas are derived from glial cells such as macroglia, astrocytes, or oligodendrocytes (18, 19). According to the World Health Organization (WHO) classification, four grades are distinguished depending on histological characteristics that are outlined in Table 1 (20). Grade III and IV gliomas are referred to as high-grade gliomas. Glioblastoma is the most common malignant primary brain tumor; accounts for more than 50% of all gliomas, and is histologically characterized by a high proliferation rate, neo-angiogenesis, and necrosis (21). The clinical course typically features rapid tumor progression or recurrence leading to death in 75% of the patients within 18 months of diagnosis (21, 22). Two major subclasses of glioblastoma are distinguished owing to their respective clinical and genetic properties (23). Primary glioblastoma arise in a de novo fashion from normal glial cells or their precursors and account for the vast majority of all glioblastoma. In contrast, secondary glioblastoma develop by progression from lower-grade gliomas and are much less frequently encountered. An exact estimation of the frequency of primary or secondary glioblastoma is difficult because rapid progression of a lower-grade tumor to a grade IV glioma might occur without prior evidence of a pre-existing lower-grade lesion (24). However, approximately 95% of all glioblastoma are estimated to be of primary origin, and only 5% may arise secondarily (24). On the molecular level, HER1/EGFR gene amplification has been shown to be five times higher in primary glioblastoma when compared with secondary glioblastoma (25). Similarly, HER1/EGFR overexpression is found in about 60% of primary glioblastoma and in only 10% of secondary glioblastoma. Inverse findings were obtained with regard to the frequency of inactivated p53 (25). Thus, a major molecular difference between both glioblastoma subtypes consists in amplification and overexpression of an oncogene versus inactivation of a tumor suppressor gene. Other abnormalities observed in both subtypes involve inactivation of the p16 tumor suppressor gene caused by various mechanisms (26). Evidently, the dysregulation of multiple molecular pathways is required to yield formation of glial tumors. Although the sequence of genetic alterations is unknown in primary glioblastoma formation, HER1/EGFR gene amplification may constitute a late event in secondary glioblastoma pathogenesis because HER1/EGFR overexpression is found in only 10% of anaplastic astrocytoma (10). The fact that HER1/EGFR represents the most often amplified gene in glioblastoma renders it an outstanding therapeutic target. Data from in vitro studies show that HER1/EGFR stimulates tumor growth, migration, and invasion (27). In addition, clinical data suggest that HER1/EGFR amplification is related to worse prognosis and decreased overall survival in patients with glioblastoma (28). It was also shown that resistance of glioblastoma cells in vitro toward radiation therapy is related to HER1/EGFR overexpression, explaining that some patients with glioblastoma show particularly poor response to this treatment modality (29, 30).

As already mentioned, EGFRvIII represents the most common HER1/EGFR mutant form and is characterized by constitutive TK activity and consecutively enhanced downstream signaling. Data from biotic glioblastoma specimens suggest

**FIGURE 1.** Schematic representation of signal transduction and cellular effects due to activation of HER1/EGFR or its mutant forms. In addition, various HER1/EGFR-targeted therapeutic approaches are illustrated. AKT, murine thymoma viral oncogene homolog; 125I, 125Iodine; mAb, monoclonal antibody; MEK, mitogen-activated protein kinase; RAF, murine leukemia viral oncogene homolog; RAS, rat sarcoma viral oncogene homolog.
concomitant overexpression of both EGFRvIII and HER1/EGFR in most of the tumors. However, in about 90% of glioblastomas, amplification and or overexpression of EGFRvIII was exceeded by that of the wild-type receptor (31). An experimental study using a model of human glioma xenografts in mice showed that EGFRvIII expression was related to increased proliferation, tumor formation, and inhibition of apoptosis (17, 32). These findings were confirmed by other studies that identified activation of the MAPK/ERK1/2 and PI3-K/Akt pathways as driving forces of cellular proliferation and tumor progression, thus explaining worse outcomes of patients with EGFRvIII-positive glioblastoma (33, 34). It was also shown in vitro that EGFRvIII-expressing tumor cells coexpress matrix metalloproteinases to a higher extent, possibly accounting for increased invasiveness of these glioma cells (35). In a murine model of intracranially xenografted glioblastoma cells, administration of a monoclonal antibody targeting EGFRvIII (mAb 806), resulted in a significant decrease of tumor growth, increase of apoptosis, and prolongation of survival (36). In this study, no adjuvant chemotherapeutic agent was given, suggesting that the antitumor effects of mAb 806 were due to inhibition of the EGFRvIII-mediated signaling cascade. In conclusion, enhanced HER1/EGFR-mediated downstream signaling due to excessive TK activity may be necessary for survival of glioma cells overexpressing HER1/EGFR or EGFRvIII, and inhibition of this cascade may lead to apoptosis or increase the cellular susceptibility toward other therapies. Therefore, a number of strategies have been developed to specifically inhibit dysregulated HER1/EGFR-mediated signaling at various levels. Among these strategies are interference with ligand binding to reduce TK activation and targeting of regulatory elements in signal transduction. Efforts were also directed toward creating individual protein- or nucleic acid-based vaccines against specific HER1/EGFR mutants or targeting toxins to HER1/EGFR using antibodies or ligands as vectors.

### Current Therapeutic Options for Glioblastoma

Despite recent developments in diagnostic techniques and treatment, the clinical outcome of patients with glioblastoma has remained poor. The vast majority of patients is not cured by surgical resection, and tumors usually recur in a distance of less than 2 cm from the initial site (37). However, the goal to attempt gross total tumor removal is generally accepted. Surgical resection should be tailored to avoid severe postoperative neurological morbidity and therefore may have to be done with limited radicality. According to current treatment standards, surgery is followed by adjuvant therapy consisting of radiotherapy combined with concomitant and subsequent chemotherapy. Irrespective of the extent of surgical tumor removal, adjuvant administration of 50 to 60 Gy of whole brain irradiation may increase survival by 14 to 36 weeks as shown by several randomized studies (3, 38, 39).

Initially, adjuvant chemotherapy was found to provide only a modest therapeutic benefit (1, 40). However, in a landmark phase III clinical trial, Stupp and colleagues showed that in patients with newly diagnosed glioblastoma administration of temozolomide (Temodar/Temodal, Schering Corporation) given concomitantly (75 mg/m² per day) with and subsequently (six cycles of 150-200 mg/m²) to radiation therapy (2 Gy per day, 5 days per week for 6 weeks) significantly increased 2-year survival from 10.4% to 26.5% and median survival from 12.1 months to 14.6 months when compared with adjuvant radiation therapy alone (41). Because of this study, a new therapeutic standard of care was established. But despite additional chemotheraphy with temozolomide, the prognosis of patients with glioblastoma remains grim as illustrated by the fact that progression-free survival and overall survival were only 6.9 and 14.6 months, respectively.

Only a small number of patients are cured despite abundant and aggressive treatment. Once tumors recur only limited therapeutic options remain. Life expectancy diminishes drastically; 25 weeks for recurrent glioblastoma and 47 weeks for recurrent anaplastic astrocytoma (42). Treatment recommendations are almost exclusively based on phase II clinical studies. Repeated extensive tumor resection is not always a reasonable approach because the risk of neurological deficits due to normal brain tissue injury increases significantly with progressive tumor invasion. However, if feasible, gross tumor resection should repeatedly be attempted. Surgery may be combined with local chemotherapy using polifeprosan 20/carmustine implants (Gliald Wafer, Guilford Pharmaceuticals), an approach associated with an increased survival of 2 to 3 months in phase III clinical trials (43, 44). Using brachytherapy or stereotactic radiosurgery to focus radiation on the tumor bed to avoid damage to healthy surrounding tissue might be an option for some patients. Survival is prolonged only modestly by this approach, and it is for the most part of only palliative character (45, 46). Conventional chemotherapy does not provide significantly better outcome either, and resistance to chemotherapy is frequently encountered. Most published studies examining the effect of chemotherapy in recurrent high-grade glioma were conducted

### Table 1. WHO Classification for Astrocytomas

<table>
<thead>
<tr>
<th>Grade</th>
<th>WHO Classification</th>
<th>Type</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>I</td>
<td>Pilocytic astrocytoma</td>
<td>Little cellularity, minimal pleomorphism</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Subependymal giant cell astrocytoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diffuse astrocytoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pilocytic astrocytoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pleomorphic xanthoastrocytoma</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>III</td>
<td>Anaplastic astrocytoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Glioblastoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Giant cell glioblastoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gliosarcoma</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Information adapted from Louis et al. (20).
Overall, despite vast technological progress in medicine as a whole, the clinical outcome of patients with glioblastoma has not substantially changed over the last five decades. However, a more profound understanding of the molecular basis of tumorigenesis and tumor progression has revealed new promising targets for a more specific and hopefully more effective therapy.

**Targeting HER1/EGFR in the Treatment of Glioblastoma**

Cellular proliferation is predominantly regulated by growth factors and their receptors. The EGF has been shown to contribute to tumorigenesis and tumor progression of various human epithelial cancers (10). Gene amplification of HER1/EGFR is found in 40% and overexpression in 60% of glioblastoma. In addition, dysregulation of HER1/EGFR is correlated to more aggressive biological behavior of the respective tumors (27, 28). Small-molecule TK inhibitors are the most clinically advanced HER1/EGFR-targeted agents for the treatment of high-grade gliomas. Antibodies have also been developed to specifically block the ligand-binding site of HER1/EGFR and to prevent TK activation. Other therapeutics such as RNA-based therapies, ligand-toxin conjugates, and radio-immuno conjugates are being evaluated. Table 2 shows HER1/EGFR-targeted agents that have been used in clinical trials.

**Small-Molecule TK Inhibitors**

HER1/EGFR TK inhibitors such as erlotinib (Tarceva, Genentech Inc.) or gefitinib (Iressa, AstraZeneca Pharmaceuticals) are small molecules that bind to the intracellular TK domain of the receptor and compete with adenosine triphosphate (9, 59). Because of reversible or irreversible binding to the TK domain, phosphorylation of the receptor as well as further downstream signaling involving PI3-K/Akt and MAPK pathways are being intercepted. Thus, HER1/EGFR-mediated cellular effects should be blocked or at least decreased. Erlotinib and gefitinib are the best explored TK inhibitors in this setting, and both specifically inhibit HER1/EGFR, whereas erlotinib additionally inhibits EGFRvIII (19). Erlotinib diminishes EGFRvIII expression in transformed glioblastoma cells on long-term exposure and also inhibits induction of certain genes encoding proinvasive proteins (35). Experimental studies showed that the extent of erlotinib-mediated inhibition of anchorage-independent growth of glioblastoma-derived cell lines correlates inversely with the cellular capability to induce HER1/EGFR mRNA, emphasizing the important role of HER1/EGFR in the pathogenesis of glioblastoma (60). In other human cell lines or xenograft models unrelated to glioma, erlotinib was shown to induce apoptosis and to inhibit cell cycle progression, resulting in decreased tumor growth (61-63). Gefitinib was found to be less effective in experimental studies evaluating its effect on glioblastoma. Interestingly, gefitinib works best against tumors expressing HER1/EGFR with mutations in exons 19 and 21 of the TK domain, an alteration that is lacking in glioblastoma and other gliomas (64-66).

Other small-molecule TK inhibitors were developed aiming at combined inhibition of multiple targets likely to play a role...
in the pathogenesis of glioblastoma. However, experience with these newer drugs is still limited, and an additional benefit derived from concomitant inhibition of multiple targets remains to be proven.

Both gefitinib and erlotinib were first applied clinically in the setting of advanced or metastatic NSCLC. Although gefitinib failed to show a survival benefit in a phase III clinical study when compared with placebo (67), treatment with erlotinib resulted in a 42.5% increase of median survival versus placebo in a randomized phase III clinical trial (68).

In various clinical trials, erlotinib and gefitinib were also studied for the treatment of glioblastoma. Both drugs were shown to fit a reasonable safety profile in phase I trials (69, 70). Moreover, it was shown that enzyme-inducing antiepileptic drugs such as phenytoin (Dilantin, Pfizer Pharmaceuticals) or carbamazepine (Tegretal, Novartis Pharmaceuticals Corporation) increase the metabolism of gefitinib and erlotinib, necessitating either changes in medication to third-generation anticonvulsive drugs or higher dosages of TK inhibitors (37). Both drugs were generally well tolerated. In a phase II study examining 16 patients with recurrent glioblastoma, Vogelbaum and colleagues showed combined partial response and stable disease rates of 25% (71). However, in another phase II trial, a median progression-free survival of only 12 weeks was reported for patients receiving erlotinib monotherapy on glioblastoma relapse, casting doubt on the efficacy of erlotinib in this setting (72). Similarly, van den Bent and colleagues currently showed in the one and only published randomized, controlled phase II trial that only 11.4% of the patients with recurrent glioblastoma treated with erlotinib were free of progression after 6 months compared with 24.1% of the patients treated with temozolomide and BCNU (73). In addition, no significant difference of overall survival was found between the two treatment groups (7.7 months for the erlotinib group versus 7.3 months for the temozolomide-BCNU group; ref. 73).

More conflicting data exist on the effects of gefitinib in patients with high-grade glioma. In one phase II trial, progression-free survival for more than 6 months was found in 13% of the patients (74). However, no objective response or significant increase in median overall survival or progression-free survival was found when compared with historical controls (42). Similar findings were observed in a second phase II study (75). Another phase I-II trial that was conducted by the North American Brain Tumor Consortium showed partial response in 13% of recurrent glioblastoma and 12% of recurrent anaplastic glioma in patients receiving prior radiotherapy (76). Noteworthy, no correlation was found between clinical response and HER1/EGFR gene amplification or EGFRvIII mutation for either gefitinib or erlotinib (71, 73-75).

The clinical findings with regard to response and stable disease rates seem promising in some trials especially for erlotinib. In addition, treatment with gefitinib or erlotinib was shown to be generally well tolerated. However, a significant breakthrough in the treatment of glioblastoma is not obvious from the existing trials. Owing to the low molecular weight of TK inhibitors, good penetration of the blood-brain barrier was initially anticipated. However, erlotinib and gefitinib both have molecular weights above 400 and thereby reach the upper limit of molecular weights that usually allow penetration of the blood-brain barrier. In addition, both compounds are relatively polar. Data on penetration of TK inhibitors into the central nervous system are sparse. In an 8-year-old glioblastoma patient who received a daily dose of erlotinib of 75 mg, a cerebrospinal fluid concentration of about 7% for erlotinib and about 9% for its active metabolite OSI-420 relative to plasma levels was reported (77). In another study, Lassman and colleagues determined steady-state concentrations by measuring levels of erlotinib and OSI-420 in tumor specimens obtained from patients with malignant glioma who had been treated with erlotinib at a dose of 150 mg/day for 1 week prior to surgical resection (78). The tumor tissue concentrations of erlotinib and OSI-420 expressed as a percentage of the corresponding plasma concentrations were 6% to 8% and 5% to 11% respectively (n = 4). Thus, the disappointing clinical responses to TK inhibitors might be a reflection of insufficient drug delivery to the target.

Further progress may be achieved by combining TK inhibitors with other targeted agents or conventional adjuvant therapies or by choosing a different mode of delivery to the target. In a first phase I trial, patients with recurrent high-grade glioma received a combined therapy with gefitinib and sirolimus, an inhibitor of mammalian target of rapamycin (mTOR), in order to simultaneously inhibit upstream (HER1/EGFR) and downstream mediators (mTOR) of PI3-K signaling (79). Thirteen out of 34 patients (38%) achieved stable disease, and two patients (6%) showed partial responses within a median follow-up period of 35.2 weeks. However, further studies are needed to examine the extent of antitumor activity or synergistic effects derived from concomitant inhibition of key regulators of upstream and downstream signaling. In addition, identification of molecular signatures of yet unknown glioblastoma subgroups might lead to the identification of patients who will benefit most.

### Table 2. HER1/EGFR-Targeted Agents for the Treatment of High-Grade Glioma Applied in Clinical Studies

<table>
<thead>
<tr>
<th>Agent</th>
<th>Substance</th>
<th>Brand Name</th>
<th>Target</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine kinase inhibitors</td>
<td>Gefitinib</td>
<td>Iressa</td>
<td>HER1/EGFR</td>
<td>AstraZeneca Pharmaceuticals</td>
</tr>
<tr>
<td></td>
<td>Erlotinib</td>
<td>Tarceva</td>
<td>HER1/EGFR, EGFRvIII</td>
<td>Genentech Inc.</td>
</tr>
<tr>
<td></td>
<td>Lapatinib</td>
<td>Tykerb</td>
<td>HER1/EGFR, HER2</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td></td>
<td>ZD6474</td>
<td>Zactima</td>
<td>HER1/EGFR, VEGFR</td>
<td>AstraZeneca Pharmaceuticals</td>
</tr>
<tr>
<td></td>
<td>AEE1788</td>
<td></td>
<td>HER1/EGFR, HER2, VEGFR</td>
<td>Novartis Pharmaceuticals Corporation</td>
</tr>
<tr>
<td></td>
<td>EKB569</td>
<td></td>
<td>HER1/EGFR</td>
<td>Wyeth Pharmaceuticals</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>Cetuximab</td>
<td>Erbitux</td>
<td>HER1/EGFR</td>
<td>ImClone Systems Inc.</td>
</tr>
<tr>
<td></td>
<td>Nimotuzumab</td>
<td>Theracol</td>
<td>HER1/EGFR</td>
<td>Oncoscience AG</td>
</tr>
</tbody>
</table>
HER1/EGFR-Targeted Vectors

Another way of inhibiting HER1/EGFR-mediated signaling is the prevention of receptor-activating ligand binding (80). For breast or colorectal cancers, application of monoclonal antibodies is an established therapeutic approach (9, 19). However, because antibodies represent molecules with high molecular weight, penetration of the blood-brain barrier might not be sufficient to effectively treat brain tumors despite increased local permeability (81). Several specific antibodies targeting the extracellular domain of HER1/EGFR have been developed and were investigated for therapeutic use in high-grade glioma. One such HER1/EGFR-specific antibody is cetuximab (Erbitux, ImClone Systems Inc. and Merck KGaA). In preclinical studies, systemic treatment with cetuximab was examined using intra- and extracranial xenograft models of highly HER1/EGFR-expressing human glioblastoma (82, 83). Increased cell death and decreased proliferation were found for both intra- and extracranial xenografts, however, more pronounced in extracranial xenografts. These data suggest that antibodies are able to cross the blood-brain barrier and legitimate the conductance of further studies. A phase I-II clinical study combining radiotherapy, chemotherapy with temozolomide, and targeting of HER1/EGFR with cetuximab for the treatment of patients with primary glioblastoma is ongoing (84). Another humanized antibody directed toward HER1/EGFR that has been studied in the setting of malignant glioma is TheraCim-hR3 (YM BioSciences Inc.), also referred to as nimotuzumab or Theralog (OncoScience AG). In a phase I-II clinical trial, 24 patients with newly diagnosed high-grade glioma received radiotherapy combined with systemic treatment with TheraCim-hR3. Sixteen percent of the patients had complete response, 21% had partial response, and 46% reached stable disease. No grade 3-4 adverse events were observed. The same research group reported a median survival of 17.47 months for 29 patients with malignant glioma, including 16 patients with glioblastoma and 13 patients with anaplastic astrocytoma who were treated concomitantly with radiotherapy and once weekly 200 mg of hR3 intravenously (85). Currently, patients with newly diagnosed glioblastoma are recruited for a multicenter, randomized phase III clinical study comparing the effectiveness of adjuvant combined radiotherapy with concomitant and subsequent temozolomide alone versus radiotherapy plus concomitant and subsequent temozolomide plus nimotuzumab (Theraloc).

Another therapeutic approach combines the specific binding properties of antibodies with the noxious effects of toxins or radioisotopes. These toxin-antibody or radio-immuno conjugates can be specifically directed against HER1/EGFR-expressing tumors. 125I-MAB 425 is a monoclonal antibody targeted against HER1/EGFR and labeled with 125I. In a phase I-II clinical trial, it was shown that administration of 125I-MAB 425 combined with radiotherapy resulted in a significantly increased median survival in patients with glioblastoma when compared with a control group receiving radiotherapy alone (86). Likewise, molecules combining toxins such as Pseudomonas exotoxin A with HER1/EGFR-binding antibodies have shown good affinity, cytotoxicity, and stability (87).

A different way of specifically targeting toxins to HER1/EGFR may be the use of specific ligands as vectors. One such ligand-toxin conjugate is TP-38, a fused protein composed of TGF-α and a mutated pseudomonas exotoxin (PE-38). In a phase I study, two out of 20 patients with glioblastoma showed partial response, and one patient had a complete response with no recurrence after 83 weeks following treatment with TP-38 (88). Another ligand-toxin conjugate that has been developed is composed of EGF and diphteria toxin (DAB389EGF; ref. 89), and preclinical data show tumor regression of subcutaneous glioblastoma xenografts in mice when treated with DAB389EGF (90).

Using conjugates specifically targeting EGFRvIII such as MAB 806 or 3C10 may offer an alternative way of destroying tumor cells with possible clinical utility in combination therapy, a prospect that has to be investigated in further clinical trials (91, 92).

Insufficient drug delivery of higher molecular weight molecules such as antibodies across the blood-brain barrier and into the central nervous system represents a serious obstacle to effective therapy. Sufficient therapeutic concentrations in the tissue of interest are often not reached even when therapeutic agents are administered during surgery at the site of tumor resection. Therefore, convection-enhanced delivery was developed using positive pressure to enhance the spatial distribution of drugs. Surgically placed catheters are used to pump the therapeutic agent into the brain, maintaining positive pressure at the site of drug administration. Preclinical evaluation of this method using immunotoxins in tumor models showed promising results (93). In clinical phase I and II studies, convection-enhanced delivery was shown to be efficient and well-tolerated by patients with high-grade glioma (93). Further studies are needed to investigate the clinical value of this mode of delivery on disease control and outcome.

Vaccination Therapy

In the past, it was postulated that the brain is an immunologically privileged organ. This concept was based on the clinical observation that cerebral metastases were often resistant to immunotherapy despite good therapeutic response of certain extracerebral cancers. In addition, experimental studies in rats showed that rejection of intracranially transplanted tumors was encountered less frequently compared with subcutaneously transplanted tumors. However, there is increasing evidence that the central nervous system is only partially privileged and rather immunologically highly active as shown by the facts that gliomas are often infiltrated by inflammatory cells and that subcutaneous tumor implants can generate resistance to a subsequent intracranial tumor challenge. The creation of antitumor vaccines, allowing the immune system to recognize and to eliminate malignant cells, seems promising. To date, little data exist on vaccination therapy targeting HER1/EGFR for the treatment of intracranial tumors. However, several experimental studies were conducted to examine if immunization against EGFRvIII may provide a benefit for the treatment of intracranial tumors. In mice, Ashley and colleagues showed that immunization with transfected allogenic 300.19/EGFRvIII cells induced a major histocompatibility complex class I-restricted response against EGFRvIII-bearing syngeneic...
B16-F10 melanoma or 560 astrocytoma cells that were implanted intracranially (94). Moreover, median survival of vaccinated animals was significantly increased on intracranial tumor challenge when compared with controls. In another experimental study, mice were vaccinated by a conjugate of a peptide comprising the tumor-specific mutated segment of EGFrVIII (PEP-3) and keyhole limpet hemocyanin (KLH; ref. 95). Animals receiving intracerebral injections with EGFr-VIII-transfected murine melanoma cells (K1735EGFrVIII) and prior immunization with PEP-3-KLH showed a 173% increased median survival time when compared with controls. In addition, vaccination with PEP-3-KLH in already established intracerebral tumors resulted in a 26% increase of median survival, thus demonstrating efficacy in a clinically more relevant setting. Overall, data from clinical trials are sparse. In a phase II trial, 21 patients with newly diagnosed EGFrVIII-positive glioblastoma underwent gross-total tumor resection followed by radiotherapy, treatment with temozolomide and vaccination with PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistently controls for treatment differences between the two study groups. Currently, Celldex sponsors a randomized, controlled, multicenter phase II-III trial for the further evaluation of the PEP-3-KLH (CDX-110, Celldex Therapeutics). A median survival of 26 months (versus 15.2 months) and a median time-to-progression of 14.2 months (versus 7.13 months) were reported (96). Although this study used historical control patients who would have met the eligibility criteria for the trial, it is unclear whether the study design consistent...
ribozymes resulted in >90% reduction of EGFRvIII mRNA (107, 108). In addition, anchorage-independent growth of U87MG.EGFRvIII cells was significantly inhibited.

**Determinants of Susceptibility to HER1/EGFR-Targeted Therapies**

As outlined above, HER1/EGFR-targeted agents such as TK inhibitors, monoclonal antibodies, or RNA-based therapeutics are promising approaches to the treatment of high-grade glioma. However, it was soon shown by clinical studies with HER1/EGFR-targeted TK inhibitors that the presence of HER1/EGFR overexpression was not sufficient to assure an individual treatment response as there was a significant discrepancy between HER1/EGFR overexpression (found in 40-50% of patients with glioblastoma) and the clinical response to TK inhibition (found in only 10-20% of the patients; refs. 71, 74, 109). Similar findings were obtained for other types of cancer. Understanding the underlying mechanisms determining individual variation of response to HER1/EGFR-targeted agents is an inevitable prerequisite to further progress in the treatment of high-grade glioma and other HER1/EGFR-expressing solid tumors.

To elucidate this phenomenon, studies have set out to examine if differences in response to TK inhibitors are due to mutational(s) within the TK. In NSCLC, responsiveness to HER1/EGFR TK inhibitors were indeed shown to be related to mutations in exons 19 or 21 of the intracellular TK domain (64, 65). However, these mutations were not found in specimens from patients with glioma. Overall, mutations of the HER1/EGFR TK domain are rarely seen in high-grade glioma (66, 74). In contrast, mutations of the extracellular ligand-binding domain such as EGFRvIII are very frequent, as coexpression of EGFRvIII is encountered in about half of all glioblastoma overexpressing HER1/EGFR.

The missing concordance between clinical response and overexpression of HER1/EGFR led to the hypothesis that susceptibility to HER1/EGFR TK inhibitors might depend on a multifactorial genetic predisposition. Various genetic markers were examined and correlated to clinical response. In a retrospective clinical study conducted by Mellinghoff and colleagues, tumor specimens of 26 patients with recurrent malignant glioma, previously treated with gefitinib or erlotinib, were examined for expression of HER1/EGFR, EGFRvIII, and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a tumor suppressor protein that causes catalytic dephosphorylation of phosphatidylinositol 3,4,5 triphosphate (PIP3) to phosphatidylinositol 3,4 biphosphate (PIP2), thereby negatively regulating the PI3-K pathway (109). Interestingly, coexpression of EGFRvIII and PTEN was significantly associated with clinical response to TK inhibitors. This finding was confirmed in another group of 33 patients with gliomas examined by the same research group, and by in vitro experiments using human U87MG glioblastoma cell lines transfected with PTEN and EGFRvIII (109). In an orthotopic xenograft model, 11 different patient-derived glioblastoma xenografts were injected intracranially into mice, followed by treatment with erlotinib at a dose of 100 or 150 mg/day (110). For two xenografts, a survival benefit from erlotinib treatment was found. Similar to the findings of the previously cited study, molecular analysis revealed that both xenografts (GBM12, GBM39) expressed wild-type PTEN along with aberrant HER1/EGFR (leucine-to-arginine change at amino acid 62 and EGFRvIII, respectively). However, response to erlotinib was missing in one xenograft (GBM6) sharing identical HER1/EGFR and PTEN properties with GBM 39. Moreover, Brown and colleagues recently showed in a phase I-II trial of patients with newly diagnosed glioblastoma receiving erlotinib in combination with radiation therapy and temozolomide that concomitant presence of PTEN expression and HER1/EGFR amplification or EGFRvIII were not predictive for survival (111). Another phase I-II trial currently recruits patients with recurrent EGFRVIII-positive and PTEN intact high-grade glioma for a combinatorial regimen of lapatinib (Tykerb, GlaxoSmithKline) and pazopanib (GlaxoSmithKline). This study uses a single-arm study design, and comparison to historical controls might lead to erroneous conclusions. In summary, although coexpression of mutated HER1/EGFR and wild-type PTEN seems to be important for the sensitivity of glioblastoma to HER1/EGFR TK inhibition, its presence does not confer obligate response. Vice versa, PTEN loss seems to be a critical molecular event associated with resistance to HER1/EGFR-targeted TK inhibitors. The fact that only 40% to 50% of glioblastoma are expressing wild-type PTEN highlights the need to identify probably more complex molecular signatures associated with sensitivity to HER1/EGFR TK inhibitors.

Preliminary studies have examined the effects of inhibition of downstream key regulators such as mTOR and PI3-K in addition to the treatment with HER1/EGFR TK inhibitors. PTEN-deficient U87 and SF295 glioblastoma cells exposed to combined treatment with erlotinib and rapamycin, an mTOR inhibitor, showed significantly increased antiproliferative effects when compared with cells receiving erlotinib alone (112). A 38% reduction in proliferation was observed for PTEN-deficient SF295 cells treated with erlotinib and rapamycin versus 14% on treatment with erlotinib alone. Treatment with rapamycin only showed similar results as erlotinib monotherapy. Another experimental study obtained similar findings (113). Moreover, additional inhibition of PI3-K using a novel mTOR/PI3-K inhibitor (PI-103) in PTEN-mutant glioma cells resulted in even more antiproliferative efficacy in combination with erlotinib when compared with erlotinib combined with either mTOR or PI3-K inhibition. Thus, resistance to HER1/EGFR-targeted treatment of high-grade gliomas may be finally overcome by using a multitarget approach. Against this background, inquiries about whether additional, yet unidentified molecules within the signal transduction cascade further modulate clinical response to treatment with HER1/EGFR-targeted agents such as TK inhibitors are warranted. In patients with NSCLC, response to gefitinib was related to higher levels of phosphorylated MAPK (pMAPK) or phosphorylated AKT (pAKT; ref. 114). However, a later study revealed conflicting data with decreased survival correlating to higher pMAPK levels in gefitinib-treated patients with bronchoalveolar carcinoma (115). Moreover, in 41 patients with glioma, clinical response to erlotinib and prolongation of time to progression significantly correlated to lower levels of phosphorylated protein kinase B/AKT (116). In view of these controversial data,
a clear-cut evaluation of the significance of pMAPK or pAKT for the sensitivity of HER1/EGFR-expressing tumors toward HER1/EGFR TK inhibitors is difficult. Other molecules that have been shown to modulate sensitivity to erlotinib in nonglioma-derived cell lines are fibroblast growth factor receptor, platelet-derived growth factor receptor, E-, and H-cadherin (117, 118).

Further studies, including clinical trials, are needed to elucidate the clinical relevance of these and yet unknown molecules that influence response of glioblastoma to HER1/EGFR-targeted therapeutics.

**Oncogene Addiction versus Oncogenic Shock**

The transformation of a noncancerous cell into a malignant cell is attributed to the acquisition of multiple genetic alterations that account for characteristic phenotypic changes. However, despite the multitude of oncogenes present in tumor cells, it was shown that tumor cells can become dependent on the activity of only one single oncogene in order to maintain cellular proliferation or even survival. Acute deprivation from that specific oncogenic input may rapidly induce growth arrest or apoptosis (119, 120). To concisely describe this phenomenon of dependency, the term “oncogene addiction” was coined (121). Although the exact nature of the mechanisms leading to reprogramming of the cellular “circuitry” toward “oncogene addiction” remains unclear, it is presumed that the homeostasis of the transformed cell is maintained by concomitant upregulation of antiproliferative/proapoptotic signaling. Switching off the proliferative signaling input from the addictive oncogene results in critical dysbalance, leading to growth arrest or apoptosis. Partly because the supposed change of the cell’s “hardwiring” is central to the concept of oncogene addiction but difficult to define, a different concept has evolved to explain the cellular decay that follows acute disruption of oncogenic signaling. It was shown for many oncoproteins, including HER1/EGFR, that their activation effects both prosurvival signaling (125). Thus, the concept of single-oncogene addiction probably will not apply to the majority of glioblastomas.

The antitumor effects of selective oncogene inhibition, e.g., by HER1/EGFR TK inhibitors, may be increased by concomitantly targeting multiple downstream prosurvival effectors (ERK, STAT, PI3-K). An additional important implication of the concept of oncogenic shock is that certain agents such as conventional chemotherapeutics that frequently cause DNA damage, cell cycle arrest, and thereby inhibition of apoptosis, may not be candidates for a rational combination therapy with HER1/EGFR TK inhibitors. The model of “oncogenic shock” provides a logical explanation for the biological response of tumor cells following oncogene inactivation and is supported by current experimental data. However, this concept needs to be further validated in the setting of drug development and subsequent clinical application.

**Conclusions and Future Perspectives**

There is compelling evidence that dysregulation of HER1/EGFR is contributing to the tumorigenesis of gliomas and various other cancers. Therefore, multiple HER1/EGFR-targeted therapeutic strategies have been developed, including TK inhibitors, monoclonal antibodies, RNA-based therapies, radioimmuno, and ligand-toxin conjugates. Clinical experience is most advanced for HER1/EGFR TK inhibitors such as erlotinib and gefitinib. The clinical data available so far for the treatment of patients with glioblastoma show more promising results for erlotinib, which is known to also inhibit the constitutively activated EGFR-VIII. However, according to a first randomized, controlled clinical trial no sufficient activity of erlotinib was found in patients with recurrent glioblastoma independent of...
HER1/EGFR expression status or presence of EGFRvIII (73). The individual therapeutic response to HER1/EGFR TK inhibitors is highly variable, which can be partly explained by subjugated differences in molecular patterns determining sensitivity or resistance. For example, PTEN deficiency was shown to be related to decreased response to treatment with HER1/EGFR TK inhibitors despite HER1/EGFR overexpression.

HER1/EGFR-directed treatments were so far mostly designed as a single-agent approach and have bestowed only modest clinical benefit. Not surprisingly, accumulating knowledge about the mechanisms that underlie individual sensitivity to HER1/EGFR-targeted agents indicates that a combination of multiple therapeutics might further improve the clinical outcome. In experimental studies, increased antineoplastic effects were obtained when therapeutics were combined to target oncogenic signal transduction at various levels. In PTEN-deficient cells, additional inhibition of downstream signaling using rapamycin to inhibit mTOR was shown to significantly improve therapeutic response (112). In a first phase I study, a combinatorial regimen of gefitinib and sirolimus was applied to patients with recurrent glioblastoma (79). Data on clinical efficacy are still awaited. Stommel and colleagues showed for 19 out of 20 glioma cell lines coactivation of multiple PI3-K activating receptor tyrosine kinases (RTK; ref. 125). Moreover, specific inhibition of only one RTK (HER1/EGFR or MET) did not alter downstream signaling whereas concomitant inhibition of both resulted in decreased PI3-K activation and significantly decreased viability of U87MG,EGFRvIII cells. These findings suggest that combined inhibition of multiple RTKs and or additional inhibition of downstream molecular key factors such as PI3-K may represent the essential step to overcome resistance toward a HER1/EGFR-targeted monotherapy.

The future discovery of other HER1/EGFR-related pathways that regulate cellular behavior such as proliferation, invasion, migration, differentiation, and apoptosis might also allow for the development of new therapeutic approaches. A recent analysis of 84 brain tumor samples including 40 WHO grade IV and 11 grade III astrocytomas revealed that the extent of co-overexpression of HER1/EGFR, insulin-like growth factor-binding protein 2 (IGFBP2) and the hypoxia-induced transcription factor 2-alpha (HIF2A) was directly correlated to the histological grade, and moreover, to survival (2-year survival of patients showing overexpression of three genes versus two genes versus one gene versus none: 6% versus 55% versus 62% versus 83%; ref. 126). Further studies are needed to evaluate if the status of the EGFR/IGFBP2/HIF2A pathway is a useful prognostic marker for patients with gliomas and if members of this pathway represent efficient therapeutic cotargets.

For more than two decades now, HER1/EGFR has been studied as a therapeutic target for the treatment of several human cancers. The knowledge about the mechanisms underlying resistance to HER1/EGFR-targeted therapeutics and related molecular determinants is steadily expanding. However, several clinically important issues such as timing of HER1/EGFR-targeted therapy or long-term side effects of HER1/EGFR signaling inhibition in normal cells have not yet been fully addressed. A multidrug approach combining different agents that target HER1/EGFR signaling at multiple levels seems to be most promising. Interfering with molecules responsible for inherent or acquired resistance to HER1/EGFR-targeted agents will likely play an important role in this combination therapy. However, our current understanding of the biological effects of HER1/EGFR-inhibiting agents on high-grade glioma cells is still profoundly incomplete. As shown by Steinbach and colleagues, human malignant glioma cells treated with HER1/EGFR inhibiting drugs were even protected from hypoxia-induced apoptosis (127). Hypoxia-induced depletion of glucose and adenosine triphosphate as well as a decrease of the mitochondrial membrane potential were significantly attenuated on treatment with HER1/EGFR-inhibiting agents. Thus, therapeutic manipulation of the molecular tumor cell homeostasis by HER1/EGFR inhibition remains a critical issue. We need to
learn more about molecular tumor signatures and synergistically acting agents for combination therapy to turn the current reality of HER1/EGFR inhibition for high-grade glioma into a success story.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
8. Earp HS III, Calvo BF, Sartor CI. The EGF receptor family - multiple roles in apoptosis and proliferation, differentiation, and neoplasia with an emphasis on HER4. Trans Am Clin Climatol Assoc 2003;114:315–33.
49. Rodriguez LA, Prados M, Silver P, Levin VA. Reevaluation of procarbazine
Therapeutic Inhibition of the Epidermal Growth Factor Receptor in High-Grade Gliomas: Where Do We Stand?


Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-08-0479

This article cites 123 articles, 58 of which you can access for free at:
http://mcr.aacrjournals.org/content/7/7/1000.full.html#ref-list-1

This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/7/7/1000.full.html#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.