

EGFR as a therapeutic target in glioblastoma

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Abstract

The tyrosine kinase receptor epidermal growth factor receptor (EGFR) can be activated by several ligands, thus triggering downstream pathways regulating cell growth and survival. Its dysregulation is particularly important for the development and progression of astrocytomas. After the description of its role in glioblastomas (WHO grade IV astrocytomas), an overview on the therapeutic strategies targeting EGFR is provided. It analyzes the past and ongoing trials concerning the small molecule tyrosine kinase inhibitors, i.e. gefitinib, erlotinib and the combination therapies, the EGFR vaccination strategies, the antibodies directed against EGFR and finally the intracranially administered EGFR-targeted therapies. As our understanding of the underlying molecular aberrancies in glioblastoma grows, our ability to better target specific subtypes of glioblastoma should improve. Molecular biomarker enriched clinical trials may lead to improved patient outcomes.

Keywords

Epidermal growth factor receptor; Glioblastoma; Tyrosine kinase inhibitors; Antibodies; Vaccines

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Introduction

Epidermal growth factor receptor (EGFR) plays a prominent role in many high-grade astrocytomas. Consequently, it is a reasonable and potentially important target in their treatment. This review will focus on its role as a therapeutic target in glioblastoma (WHO grade IV astrocytoma). We will elucidate the role of EGFR in these tumors and then provide an overview of methods currently used to evaluate EGFR status in brain tumor patients. Focus will then shift toward EGFR as a therapeutic target, covering several different types of therapies and many of their representative clinical trials.

Overview of glioblastoma and the oncogenic role of EGFR

Despite extensive investigation during the last century, glioblastoma remains a disease with an extremely poor prognosis, and it is nearly invariably fatal within a few years of diagnosis. Glioblastomas are grade IV astrocytomas (the highest grade) and are distinguished from their lower grade counterparts by the presence of necrosis and neovascularization [1]. A large body of research has shed light on a number of molecular pathways that may contribute to the generation, growth, and survival of glioblastomas. One of the most important of these dysregulated pathways is the EGFR pathway [2,3]. EGFR is a protein encoded on the short arm of chromosome 7.

A number of ligands activate EGFR, a member of the receptor tyrosine kinase superfamily. These ligands include epidermal growth factor (EGF), transforming growth factor- α (TGF- α), heparin-binding EGF, amphiregulin, betacellulin, and epiregulin of the EGF protein family [4] as well as members of the neuregulin protein family [5]. Inactive EGFR monomers dimerize when bound by ligand, leading to the activation of EGFR's tyrosine kinase activity, resulting in autophosphorylation of tyrosine residues on EGFR. Various intracellular proteins and protein complexes can associate with phosphorylated EGFR (pEGFR), activating a number of downstream pathways that promote cell growth and division [6].

One of the primary pathways activated by EGFR is the mitogen-activated protein kinase/extracellular related signal kinase (MAPK/Erk) pathway, a pathway that eventually leads to activated Ras. Activated

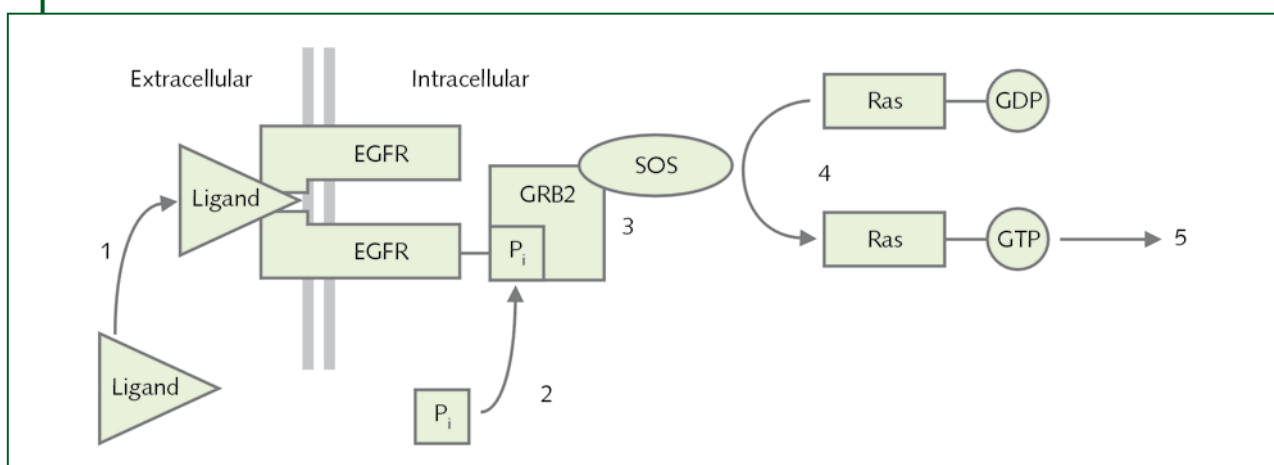


Figure 1. Ras and MAPK EGFR-mediated growth pathway. 1 – Activating ligand binds to extracellular domain of EGFR, and EGFR homodimerizes. 2 – EGFR homodimer autophosphorylates at specific tyrosine residues. 3 – GRB2/SOS protein complex associates with phosphorylated EGFR. 4 – Ras exchanges GDP for GTP. 5 – Activated Ras-GTP begins kinase cascade that leads to enhanced pro-growth gene transcription

Ras continues a phosphorylation cascade that eventually yields activated transcription regulators that promote cell growth and differentiation. Another pathway activated by pEGFR is the Akt pathway [6]. Akt promotes cell survival through inhibition of Bcl-2-associated death promoter (BAD) [7]. Akt also allows for the resumption of the cell cycle when arrested at the G1 [8] or G2 [9] stages, and overactive Akt in response to abnormally high EGFR activity could contribute to oncogenesis. A third downstream pathway activated by EGFR is the Jun N-terminal kinases (JNK) pathway. When activated by the EGFR dimer [10], JNK phosphorylates c-Jun, a transcription factor important in regulation of cell proliferation and apoptosis [11]. Through these signal cascades, EGFR has the ability to affect the growth, survival, and differentiation of many cells in the body, including those of glial origin. Two of these pathways are depicted (Figure 1 and 2).

Wild-type EGFR (wtEGFR) shows a very high prevalence of genetic variants, a phenomenon that may be important in glioblastoma. For example, a large number of inherited cytosine-adenine (CA) dinucleotide repeats in intron 1 of EGFR has been suggested to down-regulate transcription of the EGFR gene. In addition, a number of single nucleotide polymorphisms (SNPs) have been shown to be potentially associated with an increased risk of malignancy [12].

EGFR amplifications and mutations are predominantly found in what has long been described as primary glioblastoma [13]. Primary (*de novo*) glioblastoma comprises glioblastoma which arise without evidence of transformation from a lower grade glioma (secondary glioblastoma). However, recent work has identified more specific glioblastoma subtypes, each with associated sets of mutations. Four molecular subtypes have been described. The “classical” subtype is represented by tumors with paired chromosome 7 amplification/chromosome 10 deletion (100% of “classical” tumors), EGFR amplification (97%), and EGFR mutation (> 50%) [14]. Additionally, the “classical” subtype lacks some other common glioblastoma mutations. Other glioblastoma subtypes include the “mesenchymal”, “proneural”, and “neural” subtypes, each with their own unique molecular or genetic profiles. Notably, these subtypes infrequently exhibit EGFR amplification [14]. Recently, in the prognostically favorable isocitrate dehydrogenase 1 (IDH1) mutated (non-mesenchymal subtypes) glioblastomas, areas of focal amplification of EGFR have been demonstrated [15].

The EGFR mutation most commonly detected in glioblastoma is EGFRvIII [16], found in 12 of 22 “classical” glioblastomas in one study [14]. When present, it is nearly always found concurrently with EGFR gene amplification and overexpression. The reported incidence of this mutation in glioblastoma varies throughout the literature. EGFRvIII involves a deletion of exons 2-7 (amino acids 6-273) in the extracellular domain that yields a constitutively active EGFR variant [17] and also creates a unique tumor-specific epitope [18]. In addition to upregulation of EGFR activity, there is a growing literature

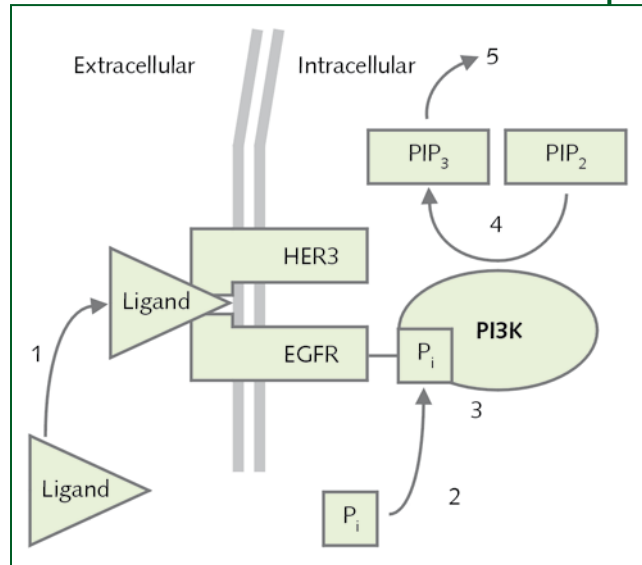


Figure 2. PI3K and Akt EGFR-mediated anti-apoptotic pathway. 1 – Activating ligand binds to extracellular domain of EGFR, allowing EGFR to form homodimers or heterodimers with other EGFR family proteins. 2 – Homodimer/heterodimer autophosphorylates at specific tyrosine residues. 3 – Phosphorylated homodimer/heterodimer activates PI3K. 4 – PI3K catalyzes PIP₃ formation from PIP₂. 5 – PIP₃ promotes Akt activation and downstream anti-apoptotic pathways

on potential EGFR downregulators, such as the leucine-rich repeats and immunoglobulin-like domain (LRIG) family of proteins, which may also be of therapeutic interest [19].

Finally, both parallel pathways and downstream components of the EGFR pathways are important to consider when analyzing the efficacy of EGFR-targeted therapies [20-23].

Other oncogenic pathways in glioblastoma

In addition to EGFR hyperactivity due to EGFR gene mutation or amplification, several other abnormal pathways have been associated with glioblastomas. For example, the p53 tumor suppressor gene is mutated or deleted in a significant percentage of glioblastomas. 10-25% of glioblastomas exhibit amplification or overexpression of murine double minute 2 (MDM2) [2], a negative regulator of p53 [24]. The phosphatase and tensin homologue (PTEN) gene, whose product is an important phosphatase involved in kinase activity regulation [25], such as that of EGFR, is abnormal or missing in up to 40% of glioblastomas [2]. Finally, recent research has shown that the constitutively active EGFRvIII possesses the ability to avoid the ubiquitin proteasome system (UPS) [26]. Phosphorylated wtEGFR is a target of Casitas-B linease (Cbl) proteins. This group of proteins polyubiquinate pEGFR, targeting it for internalization and possibly for lysosomal degradation [27]. However, EGFRvIII does not require phosphorylation for its activity, allowing it to circumvent this counterbalancing degradation cascade [26]. Though rare cases of concurrent EGFR overexpression and p53 mutation in glioblastoma have been described, these two abnormalities are essentially mutually exclusive. EGFR overexpression is strongly associated with primary glioblastoma, with one study reporting an incidence of 73.3%. When such amplification was present, it was nearly always coupled with EGFR overexpression [28]. Additionally, PTEN and MDM2 mutations are more commonly seen in primary glioblastoma [2]. On the other hand, p53 mutations are seen almost solely in secondary glioblastoma.

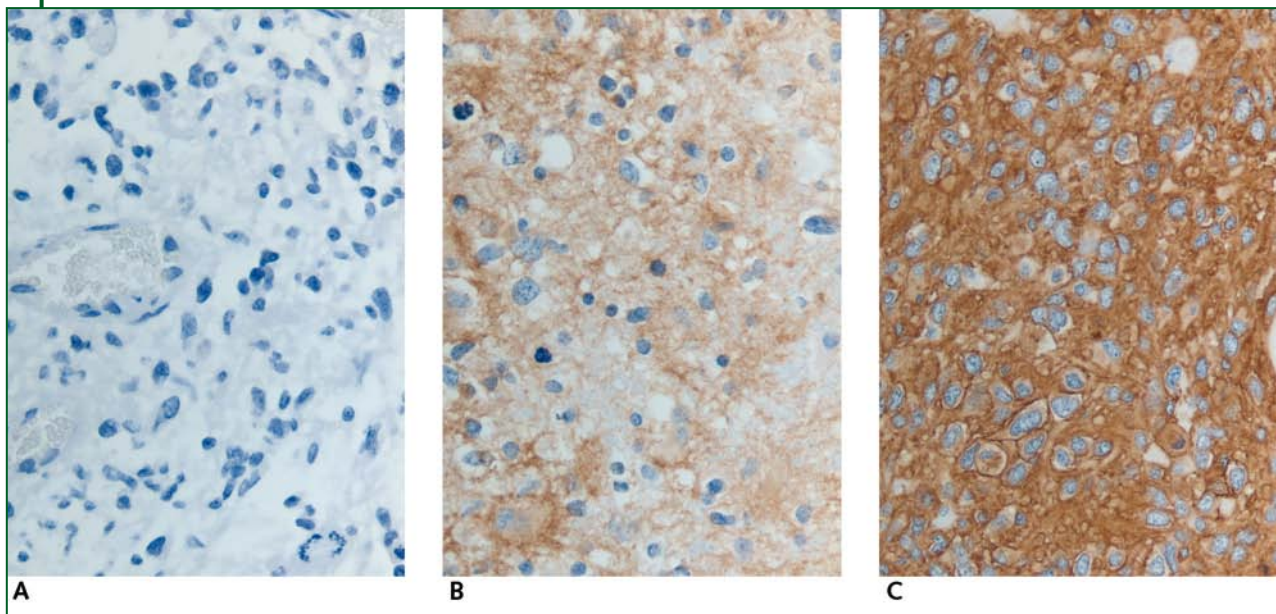


Figure 3. Microscopic images of glioblastomas illustrating absent (A), weak (B) and strong (C) immunoreactivity for wild type EGFR. Cases of strong staining often show membranous accentuation of staining as seen in image C. The interpretation of these immunohistochemical studies can be complicated by various factors including the entrapment of normal tissue elements and intra-tumoral heterogeneity of staining

Determining EGFR genotype and expression status

Various methods have been pursued to study EGFR gene amplification, EGFR gene mutations, and the expression of EGFR at the protein level. All have been applied in glioblastoma.

Fluorescence *in situ* hybridization (FISH) studies are most commonly used to look for EGFR gene amplification in a pattern of double minute chromosomes or as extra copies of the EGFR gene inserted in different loci of chromosome 7 [29-31]. Chromogenic *in situ* hybridization (CISH) has also been utilized to look for EGFR gene amplification and correlates with FISH based testing [30,32]. Other techniques, including comparative genomic hybridization (CGH) and real time PCR, also have the potential for providing information about gene copy number changes like EGFR amplification [31,33]. The presence of EGFR gene mutations, and the EGFRvIII mutation in particular, is established by PCR based techniques [31]. The feasibility of performing EGFRvIII mutation analysis on formalin fixed paraffin embedded tissue has been documented [34].

Immunohistochemistry (IHC) is widely used to look for the expression of the EGFR protein in tissue sections (Figure 3). Strong expression of EGFR detected with an antibody identifying wtEGFR is correlated with EGFR gene amplification as assessed by FISH [30,32,35]. IHC with mutation specific antibodies also offers a way to assess the expression of EGFRvIII in glioblastomas [31]. The lack of a commercially available antibody has been a challenge, but new antibodies may become available [36].

Therapeutic modalities targeting EGFR

A number of therapeutic modalities have been employed to target EGFR. Each of these modalities carries inherent benefits and limitations, and the potential benefit of each agent depends on its abil-

Author [reference]	Drug(s) investigated	Phase	Patients (n.)	Histology	Type of disease
Mellinghoff, et al [31]	Erlotinib, gefitinib	-	82	GBM, AO	Recurrent
Lassman, et al [37]	Erlotinib, gefitinib	I/II	33	GBM, AA, AMG	Recurrent or progressive
Franceschi, et al [38]	Gefitinib	II	28	GBM, AA, AO	Recurrent
Uhm, et al [39]	Gefitinib	II	98	GBM	Newly diagnosed
Rich, et al [40]	Gefitinib	II	57	GBM	First recurrence
Hegi, et al [41]	Gefitinib	II	22	GBM	Recurrent
van den Bent, et al [42]	Erlotinib vs. TMZ or carmustine	II	110	GBM	Recurrent
Yung, et al [43]	Erlotinib	II	48	GBM	First-relapse
Raizer, et al [44]	Erlotinib	II	104	GBM, AG	Recurrent or non-progressive
Sathornsumetee, et al [45]	Erlotinib plus bevacizumab	II	57	GBM, AG	Recurrent
Prados, et al [46]	Erlotinib plus TMZ and RT	II	65	GBM, GS	Newly diagnosed
Brown, et al [47]	Erlotinib plus TMZ and RT	I/II	97	GBM	Newly diagnosed
Peereboom, et al [48]	Erlotinib plus TMZ and RT	II	27	GBM	Newly diagnosed
Reardon, et al [49]	Erlotinib plus sirolimus	II	32	GBM	Recurrent
Kreisl, et al [50]	Gefitinib plus everolimus	I/II	22	GBM	Recurrent

Table I. Summary of small molecule tyrosine kinase inhibitor trials in the treatment of malignant glioma

AA = anaplastic astrocytoma; AG = Anaplastic glioma; AMG = anaplastic mixed glioma; AO = anaplastic oligodendroglioma; GBM = glioblastoma; GS = gliosarcoma; RT = radiotherapy; TMZ = temozolomide

ity to exert either a direct or indirect effect on the CNS side of the blood brain barrier (BBB). We will begin by discussing EGFR-specific small molecule TKIs. These generally well-tolerated orally administered agents directly target the intracellular component of the EGFR protein. Unfortunately, data on intra-CNS and, more importantly, intra-tumoral concentrations of these agents in humans are limited. In contrast, EGFR vaccines have an indirect effect on the CNS-side of the BBB, since vaccines function by stimulating systemic immune response with subsequent trafficking of activated immune cells across the BBB. Finally, EGFR-specific antibodies, despite their large size, must be able to cross the BBB to have an effect. This process may be facilitated by the presumed relative breakdown of the BBB in tumors, as evidenced by the robust enhancement typically noted on post-contrast imaging of glioblastoma, but the extent of antibody penetration into brain tumors is still uncertain. Direct ways to circumvent the BBB will be discussed briefly in our section on intracranial administration of therapies.

Small molecule tyrosine kinase inhibitors

We will focus our discussion of EGFR-specific small molecule TKIs on gefitinib and erlotinib, both of which block the intracellular ATP-binding domain of EGFR. Summaries of gefitinib and erlotinib trials, either alone or in tandem with other treatment agents, can be found in Table I.

Gefitinib

Gefitinib was the first drug of its class marketed for the treatment of malignant tumors, originally approved by the FDA in 2003 for the treatment of non-small cell lung cancer [51]. It has been investigated for utility in the treatment of recurrent or progressive malignant gliomas of both pure astrocytic or mixed oligo-astrocytic lineage. One study analyzed samples from 21 tumors from the North American Brain Tumor Consortium (NABTC) Trials 01-03 and 00-01. Treatment with gefitinib yielded no subject-to-subject consistencies in levels of pEGFR or two of its effector proteins, phosphorylated extracellular signal-related kinase (pERK) and pAkt [37]. However, the sole tumor found to be “sensitive” to gefitinib, defined as radiographic response per MacDonald criteria [52], did show decreased post-treatment pAkt levels, while another tumor found to be “insensitive” showed an increase in levels. The importance of this finding is unclear due to small sample size [37].

A later phase II trial of gefitinib came to similar conclusions of response inconsistencies. In a small fraction of patients with malignant gliomas, gefitinib-treated patients exhibited long-standing control of disease progression, extending to over 2 years in some. However, an overall 6 month progression-free survival (PFS6) rate of only 12.5% was found in the glioblastoma subset, and the median time to tumor progression (TTP) for the entire study was a mere 8.4 weeks; median overall survival (OS) was just 24.6 weeks. Molecular biomarker assays to determine pre-treatment EGFR and pAKT expression levels were performed on 21 out of the 28 patients enrolled; no correlation between these markers and either TTP or OS was found, highlighting the difficult nature of predicting patient-to-patient disease response to this drug. However, the study did find that a response to gefitinib therapy may be achieved at doses much lower than originally thought, noting that a daily oral dose of 250 mg produced nearly identical response rates to previous studies of daily oral doses of 500 mg while causing significantly less adverse events related to treatment, most notably diarrhea [38]. This finding is of importance in light of two studies that independently confirmed a positive correlation between the rate of development of diarrhea and OS, suggesting that there is a still unclear but specific subset of patients that may better respond to gefitinib therapy [39,40]. Despite this, and despite that gefitinib may efficiently dephosphorylate pEGFR in some cases [39], the lack of correlation between treatment response and levels of pathway proteins in these cases suggests that gliomagenesis is a much more complex process than simple overexpression of a single protein [38,41].

Erlotinib

In addition to being one of the first investigations of the molecular influences of gefitinib, the study investigating the response of patients enrolled in the NABTC Trials also analyzed the efficacy of erlotinib in the treatment of malignant gliomas. Though structurally similar to gefitinib and working through an identical mechanism of action [53], significant differences between the two small molecules were discovered. While gefitinib seems to preferentially penetrate tumor tissue over remaining in plasma, erlotinib does not. Erlotinib or its active metabolite OSI-420 reach concentrations within glioblastoma or non-small cell lung cancer tissue of a mere fraction of concurrent plasma concentrations, whereas gefitinib was found to have a three-fold preference of tumor tissue over plasma in some cases. It may be that higher tumor tissue levels may be required to achieve more consistent response, and no clear correlation between tumor penetration and prognosis has yet been identified [37].

A randomized phase II trial was carried out comparing the efficacy of erlotinib to either temozolomide (TMZ) or carmustine in the treatment of recurrent glioblastoma. This study also investigated many of the previously described biomarkers in an attempt to disclose predictors of better outcome or increased drug response. However, the study closed early, as PFS6 in the control arm was 24% but only 11.4% in the arm treated with erlotinib. However, only about half of the tumors studied were found to have clearly overactive EGFR protein pathways. Tumors not overexpressing EGFR would not likely be expected to respond to EGFR inhibitors, leaving open the likely remote possibility that a better erlotinib-treated response may have been observed if patients were selected for EGFR overactivity before being administered treatment. The study did conclude that, similar to gefitinib, patients who developed erlotinib-related adverse events, in this case skin toxicity, showed improved OS and PFS6 [42]; similar findings have also been reported in the treatment of lung cancer [54].

Other studies have reported similar underwhelming results. Yung et al. report an objective response (OR) rate of first-relapse glioblastoma to erlotinib of 8.3%. In this study, although EGFR amplification status was not used as inclusion criteria, patients were separated into EGFR-amplified and non-amplified subsets post-hoc. Somewhat surprising was the result that patients in the non-amplified group treated with erlotinib showed a longer median survival time when compared to EGFR-amplified group and also that an OR was observed in the non-amplified group [43]. Stable disease (SD) is the most commonly reported favorable response to erlotinib, and response rates vary widely in the literature. Raizer et al report SD in 13.3% of patients with recurrent glioblastoma, recurrent anaplastic glioma, or nonprogressive glioblastoma following radiotherapy. This study did not select for tumor EGFR amplification status prior to treatment [44].

Combination therapies

As stated, many of the TKI clinical trials did not use EGFR gene amplification status as inclusion criteria for subjects. Nevertheless, even in post-hoc analysis of tumors found to have overactive EGFR-dependent protein pathways, no consistent clinical response was observed. This further strengthens the notion that while TKIs may be sufficiently cytostatic in a specific and small subset of tumor types, tumor cell growth and differentiation is a multifactorial process. Because of this, TKIs have been investigated in combination with other therapies, which will be discussed below.

Bevacizumab, a monoclonal human antibody drug that inhibits angiogenesis by inhibiting vascular endothelial growth factor (VEGF), was approved for the treatment of glioblastoma in May of 2009 [55]. A phase II study of bevacizumab in conjunction with erlotinib in the treatment of recurrent glioblastoma and anaplastic glioma provided no added benefit when compared to previous bevacizumab investigations [45].

The evidence supporting concurrent use of erlotinib with TMZ and radiotherapy (RT) is conflicting. A non-randomized phase II trial combining erlotinib and TMZ during and after RT had improved PFS6 and median OS (19.3 months) in patients with newly diagnosed glioblastoma or gliosarcoma when

compared to historical controls (median OS 14.6 months) [46]. The North Central Cancer Treatment Group (NCCTG) phase I/II study N0177 reported no additional benefit of erlotinib when added to standard of care TMZ plus RT treatment regimens, with a median OS for newly diagnosed glioblastoma of 15.3 months [47]. Another phase II trial adding erlotinib to standard of care demonstrated a decreased median PFS of 2.8 months, a decreased median OS of 8.6 months, and a large number of adverse events, including severe toxicities such as thrombocytopenia, anemia, lymphopenia, and febrile neutropenia. The investigators proposed that erlotinib may be causing cell cycle arrest, limiting the effectiveness of the DNA-alkylating TMZ [48]. Also of note in this and the other two analogous studies, while EGFR gene amplification was analyzed post-hoc, gene status was again not used as inclusion criteria for subjects [46-48].

In an effort to investigate the simultaneous inhibition of EGFR and a downstream pro-cell growth protein in the Akt pathway, mammalian target of rapamycin (mTOR), the efficacy of erlotinib plus the mTOR inhibitor sirolimus was investigated in the treatment of recurrent glioblastoma. Though the treatment regimen was well tolerated, the best outcome was SD. In addition, there was no strong association between tumor marker levels and progression-free survival (PFS) time, and patients were again not selected for EGFR amplification [49]. A similar study investigating everolimus, another mTOR inhibitor, in conjunction with gefitinib also produced disappointing results. Only 1 patient of 22 with recurrent glioblastoma showed PFS6 [50].

EGFR vaccination

The idea that the human immune system can be harnessed to target and eradicate neoplastic cells was conceived over a century ago by Ehrlich and Bolduan [56,57]. Numerous studies in the ensuing years affirmed that vaccination against tumor antigens results in tumor regression [56,58]. Consequently, the potential role of immunotherapy in the treatment of glioblastoma has been pursued with great alacrity. Central nervous system (CNS) immunoprivilege, due to the presence of the BBB as well as absence of draining lymph nodes and resident antigen-presenting cells (APCs) in the brain, was thought to pose a distinct challenge to the development of CNS tumor immunotherapies [56,59,60]. In 1948, Medawar showed that allogeneic tissue grafts transplanted into the brains of experimental animals were not rejected [56,61]. However, more recent evidence demonstrates that immune cells penetrate the BBB under normal physiological conditions, antigens egress via cerebrospinal fluid (CSF) and cervical lymphatic pathways, and specialized microglia function as surrogate APCs in the CNS by mediating human leukocyte antigen (HLA) presentation [56,62-67]. Furthermore, in neuro-inflammatory disease states, including malignancy, the BBB undergoes changes that hamper its ability to block the trafficking of leukocytes and serum proteins into the CNS [56,68]. Thus, peripherally administered therapeutic antibodies and tumor-specific antigens under these circumstances are observed to access the CNS with physiologically relevant outcomes [56,69].

Immunotherapy can be divided into either active or passive approaches [56,70]. Active immunization relies on the native immune system to mount a response against antigens directly inoculated into the body or presented by autologous APCs. Passive vaccination, on the other hand, is achieved with the infusion of antibodies or antigen-specific T lymphocytes. In this section, we review the active immunotherapy strategies that target the tumor-specific EGFR mutation EGFRvIII.

PEPvIII-KLH, also known as CDX-110, is an EGFRvIII-targeted peptide vaccine. More specifically, PEPvIII is a 13-amino acid peptide with an additional terminal cysteine that spans the EGFRvIII mutation and has been shown, when coupled to keyhole limpet hemocyanin (KLH), to elicit both humoral and cellular immune responses [56,71]. In a preclinical experiment using C3H mice previously challenged with intracerebral tumors, a one-time vaccination with PEPvIII-KLH in complete Freund's adjuvant resulted in increased median survival [72]. Mice that did not respond to this vaccine were found

to have nearly absent EGFRvIII expression, suggesting that antigen escape variants may be associated with treatment failure [56,72].

Based on this preclinical data supporting the safety and efficacy of an EGFRvIII-targeted peptic vaccine, two phase II clinical trials were performed. The first such clinical study, ACTIVATE, was a multicenter trial conducted at Duke University and University of Texas, M.D. Anderson Cancer Center that enrolled 19 adults with newly diagnosed EGFRvIII-expressing glioblastoma with a gross-total radiographic resection and who had no evidence of radiographic progression after standard of care RT and concurrent TMZ chemotherapy [56,71]. Vaccinations consisted of intradermal injections of PEPvIII-KLH in combination with granulocyte macrophage-colony stimulating factor (GM-CSF). The first three vaccines were given biweekly, followed by monthly injections until radiographic evidence of tumor progression or death. The median OS of vaccinated patients was significantly better than that of patients in the matched historical cohort, 26.0 months versus 15.0 months, respectively ($p = 0.001$). IHC analysis of EGFRvIII expression among recurrent tumors revealed that 82% lost EGFRvIII expression. Interestingly, vaccinated patients with an unmethylated MGMT promoter, which confers resistance to TMZ, had a longer OS than patients with methylated MGMT ($p = 0.062$), raising the possibility that EGFRvIII-targeted vaccines may be an effective alternative for patients with unmethylated MGMT [71,73].

The second phase II clinical trial, ACT II, was undertaken by the Duke University group and enrolled 21 patients [56,74]. Participants received the PEPvIII-KLH vaccine following the same treatment protocol used in ACTIVATE, except with the addition of concomitant TMZ. Despite TMZ-induced lymphopenia, all immune responses were sustained or enhanced with successive TMZ treatments. These results were recently confirmed by the multicenter, phase II trial ACT III, which also assessed the immunogenicity and potential efficacy of the PEPvIII-KLH vaccine in the context of standard-dose and dose-intensified TMZ regimens [75].

A dendritic cell (DC)-based vaccine that targets the EGFRvIII antigen has also been studied. In preclinical experiments, intraperitoneal vaccination with DCs mixed with PEPvIII-KLH and resuspended in saline increased median survival by more than 500% (> 300 days, $p < 0.001$) in C3H mice challenged with intracerebral tumors [76]. All vaccinated mice also survived rechallenge with tumor, indicating the development of immunological memory.

The first clinical study to evaluate an EGFRvIII-targeted vaccine, which was a DC-based vaccine, was the phase I trial, VICTORI, conducted at Duke University [56,77]. This clinical trial enrolled 15 adults with newly diagnosed glioblastoma who had undergone a radiographic gross-total resection as well as standard RT and had no evidence of radiographic progression. EGFRvIII expression was not an eligibility criterion. Patients underwent leukapheresis to obtain peripheral blood mononuclear cells for DC generation. Prior to vaccination, DCs were pulsed with PEPvIII peptide. Patients received three vaccines in equal doses, two weeks apart, and were followed without additional therapy until radiographic or clinical progression. The median OS was 18.7 months after vaccination (CI95% 14.5, 25.6) and 22.8 months after histological diagnosis (CI95% 17.5, 29). Notably, blood drawn from patients after inoculation showed *ex vivo* evidence of antigen-specific cellular and humoral immune responses. This study established the EGFRvIII mutation as a safe and immunogenic tumor-specific target for immunotherapy. However, given the high cost and variability associated with autologous DC preparation, the study authors decided to pursue additional clinical trials without the use of DCs, instead administering an EGFRvIII-targeted peptide vaccine as outlined above.

In summary, these preclinical and clinical studies report encouraging PFS and OS data using a vaccine that targets the tumor-specific EGFR mutation, EGFRvIII. All of the completed clinical trials to date on EGFRvIII-target vaccines are listed in Table II. It is important to recognize that glioblastoma tumors exhibit significant antigenic heterogeneity, thereby confounding immunotherapeutic strategies aimed to target a single tumor-specific epitope [56]. Greater antitumor effects may be achieved with the de-

Clinical trial	Year	Study design	Treatment protocol
VICTORI	2009	Phase I	DCs plus PEPvIII-KLH
ACTIVATE	2010	Phase II, multicenter, single-arm	PEPvIII-KLH
ACT II	2008	Phase II, single center, single-arm	PEPvIII-KLH plus TMZ vs. TMZ alone
ACT III	2011	Phase II, multicenter, single-arm	PEPvIII-KLH plus TMZ vs. TMZ alone

Table II. Completed clinical trials on EGFRvIII-targeted vaccines.

DC = dendritic cell; KLH = keyhole limpet hemocyanin; TMZ = temozolomide

antibody, was one of the first anti-EGFR antibodies investigated. In a phase I trial, it was shown to effectively bind brain tumor tissue [81]. However, a separate phase I/II trial demonstrated no radiographic response in recurrent anaplastic astrocytomas or glioblastomas. SD was the best-reported outcome, seen in 7 of 13 patients, while the remaining 6 displayed PD [82]. A study of Mab 425 bound to ¹²⁵I concluded that the antibody plus RT had no added benefit when compared to RT alone in the treatment of anaplastic astrocytomas and glioblastomas following surgical resection. In addition, the authors observed no clear evidence of tumoral uptake of EGFR [83].

Cetuximab, another humanized murine monoclonal antibody against EGFR, is currently used for the treatment of metastatic colon cancer and squamous cell carcinoma of the head and neck. It partially blocks the ligand-binding domain of EGFR, prevents dimerization, and internalizes the EGFR receptor, effectively downregulating EGFR expression [84]. Cetuximab for recurrent high-grade glioma (HGG) after failure of standard of care has been evaluated in a phase II study. Systemic administration of cetuximab was well tolerated but achieved PFS of greater than 9 months in just 5 of 55 patients, while the remaining 50 patients had PFS of less than 6 months; median survival was 5.0 months. In this study, patients were screened for EGFR gene amplification status by FISH and then separated into amplified and non-amplified groups. However, no statistically significant correlation of EGFR amplification status and survival time could be identified [85]. Other trials of cetuximab are ongoing [86].

Nimotuzumab (hR3), a humanized antibody directed against the external domain of EGFR, has been investigated in adult and pediatric primary brain tumors. In a phase I/II trial, patients with either grade III or IV astrocytomas were treated with nimotuzumab and RT. The group reported relative drug safety as well as a median survival time of about 22 months [87].

Intracranial therapies

It has been hypothesized that direct intracranial administration of anti-EGFR therapies could allow the local delivery of a more potent drug dose. In a phase I study, nimotuzumab was delivered to post-surgical resection cavities of either recurrent anaplastic astrocytomas or glioblastomas via intracavitary administration, thereby circumventing the BBB. An average of 85.5% of the antibody remained in the tumor cavity 1 hour following injection, and it was reasonably well tolerated by patients. As 5 of 11 patients achieved at least SD, the authors suggest further investigation of the efficacy of intracavitary delivery [88].

development of multiantigenic vaccines. Moreover, RT and TMZ are themselves potent mutagens that may cause either random or systematic mutations in EGFRvIII, resulting in its apparent absence and negating the findings of clinical trials in which patients received standard of care RT and TMZ [78]. Active immunotherapy approaches for glioblastoma are promising but remain incomplete.

EGFR antibodies

EGFR-specific antibodies are used in the treatment of cancer. However, there has long been concern about the ability of these large molecules to cross the BBB and reach their intended targets [79,80].

Mab 425, a humanized murine monoclonal antibody,

In a prior phase I study, TP-38, a *Pseudomonas* toxin-TGF- α construct engineered to target EGFR was administered intracerebrally via convection-enhanced delivery (CED) to patients with recurrent glioblastoma, gliosarcoma, anaplastic oligodendroglioma, or metastatic spindle cell tumor. Only 2 of 15 patients responded to treatment, including one complete response sustained for nearly 4 years [89].

Resistance to EGFR-targeted therapy

A number of mechanisms proposed to confer resistance to EGFR-targeted therapies by glioblastoma have been elucidated [90]. First, the previously mentioned physical barrier of the BBB limits drug penetration. At the molecular level, the Akt pathway can be activated outside of EGFR-dependent processes. For instance, the phosphorylation of phosphatidylinositol 4,5-bisphosphate to active phosphatidylinositol 3,4,5-triphosphate by phosphatidylinositol 3-kinase (PI3K) allows for subsequent Akt activation. This process can occur by activation of PI3K not only by EGFR but also via proteins such as platelet-derived growth factor receptor (PDGFR) [13] and the insulin-like growth factor 1 (IGF1) receptor [91], which has been investigated as a therapeutic target [92]. Mutations of catalytic subunits of PI3K have also been demonstrated in glioblastoma [93]. Due to the pivotal role PI3K plays in a number of pathways and its ability to activate Akt without dependence on one specific activator such as EGFR, it has been proposed as a central target for glioblastoma therapy [94].

Loss of the PTEN gene product allows for unopposed Akt pathway activation via EGFR or phosphatidylinositol 3-kinase (PI3K) [90,95]. This loss has been implicated in gliomagenesis [96] as well as in tumor resistance to EGFR inhibitors [13,31,97]. Additionally, c-Met, a receptor tyrosine kinase similar to EGFR, activates a number of pro-growth pathways and has been shown to be increased in the setting of increased and decreased levels of EGFR [90,98,99]. An end product of the synthesis of cholesterol by HMG-CoA reductase, dolichol serves to glycosylate EGFR and enhance its function [90,100]. Other end products of cholesterol synthesis are used as substrates by enzymes that post-translationally modify Ras to increase its function as well [90]. Finally, the tumor suppressor STAT3 can form a nuclear complex with EGFRvIII that promotes oncogenic transformation in likely multiple but still unclear ways [90,101,102].

Conclusions

EGFR is a receptor that serves to activate numerous pathways important in the development, growth, proliferation, and survival of glioblastomas. Extensive efforts have been made in its evaluation as a potential therapeutic target for these aggressive tumors. Small molecule TKIs, antibodies, and immune targeting have all served as potential means to focus attack on EGFR. However, so far, no studies have shown significant benefit in outcome, either radiographically or clinically with prolonged PFS or OS. It is also important to note some of the limitations in the studies summarized in this review. For instance, most of the studies are limited by small sample size and lack of a randomized control group. Nevertheless, it can be concluded that the current data on EGFR-specific therapies in glioblastoma do not warrant a change in the standard of care for clinical practice. Though no approach has been clearly demonstrated yet to be effective in the majority of patients, our understanding of the role of EGFR in glioblastoma has grown in the process. This understanding will continue to grow via clinical and preclinical research.

Questions for further research

Further biomarker enriched clinical studies may allow for a better understanding of the potential benefit of EGFR-targeted therapies.

The review in brief

Clinical question	The review examines the role of EGFR in glioblastoma and provides an overview of methods currently used to evaluate EGFR status in brain tumor patients. Furthermore, it highlights the role of EGFR as a therapeutic target
Type of review	Narrative
Search of the literature	PubMed with the following keywords: epidermal growth factor receptor, tyrosine kinase inhibitors AND glioblastoma, antibodies, vaccines
Conclusions	Even if extensive efforts have been made in the evaluation of EGFR as a potential therapeutic target for glioblastoma, current data do not warrant a change in the standard of care for clinical practice
Limitations	Most of the studies are limited by small sample size and lack of a randomized control group. Non-biomarker enriched trials including patients without EGFR-pathway involvement in their tumor have a limited ability to achieve their predetermined endpoints. Further clinical and preclinical studies will increase the understanding of the role of EGFR in glioblastoma

References

1. Daumas-Duport C, Scheithauer B, O'Fallon J, Kelly P. Grading of astrocytomas. A simple and reproducible method. *Cancer* 1998; 62: 2152-65
2. Nicholas MK, Lukas RV, Chmura S, Yamini B, Lesniak M, Pytel P. Molecular heterogeneity in glioblastoma: therapeutic opportunities and challenges. *Semin Oncol* 2011; 38: 243-53
3. Gerosa MA, Talarico D, Fognani C, Raimondi E, Colombatti M, Tridente G, et al. Overexpression of N-ras oncogene and epidermal growth factor receptor gene in human glioblastomas. *J Natl Cancer Inst* 1989; 81: 63-7
4. Yarden Y. The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. *Eur J Cancer* 2001; 37 (Suppl 4): S3-S8
5. Esper RM, Pankonin MS, Loeb JA. Neuregulins: versatile growth and differentiation factors in nervous system development and human disease. *Brain Res Rev* 2006; 51(2): 161-75
6. Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* 2004; 59(Suppl 2): 21-6
7. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 1997; 91: 231-41
8. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, Roberts TM, et al. Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. *Proc Natl Acad Sci U S A* 1999; 96: 2110-5
9. Kandel ES, Skeen J, Majewski N, Di Cristofano A, Pandolfi PP, Feliciano CS, et al. Activation of Akt/protein kinase B overcomes a G(2)/m cell cycle checkpoint induced by DNA damage. *Mol Cell Biol* 2002; 22: 7831-41
10. Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, et al. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 1995; 81: 1137-46
11. Wisdon R, Johnson RS, Moore C. c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. *EMBO J* 1999; 18: 188-97
12. Andersson U, Schwartzbaum J, Wiklund F, Sjöström S, Liu Y, Tsavachidis S, et al. A comprehensive study of the association between the EGFR and ERBB2 genes and glioma risk. *Acta Oncol* 2010; 49: 767-75
13. Nicholas MK, Lukas RV, Jafri NF, Faoro L, Salgia R. Epidermal-growth factor receptor – mediated signal transduction in the development and therapy of gliomas. *Clin Cancer Res* 2006; 12: 7261-70

14. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 2010; 17: 98-110
15. Lai A, Kharbanda S, Pope WB, Tran A, Solis OE, Peale F, et al. Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. *J Clin Oncol* 2011; 29: 4482-90
16. Wikstrand CJ, McLendon RE, Friedman AH, Bigner DD. Cell surface localization and density of the tumor-associated variant of the epidermal growth factor receptor, EGFRvIII. *Cancer Res* 1997; 57: 4130-40
17. Ekstrand AJ, Longo N, Hamid ML, Olson JJ, Liu L, Collins VP, et al. Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR gene amplification. *Oncogene* 1994; 9: 2313-20
18. Kuan CT, Wikstrand CJ, Bigner DD. EGFRvIII as a promising target for antibody-based brain tumor therapy. *Brain Tumor Pathol* 2000; 17: 71-8
19. Stutz MA, Shattuck DL, Laederich MB, Carraway KL 3rd, Sweeney C. LRIG1 negatively regulates the oncogenic EGF receptor mutant EGFRvIII. *Oncogene* 2008; 27: 5741-52
20. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002; 2: 489-501
21. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* 1998; 95: 29-39
22. Liu W, James CD, Frederick L, Alderete BE, Jenkins RB. PTEN/MMAC1 mutations and EGFR amplification in glioblastomas. *Cancer Res* 1997; 57: 5254-7
23. Feldkamp MM, Lau N, Guha A. Signal transduction pathways and their relevance in human astrocytomas. *J Neurooncol* 1997; 35: 223-48
24. Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris S, Kinzler KW, Vogelstein B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* 1993; 362: 857-60
25. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast and prostate cancer. *Science* 1997; 275: 1943-7
26. Schmidt MH, Furnari FB, Cavenee WK, Bögl O. Epidermal growth factor signaling intensity determines intracellular protein interactions, ubiquitination, and internalization. *Proc Natl Acad Sci USA* 2003; 100: 6505-10
27. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, et al. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* 1998; 12: 3663-74
28. Yoon KS, Lee MC, Kang SS, Kim JH, Jung S, Kim YJ, et al. p53 mutation and epidermal growth factor receptor overexpression in glioblastoma. *J Korean Med Sci* 2001; 16: 481-8
29. Lopez-Gines C, GilpBenso R, Ferrer-Luna R, Benito R, Serna E, Gonzalez-Darder J, et al. New pattern of EGFR amplification in glioblastoma and the relationship of gene copy number with gene expression profile. *Mod Pathol* 2010; 23: 856-65
30. Fischer I, de la Cruz C, Rivera AL, Aldape K. Utility of chromogenic in situ hybridization (CISH) for detection of EGFR amplification in glioblastoma: comparison with fluorescence in situ hybridization (FISH). *Diagn Mol Pathol* 2008; 17: 227-30
31. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 2005; 353: 2012-24
32. Miyanaga T, Hirato J, Nakazato Y. Amplification of the epidermal growth factor receptor gene in glioblastoma: an analysis of the relationship between genotype and phenotype by CISH method. *Neuropathology* 2008; 28: 116-26

33. Ruano Y, Mollejo M, Ribalta T, Fiaño C, Camacho FI, Gómez E, et al. Identification of novel candidate target genes in amplicons of glioblastoma multiforme tumors detected by expression and CGH microarray profiling. *Mol Cancer* 2006; 5: 39
34. Yoshimoto K, Dang J, Zhu S, Nathanson D, Huang T, Dumont R, et al. Development of a real-time RT-PCR assay for detecting EGFRvIII in glioblastoma samples. *Clin Cancer Res* 2008; 14: 488-93
35. Tripp SR, Willmore-Payne C, Layfield LJ. Relationship between EGFR overexpression and gene amplification status in central nervous system gliomas. *Anal Quant Cytol Histol* 2005; 27: 71-8
36. Gupta P, Han SY, Holgado-Madruga M, Mitra SS, Li G, Nitta RT, et al. Development of an EGFRvIII specific recombinant antibody. *BMC Biotechnol* 2010; 10: 72
37. Lassman AB, Rossi MR, Raizer JJ, Abrey LE, Liberman FS, Grefe CN, et al. Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from North American Brain Tumor Consortium Trials 01-03 and 00-01. *Clin Cancer Res* 2005; 11: 7841-50
38. Franceschi E, Cavallo G, Lonardi S, Magrini E, Tosoni A, Grosso D, et al. Gefitinib in patients with progressive high-grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *Br J Cancer* 2007; 96: 1047-51
39. Uhm JH, Ballman KV, Wu W, Giannini C, Krauss JC, Buckner JC, et al. Phase II evaluation of gefitinib in patients with newly diagnosed grade 4 astrocytoma: Mayo/North Central Cancer Treatment Group Study N0074. *Int J Radiat Oncol Bio Phys* 2011; 80: 347-53
40. Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004; 22: 133-42
41. Hegi ME, Diserens AC, Bady P, Kamoshima Y, Kouwenhoven MC, Delorenzi M, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib--a phase II trial. *Mol Cancer Ther* 2011; 10: 1102-12
42. Van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol* 2009; 27: 1268-74
43. Yung WK, Vredenburgh JJ, Cloughesy TF, Nghiemphu P, Klencke B, Gilbert MR, et al. Safety and efficacy of erlotinib in first-relapse glioblastoma: a phase II open-label study. *Neuro Oncol* 2010; 12: 1061-70
44. Raizer JJ, Abrey LE, Lassman AB, Chang SM, Lamborn KR, Kuhn JG, et al. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. *Neuro Oncol* 2010; 12: 95-103
45. Sathornsumetee S, Desjardins A, Vredenburgh JJ, McLendon RE, Marcello J, Herndon JE, et al. Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma. *Neuro Oncol* 2010; 12: 1300-10
46. Prados MD, Chang SM, Butowski N, DeBoer R, Parvataneni R, Carliner H, et al. Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. *J Clin Oncol* 2009; 27: 579-84
47. Brown PD, Krishnan S, Sarkaria JN, Wu W, Jaeckle KA, Uhm JH, et al. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J Clin Oncol* 2008; 26: 5603-9
48. Peereboom DM, Shepard DR, Ahluwalia MS, Brewer CJ, Agarwal N, Stevens GH, et al. Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme. *J Neurooncol* 2010; 98: 93-9
49. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Friedman AH, Herndon JE 2nd, et al. Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. *J Neurooncol* 2010; 96: 219-30

50. Kreisl TN, Lassman AB, Mischel PS, Rosen N, Scher HI, Teruva-Feldstein J, et al. A pilot study of everolimus and gefitinib in the treatment of recurrent glioblastoma (GBM). *J Neurooncol* 2009; 92: 99-105
51. Cohen MH, Williams GA, Sridhara R, Chen G, Pazdur R. FDA drug approval summary: gefitinib (ZD1839) (Iressa) tablets. *Oncologist* 2003; 8: 303-6
52. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990; 8: 1277-80
53. Raymond E, Faivre S, Armand JP. Epidermal growth factor receptor tyrosine kinase as a target for anticancer therapy. *Drugs* 2000; 60 (Suppl 1): 15-23; discussion pp. 41-2
54. Faehling M, Eckert R, Kuom S, Kamp T, Stoiber KM, Schumann C. Benefit of erlotinib in patients with non-small-cell lung cancer is related to smoking status, gender, skin rash and radiological response but not to histology and treatment line. *Oncology* 2010; 78: 249-58
55. Cohen MH, Shen YL, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab (Avastin) as treatment of recurrent glioblastoma multiforme. *Oncologist* 2009; 14: 1131-8
56. Choi BD, Archer GE, Mitchell DA, Heimberger AB, McLendon ME, Bigner DD, et al. EGFRvIII-targeted vaccination therapy of malignant glioma. *Brain Pathol* 2009; 19: 713-23
57. Ehrlich P, Bolduan C. *Collected Studies on Immunity*, 1st edn. New York J. Wiley & Sons, 1906
58. Rosenberg SA. Progress in human tumour immunology and immunotherapy. *Nature* 2001; 411: 380-4
59. Fabry Z, Raine CS, Hart MN. Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. *Immunol Today* 1994; 15: 218-24
60. Hart DN, Fabre JW. Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J Exp Med* 1981; 154: 347-61
61. Medawar PB. Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br J Exp Pathol* 1948; 29: 58-69
62. Engelhardt B, Ransohoff RM. The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. *Trends Immunol* 2005; 26: 485-95
63. Hickey WF. Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. *Brain Pathol* 1991; 1: 97-105
64. Owens T, Renno T, Taupin V, Krakowski M. Inflammatory cytokines in the brain: does the CNS shape immune responses? *Immunol Today* 1994; 15: 566-71
65. Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today*. 1992; 13: 507-12
66. Goldmann J, Kwidzinski E, Brandt C, Mahlo J, Richter D, Bechmann I. T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. *J Leukoc Biol* 2006; 80: 797-801
67. Gehrman J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immune effector cell of the brain. *Brain Res Brain Res Rev* 1995; 20: 269-87
68. de Vries HE, Kuiper J, de Boer AG, Van Berkel TJ, Breimer DD. The blood-brain barrier in neuro-inflammatory diseases. *Pharmacol Rev* 1997; 49: 143-55
69. Zalutsky MR, Moseley RP, Coakham HB, Coleman RE, Bigner DD. Pharmacokinetics and tumor localization of ¹³¹I-labeled anti-tenascin monoclonal antibody 81C6 in patients with gliomas and other intracranial malignancies. *Cancer Res* 1989; 49: 2807-13
70. Rosenberg SA. Shedding light on immunotherapy for cancer. *N Engl J Med* 2004; 350: 1461-3
71. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J Clin Oncol* 2010; 28: 4722-9

72. Heimberger AB, Crotty LE, Archer GE, Hess KR, Wikstrand CJ, Friedman AH, et al. Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. *Clin Cancer Res* 2003; 9: 4247-54
73. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; 352: 997-1003
74. Sampson JH, Archer GE, Bigner D, Davis T, Friedman HS, Keler T. Effect of EGFRvIII-targeted vaccine (CDX-110) on immune response and TTP when given with simultaneous standard and continuous temozolomide in patients with GBM. *J Clin Oncol* 2011; abstr 26
75. Sampson JH, Aldape KD, Archer GE, Coan A, Desjardins A, Friedman AH, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. *Neuro Oncol* 2011; 13: 324-33
76. Heimberger AB, Archer GE, Crotty LE, McLendon RE, Friedman AH, Friedman HS, et al. Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma. *Neurosurgery* 2002; 50: 158-64
77. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE 2nd, Lally-Goss D, et al. An epidermal growth factor receptor variant-III vaccine is safe and immunogenic in patients with glioblastoma multiforme. *Mol Cancer Ther* 2009; 8: 2773-9
78. Lesniak MS. Immunotherapy for glioblastoma: the devil is in the details. *J Clin Oncol* 2011; 29: 3105
79. Jain RK. Vascular and interstitial barriers to delivery of therapeutic agents in tumors. *Cancer Metastasis Rev* 1990; 9: 253-66
80. Kemshead JT, Jones DH, Goldman A, Richardson RB, Coakham HB. Is there a role for radioimmunolocalization in diagnosis of intracranial malignancies?: discussion paper. *J R Soc Med* 1984; 77: 847-54
81. Faillot T, Magdelénat H, Mady E, Stasiecki P, Fohanno D, Gropp P, et al. A phase I study of an anti-epidermal growth factor receptor monoclonal antibody for the treatment of malignant gliomas. *Neurosurgery* 1996; 39: 478-83
82. Stragliotto G, Vega F, Stasiecki P, Gropp P, Poisson M, Delattre JY. Multiple infusions of anti-epidermal growth factor receptor (EGFR) monoclonal antibody (EMD 55,900) in patients with recurrent malignant gliomas. *Eur J Cancer* 1996; 32A: 636-40
83. Wygoda Z, Kula D, Bierzńska-Macyszyn G, Larysz D, Jarzab M, Właszczuk P, et al. Use of monoclonal anti-EGFR antibody in the radioimmunotherapy of malignant gliomas in the context of EGFR expression in grade III and IV tumors. *Hybridoma (Larchmt)* 2006; 25: 125-32
84. Harding J, Burtneß B. Cetuximab: an epidermal growth factor receptor chimeric human-murine monoclonal antibody. *Drugs Today (Barc)* 2005; 41: 107-27
85. Neyns B, Sadones J, Joosens E, Bouttens F, Verbeke L, Baurain JF, et al. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. *Ann Oncol* 2009; 20: 1596-603
86. Combs SE, Heeger S, Haselmann R, Edler L, Debus J, Schulz-Ertner D. Treatment of primary glioblastoma multiforme with cetuximab, radiotherapy and temozolomide (GERT)--phase I/II trial: study protocol. *BMC Cancer* 2006; 6: 133
87. Ramos TC, Figuerdo J, Catala M, González S, Selva JC, Cruz TM, et al. Treatment of high-grade glioma patients with the humanized anti-epidermal growth factor receptor (EGFR) antibody h-R3: report from a phase I/II trial. *Cancer Biol Ther* 2006; 5: 375-9
88. Casacó A, López G, García I, Rodríguez JA, Fernández R, Figueredo J, et al. Phase I single-dose study of intracavitary-administered Nimotuzumab labeled with 188 Re in adult recurrent high-grade glioma. *Cancer Biol Ther* 2008; 7: 333-9
89. Sampson JH, Akabani G, Archer GE, Berger MS, Coleman RE, Friedman AH, et al. Intracerebral infusion of an EGFR-targeted toxin in recurrent malignancy brain tumors. *Neuro Oncol* 2008; 10: 320-9

90. Lo HW. EGFR-targeted therapy in malignant glioma: novel aspects and mechanisms of drug resistance. *Curr Mol Pharmacol* 2010; 3: 37-52
91. LeRoith D, Werner H, Beitner-Johnson D, Roberts CT Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr Rev* 1995; 16: 143-63
92. Yin S, Girnita A, Strömberg T, Khan Z, Andersson S, Zheng H, et al. Targeting the insulin-like growth factor-1 receptor by picropodophyllin as a treatment option for glioblastoma. *Neuro Oncol* 2010; 12: 19-27
93. Mizoguchi M, Nutt CL, Mohapatra G, Louis DN. Genetic alterations of phosphoinositide 3-kinase subunit genes in human glioblastomas. *Brain Pathol* 2004; 14: 372-7
94. Lino MM, Merlo A. PI3Kinase signaling in glioblastoma. *J Neurooncol* 2011; 103: 417-27
95. Choe G, Horvath S, Cloughesy TE, Crosby K, Seligson D, Palotie A, et al. Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients *in vivo*. *Cancer Res* 2003; 63: 2742-6
96. Knobbe CB, Merlo A, Reifemberger G. Pten signaling in gliomas. *Neuro Oncol* 2002; 4: 196-211
97. Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. *J Biol Chem* 2000; 275: 8806-11
98. Blanco R, Shin I, Ritter CA, Yakes FM, Basso A, Rosen N, et al. Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. *Oncogene* 2003; 22: 2812-22
99. Reznik TE, Sang Y, Ma Y, Abounader R, Rosen EM, Xia S, et al. Transcription-dependent epidermal growth factor receptor activation by hepatocyte growth factor. *Mol Cancer Res* 2008; 6: 139-50
100. Carlberg M, Dricu A, Biegen H, Wang M, Hjertman M, Zickert P, et al. Mevalonic acid is limiting for N-linked glycosylation and translocation of the insulin-like growth factor-1 receptor to the cell surface. Evidence for a new link between 3-hydroxy-3-methylglutaryl-coenzyme a reductase and cell growth. *J Biol Chem* 1996 ; 271: 17453-62
101. De la Iglesia N, Konopka G, Puram SV, Chan JA, Bachoo RM, You MJ, et al. Identification of PTEN-regulated STAT3 brain tumor suppressor pathway. *Genes Dev* 2008; 22: 449-62
102. Lo HW, Cao X, Zhu H, Ali-Osman F. Constitutively active STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. *Clin Cancer Res* 2008; 14: 6042-54