Epidermal growth factor (EGF) was identified by embryologist Stanley Cohen in the early 1960s and its receptor (EGFR) was identified a decade later (1, 2). During that time and in the decades that followed, a family of related factors and their receptors were identified. The complex intracellular effects that follow activation of these receptors were subsequently elaborated. Together, this would prove to be of critical importance in our understanding of both normal growth and development and neoplastic transformation. This review will focus on the role of these receptor-ligand interactions in primary brain tumor biology. We will briefly discuss their importance in the development of normal brain and the implication of those roles for tumorigenesis. Following a review of specific abnormalities in brain tumors, we will conclude with a summary of brain tumor therapies that include EGFR-mediated signaling in their rationale.

Receptor-Ligand Interactions

The EGFR was the first of the human EGF receptor (HER) family members to be discovered. The HER family proteins are members of the receptor tyrosine kinase (RTK) superfamily. The platelet-derived growth factor receptor (PDGFR), another RTK family member, is also important in brain tumor biology. The ligands for the HER family receptors are also diverse. They include the EGF family and the structurally related neuregulin family. Both are produced by many cell types, including neurons and glia. All of the ligands function via autocrine and/or paracrine signaling. The EGF family includes EGF, transforming growth factor-α (TGF-α), heparin-binding EGF, amphiregulin, betacellulin, and epiregulin (3). The neuregulin (NRG) family consists of the protein products derived from the alternative splicing of four distinct genes: NRG-1, NRG-2, NRG-3, and NRG-4 (4). Several of the neuregulins were discovered by groups working in different fields and are known variously as glial growth factor, neu differentiation factor, acetylcholine receptor inducing activity, divergent of neuregulin-1, and heregulin. In this review, we will refer to them as NRG-1 to NRG-4. The receptors, their synonyms, dimerization partners, and ligands are outlined in Table 1.

Once activated by ligand binding and subsequent dimerization, these receptors activate a variety of intracellular phosphorylation-mediated signal transduction events. Those of special importance in glioma biology include the RAS/mitogen-activated protein kinase and phosphatidylinositol 3-kinase/AKT pathways.

EGFR-Mediated Signaling in Normal Brain Development

The RTK superfamily and their ligands play a critical role in the development and subsequent maintenance of the central nervous system (CNS). Although there is considerable redundancy among receptors and ligands, each plays a unique role in CNS development and/or survival. The EGFR family and its ligands are variably expressed from embryogenesis, throughout brain development, and into adulthood. These factors are
involved in the proliferation, migration, differentiation, and survival of all CNS cell types and their precursors (5). Evidence from both transgenic and knockout mice has shown unique roles for each receptor and ligand in brain development (5, 6).

Mice genetically engineered to overexpress EGFR constitutively have been studied. These animals are invariably growth retarded but lack a distinct phenotype (6). Knockout mice, lacking all EGFR expression, have also been generated. These mice display a range of phenotypes depending on the strain in which they are created, some dying during gestation, others at birth, and some surviving for the first month of life. Those mice surviving beyond birth die of a neurodegenerative process (7). This suggests a critical role for EGFR-mediated processes in neurotropism. Mice with targeted deletions of EGFR display defects in neuronal and glial precursor proliferation, differentiation, and migration (6). Similar findings are observed in mice lacking TGF-α during development (8). EGF exogenously applied to the developing mouse brain increases the number of glial cells relative to neurons (9). These observations can be extended to many species. In fact, EGFR and several of its ligands influence glial differentiation and survival in nervous systems as simple as that of Drosophila (10).

EGFR has been shown to influence the migration of neural stem cells during development (5). Furthermore, overexpression of EGFR in postnatal immobile neural progenitor cells results in their migration both in vitro and in vivo (11). These influences on cell motility are, in part, responsible for a variety of syndromes characterized by neural migration defects and hamartoma formation such as neurofibromatosis type 1 (12).

During development, cellular constituents may be differentially distributed to daughter cells during cell division. Asymmetrical distribution of EGFR during cell division influences the fate of neural progenitor cells (13). In mouse model systems, brain cortical progenitors inheriting high levels of EGFR differentiate along astrocytic lines whereas those inheriting low levels are fated to become oligodendrocytes. It has been hypothesized that this early differential EGFR expression has a corollary in oncogenesis as astrocytic gliomas are more likely to express high levels of EGFR and its mutants than are oligodendrogial cells (14).

Neuregulins play fundamental roles in brain development. They act on the developing brain and influence synapse formation, oligodendrocyte development, the cell fate of sensory and sympathetic neurons, differentiation of Schwann cells in the peripheral nervous system, and the formation of acetylcholine receptors at the neuromuscular junction (4). Abnormalities in neuregulin function at given levels of the neuraxis are associated with diseases including schizophrenia, multiple sclerosis, amyotrophic lateral sclerosis, autonomic and peripheral neuropathies, and myasthenia gravis (4).

Excepting oligodendroglialomas, evidence supporting a link between neuregulins, their role in CNS development, and subsequent gliomagenesis is less compelling than it is for the EGFR family. There is evidence that NRG-1 mediates migration of neural progenitor cells in an ErbB4-dependent manner (15). Heregulin, the alternatively spliced NRG-1 variant, has been shown in both neurons and glia during embryogenesis. Furthermore, exposure of primary rat brain cultures to heregulin resulted in a growth and survival advantage for astrocytes (16). These are but a few of the examples from developmental neurobiology about the potential effect of EGFR-mediated signaling operative in normal brain development that might influence glial tumorigenesis.

#### Classification of Glial Tumors

Generally, glial tumors are those derived from macroglia, astrocytes and oligodendroglia, as well as their precursors. They are typically classified according to the WHO criteria based on their features observed with H&E staining (17). Although this scheme allows for reliable phenotypic comparisons between pathologists, it is clear that many of these tumors, although histologically similar, differ in important ways at the molecular level. There are several subtypes, including astrocytomas, oligodendrogloma, and tumors of mixed histologies. These tumors, their grades, and the histologic criteria used to distinguish between them are outlined in Table 2. The histologic criteria used to grade these tumors have changed with time and may soon include molecular criteria. At present, however, the number of simple histologic criteria present in a tumor sample determines tumor grade.

The natural history, prognosis, and treatment responses observed in gliomas are related to both tumor category and grade. We will focus primarily on the so-called high-grade tumors (WHO grades 3 and 4) with particular reference to glioblastoma multiforme, the most common and aggressive glioma. Although EGFR family members, their ligands, and downstream signaling targets are dysregulated in many gliomas, glioblastoma multiforme remains the best characterized in this regard. Furthermore, our experience with targeted therapies is greatest in glioblastoma multiforme.

Glioblastoma multiforme arises either (a) *de novo* from normal glial cells or their precursors (primary glioblastoma multiforme) or (b) via progression from tumors of lower

---

**Table 1. Relationships between EGFR family members and their ligands**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Synonyms</th>
<th>Ligand</th>
<th>Dimerization partner</th>
<th>Kinase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>ErbB1, HER1</td>
<td>EGF, TGF-α, HB-EGF, AR, BTC, ERP, decorin</td>
<td>EGFR, HER2, HER4</td>
<td>+</td>
</tr>
<tr>
<td>HER2</td>
<td>ErbB2, neu</td>
<td>NRG-1</td>
<td>EGFR, HER3, HER4</td>
<td>+</td>
</tr>
<tr>
<td>HER3</td>
<td>ErbB3</td>
<td>NRG-1</td>
<td>HER2, HER3, HER4</td>
<td>+</td>
</tr>
<tr>
<td>HER4</td>
<td>ErbB4</td>
<td>NRG-1, NRG-2, NRG-3, NRG-4</td>
<td>EGFR, HER2, HER3, HER4</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: HB-EGF, heparin-binding EGF; AR, amphiregulin; BTC, betacellulin; EPR, epiregulin.
grade (secondary glioblastoma multiforme; ref. 18). Although primary glioblastoma multiforme is said to account for 95% of cases, this may be an overestimation, including tumors that progress rapidly to a grade 4 neoplasm without earlier histologic confirmation of a lower-grade lesion (19). Importantly, these tumors differ from one another in several ways at the molecular level. 

**EGFR** gene amplification figures most prominently in primary as opposed to secondary glioblastoma multiforme (40% versus 8%) as does EGFR receptor overexpression (>60% versus <10%; ref. 20). Inactivation of the tumor suppressor gene p53 is more often encountered in secondary glioblastoma multiforme. These two features, amplification of an oncogene (with overexpression of its product) and inactivation of a tumor suppressor gene, constitute the primary features that distinguish one group from the other.

Primary and secondary glioblastoma multiformes differ in many ways beyond their relative mutual exclusion of EGFR gene amplification and p53 gene mutation. For example, although primary glioblastoma multiformes seldom have p53 mutations, they are more likely than secondary tumors to amplify the murine double minute 2 (mdm2) gene and to overexpress its product, MDM2 (21). As MDM2 binds to and inactivates p53, many primary glioblastoma multiformes also have a mechanism that provides escape from p53-mediated growth controls. Furthermore, loss of heterozygosity on chromosome 10 and mutations in the tumor suppressor gene, phosphatase and tensin homologue on chromosome 10 (PTEN), are more common in primary than in secondary glioblastoma multiforme (22). Again, this distinction has important clinical implications as the presence or absence of functional PTEN seems to be critical in determining the effectiveness of certain EGFR-targeted molecular therapies (see below).

Abnormalities in molecular pathways that do not distinguish primary and secondary glioblastoma multiformes also exist (23). These include abnormalities in cyclin-dependent kinase pathways. For example, either mutation at the **INK4a/ARF** locus or overexpression of cyclin-dependen kinase 4 may lead to inactivation of the p16 tumor suppressor gene, encoded by the **INK4a/ARF** locus. Furthermore, RTK signaling can occur through more than one pathway (e.g., both EGFR and PDGFR in glioma; ref. 18). It seems clear from these examples that glial tumors commonly arise as a consequence of the dysregulation of several critical molecular pathways, with more than one route to that dysregulation. With time, the classification of primary and secondary glioblastoma multiformes may expand to include subcategories of tumors within these distinct groups to reflect the many potential combinations of molecular abnormalities.

**Table 2.**Histologic criteria commonly used in grading infiltrating glial neoplasms

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Tumor types</th>
<th>Histologic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO grade 2</td>
<td>Astrocytoma, oligodendroglioma, oligoastrocytoma</td>
<td>Nuclear atypia</td>
</tr>
<tr>
<td>WHO grade 3</td>
<td>Anaplastic: astrocytoma, oligodendroglioma, oligoastrocytoma</td>
<td>Nuclear atypia, increased mitotic activity</td>
</tr>
<tr>
<td>WHO grade 4</td>
<td>Glioblastoma multiforme, gliosarcoma</td>
<td>Nuclear atypia, endothelial proliferation and/or necrosis</td>
</tr>
</tbody>
</table>

**Gliomas and Their Cell(s) of Origin**

Controversy exists about the cell of origin in glial tumor formation. It is clear from a review of normal brain development that glial cells derive from a common precursor, or neural stem cell, and that many factors influence their fate. Pluripotent neural stem cells capable of generating both neurons and glia persist in distinct regions of the adult brain throughout life. Several distinct neural stem cell – derived glial restricted precursor cells have been identified. These include the oligodendrocyte-type 2 astrocyte progenitor, first derived from the rat optic nerve (24), and the A2B5 precursor, first derived from the developing rat spinal cord (25). Others are also described, including neural stem cells that persist in the mature (adult) nervous system. Whereas EGFR-mediated influences allow for continued division and differentiation of various neural stem cells during life, in vitro modeling suggests that this capacity diminishes with age (26).

The concept of a self-renewing cancer stem cell developed from the observation of the shared properties of stem cells and cancer cells (27). Substantial cellular heterogeneity exists within a given glial tumor and a small proportion of those cells have characteristics of neural stem cells. This has given rise to the idea that glial tumors arise from brain tumor stem cells, which, in turn, arise from neural stem cells (reviewed in ref. 28). CD133-expressing brain tumor stem cells are characterized by their ability to recapitulate the phenotype of a morphologically heterogeneous glial tumor in cell culture, thus supporting their role in glial tumor formation (28).

The cancer stem cell hypothesis has challenged the view that tumors arise from the dedifferentiation of more mature cell types. Modeling in vitro has shown the capability for both differentiated astrocytes and neural stem cells to acquire malignant features following loss of key cell cycle regulators (Ink4a/Arf) in the presence of constitutive EGFR expression (29). Thus, it seems that dysregulation of specific molecular pathways can influence tumor phenotype independent of the cell of origin. Furthermore, transgenic modeling of gliomagenesis directed towards the formation of oligodendrogliomas has resulted in astrocytomas (30). Conversely, transgenic strategies directed towards the formation of astrocytoma have generated oligodendrogliomas (31), supporting the view that astrocytes and oligodendrocytes have a common origin, their bidirectional movement along differentiating pathways subject to influence by a host of factors.

**EGFR-Mediated Signaling in Glial Tumorigenesis**

As noted, activation of EGFR-mediated signal transduction pathways is common in many malignancies, including glial neoplasms, and may be caused by perturbations at several levels. The four common pathways include (a) increased...
expression of EGFR ligands; (b) amplification of EGFR genes; (c) overexpression of wild-type and mutant EGFR family members; and (d) activation of downstream pathways via EGFR-independent mechanisms. Many of these factors operate simultaneously in glial tumors and often act in concert to confer the malignant phenotype.

**Ligand-Mediated Mechanisms of Activation in Gliomagenesis**

EGFR and other RTK family ligands are often overexpressed in gliomas. For example, neoplastic glial cells produce increased levels of both TGF-α and heparin-binding EGF, leading to overactivity of EGFR via an autocrine loop. A similar mechanism is also observed with PDGF (32). The effects of TGF-α and heparin-binding EGF become more apparent as tumors progress from lower to higher grades. PDGF, on the other hand, seems to play a role early in tumorigenesis (33). Gliomagenesis is now recognized as a multistep process, and although increased ligand expression may influence the transition of a glial cell from a quiescent to an activated state, this alone is probably insufficient to confer a malignant phenotype.

**Gene Amplification, Overexpression, and Mutation of EGFR Family Members in Glioma**

EGFR is best characterized among the HER family members that are frequently overexpressed in glioma. In glioblastoma multiforme, EGFR overexpression is almost always associated with gene amplification. Most series note an incidence of ~40%. Mutation is also common. This is in contrast to other EGFR family members that may be present but are not commonly amplified, overexpressed, or mutated. Mutations in gliomas typically lead to truncated proteins, with a very low prevalence of point mutations. Furthermore, different EGFR mutations may be present in the same glioblastoma multiforme (34). In other categories of gliomas (e.g., oligodendroglioma), increased EGFR expression often occurs independent of amplification, with rare evidence for mutation (14). EGFR aberrancy is not, however, typically seen in lower-grade glial tumors of astrocytic histology. EGFR gene amplification is found in one third to one half of glioblastoma multiforme and 90% of these show protein overexpression (19, 34). Many EGFR mutations result in constitutive activation (35). EGFR mutations seem to be restricted to those tumors with gene amplification (34). This differs from other tumors (e.g., non–small-cell lung cancer) where amplification of mutant EGFR genes is uncommon (36).

HER2/neu overexpression is less common in glioma than it is in other solid tumors, such as breast cancer. However, one retrospective study involving 149 subjects with glioblastoma multiforme revealed HER2/neu overexpression in 15% (37). A subsequent study involving 347 subjects found an incidence of HER2/neu overexpression of 10% (38). In both studies, HER2/neu overexpression was associated with poor survival.

**Specific EGFR Mutations in Glioblastoma Multiforme**

EGFR gene rearrangements and expression of their aberrant protein products are common in glioblastoma multiforme (39). The EGFR deletion mutations are outlined in Fig. 1. Of the five common deletion mutations, EGFRvII, EGFRvIII, and EGFRvV are commonly found in glioblastoma multiforme. One study reviewing tissues from 44 glioblastoma multiformes identified mutations in 17 (38%) of the tumors, all of which had amplified EGFR (34). The EGFR mutations most commonly found in glioblastoma multiforme, EGFRvII and EGFRvIII, are in-frame deletion of regions of the extracellular domain. The most common deletion, accounting for 60% to 70% of EGFR mutations in glioblastoma multiforme, involves exons 2 to 7 of the extracellular domain, forming a receptor known as EGFRvIII (ref. 40; see below). EGFRvII and EGFRvV each account for ~15% of the EGFR mutations in glioblastoma multiforme. The first of these is EGFRvII, a deletion of exons 14 and 15 (amino acids 521-603) in the cysteine-rich portion of the extracellular region. This mutation may lend a growth advantage to the tumor cells (34). The other relatively common mutation seen in glioblastoma multiforme is a truncation of the intracellular region at amino acid 958, EGFRvV. Cells expressing this mutation exhibit increased ligand-dependent kinase activity (34). Mutations of the intracellular portion of EGFR are more common in other neoplasms (e.g., non–small-cell lung cancer). In-frame tandem duplications of selected EGFR exons occur at a low frequency, as do missense mutations of the extracellular juxtamembrane region. Their significance is unclear. Of note, multiple mutations are sometimes seen in the same amplified EGFR gene, a finding unique to glioblastoma multiforme (34).

As noted above, the most common in-frame deletion involves exons 2 to 7 (amino acids 6-273) creating the 145-kDa EGFRvIII mutant (41). Regions of multiple Alu repeats in introns 1 and 7 may play a role in mediating susceptibility to this specific gene rearrangement (42). The same mutation is also found in cancers of the breast, ovary, lung, and prostate (43–47). The joining of exons 1 to 8 creates a novel tertiary conformation of the extracellular domain, leaving the transmembrane and intracellular portions of the receptor intact. Although this mutant protein is unable to bind either EGF or TGF-α (and presumably its other ligands), it is constitutively active, phosphorylating downstream targets in the absence of ligand binding. Targets include those mediated by phosphatidylinositol 3-kinase and Ras-GTP, both of which are thought to
confer a growth advantage on EGFRvIII-expressing cells (48, 49). In vitro, EGFRvIII-expressing glioblastoma multiforme cells have elevated expression of matrix metalloproteinases and extracellular matrix components when compared with those that do not express the variant receptor. These findings support a role for EGFR mutations in the increased invasiveness of tumor cells (50).

Alternate Mechanisms for Activating EGFR-Mediated Signal Transduction Pathways in Glioma

The activation of signal transduction pathways in glioblastoma multiforme by EGFR family receptor phosphorylation is also influenced by other factors. For example, phosphatidylinositol 3-kinase is an enzyme critical to the activation of Akt. Activation occurs as a consequence of phosphatidylinositol 3-kinase-mediated phosphorylation of phosphatidylinositol 4,5-bisphosphate to its triphosphate form, phosphatidylinositol 3,4,5-trisphosphate. Activated Akt inhibits apoptosis while promoting cell division (51). Phosphatidylinositol 3-kinase can be activated by PDGFR, thus bypassing EGFR-mediated signaling. Furthermore, PTEN converts phosphatidylinositol 3,4,5-trisphosphate to phosphatidylinositol 4,5-bisphosphate, inhibiting Akt activity (52). The PTEN gene is frequently deleted in glioblastoma multiforme, thus favoring activation of Akt signaling independent of and in concert with RTK-mediated signal transduction (52). This finding has important implications in the prediction of glioblastoma multiforme responsiveness to several tyrosine kinase inhibitors (see below).

RAS mutations lead to its constitutive activation in many tumors, resulting in cell proliferation. This is not the case for glioblastoma multiforme, where activation of the RAS/mitogen-activated protein kinase pathway is dependent on RTK overactivity (53). However, like phosphatidylinositol 3-kinase, RAS activation can occur through PDGFR phosphorylation, thus bypassing EGFR-mediated signaling. Furthermore, although targeted therapies directed at EGFR family members have not been devised to directly affect RAS/mitogen-activated protein kinase pathways, inhibitors of key enzymes important in the posttranslational modification of RAS are being investigated in glioblastoma multiforme (54). These agents, farnesyl transferase inhibitors, may prove synergistic when combined with tyrosine kinase inhibitor directed at EGFR family members.

The EGFR Family and Targeted Therapies in Malignant Glioma

The information outlined above suggests that glioblastoma multiforme is a reasonable tumor for the application of targeted molecular therapies. A number of EGFR-family–associated targets have been studied for possible therapeutic interventions. Strategies have been aimed at (a) interference with ligand binding; (b) the reduction of tyrosine kinase activity; (c) interference with key regulatory elements of downstream signal transduction pathways; (d) using antibodies and ligands as vectors to target toxins to EGFR family proteins; and (e) the use of selectively expressed EGFR mutants as antigens to generate vaccines.

Many of these strategies have only been tested in preclinical settings. For example, antibodies directed against the EGFR ligand, TGF-α, have been evaluated in human glioma cell lines and have been shown to inhibit their growth (55). However, because EGFRvIII, the most common mutant EGFR family member in glioblastoma multiforme, is ligand independent, such strategies, when applied alone, are likely to show only modest benefit.

Antibodies. A number of monoclonal antibodies directed against HER family proteins have been developed and tested in preclinical models. Proof of principle for this application is seen with Herceptin, an anti-HER2 antibody, effective in the treatment of some breast cancer patients with systemic disease (i.e., not involving CNS) whose tumors overexpress this receptor. Herceptin has been shown to mediate cell death of HER2/neu–expressing glioblastoma cell lines in vitro, but in vivo activity against intra-CNS tumors is lacking, perhaps due to impaired uptake in the CNS (56). These include unmodified and modified antibodies, targeted against EGFRvIII. Significant anti–glioblastoma multiforme responses have also been shown with anti-EGFRvIII antibodies in s.c. animal models of glioblastoma multiforme. Potential mechanisms of action include antibody-dependent cellular cytoxicity and receptor internalization (57).

The blood-brain barrier represents a significant obstacle to the successful treatment of intra-CNS tumors with systemically administered antibodies, despite the apparent increased region permeability within brain tumors (58–60). Most of the clinical trials to date have attempted to circumvent the blood-brain barrier via intracavitary antibody administration. None of these studies has yet employed an anti-EGFR product. However, responses have been seen in animal studies following the intracranial administration of boronated EGFRvIII-specific antibodies (61). Objective responses have been seen in humans following the intracavitary administration of monoclonal antibodies conjugated to radioisotopes (62). Antibodies can also be used as vectors to deliver other conjugated toxins to tumor cells. Potential toxins (e.g., tyrosine kinase inhibitors or bacterial toxins) can be either linked to the antibodies themselves or packaged in liposomes linked to antibody fragments (63). Although these antibody-based constructs will affect tumor cells adjacent to the resection cavity, the most common site of early tumor recurrence, they may not reach tumor cells that lie at a distance from the site of their administration.

Results of a single phase I/II clinical trial with i.v. EGFR antibodies in malignant glioma have been reported from the Center of Molecular Immunology, Havana, Cuba (64). Twenty-nine patients with newly diagnosed malignant glioma received weekly infusions of a humanized monoclonal antibody (h-R3) directed against the EGFR extracellular domain. Sixteen of these patients had glioblastoma multiforme and, at the time of publication, median survival was reported to be 17.47 months. Immunoscintigraphy studies did show uptake of a technitium-99m-labeled anti-EGFR antibody in some patients in the study, suggesting that the antibodies may be crossing from the systemic circulation into the CNS.
Another trial in development will combine temozolomide, radiation therapy, and cetuximab (IMC-C225), a humanized monoclonal antibody approved by the Food and Drug Administration in 2004 for the treatment of colorectal cancer. Cetuximab binds to both EGFRwt and EGFRvIII (65). Thus, it may be useful in tumors that express only the wild-type receptor.

**Vaccines.** EGFRvIII is currently being used as an antigen in a vaccine-based clinical trial for glioblastoma multiforme (66). Subjects with newly diagnosed glioblastoma multiforme receive s.c. injections of an EGFRvIII vaccine every 2 weeks for 8 weeks and then monthly until evidence for tumor progression is shown. Results are pending.

**Tyrosine kinase inhibitors.** Several small molecules that inhibit the tyrosine kinase activity (tyrosine kinase inhibitors) of HER family receptors have been developed and tested for activity against tumors, including malignant glioma (reviewed in refs. 67, 68). The greatest experience to date is with the reversible tyrosine kinase inhibitors erlotinib (OSI-774, Tarceva) and gefitinib (ZD1839, Iressa). Both of these agents were approved by the Food and Drug Administration for patients with advanced non–small-cell lung cancer who had failed prior cytotoxic chemotherapies. Sensitive tumors harbor specific in-frame deletions or amino acid substitutions that allow these drugs to act within EGFR ATP binding domains. Of note, gliomas do not carry the EGFR kinase domain mutations associated with tyrosine kinase inhibitor responses.

Given the high incidence of EGFRvIII mutations and associated amplification in gliomas, the activity of tyrosine kinase inhibitor in tumors with these mutations warranted investigation. The relative effects of gefitinib have been tested on cell lines that harbor wild-type EGFR and EGFRvIII (69). Higher
doses of gefitinib were needed to inhibit downstream EGFR phosphorylation and signaling events from EGFRvIII. Furthermore, low levels of gefitinib actually seemed to confer a growth advantage to cells with EGFRvIII overexpression, measured both by rates of proliferation and anchorage-independent growth in this study. Erlotinib showed only modest efficacy in a mouse model of EGFRvIII-driven lung tumors (70). Erlotinib treatment resulted in decreased total EGFRvIII and phosphorylated EGFR levels. More robust responses were seen with the irreversible EGFR/HER2 inhibitor HKI-272.

As noted above, EGFRvIII is constitutively active, allowing phosphorylation and downstream activation of signal transduction cascades independent of ligand binding. Thus, one might predict resistance to agents designed to work through receptor-mediated blockade. Several lines of investigation support this view (71, 72). Furthermore, EGFRvIII is relatively resistant to degradation, allowing for prolonged activity at the cancer cell surface (73). Resistance to the current generation of tyrosine kinase inhibitors may be mediated, in part, by this effect.

Despite these findings, gefitinib and erlotinib have been evaluated for their safety and efficacy in the treatment of both newly diagnosed and recurrent malignant glioma, either alone or in combination with radiotherapy or chemotherapy. Some of these trials are ongoing, others closed to accrual with either no or only preliminary data available (67). Results of several completed trials have been published and summarized (74–77). The conclusions drawn from these studies to date are (a) these agents can be safely administered to glioma patients; (b) anticonvulsants that induce hepatic cytochrome P450 enzymes, commonly used in brain tumor patients, alter the metabolism of these agents; (c) the proportion of clinical responses are disappointing; (d) occasional long-lasting clinical responses are seen; and (e) complex mechanisms involving communication between various levels of EGFR-mediated signal transduction cascades account for responses to treatment.

Evaluation of tumor samples from two malignant glioma trials done by the North American Brain Tumor Consortium, one using gefitinib and the other using erlotinib, failed to show consistent and effective inhibition of phosphorylation both of EGFR and its downstream effectors, AKT and extracellular signal–regulated kinase (78). It is important to recognize that more than 100 subjects were enrolled in these studies and only a fraction had tumor available for molecular analysis (21 samples). Only one of the patients had a major response based on radiographic review. Of note, none of the EGFRvIII mutations were detected in this study and there was a lower incidence of EGFR gene amplification than expected. Rather, several new missense mutations were detected. Finally, tissue analysis of drug accumulation suggested that erlotinib penetration into tumors was disappointingly low in most cases and, at its highest, failed to correlate with inhibition of EGFR phosphorylation. Gefitinib, on the other hand, seemed to have higher penetration. Whereas the small numbers made the results from this preliminary study complex, systematic molecular analyses of this type will be important in future trials attempting to interpret the activity of these agents in glioma.

Occasional sustained responses to either gefitinib or erlotinib in select cases have led some to hypothesize that a multifactorial genetic predisposition involving both EGFR signaling and AKT activation was responsible for the beneficial responses to tyrosine kinase inhibitor observed in some patients (71, 72). EGFR-mediated phosphorylation of phosphatidylinositol 4,5-bisphosphate to its active state, phosphatidylinositol 3,4,5-trisphosphate, leads to activation of AKT and cell proliferation while simultaneously inhibiting apoptosis. PTEN ends the phosphatidylinositol 3,4,5-trisphosphate signal, converting the second messenger back to phosphatidylinositol 4,5-bisphosphate. Some patients both have constitutively active EGFR-mediated signaling and have also lost the PTEN tumor suppressor gene. This combination of molecular events is associated with high AKT activity and a poor prognosis. In contrast, preservation of PTEN, according to a study by Mellinghoff et al., provides a setting in which response to a tyrosine kinase inhibitor could be observed.

Mellinghoff et al. (79) retrospectively analyzed pretreatment tissue from 26 of 49 subjects with recurrent malignant glioma treated with either erlotinib or gefitinib. Response was defined as >25% reduction in the bidirectional area of contrast enhancing tumor on brain magnetic resonance imaging scans. Three of nine patients with a clinical response were noted to have the EGFRvIII mutation. They found and verified, in a second cohort of 33 patients, a significant association between coexpression of EGFRvIII and PTEN and clinical response (P < 0.001). This study provides a strong rationale for prospective testing of the molecular phenotype in malignant glioma, with a future goal of guiding treatment decisions.

**Conclusions and Future Directions**

The EGFR family plays a central role in both development and tumorigenesis within many organ systems, including the CNS. In this article, we have reviewed some of the relationships between

---

**Table 3. EGFR and PDGFR inhibitors under investigation for the treatment of malignant glioma**

<table>
<thead>
<tr>
<th>EGFR inhibitor</th>
<th>PDGFR inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlotinib (OSI 774, Tarceva; Genentech)</td>
<td>Imatinib mesylate (Gleevec, STI1571; Novartis)</td>
</tr>
<tr>
<td>Gefitinib (ZD1839, Iressa; AstraZeneca)</td>
<td>Sorafenib (Bay 43-9006; ONYX/Bayer)</td>
</tr>
<tr>
<td>Cetuximab (C225, Erbitux; Merck)</td>
<td>SU011248 (Sutent; Pfizer)</td>
</tr>
<tr>
<td>ZD6474 (Zactima) AstraZeneca</td>
<td>Pazopanib (GW786034; GlaxoSmithKline)</td>
</tr>
<tr>
<td>Laptatinib (GW-572016; GlaxoSmithKline)</td>
<td>PTK787 (Novartis/Schering)</td>
</tr>
<tr>
<td>AEE788 (Novartis)</td>
<td>AMG706 (Amgen)</td>
</tr>
<tr>
<td>EKB569 (Wyeth Ayerst)</td>
<td></td>
</tr>
</tbody>
</table>
normal brain development and glial tumorigenesis. These are summarized in Fig. 2. The mechanisms whereby EGFR-mediated signal transduction influences normal brain and the glioma phenotype differ significantly from those of other organs and tumor types. This is not surprising, given the complexity of EGFR-mediated signaling and its diverse roles in normal growth and development as well as neoplasia. Because the EGFR family mediates so many interactions between cells and their environment, there are many ways in which these processes might be influenced. Regarding the CNS, normal development is highly dependent on temporal and spatial restrictions of progenitor cells of which the ultimate differentiation results in tremendous variability in function within a compact anatomic space. EGFR-mediated events are critical in this process.

In normal CNS development, neurotransmitters are known to interact with EGFR-mediated processes to control growth and differentiation not only of the brain but also of the organism as a whole. This is well characterized in the events surrounding the initiation and subsequent inhibition of puberty (80). To date, the effects of neuron-to-glia signaling in gliomagenesis remain unstudied. It is possible that such inputs might contribute to the regional and temporal incidence of particular glial tumors characterized, to a large extent, by their location within the neuraxis and their usual age of onset.

Most EGFR-directed glioma therapies used to date have focused on single agents, with modest results at best. However, when the molecular mechanisms underlying the observed responses are understood, the possibility will exist that the overall effectiveness of a given therapy can be predicted a priori. A number of agents that target the EGFR family and other RTK family members important in glial tumorigenesis are under investigation and summarized in Table 3. These agents, in addition to the other anti-EGFR strategies outlined above, should provide further insights into the complex physiology that underlies malignant glioma formation while offering new hope to brain tumor patients. Our current level of understanding of these pathways suggests the need to combine agents with different mechanisms of action, targeting signal transduction at several levels simultaneously, with close attention to pharmacodynamic constraints present in the unique environment of the CNS.

References

37. Koka V, Potti A, Forseos SF, et al. Role of Her-2/neu in...


