High-grade gliomas are the most common primary intracranial malignancies and account for over half of all malignant brain tumors in adults. Glioblastoma is defined by WHO as grade IV tumors, the highest grade and most lethal of all gliomas. Despite recent developments in the treatment of high-grade gliomas, only few patients are cured (1). The limited treatment options for patients with recurrent gliomas (2–4) underscore the need for clinical investigations into new agents with novel mechanisms of action.

Epidermal growth factor receptor (EGFR) amplification and overexpression, present in ~50% of glioblastomas, are associated with a poor prognosis, especially when occurring in younger patients (5, 6). Nevertheless, the prognostic value of the EGFR remains controversial (7). EGFR signaling promotes proliferation, migration, and invasion, and inhibits glioma cell apoptosis (8). Frequently, in the context of gene amplification, the EGFR gene presents structural rearrangements and some mutations, the most common being EGFRvIII (9, 10), characterized by the lack of a portion in the extracellular domain. The resulting mutant protein is ligand independent and constitutively phosphorylated. EGFRvIII-positive tumors are also reported to be associated with a worse prognosis and shorter life expectancy (6), and have been targeted by antitumor vaccines in preclinical studies (11, 12) and in a few patients (13, 14). More recently novel missense mutations in the extracellular domain of the EGFR, again associated with increased EGFR gene dosage, have been reported from 14% (18 of 132) of glioblastoma with potentially activating properties (15). Taken together, EGFR and the truncated form, EGFRvIII, activate a signaling cascade and are playing a key role in the development of an aggressive phenotype with a dismal prognosis and resistance to therapy.

Monoclonal antibodies against EGFR were first introduced in the 1980s—first-generation murine monoclonal antibodies later generations using humanized antibodies. However, a prospective phase I/II trial on patients with recurrent malignant gliomas failed to show that these antibodies were of therapeutic efficacy (16); a trial using the intratumoral delivery of monoclonal antibodies was terminated early because they caused a severe intracranial inflammatory reaction (17). Ongoing studies test a vaccination approach targeting the tumor-specific EGFRvIII (13).

In recent years, small molecule inhibitors targeting tyrosine kinases, such as erlotinib (Tarceva) and gefitinib (Iressa), binding to the intracellular part of the receptor have been introduced in clinical practice. In non–small cell lung cancer, a particular sensitivity was observed in female patients with adenocarcinoma, never smokers and Japanese origin (18, 19). Sequencing of the EGFR receptor identified mutations in the tyrosine kinase pocket that were associated with both tyrosine kinase inhibitor response and prolonged survival (20–22). Subsequent functional analysis suggested mutation-mediated enhanced binding of the tyrosine kinase inhibitors (23, 24). These mutations do not fully explain the whole spectrum of activity of EGFR tyrosine kinase inhibitors. The first generations of studies on EGFR inhibitors have not found significant activity of these agents in high-grade gliomas. Furthermore, no clear molecular or clinical predictors have been identified. As with other targeted agents, prospective trials using specific criteria and standardized methods to evaluate tissue biomarkers are required to find predictors of EGFR inhibitors activity in high-grade glioma patients.
of gefitinib activity: few cases of response, and some cases of disease stabilization, have been observed in patients with wild-type \( \text{EGFR} \), and rarely have \( \text{EGFR} \) mutations been described in patients with treatment-induced disease stabilization, which probably contributes substantially to the survival benefit of tyrosine kinase inhibitors (25). However, in malignant glioma, such a relationship could not be shown (26–30).

Mutations in the tyrosine kinase domain could not be shown in malignant glioma (29, 31–33); however, some point mutations in the \( \text{EGFR} \) extracellular domain have been identified, and a potential effect on tyrosine kinase inhibitors response in glioblastoma has been hypothesized (15). Other determinants for \( \text{EGFR} \) tyrosine kinase inhibitor sensitivity, such as \( \text{EGFR} \) copy number, Akt activation (phosphorylation), and phosphatase and tensin homologue (PTEN) protein expression have therefore been investigated.

In non–small cell lung cancer, gefitinib seems to confer a clinical benefit in tumors with a high \( \text{EGFR} \) gene copy number, identified by fluorescent \textit{in situ} hybridization (34–37). Akt, a serine/threonine kinase that acts downstream of \( \text{EGFR} \) regulates cellular processes including cell survival, proliferation, and growth, is activated by phosphorylation. PTEN loss has been associated with Akt activation (38) and with an \textit{in vitro} resistance to gefitinib (39, 40). However, in recent reports, no association has been found between PTEN expression and gefitinib sensitivity in non–small cell lung cancer cells (41, 42). \( \text{K-ras} \) mutation or p-Akt overexpression in non–small cell lung cancer is associated with a low response rate and a shorter time to disease-progression in the absence of \( \text{EGFR} \) mutations (43). In a phase II trial of gefitinib on a series of 53 patients with recurrent glioblastoma, none presented objective responses; however, only 21% of patients had measurable disease at treatment initiation (26). The progression-free survival at 6 months was only 13% not different from historical controls (26). The a progression-free survival at 6 months in the erlotinib arm was 12% only compared with 24% in the control arm. No responses were observed with erlotinib, and no correlations could be shown for treatment effect and \( \text{EGFR} \) expression or amplification and \( \text{EGFRvIII} \) expression. Moreover, \( \text{EGFRvIII} \) expression correlated with a decreased overall survival and progression-free survival in all patients, all patients with \( \text{EGFRVIII} \), and \( \text{PTEN} \) protein coexpression with gefitinib rapidly progressed. These findings are in clear contradiction of the previous retrospective analyses and underline the need for randomized controlled trials and prospective analyses of molecular markers.

The above studies differ greatly in frequencies of biomarkers analyzed and respective predictive values (Table 1). These discrepancies may originate in the different assays used to

Similarly, 28 patients with recurrent or progressive high-grade glioma were prospectively treated with gefitinib within the Gruppo Italiano Cooperativo di Neuro-Oncologia network. No objective responses were observed, and a progression-free survival at 6 months of 14% was reported (27). On analyzing phospho-Akt, \( \text{EGFR} \) gene copy number, and protein expression, no significant correlations with survival and response variables were found.

However, in two retrospective series, correlations have been suggested between molecular biomarkers and responses or survival. Haas-Kogan et al. (30) observed that the response to erlotinib treatment was higher in glioblastoma with high \( \text{EGFR} \) expression and low phospho-Akt than those with low \( \text{EGFR} \) expression and high phospho-Akt levels. The authors found no correlation between \( \text{EGFRvIII} \) expression and response. In their study on 49 glioblastoma patients treated with erlotinib or gefitinib, Mellinghoff et al. (28) found that \( \text{EGFRvIII} \) and \( \text{PTEN} \) protein coexpression was correlated with response to treatment.

Most recently, a large, well-conducted, randomized phase II study by the European Organisation for Research and Treatment of Cancer (EORTC 26034 trial) compared first-line erlotinib with either temozolomide or 1,3-bis(2-chloroethyl)-1-nitrosourea as standard treatments (44). This study confirmed the disappointing results with the \( \text{EGFR} \) inhibitor as a single agent in recurrent disease. The a progression-free survival at 6 months in the erlotinib arm was 12% only compared with 24% in the control arm. No responses were observed with erlotinib, and no correlations could be shown for treatment effect and \( \text{EGFR} \) expression or amplification and \( \text{EGFRvIII} \) expression. Moreover, \( \text{EGFRvIII} \) expression correlated with a decreased overall survival and progression-free survival in all patients, all patients with \( \text{EGFRVIII} \), and \( \text{PTEN} \) protein coexpression treated with erlotinib rapidly progressed. These findings are in clear contradiction of the previous retrospective analyses and underline the need for randomized controlled trials and prospective analyses of molecular markers.

The above studies differ greatly in frequencies of biomarkers analyzed and respective predictive values (Table 1). These discrepancies may originate in the different assays used to

Table 1. Biomarkers and their predictive values in erlotinib/gefitinib studies in high-grade gliomas

<table>
<thead>
<tr>
<th>Patients in study</th>
<th>Rich (26)</th>
<th>Haas-Kogan (30)</th>
<th>Mellinghoff (28)</th>
<th>Franceschi (27)</th>
<th>van den Bent (44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N = 53 )</td>
<td>( N = 52 )</td>
<td>( N = 49 )</td>
<td>( N = 28 )</td>
<td>( N = 110 )</td>
</tr>
<tr>
<td>( \text{EGFR} ) expression</td>
<td>79%</td>
<td>27%</td>
<td>NA</td>
<td>38%</td>
<td>58%</td>
</tr>
<tr>
<td>Tumors analyzed, ( n )</td>
<td>53</td>
<td>41</td>
<td>NA</td>
<td>21</td>
<td>99</td>
</tr>
<tr>
<td>Predictive value</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>( \text{EGFR} ) amplification</td>
<td>36%</td>
<td>26%</td>
<td>48%</td>
<td>47%</td>
<td>46%</td>
</tr>
<tr>
<td>Tumors analyzed, ( n )</td>
<td>42</td>
<td>41</td>
<td>25</td>
<td>19</td>
<td>91</td>
</tr>
<tr>
<td>Predictive value</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>( \text{EGFRvIII} ) expression</td>
<td>49%</td>
<td>5%</td>
<td>46%</td>
<td>21%</td>
<td>21%</td>
</tr>
<tr>
<td>Tumors analyzed, ( n )</td>
<td>53</td>
<td>41</td>
<td>25</td>
<td>NA</td>
<td>99</td>
</tr>
<tr>
<td>Predictive value</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>( \text{P-Akt} ) expression</td>
<td>NA</td>
<td>55%</td>
<td>NA</td>
<td>48%</td>
<td>19%</td>
</tr>
<tr>
<td>Tumors analyzed, ( n )</td>
<td>41</td>
<td>NA</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Predictive value</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>( \text{PTEN} ) expression</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tumors analyzed, ( n )</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predictive value</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.
evaluate biomarkers, difference in stringency of response criteria, and very small numbers of samples analyzed.

Mellinghoff and coworkers (45) defined response as a decrease in the bidirectional diameters by 25%, whereas the established standard WHO or Macdonald criteria require a 50% decrease. The differences for the reported frequency for EGFRvIII (Table 1) may be partly due to different specificity and sensitivity of the antibodies against EGFRvIII for immunohistochemistry used (clone L8A4 and clone G100 in Mellinghoff and Haas-Kogan studies, respectively). Although in the report by Rich et al. (26), the same antibody for EGFRvIII (Clone L8A4) was used, and the authors came to different conclusions (28).

In summary, findings reported in studies evaluating EGFR inhibitors are surprisingly contradictory: three prospective trials with biomarker reported negative findings, whereas two other retrospective trials suggested that these inhibitors had some sort of activity in a subset of patients with particular molecular characteristics. However, the retrospective nature of the latter studies may have been affected by variations in patient selection.

Due to the lack of a sound demonstration of the efficacy of EGFR tyrosine kinase inhibitors and the absence of validated predictive factors, these drugs should not be administered as single agents to patients with high-grade gliomas. The complexity of signaling pathways regulated by tyrosine kinases and the ignorance, until recently, of the relevance of negative feedback loops altered by targeted treatments, suggests requirement of a more in-depth molecular analysis (46–48). Moreover, well-designed prospective trials using specific criteria to evaluate tyrosine kinase inhibitor activity and standardized methods to evaluate tissue biomarkers are required to confirm molecular predictors of the clinical activity of EGFR tyrosine kinase inhibitors in patients with high-grade gliomas.

References


Epidermal Growth Factor Receptor Inhibitors in Neuro-oncology: Hopes and Disappointments

Alba A. Brandes, Enrico Franceschi, Alicia Tosoni, et al.


Updated version: Access the most recent version of this article at: [http://clincancerres.aacrjournals.org/content/14/4/957](http://clincancerres.aacrjournals.org/content/14/4/957)

Cited articles: This article cites 47 articles, 29 of which you can access for free at: [http://clincancerres.aacrjournals.org/content/14/4/957.full.html#ref-list-1](http://clincancerres.aacrjournals.org/content/14/4/957.full.html#ref-list-1)

Citing articles: This article has been cited by 16 HighWire-hosted articles. Access the articles at: [http://clincancerres.aacrjournals.org/content/14/4/957.full.html#related-urls](http://clincancerres.aacrjournals.org/content/14/4/957.full.html#related-urls)

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

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

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