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 tumor, 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 receptor 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 anti-tumor 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 gefitinib activity: few cases of response, and some cases of
disease stabilization, have been observed in patients with
wild-type EGFR, and rarely have EGFR mutations been
described in patients with treatment-induced disease stabiliza-
tion, which probably contributes substantially to the survival
benefit of tyrosine kinase inhibitors (25). However, in mali-
gnant 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 EGFR extracellular domain have been
identified, and a potential effect on tyrosine kinase inhibitors
response in glioblastoma has been hypothesized (15). Other
determinants for EGFR tyrosine kinase inhibitor sensitivity,
such as 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 EGFR gene copy number,
defined by fluorescent in situ hybridization (34–37). Akt, a
serine/threonine kinase that acts downstream of 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 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).
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 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
with other agents considered inactive. In this trial, EGFR pro-
tein expression and gene status, and EGFRvIII protein expres-
sion were not significantly correlated with progression-free
survival at 6 months and survival, and gefitinib as a single agent
is considered inactive in this setting.

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, 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 EGFR
expression and low phospho-Akt than those with low EGFR
expression and high phospho-Akt levels. The authors found no
correlation between EGFRvIII expression and response. In their
study on 49 glioblastoma patients treated with erlotinib or
gefitinib, Mellinghoff et al. (28) found that EGFRvIII and 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 Treat-
ment 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 con-
fi rmed the disappointing results with the 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 erlo-
tinib, and no correlations could be shown for treatment effect
and EGFR expression or amplification and EGFRvIII expres-
sion. Moreover, EGFRvIII expression correlated with a decreased
overall survival and progression-free survival in all patients, all
patients with EGFRvIII, and PTEN protein coexpression treated
with erlotinib rapidly progressed. These findings are in clear
contradiction of the previous retrospective analyses and under-
line 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 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patients in study | Rich (26) | Haas-Kogan (30) | Mellinghoff (28) | Franceschi (27) | van den Bent (44) |
| N = 53 | N = 52 | N = 49 | N = 28 | N = 110 |
| EGFR expression | 79% | 27% | NA | 38% | 58% |
| Tumors analyzed, n | 53 | 41 | NA | 21 | 99 |
| Predictive value | No | Yes | NA | No | No |
| EGFR amplification | 36% | 26% | 48% | 47% | 46% |
| Tumors analyzed, n | 42 | 41 | 25 | 19 | 91 |
| Predictive value | No | Yes | No | No | No |
| EGFRvIII expression | 49% | 5% | 46% | NA | 21% |
| Tumors analyzed, n | 53 | 41 | 25 | 19 | 91 |
| Predictive value | No | No | Yes | No | No |
| P-Akt expression | NA | 55% | NA | 48% | 19% |
| Tumors analyzed, n | 41 | NA | 21 | 21 | |
| Predictive value | Yes | NA | Yes | No | No |
| PTEN expression | NA | NA | NA | NA | |
| Tumors analyzed, n | 26 | NA | NA | NA | |
| Predictive value | Yes | NA | NA | NA | |

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 L844 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 L844) 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 confirm molecular predictors of the clinical activity of EGFR tyrosine kinase inhibitors in patients with high-grade gliomas.

References


41. Panigrahi AR, Pinder SE, Chan SY, Paish EC, Robertson JF, Ellis IO. The role of PTEN and its signalling pathways, including AKT, in breast cancer; an assessment of relationships with other prognostic factors and with outcome. J Pathol 2004;204:93–100.


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