

REVIEW ARTICLE

# Predictive and Prognostic Markers in Neuro-Oncology

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## Abstract

Over the past few years molecular assays have been introduced to aid in typing and grading of gliomas. This is the result of improved understanding of these tumors at the molecular level. In particular, the presence or absence of combined 1p/19q loss in oligodendroglial tumors, epidermal growth factor receptor amplification, epidermal growth factor receptor vIII mutations in grade III tumors and glioblastoma multiforme, and *MGMT* promoter gene methylation in glioblastoma multiforme are now being used to tailor treatment decisions in patients. However, the application of these tests is far from straightforward, and certain standards are required before any test can be introduced in the daily management of patients. Some of these requirements concern inter- and intratest variability, including whether a test gives the same results if repeated in the same or in another laboratory or when different methodologies are used (e.g. loss of heterozygosity vs fluorescence in situ hybridization and a polymerase chain reaction-based test vs immunohistochemistry). The sensitivity and specificity of a test (or negative and positive predictive value) indicate the likelihood that the test results are positive if the disease is present and the likelihood that the disease is present if the test results are positive. Studies on these test characteristics usually require the presence of a gold standard to which new tests should be compared. Last but not least there is the question of what added value the test has; this criterion determines the clinical usefulness of the assay and why some recently introduced molecular assays need to be scrutinized.

**Key Words:** 1p, 19q, Epidermal growth factor receptor, Epidermal growth factor receptor vIII mutant, *MGMT*, Predictive significance, Prognostic significance.

## INTRODUCTION

Any marker of a disease can help identify the disease or help establish the prognosis of an individual patient. With respect to prognostic tests it is important to make a distinction between markers of prognostic and predictive significance. Prognostic significance refers to the presence of a relation between the overall outcome of the patient and the test result, regardless of treatment. In contrast, predictive significance implies that a test predicts the outcome after a specific

treatment: the relationship between overall outcome and the test result exists only after a specific treatment. For instance, the presence of a high lactate dehydrogenase count confers a poor prognosis to patients with lymphoma: elevated lactate dehydrogenase has prognostic significance. Treatment with the anti-CD20 antibody rituximab improves the outcome only in patients with B cell non-Hodgkin's lymphoma expressing the CD20 receptor. Therefore, for treatment with rituximab, an assay for CD20 has predictive significance. An ideal predictive marker should allow selection of patients for a certain therapy, which would be useless in the absence of that test result.

Regardless of the predictive test used to select patients for specific treatments, the assay needs to be reliable in terms of intra- and intertest variability and sensitive and predictive for outcome as to result of that treatment. Here, sensitivity may be more important than specificity: a test can be very specific, but if it misses most of the cases it becomes useless in daily practice. Admittedly, any test that has too many false-positive results fails its purpose, but in general most clinicians would rather treat some patients in whom the treatment will fail than withhold an active treatment in patients on the basis of an insensitive test (i.e. "better safe than sorry").

Currently, patients with gliomas are treated with a varying combination of surgery, radiotherapy (RT), and chemotherapy. In addition, a number of novel targeted agents that will only work if their specific (molecular) target is present are entering the clinical arena. In this article we review the predictive and prognostic properties of 3 molecular markers that are currently used to select specific treatments for patients.

## THE USEFULNESS OF 1P/19Q DETERMINATION FOR THE DAILY MANAGEMENT OF PATIENTS

The classification and grading of glial tumors is based primarily on histologic criteria, usually the World Health Organization criteria. The most common glial tumors are astrocytic tumors, oligodendroglioma, and mixed oligoastrocytoma. Although the clinical value of using the World Health Organization-based diagnoses was proven in various studies, it was also shown that a considerable interobserver disagreement exists with respect to tumor typing and grading, particularly in the diagnosis of grade II and grade III tumors (1–3). In the past this disagreement had little therapeutic implication because the treatment options for these tumors were more or less the same (i.e. surgery, RT, and chemotherapy). However, this situation changed with

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the demonstration of the sensitivity of anaplastic oligodendrogliomas and oligoastrocytomas to PCV (i.e. procarbazine, lomustine [CCNU], and vincristine) chemotherapy, with two-thirds of patients responding to the treatment (4). Both before and after treatment, an increase in the percentage of glial tumors that were considered to be of oligodendroglial lineage was noted, together with a loosening of the histologic criteria for oligodendroglioma (5, 6).

At the same time, genetic analysis of oligodendroglioma revealed that the combination of loss of the long arm of chromosome 1 and the short arm of chromosome 19 caused by an unbalanced translocation of 19p to 1q is the most frequent genetic event in oligodendroglioma (7–9). The first indication of clinical relevance of 1p/19q loss was the finding that low-grade oligoastrocytomas had either TP53 mutations or combined 1p/19q loss but not both (10). This finding suggested that true mixed low-grade oligoastrocytomas do not exist, but at the molecular level these tumors are either oligodendrogliomas (1p/19q loss) or astrocytomas (TP53 mutation). These observations also did not have therapeutic implications, but the situation changed when correlative studies showed a strong association with 1p/19q loss and response to PCV chemotherapy, with virtually all patients with a 1p/19q co-deletion responding to the treatment (11, 12). In contrast, the response rate for patients with no loss or 1p loss only was 25% or less. There is now an abundance of data confirming the relation between 1p/19q status and response to chemotherapy, not only to PCV but also to temozolomide (TMZ), which is not limited to anaplastic tumors only but applies to low-grade oligodendrogliomas as well (13–16).

Other studies showed that combined 1p/19q loss is usually found in classic oligodendroglioma and is much less frequent in atypical oligodendroglioma or oligoastrocytoma (17). Because several studies have suggested a stronger correlation between response to chemotherapy and 1p/19q status compared with histology, the determination of 1p/19q loss appears to be a better way to identify chemosensitive oligodendroglioma (11, 12). An important consequence of these findings was the return to more stringent criteria for the histologic diagnosis of oligodendroglioma and testing for the 1p/19q status to establish the diagnosis of “chemotherapy-

sensitive oligodendroglioma.” Currently, treatment decisions in oligodendrogliomas are increasingly based on the absence or presence of 1p/19q loss. Despite the absence of a formal trial that justifies this change, an increasing number of clinicians are now inclined to use chemotherapy for tumors with combined 1p/19q loss.

Other retrospective studies suggested a better prognosis in patients with 1p/19q loss tumors (with longer survival after RT) and a longer symptomatic period (usually with seizures) before the start of treatment (15, 18–20). The link between overall prognosis and 1p/19q status was recently firmly established with 2 prospective randomized clinical studies on adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors. These trials have shed new light on the clinical significance of 1p/19q loss and its use for treatment decisions. One trial on 369 patients was conducted by the European Organization for Research and Treatment of Cancer (EORTC) and investigated standard adjuvant PCV chemotherapy after RT in anaplastic oligodendroglioma and anaplastic oligoastrocytoma (21). The other study of 299 patients performed by the Radiation Therapy Oncology Group (RTOG) investigated 4 cycles of intensified PCV given before RT (22). Both studies showed that (neo)-adjuvant PCV does not improve overall survival but does increase progression-free survival. Most likely, this is explained by the fact that in both studies the majority of patients randomly assigned to the RT-only arm received PCV at progression, which was intended in both studies. Thus, both studies actually compared early versus delayed PCV chemotherapy. In both studies combined 1p/19q loss had a major impact on survival: survival without combined 1p/19q loss was 2 to 3 years but was more than 5 to 6 years in patients with 1p/19q loss (Table 1). However, in both studies even the patients with combined 1p/19q loss did not benefit from early PCV chemotherapy. Therefore, the absence or presence of 1p/19q loss does not give guidance in the choice for early versus delayed PCV chemotherapy, despite the proven chemosensitivity of 1p/19q co-deleted tumors.

More data can be obtained from these studies. First, in both studies progression-free survival after RT only (the control arm) was far better in the patients with the 1p/19q co-deletion (Table 2). In the EORTC study, progression-free

**TABLE 1.** Median Survival and 5-Year Overall Survival According to Combined 1p/19q Loss Status in EORTC 26951 and RTOG 9402 With (Neo)Adjuvant PCV Chemotherapy in Anaplastic Oligodendroglial Tumors

Chromosomal Loss	Overall Survival			
	Median (months)		5-Year (%)	
	RT/PCV	RT	RT/PCV	RT
Combined 1p/19q loss				
EORTC	NR	NR	74 [57–85]	75 [55–87]
RTOG	NR	NR (5.4, NA)	72 [54–83]	66 [50–78]
No combined 1p/19q loss				
EORTC	25.2 [18.9–42.6]	21.4 [17.6–30.0]	34 [24–43]	28 [19–36]
RTOG	2.7 [2.0–5.5]	2.8 [1.9–4.4]	37 [24–50]	31 [19–45]

95% confidence interval is shown in brackets.

EORTC, European Organization for Research and Treatment of Cancer; RTOG, Radiation Therapy Oncology Group; RT, radiation therapy; PCV, procarbazine, lomustine (CCNU), and vincristine; NR, not reached; NA, not applicable.

**TABLE 2.** Median and 5-Year Progression-Free Survival and Overall Survival in Months According to Combined 1p/19q Loss Status in EORTC 26951 and RTOG 9402 on (Neo)Adjuvant PCV Chemotherapy in Anaplastic Oligodendroglial Tumors

Chromosomal Loss	Progression-Free Survival			
	Median (years)		5-Year (%)	
	RT/PCV	RT	RT/PCV	RT
Combined 1p/19q loss				
EORTC	NR	5.2 [3.6, NR]	70 [52–82]	50 [32–66]
RTOG	NR	2.6 [1.5–4.1]	57 [40, 71]	27 [15–41]
No combined 1p/19q loss				
EORTC	1.3 [1.0–1.9]	0.7 [0.6–1.2]	27 [19–36]	14 [8–21]
RTOG	1.4 [0.9–2.6]	1.0 [0.6–1.9]	20 [11–33]	8 [2–18]

95% confidence interval is shown in brackets.

EORTC, European Organization for Research and Treatment of Cancer; RTOG, Radiation Therapy Oncology Group; RT, radiation therapy; PCV, procarbazine, lomustine (CCNU), and vincristine; NR, not reached.

survival after RT was 62 months for patients with co-deleted tumors, but only 9 months for the patients without 1p/19q loss. Thus, the increased responsiveness to treatment of 1p/19q co-deleted tumors is not limited to chemotherapy. Second, in the RTOG study the progression-free survival in the non-1p/19q co-deleted group after no statistically superior progression-free survival was observed after neoadjuvant dose-intensified PCV followed by RT compared with RT only (although a trend is visible). This result suggests that in this group the addition of dose-intensified PCV did little to improve the outcome with RT only.

Why is 1p/19q loss only prognostic and not predictive? Oligodendrogliomas with 1p/19q loss are sensitive to both RT and PCV or TMZ chemotherapy. These treatments are active, however, in most glial tumors regardless of 1p/19q status, albeit much more so in patients with 1p/19q co-deleted tumors. Most patients with a glial tumor—regardless of histology and grade—will receive these treatments sometime in the course of their disease. For specific treatment decisions the determination of 1p/19q is of limited value, the exception being patients in whom chemotherapy is considered: this approach is unlikely to yield long-lasting responses in patients without 1p/19q co-deleted tumors. For these patients, RT alone is as effective as neoadjuvant PCV followed by RT. The most important information learned from the determination of 1p/19q loss is the prognostic information this test yields. Whether classical histology still plays a role in the determination of the prognosis is controversial; 1 article suggested that 1p/19q status affected outcome in pure oligodendroglial tumors but not in oligoastrocytomas (17). Further prospective studies are required to answer this question.

### THE *MGMT* GENE AND ALKYLTRANSFERASE EXPRESSION IN CHEMOIRRADIATED GLIOBLASTOMA MULTIFORME

In a study conducted by the EORTC and the National Cancer Institute of Canada (NCI-C) Brain Group, chemoradiation with TMZ was shown to provide a superior outcome in patients with glioblastoma multiforme (GBM) compared with treatment with RT only (23). That trial was,

in fact, the first to show a clinically meaningful increase in survival with the addition of chemotherapy to RT. With this well-tolerated treatment, 2-year survival increased from 10% to 26%, and combined chemoradiation with TMZ is now considered the standard of care for patients with GBM. Perhaps even more important was the result of a companion translational research study suggesting that patients with a methylated promoter gene of the O<sup>6</sup>-methylguanine-DNA methyltransferase (*MGMT*) gene, in particular, benefited from combined chemoradiation.

*MGMT* encodes for the nuclear repair enzyme alkyltransferase, which removes alkylating adducts from the O<sup>6</sup> position of guanine and to a lesser extent the O<sup>6</sup> position of thymine (24). Physiologically, this enzyme protects cells against potentially mutagenic DNA lesions and prevents tumor formation after exposure to alkylating and methylating substances. During this process the alkyltransferase enzyme is irreversibly inactivated, and the cell depends on new enzyme synthesis to maintain its protection against methylating and alkylating agents.

This DNA repair enzyme plays a role in maintaining the integrity of the DNA in normal cells but also protects tumor cells against alkylating (e.g. BCNU [*N,N*′-bis(2-chloroethyl)-*N*-nitrosourea]) and methylating (e.g. procarbazine and TMZ) chemotherapeutic agents. These agents add methyl groups at various positions of the DNA in 7% of cases to the O<sup>6</sup> position of guanine. In particular, the presence of adducts at the O<sup>6</sup> position of guanine is mutagenic; if present, thymine will not be paired to adenosine but will be mispaired to O<sup>6</sup> guanine. Subsequent DNA repair of this lesion by alkyltransferase yields a G:T mismatch, which will be recognized by mismatch repair enzymes MSH2 and MSH6 and further repaired. However, if a mismatch O<sup>6</sup>-MG is present, the mismatch with thymine will repeat itself and after several futile cycles of attempted DNA repair the cell will undergo apoptosis.

The *MGMT* gene product is the primary cell mechanism of resistance against alkylating and methylating agents (although it requires intact mismatch repair). In tumors, the *MGMT* promoter gene is frequently methylated at CpG islands and thus silenced; this silencing is often associated with the methylation of a number of other cancer-associated

genes. Tumor cells that lack *MGMT* expression are much more sensitive to alkylating and methylating agents. Thus, theoretically *MGMT* promoter methylation and reduced alkyltransferase expression will make tumor cells susceptible to alkylating and methylating agents, potentially making an assay of the activity of the alkyltransferase DNA repair mechanism a predictive test for outcome to alkylating and methylating agents.

Early studies showed that patients with GBM who were treated with RT and BCNU/ACNU [1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride] had a better outcome in the absence of alkyltransferase or in the presence of a methylated *MGMT* promoter gene (25–27). Because all patients received nitrosoureas and RT, it was impossible to establish whether *MGMT* expression was a predictive factor for response to treatment or merely a prognostic factor. In the EORTC study patients were randomly assigned to either RT only or to RT with TMZ; therefore, this study showed that the improved outcome in patients with *MGMT* promoter methylation depended on the type of treatment (i.e. the addition of TMZ) (28). In the EORTC study about half of the investigated GBMs had a methylated *MGMT* gene promoter. Indeed, survival in the RT plus TMZ-treated patients was better in the presence of a methylated *MGMT* promoter gene. In this group the addition of TMZ to RT increased 2-year survival from 23% with RT only to 46% for patients treated with RT plus TMZ (Table 3). Some improvement of outcome was also observed in the patients randomly assigned to RT only, which was attributed by the authors to the administration of TMZ at the time of progression. Indeed, if only progression-free survival was taken into consideration, only the patients with a *MGMT* promoter gene methylated tumor treated with RT and TMZ had a better outcome.

Since the publication of that study a large number of articles on this topic have been published. Unfortunately, no other series reported on a randomized study, and most reported on retrospective series of patients. A preclinical study using both in vivo and in vitro models confirms the pivotal role of *MGMT* promoter methylation in the outcome after combined chemoradiation (29). In that study, the combination of RT and TMZ proved synergistic in the presence of a methylated *MGMT* promoter, but only if TMZ was administered during RT. No benefit was observed with the addition of TMZ to RT in cell lines and xenografts without *MGMT* promoter methylation. The administration of

TMZ after RT also did not affect outcome. In retrospective studies on alkyltransferase expression in grade III tumors and GBM a relation between alkyltransferase expression and outcome was found only in the patients treated with adjuvant chemotherapy (30, 31). Studies on upfront TMZ chemotherapy in GBM and in low-grade glioma also showed a relation between response and *MGMT* promoter methylation or alkyltransferase expression (32, 33). Other recent studies confirmed the strong relationship between outcome after RT and alkylating agents and *MGMT* status (34).

Assays for *MGMT* activity seem predictive for the outcome of TMZ-based therapy, which raises the question of whether tests for *MGMT* expression should now be used to select patients with GBM for combined chemoradiation with TMZ. And if so, which test should be used? This answer is less straightforward than it seems though. In the EORTC study, in only half of the patients was enough material present to test for *MGMT* promoter status, and in only 206 of these 307 patients did the test yield a result. Moreover, interaction tests between the magnitude of the treatment effect and *MGMT* status remained negative because of the limited sample size (the study was not designed to answer this question). Therefore, a confirmation in a larger independent and preferably prospective data set remains crucial.

But more issues exist. It is not clear which test should be used. Many investigators used immunohistochemistry (IHC) with antibodies against the alkyltransferase protein, whereas others have relied on quantitative polymerase chain reaction techniques or on promoter methylation assays. The latter has the advantage that even if alkyltransferase is absent, the gene can still be upregulated and expressed if the promoter is functional. This is not the case once the promoter gene is methylated. There are, however, reports on the lack of correlation between IHC for the *MGMT* protein and *MGMT* promoter gene hypermethylation (30, 35). In addition, inconsistent results between assays on frozen samples and paraffin samples have been mentioned (33). Data on intra- and interobserver variation of the various tests are not available. For use in other tumor types (low-grade tumors and oligodendrogliomas) even fewer data are available. Some studies suggested that *MGMT* promoter methylation occurs in up to 80% of these tumors. Studies on the outcome after treatment with TMZ have yielded conflicting results, with some studies showing a relation between *MGMT* status and outcome but others failing to do so (14). This result could be due in part

**TABLE 3.** Survival in EORTC Study 26981 With Combined Chemoradiation in Relation to *MGMT* Promoter Gene Methylation Status

Survival	Unmethylated <i>MGMT</i> Promoter (n = 114)		Methylated <i>MGMT</i> Promoter (n = 92)	
	RT	RT + TMZ	RT	RT + TMZ
Median (months)	11.8 [9.7–14.1]	12.7 [11.6–14.4]	15.3 [13.0–20.9]	21.7 [17.4–30.4]
2 year (%)	<2	13.8 [4.8–22.7]	22.7 [10.3–35.1]	46.0 [31.2–60.8]

95% confidence interval in brackets.  
RT, radiotherapy; TMZ, temozolomide.

**TABLE 4.** Outcome of Treatment With the Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitors Erlotinib and Gefitinib in Glioblastoma

Agent	Author	Number of Patients	Response Rate (%)	MTP (weeks)	6-Month PFS (%)
Gefitinib	Rich et al (56)	53		8.1	13.2
	Lieberman (57)	38	13	8	9
Erlotinib	Vogelbaum et al (55)	31	6	NS	25.8
	Raizer et al (54)	31	0	12	0
	Cloughesy et al (58)	48	8	NS	17

MTP, median time to progression; 6 month PFS, 6-month progression-free survival; NS, not stated.

to sample size issues, but it also suggests that other factors are of relevance.

To conclude, both preclinical and clinical data suggest that an MGMT assay is likely to be of predictive significance for the outcome of GBM to chemoradiation with TMZ. However, despite the strong biologic rationale, because of the absence of a confirmation in an independent data set and the absence of a validated test assay, it is too early to rely on MGMT testing for clinical decision-making. Currently, MGMT assays should be used for scientific purposes only, and institutions in which these tests are applied are encouraged to develop a program to establish the reliability of these tests.

## EPIDERMAL GROWTH FACTOR RECEPTOR AND EPIDERMAL GROWTH FACTOR RECEPTOR-INHIBITING AGENTS

The epidermal growth factor receptor (EGFR) is a 170-kDa receptor tyrosine kinase located on chromosome 7p11.2. EGFR is composed of an extracellular domain, a transmembrane lipophilic segment, and an intracellular domain that has protein kinase activity. The ligands for this receptor are epidermal growth factor and transforming growth factor- $\alpha$ . After binding with its ligand the EGFR dimerizes, and the tyrosine kinase receptor is activated, which triggers a downstream cascade involving the RAS/

RAF/mitogen-activated protein kinase (MAPK) and, in particular, the phosphatidylinositol 3-kinase/AKT pathway. *EGFR* gene amplification is a common event in glioblastoma, occurring in 40% to 50% of cases. Amplified *EGFR* is manifested cytogenetically as double minutes, each of which contains multiple copies of the amplification repeat unit. If amplified, *EGFR* appears to be the sole amplification target, although additional 7p11.2 genes can be co-amplified and overexpressed (36). In the absence of *EGFR* amplification, *EGFR* overexpression is rare (37). Mutant forms of *EGFR* are expressed in approximately 40% of GBMs with an amplified *EGFR* gene, most frequently the EGFRvIII mutant (38). This mutant lacks the extracellular ligand-binding domain as a result of the deletion of exons 2 to 7, probably through alternative splicing. The result is an in-frame deletion of 801 base pairs of the coding sequence of the extracellular domain, which causes constitutive phosphorylation and thus activation of the receptor, leading to constant downstream signaling. Although EGFRvIII may be the predominant amplification, wild-type EGFR overexpression is more extensive in most tumors. If EGFRvIII mutants are present, they are usually manifested as focal areas of positive IHC staining. Introduction of EGFRvIII in glioma cells mediates a growth advantage and confers resistance to radiation therapy and to chemotherapy (39, 40).

*EGFR* amplification is seen in 40% to 50% of GBMs and in up to only 10% of anaplastic astrocytomas (AA), but not in low-grade astrocytomas. In patients with GBM the presence of *EGFR* expression, *EGFR* amplification, or EGFRvIII expression is not associated with a poor prognosis (41–44). In AAs and other grade III tumors, however, the presence of *EGFR* amplification or EGFRvIII mutations does have a poor prognostic significance (41, 45, 46). This finding suggests that at the molecular level these tumors are in fact GBMs, an assumption that is supported by a study on AAs in which half of the EGFRvIII-positive tumors were reviewed and reclassified as GBMs (46). Clinical studies have shown that the interobserver variation in the diagnosis of AAs is larger than that in GBMs; perhaps the conclusion here is that tests for *EGFR* amplification may help to distinguish between AAs and GBMs (1).

EGFR is abundantly expressed in non-small cell lung cancer and therefore trials with EGFR tyrosine kinase inhibitors erlotinib and gefitinib were initiated in this disease. These trials showed various response rates, and the

**TABLE 5.** Presence or Absence of a Relationship Between Molecular Findings and Outcome of Treatment With EGFR Tyrosine Kinase Inhibitors

Study	EGFR	pEGFR	<i>EGFR</i> amplification	EGFRvIII	pAkt	<i>PTEN</i>
Rich et al (56)	–			–	ND	ND
Vogelbaum et al (55)			–		ND	ND
Haas-Kogan et al (59)	+		+	–	+	ND
Mellinghoff et al (51)	ND		–	+	ND	+
Lassman et al (50)		–	–		–	ND
Cloughesy et al (58)	±	ND	–	–	ND	–

EGFR, epidermal growth factor receptor; p, phosphorylated; ND, not done; +, positive relation between outcome and a parameter; ±, borderline relation between outcome and parameter; –, no relation between outcome and a parameter.

responses were subsequently found to be related to the presence of mutations in exons 19 to 21 of the *EGFR* gene. These exons encode for the ATP binding pocket of the tyrosine kinase domain, but these domains virtually never show mutations in GBM (47–51). In vitro models of non-small lung cancer showed that the presence of these mutations made cell lines sensitive to erlotinib or gefitinib. These findings are in contrast with research on GBM cell lines, in which gefitinib inhibited cell cycle progression, growth, and invasion of cells expressing wild-type *EGFR*, but not in cells expressing the mutant *EGFRvIII* (52). Perhaps this result is due to differential regulation of Akt activity and other functionally redundant promitotic signaling pathways in cells expressing mutant *EGFRvIII*.

Because of the frequent *EGFR* amplification in GBM, the activity of the *EGFR* tyrosine kinase inhibitors gefitinib and erlotinib on recurrent GBM was investigated in several trials. In particular, one phase I trial on erlotinib with or without TMZ showed promising results. Of 49 patients evaluated 8 responses were observed; 7 patients had a GBM and 6 of the responding patients had been treated with erlotinib only (53). A pivotal article on the molecular determinants of response to *EGFR* tyrosine kinase inhibitors suggested that tumors having both *EGFRvIII* mutations and expression of *PTEN* are likely to respond to these agents (51). Whereas 6 of 7 tumors with coexpression of *EGFRvIII* and *PTEN* responded, only 2 of 19 tumors without this coexpression were responsive (51). This study also presented an independent data set from another study in which this relationship was confirmed. Moreover, in an in vivo model the induction of coexpression of *EGFRvIII* and *PTEN* made glioma cell lines sensitive to erlotinib. In these cell lines it appeared that Akt-independent branches of the *PTEN* pathway contributed to the effects of *PTEN* on the sensitivity of *EGFR* inhibitors. If true, these findings would have major implications for the further development of the treatment of high-grade gliomas.

The question is whether these data are solid enough to take treatment decisions. The other available clinical trials showed various results (Table 4), with 2 trials showing limited or no activity of *EGFR* tyrosine kinase inhibitors (54–56). The results of most of these trials have not yet been fully reported, and the molecular studies aiming to identify GBMs responsive to *EGFR* tyrosine kinase I have also failed to show consistent results (Table 5). This is also the case for the individual studies that were used in the pivotal article mentioned above (37, 58). In 1 of those studies the response to erlotinib was associated with high levels of *EGFR* expression and low levels of pAkt (37). IHC for pAkt was strongly associated with response; none of the patients with positive IHC for pAkt responded. Time to progression was associated with pAkt but not with *EGFR* expression. Other studies failed to find a relation between response and *EGFR* expression (IHC and/or at the DNA level) or *EGFRvIII* IHC (55, 56).

There are some more remarkable discrepancies between the results of these molecular studies. One study noted *EGFRvIII* expression in 49% of GBMs and another in only 2 of 29 GBMs (56, 59). In 1 study *EGFRvIII* was

expressed in 15 of 27 tumors without *EGFR* amplification; however, most studies found *EGFRvIII* mutants only in tumors with *EGFR* amplification or with gain of chromosome 7 (51, 56). One study found only 7% of 268 GBMs and another found 38% of 29 GBMs negative for pAkt (37, 60). Are these differences true differences or artificial differences and due to limited test reliability or to differences in methodology and inherent to IHC? Moreover, a control group without *EGFR* tyrosine kinase inhibitors was not a part of any of these studies, implying that any observed outcome would have prognostic significance. For example, in 1 study activated substrates of the MAPK and Akt pathways were associated with a worse prognosis (59).

What conclusions can be drawn from these data? At this point it is fair to conclude that the full reports on most of the trials on tyrosine kinase inhibitors in GBM are still pending, and the outcome of the molecular analyses so far are contradictory. Yet, *EGFR* tyrosine kinase inhibitors are used for treatment of recurrent GBM, despite the meager preliminary results in the clinical trials. Why has the activity of these agents so far been disappointing, even in patients with *EGFR* amplification? One study on erlotinib- and gefitinib-treated GBM observed no clear changes in *EGFR* phosphorylation or downstream signalling (on pErk and pAkt) compared with control tissue (50). The *EGFR* mutations in exons 18 to 21 related to response in non-small cell lung cancer appeared to be virtually absent in GBM, and cell lines with *EGFRvIII* mutations failed to respond to *EGFR* tyrosine kinase inhibition. A mutation or overexpression in the *EGFR* gene per se is not enough to respond to *EGFR* tyrosine kinase inhibitors.

In summary, tests for *EGFR* amplification and *EGFRvIII* mutations do not have prognostic significance in GBM but may have prognostic significance in grade III tumors. Perhaps this result is due to the known difficulties in the histologic diagnosis of grade III tumors. Therefore, *EGFR* amplification may be used to identify GBM among tumors with histologic features resembling grade III tumors. Despite the clear biologic rationale for studies with *EGFR* tyrosine kinase inhibitors, their role is still unclear in GBM because of contradictory results in clinical trials and because the predictive value of some of the identified molecular markers remains to be clarified.

## CONCLUSION

Combined 1p/19q loss is mainly a prognostic factor, but it can be used in individual patients to assess whether chemotherapy is a therapeutic option. Most likely, *MGMT* promoter gene methylation is predictive for outcome after combined chemoradiation in patients with GBM, but its clinical use still requires further confirmatory studies. *EGFR* amplification does not have prognostic significance in GBM, but may have prognostic significance in grade III gliomas. The presence of *EGFR* amplification should be considered for inclusion in the criteria for the diagnosis of GBM. *EGFR*-inhibiting strategies have so far not been very successful, and it is unclear whether predictive markers exist that identify responding tumors.

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## REFERENCES

- Scott CB, Nelson JS, Farman NC, et al. Central pathology review in clinical trials for patients with malignant glioma. *Cancer* 1995;76:307–13
- Smith JS, Alderete B, Minn Y, et al. Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. *Oncogene* 1999;18:4144–52
- van den Bent MJ, Afra D, De Witte O, et al. Long term results of EORTC study 22845: A randomized trial on the efficacy of early versus delayed radiation therapy of low-grade astrocytoma and oligodendroglioma in the adult. *Lancet* 2005;366:985–90
- Cairncross G, Macdonald D, Ludwin S, et al. Chemotherapy for anaplastic oligodendroglioma. *J Clin Oncol* 1994;12:2013–21
- Coons SW, Johnson PC, Scheithauer BW, et al. Improving diagnostic accuracy and interobserver concordance in the classification and grading of primary gliomas. *Cancer* 1997;79:1381–91
- Burger PC. What is an oligodendroglioma? *Brain Pathol* 2002;12:257–59
- Reifenberger J, Reifenberger G, Liu L, et al. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. *Am J Pathol* 1994;145:1175–90
- Jenkins RB, Blair H, Ballman KV, et al. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer Res* 2006;66:9852–61
- Griffin CA, Burger P, Morsberger L, et al. Identification of der(1;19)(q10;p10) in five oligodendroglomas suggests mechanism of concurrent 1p and 19q loss. *J Neuropathol Exp Neurol* 2006;65:988–94
- Maintz D, Fiedler K, Koopmann J, et al. Molecular genetic evidence for subtypes of oligoastrocytomas. *J Neuropathol Exp Neurol* 1997;56:1098–1104
- Cairncross JG, Ueki K, Zlatescu MC, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendroglomas. *J Natl Cancer Inst* 1998;90:1473–79
- van den Bent MJ, Looijenga LHJ, Langenberg K, et al. Chromosomal anomalies in oligodendroglial tumors are correlated with clinical features. *Cancer* 2003;97:1276–84
- Kouwenhoven MC, Kros JM, French PJ, et al. 1p/19q loss within oligodendroglioma is predictive for response to first line temozolomide but not to salvage treatment. *Eur J Cancer* 2006;42:2499–503
- Brandes AA, Tosoni A, Cavallo G, et al. Correlations between O6-methylguanine DNA methyltransferase promoter methylation status, 1p and 19q deletions, and response to temozolomide in anaplastic and recurrent oligodendroglioma: A prospective GICNO study. *J Clin Oncol* 2006;24:4746–53
- Biemond-ter Stege E, Kros JM, de Bruin HG, et al. Treatment of low grade oligodendroglial tumors with PCV chemotherapy. *Cancer* 2005;103:802–809
- Hoang-Xuan K, Capelle L, Kujas M, et al. Temozolomide as initial treatment for adults with low-grade oligodendroglomas or oligoastrocytomas and correlation with chromosome 1p deletions. *J Clin Oncol* 2004;22:3133–38
- McDonald JM, See SJ, Tremont IW, et al. The prognostic impact of histology and 1p/19q status in anaplastic oligodendroglial tumors. *Cancer* 2005;104:1468–77
- Walker C, du Plessis DG, Joyce KA, et al. Molecular pathology and clinical characteristics of oligodendroglial neoplasms. *Ann Neurol* 2005;57:855–65
- Smith JS, Perry A, Borell TJ, et al. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendroglomas, astrocytoma, and mixed oligoastrocytomas. *J Clin Oncol* 2000;18:636–45
- Bauman GS, Ino Y, Yeki K, et al. Allelic loss of chromosome 1p and radiotherapy plus chemotherapy in patients with oligodendroglioma. *Int J Radiat Oncol Biol Phys* 2000;48:825–30
- van den Bent MJ, Carpentier AF, Brandes AA, et al. Adjuvant PCV improves progression free survival but not overall survival in newly diagnosed anaplastic oligodendroglomas and oligoastrocytomas: A randomized EORTC phase III trial. *J Clin Oncol* 2006;24:2715–22
- Cairncross JG, Berkey B, Shaw E, et al. Phase III trial of chemotherapy plus radiotherapy (RT) versus RT alone for pure and mixed anaplastic oligodendroglioma (RTOG 9402): An intergroup trial by the RTOG, NCCTG, SWOG, NCI CTG and ECOG. *J Clin Oncol* 2006;24:2707–14
- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96
- Gerson SL. MGMT: Its role in cancer aetiology and cancer therapeutics. *Nat Rev* 2004;4:296–307
- Jaecckle KA, Eyre HJ, Townsend JJ, et al. Correlation of tumor O6 methylguanine-DNA methyltransferase levels with survival of malignant astrocytoma patients treated with bis-chloroethylnitrosourea: A Southwest Oncology Group Study. *J Clin Oncol* 1998;16:3310–15
- Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* 2000;343:1350–54
- Tanaka S, Kobayashi I, Utsuki S, et al. O6-methylguanine-DNA methyltransferase gene expression in gliomas by means of real-time quantitative RT-PCR and clinical response to nitrosoureas. *Int J Cancer* 2003;103:67–72
- Hegi ME, Diserens A-C, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352:997–1003
- Chakravarti A, Erkinen MG, Nestler U, et al. Temozolomide-mediated radiation enhancement in glioblastoma: A report on underlying mechanisms. *Clin Cancer Res* 2006;12:4738–46
- Brell M, Tortosa A, Verger E, et al. Prognostic significance of O6-methylguanine-DNA methyltransferase determined by promoter hypermethylation and immunohistochemical expression in anaplastic gliomas. *Clin Cancer Res* 2005;11:5167–74
- Criniere E, Kaloshi G, Laigle-Donadey F, et al. MGMT prognostic impact on glioblastoma is dependent on therapeutic modalities. *J Neurooncol* 2007;83:173–79
- Friedman HS, McLendon RE, Kerby T, et al. DNA mismatch repair and O6-alkylguanine-DNA alkyltransferase and response to temodal in newly diagnosed malignant glioma. *J Clin Oncol* 1998;16:3851–57
- Everhard S, Kaloshi G, Criniere E, et al. MGMT methylation: A marker of response to temozolomide in low-grade gliomas. *Ann Neurol* 2006;60:740–43
- Herrlinger U, Rieger J, Koch D, et al. Phase II trial of lomustine plus temozolomide chemotherapy in addition to radiotherapy in newly diagnosed glioblastoma: UKT-03. *J Clin Oncol* 2006;24:4412–17
- Möller M, Wolter M, Felsberg J, et al. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. *Int J Cancer* 2004;113:379–85
- Eley GD, Reiter JL, Pandita A, et al. A chromosomal region 7p11.2 transcript map: Its development and application to the study of EGFR amplicons in glioblastoma. *Neuro Oncol* 2002;4:86–94
- Haas-Kogan DA, Prados MD, Tihan T, et al. Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. *J Natl Cancer Inst* 2005;97:880–87
- Frederick L, Wang XY, Eley G, et al. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Res* 2000;60:1383–87
- Nishikawa R, Ji X, Harmon RC, et al. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. *Proc Natl Acad Sci USA* 1994;91:7727–31
- Ekstrand AJ, Longo N, Hamid ML, et al. Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR amplification. *Oncogene* 1994;9:2313–20
- Liu L, Backlund LM, Nilsson BR, et al. Clinical significance of EGFR amplification and the aberrant EGFRvIII transcript in conventionally treated astrocytic gliomas. *J Mol Med* 2005;83:917–26
- Heimberger AB, Suki D, Yang D, et al. The natural history of EGFR and EGFRvIII in glioblastoma patients. *J Transl Med* 2005;3:38
- Quan AL, Barnett GH, Lee SY, et al. Epidermal growth factor receptor amplification does not have prognostic significance in patients with glioblastoma multiforme. *Int J Radiat Oncol Biol Phys* 2005;63:695–703
- Chakravarti A, Seiferheld W, Tu X, et al. Immunohistochemically determined total epidermal growth factor receptor levels not of prognostic value in newly diagnosed glioblastoma multiforme: Report

- from the Radiation Therapy Oncology Group. *Int J Radiat Oncol Biol Phys* 2005;62:318–27
45. Dehais C, Laigle-Donadey F, Marie Y, et al. Prognostic stratification of patients with anaplastic gliomas according to genetic profile. *Cancer* 2006;107:1891–97
  46. Aldape KD, Ballman K, Furth A, et al. Immunohistochemical detection of EGFRvIII in high malignancy grade astrocytomas and evaluation of prognostic significance. *J Neuropathol Exp Neurol* 2004; 63:700–7
  47. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–39
  48. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* 2004; 304:1497–1500
  49. Marie Y, Carpentier AF, Omuro AM, et al. EGFR tyrosine kinase domain mutations in human gliomas. *Neurology* 2005;64:1444–45
  50. Lassman AB, Rossi MR, Raizer JJ, 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
  51. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 2005;353:2012–24
  52. Learn CA, Hartzell TL, Wikstrand CJ, et al. Resistance to tyrosine kinase inhibition by mutant epidermal growth factor receptor variant III contributes to the neoplastic phenotype of glioblastoma multiforme. *Clin Cancer Res* 2004;10:3216–24
  53. Prados M, Chang S, Burton E, et al. Phase I study of OSI-774 alone or with temozolomide in patients with malignant glioma (Abstract 394). *Proc Am Soc Clin Oncol* 2003;22:99
  54. Raizer JJ, Abrey LE, Wen P, et al. A phase II trial of erlotinib (OSI-774) in patients with recurrent malignant gliomas not on EIAEDs (Abstract 1502). *Proc Am Soc Clin Oncol* 2004;40:107
  55. Vogelbaum MA, Peereboom D, Stevens GHJ, et al. Response rate to single agent therapy with the EGFR tyrosine kinase inhibitor erlotinib in recurrent glioblastoma multiforme: Results of a phase II study (Abstract A-59). *Neuro Oncol* 2004;6:384
  56. Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004;22:133–42
  57. Lieberman FS, Cloughesy I, Malkin M, et al. Phase I–II study of ZD-1839 for recurrent malignant gliomas and meningiomas progressing after radiation therapy [abstract 421]. *J Clin Oncol* 2003;22:105
  58. Cloughesy T, Yung A, Vredenberg J, et al. Phase II study of erlotinib in recurrent GBM: Molecular predictors of outcome (Abstract #1507). *Proc Am Soc Clin Oncol* 2005;41:115
  59. Haas-Kogan DA, Prados MD, Lamborn KR, et al. Biomarkers to predict response to epidermal growth factor receptor inhibitors. *Cell Cycle* 2005;4:1369–72
  60. Pelloski CE, Lin E, Zhang L, et al. Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma. *Clin Cancer Res* 2006;12:3935–41