Temozolomide for low-grade gliomas

Predictive impact of 1p/19q loss on response and outcome

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ABSTRACT **Objective:** To evaluate the predictive impact of chromosome 1p/19q deletions on the response and outcome of progressive low-grade gliomas (LGG) treated with up-front temozolomide (TMZ) chemotherapy. **Methods:** Adult patients with measurable, progressive LGG (WHO grade II) treated with TMZ delivered at the conventional schedule (200 mg/m^2/day for 5 consecutive days, repeated every 28 days) were retrospectively evaluated for response by central review of MRI-s. Chromosome 1p and 19q deletions were detected by the loss of the heterozygosity technique (LOH). **Results:** A total of 149 consecutive patients were included in this retrospective, single center observational study. The median number of TMZ cycles delivered was 14 (range 2 to 30). Seventy-seven patients (53%) experienced an objective response (including 22 [15%] cases of partial response and 55 [38%] cases of minor response), 55 (37%) patients had stable disease, and 14 (10%) had a progressive disease. The median time to maximum tumor response was 12 months (range 3 to 30 months). The median progression-free survival (PFS) was 28 months (95% CI: 23.4 to 32.6). Material for genotyping was available for 86 patients. Combined 1p/19q LOH was present in 42% of the cases and was significantly associated with a higher rate ($p = 0.02$) and longer objective response to chemotherapy ($p = 0.017$), and both longer PFS ($p = 4.10^{-5}$) and overall survival ($p = 0.04$). **Conclusion:** Low-grade gliomas respond to temozolomide and loss of chromosome 1p/19q predicts both a durable chemosensitivity and a favorable outcome. **NEUROLOGY 2007;68:1831–1836**

Diffuse low-grade gliomas (LGG) are slow-growing tumors, including grade II oligodendrogliomas, astrocytomas, and oligo-astrocytomas, according to the WHO classification.[1,2] These tumors share an invasive and malignant potential. Gross total surgical resection, whenever possible, is recommended. Radiotherapy is considered as a postoperative standard treatment for LGG, although the optimal timing of this treatment (i.e., immediate vs at progression) remains debatable.[3,4] We and others have recently provided some evidence based on small studies that temozolomide (TMZ), an oral alkylating agent, may represent an interesting alternative option as a primary treatment after surgery in diffuse LGG.[5-7] We have reported preliminary results suggesting that chromosome 1p deletion is correlated with radiographic response of LGG to TMZ and might represent a helpful molecular tumor marker for guiding therapeutic decision-making.[6] In the present study we report additional data from an extended series of patients with longer follow-up confirming the efficacy of TMZ in progressive LGG and the predictive value of 1p/19q loss both in terms of prognosis and chemosensitivity.

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METHODS Since 1999, all patients in our institution with progressive diffuse LGG have been offered up-front TMZ chemotherapy. The patients who met the following criteria were included in this retrospective, single center observational study: 1) histologically confirmed WHO grade II astrocytoma, oligoastrocytoma, or oligodendroglioma after central review; 2) evidence of progressive disease, clinically or radiologically; 3) 18 years of age or older; 4) Karnofsky performance score > 40; 5) supratentorial tumor; 6) no prior specific treatment other than surgery; and 7) measurable disease on MRI. All patients received the standard TMZ schedule consisting of 200 mg/m^2 orally once daily on days 1 through 5. Treatment cycles were repeated every 28 days.

Response to TMZ was evaluated by MRI (repeated every 2 or 3 months). We used modified Mac Donald criteria adapted to low-grade gliomas, as previously reported.[6,8] Such criteria are based on modifications in tumor size defined as the product of the two largest perpendicular diameters of the T2 hypersignal lesion in nonenhancing tumors. In tumors displaying contrast enhancement, response criteria take into consideration both the size of T2 hypersignal and T1 post-contrast enhancement. In brief, a complete response (CR) was defined as complete disappearance of all T2 hypersignal and T1 postcontrast enhancing lesions on consecutive MRIs preformed at least 8 weeks apart. A partial response (PR) was defined as >50% reduction in size in both nonenhancing and enhancing (when present) lesions from baseline, lasting for at least 8 weeks. Minor response (MR) was defined as a 25% to 50% reduction in the size of nonenhancing tumors. In patients with enhancing tumors, disappearance of all contrast enhancement lasting more than 8 weeks and stable T2 hypersignal lesion size was also considered a MR. Patients must be on stable or reduced doses of corticosteroids and stable or improved neurologic status for characterization of CR, PR, or MR. Progressive disease (PD) was defined as greater than 25% increase in size of T2 hypersignal or contrast enhancement, or any new tumor on MRI scans, or tumor-related neurologic deterioration, in patients on stable or increased doses of corticosteroids. Stable disease (SD) was defined as any other clinical status not meeting the criteria for CR, PR, MR, or PD, lasting for at least 6 months (prior to 6 months, such patients were considered nonevaluable for response). The radiographic responses were reviewed independently by two investigators who were kept unaware of the tumor genotyping.

DNA from both blood and tumor tissue was extracted using a standard protocol (Qiagen, QIAmp DNA mini Kit). Chromosome 1p and 19q deletions were screened by the loss of heterozygosity technique (LOH) using microsatellite polymorphism markers as previously described.[6,9] All patients gave their written informed consent.

The χ^2 test was used to test the association between the radiologic response and 1p/19q codeletion status. Tumors with isolated 1p or 19q loss were included in the 1p/19q intact group. Progression-free survival (PFS) was defined as the time from the start of chemotherapy until the first unequivocal sign of radiologic or clinical progressive disease. Patients who had no evidence of disease progression were treated as censored for the analysis of PFS. Probability estimates for PFS were calculated utilizing the Kaplan-Meier method. The log-rank test was used to test for equality of PFS distribution. Two-sided p values less than 0.05 were considered significant.

Table Patient characteristics

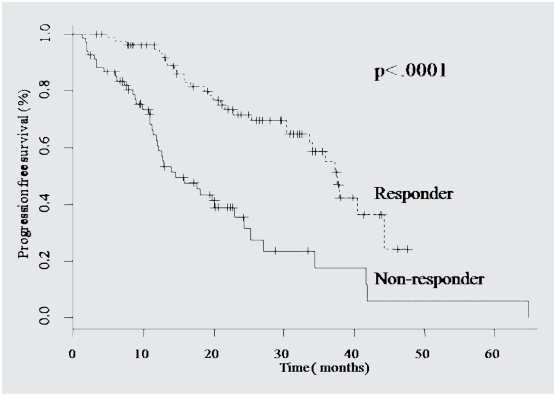
Characteristics	No. (%) of patients (n = 149)
Age, y	
Median	44
Range	24–72
Sex	
Male	84 (56)
Female	65 (44)
Histology	
Pure oligodendroglioma	105 (70)
Other	44 (30)
Karnofsky score	
Median	90
Range	40–100
Surgery	
Biopsy	81 (54)
Resection	68 (46)
Time interval from surgery to chemotherapy, mo	
Median	3
Range	0.2–125
MRI	
Nonenhancing	126 (85)
Enhancing (scant)	23 (15)
Tumor size, mm*	
Median	50.8
Range	19.6–96

*Longest diameter.

RESULTS Between September 1999 and May 2005, 149 consecutive patients were treated with up-front TMZ chemotherapy and fulfilled the inclusion criteria of the study. The main clinical characteristics of the patients are summarized in the table. The preliminary data of the first 60 patients of this series have been previously reported.[6]

The median follow-up was 30.4 months (range 2 to 70 months). The median number of cycles delivered was 14 at the time of analysis (range 2 to 30 cycles). TMZ chemotherapy was well-tolerated with 7% grade 3 and 8% grade 4 myelosuppression. There was no toxic death. At the time of analysis, all but one (due to an early grade 4 thrombopenia) were evaluable for response: 22 patients (15%) achieved a PR, 57 patients (38%) achieved a MR, 55 patients (37%) were stable, and 14 patients (10%) had progressive disease. Hence the objective response rate was 53%. The median time to the onset of radiographic response (>25% reduction in the tumor size) was 9 months (range 2 to 23 months). The median time to maximum radiographic re-

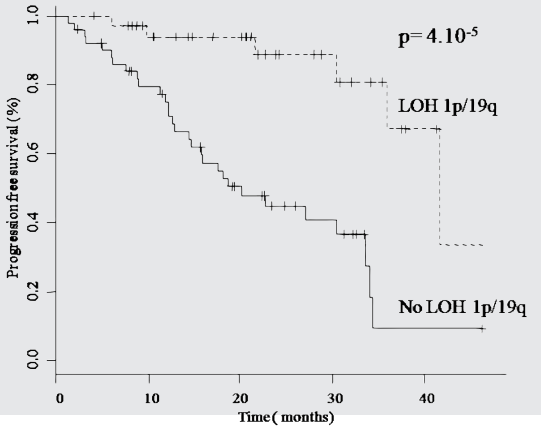
Figure 1 Progression-free survival according to the response status



sponse was 12 months (range 3 to 30 months). A clear clinical improvement, especially in seizure control (50% or more reduction in seizures frequency), was observed in 87 patients (58%). The median PFS was 28 months (95% CI 23.4 to 32.6 months); rates of PFS at 1 and 2 years were 79.5% (95% CI 73 to 86.6) and 55.8% (95% CI 47.5 to 65.6). The median overall survival was not reached; 1-year, 2-year, and 3-year survival were 95% (CI 81.5 to 98.7%), 85.9% (CI 79.8 to 92.5%), and 69.8% (CI 60 to 81.2%). A correlation between the response and PFS was identified ($p < 0.0001$) (figure 1). We did not find any difference between pure oligodendrogliomas and other types of tumor in terms of either response rates ($p = 0.72$) or PFS ($p = 0.4$).

Tumor and blood DNA pairs from 86 patients were available for LOH analysis. The outcomes in patients who had molecular genetic testing (n = 86) were similar to those who did not (n = 63) in terms of response rates ($p = 0.49$) and PFS ($p = 0.2$). Combined 1p/19q loss was detected in 36 cases (42%); there was a higher rate of 1p/19 q loss in pure oligodendrogliomas (30/60 = 50%) than in other gliomas (6/26 = 25%) ($p = 0.02$). In the 36 patients with 1p/19q loss, we observed 26 objective responses (7 PR, 19 MR), 10 stable disease, and zero progressive disease. In the 50 tumors (58%) without combined 1p/19q loss, 23 objective responses (7 PR, 19 MR), 22 stable disease, and 5 progressive disease were noted. The objective response rate was higher in the 1p/19q loss group (26/36 patients: 72%) than in the 1p/19q intact group (23/50 patients: 46%) ($p = 0.02$). In addition, 1p/19q loss was also associated with a better PFS ($p = 0.00004$) and overall survival ($p = 0.04$ as a result of very few events, only two, among 1p/19q group) (figures 2 and 3). Among responders to TMZ and at the time of analysis, 4 of 26 patients with 1p/19q deleted tumors had progressed while

Figure 2 Progression-free survival according to the 1p/19q loss of heterozygosity status



11 of 23 patients without 1p/19q codeletion had progressed. Hence, the duration of response was longer in the 1p/19q deleted responders (median not reached) than in the 1p/19q retained responders (33.6 months) ($p = 0.017$). We found no correlation between response to treatment or PFS and other potential prognostic factors such as age (<40 vs ≥40 years), histology (pure oligo vs other), tumor size (<5 cm vs ≥5 cm), or Karnofsky score. Presence of contrast enhancement had a negative impact on PFS ($p = 0.02$) but not on response.

DISCUSSION This study is the largest to date in progressive diffuse LGG treated with up-front chemotherapy and confirms that TMZ induces tumor response in a substantial number of patients with progressive LGG and that loss of chromosome 1p/19q predicts a better outcome and a higher likelihood of TMZ response.

In the present series of 149 patients, up-front

Figure 3 Overall survival according to the 1p/19q loss of heterozygosity status

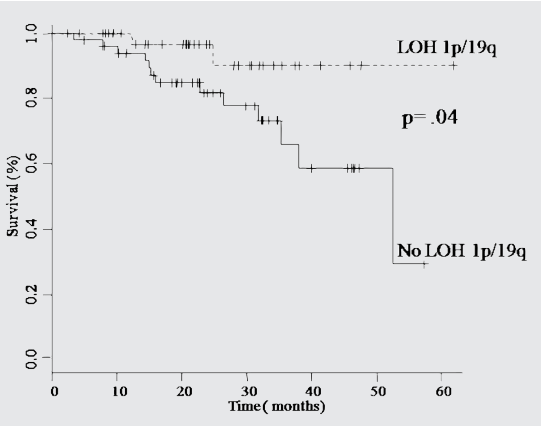
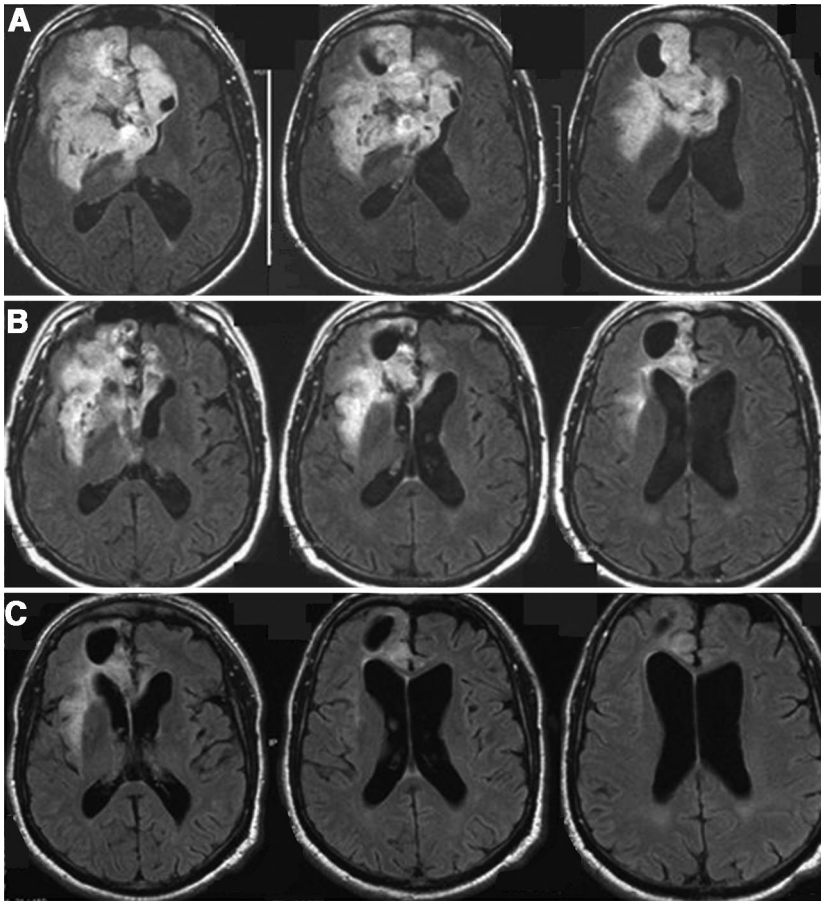


Figure 4 Continuous response to temozolomide



An example of continuous response to temozolomide (TMZ) of a 51-year-old man with a low-grade oligodendroglioma. (A) MRI before TMZ, (B) after 12 cycles of TMZ, (C) after 24 cycles of TMZ.

TMZ treatment resulted in a 53% objective radiographic response rate, which is similar to that of previous smaller studies which show a 58% (17/29 patients)[5] and 61% (17/28 patients) response rate.[7] However, this rate is higher than our preliminary findings (18/59 patients, 31%),[6] probably because the median duration of treatment and follow-up at the time of analysis is longer in the present study. Indeed, the response to TMZ was often delayed with a median time at the onset of response of 9 months (ranging from 2 to 23 months). Hence, one should be aware to not stop the treatment in the absence of apparent radiographic response after the first cycles prematurely, especially in the case of clinical improvement, such as a marked reduction in seizures frequency (observed in 58% of our patients). In addition, the response may continue to improve slowly in subsequent months. While the median time to best response was 12 months, many patients experienced longer progressive shrinkage of the tumor, up to 24 months (figure 4). Concerning PFS of patients treated for progressive LGG with up-front TMZ, the literature is limited primarily because of lack of prolonged follow-up.[5,6] In the present study, the median PFS was 28 months and the 1- and 2-year PFS was 79.5% and 55.8% (CI 73 to 86.6% and CI 47.5 to 65.6%). The treatment was well-tolerated with a 15% reversible grade III and IV toxicity.

Up-front chemotherapy with the PCV (procarbazine-CCNU-vincristine) regimen seems to be also active in progressive LGG. In a series of 16 low-grade oligodendroglial tumors (with little or almost no contrast enhancement), 3 (19%) demonstrated a PR and 10 (62%) demonstrated a minor response (defined as "radiologic improvement" and "decrease in mass effect").[10] In another study, a 29% (8/28 patients) radiographic response rate was obtained after a PCV neoadjuvant chemotherapy regimen, which was followed immediately by radiotherapy.[11] However, PCV regimen is associated with higher toxicity than TMZ, mainly related to myelosuppression. Hence, in the two latter studies, a majority of patients could not receive the fully intended six cycles or the complete drug combination. In addition, there is a well-known cumulative toxicity with nitrosourea-based chemotherapy when delivered in a more prolonged treatment, which seems to represent an important factor to achieve the best response in LGG.

Combined 1p/19q deletion represents a strong and independent prognostic factor in anaplastic oligodendroglial tumors as clearly shown in two recent phase III trials.[12,13] In addition, a few retrospective studies suggest that 1p/19q loss might also predict the chemosensitivity of these tumors to nitrosourea-based chemotherapy.[14,15] Since recurrent deletions of 1p/19q are also observed in LGG, preferentially in tumors demonstrating an oligodendroglial phenotype, they may have a clinical impact in LGG. However, studies specifically devoted to evaluating the predictive impact of 1p/19q loss on prognosis and response to chemotherapy in LGG are scarce. Retrospective data suggest that, as for anaplastic oligodendroglial tumors, 1p/19q loss is associated with a significantly longer PFS in LGG and represents an independent prognostic factor.[16] However, the use of 1p/19q status as a surrogate marker of an intrinsic favorable biologic behavior or a predictor of favorable outcome after specific treatments, or both, remains unclear.

In a preliminary study, we reported a significant correlation between 1p loss and radiographic response in a limited series of 26 patients who received TMZ as an initial treatment.[6] Other authors[7] found a similar statistical association in a series of 15 progressive LGG treated by TMZ. In the present study, we confirmed this correlation in a much larger series of 86 LGG for which genotyping was available. Apart from 1p/19q loss, we did not find any other potential clinico-pathologic predictive factors correlated with tumor response (i.e., age, KPS, histol-

ogy, tumor size, contrast enhancement). Since the tumor suppressor genes targeted by the 1p/19q deletions are still unidentified, the molecular mechanisms underlying the association between 1p/19q loss and tumor chemosensitivity remain unknown. Interestingly, 1p/19q loss has been recently shown to be correlated with the MGMT (O6-methylguanine-methyltransferase) gene silencing[17] and reduced protein expression.[7] This raises the question of the contribution of MGMT, which is a DNA repair enzyme conferring resistance to DNA-alkylating agents, in the chemosensitivity of LGG to TMZ. Although we found that 1p/19q loss was statistically associated with radiographic response, it is of note that TMZ may also induce response in tumors without 1p/19q alteration. However, the duration of response time in responding patients without 1p/19q codeletion was shorter than that of the patients with 1p/19q deleted tumors ($p = 0.017$), contributing, therefore, to the shorter PFS. Hence, in a clinical point of view, it could be more appropriate to consider the 1p/19q codeletion as a predictor of delayed acquisition of chemoresistance than a mere response predictor to TMZ.

Despite some study limitations such as the retrospective nature and the possibility of analyzing the genotype in only 60% of patients, our findings provide additional evidence that TMZ as primary treatment is a therapeutic option for progressive LGG both in terms of tolerance and activity, and contribute to validate the fact that 1p/19q loss is both a predictor of chemosensitivity (response rate and duration of response) and favorable prognosis (PFS). Comparison with radiotherapy, which is considered standard treatment for progressive LGG, is not easy and justifies the ongoing EORTC randomized phase III trial (22033) comparing radiotherapy and TMZ in patients with progressive LGG. That study is stratified according to the 1p status. Already, chemotherapy with TMZ is an interesting alternative to radiotherapy in patients with very large tumors or in the elderly who are exposed to a higher risk of delayed neurotoxicity. Anecdotal observations suggest that preoperative chemotherapy may enable radical surgery after response in initially unresectable large tumors.[18,19]

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