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# Distinct Responses of Xenografted Gliomas to Different Alkylating Agents Are Related to Histology and Genetic Alterations

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## ABSTRACT

**A series of 12 human gliomas was established as xenografts in *nude* mice and used to evaluate the relationship between histology, genetic parameters, and response to alkylating agents. Eight were high-grade oligodendroglial tumors, and four were glioblastoma. They were characterized for their genetic alterations, including those considered as "early" alterations, namely loss of chromosome 1 ± loss of chromosome 19q, *TP53* mutation, and those considered as "late" alterations, namely loss of chromosome 10, loss of chromosome 9p, *EGFR* genomic amplification, *PTEN* mutation, *CDKN2A* homozygous deletion, and telomerase reactivation. Chemosensitivity of xenografts to four alkylating agents, temozolomide (42 mg/kg, days 1–5, p.o.), 1,3-bis(2-chloroethyl)-1-nitrosourea (5 mg/kg, day 1, i.p.), Ifosfamide (90 mg/kg, days 1–3, i.p.), and carboplatin (66 mg/kg, day 1, i.p.) was tested by administration of drugs to tumor-bearing mice. Although each tumor presented an individual response pattern, glioblastoma had a lower chemosensitivity than oligodendrogliomas, and temozolomide was the most effective drug. Deletion of 1p ± 19q was associated with higher chemosensitivity, whereas late molecular alterations, particularly *EGFR* amplification, were associated with chemoresistance. These results suggest that the combined use of histology and molecular markers should eventually be helpful selecting the most appropriate agents for treatment of malignant oligodendrogliomas and astrocytomas.**

## INTRODUCTION

According to the WHO, gliomas are divided into two main subtypes depending on their putative cells of origin (astrocyte or oligodendrocyte), and they are graded according to histopathological criteria (1). Survival varies between >10 years for oligodendroglioma to <1 year for the majority of grade IV astrocytomas [glioblastoma (GBM)]. This wide difference is also influenced by clinical parameters, including age (2–4), Karnofsky performance score (4), tumor location, extent of surgical resection (5–7), radiotherapy (5, 8), and chemotherapy (9). Surgical resection is limited to the main tumor mass and does not remove tumoral cells invading the surrounding parenchyma. Consequently, the present treatment for malignant gliomas includes adjuvant radiotherapy, which may or may not be followed by chemotherapy.

Response of gliomas to chemotherapy varies mainly according to histology, with GBM being resistant whereas oligodendrogliomas are more chemosensitive. Recently developed molecular biology methods showed that oligodendrogliomas could be divided into two sets of tumors, those with the double loss of chromosomes 1p and 19q and those without these deletions. Cairncross *et al.* (10) recently found that loss of 1p and 19q was related to a better response to combined treatment with procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, and vincristine in anaplastic and progressive oligodendrogliomas. In subsequent studies, the 1p-19q loss was also related to an overall better prognosis (11, 12). In contrast, several studies have identified genetic alterations related with a poor prognosis, such as epidermal growth factor receptor (*EGFR*) amplification (13–16), phosphatase and tensin homolog tumor suppressor gene (*PTEN*) mutation or loss of 10q (16–19), and cyclin-dependent kinase inhibitor 2A (*CDKN2A*) homozygous deletions (10, 20, 21). In low-grade gliomas, telomerase reactivation also seems to be associated with a shorter survival (22, 23). Nevertheless, no clear correlation with chemoresistance has been established. To further study the molecular alterations underlying response to chemotherapy, we established a series of 12 human gliomas, derived from surgical specimens, s.c. xenografted into *nude* mice. These models have recently been developed and characterized for molecular alterations (24). The tumors were tested *in vivo* for their response to four alkylating drugs generally used in malignant gliomas [1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), carboplatin (CP), ifosfamide (IFO), and temozolomide (TMZ)], and correlations between response to treatment and genetic patterns were established.

## MATERIALS AND METHODS

### *In Vivo* Studies

**Xenografted Tumors.** *In vivo* chemosensitivity tests were performed with 12 xenografts, 8 derived from oligodendroglial tumors (ODA4-Gen, ODA5-Rai, ODA14-Rav, ODA17-Gir, ODA20-Tho, ODA23-Nan, ODA24-Nan, and ODA25-Nan) and 4 from GBM tumors (GBM1-Ham, GBM7-Rom, GBM14-Cha, and GBM21-Nan). Xenografts were established by s.c. implantation of small fragments (30 ± 10 $mm^3$) of human glioma into the s.c. scapular area of *nude* mice and were serially transplanted. Each mouse received one fragment. Tumor-bearing mice were randomly divided into control and treated groups. For each experiment, 5–12 mice (median, 8) were treated per group. Treatments were started as soon as local tumor reached a volume of 300 ± 100 $mm^3$. Each mouse was identified by a code number.

**Animals.** Swiss (*nu/nu*) mice, 6–8 weeks of age, were bred in the animal facilities of Institut Curie (Paris, France). The animals were maintained under specified pathogen-free sterile conditions with rodent food and water *ad libitum*. Their care and housing were in accordance with institutional guidelines established by the Ministère de l'Agriculture et de la Forêt, Direction de la Santé et de la Protection Animale (Paris, France).

**Formulation and Administration of Drugs.** BCNU (Carmustine) was provided by Bristol-Myers Squibb (Princeton, NJ). BCNU was administered at a single i.p. injection at a dose of 5 mg/kg in a 0.2-ml volume per tumor-bearing mouse. CP (Paraplatin) was provided by Bristol-Myers Squibb. CP was administered as a single i.p. injection at a dose of 66 mg/kg in a 0.2-ml

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**Note:** P. Leuraud and L. Taillandier contributed equally to this work.

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volume per tumor-bearing mouse. IFO (Holoxan) was provided by Baxter Oncology (Frankfurt, Germany). IFO was administered daily for three doses by i.p. route. The daily dose was 90 mg/kg/day in a 0.2-ml volume per tumor-bearing mouse. TMZ (Temodar in the United States; Temodal elsewhere), provided by Schering-Plough (Kenilworth, NJ), was administered p.o. daily for 5 days. The daily dose was 42 mg/kg/day in a 0.1-ml volume per tumor-bearing mouse.

***In Vivo* Assay of Chemosensitivity of Xenografted Gliomas.** Xenografts were measured three times a week from the first day of treatment. Two perpendicular diameters were measured with a caliper, and their volumes were calculated using the following formula: $(\text{width})^2 \times (\text{length})/2$. Growth of xenografts was evaluated by the relative tumor volume (RTV), which was expressed as $RTV_X = V_X/V_1$, where $V_X$ is the tumor volume on day $X$, and $V_1$ is the initial tumor volume when the treatment was started (day 1).

Mice were sacrificed by prolonged anesthesia when the tumor volumes reached 2000 $mm^3$.

Complete regression was defined as the absence of any palpable nodule at the graft site and was followed by either "cure," *i.e.*, no tumor regrowth up to 3 months, or relapse. In the latter case, the duration of regression was noted. All data are presented as the median values and are reported in tables and figures.

The tumor growth inhibition (TGI) was calculated according to the following formula: % growth inhibition = (RTVt/RTVc), where RTVt were calculated for individual tumors and RTVc was the mean of the relative tumor volumes in the control group at a given time; the medians of growth inhibition were calculated and are reported in the tables. Calculation of TGI was made as a function of time until the day of sacrifice of the first mouse. Tumors were considered as responders to chemotherapy if the tumor growth inhibition was >50%.

Individual tumor growth delay (TGD) was calculated as the time in days required for individual tumor to reach a 5-fold increase in volume, as published previously (25); the ratio of median growth delay per group was calculated and is reported in the tables.

The TGD index was calculated as the median growth delay in the treated group divided by the median growth delay in the control group; a TGD index of 2 was considered the limit of meaningful effect.

### Molecular Parameters of Patient Tumors and Corresponding Xenografts

**DNA Extraction.** Paired blood samples from the patient and xenografted tumor were used. Blood DNA was extracted by use of the Nucleon BACC3 DNA Extraction kit (Amersham Bioscience, Piscataway, NJ), and tumoral DNA was extracted by use of the QIAamp DNA minikit, as described by the manufacturer (Qiagen, Venlo, the Netherlands).

**Microsatellite Analysis for Loss of Heterozygosity (LOH) on Chromosomes 1, 9p, 10, and 19q.** Blood and tumor DNA from the patients and tumor DNA from the xenografts were screened for LOH on chromosome 1p with use of the polymorphic markers D1S450, D1S2667, D1S234, D1S255, D1S2797, and D1S2890, and on chromosome 1q with use of the markers D1S2878, D1S249, and D1S2785 (18, 24, 26). On chromosome 9p, LOH was screened with use of the markers D9S286, D9S168, D9S1870, D9S156, and D9S1687, which span the region located near *CDKN2A* (24). On chromosome 10p, LOH was screened with use of the markers D10S249, D10S189, D10S547 (near hTR repressor), D10S585 (near hTR repressor), D10S548, and D10S204 (27); on chromosome 10q, LOH was scanned with use of the markers D10S537, D10S219, D10S1744 (near *PTEN*), D10S541, D10S579, D10S1755, D10S1671, D10S597, D10S1693, D10S209, D10S587 (near *DMBT1*), D10S1723, D10S212, D10S537, D10S541, D10S597, D10S1693, and D10S212, which span the region located between 10q21.22 and 10qter (18, 24, 26). For chromosome 19q, LOH was screened with use of the markers D19S425, D19S219, D19S888, D19S412, and D19S418 (18, 24, 26). One of the primers was labeled with the fluorochromes hexachlorofluorescein, 6-carboxyfluorescein, or Ned (Applied Biosystems, Foster City, CA). The samples were sequenced on an automatic sequencer and analyzed with the GeneScan program (Applied Biosystems).

**Screening of the *PTEN/MMAC1* and *TP53* Gene Mutations.** *PTEN/MMAC1* mutations were screened by denaturing gradient gel electrophoresis over the entire coding sequence of the nine exons and their corresponding splice junctions, with use of previously described primers (28). *TP53* mutations were screened by denaturing gradient gel electrophoresis for exons 5–8 and their corresponding splice junctions, with use of previously described primers (29). DNA showing an altered denaturing gradient gel electrophoresis profile was sequenced bidirectionally with use of the Perkin Kit and sequencer. When a DNA variant was found, the corresponding blood DNA was sequenced to differentiate somatic events from constitutional variants (polymorphism or germline mutation).

**Screening of *EGFR* Gene Amplification and *P16/CDKN2A* Gene Homozygous Deletion.** *EGFR* amplification was screened by semiquantitative PCR with previously described primers and protocol (30). *P16/CDKN2A* homozygous deletions were screened by semiquantitative PCR with previously described primers and protocol (31).

**Screening of Telomerase Activity.** Telomerase activity was screened by the telomeric repeat amplification protocol technique using the TRAPeze kit (Serologicals Corporation, Norcross, GA) as described by the manufacturer.

### Statistical Analyses

Descriptive analyses of the tumor response were performed by treatment and by tumor. The mean tumor growth inhibition rate, SD, and range were calculated per groups of control and treatment mice.

For calculation of the TGD index, median survival times were estimated according to the Kaplan–Meier method. The first day was the day of treatment of tumor-bearing mice. The date of last follow-up was the first day on which a RTV >5 was attained. If a RTV >5 was not reached, data were censored at the date of last follow-up. This situation occurred in case of death from treatment toxicity or because of lack of tumor regrowth at the end of the study (180 days of follow-up).

Correlations between global response to treatment and genetic alterations were first studied using the TGI. This analysis was performed in keeping with univariate and multivariate Cox proportional hazard regression models. The relationship between response and molecular biological biomarkers was studied for each treatment.

Correlations between response to a given drug and genetic alterations were then studied using the TGD index. This analysis involved both univariate and multivariate stepwise logistic regression analyses. Effects related to the inoculation of the same tumor into several mice were taken into account. Comparison of treatments was performed with log likelihood ratio tests.

All tests were considered significant at the 0.05 significance level. Odds ratios are presented with their 95% confidence intervals.

All statistical analyses were performed with SAS software, Version 8 (SAS Institute, Cary, NC).

## RESULTS

**Histological Characteristics of Original and Xenografted Gliomas.** We studied a set of 12 human gliomas xenografted into *nude* mice. All initial tumors were analyzed and classified according to the current WHO guidelines (1), except for GBM with an oligodendroglial component, which is considered to be a separate entity (26). Tumors were classified into two main subtypes: eight displayed oligodendroglial features (seven anaplastic oligodendrogliomas and one GBM with an oligodendroglial component) and four with astrocytic features (four GBM).

After being grafted into *nude* mice, tumors presented a high density of dedifferentiated cells devoid of any particular pattern with small round or fusiform cells, as described previously (24).

**Antitumor Effect of Alkylating Agents in Xenografts.** Tumor-bearing mice were treated as soon as their tumors reached a volume of $300 \pm 100$ $mm^3$. No spontaneous regression was observed.

The growth delay (TGD) of oligodendrogliomas was increased by a factor of 2 or more, and TGI exceeded 50% in 8 of 8 oligodendrogliomas after treatment with TMZ, 6 of 8 after IFO, 5 of 8 after BCNU, and 4 of 8 after CP (Table 1). As also shown in Table 1, the range of increased TGD varied widely (from 2- to 17.6-fold) according to the tumor and the agent tested. The rate of complete regression

Table 1 *Distinct responses to four different alkylating agents of a series of oligodendroglioma xenografts*

A TGDI[a] of 2 and tumor growth inhibition >50% were considered as the limits of meaningful effect.

| | Tumor growth delay control group (in days) | Drugs | TGDI in treated group | Tumor growth inhibition (%) | | Mice/group | No. of PR | No. of CR | No. of cured |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean ± SD | Range | | | | |
| ODA4-Gen | 10.5 | CP | 1.3 | 40 ± 14 | 20–62 | 8 | 2 | | |
| | | IFO | 2.8 | 88 ± 6 | 77–93 | 7 | 7 | | |
| | | BCNU | 1.5 | 52 ± 27 | 48–88 | 8 | 6 | | |
| | | TMZ | 9.3 | 93 ± 4 | 89–100 | 8 | 2 | 4 | 2 |
| ODA5-Rai | 11.5 | CP | 2.3 | 54 ± 20 | 0–72 | 9 | 9 | | |
| | | IFO | 2.5 | 65 ± 21 | 62–81 | 12 | 12 | | |
| | | BCNU | 3.6 | 98 ± 3 | 94–100 | 9 | | 3 | 6 |
| | | TMZ | 2.6 | 92 ± 4 | 86–98 | 10 | 9 | | |
| ODA14-Rav | 10 | CP | 1.5 | 71 ± 30 | 0–100 | 8 | 6 | 1 | |
| | | IFO | 1.6 | 57 ± 17 | 35–80 | 8 | 5 | | |
| | | BCNU | 1.4 | 48 ± 14 | 28–65 | 7 | 4 | | |
| | | TMZ | 6 | 95 ± 5 | 89–100 | 8 | | 7 | 1 |
| ODA17-Gir | 7 | CP | 1.6 | 50 ± 32 | 32–89 | 11 | 8 | | |
| | | IFO | 5.4 | 92 ± 4 | 85–96 | 8 | 1 | 6 | 1 |
| | | BCNU | >11.6 | 94 ± 3 | 90–97 | 9 | 8 | | |
| | | TMZ | 17.6 | 93 ± 7 | 76–100 | 8 | | 3 | 5 |
| ODA20-Tho | 12.5 | CP | 2.1 | 56 ± 44 | 7–98 | 9 | 4 | 1 | |
| | | IFO | 3.3 | 82 ± 16 | 58–100 | 8 | 8 | | |
| | | BCNU | 2 | 64 ± 16 | 45–89 | 8 | 7 | | |
| | | TMZ | 4.6 | 83 ± 13 | 65–100 | 11 | 11 | | |
| ODA23-Nan | 6 | CP | >4.2 | 93 ± 2 | 90–96 | 7 | 7 | | |
| | | IFO | 3.8 | 88 ± 7 | 85–100 | 5 | 5 | | |
| | | BCNU | 2.1 | 76 ± 13 | 72–100 | 7 | 7 | | |
| | | TMZ | 13.3 | 97 ± 1 | 97–99 | 7 | 1 | 3 | 3 |
| ODA24-Nan | 24 | CP | 1.2 | 11 ± 16 | 0–45 | 7 | 5 | | |
| | | IFO | 1 | 0 ± 3 | 0–80 | 6 | 3 | | |
| | | BCNU | 0.9 | 0 ± 18 | 0–44 | 7 | 2 | | |
| | | TMZ | 3.5 | 64 ± 11 | 55–86 | 6 | 6 | | |
| ODA25-Nan | 25.5 | CP | 2.9 | 66 ± 17 | 40–78 | 5 | 4 | | |
| | | IFO | 2.1 | 59 ± 3 | 29–83 | 6 | 5 | | |
| | | BCNU | 2 | 67 ± 18 | 39–91 | 6 | 4 | | |
| | | TMZ | 2.2 | 59 ± 11 | 32–74 | 7 | 5 | | |

[a] TGDI, tumor growth delay index; PR, partial response; CR, complete response; CP, carboplatin; IFO, ifosfamide; BCNU,1,3-bis(2-chloroethyl)-1-nitrosourea; TMZ, temozolomide.

and of cure at the time of sacrifice were 26% (17 of 65) and 14% (11 of 65) after TMZ, 5% (3 of 61) and 10% (6 of 61) after BCNU, 10% (6 of 60) and 2% (1 of 60) after IFO, and 3% (2 of 64) and 0% (0 of 64) after CP.

The growth delay (TGD) of GBMs was increased by a factor of 2 or more, and TGI exceeded 50% in 2 of 4 GBM after treatment with IFO, 1 of 4 after CP, 1 of 4 after TMZ, and none after treatment with BCNU (Table 2). The rates of complete regression and of cure at the time of sacrifice were zero after TMZ, BCNU, or CP and 23% (7 of 31) after IFO treatment. These results confirmed the relative chemoresistance of GBMs compared with oligodendrogliomas ($P < 10^{-4}$, Table 3).

When both histological groups were studied together by uni- and multivariate analysis, only TMZ had a positive impact on TGD ($P < 10^{-4}$), whereas the other drugs did not affect it even when these agents were clearly active in individual tumors.

The growth delay induced by TMZ in two different xenografts [one GBM (GBM14-Cha) and one oligodendroglioma (ODA14-Rav)] is shown in Fig. 1. The GBM was chemoresistant (Fig. 1, *top*), whereas the oligodendroglioma (Fig. 1, *bottom*) responded transiently but significantly to TMZ.

**Molecular Characteristics of the Xenografted Tumors.** As shown previously (24), genetic alterations detected in xenografts were remarkably stable after growth in *nude* mice compared with the

Table 2 *Distinct responses to four different alkylating agents of a series of glioblastoma xenografts*

A TGDI[a] of 2 and tumor growth inhibition >50% were considered as the limits of meaningful effect.

| Xenografts | Tumor growth delay control group (in days) | Drugs | TGDI in treated group | Tumor Growth Inhibition (%) | | Mice/group | No. of PR | No. of CR | No. of cured |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean ± SD | Range | | | | |
| GBM1-Ham | 11 | CP | 1.5 | 45 ± 15 | 34–72 | 8 | 1 | | |
| | | IFO | 0.9 | 0 ± 8 | 0–22 | 8 | | | |
| | | BCNU | 1.6 | 49 ± 8 | 38–60 | 8 | 1 | | |
| | | TMZ | 1.1 | 22 ± 19 | 7–55 | 8 | | | |
| GBM7-Rom | 9 | CP | 0.9 | 0 ± 10 | 0–27 | 8 | | | |
| | | IFO | 5.2 | 94 ± 3 | 89–99 | 8 | 1 | 7 | |
| | | BCNU | 1 | 17 ± 24 | 0–58 | 8 | 1 | | |
| | | TMZ | 0.9 | 0 ± 21 | 0–48 | 8 | | | |
| GBM14-Cha | 9 | CP | 1.3 | 39 ± 25 | 0–74 | 8 | 3 | | |
| | | IFO | 1.2 | 31 ± 18 | 9–60 | 8 | 2 | | |
| | | BCNU | 1.2 | 21 ± 19 | 0–45 | 8 | | | |
| | | TMZ | 1.1 | 12 ± 34 | 0–39 | 7 | | | |
| GBM21-Nan | 12 | CP | 2.5 | 73 ± 10 | 57–83 | 7 | 7 | | |
| | | IFO | 2.7 | 86 ± 11 | 69–100 | 7 | 4 | | |
| | | BCNU | 1.7 | 57 ± 7 | 49–68 | 6 | 6 | | |
| | | TMZ | 6.3 | 98 ± 1 | 98–99 | 7 | 7 | | |

[a] TGDI, tumor growth delay index; PR, partial response; CR, complete response; CP, carboplatin; IFO, ifosfamide; BCNU,1,3-bis(2-chloroethyl)-1-nitrosourea; TMZ, temozolomide.

Table 3 *Comparison of TGDI[a] of the xenografts treated with four different drugs*

Oligodendrogliomas were the most chemosensitive and responded at least to temozolomide. Glioblastomas were almost all chemoresistant, except for one tumor (GBM21-Nan), which responded to carboplatin, ifosfamide, and temozolomide. Gray shading indicates a responding tumor; no shading indicates an unresponsive tumor; a TGDI of 2 was considered as the limit of meaningful effect.

| | TGDI[a] | | | |
|---|---|---|---|---|
| | CP | IFO | BCNU | TMZ |
| Oligodendroglioma xenografts | | | | |
| ODA4-Gen | 1.3 | 2.8 | 1.5 | 9.3 |
| ODA5-Rai | 2.3 | 2.5 | 3.6 | 2.6 |
| ODA14-Rav | 1.5 | 1.6 | 1.4 | 6 |
| ODA20-Tho | 2.1 | 3.3 | 2 | 4.8 |
| ODA17-Gir | 1.6 | 5.4 | >11.6 | 17.6 |
| ODA25-Nan | 2.9 | 2.1 | 2 | 2.2 |
| ODA23-Nan | >4.2 | 3.8 | 2.1 | 13.3 |
| ODA24-Nan | 1.2 | 1 | 0.9 | 3.5 |
| GBM xenografts | | | | |
| GBM21-Nan | 2.5 | 2.7 | 1.7 | 6.3 |
| GBM1-Ham | 1.5 | 0.9 | 1.6 | 1.1 |
| GBM7-Rom | 0.9 | 5.2 | 1 | 0.9 |
| GBM14-Cha | 1.3 | 1.2 | 1.2 | 1.1 |

[a] TGDI, tumor growth delay index; CP, carboplatin; IFO, ifosfamide; BCNU,1,3-bis(2-chloroethyl)-1-nitrosourea; TMZ, temozolomide; GBM, glioblastoma.

original tumor (postsurgical sample). The patterns of molecular alterations of the 12 tumors are summarized in Table 4. Genetic alterations were divided into "early" genetic changes (LOH at 1p ± 19q, *TP53* mutations) and "late" alterations, related to higher-grade gliomas (loss of chromosome 10, *PTEN* mutation, *CDKN2A* deletion, *EGFR* amplification, and telomerase reactivation; Refs. 16–27).

Five of eight oligodendroglioma xenografts displayed early alterations (including LOH on chromosomes 1p ± 19q in one tumor, *TP53* mutations in one tumor, and both changes in three tumors), constantly associated with late-occurring alterations. In two of eight tumors, late molecular alterations were isolated (*i.e.,* without loss of 1p-19q or *TP53* mutation), whereas the molecular profile was normal in one of eight tumors.

In GBM, two of four tumors had early alterations (LOH at 1p-19q in one and *TP53* mutation in one) associated with late changes in both cases, and two of four had only late alterations.

When molecular profiles of both histological groups were compared, oligodendroglial tumors displayed a significantly lower number of late-occurring alterations than did GBMs ($P = 0.007$).

**Correlation between Genetic Alterations and Overall Response to Treatment.** A total of 502 mice were used for the whole study. Each tumor was xenografted into 42 ± 11 mice. The different alkylating drugs were injected into a total of 372 mice. CP and TMZ were injected into 95 mice, respectively. Both IFO and BCNU were injected into 91 mice. The antitumoral effect of each drug was tested in groups of 5–12 mice (median, 8) grafted with the same tumor. Univariate analysis showed that loss of 1p alone ($P < 10^{-4}$), loss of 19q alone ($P = 0.008$), loss of 1p ± 19q ($P = 0.008$), and *TP53* mutation ($P = 0.03$) were significantly related with a better response to treatment. Tumors with *EGFR* amplification were poorer responders ($P < 10^{-4}$) than tumors without *EGFR* amplification. After multivariate analysis, only the loss of 1p, with or without an associated loss of 19q, remained related to a better response rate ($P < 10^{-4}$), and EGFR amplification remained related to a poorer response to treatment ($P = 0.002$).

**Correlation between Genetic Alterations and Individual Response to Treatment.** Despite the limited sizes of the groups, their great homogeneity prompted us to do a statistical analysis to relate genetic alterations and individual drug responses. Alterations were first considered independently and then stratified depending on the number of early and late alterations present in the tumor. Independent analysis showed that, after multivariate analysis, no individual alteration was related to resistance to CP and IFO, whereas *PTEN* alteration and 9p LOH were related to resistance to TMZ and BCNU, respectively ($P < 10^{-4}$ each). Grouped analysis showed that, after multivariate analysis, the presence of at least four late alterations was significantly associated with resistance to CP and IFO ($P < 10^{-4}$ each). The presence of four late alterations was significantly associated with resistance to TMZ in univariate analysis but was not significant after multivariate analysis.

## DISCUSSION

Using a series of 12 malignant human gliomas growing in *nude* mice, we showed that chemosensitivity to four different alkylating agents, BCNU, IFO, CP, and TMZ, varied considerably according to the tested agent, the histological type, and the molecular profile of the tumors.

Oligodendrogliomas were clearly more responsive than GBM despite selection of the most anaplastic subtypes by the grafting process (24). Oligodendrogliomas responded variously to the four tested drugs, and chemosensitivity to TMZ was higher.

In contrast, as expected, GBMs were more resistant. One GBM (GBM7-Rom) responded to IFO, and only one (GBM21-Nan) exhibited a significant response to three of the four tested agents. These observations are in agreement with the clinical observation that GBMs are globally less sensitive than oligodendrogliomas and also showed that histology of the original tumor is an important, but not sufficient, factor to predict response to chemotherapy.

Loss of 1p ± 19q was the only factor associated with chemosensitivity. Indeed, TGI was much higher in tumors with 1p ± 19q LOH (73 ± 4%) than in tumors with intact 1p-19q chromosomes (49 ± 7%;

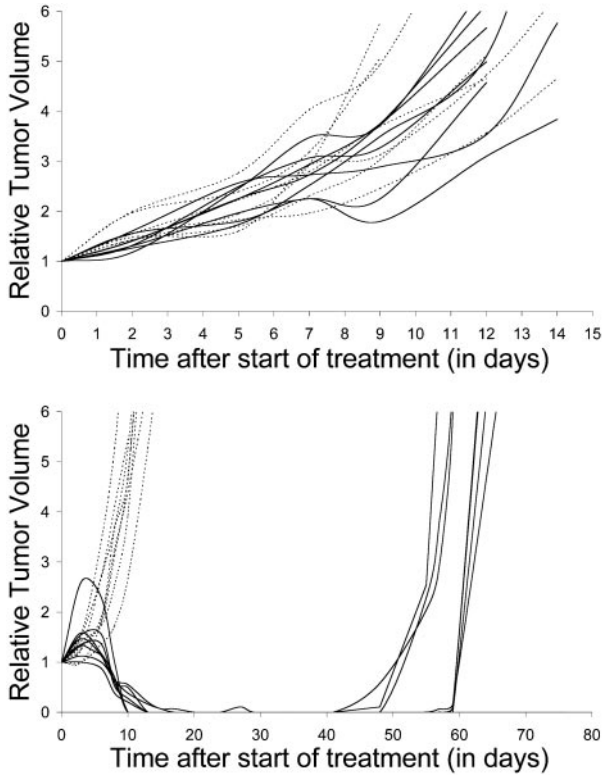


Fig. 1. Representative response of two tumor types to temozolomide (TMZ) treatment. The *top panel* shows that none of the treatment group for GBM14-Cha responded to TMZ [tumor growth delay index = 1.1 (control group, $n = 7$ mice; treated group, $n = 8$ mice)]. The *bottom panel* shows that all of the treated group for ODA14-Rav had significant responses to TMZ [tumor growth delay index = 6 (control group, $n = 8$ mice; treated group, $n = 8$ mice)]. *Dotted lines,* control group; *solid lines,* TMZ-treated group.

Table 4 *Molecular characteristics of xenografts*

As reported previously (24), molecular profiles of xenografted tumors show many alterations, which correspond to the most malignant gliomas from a molecular point of view. None of the oligodendrogliomas exhibited loss of 1p + 19q alone. These LOH[a] lesions are often associated with other alterations, such as loss of 10q or EGFR amplification. GBMs exhibited a constant loss of 10q, and one (GBM21-Nan) had a loss of 1p + 19q, suggesting an oligodendroglial origin.

| | 1p | 1q | 19q | 9p | 10p | 10q | *TP53* | *PTEN* | *EGFR* | *CDKN2A* | Telomerase activity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oligodendroglioma xenografts | | | | | | | | | | | |
| ODA4-Gen | LOH | – | LOH | LOH | LOH | LOH | – | – | Amp | HD | Detectable |
| ODA5-Rai | LOH | LOH | LOH | – | LOH | – | Mut | – | – | – | Undetectable |
| ODA14-Rav | LOH | LOH | – | – | LOH | LOH | Mut | – | Amp | – | Detectable |
| ODA20-Tho | –[b] | – | LOH | LOH | – | LOH | Mut | HD | – | – | Undetectable |
| ODA17-Gir | – | – | – | – | LOH | LOH | – | – | Amp | HD | Detectable |
| ODA25-Nan | – | – | – | – | LOH | LOH | – | – | – | – | ND |
| ODA23-Nan | – | – | – | – | – | – | Mut | – | – | – | ND |
| ODA24-Nan | – | – | – | – | – | – | – | – | – | – | ND |
| GBM xenografts | | | | | | | | | | | |
| GBM21-Nan | LOH | LOH | LOH | LOH | LOH | LOH | – | – | Amp | HD | ND |
| GBM1-Ham | – | – | – | LOH | LOH | LOH | Mut | HD | – | HD | Detectable |
| GBM7-Rom | – | – | – | – | LOH | LOH | – | – | – | – | Detectable |
| GBM14-Cha | – | – | – | LOH | LOH | LOH | – | Mut | – | HD | Detectable |

[a] LOH, loss of heterozygosity; *PTEN*, phosphatase and tensin homolog tumor suppressor gene; *EGFR*, epidermal growth factor receptor; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; Amp, genomic amplification; HD, homozygous deletion; Mut, mutation; ND, not determined; GBM, glioblastoma.
[b] Not altered.



$P = 0.003$). The results were less clear when individual agents were considered (response to IFO was the only one to be significantly linked to 1p ± 19q deletion), but this probably reflects the lack of statistical power in our subgroup analysis. Interestingly, one GBM (GBM21-Nan), which was the only responder to three of the four tested agents, also had 1p-19q deletions in addition to the genetic alterations usually associated with GBM.

Several clinical studies have investigated the prognostic value of *EGFR* genomic status, but the results have been rather conflicting (13–18, 26–28). Moreover, the question of chemosensitivity has never been specifically addressed. In our series, *EGFR* amplification was the only single alteration associated with drug resistance in the global analysis. However, when the response to each drug was analyzed separately, the number of genetic alterations known to be associated with malignant progression (at least four late alterations) was predictive of chemoresistance. These data strongly suggest that *EGFR* amplification is predictive of chemoresistance regardless of the histological subtypes of tumors.

The influence of *TP53* on outcome and response to treatment is controversial (15, 32–38). In our series, *TP53* mutations were correlated with a better response to overall chemotherapy in univariate analysis, but this correlation was not confirmed in multivariate analysis. *TP53* status was not correlated with the response to individual treatments in either univariate or multivariate analysis (CP, $P = 0.81$; BCNU, $P = 0.39$; IFO, $P = 0.1$; TMZ, $P = 0.62$), suggesting that *TP53* status is not predictive of response to chemotherapy with alkylating drugs.

Although much work remains to be done to reliably predict response to a given agent even in this simplified model, this study supports the view that combining histological analysis (anaplastic oligodendrogliomas are more chemosensitive than glioblastomas) and molecular profiling (loss of 1p ± 19q, *EGFR* amplification, and the number of late alterations) could eventually be useful in the selection of potentially sensitive tumors as well as the choice of therapy.

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