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Patients with High-Grade Gliomas Harboring Deletions of Chromosomes 9p and 10q Benefit from Temozolomide Treatment[1]

Silke Wemmert[*,†,2], *Ralf Ketter*[†,2], *Jörg Rahnenführer*[‡], *Niko Beerenwinkel*[‡], *Martin Strowitzki*[†], *Wolfgang Feiden*[§], *Christian Hartmann*[¶], *Thomas Lengauer*[‡], *Florian Stockhammer*[#], *Klaus D. Zang*[*], *Eckart Meese*[*], *Wolf-Ingo Steudel*[†], *Andreas von Deimling*[¶] *and Steffi Urbschat*[*,†]

*Institute of Human Genetics, Saarland University, Homburg/Saar D-66421, Germany; †Department of Neurosurgery, Saarland University, Homburg/Saar D-66421, Germany; ‡Max-Planck-Institute for Informatics, Stuhlsatzenhausweg 85, Saarbrücken D-66133, Germany; §Institute of Neuropathology, Saarland University, Homburg/Saar D-66421, Germany; ¶Institute of Neuropathology, Charité, Universitaetsmedizin Berlin, Berlin D-13353, Germany; #Department of Neurosurgery, Charité, Universitaetsmedizin Berlin, Berlin D-13353, Germany

Abstract

Surgical cure of glioblastomas is virtually impossible and their clinical course is mainly determined by the biologic behavior of the tumor cells and their response to radiation and chemotherapy. We investigated whether response to temozolomide (TMZ) chemotherapy differs in subsets of malignant glioblastomas defined by genetic lesions. Eighty patients with newly diagnosed glioblastoma were analyzed with comparative genomic hybridization and loss of heterozygosity. All patients underwent radical resection. Fifty patients received TMZ after radiotherapy (TMZ group) and 30 patients received radiotherapy alone (RT group). The most common aberrations detected were gains of parts of chromosome 7 and losses of 10q, 9p, or 13q. The spectrum of genetic aberrations did not differ between the TMZ and RT groups. Patients treated with TMZ showed significantly better survival than patients treated with radiotherapy alone (19.5 *vs* 9.3 months). Genomic deletions on chromosomes 9 and 10 are typical for glioblastoma and associated with poor prognosis. However, patients with these aberrations benefited significantly from TMZ in univariate analysis. In multivariate analysis, this effect was pronounced for 9p deletion and for elderly patients with 10q deletions, respectively. This study demonstrates that molecular genetic and cytogenetic analyses potentially predict responses to chemotherapy in patients with newly diagnosed glioblastomas.

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Keywords: CGH, LOH, glioblastoma multiforme, survival, TMZ.

Introduction

Treatment of diffuse gliomas still remains one of the most disappointing tasks in oncology. Surgical cure of these infiltrating brain tumors is virtually impossible. The clinical course is determined by the biologic behavior of the tumor, including growth rate and response to radiation and chemotherapy.

Neuropathologic diagnosis is the most valuable tool for the classification of human tumors. However, within neuropathologic entities, morphologic and immunohistochemical analyses usually cannot predict prognosis and response to therapy. However, molecular genetic analysis has succeeded in determining distinct genetic subgroups that may exhibit different biologic behavior [1–3].

Trials on the effects of systemic chemotherapy on survival and recurrence in adults with high-grade gliomas showed extended survival times, but there was no evidence that the effect of chemotherapy depended on age, sex, histology, Karnofsky performance status (KPS), or extent of resection [4]. However, patients of young age and with complete resection have a considerably better prognosis [5–7]. Temozolomide (TMZ), an orally administered second-generation imidazotetrazine, has been demonstrated to increase the survival time of glioma patients [8]. Phase II studies of TMZ (*versus* procarbazine) showed that TMZ has an acceptable safety profile and can improve the quality of life [8–10].

Abbreviations: CGH, comparative genomic hybridization; GBM, glioblastoma multiforme; KPS, Karnofsky performance status; LOH, loss of heterozygosity; *MGMT*, methylguanine methyltransferase; MRI, magnetic resonance imaging; sGBM, secondary glioblastoma multiforme; SSC, sodium saline citrate; ST, survival time; TMZ, temozolomide; WHO, World Health Organization

Address all correspondence to: Steffi Urbschat, PhD, Neurooncology, Institute of Human Genetics, Saarland University, Homburg/Saar D-66421, Germany.
E-mail: hgsmur@uniklinik-saarland.de

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[2]S. Wemmert and R. Ketter contributed equally to this work.

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Numerous studies revealed that the most common somatic chromosomal changes in malignant gliomas are complete or partial loss of chromosome 10 and gain of chromosome 7. Various molecular genetic alterations have been identified, including the amplification of *EGFR*, *CDK4*, and *MDM2*, as well as the deletion of tumor-suppressor genes like *TP53* (17p), *RB* (13q), *CDKN2A* (9p), *CDKN2B* (9p), *PTEN* (10q), and *DMBT1* (10q) [2,11–15]. These tumor-suppressor genes play crucial roles in the regulation of cell proliferation and apoptosis. The *TP53* gene product, p53, is involved in the regulation of cell repair, apoptosis, and cell cycle. Cyclin-dependent kinases (cdk), such as CDK4 and their inhibitors, p16 and p15, proteins from *CDKN2A* and *CDKN2B*, and pRB, are key regulators of the cell cycle. The gene products of *CDKN2A*/*B* locus on 9p also participate in the *TP53* pathway through a protein encoded by an alternate reading frame, $p14^{arf}$, which binds to the p53/MDM2 complex and inhibits MDM2-mediated degradation of p53. Therefore, homozygous deletion of the *CDKN2A*/*B* locus affects both *Rb* and *TP53* pathways [16].

In recent years, studies have identified a correlation between alterations on chromosome 10q and shorter survival in patients with high-grade glioma. Tada et al. [3] reported significantly shorter survival rates of patients with glioblastoma multiforme (GBM) with loss of heterozygosity (LOH) on 10q containing the *PTEN*/*MMAC1* gene, and in anaplastic astrocytoma patients with LOH on 10q in the region containing *DMBT1*. Several authors [3,17–19] detected poorer survival rates associated with LOH on 10q in glioblastomas, but showed that *PTEN* mutation is only marginally associated with survival [17,20].

A further candidate on chromosome arm 10q is *MGMT*. The *MGMT* gene encodes for the DNA repair enzyme O^6-alkylguanine-DNA-alkyltransferase, which is responsible for protecting cells from alkylating agents. The enzyme efficiently removes methyl adducts at the O-6 position of guanine, which is an important target of alkylating agents [21]. It could been shown that the sensitivity to alkylating agents like BCNU correlates inversely to *MGMT* activity [22,23]. The responsiveness to BCNU is associated with an increase in overall survival rate [24]. Further on, the presence of aberrant promoter hypermethylation of *MGMT* was associated with loss of the MGMT protein, in contrast to retention of protein in the majority of tumors without hypermethylation [25]. Further clinical trials suggested that methylation of the *MGMT* promoter is predictive for better outcome in patients with malignant gliomas treated with alkylating agents such as TMZ [26–28].

Gains of chromosome 7 are known to be associated with shorter patient survival in anaplastic astrocytomas and low-grade astrocytomas [29,30], but, to our knowledge, no correlation between additional copies of chromosome 7 and survival in GBM has been found so far. However, *EGFR* amplification is considered to be an unfavorable marker for survival [31,32]. Further indicators of poor prognosis are LOH on 9p [17,33] and *p16* mutations [34].

Chemosensitivity and prolonged overall survival of patients with anaplastic oligodendroglioma have recently been linked to specific genetic alterations, namely LOH on 1p or combined LOH on 1p and 19q, and the absence of homozygous deletion of the *CDKN2A* tumor-suppressor gene on 9p21 [19,35]. Apart from these data on the effect of genetic changes on the overall prognosis of gliomas, there is no information at the moment on the significance of further genetic changes on therapy response. Therefore, we analyzed a series of TMZ-treated patients in comparison to a retrospective, conventionally treated control group with newly diagnosed glioblastoma with respect to the above-mentioned typical chromosomal alterations in glioblastomas.

The aim of this study was to determine whether specific genetic markers predict response to TMZ chemotherapy and may serve as parameters for the rational design of chemotherapy.

Materials and Methods

Patients

In total, 80 cases of newly diagnosed glioblastomas operated on during the period of 1997 to 2003 were studied (Table 1). The patients were treated in two centers: 48 patients in the Department of Neurosurgery of the Saarland University and 32 patients in the Department of Neurosurgery, Charité, University Berlin. Patients eligible for this nonrandomized study were 18 to 70 years of age, with a histologically proven GBM (World Health Organization [WHO] grade IV astrocytoma) [2] and a KPS of 70 or better. Patients with renal, hepatic, or bone marrow impairment; HIV infection; prior chemotherapy; or stereotactic biopsy were excluded. All patients underwent radical resection followed by radiotherapy within 4 weeks of surgery. Radiotherapy consisted of fractionated focal irradiation at a dose of 1.8 to 2 Gy per fraction, given once daily 5 days per week over a period of 6 weeks, for a total dose of 60 Gy. Radiotherapy was delivered to the gross tumor volume with a 2-cm margin volume for the clinical target volume on a preoperative magnetic resonance imaging (MRI).

Two groups of patients were defined. The first group included patients from March 1997 to April 1999, treated after radical resection with radiotherapy alone (control group). In the second group, patients from the time period from April

Table 1. Patient Characteristics.

Patients	TMZ (n = 50)	Conventionally Treated (n = 30)
Age (years)		
Median	53	58
Range	26–72	24–77
Still alive	17	3
Gender		
Male	33	20
Female	17	10
KPS		
100	27	6
90	19	16
80	4	2
70		6

1999 to November 2003 were included. This group received, after radical resection, the abovementioned radiotherapy regime and an adjuvant chemotherapy consisting of TMZ (TMZ group). TMZ was administered as a test dosage of 150 mg/m^2 body surface area per day (750 mg/m^2 total dose per cycle) on days 1 to 5 in the first cycle because of its dose-limiting toxic effect resulting in thrombocytopenia. The following cycles were performed at a dosage of 200 mg/m^2 per day (1000 mg/m^2 total dose per cycle). Treatment cycles were repeated every 28 days. Altogether, eight cycles were carried out. Patients were seen at least every 3 months in the first 2 years. At each follow-up visit, clinical performance status, neurologic status, and MRI were recorded.

Specimens of resected tumors were immediately frozen and stored at −80°C, or fixed in formalin and embedded in paraffin. All patients gave written informed consent for the use of the tumor samples for genetic analysis.

Comparative Genomic Hybridization (CGH)

DNA was obtained using standard protocols. Reference DNA from the blood of a healthy donor and tumor DNA from frozen tumor tissues were labeled with biotin and digoxigenin by standard nick translation (Roche Diagnostics, Mannheim, Germany). Six hundred nanograms of each tumor and reference DNA was hybridized together with COT1 DNA (Roche Diagnostics) to normal chromosome metaphase spreads from peripheral blood lymphocytes prepared following standard procedures. After 3 to 4 days of hybridization at 37°C, posthybridization washes were performed at a stringency of 50% formamide/2× standard saline citrate (SSC), 2× SSC, and 0.1× SSC at 45°C. Tumor DNA was visualized with fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA) and reference DNA with rhodamine (Roche Diagnostics). Fluorescence images were captured using a fluorescence microscope Olympus AX 70 (Olympus, Hamburg, Germany) with a cooled charged-coupled device camera. Image processing was performed by use of ISIS (MetaSystems, Altlussheim, Germany). Average ratio profiles were determined from analyses of 10 to 15 metaphases. The thresholds used for ratio profiles were 1.2 for gain and 0.8 for loss.

Because of suppression with COT1 DNA, the fluorescence intensities were not representative at chromosome regions with tandem repetitive DNA clusters (i.e., at the heterochromatic blocks on chromosomes 1, 9, 16, and Y, at the centromeric regions, and along the short arms of acrocentric chromosomes). These areas were excluded from evaluation. Chromosome 19 and the chromosomal segment 1p34-pter were also excluded from the analysis because results for 19 and 1p34-pter have been observed to be prone to artifacts in our and others' laboratories [36].

Microsatellite Analysis for LOH

DNA from frozen tumor tissues and, if not available, DNA from paraffin-embedded tumor sections (n = 24) was separated using previously published protocols [37,38].

The following regions were examined for allelic losses by nonradioactive microsatellite analysis: chromosomal arm 10p with markers D10S1172, D10S1159, D10S527, and D10S506; chromosomal arm 10q with markers D10S1419, D10S1171, D10S523, D10S1765, D10S1143, D10S 1173, D10S520, D10S521, D10S1141, D10S503, D10S1165, D10S1439, D10S505, D10S1134, and D10S1248; chromosomal arm 13q with markers D13S326, D13S887, D13S788, and D13S773; and chromosomal arm 9p with markers D9S759, D9S925, D9S1121, and D9S319. Nucleotide sequences and mapping information were retrieved from the Human Genome Database (www.gdb.org) or the Cooperative Human Linkage Center (www.chlc.org) database files. Polymerase chain reaction (PCR) was performed in a final volume of 10 μl containing 10 ng of DNA, 50 mM KCl, 10 mM Tris−HCl (pH 8.3), 200 μM deoxynucleotides, 0.1% gelatin, and 20 pmol of each primer. Taq polymerase (Gibco BRL/Life Technologies, Karlsruhe, Germany) was used and the $MgCl_2$ concentration ranged from 1.0 to 2.0 mM, depending on the primer pair. Initial denaturation at 95°C for 3 minutes was followed by 29 cycles on a thermocycler (Biometra, Goettingen, Germany), denaturation at 95°C for 30 seconds, annealing at temperatures ranging from 52°C to 64°C for 40 seconds, and extension at 72°C for 30 seconds. A final extension step of 10 minutes at 72°C was added. PCR products were separated on 8% denaturating acrylamide gels and visualized by silver staining [39]. LOH was scored as previously described [38].

Statistical Analyses

Subgroups of patients were defined by genetic status, therapy, and age (old patients, > 53 years; young patients, ≤ 53 years). We chose this classification because the median age was 54 years. Comparisons between groups were performed by Kaplan-Meier curves and Cox regression analysis.

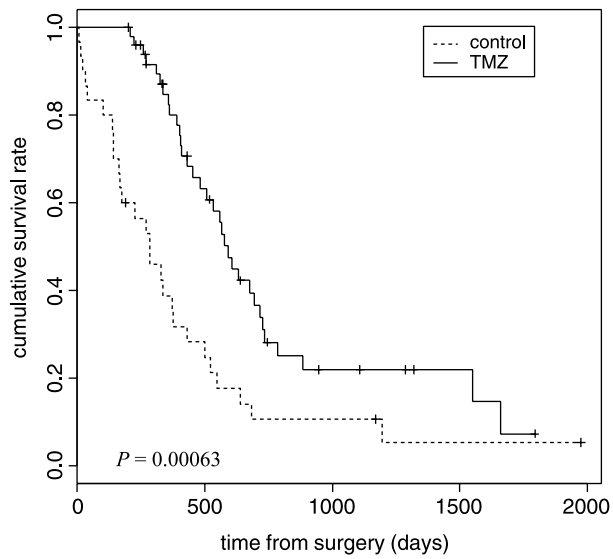

Figure 1. *Kaplan-Meier estimates for patients with GBM treated with TMZ and radiotherapy (TMZ; solid lines)* versus *the untreated control group (radiotherapy alone; dashed lines) had significantly higher overall survival. Censored data (patients still alive) are plotted as hash marks.*

Table 2. Clinical Characteristics and CGH Results of TMZ-Treated and Conventionally Treated High-Grade Gliomas.

Case Number	Tumor	Age/Sex	Chromosomal Imbalances by CGH		
			ST (months)	Gains	Losses
TMZ-treated high-grade gliomas					
1782	sGBM	26/M	24.2	19p, X	16p13.1p13.3
265	GBM	46/F	23.1	None	10q25.3qter, 16p12p13.3
1349	GBM	54/M	33.8	X	16p, Y
1099	GBM	49/M	23.8	7, 12q13.2q13.3	1p31.3p32.3, 1q32.1q41, 10q
369	sGBM	37/M	14.3	1q, 5p15.1pter, 7q31.3qter, 8q21.1qter, 9p, 10p, 12p, 15q25qter, 18p11.2pter	1p33p36.1, 4q21.1qter, 5q21qter, 10q21.3qter, 11p12p15.3, 13q12.1q31, 15q14q21.2, 19
1534	GBM	62/M	18.4	7q11.2q21, 19p13.1p13.3, X	4p16pter, 10q24.1qter, 13q21.3qter
1106	GBM	70/M	19.8	7q21.1qter, 12q13.1q14, 19p	10q, 13q12.3q22, Y
662	GBM	70/M	13.4	3q24q26.3, 7p11.1p12, 7q31.1q34, 18q12.1q21.1, X	4p16, 8p22pter, 10q, 16p11.2p13.2, Y
1326	GBM	56/M	19.2	7q34qter, 12p13.1pter	10q21.3qter, 22q13.1q13.3
1515	sGBM	40/F	11.2	19p	2p23pter, 3p24.2p26, 21q21qter
1707	GBM	47/M	29.4	7, 12q13.3q14	10q21.1qter
1691	sGBM	39/F	17.4	None	1p35p36.1, 4q27q32, 9p21, 9q21.2q32, 14
497	GBM	58/F	16.9	7p11.2p13, amp 1p36pter	6q12q21, 9p23p24
1460	GBM	31/F	11.9	2q34q37.1, 3p26pter, 3q23.2qter, 4p11p16, 4q11q13.3, 8p22pter, 9, 15q22.3qter, 18q12.3qter, 19q11q13.1, 20p, 22	3q11.1q25.2, 5p, 13q12.3qter, 16q, 20q11.2qter, Y
896	GBM	51/M	13.0	7	6q22.2qter, 9p21pter, 10p12.2pter, 10q23.1qter, 13q13qter, 14, 16q13qter, 22q12.2
1405	GBM	53/M	7.4	4q31.3q33	8p23.1pter, 16p, 17, 19q13.2qter
643	GBM	54/F	15.3	4p16pter, 7p11.1p13, 7q32qter, 16p12pter, 19q, 22	10, 13q14.3q22.1
1795	sGBM	31/M	6.9	None	5p15.1pter, 6q16.3qter, 8p23.2pter, 9p13pter 18q12.1qter
T4789	GBM	38/M	44.1	8p, Xq23q28	3q13.1q25.3, 9p
T5958	GBM	63/F	14.4	2q22q35, 3p11.1p14.1, 3p25pter, 3q13.1q21, 3q24q26.3, 4p14p15.3, 4q21.2q34, 5p14, 5q13.1q22, 6q14q23.1, 7, 8q22.3, 11p13p15.3, 11q14.1qter, 13q14.1q33, 14q12q22, 20p12pter, amp 7p11.1p11.2	1p32.3pter, 10, 12q23q24.3, 16p, 17p, 17q24qter, 18p11.2, 19, 22, Y
784	GBM	52/M	22.5	7p13p22, 7q11.1q22, 19p, X, amp 7p11.1p13	4p16, 8p22pter, 9p11p13, 9p23pter, 10p13pter, 10q11.2q21.2, 16p
T6002	GBM	53/M	11.2	5, 6, 7, 12p, 12q11q14, 12q24.1qter, 15, 19, 20, amp 7p11.1p11.2	1p21p31.3, 3p, 3q23q26.3, 10, 13, 17p11.1p13, 18, 22
1536	GBM	53/M	15.1	3p11.1p14.1, 3q11.1q26.3, 7, 9q, 21q21qter, X	1p33p36.1, 4p15.1pter, 10, 12p12.1pter, 12q11q21.3, 15, 17q12q23, 18p11.1p11.2, 19q13.3qter, 20p11.1p11.2
1940	GBM	54/F	17.3	5p13.3p15.2, 7p15.3pter, 18p	8q22.1q24.1, 10, 12q14q23, Xp11.4pter, Xq13q21.3
6	GBM	41/M	26.2	6q12q15, 6q25.1qter, 7, 12q14q21.1, X	4p16, 9p11p23, 10, 11q12q14.3, 15q11.1q22.2, 20p11.1p11.2, Y
596	GBM	32/M	21.1	7, 12q13.3q21.1, 17p11.1p12, 19, Y	3q26.1q36.3, 10, X
947	GBM	46/F	17.7	7, 15q24q26.1, 18p, 19, 20q	9p21p23, 10q21.1q25.2
XXL	GBM	45/M	8.6	7p15.1pter, 7q11.1q31.3, amp 2p22pter	1p36pter, 10, 13q13q21.1, 17p12pter, 22q13.1qter, X
T6044	GBM	37/M	7.6	18p11.1p11.2, 19p, X	6q25.2qter, 7q36
Conventionally treated high-grade gliomas					
861	GBM	70/F	22.7	2p21p23, 7p12, 12q14q21.3, 13q21.3q32	4p15.3pter, 8p22pter, 16p, 19p, 20q13.1qter
1856	GBM	46/M	65.8	13q21.1q32	1p34pter, 17q12q21.3, 19q, 22, Y
1028	GBM	62/M	5.6	6q11q14, 7, 19p, X, Y	10, 14, 15, 18
2046	GBM	39/M	39.8	1p13.3p31.1, 1q25q41, 3p24.1pter, 3p11.1p13, 3q11.2q13.3, 3q24q26.3, 4p11p15.2, 4q12q13.3, 4q22q28, 5p13.3pter, 5q12q23.3, 6q12q23.1, 7, 8p22pter, 9p, 14q11.2q24.1, 18p11.3pter, 20p13, 21q11.1q22.1	1p35pter, 10, 11, 12q22qter, 13q12.1q14.1, 13q32qter, 15q21.3qter, 17, 19, 20q11.2qter, 22
T6025	GBM	73/M	6.3	1p34.3p36.1, 1q31q43, 3p14.1p21.1, 7, 16p11.1p13.2, 17p11.1p12, 17q, 18p11.1p11.3, 19p, Y, amp 7p11.1p12	2p24pter, 4p15.1p15.3, 5p15.2pter, 8p21.3pter, 8q22.1q24.1, 10, 12q15q21.3, 13q14.3qter, 14, 15q21.3qter, 20p12pter
393	GBM	65/M	4.6	1p, 3, 7, 12q11q13.2, 12q23q24.3, 16p, 17q, 19, 20q, 22. amp 7p11.1p12	1q32.2qter, 9p, 10, 13q22q31, 21q11.1q21
T5954	GBM	61/M	3.3	1p24.1pter, 11q11q14.1, 16p, 18p11.1p11.2, 19, 20q, 22q12.3q13.3, Xp, Xq11.1q21.2	9p13pter, 13, 14q11.1q12, 18q12.1qter
1819	GBM	76/F	1	4q32qter, 5p14pter, 5q23.3q35.1, 6p23pter, 6q22.3qter, 7q33q36, 8q23q24.2, 13q21.3q33, 18q12.2qter	17q11.2q21.3, 19p
63	GBM	77/F	10.9	7p11.1p12, 7q11.1q11.2	5p, 5q11.2q23.1, 6p23pter, 6q15q22.3, 9p23pter, 18p11.3pter
T6052	GBM	24/F	4.8	5, 7, 12, 18, 19p, amp 12p13.1, 18q11.1q11.2	3p25pter, 3q26.3qter, 6p23pter, 8q22.1qter, 9p21pter, 11q23.3qter, 13q21.3q31, 20q13.2qter
832	GBM	57/M	8.9	4, 5, 7, 8q22.1q24.3, 14q11.1q12, 14q24.3q31, 17q24q25, 19, 20, 22, X	3p, 9p13p24, 9q22.1q31, 10, 21q22

(*continued on next page*)

Table 2. (*continued*)

Case Number	Tumor	Age/Sex	Chromosomal Imbalances by CGH		
			ST (months)	Gains	Losses
Conventionally treated high-grade gliomas					
T4795	GBM	66/M	0.2	4p15.1p16, 6q24q27, 7p15.1p22, 18q12.3q23, 20p13	10, 11p, 11q11q23.1, 13, 14, 19, 22, X, Y
838	GBM	56/w	1.3	7, 16, 17q12q21.3, 19, X, amp 7p11.2p12	9p21p24, 10, 13, 14, 22
T4803	GBM	59/M	9.4	1p32.1p36.3, 7, 9q32q34.2, 12q13.1q14, 15q22.1q24, 17, 20q11.1q13.1	10, 14
H549	GBM	38/M	5.5	3q21q23, 3q26.2qter, 7q21.3q31.1, amp 12q11q14	6q21q23.3, 10q, 11p, 11q11, 13q12.1qter, 14
H321	sGBM	48/M	18.2	19p, 20q11.2q13.1, X	5p15.2pter, 9p21pter, 10q, Y
H147	GBM	48/M	7.5	7q11.1q11.2, 19, 20q11.1q13.1	10p13pter, 10q11.2q21.3
H281	GBM	56/M	12.5	7p11.1p12	None
N111	sGBM	24/M	17.4	1p32pter, 11q13, 17, 19, 20, 22	6q16q23

sGBM, secondary glioblastoma multiforme; M, male; F, female; ST, survival time; amp, amplification.

In the univariate Cox proportional hazards model, for a given genetic status, the main effect of therapy on survival was tested. In the first multivariate Cox model, for a given genetic status, main effects of therapy, age, gender, and KPS were estimated. In the second multivariate Cox model, main effects of therapy and age and an interaction effect between therapy and age were estimated. All effects were quantified by hazard ratio estimates with 95% confidence intervals (CIs). All *P* values were calculated with two-sided tests. Median survival rates were calculated using the Kaplan-Meier method. Association between dichotomous variables was tested by chi-square analysis.

LOH data were summarized per chromosomal arm. LOH was defined as present if at least one loss on the respective chromosomal arm was detected. For chromosome arm 10q with a large number of primer, LOH was defined as present if the majority of the considered regions were lost.

Results

Univariate and Multivariate Prognosis Analyses

Treatment, gender, and age In total, 60 patients had died and 20 were alive during the last follow-up (20 censored data). Median follow-up was 17.2 months and median age at diagnosis was 54 years.

Median overall survival for the TMZ group ($n = 50$; median age, 53 years) was 19.5 months and for the control group ($n = 30$; median age, 58 years) was 9.3 months. Univariate analysis showed that TMZ chemotherapy was significantly associated with longer survival rates ($P = .00063$; Figure 1).

Multivariate analysis indicated that the strongest factors associated with survival were chemotherapy and age. Younger patients survived longer than older patients (> 53 years of age) in the control group ($P = .024$). However, for older patients, a prolonged survival time under adjuvant chemotherapy treatment was detectable ($P = .00014$), but not for younger patients ($P = .96$). There was no significant difference in survival time by age in the TMZ-treated group ($P = .83$).

Genetic status, treatment, gender, and age Clinical data, CGH, and LOH results of patients in the TMZ and control groups are summarized in Tables 1–3. Both groups showed a similar genetic pattern. The most frequent genetic aberrations were complete or partial losses of chromosome 10 (63%) and complete or partial gains of chromosome 7 (58%). Further deletions were detectable on 9p (29%) and 13q (35%) (Figure 2).

Subgroups of patients were defined by their genetic pattern. In the univariate model, patients with deletions of 9p and 10q, respectively, did associate with better prognosis under treatment (Figure 3; Table 4). No correlation with treatment was observed for gains of chromosomes 7p and 12, losses of chromosome 13, and gender. Due to the methodical limitations of LOH, only deletions could be detected. A positive effect on survival was observed for better KPS and younger age (Table 5).

In the multivariate Cox proportional hazards models, for the genetic subgroups, the main effects of TMZ treatment, gender, KPS, and age, as well as an interaction effect between treatment and age, were estimated. Patients with deletions of 9p showed a positive correlation between TMZ chemotherapy and survival also in multivariate analysis, whereas gender, age, and the interaction of age and treatment had no effect (Table 6). Patients with deletions on 10q and higher age had a poorer prognosis ($P = .0076$). However, the negative effect of the genetic status and age was compensated by TMZ treatment ($P = .0042$) (Table 7).

LOH analysis of the *CDKN2A* region, as well as LOH on chromosome arm 10q and the *MGMT* region, respectively, produced results similar to that of CGH (Tables 6 and 7; Figures 4 and 5). The only difference was the association of high KPS and better prognosis in patients with losses of 9p and 10q, in contrast to the corresponding CGH data.

Discussion

Today, the aim of chemotherapy in patients with glioblastomas is palliation and improvement of health-related quality of life (HQL), as well as prevention of neurologic

Table 3. LOH Results and Comparison with CGH Data of TMZ-Treated and Conventionally Treated High-Grade Gliomas.

Tumor	ST (months)	CGH 10q*	*PTEN* LOH/Inf†	*MGMT* LOH/Inf†	CGH 13q*	*RB* LOH/Inf†	CGH 9p*	*p16* LOH/Inf†
TMZ-treated high-grade gliomas								
1795	6.9	0	2/2		0	0/3	1	3/3
1534	40.9	1	1/4	2/4	0	0/2	0	0/2
T4789	55.3	0	0/2		0	0/2	1	1/1
1691	39.9	0	1/4		0	0/2	1	2/2
1326	19.2	1	1/4	0/2	0	0/3	0	0/2
1460	11.9	0	0/5	1/5	1	2/2	0	0/2
643	35.3	1	3/4		1	0/3	0	3/3
369	20.2	1	3/3	3/4	1	2/2	0	0/1
1515	11.2	0	1/1		0		0	1/1
1106	19.8	1	1/4	2/2	1	0/1	0	0/2
497	16.9	0	1/3	2/5	0	0/2	1	
662	13.4	1	2/2	2/6	0	0/3	0	0/3
1782	24.2	0	3/3	4/5	0	0/2	0	2/2
1349	56.8	0	0/5		0	0/3	0	0/2
1099	23.8	1	4/4	4/6	0	0/1	0	1/2
1707	29.4	1	5/5	3/4	0	0/1	0	1/2
596	21.1	1	3/3	4/4	0	0/1	0	0/3
398	14.4		1/4			0/1		2/3
1777	51.6		0/5			0/3		0/1
1536	15.1	1	4/4	4/4	0	1/1	0	0/1
90	12.0		0/0	4/5		0/1		
947	17.7	1	5/5		0	0/3	1	2/3
XXL	8.6	1	1/1	4/5	1		0	0/2
784	22.4	1	1/1		0		0	
1921	3.4	1	5/5		0	0/2	0	0/2
N529/02	21.4		0/5			0/2		0/1
N20/02	24.8		2/2	4/4		0/3		1/2
N690/02	18.6		1/2			0/1		0/3
N1082/02	13.5		1/1			0/1		
N1421/01	13.6		0/1	1/2		0/1		
N934/02	24.5		1/1					
N1124/01	9.0		0/1	0/1				
N1507/01	16.0		5/5			0/3		0/3
N363/02	18.9		0/4	0/4		0/1		1/3
N449/03	8.2		2/3	3/3		2/4		2/3
N1443/02	15.1		6/6					2/2
N528/03	6.7		4/4			0/4		1/2
N438/03	8.9		6/6			0/3		0/2
N202/03	10.8		4/4	2/2		0/2		1/2
N412/03	9.0		0/5			0/3		0/2
N759/01	31.5		0/1	0/3		1/3		0/2
N193/03	11.0		3/3	3/3		1/2		0/2
N1618/02	10.3		1/2	1/2				0/1
Conventionally treated high-grade gliomas								
1856	58.5	0	0/3		0	0/1	0	0/3
1394	0.5		5/5	2/4		2/2		3/3
1819	1.0	0	0/4		0	0/3	0	0/2
2046	39.8	1	4/4		1		0	0/1
63	10.9	0	0/6	2/5	0		1	0/1
T4803	9.4	1	3/3		0	0/3	0	0/3
N111	17.4	0	0/4		0	0/1	0	0/3
2064	18.2	1	0/3	3/3	0	0/2	1	1/2
393	4.6	1	4/4	4/4	1	0/4	1	2/2
1028	5.6	1	0/5		0	0/3	0	0/2
N28/99	5.8		2/4	2/6		0/1		2/2
N1106/00	39.0		1/3	3/4		1/2		0/2
N113/99	9.5		3/4			0/2		2/3
N600/98	16.7		3/3	5/5		0/1		2/3
N1047/00	0.7		4/4			1/1		1/1
N982/00	14.4		1/3			0/2		1/1
N823/01	14.4		2/2			0/1		1/1
N894/00	21.3		0/2			0/2		0/2
N860/01	21.3		0/3	1/2		0/3		0/3
N1320/99	4.7		1/2					
N231/00	12.4		0/2	2/7		1/2		0/2
N358/00	11.2		3/3					1/1

*1, deletion; 0, no deletion determined by CGH.
†LOH at available informative markers.

deterioration. Multimodal therapy approaches had limited success in the treatment of patients with malignant glioma [40–42]. The present studies demonstrate that TMZ chemotherapy after prior radiotherapy significantly prolongs survival among patients with newly diagnosed glioblastomas. Their median overall survival time was 19.5 months, compared to 9.3 months of patients with radiotherapy alone. Due to the retrospective nature of our study, patient selection cannot be excluded. However, the outcome of patients with radiotherapy alone in our study compares to the outcome in the literature. These patients had an overall survival of 7.7 and 12.1 months, respectively [43,44], whereas patients with TMZ chemotherapy and radiotherapy had an overall survival of 13.4 to 16 months [23,43,44]. The survival time of our TMZ-treated group is better than that reported in the papers before, but is in line with the study of Hegi et al. [28]. They reported a median survival of 21.7 months for patients treated with radiation and chemotherapy when defining subgroups according to the *MGMT* promoter methylation status.

Several clinical and histopathologic features are known to be of prognostic significance for survival in patients with glioblastomas. Favorable features include young age and

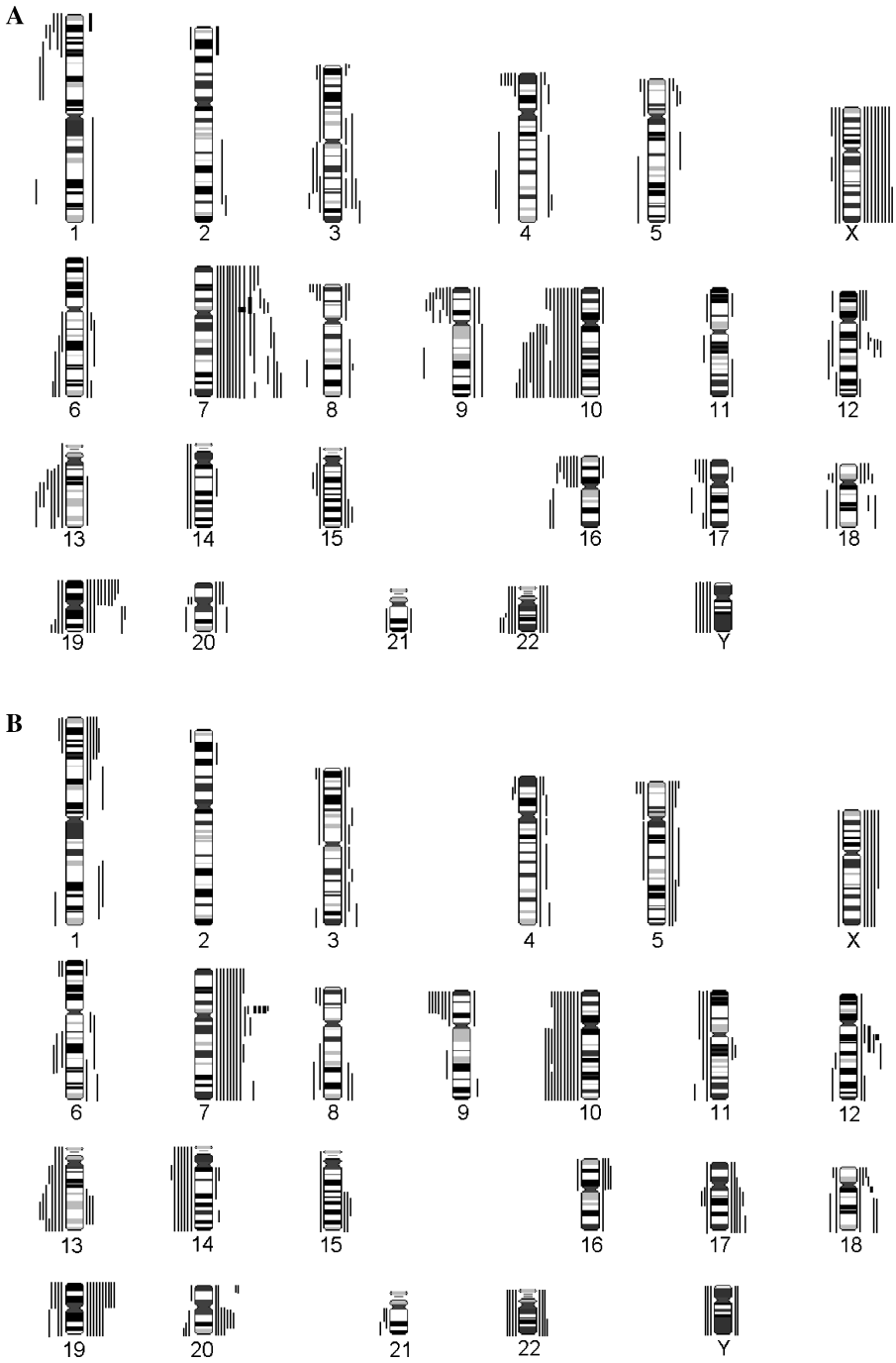

Figure 2. *Overview of genetic imbalances of (A) TMZ-treated GBMs and (B) conventionally treated GBMs. Lines on the left represent losses, and lines on the right represent gains; amplifications are in bold.*

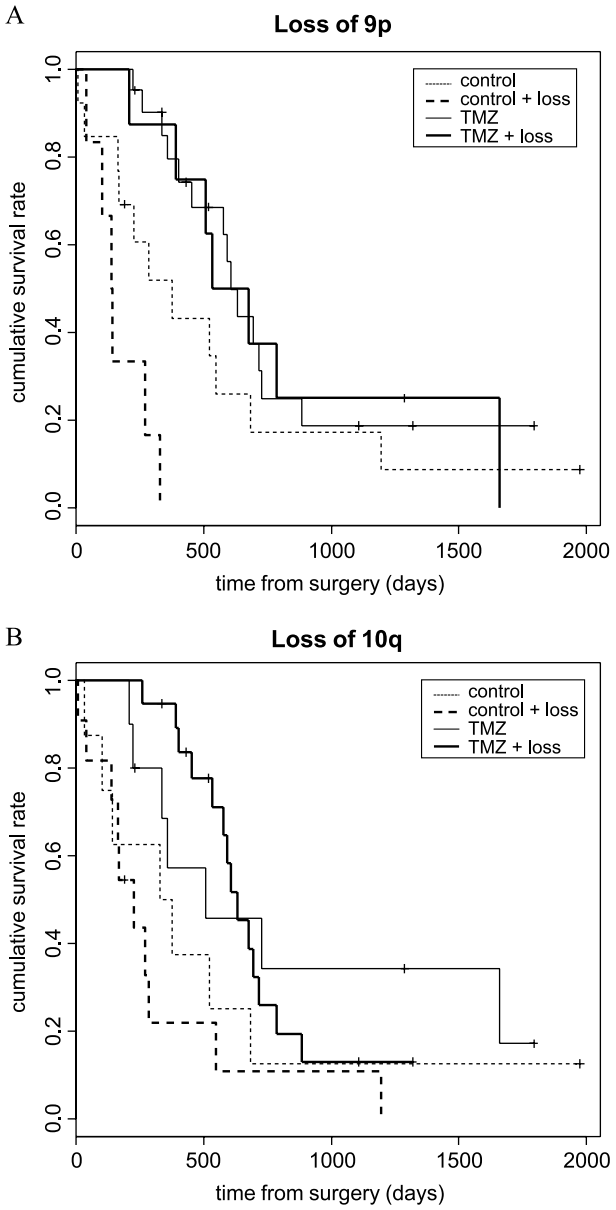

Figure 3. *Kaplan-Meier estimates of overall survival, according to genetic alteration and assignment to TMZ and radiotherapy or radiotherapy alone. (A) Survival curves for patients with loss of 9p (thick lines) and without loss of 9p (thin lines). (B) Survival curves for patients with loss of 10q (thick lines) and without loss of 10q (thin lines), determined by CGH. The Kaplan-Meier estimates for overall survival indicate that the patients with losses of 9p and 10q had significantly longer overall survival rates under TMZ treatment.*

good KPS [2,7,19]. For lymphomas, it was already suggested that increased perioperative and postoperative morbidity and mortality, as well as diminished tolerance to therapy of elderly patients, may be caused by the presence of concomitant diseases [45]. For gliomas, to our knowledge, there are no appropriate results concerning this matter.

In our present study, young age also correlated with better prognosis, but interestingly, the negative impact of age on survival was compensated by the TMZ treatment.

Recent studies suggest that there are genetic subtypes of diffuse gliomas associated with survival time [3,29], and that their clinical course as well as their response to radiation and chemotherapy are primarily determined by the biologic behavior of the tumor cells. Attention has already been drawn to gliomas with LOH on 1p and 19q. These alterations are typical for oligodendroglial tumors [46–49] and have been proposed to be a powerful independent factor for prolonged survival time and favorable response to chemotherapy in WHO grade III anaplastic oligodendrogliomas [35,50]. These genomic alterations are also observed in GBM, however, at lower frequencies, with approximately 10% for LOH 1p and up to 30% for LOH 19q [19,51], but indicating better survival [19].

Uniform numerical and/or structural alterations affecting chromosomes 7, 9, 10, 12, and 13 represent the most common genetic abnormalities in high-grade gliomas [11–13,51] despite genetic heterogeneity [52,53]. Several investigators have noted that LOH on 9p and 10q is associated with shorter survival time in these tumors [3,17–19]. The candidate genes *CDKN2A* and *CDKN2B* on the short arm of chromosome 9, and *PTEN* and *DMBT1* on 10q are discussed to influence survival time. LOH around *PTEN* has been associated with

Table 4. Univariate Analysis of the TMZ Effect on Overall Survival.

Alteration	Hazard Ratio CGH and *LOH* (95% CI)	*P* Value CGH and *LOH* (Two-Sided)
Loss 9p	0.55 (0.0064–0.48)	.0085
	0.16 (0.053–0.5)	*.0015*
Loss 10q	0.31 (0.134–0.729)	.0071
	0.63 (0.28–1.43)	*.27*

Table 5. Univariate Analysis of Effects of Predictors on Overall Survival.

Predictor	Hazard (95% CI)	*P* Value (Two-Sided)
TMZ	0.409 (0.245–0.683)	.00063
Sex	1.03 (0.589–1.79)	.93
Age	1.03 (1.00–1.05)	.017
KPS	0.955 (0.923–0.988)	.0074

Table 6. Subgroup Analysis of Differential TMZ, Age, Gender, and KPS Effects Depending on Genetic Aberration with Respect to Survival Assessed by Cox Proportional Hazard Regression.

Alteration	Variable	Hazard Ratio CGH and *LOH* (95% CI)	*P* Value CGH and *LOH* (Two-Sided)
Loss 9p	TMZ	0.065 (0.0043–0.98)	.048
		0.12 (0.031–0.47)	*.0024*
	Age (> 53 years)	1.63 (0.21–12.76)	.64
		1.04 (0.33–3.25)	*.95*
	Gender	0.81 (0.23–2.87)	.75
		0.61 (0.16–2.34)	*.47*
	KPS (≥ 90)	1.32 (0.21–8.30)	.76
		0.086 (0.017–0.43)	*.003*
Loss 10q	TMZ	0.32 (0.13–0.80)	.014
		0.74 (0.30–1.85)	*.52*
	Age (> 53 years)	1.20 (0.46–3.17)	.71
		2.39 (1.00–5.73)	*.051*
	Gender	0.77 (0.21–2.87)	.77
		0.46 (0.13–1.61)	*.22*
	KPS (≥ 90)	0.41 (0.075–2.28)	.31
		0.028 (0.0048–0.16)	*.000068*

In the multivariate Cox model, all predictors are included and only main effects are estimated.

Table 7. Subgroup Analysis of Differential TMZ and Age Effects Depending on Genetic Aberration with Respect to Survival Assessed by Cox Proportional Hazard Regression.

Alteration/ Normal	Variable	Hazard Ratio CGH and *LOH* (95% CI)	*P* Value CGH and *LOH* (Two-Sided)
Loss 9p	TMZ	0.037 (0.0017–0.78)	.034
		0.49 (0.054–4.51)	*.53*
	Age (> 53 years)	0.81 (0.081–8.09)	.86
		4.31 (0.47–39.36)	*.20*
	TMZ × age (> 53 years)	3.57 (0.13–99.15)	.45
		0.20 (0.015–2.71)	*.23*
Loss 10q	TMZ	0.85 (0.25–2.91)	.79
		5.64 (0.71–44.57)	*.10*
	Age (> 53 years)	10.47 (1.87–58.65)	.0076
		33.56 (3.67–307.35)	*.0019*
	TMZ × age (> 53 years)	0.040 (0.0044–0.36)	.0042
		0.017 (0.0014–0.21)	*.0015*

shorter survival time [3,18]; however, mutations of *PTEN* did not affect prognosis for survival [20,34].

Esteller et al. [25] were able to show that aberrant *MGMT* promoter hypermethylation was associated with loss of MGMT protein, in contrast to retention of protein in the majority of tumors without aberrant hypermethylation. Recent studies suggested that methylation of the *MGMT* promoter is predictive for good outcome in patients with glioblastomas treated with alkylating agents [24,26–28]. Our data support the results mentioned above because *MGMT* is included in the deleted regions on 10q. The results for our control group showed that deletions on chromosome 10 indicate a trend to shorter survival. The same results are observed for LOH of *MGMT*, whereas LOH of *PTEN* showed no association with survival as reported before [17,20]. Interestingly, patients with deletion on 10q benefited significantly from adjuvant chemotherapy in univariate analysis.

Therefore, deletion as well as inactivation by hypermethylation of *MGMT* will predict responses to alkylating chemotherapy, probably in a dose-related manner. Further on, multivariate analysis showed that patients benefited significantly from the therapy especially if they belonged to the older age group (Table 7). Hence, our data suggest that the negative effect of age was compensated by TMZ treatment.

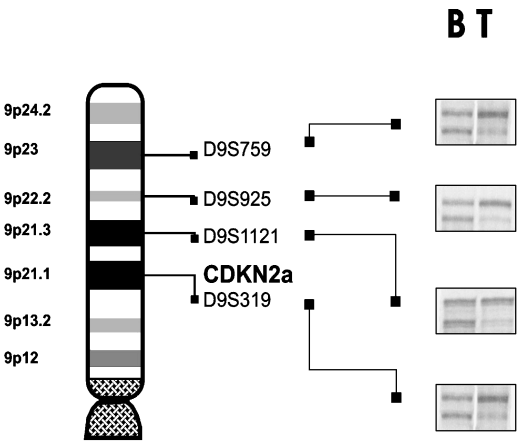

Figure 4. *Microsatellite analysis for LOH on chromosome arm 9p.*

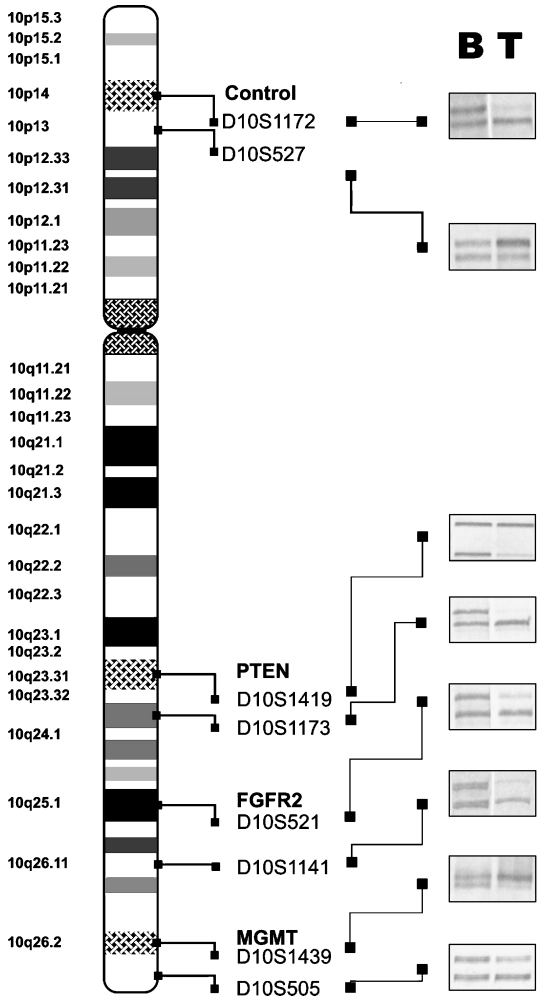

Figure 5. *Microsatellite analysis for LOH on chromosome 10.*

Homozygous deletion of *CDKN2A* gene mapping on chromosome 9p21 has been reported as an indicator of poor prognosis and resistance to chemotherapy for patients with anaplastic oligodendroglioma. However, homozygous *CDKN2A* losses were observed exclusively in tumors without LOH on 1p [35]. In glioblastomas with oligodendroglial components, similar results were found [54,55].

An association of tumor necrosis and microvascular proliferation with 9p deletion and *CDKN2A* alterations was observed in oligodendrogliomas. The higher vascularization of tumors harboring 9p deletion may explain the reasons for better response to chemotherapy [56]. This may also hold true for glioblastomas. However, this effect has never been proven in trials.

To our knowledge, this is the first report demonstrating an effect of 9p deletion on chemotherapy response in glioblastomas. The unfavorable prognostic factor of this alteration [15,17,33,34,57] was confirmed by CGH and LOH analyses in our control group. Further on, the effectivity of TMZ is enhanced by deletion of 9p by CGH and LOH of *CDKN2A*,

respectively, indicating also a crucial role of *CDKN2A* in chemotherapy response to alkylating agents.

Further, our present data indicate that gains of chromosomes 7 and 12 have no influence on response and survival in TMZ treatment. Therefore, our results suggest that there is no association between frequently amplified regions on these chromosomes (*EGFR*, *CDK4*, and *MDM2*) and TMZ response. Deletions on chromosome arm 13q did not correlate with TMZ chemotherapy either.

In conclusion, we demonstrate a positive effect of TMZ treatment on survival in patients with newly diagnosed glioblastoma. Although deletions on 9p and 10q indicate poorer survival in patients without adjuvant therapy, patients with these molecular alterations benefit from TMZ treatment. This effect was pronounced also in elderly patients with 10q deletion having a very poor prognosis with conventional treatments. Thus, a controlled prospective study should be performed to confirm that TMZ chemotherapy is effective in patients with major factors for poor prognosis, deletions on 9p and 10, and older age.

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