Long-term survival with glioblastoma multiforme

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The median survival of glioblastoma patients is ~12 months. However, 3–5% of the patients survive for more than 3 years and are referred to as long-term survivors. The clinical and molecular factors that contribute to long-term survival are still unknown. To identify specific parameters that might be associated with this phenomenon, we performed a detailed clinical and molecular analysis of 55 primary glioblastoma long-term survivors recruited at the six clinical centres of the German Glioma Network and one associated centre. An evaluation form was developed and used to document demographic, clinical and treatment-associated parameters. In addition, environmental risk factors, associated diseases and occupational risks were assessed. These patients were characterized by young age at diagnosis and a good initial Karnofsky performance score (KPS). None of the evaluated socioeconomic, environmental and occupational factors were associated with long-term survival. Molecular analyses revealed MGMT hypermethylation in 28 of 36 tumours (74%) investigated. TP53 mutations were found in 9 of 31 tumours (29%) and EGFR amplification in 10 of 38 tumours (26%). Only 2 of 32 tumours (6%) carried combined 1p and 19q deletions. Comparison of these data with results from an independent series of 141 consecutive unselected glioblastoma patients registered in the German Glioma Network revealed significantly more frequent MGMT hypermethylation in the long-term survivor group. Taken together, our findings underline the association of glioblastoma long-term survival with prognostically favourable clinical factors, in particular young age and good initial performance score, as well as MGMT promoter hypermethylation.

Keywords: EGFR; glioblastoma; long-term survival; MGMT, TP53

Abbreviations: LOH = loss of heterozygosity; WHO = World Health Organization


Introduction

Glioblastoma multiforme is the most common and most malignant primary tumour of the brain and associated with one of the worst 5-year survival rates among all human cancers. Despite multimodal aggressive treatment, comprising surgical resection, local radiotherapy and systemic chemotherapy, the median survival time after diagnosis is still in the range of just 12 months (Smith and Jenkins, 2000), with population-based studies indicating even shorter median survival (Ohgaki et al., 2004). Nevertheless, a small fraction of glioblastoma patients survive for more than 36 months. These patients are referred to as long-term survivors.

With the exception of rare instances of glioblastoma in patients with a hereditary tumour syndrome, e.g. Turcot’s syndrome (Hamilton et al., 1995) or Li–Fraumeni syndrome (Kleihues et al., 1997), most tumours originate in a sporadic fashion without any known genetic predisposition. Several studies have evaluated the impact of various exogenous factors, such as smoking (Zheng et al., 2001), diet (Huncharek et al., 2003; Lee et al., 1997), ionizing radiation (Brustle et al., 1992; Neglia et al., 1991), cellular phones (Inskip et al., 2001), electromagnetic fields (Savitz et al., 1998; Theriault et al., 1994), socioeconomic status and education level (Schlehofer et al., 2005), as well as medical risk factors, such as allergy (Wiemels et al., 2002),
immunological status (Schlehofer et al., 1999) and viral infections (Vilchez et al., 2003). However, no unequivocal evidence linking specific risk-factors to glioblastoma has emerged from these studies, except for an association with the exposure to ionizing radiation (for review see (Cavenee, 2000)). In addition, younger age and a good Karnofsky performance score (KPS) at the time of diagnosis are established clinical parameters associated with longer survival (Curran Jr et al., 1993).

Despite the enormous progress in the understanding of the genetic alterations in glioblastomas, clinically useful molecular markers that help to predict response to therapy and prognosis are still rare. To date, only the methylation status of the O6-methylguanine methyltransferase (MGMT) gene has become a molecular marker of clinical significance. MGMT encodes a DNA repair protein that causes resistance to DNA alkylating agents, such as nitrosoureas and temozolomide. Transcriptional silencing of the MGMT gene by promoter hypermethylation is seen in ~50% of glioblastomas and has been linked to prolonged progression-free and overall survival in glioblastoma patients treated with alkylating agents (Esteller et al., 2000b; Hegi et al., 2005). Combined deletions of the chromosomal arms 1p and 19q have been shown to be associated with a favourable prognosis in oligodendrogial tumours (Cairncross et al., 2006; van den Bent et al., 2006).

In glioblastoma, however, this aberration is rare and its prognostic significance is less clear (Ino et al., 2000; Schmidt et al., 2002; Brat et al., 2004; Houllier et al., 2006).

The investigation of glioblastoma long-term survivors could help to identify yet unknown clinical, environmental and/or molecular factors that are associated with favourable prognosis. Here, we report on a retrospective analysis of 55 glioblastoma long-term survivors recruited within the German Glioma Network. In addition to basic clinical data, we evaluated the prevalence of environmental, occupational and socioeconomic risk factors and screened for glioma-associated genetic aberration, i.e. TP53 mutation, EGFR amplification and 1p/19q deletion, as well as MGMT hypermethylation.

Patients and Methods

Patient recruitment and data acquisition

Primary glioblastoma patients with survival longer than 36 months after diagnosis were retrospectively identified by the six clinical centres of the German Glioma Network (www.gliomnetzwerk.de). In addition, 13 patients from an associated centre (Heinrich-Heine University Hospital Düsseldorf) were included in the study. Five of these patients were included in a previous report on glioblastoma patients surviving for more than 5 years (Steinbach et al., 2006). The study was approved by the local ethics committees at the contributing clinical centres. A histological diagnosis of glioblastoma according to the World Health Organization (WHO) classification of brain tumours was confirmed by central pathology review at the Brain Tumour Reference Centre of the German Society of Neuropathology and Neuroanatomy (T. Pietsch). For this purpose, immunohistochemical characterization with antibodies against lineage-associated antigens including glial fibrillary acidic protein was performed in addition to conventional haematoxylin/eosin stainings. Possible sarcomatous areas were illustrated by silver staining of reticulin fibres. The clinical data were evaluated by carefully scrutinizing the patients’ clinical charts and by interviews with the patients. Relatives were contacted in case that the patient has already deceased. A standardized basic evaluation form was used for demographic, clinical and treatment-associated parameters.

In addition, a special long-term survivor form was completed in which more details like environmental risk factors, associated diseases and occupational risks were assessed. Both forms are available at www.gliomnetzwerk.de. Data were collected at the Department of Neurosurgery, University Hospital Dresden. As a control population for the molecular analysis, we investigated an independent series of primary glioblastomas (137 classic glioblastomas, one gliosarcoma and three giant cell glioblastoma) from 141 unselected patients operated at the clinical centres of the German Glioma Network between October 2004 and December 2005. These tumours were derived from 83 male and 58 female patients with a median age at operation of 60.9 years.

DNA extraction

Tumour DNA useful for molecular analysis of one or more of the selected genes could be extracted from tumours of 38 glioblastoma long-term survivors. In 13 of these tumours, as well as in the 141 glioblastomas of the independent control series, high-molecular weight DNA was extracted from unfixed frozen tissue samples as described before (van den Bent et al. 2003). From the remaining 28 long-term survivor tumours, DNA was extracted from formalin-fixed paraffin-embedded tumour samples as reported (Reifenberger et al. 1996). A tumour cell content of at least 80% was histological assured for each specimen used for nucleic acid extraction.

MGMT promoter methylation analysis

MGMT promoter methylation was analysed by methylation-specific PCR as reported before (Mollemann et al., 2005). The primer sequences used to detect methylated MGMT promoter sequences were 5’-gtttttagaagtttggctgc-gac-3’ and 5’-caaggctccgg aaaaaaactcg-g-3’ (corresponding to nucleotides 46912-47033, GenBank accession no. AL355531; fragment size: 122 bp). The primer sequences used to detect unmethylated MGMT promoter sequences were 5’-tgttttttagaagtttggctgc-gac-3’ and 5’-ctacacccac tccaaaaaactcca-g-3’ (corresponding to nucleotides 46909-47037; fragment size: 129 bp). As positive control sample, we used the A172 glioma cell line, which has a completely methylated MGMT promoter. Genomic DNA from non-neoplastic brain tissue served as an unmethylated control sample.

Detection of EGFR gene amplification

EGFR gene dosage was determined by real-time PCR analysis using the ABI PRISM 5700 (Applied Biosystems, Darmstadt, Germany) sequence detection system. Continuous quantitative measurement of the PCR product was achieved by labelling of double-stranded DNA with SYBR Green fluorescent dye (Applied Biosystems). The following EGFR-specific primers were used: 5’-cactgctcatctcttcaccatc-3’ (sense) and 5’-gacatcctgtagcttcagac-3’ (antisense). The anonymous marker D2S1743 at chromosome 5p15.3 was used as an internal control.
2q21.2 was used as reference locus employing the primers 5′-catgactgcgacccaaagt-3′ (sense) and 5′-cagttttgttcactcaga t-3′ (antisense). As constitutional references, we used DNA extracted from peripheral leukocytes as well as non-neoplastic brain tissue samples from different patients. DNA from a glioblastoma with known EGFR amplification served as a positive control. Only tumours showing a normalized increase in EGFR gene dosage of more than 3-fold relative to constitutional DNA were considered as showing EGFR amplification.

**Multiplex ligation-dependent probe amplification (MLPA)**

MLPA was employed for the determination of allelic losses at 1p and 19q in the long-term survivor group because constitutional DNA was not available from the majority of patients. Analysis was performed using the SALSA MLPA KIT P088 lots 0305 and 0705 (MRD Holland, Amsterdam, the Netherlands). MLPA was performed according to the protocol of Jeuken et al. (Jeuken et al., 2006). PCR was performed with 35 cycles (95°C, 30 s; 60°C, 30 s; 72°C, 1 min; final extension 20 min, 72°C). Fragments were separated and quantified on an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA) and Genemapper 3.7 software utilizing the AFLP algorithm (Applied Biosystems). In each run at least four reference samples were included.

The allelic status for each sample was determined as follows: Peak areas of 19 control probes not from chromosomal arms 1p and 19q were evaluated. An average peak area for the 19 control genes was determined. Then peak areas from each individual probe on 1p and 19q were divided by the value for average peak area. We determined this average ratio on DNA samples from seven non-neoplastic tissues for each probe on 1p and 19q and repeated this experiment four times. Standard deviations of this ratio for each probe were determined for the data of the multiple rounds of analyses on these seven control tissues. A ratio deviating for >2 SDs from that established for each of the probes in control tissues was scored as reduced gene dosage. Because SDs for all data from controls were within narrow limits, data from tumours with a ratio smaller than 0.7 fulfilled these criteria for reduced gene dosage. DNA from tumours was scored as deleted if two or more probes on 1p or 19q adjacent to each other exhibited a ratio smaller than 0.7.

**Loss of heterozygosity (LOH) analysis at microsatellite markers on 1p and 19q**

Allelic losses in the control series were determined by microsatellite-based LOH analysis as reported (Felsberg et al., 2004; Hartmann et al., 2005). The following microsatellite loci were evaluated: D19S211, D19S489 and D19S469 (all located on 1p), as well as D19S572, D19S1182 and D19S596 (all located on 19q). Electrophoresis on denaturing 10% polyacrylamide gels, silver staining and assessment for LOH was carried out as described elsewhere (Felsberg et al., 2004; Hartmann et al., 2005).

**Single strand conformation polymorphism (SSCP) analysis and direct sequencing**

Exons 4–9 of the TP53 gene were screened for mutations using SSCP analysis as reported before (Wellenreuther et al., 1995; von Deimling et al. 2000). Aberrant SSCP bands were excised and the DNA was extracted as described (Wellenreuther et al., 1995).

### Table I Patient characteristics

<table>
<thead>
<tr>
<th>Total population of primary glioblastoma</th>
<th>n = 55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>n = 28</td>
</tr>
<tr>
<td>Female</td>
<td>n = 27</td>
</tr>
<tr>
<td>Median age at diagnosis</td>
<td>51 (21–72) years</td>
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<tr>
<td>Median KPS</td>
<td>80 (30–100)</td>
</tr>
<tr>
<td>Median survival</td>
<td>4.6 years (3.0–15.3 years)</td>
</tr>
<tr>
<td>Tumour localization by location</td>
<td></td>
</tr>
<tr>
<td>Right hemisphere</td>
<td>n (%)</td>
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<tr>
<td>Left hemisphere</td>
<td>31 (56.4)</td>
</tr>
<tr>
<td>Both hemispheres</td>
<td>23 (41.8)</td>
</tr>
<tr>
<td>Frontal</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Temporal</td>
<td>24 (43.6)</td>
</tr>
<tr>
<td>Parietal</td>
<td>17 (30.9)</td>
</tr>
<tr>
<td>Occipital</td>
<td>16 (29.1)</td>
</tr>
<tr>
<td>Occipital</td>
<td>11 (20.0)</td>
</tr>
<tr>
<td>Treatment of glioblastoma</td>
<td></td>
</tr>
<tr>
<td>Tumour resection</td>
<td>55 (100.0)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>55 (100.0)</td>
</tr>
<tr>
<td>Any chemotherapy</td>
<td>37 (67.3)</td>
</tr>
</tbody>
</table>

KPS = Karnofsky performance score, partly retrospectively evaluated.

*a*13 Tumours involved more than one lobe.

*b*All patients had gross total resection or partial resection, a biopsy was performed only once for recurrent tumour.

Following reamplification with the same set of primers the PCR products were sequenced on a semiautomated sequencer (Applied Biosystems, model 377, Foster City, CA) using the Taq cycle sequencing kit (Applied Biosystems, Foster City, CA). Each amplicon was sequenced bidirectionally.

### Statistical analysis

The Fisher’s exact test or the χ² tests were used to compare the frequencies of molecular aberrations in the long-term survivor group versus the control group.

### Results

#### General demographics

The study population of glioblastoma long-term survivors consisted of 55 patients (male 28; female 27) with a median age at diagnosis of 51 years (range 21–72 years). In comparison, the cohort of 141 unselected glioblastoma showed a significantly higher age (median 60.9 years, P < 0.001). The median follow-up time in the long-term survivor group was 7 years and the median survival time was 4.6 years (range 3.0–15.3 years). Median KPS at diagnosis for the entire population of 55 long-term survivors was 80 (range 30–100), with five patients having scores of <70 (Table 1).

Patients were married (49%), single (18%) or divorced (2%); data on marital status were not available for 17 patients (31%). The average number of children was two. There were nine (16%) current smokers among the entire study population, consuming 3–45 cigarettes per day [no data in 22 cases (40%)]. Occasional alcohol consumption was admitted by 10 (18%) patients,
while there were no data available for 23 patients (42%). No other drug consumption was recorded.

History
Inborn defects or inherited diseases were recorded in one case each; one patient had an anomaly of the ear and one had thalassaemia minor. Regarding childhood diseases and vaccination status, only data from 10 (18%) and 7 (13%) patients were available. Within these groups the prevalence of measles, small-pox, scarlet fever, whooping-cough, mumps and German measles was identical with those found in the general population. Similarly, 7/7 patients had vaccination for tetanus, 6/7 for diphtheria, and 4/7 for whooping-cough, which is comparable with the status in the general population (Reiter, 2004).

Associated risk factors
There was no preponderance of any occupation among the study population. Twenty-four different occupations were documented, in addition to retired patients, homemakers and patients with no occupation. One profession (book-keeper) was represented four times, another one (farmer) three times and six jobs were represented twice.

Coexisting tumour within the family was found in nine (16%) patients. The recorded tumour entities were: hepatocellular carcinoma, stomach carcinoma, breast carcinoma (twice), renal carcinoma, lung carcinoma, colon carcinoma and abdominal wall carcinoma.

An accident in the history was found in 12 (22%) patients resulting in fractures in nine (16%), tendon rupture in two (4%), and in incision injury in one patient. There was only one patient with a significant head injury in history (skull fracture).

Any other operation not for repair of accident related injuries was reported in 27 (49%) patients. There were 13 different procedures including cholecystectomy (three times), endoscopic knee joint exploration (four times) and appendectomy (twice). No patient had undergone prior surgery for an intracranial tumour.

Sixteen different diagnoses in 22 patients were registered as co-morbidities. Diabetes mellitus was found in four and depression in two patients. Gall bladder disease and arterial hypertension were found in two patients each. Among all co-morbidities there was no preponderance of any organ system. In order to evaluate disorders associated with intracranial location, only the two with depression were identified.

Tumour localization and histological features
Tumour localization was in the left cerebral hemisphere in 23 (42%), right cerebral hemisphere in 31 (56%) patients and in both frontal lobes in one patient. Thirteen (23%) tumours involved more than one lobe. The frontal lobe was involved in 24 (44%) patients, followed by temporal (31%), parietal (29%) and occipital (20%) lobe affliction.

In five of the tumours, the histology corresponded to giant cell glioblastoma (WHO grade IV). Two additional cases were classified as glioblastoma with sarcomatous component (gliosarcoma WHO grade IV). Two cases exhibited a significant oligodendroglial component in addition to the typical areas of glioblastoma with astrocytic differentiation. These cases were classified as glioblastoma with oligodendroglial component. All other tumours corresponded histological to classic glioblastoma multiforme (WHO grade IV).

Surgical treatment
All patients had planned gross total resection during the first surgery. The extent of surgery was determined on postoperative MR images. Due to the multicentric retrospective evaluation of data, the time between surgery and MRI data acquisition was not uniform. However, the first post-operative MRI data were recorded. Accordingly, 16 (29%) complete resections, i.e. no contrast-enhancing residual tumour, and 25 (45%) partial resections, i.e. contrast-enhancing tumour detectable irrespective of size were documented whereas no data were available in 14 (25%) patients. Twenty-five patients (45%) had a second operation (seven complete and 10 partial resections, one biopsy, seven no data), 12 patients (22%) had a third operation (three complete, six partial resections, three no data), and four patients (7%) were operated on a fourth time (two partial resections, two no data).

Adjuvant treatment
Following surgery, all patients had involved-field radiotherapy. A total dose between 59 and 60 Gy was applied to 46 out of 55 patients (84%); in seven patients, a lower dose (between 46 and 56 Gy) was applied because of side effects and in two patients higher doses of 70 and 80 Gy were applied. A combination of involved-field radiotherapy and stereotactic convergence radiotherapy was implemented in two patients; one patient had stereotactic convergence radiotherapy only. Any kind of chemotherapy was applied to 37 patients (67%). Some patients received more than one protocol (Table 2). Chemotherapy was applied to 31 (56%) patients after the first tumour resection and radiotherapy, to nine patients after the second, to five patients after the third, and to one patient after the fourth operation. Nimustine (ACNU) was the most frequently (16 out of 31 patients) used drug in first-line chemotherapy protocols as monotherapy in one, or in combination either with teniposide in thirteen, or with cytarabine in three patients. Temozolomide was applied to 21 patients, including five patients who received combined radio-chemotherapy. The PCV (procarbacine, lomustine, vincristine) protocol was applied to seven patients after radiotherapy. ACNU, temozolomide and PCV were also used for chemotherapy...
after the second to fourth operation. A total of 13 patients received more than one chemotherapy regime (Table 2).

Molecular analyses
DNA suitable for molecular analysis could be extracted from 38 of 55 long-term survivors. However, due to DNA degradation and limited amounts of DNA, not all 38 tumours could be investigated for aberrations in each of the four selected genes. Exons 4–9 of the TP53 gene were screened for mutations in 31 tumours. TP53 mutations were identified in tumours of nine patients (16%). The detected mutations included nine distinct missense mutations (c.451C>T/p.P151S, c.481G>C/p.A161T, c.568C>A/p.P190T, c.733G>A/p.G245S, c.736A>G/p.M256V, c.742C>T/p.R248W, c.817C>T/p.R273C, c.823T>C/p.R273H) and one nonsense mutation (c.135G>delG p.45fs). The EGFR gene dose could be assessed in all 38 tumours and revealed EGFR gene amplification in 10 patients (26%). Combined allelic losses on chromosome arms 1p and 19q were detected in 2 of 32 tumours investigated by MLPA (6%). In both cases, histological evaluation did not reveal an oligodendroglial component. The methylation status of the MGMT promoter was determined in 36 patients and revealed MGMT hypermethylation in tumours of 28 patients (74%). In 10 patients, tissue samples from recurrent tumours were additionally analysed for MGMT methylation. In all instances, the MGMT status in the primary tumour was retained in the recurrent tumour (eight methylated, two unmethylated). There was no correlation of molecular features with tumour location.

Similar to the long-term survivor group, not all 141 tumours of the control group of unselected consecutive primary glioblastomas could be studied for each of the genes. Data on the EGFR copy number were obtained from 138 tumours and revealed EGFR gene amplification in 60 tumours (44%). A total of 136 tumours from this series were studied for allelic losses on 1p and 19q, which revealed combined losses on both chromosome arms in 14 tumours (10%). MGMT hypermethylation was detected in 59 of 136 tumours investigated (43%). Mutational analysis of 135 tumours for TP53 aberrations identified mutations in 30 tumours (22%). Statistical comparisons revealed that MGMT hypermethylation was significantly more common in glioblastomas from the long-term survivor group as compared to glioblastomas from the unselected patient group (P = 0.0002, χ² test). In contrast, the frequencies of TP53 mutation, EGFR amplification and 1p/19q losses did not significantly differ between both groups, although there was a trend towards less frequent EGFR amplification in the long-term survivor group (Table 3).

Discussion
This is the largest series of glioblastoma long-term survivors reported to date. We provide a clinical characterization and report on molecular analyses of 55 primary glioblastoma patients with a survival time of >36 months. There was no association between socioeconomic, environmental and occupational factors and long-term survival. Molecular analyses indicated a higher proportion of MGMT methylation in the long-term survivor group when compared with a reference sample of an unselected consecutive series of glioblastomas.

Table 2 Chemotherapy in primary glioblastoma

<table>
<thead>
<tr>
<th></th>
<th>After First OP</th>
<th>After second OP</th>
<th>After third OP</th>
<th>After fourth OP</th>
</tr>
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<td>Nimustine</td>
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<tr>
<td>PCV</td>
<td>7</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>Temozolomide</td>
<td>12</td>
<td>7</td>
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<tr>
<td>More than one chemotherapy regimen in total n (%)a</td>
<td>4 (7.2)</td>
<td>7 (12.7)</td>
<td>13 (23.6)</td>
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<tr>
<td>No chemotherapy n (%)a</td>
<td>24 (43.6)</td>
<td>19 (34.5)</td>
<td>18 (32.7)</td>
<td>18 (32.7)</td>
</tr>
</tbody>
</table>

Total number of patients treated n (%)a 31 (56.4) 36 (65.5) 37 (67.3) 37 (67.3)

Table 3 Incidences of molecular aberrations detected in glioblastomas from long-term survivors and from an unselected control series

<table>
<thead>
<tr>
<th></th>
<th>Glioblastomas from long-term survivors</th>
<th>Glioblastomas from control series</th>
<th>Statistical results P</th>
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<tbody>
<tr>
<td>TP53 mutation</td>
<td>9/31 (29%)</td>
<td>30/135 (22%)</td>
<td>0.4199 (χ² test)</td>
</tr>
<tr>
<td>EGFR amplification</td>
<td>10/38 (26%)</td>
<td>60/138 (44%)</td>
<td>0.0056 (χ² test)</td>
</tr>
<tr>
<td>Combined 1p/19q loss</td>
<td>2/32 (6%)</td>
<td>14/136 (10%)</td>
<td>0.7394 (Fisher’s exact test)</td>
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<tr>
<td>MGMT hypermethylation</td>
<td>28/36 (74%)</td>
<td>59/136 (43%)</td>
<td>0.00002 (χ² test)</td>
</tr>
</tbody>
</table>

aPercentage of all glioblastoma patients (n = 55).
The median age of glioblastoma patients is >60 years according to population-based studies (www.cbtrus.org) (Ohgaki and Kleihues, 2005; Chakrabarti et al., 2005). In line with these data, the median age of our control group of unselected consecutive glioblastoma patients was 60.9 years. In contrast, the median age of the long-term survivors in our study was considerably lower, i.e. 51 years ($P<0.001$). This is in accordance with numerous clinical studies indicating that young age at the time of diagnosis is an important parameter associated with longer survival (Burger and Green 1987; Curran Jr et al., 1993; Devaux et al., 1993; Chang et al., 2005). Taking data from all 281 published glioblastoma long-term survivors (Table 4), their median age is 36.9 years, which supports that age is of

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Mean age</th>
<th>male</th>
<th>female</th>
<th>Mean KPS score</th>
<th>GTR</th>
<th>RT</th>
<th>Chemotherapy</th>
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<td>105</td>
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Data from a recent review for glioblastoma patients living longer than 5 years (Shinojima et al., 2004) are included.

aCalculated on the basis of data that were provided in the concerning paper.

bThe study population is also published in another paper by Burton et al., 2002b.

na = not available. Data were not provided in the paper.

KPS = Karnofsky Performance score; GTR = gross total resection; RT = radiotherapy.
predictive value in glioblastoma. On the other hand, four
of our patients were 65 years or older, indicating that older
age does not exclude long-term survival of glioblastoma.
Interestingly, the median age in our study is almost 15 years
above to that of all published cases. This might in part
be explained by the fact that older studies are possibly
contaminated with low-grade tumours in young adults that
were mistaken for glioblastoma, in particular pleomorphic
oxanthoastrocytomas. In our study population we had
five tumours characterized as giant cell glioblastoma.
In comparison to the control cohort of 141 patients,
which included three cases of giant cell glioblastoma,
this histological variant was overrepresented in the group
of long-term survivors (9.1% versus 2.1%, $P = 0.041$) support-
ing the hypothesis that this histological variant is associated
with longer survival (Shinojima et al., 2004). A contamina-
tion of glioblastoma patients’ cohorts by high grade
tumours of the oligodendroglial lineage may also occur.
However, in our series, only two cases showed a minor
oligodendroglial component in an otherwise typical
glioblastoma.

In general, glioblastomas are more frequent in males,
with a male/female ratio between 1.3 and 1.45, correspond-
ing to a proportion of female patients of 43 and 41%,
respectively (Barnholtz-Sloan et al., 2003; Ohgaki and
Kleihues 2005). In line with these data, the male to
female ratio in our control series of 141 unselected
glioblastoma patients was 1.43. The long-term survivors
showed a trend to a higher proportion (50%, 95% CI 38–
62%) of female patients, although the proportion reported
in the literature is still within the limits of the confidence
interval. Nevertheless, it seems that glioblastoma long-term
survival is favoured by the combination of two basic clinical
parameters—young age and female gender. Unfortunately,
the median follow up time in the control group did not
allow validation of these findings. Several environmental
and socioeconomic risk factors have been associated with
the development of malignant glial tumours (Lee et al.,
1997; Inskip et al., 2001; Huncharek et al., 2003; Schlehofer
et al., 2005). One might assume that the absence of
such factors could be associated with a better prognosis in
those tumours. However, in the present study we failed
to identify any of those factors evaluated by our question-
naire to be obviously under- or overrepresented in long-
term survivors. Although such data are difficult to obtain in
a retrospective setting, our results support the hypothesis
that socioeconomic, environmental and occupational
factors do not play major roles in determining whether a
glioblastoma patient becomes a long-term survivor or not.

All patients of our series were initially treated by
(gross total) tumour resection. Recent data have confirmed
that the extent of resection is associated with improved
progression-free survival (Stummer et al., 2006). Although
we had no uniform early postoperative MRI scanning
to document the extent of resection in our patients,
the fact that all 55 glioblastoma long-term survivors had
intended gross total resection as initial treatment confirms
that tumour total resection enhances the chances for a favourable
outcome.

All patients had adjuvant radiotherapy. Standard treat-
ment for glioblastoma includes postoperative radiotherapy;
hence radiotherapy is unlikely to be a positive selection
factor. Chemotherapy for malignant gliomas has experi-
cenced a renaissance since the publication of the EORTC
26981/22981-NCIC CE3 phase III randomized trial, which
demonstrated that concomitant and adjuvant temozolo-
mide chemotherapy has a positive effect on survival of
patients with glioblastoma (Stupp et al., 2005). In our
study, patients were treated between 1986 and 2003.
Therefore, only 21 patients received temozolomide treat-
ment, among these were 12 patients who had temozolomide
for initial adjuvant treatment. A total of 37 patients (67%) had at least one or up to three different adjuvant chemo-
therapy regimens, although chemotherapy was not included
in standard of care when those patients were treated.
Therefore, these data are in support of a positive role for
chemotherapy in glioblastoma with respect to long-term
survival. However, the study design does not allow to define
chemotherapy as a prognostic factor. Here, the same
limitations apply as for the number of surgical interven-
tions. Patients who have a less malignant course of disease
also have more options to undergo multiple surgical and
chemotherapeutic interventions. Yet, nitrosourea-
based chemotherapy has previously been defined as a
factor favouring long-term survival in the classical
meta-analyses published in 1993 by Fine and colleagues
and in 2002 by the Glioma Meta-analysis Trialists group.
These authors reported that there are consistently more
long-term surviving patients in the experimental trial arms
of adjuvant nitrosourea-based chemotherapy (Fine et al.,
1993; Stewart 2002).

Recent translational studies have reported that hyper-
methylation of the MGMT promoter is associated with
prolonged progression-free and overall survival in glioblas-
toma patients treated with alkylating agents (Esteller et al.,
2000a; Hegi et al., 2005). In line with these data, MGMT
promoter methylation was observed in the majority of
glioblastomas from long-term survivors. We found MGMT
hypermethylation to be significantly (almost two-fold)
more common in long-term survivors (74%) as compared
to an unselected consecutive series of glioblastoma patients
in the classical centres of the German Glioma Network (43%). On the other hand, it is also important to
note that the absence of MGMT promoter methylation is
still compatible with long-term survival in individual
patients.

In addition to MGMT methylation, we investigated
genetic alterations that are characteristic of either primary
(EGFR amplification) or secondary glioblastomas (TP53
mutation) (Kleihues and Ohgaki, 2000), as well as allelic
losses on 1p and 19q, which are an established prog-
nostic marker for anaplastic oligodendrogial tumours
Long-term survival with glioblastoma

(Cairncross et al., 2006; van den Bent et al., 2006). In comparison to our control group of unselected consecutive glioblastoma patients, there was a trend towards less common EGFR amplification in glioblastomas from long-term survivors. However, statistical analysis did not confirm a significant difference in EGFR amplification frequency between the long-term survivor group and the control group (Table 3). In the literature, conflicting data on the prognostic relevance of EGFR amplification in glioblastomas have been reported. While EGFR amplification and/or overexpression has been associated with a poor prognosis in some studies (Hiesiger et al., 1993; Zhu et al., 1996; Etienne et al., 1998; Korshunov et al., 1999), other authors could not substantiate a prognostic significance (Quan et al., 2005), or even reported an association with better prognosis (Houillier et al., 2006; Smith et al., 2001). Our finding suggests that the likelihood of long-term survival is higher in glioblastomas without EGFR amplification. However, it has to be considered that EGFR amplification is more common in glioblastomas of older patients (von Deimling et al., 2000). Therefore, the lower percentage of EGFR amplified tumours among the long-term survivors might be due to the significantly lower median age of this patient group.

Similar to EGFR amplification, the prognostic significance of TP53 mutations in glioblastoma is unclear. In glioblastomas, some studies reported a better prognosis of patients with TP53-mutant tumours (Chen et al., 2006) while others did not detect any prognostic significance (Kraus et al., 2001; Shiraishi et al., 2002; Rich et al., 2005). In a population-based study of glioblastoma patients, TP53 mutation was predictive of longer survival but not significant when adjusted for age at diagnosis (Ohgaki et al., 2004). In our study, the frequencies of TP53 mutation did not differ significantly between the long-term survivor group and the control group, which does not support an association between the TP53 mutation status and long-term survival.

In contrast to anaplastic oligodendrogliomas and oligoastrocytomas (Cairncross et al., 2006; van den Bent et al., 2006), the prognostic significance of 1p/19q deletion in glioblastomas is not clear yet (Ino et al., 2000; Schmidt et al., 2002; Brat et al., 2004; Houillier et al., 2006). While the incidence of combined 1p/19q losses is low in glioblastomas (von Deimling et al., 2000), some studies reported that the presence of an oligodendroglial component is associated with more frequent 1p/19q deletion and better prognosis (He et al., 2001; Kraus et al., 2001). Other authors did not confirm a better outcome of glioblastoma patients with oligodendrogial component in a multivariate analysis adjusted for age and gender (Homma et al., 2006). However, these authors found that allelic losses on 1p were associated with longer survival in glioblastoma. Our study did not identify an increased frequency of 1p/19q losses in glioblastoma long-term survivors when compared to our control series of unselected glioblastoma patients.

The central pathology review confirmed classic glioblastoma histology in the three cases with 1p/19q loss. These data suggest that long-term survival of classic glioblastoma is unrelated to 1p/19q loss. Furthermore, we can exclude a major contamination of our series with malignant oligodendroglial neoplasms.

We conclude that although we confirmed certain clinical factors and MGMT promoter hypermethylation to be associated with prolonged survival, our current study does not explain why the 55 glioblastoma patients reported here became long-term survivors. Thus, our results do not allow refining current treatment and overall counselling strategies for patients with glioblastoma. We assume that further molecular analyses employing large-scale microarray-based genomic and expression profiling approaches will identify molecular features that are specific to glioblastomas of long-term survivors. For this purpose, the German Glioma Network is prospectively collecting fresh tissue from all glioblastoma patients operated at its participating centres and shall thus be able to perform comparative profiling experiments on a reasonable number of long-term survivors from this large population within the next few years.

Acknowledgements

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