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REVIEW ARTICLE

Population-Based Studies on Incidence, Survival Rates, and Genetic Alterations in Astrocytic and Oligodendroglial Gliomas

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Abstract

Published data on prognostic and predictive factors in patients with gliomas are largely based on clinical trials and hospital-based studies. This review summarizes data on incidence rates, survival, and genetic alterations from population-based studies of astrocytic and oligodendrogliomas that were carried out in the Canton of Zurich, Switzerland (approximately 1.16 million inhabitants). A total of 987 cases were diagnosed between 1980 and 1994 and patients were followed up at least until 1999. While survival rates for pilocytic astrocytomas were excellent (96% at 10 years), the prognosis of diffusely infiltrating gliomas was poorer, with median survival times (MST) of 5.6 years for low-grade astrocytoma WHO grade II, 1.6 years for anaplastic astrocytoma grade III, and 0.4 years for glioblastoma. For oligodendrogliomas the MST was 11.6 years for grade II and 3.5 years for grade III. *TP53* mutations were most frequent in gemistocytic astrocytomas (88%), followed by fibrillary astrocytomas (53%) and oligoastrocytomas (44%), but infrequent (13%) in oligodendrogliomas. LOH 1p/19q typically occurred in tumors without *TP53* mutations and were most frequent in oligodendrogliomas (69%), followed by oligoastrocytomas (45%), but were rare in fibrillary astrocytomas (7%) and absent in gemistocytic astrocytomas. Glioblastomas were most frequent (3.55 cases per 100,000 persons per year) adjusted to the European Standard Population, amounting to 69% of total incident cases. Observed survival rates were 42.4% at 6 months, 17.7% at one year, and 3.3% at 2 years. For all age groups, survival was inversely correlated with age, ranging from an MST of 8.8 months (<50 years) to 1.6 months (>80 years). In glioblastomas, LOH 10q was the most frequent genetic alteration (69%), followed by *EGFR* amplification (34%), *TP53* mutations (31%), $p16^{INK4a}$ deletion (31%), and *PTEN* mutations (24%). LOH 10q occurred in association with any of the other genetic alterations, and was the only alteration associated with shorter survival of glioblastoma patients. Primary (de novo) glioblastomas prevailed (95%), while secondary glioblastomas that progressed from low-grade or anaplastic gliomas were rare (5%). Secondary glioblastomas were characterized by frequent LOH 10q (63%) and *TP53* mutations (65%). Of the *TP53* mutations in secondary glioblastomas, 57% were in hot-spot codons 248 and 273, while in primary glioblastomas, mutations were more evenly distributed. G:C → A:T mutations at CpG sites were more frequent in secondary than primary glioblastomas, suggesting that the acquisition of *TP53* mutations in these glioblastoma subtypes may occur through different mechanisms.

Key Words: Astrocytoma, *EGFR* amplification, Glioblastoma, Oligodendroglioma, LOH 10, LOH 1p, LOH 19q, Population-based study, *PTEN* mutation, $P16^{INK4a}$ deletion, *TP53* mutation.

INTRODUCTION

Gliomas of astrocytic, oligodendroglial and ependymal origin account for more than 70% of all brain tumors, but current knowledge on the survival of glioma patients and on factors that are predictive of outcome is based largely on clinical trials on patients with malignant gliomas (anaplastic astrocytoma and glioblastoma). However, clinical trials have a strong bias towards the recruitment of patients with better prognosis, i.e. high preoperative Karnofsky performance score and younger age (1, 2). Therapeutic trials have less frequently addressed the outcome of patients with low-grade diffuse astrocytoma or oligodendroglioma, since in many centers these patients are not subjected to adjuvant radio- and chemotherapy. Similarly, studies on genetic alterations and how they influence response to therapy and survival are usually based on small numbers of patients, often contradictory and difficult to validate. In recent years, it has been established that primary (de novo) glioblastomas and secondary glioblastomas derived from low-grade or anaplastic gliomas develop through different genetic pathways (3–5). However, these studies, too, were based on small cohorts of selected cases and the relative frequencies of these glioblastoma subtypes remained unclear. In order to overcome these problems, we conducted a large, population-based study on close to 1,000 patients (6–8).

Patient Population and Review of Histology

We carried out a population-based study of patients who were diagnosed with an astrocytic or oligodendroglial tumor (total 987 cases) in the Canton of Zurich, Switzerland (population approximately 1.16 million) during the 15-year period from 1980 to 1994 (6, 8). Patients were followed up until death or at least until the end of 1999. The mean patient follow-up periods were 12 years for pilocytic astrocytoma (6), 90.2 ± 57.8 months for low-grade diffuse glioma (7), 37.6 ± 45.2 months for anaplastic glioma, and 7.2 ± 7.6 months for glioblastoma (8). Of 987 cases, 80% were histologically diagnosed after surgical intervention (611 cases, 62%) or at autopsy

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(180 cases, 18%); the remaining 20% were diagnosed by CT or MRI without histopathologic verification. Histologic diagnoses were reevaluated according to the new WHO classification of tumors of the nervous system (9, 10).

Incidence Rates

Incidence rates for histologic subtypes of CNS tumors are not readily available, since most cancer registries give combined data for all brain tumors. More detailed information is collected in certain regions; in the United States, registration of all brain neoplasms, including benign lesions, in the SEER database has recently become mandatory (11, 12).

Incident cases included in this population-based study are summarized in Table 1. With 3.55 new cases per 100,000 persons per year, adjusted to the European Standard Population, the glioblastoma is the most frequent histologic type, and accounts for 69% of all incident cases of astrocytic and oligodendroglial tumors. The incidence rate of glioblastomas in the USA, adjusted to the US Standard Population, is 29.6 new cases per million population per year (11). These incident cases do not include secondary glioblastomas that progressed from low-grade or anaplastic gliomas, since only the first diagnosis is considered as an incident case. Incidence rates of all astrocytic and oligodendrogliomas combined, adjusted to the European and US Standard Populations were 5.27 and 5.17 per 100,000 persons per year, respectively (Table 1).

Age and Sex Distribution

For pilocytic astrocytomas there was a clear correlation between the age of patients and the location of tumors (6): the cerebellum was the most frequent tumor site in children (67%); supratentorial examples were not observed in patients younger than 12 years of age. There was no significant difference between cerebellar and supratentorial locations in patients older than 35 years of age (6). Low-grade and anaplastic gliomas developed in middle-aged patients (Table 1).

Among low-grade astrocytomas, fibrillary astrocytomas were diagnosed at a mean age of 39 years, while the gemistocytic variant developed in older patients (mean, 50 years; $p = 0.0072$) (7).

The mean age of all glioblastoma patients (including secondary glioblastomas) was 61.3 ± 14.0 years. Their age distribution at the population level (including secondary glioblastomas) was as follows: 6.9% $<$ 39 years, 12.5% 40 to 49 years, 21.1% 50 to 59 years, 29.9% 60 to 69 years, 22.1% 70 to 79 years, and 7.6% $>$ 80 years. It is notable that the age of glioblastoma patients is significantly higher than that of patients recruited in clinical trials (1, 2). Older patients are often not eligible in the setting of a therapeutic trial due to a low Karnofsky performance score, and they are less likely to be treated by surgery and radiotherapy. Most histologic types of astrocytic and oligodendroglial gliomas developed more frequently in males than in females, with an overall male female ratio of 1.26 (Table 1).

Treatment

All the pilocytic astrocytoma patients underwent microsurgery, which aimed at maximum tumor removal. Of the 55 patients, only 7 (13%) underwent postoperative radiotherapy. Irrespective of radiotherapy, the prognosis of patients with pilocytic astrocytoma was excellent (6). Most patients (116/122, 95%) with low-grade gliomas also

TABLE 1. Incident Cases of Astrocytic and Oligodendroglial Tumors in Population-Based Study in the Canton of Zurich, Switzerland

Histologic Type	WHO Grade	ICD-O Code*	No. of Cases (%)	Mean Age	M/F Ratio	Incidence Rate†
Pilocytic astrocytoma	I	94211	53 (5.4%)	18.2 ± 12.2	1.12	0.37
Anaplastic pilocytic astrocytoma	III	94211	3 (0.3%)	31.3 ± 17.6	2.00	0.02
Subependymal giant cell astrocytoma	I	93841	1 (0.1%)	26.0	nd	0.01
Pleomorphic xanthoastrocytoma	II	94243	3 (0.3%)	19.0 ± 4.6	2.00	0.02
Fibrillary astrocytoma	II	94203	43 (4.4%)	39.5 ± 13.0	1.44	0.22
Gemistocytic astrocytoma	II	94113	9 (0.9%)	50.1 ± 21.9	3.50	0.04
Oligoastrocytoma	II	93823	20 (2.0%)	41.1 ± 9.9	1.00	0.10
Oligodendroglioma	II	94503	50 (5.1%)	40.9 ± 15.1	0.92	0.27
Anaplastic astrocytoma	III	94013	47 (4.8%)	45.5 ± 16.2	1.09	0.25
Anaplastic oligoastrocytoma	III	93825	11 (1.1%)	48.2 ± 17.4	0.57	0.06
Anaplastic oligodendroglioma	III	94513	13 (1.3%)	50.4 ± 13.9	3.33	0.07
Glioblastoma‡	IV	94403, 94413, 94423	680 (69.0%)	62.2 ± 13.4	1.34	3.55
Diffuse astrocytoma NOS	nd	94003	23 (2.3%)	46.9 ± 23.8	0.92	
Glioma NOS	nd	nd	14 (1.4%)	41.3 ± 20.5	1.00	
High-grade glioma NOS	III/IV	93803	17 (1.7%)	64.9 ± 16.6	0.70	
Total			987 (100%)	55.2 ± 18.6	1.26	5.27

*, See (67)
†, Incidence rates adjusted to the European Standard Population (per 100,000 persons per year).
‡, Secondary glioblastomas are not included since they are not incidence cases.
nd, not determined; NOS, not otherwise specified.

underwent surgical intervention. The management policy of treatment for low-grade gliomas at the University of Zurich at that time was surgical intervention aiming at maximum tumor removal without adjuvant radio-chemotherapy. Radiotherapy was standard procedure for anaplastic astrocytomas and anaplastic oligodendrogliomas (WHO grade III) and for glioblastomas (WHO grade IV). For the latter, it typically consisted of 2 Gy fractions and a total dose of 60 Gy.

We were surprised to see that at the population level, only 54% of glioblastoma patients underwent surgical intervention, either partial or complete resection (8). The mean age of these patients was significantly younger (56.1 years) than those who did not receive an operation (67.5 years; $p < 0.0001$) and their survival times were longer (median, 7.9 months) than those of patients without surgery (2.5 months; $p < 0.001$). Similarly, the mean age of patients who received radiotherapy (54.6 years) was significantly lower than that of patients who did not receive radiotherapy (68.4 years; $p < 0.0001$) and their survival time was longer (median, 10 months vs. 2 months; $p < 0.001$) (8). However, these differences probably reflect the better prognosis of younger patients more than the efficacy of treatment.

Histologic Types and Survival

Except for patients with pilocytic astrocytoma, who had excellent outcome irrespective of radiotherapy (6), survival of patients with an astrocytic tumor is still very poor. The median survival time (MST) of patients with a low-grade astrocytoma was 5.6 years, with anaplastic astrocytoma 1.6 years and with glioblastoma only 4.9 months (Fig. 1). The observed survival rates of glioblastoma patients at the population level were 42.4% at 6 months, 17.7% at one year, 3.3% at 2 years, and 1.2% at 3 years (8). This is consistent with population-based data from Canada, which showed that only 15 out of 689 glioblastoma patients (2.2%) diagnosed during 1975–1991 survived 3 years or longer (13).

For low-grade astrocytomas, survival of patients with gemistocytic astrocytoma (MST = 3.8 years; 16% survival at 5 years) was significantly shorter than that of fibrillary astrocytoma (MST = 5.9 years; 31% survival at 10 years; $p = 0.007$) (7). The survival of patients with gemistocytic astrocytoma was longer than that of anaplastic astrocytoma, but the difference was not significant ($p = 0.2$). This population-based finding is consistent with previous observations in several hospital-based studies, which indicate a tendency towards rapid recurrence and malignant progression in patients with gemistocytic astrocytoma (9, 14). It may, therefore, be justified to assign the grade III to the gemistocytic variant in future editions of the WHO classification. The biologic basis of the poor outcome in patients with gemistocytic astrocytoma has not been elucidated; gemistocytes themselves show very low proliferative activity (14, 15), although they are truly neoplastic cells, typically with a *TP53* mutation (16, 17).

The presence of oligodendroglial components in low-grade gliomas is associated with longer survival: oligodendroglioma patients (MST = 11.6 years; 51% at 10 years) survived longer than patients with oligoastrocytoma (MST = 6.6 years; 49% at 10 years) or fibrillary astrocytoma (MST = 5.9 years; 31% at 10 years) (7). For oligodendrogliomas the MST was 11.6 years for grade II (7) and 3.5 years for grade III (unpublished data).

Age and Survival

Age of patients is a significant predictive factor of survival among patients with low-grade glioma or glioblastoma at the population level (7, 8). In patients with glioblastoma, older age was significantly associated with shorter survival at the population level in both univariate (Fig. 2) and multivariate analyses (Table 2). Older patients are less frequently treated by surgical intervention and radiotherapy, and those who are treated show better survival than those who are not (see above) (8). Thus, the poor survival of many older patients could be

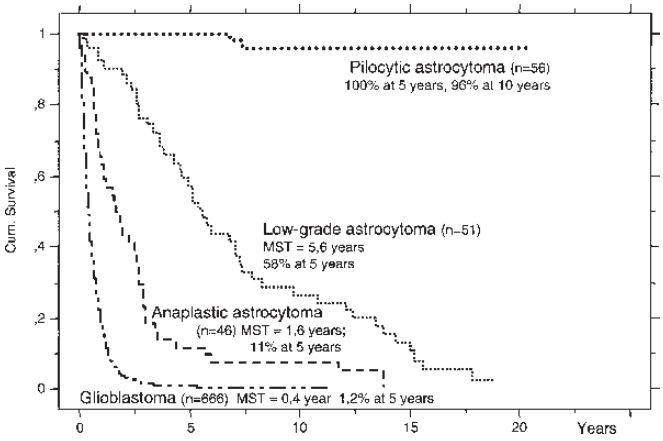

FIGURE 1. Survival of patients with pilocytic astrocytoma (WHO grade I), low-grade astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), and glioblastoma (WHO grade IV). Except for patients with pilocytic astrocytoma, who have excellent survival, outcome of other diffuse astrocytomas (in particular glioblastomas) is poor (6–8).

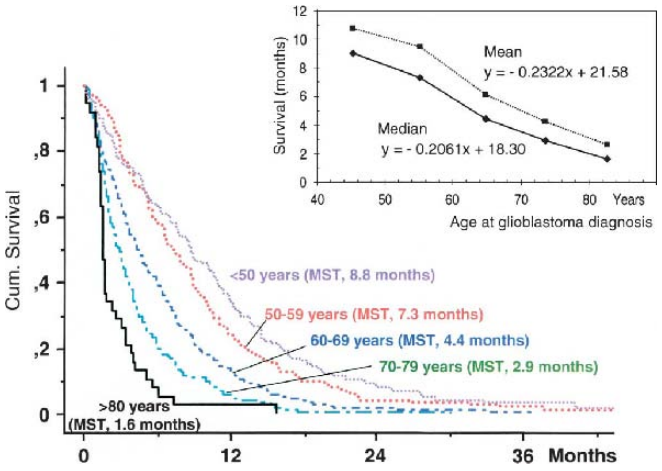

FIGURE 2. Kaplan–Meier curves showing that younger age of patients with glioblastoma is a significant predictive factor for longer survival. On the basis of these data, formulae were established to calculate the mean and median survival time from the date of glioblastoma diagnosis (upper right). Modified from Ohgaki et al (8).

TABLE 2. Multivariate Analysis for the Effect of Age, Sex, and Genetic Alterations on Survival of Glioblastoma Patients

Variable	No. of Cases	Age-adjusted Hazard Ratio (95% confidence interval)	p Value
Age			
<39 years	44	0.53 (0.39–0.75)	<0.001
40–49 years	87	0.55 (0.43–0.71)	<0.001
50–59 years	144	0.63 (0.50–0.78)	<0.001
60–69 years	204	1	
>70 years	186	1.65 (1.35–2.01)	<0.001
Female versus male	665	0.91 (0.78–1.07)	ns
Genetic alterations			
TP53 Mut versus *TP53* wild-type	386	0.86 (0.69–1.07)	ns
PTEN Mut versus *PTEN* wild-type	324	0.99 (0.76–1.28)	ns
$p16^{INK4a}$ Del versus no $p16^{INK4a}$ Del	328	0.90 (0.71–1.14)	ns
EGFR Amp versus no *EGFR* Amp	371	1.08 (0.87–1.34)	ns
LOH 10q versus no LOH 10q	269	1.28 (0.98–1.67)	0.067

Mut, mutation; Del, homozygous deletion; Amp, amplification; ns, not significant.

due to lack of treatment. However, this is not likely; even among treated patients older age remained a significant predictor for poorer survival (unpublished data).

Recurrence and Progression

Five of 55 patients (9%) with pilocytic astrocytoma underwent a second operation because of recurrence 1 to 12 years after the first intervention. In all cases, the diagnosis was again of a pilocytic astrocytoma, and no case progressed from pilocytic astrocytoma (WHO grade I) to anaplastic pilocytic astrocytoma (WHO grade III) (8).

During the study period, at least one recurrence was observed in 43% patients with low-grade glioma (low-grade astrocytoma, oligoastrocytoma, and oligodendroglioma), and in 68% of these cases this was associated with progression to a more malignant histologic grade. At least one recurrence was observed in 42% patients with anaplastic glioma (anaplastic astrocytoma, anaplastic oligoastrocytoma, and anaplastic oligodendroglioma), and in 66% of these cases this was associated with progression to glioblastoma. The mean time from progression from low-grade astrocytoma or oligoastrocytoma WHO grade II) to anaplastic astrocytoma or anaplastic oligoastrocytoma (WHO grade III) was 5.9 + 3.0 years, from anaplastic astrocytoma or anaplastic oligoastrocytomas to glioblastoma (WHO grade IV) 1.4 + 1.0 years, and that from low-grade astrocytoma or oligoastrocytoma to glioblastoma 5.3 + 5.1 years. The mean time till progression from oligodendroglioma to anaplastic oligodendroglioma was 6.6 + 4.2 years.

Genetic Alterations and Their Predictive Value in Low-Grade Gliomas

TP53 mutations were most frequent in gemistocytic astrocytomas (88%), followed by fibrillary astrocytomas and oligoastrocytomas, but infrequent (13%) in oligodendrogliomas (7). Conversely, LOH 1p/19q was frequent in oligodendrogliomas (69%), less frequent in oligoastrocytomas (44%), rare in fibrillary astrocytomas and absent in gemistocytic astrocytomas (Fig. 3) (7). LOH 1p + 19q occurred in 19/35 (54%) of oligodendrogliomas. Except for 2 cases, *TP53* mutation and LOH 19q were mutually exclusive in low-grade gliomas and there was not a single case with both *TP53* mutation and LOH 1p (7). These findings indicate that LOH 1p/19q and *TP53* mutations are genetic alterations that clearly distinguish 2 pathways leading to oligodendrogliomas and to low-grade astrocytomas, respectively. Although oligoastrocytomas may carry LOH 1p/19q and *TP53* mutations, it is extremely rare that both LOH 1p/19q and *TP53* are detected in the same tumor (7, 18).

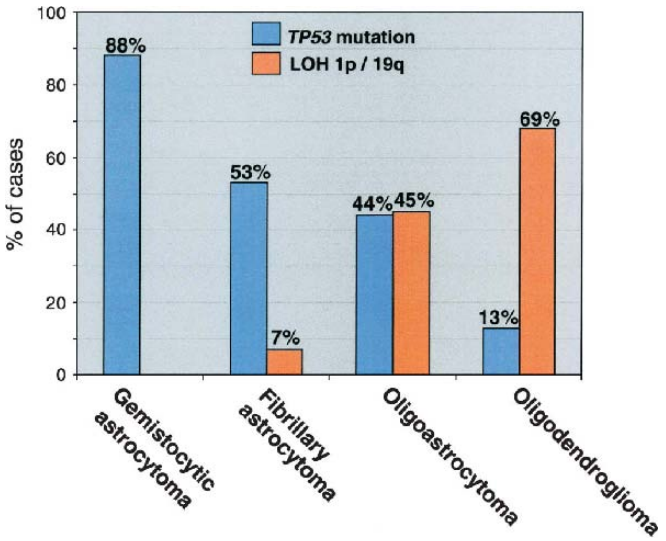

FIGURE 3. *TP53* mutations and LOH 1p/19q in low-grade diffuse gliomas. *TP53* mutations are most frequent in gemistocytic astrocytomas, followed by fibrillary astrocytomas, and lowest in oligodendrogliomas. LOH 1p/19q is most frequent in oligodendrogliomas, but rare in low-grade diffuse astrocytomas. Oligoastrocytomas show a genetic status between those of low-grade diffuse astrocytomas and oligodendrogliomas (7).

Multivariate analysis adjusting for histologic type, age, sex, and genetic alterations revealed that the presence of neither *TP53* mutations nor LOH 1p/19q was significantly predictive of survival in low-grade gliomas (7). These findings suggest that although *TP53* mutations and LOH 1p/19q may be key events in the development of low-grade gliomas, their presence or absence alone does not predict clinical outcome. Combining histologic diagnosis and genetic alterations with gene expression patterns may yield data of higher predictive value (19).

Primary and Secondary Glioblastomas

Tumors were considered primary (de novo) if a glioblastoma diagnosis was made at the first biopsy, without clinical or histopathologic evidence of a less malignant precursor lesion. The diagnosis of secondary glioblastoma was made only in cases with histopathologic evidence of preceding low-grade or anaplastic glioma. Using these criteria, secondary glioblastomas were rare at the population level, amounting to only 5% of all glioblastomas (8). This is consistent with the finding by Dropcho et al at the University of Alabama (20), who observed that 19 of 392 patients (5%) with glioblastomas had histologically proven prior low-grade gliomas. However, in population-based data in the USA, low-grade and anaplastic astrocytomas and mixed gliomas comprised approximately 25% of incident glioblastoma cases (11, 21). Similarly, in the present population-based study, the incidence rate of low-grade and anaplastic gliomas is approximately 2 to 3 times higher than that of secondary glioblastoma (7). The higher frequency of precursor lesions may be explained at least in part by the fact that a significant fraction of patients with low-grade or anaplastic astrocytoma die before progression to glioblastoma occurs. However, some cases with very rapid progression from low-grade or anaplastic astrocytoma may have been misclassified as primary glioblastoma. Even taking into account this possibility, at the population level secondary glioblastomas constitute a rare disease compared to primary glioblastoma.

There was a striking difference in the age distribution (Fig. 4). The mean age of primary glioblastoma patients was 62 years, while secondary glioblastomas developed in younger patients (45 years). The median survival of secondary glioblastoma patients was 7.8 months, significantly longer than that of primary glioblastoma patients (4.7 months; p = 0.003) (Fig. 5). However, this was largely due to the younger age of secondary glioblastoma patients; after age adjustment, there was no significant difference in survival.

Primary glioblastomas developed more frequently in men (M/F ratio, 1.33), while secondary glioblastomas developed more frequently in women (M/F, 0.65). This corroborates a previous observation that glioblastomas with *TP53* mutations (a genetic hallmark of secondary glioblastomas) are more common in women (22). This is surprising, since in previous hospital-based studies (9, 23) and population-based studies (7, 11), the incidence of low-grade or anaplastic gliomas in males was similar to or higher than that in females. The possibility exists that in female patients, gliomas progress more frequently or more rapidly to glioblastoma.

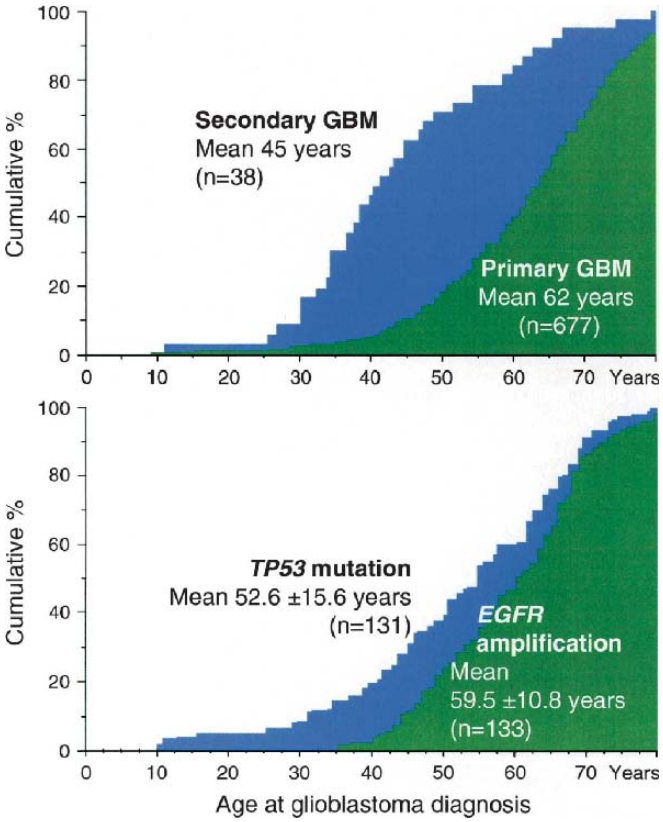

FIGURE 4. Secondary glioblastomas develop significantly in younger patients than primary glioblastomas ($p < 0.0001$). *TP53* mutations occur in patients of any age group, while *EGFR* amplification occurs in older patients. Note that there is no single case of glioblastoma with *EGFR* amplification that developed in a patient younger than 35 years of age. Modified from Ohgaki et al (8).

Genetic Pathways to Glioblastomas

At the population level, the most frequent genetic alteration in glioblastomas was LOH 10q (69%), followed by *EGFR* amplification (34%), *TP53* mutations (31%), $p16^{INK4a}$

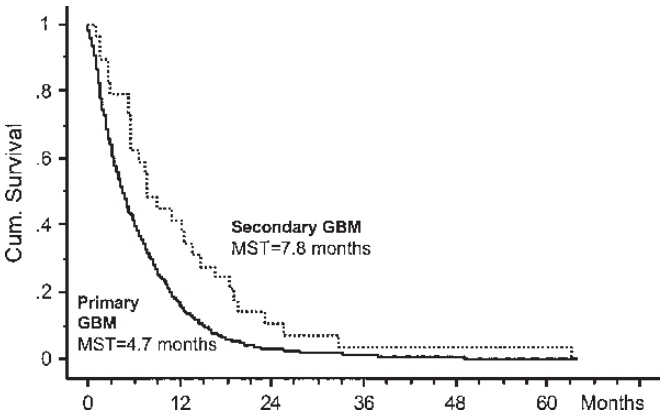

FIGURE 5. Univariate analysis shows that survival of secondary glioblastoma patients is significantly longer than that of primary glioblastoma patients (logrank test; p = 0.003). Modified from Ohgaki et al (8).

homozygous deletion (31%), and *PTEN* mutations (24%) (8). Similar frequencies of genetic alterations were observed in primary glioblastomas (Fig. 6). In secondary glioblastomas, LOH 10q and *TP53* mutations were frequent (63% vs. 65%), but other genetic alterations were infrequent (4%–19%) (Fig. 6).

Genetic alterations that are significantly more frequent in primary than in secondary glioblastomas are *EGFR* amplification and *PTEN* mutations, whereas *TP53* mutations are significantly more frequent in secondary than primary glioblastomas (Fig. 6). In the pathway leading to secondary glioblastomas, *TP53* mutations are early genetic events, since they are already present in low-grade and anaplastic gliomas at similar frequencies (Fig. 6), while LOH 10q is a late genetic event. In the absence of identifiable precursor lesions, the sequence of genetic alterations in primary glioblastomas remains enigmatic.

Some patients with glioblastomas have an extended preoperative clinical history but without radiologic or histopathologic evidence of less malignant precursor lesions. Thus, the possibility exists that these glioblastomas are clinically and genetically intermediate between primary and secondary glioblastomas. However, the pattern of genetic alterations was similar in primary glioblastomas with long and short preoperative histories (unpublished results), making the existence of hybrid subtypes unlikely. Even among primary glioblastomas, some tumors grow more rapidly than others, possibly due to different combinations of genetic alterations and other factors, such as the genetic background of the individual.

Age and Genetic Alterations in Glioblastomas

Glioblastomas with a *TP53* mutation were observed in younger patients (mean, 53 years; Table 3), in particular in patients younger than 35 years (Fig. 7). The mean age of patients with glioblastomas carrying a *PTEN* mutation and LOH 10q were older than those without these alterations (Table 3). *EGFR* amplification closely reflects the age distribution of primary glioblastomas; most interestingly, *EGFR* amplification was never observed in any glioblastoma that developed in patients below 35 years of age (Figs. 4, 7). Among patients older than 35 years, the overall frequencies of key genetic alterations in glioblastoma were similar among different age groups (Fig. 7). Thus, the poor prognosis of older patients cannot be explained by the frequency of specific genetic alterations or the combination. Although there may be as yet unidentified transformation-associated genes that are more frequently altered in glioblastomas of older patients and may affect susceptibility to therapy, it is also possible that the sum of all changes, i.e. the level of genomic instability, is more relevant.

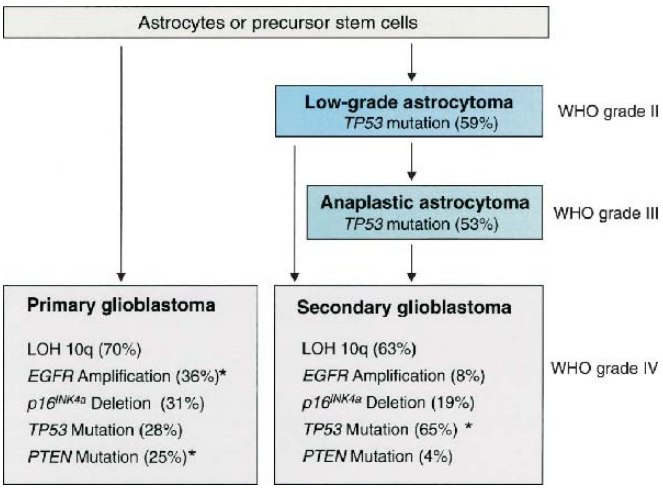

FIGURE 6. Genetic pathways to primary (de novo) and secondary glioblastomas. LOH 10q is frequent in both primary and secondary glioblastomas. *TP53* mutations are early and frequent genetic alterations in the pathway leading to secondary glioblastomas. Asterisks (*) indicate genetic alterations that are significantly different in frequency between primary and secondary glioblastomas. Modified from Ohgaki et al (8).

Predictive Value of Genetic Alterations in Glioblastomas

LOH 10 is the most frequent genetic alteration in glioblastomas and occurs in 60% to 80% of cases (24–26). Many glioblastomas appear to have lost one entire copy of chromosome 10. LOH occurs most frequently at 3 common loci (i.e. 10p14-p15, 10q23-24 *[PTEN]*, and 10q25-pter), suggesting the presence of several tumor suppressor genes (24–27). LOH 10q was the only genetic alteration associated with shorter survival of glioblastoma patients at the population level (Tables 2 and 3) (8), in accordance with results from several previous hospital-based studies (28–30).

The *PTEN* gene encodes a protein that plays important roles in the regulation of cell proliferation, apoptosis, and tumor invasion (31, 32). *PTEN* mutations have been reported in 15% to 40% of glioblastomas (32, 33). In several hospital-based studies, *PTEN* mutations were not associated with prognosis of glioblastoma patients (28, 34, 35), and this was confirmed at the population level (8). Most missense mutations were located in exons 1 to 6, the region homologous to tensin, auxilin, and dual-specificity phosphatases, whereas nonsense mutations and deletions or insertions leading to stop codons and protein truncation were located more evenly throughout the gene, suggesting that cells with PTEN truncation at any site or *PTEN* missense mutations in the region homologous to tensin/auxilin and dual-specificity phosphatases acquire a transformed phenotype (8).

The *TP53* gene plays important roles in several cellular processes including the cell cycle, response to DNA damage, apoptosis, cell differentiation, and neovascularization (36). Data on the predictive value of *TP53* mutations in glioblastomas have been contradictory. While some hospital-based studies showed no association between *TP53* status and outcome of glioblastoma patients (34, 37), one study showed that the presence of *TP53* mutations was a favorable prognostic factor (28). At the population level, univariate analysis revealed that the presence of *TP53* mutations was predictive of longer survival (8). However, age-adjusted multivariate analysis revealed no difference in survival between patients with and without *TP53* mutations (8).

Epidermal growth factor receptor (EGFR) is a transmembrane receptor that binds to extracellular ligands such as EGF and transforming growth factor alpha (TGFα), and transduces a mitotic signal (38). The predictive value of *EGFR*

TABLE 3. Univariate Analysis for the Effect of Genetic Alterations on Survival and Mean Age of Glioblastoma Patients

	Median Survival Time	Mean Age of Patients
TP53 mutation	8.2 months† (n = 126)	52.5 ± 15.8 years§ (n = 126)
Wild-type	7.2 months (n = 259)	59.0 ± 11.9 years (n = 271)
EGFR amplification	6.8 months (n = 127)	59.5 ± 10.8 years (n = 133)
No amplification	7.6 months (n = 244)	57.9 ± 15.0 years (n = 260)
$p16^{INK4a}$ deletion	8.5 months (n = 102)	56.9 ± 10.9 years (n = 102)
No deletion	8.0 months (n = 226)	55.0 ± 13.3 years (n = 228)
PTEN mutation	8.8 months (n = 77)	58.6 ± 11.4 years‖ (n = 77)
Wild-type	8 months (n = 247)	54.2 ± 13.3 years (n = 250)
LOH 10q	7.7 months‡ (n = 185)	56.4 ± 12.1 years¶ (n = 185)
No LOH 10q	9.3 months (n = 84)	52.7 ± 14.6 years (n = 84)

Significantly different in log-rank test (†, p = 0.0173; ‡, p = 0.0561).
Significantly different in Student *t*-test (§, p < 0.001; ‖, p = 0.0091; ¶, p = 0.0313).

amplification has been unclear. In earlier hospital-based studies (<40 cases), *EGFR* amplification was associated with poorer survival of glioblastoma patients (39, 40). In contrast, a meta-analysis of 7 previous studies (total 395 glioblastoma cases) did not reveal a significant predictive value of *EGFR* amplification (41). Several studies have suggested that *EGFR* amplification is predictive for certain age groups of glioblastoma patients (34, 37, 42, 43). Shinojima et al (42) reported that *EGFR* amplification was a significant predictor of poorer overall survival in glioblastoma patients and that the *EGFR* gene status was a more significant prognostic factor in younger patients (<60 years). Simmons et al (37) reported that EGFR overexpression was associated with poorer survival of glioblastoma patients younger than the median age, and that EGFR overexpression was negatively associated with survival in cases without *TP53* mutation. Other studies found *EGFR* amplification to be a predictor of longer survival only in older glioblastoma patients (34, 43). At the population level, *EGFR* amplification did not affect survival of glioblastoma patients at any age (8).

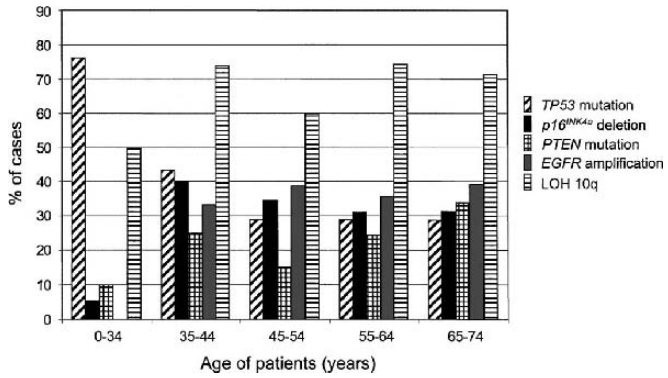

FIGURE 7. Genetic alterations versus age of patients with all glioblastoma. Glioblastomas developed in patients younger than 35 years are genetically characterized by frequent *TP53* mutations and absence of *EGFR* amplification, while there is no significant difference in genetic alterations between different age groups of patients older than 35 years.

p16^{INK4a} binds to cyclin-dependent kinase 4 (CDK4) and inhibits the CDK4/cyclin D1 complex (44, 45). This complex phosphorylates the RB1 protein (pRb), thereby inducing release of the E2F transcription factor that activates genes involved in the late G_1 and S phases (44, 45). In glioblastomas, disruption of the $p16^{INK4a}$ gene occurs through homozygous deletion or promoter methylation (9, 46). Findings regarding the predictive value of $p16^{INK4a}$ homozygous deletion in glioblastomas have been inconsistent. One study showed that homozygous $p16^{INK4a}$ deletion was a significantly unfavorable predictor for survival of glioblastoma patients (47), while another study showed that homozygous $p16^{INK4a}$ deletion was associated with shorter survival only in glioblastoma patients older than 50 years of age (48). At the population level, both univariate and multivariate analyses failed to show any predictive value of homozygous $p16^{INK4a}$ deletion (8).

Copresence of Genetic Alterations in Glioblastomas

LOH 10q is not only most the frequent genetic alteration in both primary and secondary glioblastomas, but is also frequently copresent with additional genetic alterations, i.e. with *EGFR* amplification (25.8%), *TP53* mutations (23.7%), $p16^{INK4a}$ deletion (23.3%), and *PTEN* mutations (16.2%) (8). Other combinations of genetic alterations were less frequent; *TP53* mutations showed significant inverse correlations with $p16^{INK4a}$ deletion and *EGFR* amplification (8). There were many different combinations of genetic alterations (Fig. 8), the most frequent being 2 alterations (36.1%), followed by one alteration (26.2%) and 3 alterations (24.1%), while more than 4 were rarely observed (4.8%) (Fig. 8). Taken together, LOH 10q plus at least one or 2 other genetic alterations appear to be operative in the development of the majority of glioblastomas. LOH 10q25-qter distal to the *PTEN* appears to be associated with acquisition of the glioblastoma phenotype (49), suggesting that a tumor suppressor gene in this region may be crucial in the development of glioblastomas. Candidate genes include

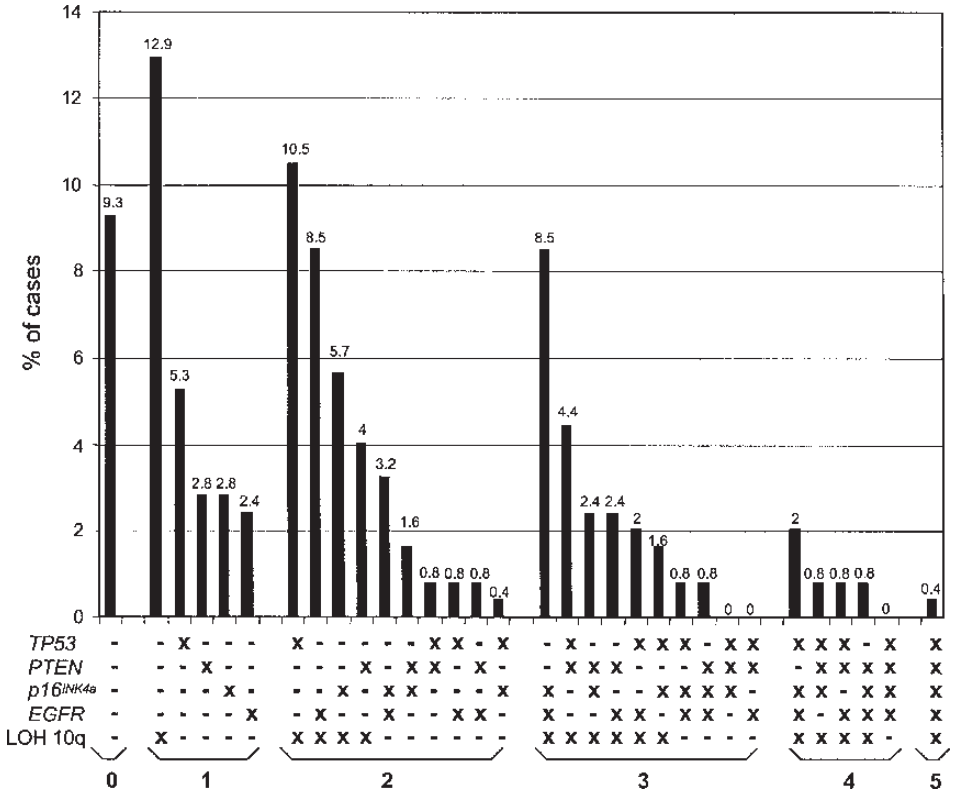

FIGURE 8. Combinations of genetic alterations in glioblastomas, including secondary glioblastomas. There are many combinations of genetic alterations, but it is clear that the majority of glioblastomas (62.9%) show LOH 10q alone and LOH 10q plus one or 2 additional genetic alterations.

DMBT1 (29, 50) and *FGFR2* (29). Copresence of *EGFR* amplification and *p16*^{INK4a} deletion was also frequent (17%), with a significant positive association in glioblastomas (8), in agreement with the findings of previous hospital-based studies based on small numbers of cases (51, 52).

TP53 Mutations as Molecular Fingerprints

Several familial cancer syndromes are associated with tumors of the nervous system, the most important ones being the Li-Fraumeni syndrome (*TP53* germline mutations), neurofibromatosis 1 (*NF1*), neurofibromatosis 2 (*NF2*) and Turcot syndrome (*APC* and *hMLH1*/*hPSM2*). However, such syndromes resulting from mutations in high-penetrance genes are rare (9, 21). The etiology of sporadic gliomas is largely unknown. Several occupations, environmental carcinogens, and diet (*N*-nitroso compounds) have been implicated, but the only environmental factor unequivocally associated with an increased risk of brain tumors, including gliomas, is therapeutic X-irradiation. In particular, children treated with X-irradiation for acute lymphoblastic leukemia show a significantly elevated risk of developing gliomas and primitive neuroectodermal tumors (PNET), often within 10 years after therapy (21, 53, 54).

Mutations induced in transformation-associated genes by environmental carcinogens (55) may give clues to the etiology of the respective tumor type, e.g. G:C→T:A mutations in codon 249 of *TP53* in hepatocellular carcinomas induced by aflatoxin B_1 (56) and G:C→T:A transversions in lung carcinomas of smokers (57). Among *TP53* mutations identified in astrocytic brain tumors, G:C→A:T transitions are the most frequent and are located predominantly at CpG sites (8, 58). A similar pattern was seen in colon cancer, sarcomas and lymphomas and among germline *TP53* mutations (58).

At the population level, the type and distribution of *TP53* mutations were different in the pathways leading to primary and secondary glioblastomas. A total of 47 mutations were detected in 40 patients with low-grade diffuse glioma and these were most frequently located in the 2 hot-spot codons 248 and 273, corresponding to 47% of all mutations. Of all *TP53* mutations identified in low-grade diffuse gliomas, 64%

TABLE 4. Nature of *TP53* Mutations in Primary Glioblastomas, Secondary Glioblastomas, and Low-Grade Gliomas

	Mutations at G:C				Mutations at A:T				
	>A:T at CpG	>A:T not at CpG	>T:A	>C:G	>T:A	>G:C	>C:G	Deletion Insertion	Splicing
Primary glioblastoma	44 (30%)	42 (29%)	17 (12%)	5 (3%)	2 (1%)	14 (10%)	2 (1%)	16 (11%)	3 (2%)
Secondary glioblastoma	14 (56%)*	3 (12%)	0	2 (8%)	0	2 (8%)	2 (8%)	2 (8%)	0
Low-grade glioma	27 (58%)	3 (6%)	4 (9%)	1 (2%)	1 (2%)	6 (13%)		5 (11%)	0

*, Significantly higher in secondary glioblastomas than primary glioblastomas.

were G:C → A:T transitions and of these, 90% were located at CpG sites (Table 4) (7). A similar pattern was seen in secondary glioblastomas (Table 4) (8). In contrast, *TP53* mutations were distributed more evenly among exons 5 to 8 of the *TP53* gene (only 17% at codons 248 and 273) and only 30% of *TP53* mutations in primary glioblastomas were G:C → A:T transitions at CpG sites (Table 4) (8). These observations suggest that the mechanisms of acquisition of *TP53* mutations in these glioblastoma subtypes may differ (8).

The best-characterized mechanism of G:C → A:T transitions at CpG sites is deamination of 5-methylcytosine at a CpG site, resulting in substitution of 5-methylcytosine by thymine. This occurs spontaneously or is factor-mediated, for example, through the action of oxygen radicals or nitric oxide produced by nitric oxide synthase under conditions of chronic inflammation (59). Since all CpG sites in exons 5 to 8 of the *TP53* gene have been reported to be methylated in normal human tissues (60), factors that affect the rate of deamination may be critical for the acquisition of G:C → A:T mutations.

In addition, the involvement of promutagenic alkyl groups in the O^6 position of guanine in DNA cannot be ruled out, since in low-grade astrocytomas there is a significant correlation between the presence of *TP53* mutations and promoter methylation of the O^6-methylguanine-DNA methyltransferase (*MGMT*) gene (61). MGMT specifically removes promutagenic alkyl groups from the O^6 position of guanine in DNA (62). Lack of MGMT in histologically normal perifocal brain is frequent and is age-dependent in brain tumor patients, while lack of MGMT in normal brain is infrequent in nontumor patients (63). DNA methylation may be caused by *N*-nitroso compounds in food or tobacco (21, 64), and through nonenzymatic methylation by *S*-adenosylmethionine (SAM) (65). Kang et al reported that O^6-methylguanine is present in leukocytes (0.7–4.6 adducts/10^8 guanine) and in liver (1.1–6.7 adducts/10^7 guanine) from healthy volunteers (66). The levels of O^6 -methylguanine in normal human brain tissue remain to be assessed.

Conclusions

Population-based analyses provide epidemiological data without the selection bias typically associated with clinical cancer registration. Based on a large number of patients diagnosed with astrocytic or oligodendroglial tumors in the Canton of Zurich during 1980–1994, the following conclusions emerged:

Patients with pilocytic astrocytoma (WHO grade I) have an excellent prognosis after surgical intervention (96% 5-year survival), suggesting that adjuvant radio- or chemotherapy is unnecessary in the management of this generally benign neoplasm.

In low-grade gliomas (WHO grade II), age and histologic type are significant predictors of clinical outcome. *TP53* mutations and LOH 1p/19q are genetic hallmarks of astrocytomas and oligodendrogliomas, respectively, and these alterations are mutually exclusive. With respect to gene status and survival, oligoastrocytomas were intermediate between low-grade diffuse astrocytomas and oligodendrogliomas.

The prognosis of glioblastoma patients at the population level is even worse than generally assumed from clinical trials, with less 2% surviving more than 3 years. Through the entire age range, higher age is the strongest predictor of poor outcome. Patients older than 70 years amount to 30% of all GBM patients and are underrepresented in clinical trials. Older patients are also less likely to receive surgical intervention and radiotherapy but there is little evidence to suggest that this is a major factor for their low survival rates.

Secondary glioblastomas originating from low-grade or anaplastic astrocytoma are rare, accounting for only about 5% of all glioblastomas. However, they are a distinct entity, based on their occurrence in younger patients, a higher proportion of females and a genetic pathway characterized by frequent and early *TP53* mutations. When corrected for their different age distribution, there was no difference in survival after glioblastoma diagnosis.

EGFR amplification, *TP53* mutations, $p16^{INK4a}$ homozygous deletion, and *PTEN* mutations are considered key genetic events in the evolution of glioblastomas, but the presence or absence of any of these changes does not affect survival.

LOH 10q is the most frequent genetic alteration in both pathways to primary and secondary glioblastomas and the only one associated with poorer survival of glioblastoma patients. LOH 10q plus any of one or 2 additional alterations are the most frequent combination, suggesting that LOH 10q plus at least one or 2 additional genetic alterations is a major player in the development of glioblastomas.

In several studies, including our population-based survey, LOH 10q has been associated with poorer survival of glioblastoma patients (8, 28–30), while *PTEN* mutations were not associated with prognosis (8, 28, 34, 35). This suggests that an additional, not yet unequivocally unidentified, tumor suppressor on chromosome 10q25-qter may be critically involved in the evolution of the glioblastoma phenotype. Identification and validation of such a gene could greatly advance in our understanding of the pathogenesis of glioblastomas, and in the development novel strategies for the treatment of this most malignant brain tumor.

G:C → A:T *TP53* mutations at CpG sites, particularly in the hotspot codons 248 and 273, constitute an early genetic event associated with malignant transformation in the pathway to secondary glioblastoma. In primary glioblastomas they are less frequent and may, at least in part, reflect increased genomic instability during tumor progression.

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