A t(1;19)(q10;p10) Mediates the Combined Deletions of 1p and 19q and Predicts a Better Prognosis of Patients with Oligodendroglioma

Robert B. Jenkins,[1] Hilary Blair,[1] Karla V. Ballman,[1] Caterina Giannini,[1] Robert M. Arusell,[2] Mark Law,[1] Heather Flynn,[1] Sandra Passe,[1] Sara Felten,[1] Paul D. Brown,[1] Edward G. Shaw,[3] and Jan C. Buckner[1]

[1]Mayo Clinic, Rochester, Minnesota; [2]Merit Care Community Clinical Oncology Program, Fargo, North Dakota; and [3]Wake Forest University School of Medicine, Winston-Salem, North Carolina

Abstract

Combined deletion of chromosomes 1p and 19q is associated with improved prognosis and responsiveness to therapy in patients with anaplastic oligodendroglioma. The deletions usually involve whole chromosome arms, suggesting a t(1;19)(q10;p10). Using stem cell medium, we cultured a few tumors. Paraffin-embedded tissue was obtained from 21 Mayo Clinic patients and 98 patients enrolled in 2 North Central Cancer Treatment Group (NCCTG) low-grade glioma trials. Interphase fusion of CEP1 and 19p12 probes detected the t(1;19). 1p/19q deletions were evaluated by fluorescence *in situ* hybridization. Upon culture, one oligodendroglioma contained an unbalanced 45,XX,t(1;19)(q10;p10). CEP1/19p12 fusion was observed in all metaphases and 74% of interphase nuclei. Among Mayo Clinic oligodendrogliomas, the prevalence of fusion was 81%. Among NCCTG patients, CEP1/19p12 fusion prevalence was 55%, 47%, and 0% among the oligodendrogliomas, mixed oligoastrocytomas, and astrocytomas, respectively. Ninety-one percent of NCCTG gliomas with 1p/19q deletion and 12% without 1p/19q deletion had CEP1/19p12 fusion ($P < 0.001$, χ^2 test). The median overall survival (OS) for all patients was 8.1 years without fusion and 11.9 years with fusion ($P = 0.003$). The median OS for patients with low-grade oligodendroglioma was 9.1 years without fusion and 13.0 years with fusion ($P = 0.01$). Similar significant median OS differences were observed for patients with combined 1p/19q deletions. The absence of alterations was associated with a significantly shorter OS for patients who received higher doses of radiotherapy. Our results strongly suggest that a t(1;19)(q10;p10) mediates the combined 1p/19q deletion in human gliomas. Like combined 1p/19q deletion, the 1;19 translocation is associated with superior OS and progression-free survival in low-grade glioma patients. (Cancer Res 2006; 66(20): 9852-61)

Introduction

Among gliomas, deletions of 1p and 19q are associated with tumors with oligodendroglial components (1, 2). Combined deletions of both arms have been observed in up to 70% of oligodendrogliomas and 50% of mixed oligoastrocytomas (3–5). The prevalence of deletions is similar in both low-grade (WHO grade 1) and anaplastic (WHO grade 3) oligodendroglial tumors (3–6). In addition to their diagnostic relevance, deletions of 1p and 19q have been associated with a prolonged survival in patients with anaplastic oligodendrogliomas and mixed oligoastrocytomas using retrospectively collected cohorts (3–5, 7–9). The prognostic relevance of the deletions has been validated recently by two prospective clinical trials, one led by the Radiation Therapy Oncology Group (RTOG) and another led by the European Organization for Research and Treatment of Cancer (EORTC; refs. 10, 11). Both retrospective and prospective data also suggest that 1p and 19q deletion also predict the responsiveness of anaplastic oligodendroglial tumors to combined radiation therapy (RT) and chemotherapy (7, 10, 11).

Whereas the prognostic relevance of 1p and 19q deletions is well established for anaplastic oligodendrogliomas and mixed oligoastrocytomas, the prognostic relevance of the deletions for low-grade gliomas is more controversial. In this report, using patients enrolled on two NCCTG trials for newly diagnosed low-grade gliomas, we show that combined deletion of 1p and 19q independently predicts a prolonged survival for patients with these tumors.

The majority of 1p and 19q deletions have appeared to involve the entire 1p and 19q arms (1, 2, 4, 6). Based on this observation, we hypothesized that an unbalanced translocation might be the etiology of both deletions. In this report, we show that an unbalanced t(1;19)(q10;p10) likely mediates the combined 1p and 19q deletion in gliomas. Furthermore, we show that the translocation is associated with a significantly better prognosis as well as response [as measured by overall survival (OS) and progression-free survival (PFS)] to radiation in patients with low-grade gliomas, especially gliomas with oligodendroglial components.

Materials and Methods

Patients. The initial discovery and validation studies used material from 21 patients enrolled in an Institutional Review Board–approved Mayo Clinic Neuro-Oncology project entitled "Molecular Markers of Glioma Initiation and Progression." Further clinical validation used 125 patients enrolled on North Central Cancer Treatment Group (NCCTG) trial 94-72-53 "Diagnostic and Prognostic Markers in Low-Grade Gliomas." NCCTG 94-72-53 is a correlative translational biomarker study of the NCCTG. Eligible patients were those enrolled in two prospective NCCTG trials for newly diagnosed low-grade glioma: 86-72-51, a phase III trial of 50.4 Gy versus 64.8 Gy RT (12), and 93-72-02, a phase II trial of six cycles of procarbazine, lomustine, and vincristine (PCV) chemotherapy followed by 54.0 Gy RT (13). Details of the trial designs, end points, and outcomes are provided in refs. 12 and 13. Paraffin-embedded tumor tissue was available and obtained for 98 patients

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Robert B. Jenkins, Division of Laboratory Genetics, Mayo College of Medicine, 200 First Street Southwest, Rochester, MN 55905. Phone: 507-284-9617; Fax: 507-284-0043; E-mail: rjenkins@mayo.edu.

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from these two trials. Table 1 describes the clinical and pathologic features of the patients enrolled on 94-72-53 for whom paraffin tissue was available. Two patients had WHO grade I gliomas; the remaining 96 gliomas were WHO grade II. Pilocytic astrocytomas were not included.

Culture. A fresh oligodendroglioma sample was received and dissociated in collagenase. Cells were rinsed in 10% fetal bovine serum/MEM and cultured in human neuroproliferative medium (StemCell Technologies, Vancouver, British Columbia, Canada) at 37°C in a gassed incubator (5% CO_2, 5% O_2) using previously reported methods for culturing neural stem cells (14). Tumor cells grew as suspended clumps and were harvested and fixed using standard cytogenetic techniques. Metaphase spreads were obtained and G banded using standard cytogenetic techniques. The International System for Cytogenetic Nomenclature was used to describe the cytogenetic anomaly (15).

Fluorescence *in situ* hybridization. Fluorescence *in situ* hybridization (FISH) analyses of fixed cytogenetic preparations from the fresh oligodendroglioma followed standard methods.

1p and 19q deletion analysis of formalin-fixed paraffin-embedded gliomas by FISH was done as described previously (9).

The prehybridization, hybridization, and posthybridization procedures for the t(1;19) translocation analysis of formalin-fixed paraffin-embedded tissues were similar to the above method.

FISH probes: for 1p and 19q deletion analysis of metaphase and interphase nuclei (for both fresh and paraffin-embedded tumor specimens), nick-translated bacterial artificial chromosome (BAC) contig probes for the target region 1p36 (Spectrum Orange, Vysis, Downers Grove, IL) with a control region at 1q24 (Spectrum Green, Vysis) and the control region 19p13 (Spectrum Green) with the target region 19q13 (Spectrum Orange) were used as described previously (9). Whole chromosome painting (WCP) probes (Vysis) for 1 and 19 were used in the analysis of metaphase spreads from the primary tumor. For the t(1;19) translocation (fusion) analysis, a chromosome 1 α satellite probe CEP1 in Spectrum Orange (Vysis) was cohybridized with a BAC contig probe for 19p12 that was nick translated and labeled in Spectrum Green. The BAC clones included in the 19p12 probe were the following: RP11-771C12, RP11-587H3, RP11-460G17, CTD-3173A10, CTD-3074B13, and RP11-677G1. All BAC clones were supplied by Invitrogen (Carlsbad, CA).

Scoring criteria. For the deletion portion of the study, 100 nuclei were scored per specimen for number of red and green signals per nuclei. The specimen was considered deleted if the red/green ratio was <0.85 and aneusomy if there were >30% of the nuclei with three green signals.

For the translocation portion of the study, the definition of fusion required that the red and green signals be within two signal widths of each other (16). This less strict fusion requirement was due to the potential 4.0 to 6.5 Mb gap between the probes when the hypothesized translocation was present. The range in estimated gap size is based on the distance from the position of BAC RP11-587H3 (the 19p BAC closest to the centromere that did not show cross-hybridization) to the center and q-arm end of the chromosome 19 centromere (17). The two-signal width definition was selected after measurement of the interphase distance in paraffin-embedded nuclei from the tumor with the known t(1;19) (see Results). Fifty nuclei were scored by each of two independent scorers. Diploid nuclei were classified as abnormal if one or more fusion signals were identified. Aneuploid nuclei were classified as abnormal if apparently triploid cells had one or more fusion signals and apparently tetraploid cells had two or more fusion signals. The criteria for translocation was established by scoring 12 normal glial specimens and 5 abnormal astrocytoma and glioblastoma multiforme specimens known not to contain combined 1p and 19q deletion. For a case to be classified as containing the t(1;19), 60% or greater of the nuclei were required to have fusion of the red and green signals. This relatively high criteria is necessary because the two-signal width definition for fusion results in a large random colocalization volume.

Statistical methods. Counts were summarized as frequencies and relative frequencies. Distributions of counts were compared among groups with a χ^2 test. Time-to-event data (survival and PFS) were summarized by Kaplan-Meier estimation. Differences in time-to-event experiences between

Table 1. Clinical and histologic characteristics of low-grade glioma patients eligible for NCCTG 94-72-53

Variables	All eligible patients (n = 125)	Deletion successful patients (n = 91)	P*	Fusion successful patients (n = 84)	P †
Age (y)					
Median (range)	39 (18-72)	38 (18-72)	0.46	38 (18-72)	0.27
Gender, n (%)			0.92		0.92
Male	75 (60)	54 (59)		51 (60)	
Female	50 (40)	37 (41)		33 (40)	
MMSE score (out of 30)			0.83		0.94
Median (range)	29 (21-30)	30 (21-30)		29 (21-30)	
Performance score, n (%)			0.86		0.56
0	42 (34)	29 (32)		29 (35)	
1	14 (11)	9 (10)		10 (12)	
2	3 (2)	1 (1)		0 (0)	
Missing	66 (53)	52 (57)		45 (53)	
Trial/treatment, n (%)			0.76		0.87
867251/64.8 Gy	43 (34)	30 (33)		27 (32)	
867251/50.4 Gy	46 (37)	38 (42)		34 (41)	
937202/PCV+50.4 Gy	34 (27)	22 (24)		22 (26)	
867251/observation	2 (2)	1 (1)		1 (1)	
Histologic type, n (%)			0.38		0.41
Oligodendroglioma	57 (45)	47 (52)		42 (50)	
Mixed oligoastrocytoma	41 (33)	31 (34)		30 (36)	
Astrocytoma	27 (22)	13 (14)		12 (14)	

*Ps comparing deletion successful patients with all eligible patients.
† Ps comparing fusion successful patients with all eligible patients.

groups (i.e., those with deletion versus those without deletion, those with fusion and those without fusion) were compared with a Wilcoxon test. Univariable and multivariable analyses of time-to-event data were conducted using Cox proportional hazards modeling. The following variables were included in the modeling: age, gender, histologic type, mini-mental status exam (MMSE) status, 1p/19q deletion status, and CEP1/19p12 fusion status. The intent of the multivariable models was to ascertain whether the univariable association between deletion status and fusion and the time-to-event end points (survival and PFS) remained after adjusting for known prognostic factors.

Results

Identification of a t(1;19)(q10;p10) in an oligodendroglioma. Using modifications of various stem cell medium, we have been able to do short-term culture and recovery of metaphases from a few oligodendrogliomas. In one oligodendroglioma, we observed an unbalanced 45,XX,der(1;19)(q10;p10) karyotype in all abnormal metaphases (Fig. 1*A*). Other nonclonal abnormalities, such as an extra chromosome 7 (see Fig. 1*A*), were also observed in single metaphases. Initially, the translocation was verified in metaphases using WCP probes for chromosomes 1 and 19 (Fig. 1*B*). We then developed a BAC contig FISH probe for proximal 19p12 in green and combined it with CEP1 in orange. Using a single-fusion strategy, this probe mixture was applied to metaphase cells and interphase nuclei from this glioma (Fig. 1*C* and *D*). Fusion of the CEP1 and 19p12 probes was observed in all abnormal metaphases as well as 74% of interphase nuclei.

Initial validation of the CEP1/19p12 FISH fusion probe set. The translocation probe was then applied to 4 nontumor gliosis specimens, 5 oligodendroglioma paraffin specimens with (3) and without (2) 1p and 19q deletion, and a tissue microarray containing core biopsies from paraffin blocks from 16 oligodendrogliomas with (12) and without (4) 1p and 19q deletion. As described in Materials and Methods, at least 60% of interphase nuclei were required to have colocalization of the CEP1 and 19p12 signals for the tumor to be classified as having the t(1;19). Using this criterion, 17 (81%) oligodendrogliomas had evidence of fusion and there was 90% concordance between codeletion of 1p and 19q with fusion of the CEP1 and 19p12 probes on the tumor specimens ($P < 0.001$). These included all 15 patients with codeletion of 1p and 19q. In this group, the proportion of nuclei with fusion ranged from 62% to 98%. Two patients with fusion (respectively in 62 and 68% of nuclei) had no evidence of 1p and 19q codeletion. Four patients had evidence of neither deletion nor fusion. The proportion of nuclei with fusion ranged from 4% to 35% in this group. The four nontumoral control specimens showed no evidence of the translocation, whereas the proportion of nuclei having fusion ranged from 4% to 29%.

1p and 19q deletion as well as CEP1 and 19p12 fusion predict PFS and OS in patients with low-grade gliomas. Of 125 patients enrolled in NCCTG 94-72-53, paraffin blocks were available for 98 patients, and FISH studies for 1p/19q deletion were successful for 91 (93%) patients. FISH studies for CEP1 and 19p12 fusion were successful for 84 (86%) patients. The reasons for unsuccessful FISH studies were block depletion (two patients, deletion; three patients, fusion) and hybridization failure (5 patients, deletion; 11 patients, translocation). Table 1 compares characteristics of all eligible patients enrolled on the NCCTG trials to those of patients for whom FISH studies were successful. There were no significant differences in age, gender, MMSE, performance scores, treatment, and histologic type among groups. Supplementary Table S1 summarizes the 1p and 19q deletion and the CEP1/19p12 fusion results for each patient enrolled on 94-72-53.

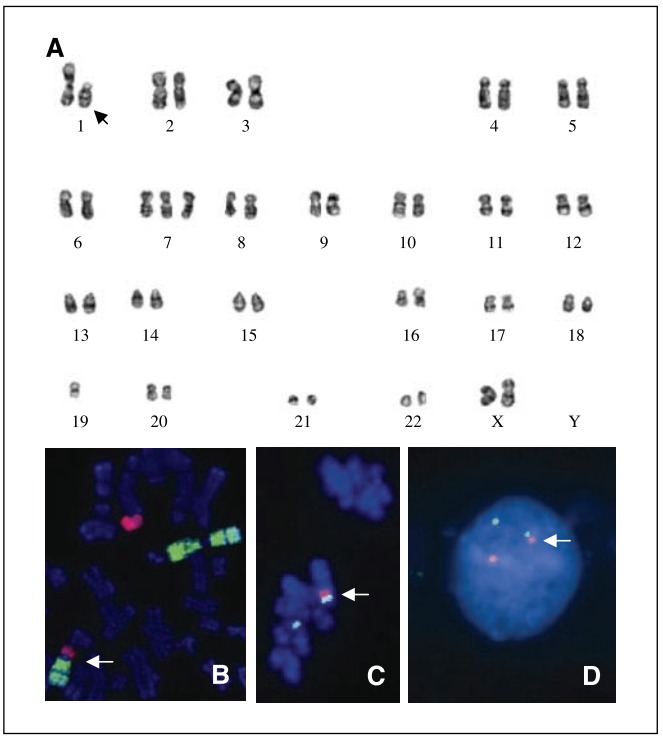

Figure 1. *A,* karyotype of oligodendroglioma patient. *Arrow,* t(1;19)(q10;p10). *B,* metaphase FISH using whole chromosome painting probes. *Arrow,* t(1;19). Spectrum Green = 1 WCP, Spectrum Orange = 19 WCP. *C,* metaphase FISH using 1CEP in Spectrum Orange and a BAC contig for 19p12 in Spectrum Green. *Arrow,* fusion of the probes at the translocation site. *D,* interphase FISH using 1CEP in Spectrum Orange and a BAC contig for 19p12 in Spectrum Green. *Arrow,* fusion signal.

Table 2A summarizes the 1p and 19q deletion results. Of 91 patients with low-grade glioma, 37 (41%) showed 1p and 19q codeletion, 6 (6%) showed 19q deletion alone, and 48 (53%) showed neither. In this group of patients, no case showed 1p deletion alone.

The prevalence of combined 1p and 19q deletion was 57% among 47 oligodendrogliomas, 32% among 31 mixed oligoastrocytomas, and 0% among 13 astrocytomas. The prevalence of combined 1p and 19q deletion was significantly different between all three histologic groups ($P < 0.001$, χ^2 test).

Patients with 1p and 19q deletion had a median OS time of 11.9 years and a 5-year OS rate of 95% versus 8.1 years and 59%, respectively, for those without deletion (Wilcoxon $P = 0.004$). The median PFS time and 5-year PFS rate for patients with 1p and 19q deletion were 8.2 years and 84% versus 3.3 years and 48%, respectively, for those without deletion (Wilcoxon $P = 0.002$).

In oligodendroglioma patients, median OS time and 5-year OS rate were 13 years and 96%, respectively, in patients with 1p/19q–codeleted tumors versus 10.8 years and 70% in those without deletion (Wilcoxon $P = 0.06$). Median PFS time and 5-year PFS rate were 8.2 years and 85%, respectively, in patients with 1p/19q–codeleted tumors versus 7.0 years and 70% in those without deletion (Wilcoxon $P = 0.26$).

We also did an analysis of the combined set of 78 oligodendrogliomas and mixed oligoastrocytomas. Patients with oligodendrogliomas or mixed oligoastrocytomas with 1p and 19q deletion had a median OS time of 11.9 years and a 5-year OS rate

of 95% versus 10.3 years and 66%, respectively, for those without deletion (Wilcoxon $P = 0.02$). The median PFS rate and 5-year PFS rate for patients with 1p and 19q deletion was 8.2 years and 84% versus 5.6 and 59%, respectively, for those without deletion (Wilcoxon $P = 0.04$).

Table 2B summarizes the CEP1 and 19p12 fusion results. Of the 84 patients, 37 (44%) had CEP1/19p12 fusion, whereas 47 (56%) did not. The prevalence of fusion was 55% among 42 oligodendrogliomas, 47% among 30 mixed oligoastrocytomas, and 0% among 12 astrocytomas. The prevalence of fusion was significantly different between all three histologic groups ($P < 0.001$, χ^2 test).

1p and 19q deletion results were available for 81 of the 84 patients. Supplementary Table S2 summarizes the association of CEP1/19p12 fusion with chromosome 1p and 19q deletion data. Briefly, of 33 gliomas with combined 1p and 19q deletion, 91% had CEP1/19p12 fusion. Of 48 gliomas without combined 1p and 19q

Table 2. Association of (A) combined 1p and 19q deletion and (B) t(1;19)(q10;p10) with OS and PFS in patients with low-grade gliomas enrolled on NCCTG 94-72-53

A. OS and PFS by 1p and 19q deletion status

Histologic type	Oligodendroglioma (n = 47)	Mixed oligoastrocytoma (n = 31)	Astrocytoma (n = 13)	Total (N = 91)
Prevalence (%)				
1p− and 19q−	27 (57)	10 (32)	0 (0)	37 (41)
Not 1p− and 19q−	20 (43)	21 (68)	13 (100)	54 (59)
OS				
5 y (%)				
1p− and 19q−	96	90	—	95
Not 1p− and 19q−	70	62	38	59
Median, y (95% CI)				
1p− and 19q−	13.0 (11.2 to NR)	11.0 (7.2 to NR)	—	11.9 (10.4 to NR)
Not 1p− and 19q−	10.8 (5.0-12.7)	8.3 (3.9-13.0)	3.1 (2.2 to NR)	8.1 (4.1-11.2)
P*	0.06	0.34	—	0.004
PFS				
5 y (%)				
1p− and 19q−	85	80	—	84
Not 1p− and 19q−	70	48	15	48
Median, y (95% CI)				
1p− and 19q−	8.2 (5.5-11.1)	8.1 (5.2-13.3)	—	8.2 (5.8-10.4)
Not 1p− and 19q−	7.0 (3.3-10.3)	3.5 (2.7-6.2)	1.4 (0.8-2.6)	3.3 (2.5-6.2)
P*	0.26	0.18	—	0.002

B. OS and PFS by CEP1/19p12 fusion status

Histologic type	Oligodendroglioma (n = 42)	Mixed oligoastrocytoma (n = 30)	Astrocytoma (n = 12)	Total (N = 84)
Prevalence (%)				
CEP1/19p12 fusion	23 (55)	14 (47)	0 (0)	37 (44)
No CEP1/19p12 fusion	19 (45)	16 (53)	12 (100)	47 (56)
OS				
5 y (%)				
CEP1/19p12 fusion	96	93	—	95
No CEP1/19p12 fusion	68	50	58	60
Median, y (95% CI)				
CEP1/19p12 fusion	13.0 (11.2 to NR)	10.4 (7.3 to NR)	—	11.9 (10.4 to NR)
No CEP1/19p12 fusion	9.1 (5.0-12.7)	6.4 (3.3-13.0)	6.0 (3.1 to NR)	8.1 (4.1-12.3)
P*	0.01	0.17	—	0.003
PFS				
5 y (%)				
CEP1/19p12 fusion	83	71	—	78
No CEP1/19p12 fusion	63	50	33	51
Median, y (95% CI)				
CEP1/19p12 fusion	8.2 (5.5-10.3)	7.2 (2.7-13.3)	—	8.1 (6.2-10.3)
No CEP1/19p12 fusion	5.6 (2.4-8.1)	3.3 (1.7-11.0)	1.6 (1.2-6.4)	3.3 (2.4-5.8)
P*	0.06	0.35	—	0.006

Abbreviation: NR, not reached.
*Wilcoxon test.

deletion, 13% had CEP1/19p12 fusion. This difference in proportion was significant ($P < 0.001$, χ^2 test).

Figure 2*A* and *B* shows the Kaplan-Meier survival curves for median OS and PFS time for patients with grade II gliomas with and without CEP1/19p12 fusion. Patients with fusion had a median OS time of 11.9 years and 5-year OS rate of 95% versus 8.1 years and 60%, respectively, for those without fusion (Wilcoxon $P = 0.003$). The median PFS time and 5-year PFS rate for patients with fusion was 8.1 years and 78%, versus 3.3 years and 51%, respectively, for those without the translocation (Wilcoxon $P = 0.006$).

Figure 2*C* and *D* shows the Kaplan-Meier survival curves for median OS and PFS time for patients with grade II oligodendrogliomas with and without CEP1/19p12 fusion. Patients with oligodendrogliomas with fusion had a median OS time of 13.0 years and 5-year OS rate of 96%, versus 9.1 years and 68%, respectively, for those without fusion (Wilcoxon $P = 0.01$). The median PFS time and 5-year PFS rate for patients with fusion was 8.2 years and 83%, versus 5.6 years and 63%, respectively, for those without the translocation (Wilcoxon $P = 0.06$).

We also did an analysis of the combined set of 72 oligodendrogliomas and mixed oligoastrocytomas. Patients with oligodendrogliomas or mixed oligoastrocytomas with CEP1/19p12 fusion had a median OS time of 11.9 years and a 5-year OS rate of 95% versus 8.7 years and 60%, respectively, for those without fusion (Wilcoxon $P = 0.005$). The median PFS time and 5-year PFS rate for patients with CEP1/19p12 fusion was 8.1 years and 78%, versus 4.4 years and 57%, respectively, for those without fusion (Wilcoxon $P = 0.05$).

The t(1;19) or combined 1p and 19q deletion status is associated with treatment response. One of the trial components of NCCTG 94-72-53, NCCTG 86-72-51, compared two doses of RT for low-grade gliomas: 50.4 Gy versus 64.8 Gy (12). Table 3 summarizes the survival associations of combined 1p and 19q deletion or CEP1/19p12 fusion with these two radiation doses. Although no difference in OS between the two treatment arms was observed (12), there was evidence that patients without fusion had a significantly shorter OS when treated with higher doses of radiation (Fig. 3*A*; Table 3). The hazard ratio (HR) for death after 64.8 Gy of radiation, comparing patients with and without fusion, was 2.75 [95% confidence interval (95% CI), 1.14-6.65; likelihood ratio $P = 0.03$; median OS, 11.6 years versus 5.0 years]. Conversely, there was no significant difference in OS whether the patients with fusion received either a lower or higher dose of radiation (Fig. 3*A*; Table 3). The HR for death after 64.8 Gy versus 50.4 Gy of radiation for patients with fusion was 1.59 (95% CI, 0.57-4.42; likelihood ratio $P = 0.37$; median OS, 11.6 years versus not reached years). Similar results were observed for the association of PFS after RT with fusion status (Fig. 3*B*; Table 3) and associations of OS and PFS after RT with combined 1p and 19q deletion status (Table 3; data not shown).

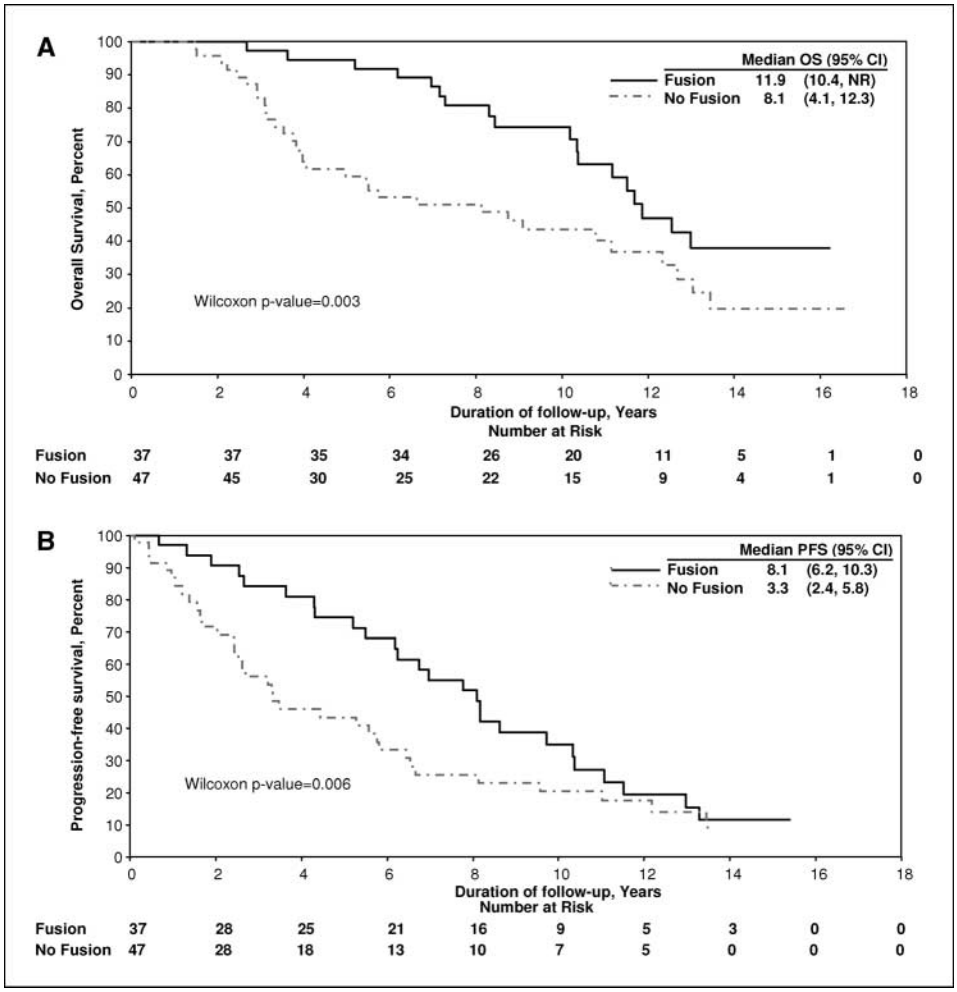

Figure 2. *A,* Kaplan-Meier curve for OS for patients with grade II gliomas with and without CEP1/19p12 fusion. *B,* Kaplan-Meier curve for PFS for patients with grade II gliomas with and without CEP1/19p12 fusion.

The second trial component of NCCTG 94-72-53, the phase II trial 93-72-02, evaluated six cycles of PCV chemotherapy followed by 54.0 Gy RT (13). Table 3 summarizes the survival associations of combined 1p and 19q deletion or CEP1/19p12 fusion with this therapeutic approach. There was a trend for patients without fusion to have a shorter PFS compared with patients with fusion. The HR for progression after 54.0 Gy, comparing patients with and without fusion, was 7.39 (95% CI, 0.84-65.03; likelihood ratio P = 0.03; median PFS, 3.3 years versus 9.5 years). Similar trends were observed for the association of OS after RT and PCV with fusion status and for the associations of OS and PFS after RT and PCV with combined 1p and 19q deletion (Table 3; data not shown), but the Ps were not significant.

Multivariable modeling. We did multivariable modeling to assess whether the tumor deletion and fusion status remained associated with survival among the patients enrolled on 94-72-53 after adjusting for known prognostic factors (see Statistical Methods). Supplementary Table S3 summarizes the univariable and multivariable Cox modeling. Briefly, in multivariable modeling, the HRs for 1p and 19q deletion status did not change considerably from those in the univariable model, indicating that the association still remained after adjustment. The Ps did not achieve significance at the 0.05 level, likely due to the number of variables in the model and the relatively small sample size. However, after adjustment for known prognostic variables, the association between CEP1/19p12 fusion status and OS remained significant in the whole cohort of low-grade gliomas and in the subset of mixed oligoastrocytomas plus oligodendrogliomas (P = 0.04 and 0.03, respectively). This indicates that there is likely an independent association between fusion status and OS.

Discussion

There have been several prior routine cytogenetic and molecular cytogenetic (loss of heterozygosity and comparative genomic hybridization) studies of human gliomas (1–6, 18–23). Whereas the molecular cytogenetic studies have consistently described the prevalence of 1p and 19q deletions as between 50% and 70% in oligodendrogliomas and anaplastic oligodendrogliomas, routine cytogenetic studies have not identified recurrent abnormalities of 1p and 19q in these tumors (1, 18). We identified an unbalanced whole-arm (centrosomal or pericentrosomal) t(1;19)(q10;p10) in a single oligodendroglioma. A similar t(1;19) was also observed by Magnani et al. (24) in a single oligodendroglioma. These two cytogenetic results and the association of whole-arm 1p and 19q deletions in oligodendrogliomas suggested to us that the unbalanced t(1;19)(q10;p10) translocation might be more common in these tumors than previously appreciated. Our interphase FISH studies show that the combined deletion of 1p and 19q is most likely mediated by this unbalanced translocation.

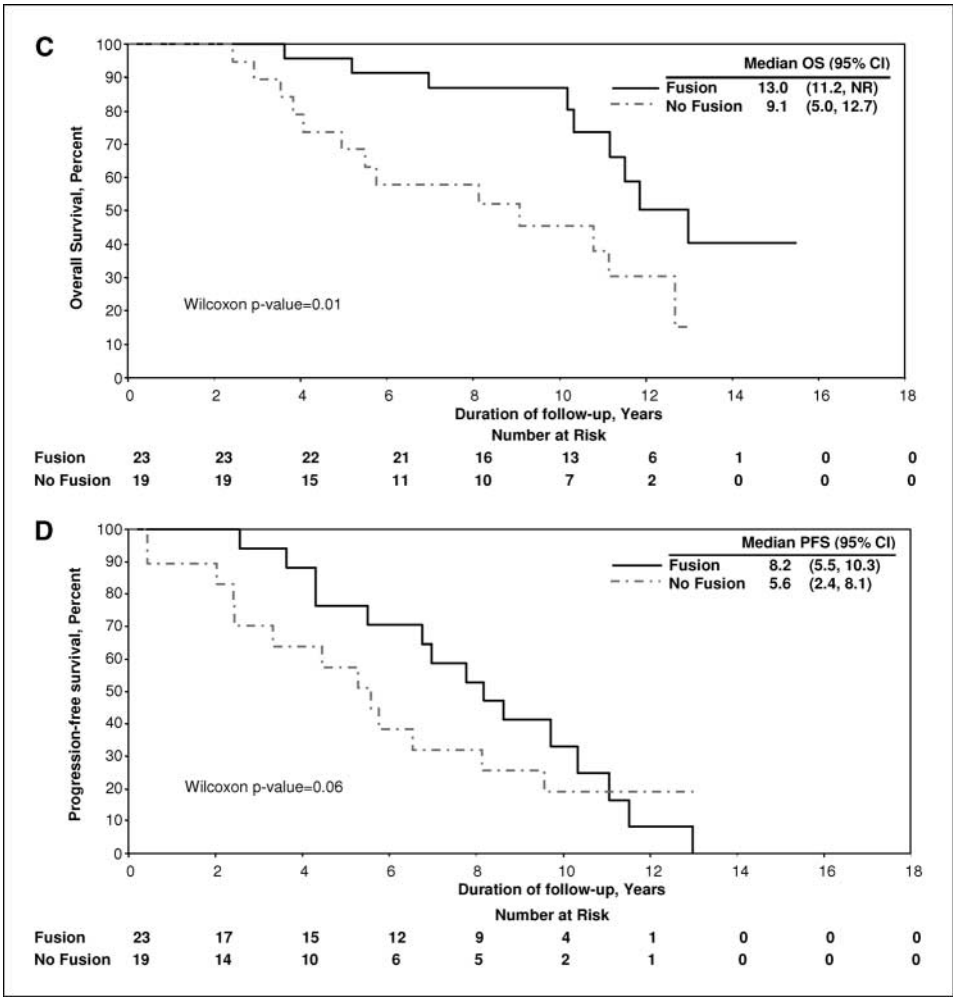

Figure 2 *Continued. C,* Kaplan-Meier curve for OS for patients with oligodendroglioma with and without CEP1/19p12 fusion. *D,* Kaplan-Meier curve for PFS for patients with oligodendroglioma with and without CEP1/19p12 fusion.

The FISH method used for detection of the t(1;19) within interphase nuclei was somewhat different from prior interphase translocation strategies. The abnormal chromosome has the cytogenetic appearance of being the result of a whole-arm (centrosomic or pericentrosomic) translocation. The initial probe choice to detect such alterations would be to use centromere-specific probes for chromosomes 1 and 19 (e.g., using a CEP1 and CEP19 in two different colors). However, there is significant sequence homology between the centromeres of chromosomes 1 and 19 (and 5; ref. 25). Much of 19p12 and the entire 19cen (including the α satellite region) band is homologous to both 5cen and 1cen (25). Although it has been possible to develop centromere 1 probes that do not cross-hybridize with chromosome 19, it has been difficult to develop centromere 19 probes that do not cross-hybridize with chromosome 1. Thus, we developed a FISH probe for proximal 19p12, using BACs that mapped close to the 19 centromere, but which lacked cross-hybridization with other genomic sequences, including chromosomes 1 and 5. Given the hypothesized size of most human centromeres (17), the CEP1 probe and the 19p12 probe would be separated by at least 4.0 Mb (and at most 6.5 Mb) by the translocation. Thus, we did not expect to observe actual fusion of the red and green signals (e.g., a merged yellow signal) within interphase nuclei that contained the translocation. We empirically set the criteria for signal fusion to require that at least one red and one green signal be within two signal diameters of each other. The resulting potential colocalization volume is large enough to result in random fusion events. The rate of random fusion is also increased by tumor aneuploidy and polyploidy. For example, trisomy 19 is a common alteration in glioblastomas (18, 23). Our final criteria for fusion, which minimized the false-positive and false-negative rates, required at least 60% of nuclei show colocalization of the red and green signals. Because the two-signal width definition will be relatively difficult to implement into clinical practice, we are currently developing a three-color probe strategy to simultaneously detect 1p or 19q deletion and the t(1;19).

The strong homology of the chromosome 1 and 19 centromeric regions suggests a mechanism, centromeric or pericentromeric fusion, for the translocation. Similar whole-arm translocations have been observed in several congenital and neoplastic disorders [e.g., translocation Down syndrome (trisomy 21) and translocation Patau syndrome (trisomy 13)] in the t(1;7)(p10;q10) observed in secondary myelodysplastic syndromes (26) and in many carcinomas (reviewed in ref. 27). Abnormalities of the chromosome 1, 9, and 16 centromeres (containing α satellite DNA) and qh regions (containing satellite 2 and 3 sequences) are also common in ICF syndrome (28), a disorder with immunodeficiency, centromere region instability, and abnormal facies. Breakage and rejoining of homologous satellite sequences appear to mediate these centromeric alterations (29). Similar centromeric instability has been hypothesized to underlie many of the whole-arm translocations observed in solid tumors (27). Recent evidence suggests that chromosomes (including chromosomal centromeres) are organized into a specific intranuclear anatomy that is cell type and developmental stage specific (30, 31). Because the t(1;19) seems to be relatively specific for gliomas, and especially oligodendrogliomas, it is reasonable to speculate that regions of chromosomes 1 and 19 are colocalized, within early or more mature glial precursor cells and/or within mature oligodendroglial cells. We observed seven gliomas (two retrospective and five NCCTG tumors) with evidence of fusion but without deletion. Although the discordant cases could be measurement errors, these gliomas may be evidence that colocalization precedes translocation or deletion.

The cancer-specific nature of the translocation may also be facilitated by alterations of DNA methylation and/or histone modification. Whereas the promoters of many genes are hypermethylated in cancer (32), several DNA repeat sequences, including centromeric and pericentromeric repeats, are usually hypomethylated in cancer (33). These epigenetic alterations may underlie the centromeric instability in cancer. It has been reported recently that mutations in the *DNMT3B* gene are associated with the development of chromosome 1qh region alterations in hepatocellular carcinoma (34, 35). Similarly, hypomethylation of the chromosome 9qh region is associated with abnormalities of chromosome 9 in urothelial cancers (36). A relevant observation supporting our proposed mechanism is that combined deletions of 1p and 19q are highly correlated with hypermethylation of a large number of genes (and putatively with centromeric hypomethylation; ref. 37).

Molecular cytogenetic deletion mapping studies have suggested that the minimal regions of deletion and, by implication, the putative candidate genes reside within 1p36 and 19q13.3 (4, 6, 23, 38). Isolated deletions of 19q are relatively common in astrocytic and oligodendroglial tumors (6, 9, 10, 23). We observed six such cases in the low-grade NCCTG cohort described in this report; one of these had evidence of CEP1/19q12 fusion. Isolated deletions of 1p are rarer in gliomas and are associated with a poorer prognosis (4, 8–10, 23). It should be noted that the smaller deletions have usually been described in high-grade gliomas, especially glioblastomas (6, 21). The prevalence of the translocation strongly suggests that the combined loss of two or more genes on 1p and 19q are required for the development of oligodendrogliomas. Our results do not exclude the possibility that small single-copy regions of DNA within the pericentromeric repeats might be involved in the translocation. Because of their location, such regions would be difficult to clone and would likely be underrepresented in the current versions of the human genome map.

In 1998, using retrospectively collected material, Cairncross et al. (7) showed that 1p and 19q deletions were associated with anaplastic oligodendrogliomas that had better prognosis. Cairncross et al. also suggested that the deletions were associated with responsiveness to adjuvant chemotherapy and RT. Recently, using prospectively collected anaplastic oligodendrogliomas and mixed oligoastrocytomas from patients enrolled on RTOG trial 9402, Cairncross et al. (10) have confirmed that combined 1p and 19q deletions are associated with significantly better prognosis (median survival of 7 years versus 2.8 years, comparing patients whose tumors contained or did not contain combined 1p and 19q deletion). The prospective trial data also suggest that patients with combined 1p and 19q deletion may have a better initial response to PCV chemotherapy and radiotherapy. Similar prognostic but not predictive conclusions were drawn by the simultaneous EORTC trial of PCV and radiation in patients with anaplastic oligodendrogliomas (11).

The prognostic relevance of combined 1p and 19q deletion of low-grade gliomas is somewhat more controversial. However, the majority of reports suggest that patients whose low-grade glioma, especially oligodendroglioma, with combined 1p and 19q deletion have a better prognosis than those patients whose tumor lack the deletions (4–6). Two recent reports also suggest that low-grade oligodendrogliomas with 1p deletions may be associated with

Table 3. Association of (A) combined 1p and 19q deletion and (B) t(1;19)(q10;p10) with OS and PFS by treatment arm in patients with low-grade glioma enrolled on NCCTG 94-72-53

A. OS and PFS by treatment arm by 1p and 19q deletion status

Treatment	50.4 Gy 86-72-51 arm A (*n* = 38)	64.8 Gy 86-72-51 arm B (*n* = 30)	54.0 Gy/PCV 93-72-02 (*n* = 22)
OS			
5 y (%)			
1p− and 19q−	93	93	100
Not 1p− and 19q−	63	44	69
Median, y (95% CI)			
1p− and 19q−	13.4 (6.1 to NR)	11.6 (8.4-12.6)	9.7 (9.1-10.4)
Not 1p− and 19q−	8.2 (3.9-12.7)	4.5 (3.7-11.1)	10.3 (3.3 to NR)
*P**	0.09	0.02	0.12
PFS			
5 y (%)			
1p− and 19q−	71	93	89
Not 1p− and 19q−	54	25	62
Median, y (95% CI)			
1p− and 19q−	7.3 (4.3-13.0)	8.2 (5.5-11.5)	8.6 (4.4-10.4)
Not 1p− and 19q−	5.7 (2.6-7.2)	2.2 (0.8-2.5)	3.3 (1.1-6.7)
*P**	0.34	0.002	0.08

B. OS and PFS by treatment arm by CEP1/19p12 fusion status

Treatment	50.4 Gy 86-72-51 arm A (*n* = 34)	64.8 Gy 86-72-51 arm B (*n* = 27)	54.0 Gy/PCV 93-72-02 (*n* = 22)
OS			
5 y (%)			
CEP1/19p12 fusion	92	93	100
No CEP1/19p12 fusion	57	46	75
Median, y (95% CI)			
CEP1/19p12 fusion	NR (7.0 to NR)	11.6 (8.4-12.6)	10.4 (10.3-10.4)
No CEP1/19p12 fusion	8.1 (3.1-12.7)	5.0 (3.7-6.6)	NR (9.1 to NR)
*P**	0.07	0.004	0.10
PFS			
5 y (%)			
CEP1/19p12 fusion	62	86	90
No CEP1/19p12 fusion	52	31	67
Median, y (95% CI)			
CEP1/19p12 fusion	6.7 (4.3-9.7)	8.2 (5.5-11.5)	9.5 (2.7-10.4)
No CEP1/19p12 fusion	5.6 (2.5-8.1)	2.4 (0.8-5.3)	3.3 (1.1-6.7)
*P**	0.43	0.003	0.09

Abbreviation: NR, not reached.
*Wilcoxon test.

response to temozolomide chemotherapy (39, 40). In this report, using prospectively collected patients enrolled on cooperative group trials, we confirm that combined deletion of 1p and 19q is associated with significantly prolonged OS and PFS in patients with low-grade oligodendrogliomas. In the parent trials, there was no significant difference in the response of the patients by different radiotherapeutic arm (12, 13). However, like the response of anaplastic oligodendrogliomas to PCV (10), the presence of combined 1p and 19q deletion seemed to be associated with responsiveness to RT. The RT results suggest that higher doses (64.8 Gy) of radiation are associated with shorter survival in patients who lack combined 1p and 19q deletion. Patients with combined deletion fare equally with both 50.4 and 64.8 Gy. It should be noted that these observations may be an artifact of subset analysis.

In this report, we also show that the t(1;19) that underlies the majority of the combined deletions is also associated with a prolonged OS and PFS and response to radiation (as measured by survival). Multivariable analysis suggested that, in this group of patients, the t(1;19) is independently associated with OS (compared with combined deletion). Although the multivariable results may be due to differences in FISH assay performance, they may also mean that the mechanism of combined deletion is of clinical and biological relevance (e.g., that loss of 1p and 19q by translocation has different consequences compared with loss of 1p and 19q by other mechanisms). It is likely that the t(1;19) will also predict the prognosis of patients with anaplastic oligodendrogliomas and perhaps predict the initial response to chemotherapy in such patients.

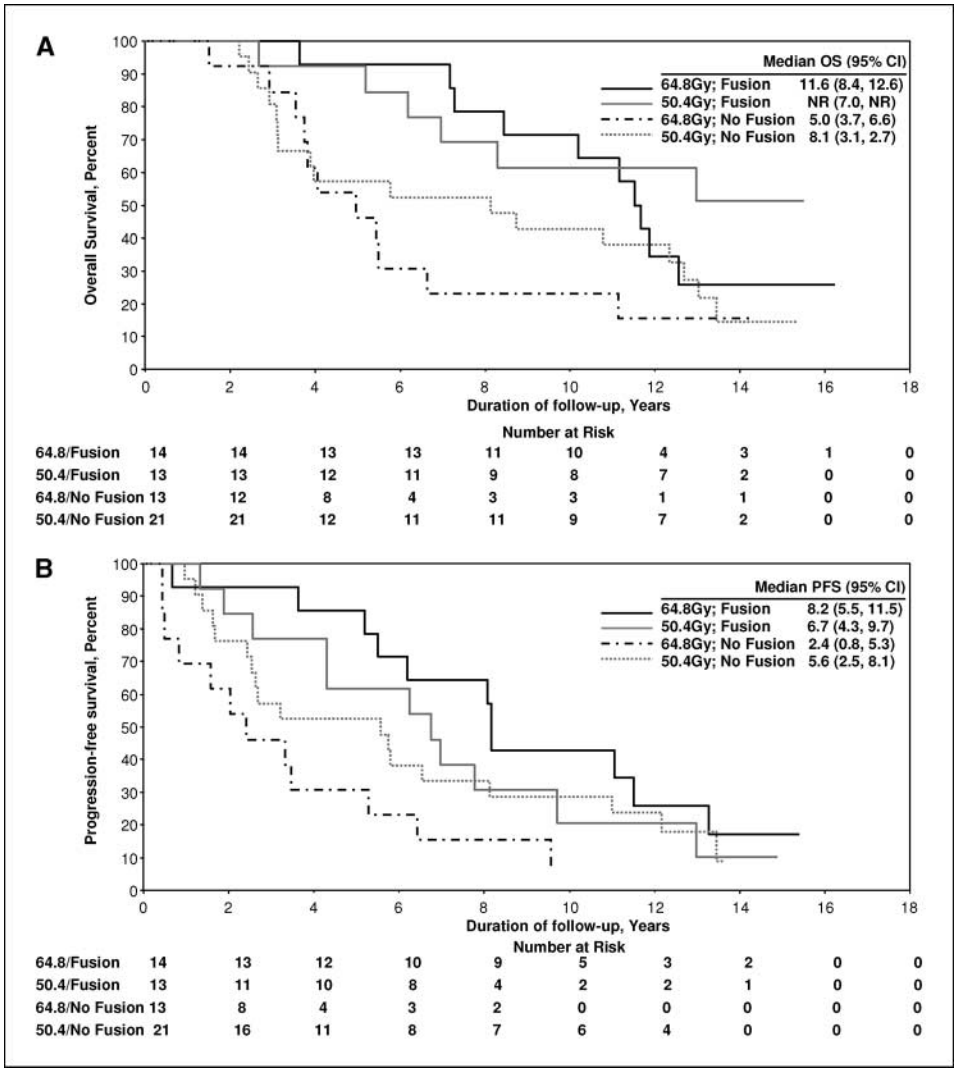

Figure 3. *A,* Kaplan-Meier curves of OS by treatment and CEP1/19p12 fusion status for 61 patients with grade II gliomas enrolled on NCCTG 86-72-51. *B,* Kaplan-Meier curves of PFS by treatment and CEP1/19p12 fusion status for 61 patients with grade II gliomas enrolled on NCCTG 86-72-51.

In summary, we report that the majority of combined 1p and 19q deletions associated with oligodendrogliomas are mediated by a single genetic event, a t(1;19)(q10;p10). The prevalence of this translocation in low-grade oligodendrogliomas is 44%. Combined with the recent observation that *ERG1:TMPRSS2* and *ETV1:TMPRSS2* translocations are highly specific for and prevalent in prostate cancer (41), the results suggest that recurrent translocations are more common in solid tumors than previously appreciated. Finally, our results confirm that combined 1p and 19q deletions as well as the t(1;19) that mediates these translocations are independently associated with a significantly better prognosis in patients with low-grade gliomas, especially oligodendrogliomas.

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