Alterations of Chromosome Arms 1p and 19q as Predictors of Survival in Oligodendrogliomas, Astrocytomas, and Mixed Oligoastrocytomas

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Purpose: **A recent report suggests that alterations of chromosome arms 1p and 19q are associated with chemotherapeutic response and overall survival in anaplastic oligodendroglioma patients treated with procarbazine, lomustine, and vincristine chemotherapy. We set out to further clarify the diagnostic and prognostic implications of these alterations in a broader set of diffuse gliomas, including astrocytic neoplasms and low-grade oligodendrogliomas.**

Patients and Methods: **Fluorescence in situ hybridization (FISH) signals from DNA probes mapping to 1p and 19q common deletion regions were enumerated in 162 diffuse gliomas (79 astrocytomas, 52 oligodendrogliomas, and 31 mixed oligoastrocytomas), collected as part of an ongoing prospective investigation of CNS tumors.**

Results: **The oligodendroglial phenotype was highly associated with loss of 1p ($P = .0002$), loss of 19q ($P < .0001$), and combined loss of 1p and 19q ($P < .0001$). Combined loss of 1p and 19q was identified as a univariate predictor of prolonged overall survival among patients with pure oligodendroglioma (log-rank, $P = .03$) and remained a significant predictor after adjusting for the effects of patient age and tumor grade ($P < .01$). This favorable association was not evident in patients with astrocytoma or mixed oligoastrocytoma.**

Conclusion: **Combined loss of 1p and 19q is a statistically significant predictor of prolonged survival in patients with pure oligodendroglioma, independent of tumor grade. Given the lack of this association in patients with astrocytic neoplasms and the previously demonstrated chemosensitivity of oligodendrogliomas, a combined approach of histologic and genotypic assessment could potentially improve existing strategies for patient stratification and management.**

J Clin Oncol 18:636-645. ***© 2000 by American Society of Clinical Oncology.***

DIFFUSE GLIOMAS are the most common primary malignancies of the CNS and are comprised of astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas. Though all glioma subtypes are often grouped together, differences in clinical behavior and response to therapy warrant separation. Furthermore, within each subtype there is a wide spectrum of clinical behavior among individual patients. However, distinguishing glial subtypes based on nuclear and cellular morphology alone is subjective, with significant interobserver variability, even among highly experienced neuropathologists.[1-4]

The importance of further stratifying patient populations is exemplified by the often remarkable chemosensitivity of a subset of oligodendrogliomas and mixed oligoastrocytomas.[5-9] As many as two thirds of oligodendrogliomas have been shown to be chemosensitive, particularly to the combination of procarbazine, lomustine (1-[chloroethyl]-3-cyclohexyl-1-nitrosourea), and vincristine (PCV). A recent report by Cairncross et al[10] suggests that alterations of chromosome arms 1p and 19q are associated with both chemotherapeutic response and prolonged overall survival in anaplastic (grade 3) oligodendrogliomas treated with PCV chemotherapy.

Though combined loss of 1p and 19q is associated with the oligodendroglial phenotype, these alterations are occasionally encountered in other glioma subtypes, both individually and in combination.[11-17] Recently, we studied chromosomes 1 and 19 in a large collection of gliomas, finding loss of 1p, 19q, and combined loss of 1p and 19q in 18%, 38%, and 11% of astrocytomas ($n = 55$), respectively.[3]

In the present study, we set out to further understand the potential diagnostic and prognostic significance of 1p and 19q alterations in the three diffuse glioma subtypes: oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. The prognostic significance of these alterations has not been pre-

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Submitted March 5, 1999; accepted September 15, 1999.

Supported by National Institutes of Health grants no. CA50905, CA64928, and CA50910, the American Brain Tumor Association, and the Sydney Luckman Endowed Physician Scientist Scholarship.

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0732-183X/00/1803-636

viously reported for the latter two subtypes, and, in contrast to Cairncross et al,[10] our oligodendrogliomas were predominantly low-grade (World Health Organization grade 2).

PATIENTS AND METHODS

Tumor Selection and Pathology Review

A set of 162 surgically resected diffuse gliomas was selected, based on the availability of tissue for analysis, from cases collected as part of the Glioma Markers Network, an ongoing multi-institutional prospective investigation of CNS tumors. Specimens used for the present analysis were surgically resected at the Mayo Clinic in Rochester, MN (142 cases), or at the Johns Hopkins Hospital in Baltimore, MD (20 cases). Tumors were classified morphologically and graded according to the World Health Organization system[1] by three independent neuropathologists. Consensus morphology and grade were established for each glioma, as previously described.[3] Specimens derived from initial surgical resection were classified as primary (n = 116, 72%), whereas those from subsequent resections were classified as recurrent (n = 46, 28%).

Clinical Parameters

Survival analyses were performed using 116 primary specimens. As part of the Glioma Markers Network and in accordance with the Institutional Review Boards of the Mayo Clinic and the Johns Hopkins Hospital, clinical parameters were collected and are regularly updated for these patients through follow-up and questionnaires. These data include: date of birth, sex, date of surgical resection or biopsy and pathology of the specimen, whether treated with chemotherapy and/or radiation therapy for primary and/or recurrent lesions, pathology and date of surgically resected recurrent tumors, date of last follow-up, and status of patient (living/deceased) at the time of last follow-up.

Typical treatment for patients with astrocytoma, either low-grade (grade 2) or high-grade (grades 3 and 4), included surgical resection followed by radiation (approximately 50 to 60 Gy). Most patients with high-grade astrocytoma also received adjuvant chemotherapy that included a nitrosourea compound. At present, however, there is no conclusive evidence that differences in chemotherapy regimens affect the survival of patients with high-grade astrocytomas.[18]

Approximately half of the patients with low-grade oligodendroglioma or low-grade mixed oligoastrocytoma (grade 2) were treated by radiation (approximately 50 to 60 Gy); the remaining patients were observed. High-grade oligodendrogliomas and high-grade oligoastrocytomas (grades 3 and 4) were typically treated with radiation and adjuvant chemotherapy, consisting of either PCV or a different regimen including a nitrosourea compound.

Flourescence In Situ Hybridization (FISH) Probe Selection and Analysis

Five bacterial artificial chromosomes (BACs) were labeled to generate locus-specific FISH probes mapping to 1p36, 1q24, 19p13.1, 19q13.1-q13.2, and 19q13.3. The BACs mapping to 1p36 and 19q13.3 were selected based on previous reports that these BACs map to regions of common allelic loss in gliomas.[3,19,20] The BAC mapping to 19q13.1-q13.2 was selected using primers for the *AKT2* oncogene (Genome Systems, St Louis, MO). All FISH probes used for these analyses were previously developed by our group, and the capacities of these probes to detect alterations of chromosomes 1 and 19 have been validated by comparison with loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) analyses.[3]

For each case, a paraffin-embedded tumor block was selected based on tumor content, including the highest grade component and representation of the predominant morphology of the individual case. Approximately 20 5-μm sections were prepared for various studies from each selected tumor block. The first and last sections were hematoxylin and eosin stained, regions representing tumor and normal tissue were delineated, and the first section was examined to ensure that it met the standards by which the block was selected. Importantly, for the present study, FISH analyses were performed using the sections immediately adjacent to the first hematoxylin and eosin stained slide to minimize the effects of tumor heterogeneity.

FISH analysis was performed, as previously described.[21] Briefly, tumor sections were deparaffinized, dehydrated, microwave treated in citrate buffer (pH 6.0) for 10 minutes, digested in pepsin solution (4 mg/mL in 0.9% NaCl, pH 1.5) for 15 minutes at 37°C, rinsed in two times standard saline citrate (SSC) at room temperature for 5 minutes, and air dried. Dual-probe hybridization was performed using a digoxigenin-labeled locus-specific 1p or 19q probe and a SpectrumGreen-labeled probe (Vysis, Downers Grove, IL) mapping to 1q and 19p, respectively. Probes and target DNA were denatured simultaneously in an 80°C oven for 5 minutes, followed by overnight incubation at 37°C. Slides were then washed in 1.5 mol urea/0.1 times SSC at 45°C for 10

Table 1. Number of Patients Examined by Histologic Subtype and Grade in Primary Versus Recurrent Gliomas

Subtype and Grade	No. of Patients		
	Primary	Recurrent	Total
Oligodendrogliomas	36	16	52
Grade 2	26	8	34
Grade 3	6	6	12
Grade 4	4	2	6
Mixed oligoastrocytomas	19	12	31
Grade 2	8	2	10
Grade 3	7	8	15
Grade 4	4	2	6
Astrocytomas	61	18	79
Grade 2	1	1	2
Grade 3	7	8	15
Grade 4	53	9	62
Total	116	46	162

Table 2. Clinical Parameters of 116 Patients With Primary Gliomas

Variable	Oligodendrogliomas	Mixed Gliomas	Astrocytomas
No. of patients	36	19	61
Sex, n			
Male	24	11	41
Female	12	8	20
Age, years			
Median	44	43	58.5
Range	8-74	20-68	25-82
Follow-up, months*			
Median	49.9	30.6	18.3
Range	6.4-124.6	1.2-45.9	0.6-92.8

*Does not include deceased patients.

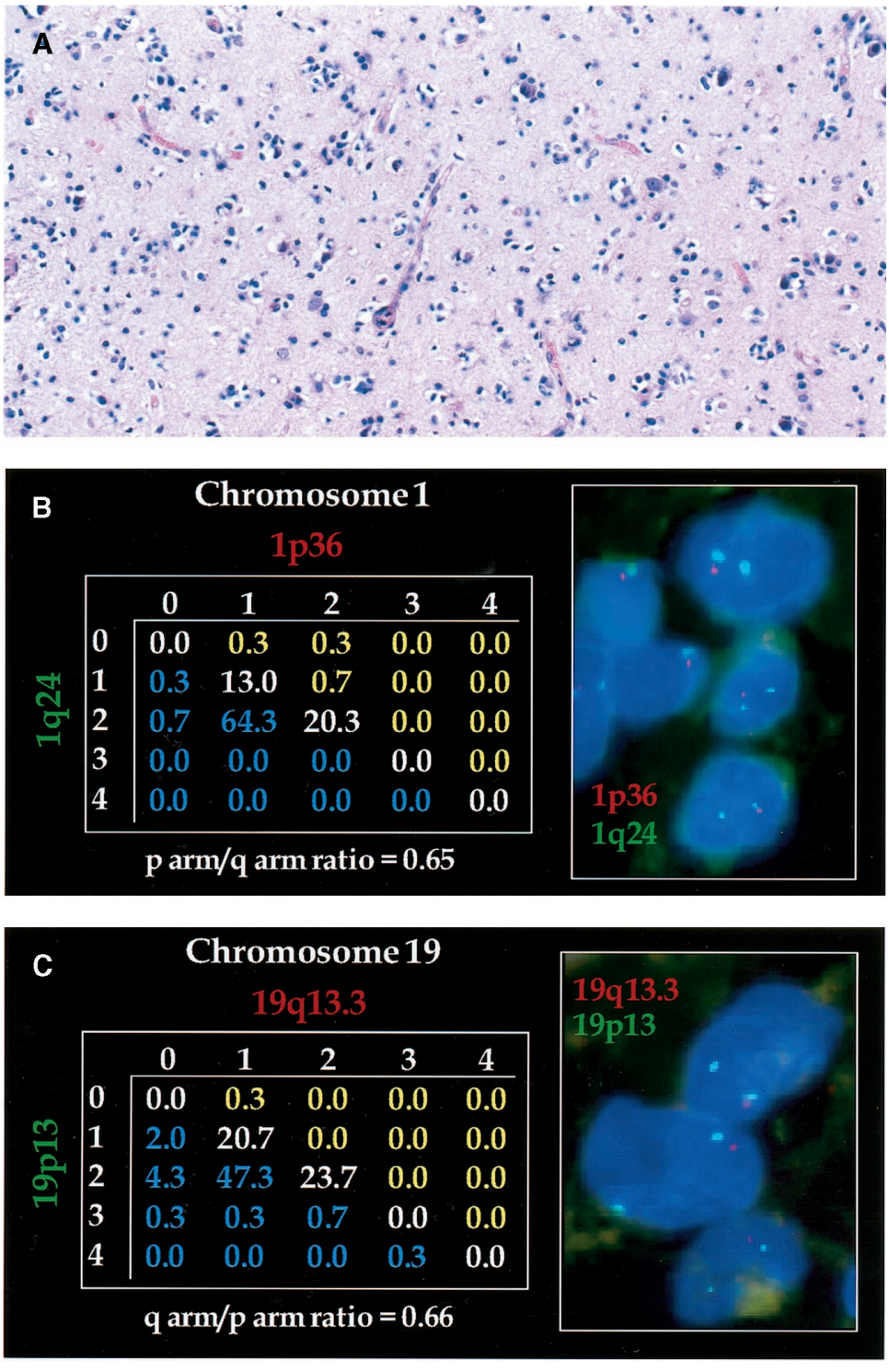

Fig 1. (A) Representative histology for case 62, a grade 2 oligodendroglioma. (B) Corresponding FISH signal enumeration (% nuclei) and dual probe hybridization images show loss of 1p36 relative to 1q24 and (C) loss of 19q13.3 relative to 19p13.

minutes (three times) and in two times SSC at room temperature for 2 minutes. After washing, the digoxigenin-labeled probes were detected using a rhodamine detection kit (Oncor, Gaithersburg, MD). Nuclei were counterstained with 4,6-diamidino-2-phenylindole and the antifade compound *p*-phenylenediamine. A Zeiss (Thornwood, NY) Axioplan microscope equipped with a triple-pass filter (DAPI/Green/

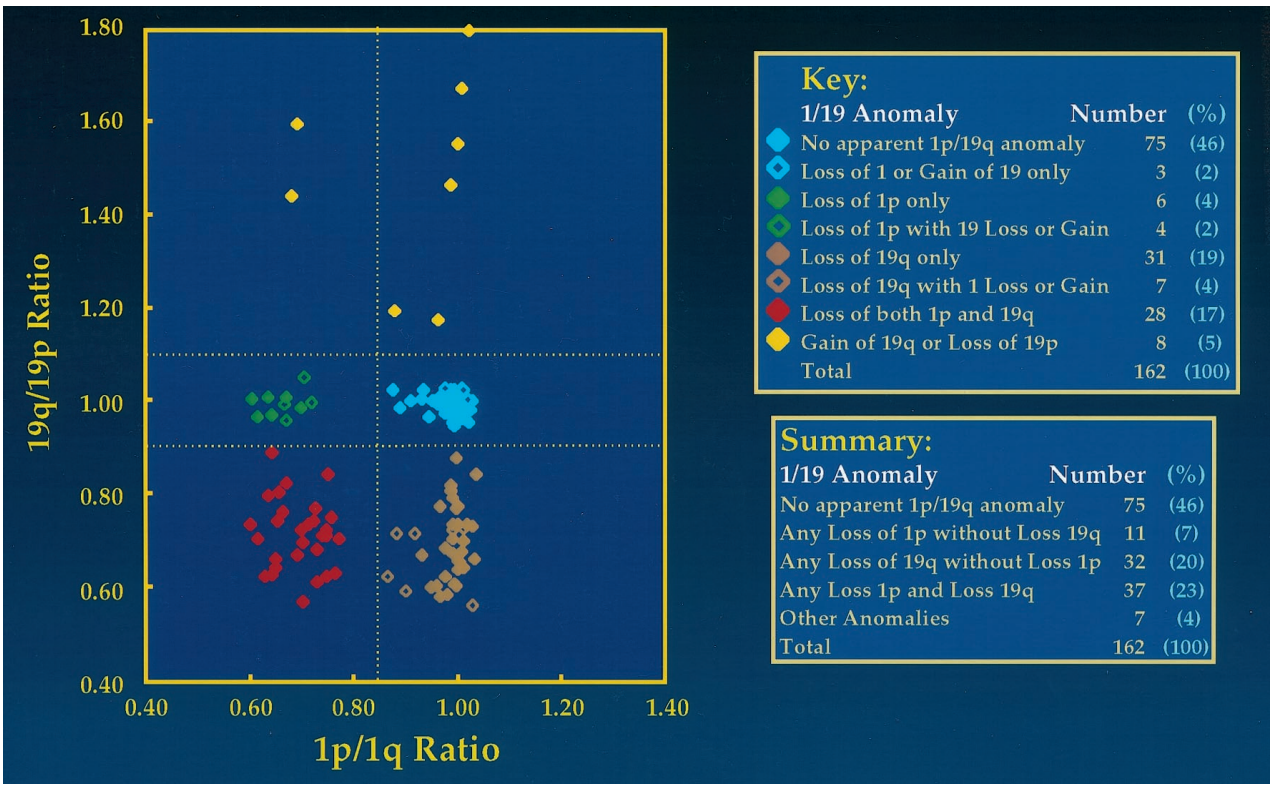

Fig 2. Plot of 19q/19p *v* 1p/1q FISH signal ratios for 162 gliomas. Dashed lines represent cutoff points for normal probe copy number ratios for 19q/19p (< 0.9 for 19q loss and > 1.1 for 19q gain or 19p loss) and 1p/1q (< 0.85 for loss of 1p).

Orange; Vysis, Downers Grove, IL) was used to assess the number of FISH signals for each locus-specific FISH probe. Approximately 300 nonoverlapping nuclei were enumerated per hybridization.

Ranges for normal FISH probe copy number were established by hybridizing and enumerating gliosis specimens (data not shown) and through extensive comparisons of FISH, LOH, and CGH data.[3] These ranges are delineated in Fig 2.

Statistical Analysis

Data for both primary and recurrent tumors were used to evaluate the association between molecular alterations and histologic subtype. These associations were assessed using χ^2 tests. Pearson's and Fisher's exact tests were used to calculate P values for comparison of these binomial proportions. Survival analyses were performed using the 116 specimens from initial surgical resection, with survival defined as the time between tissue acquisition and patient death. Survival distributions were estimated with Kaplan-Meier curves[22] and compared among patient subsets with log-rank tests.[23] Multivariate analyses were performed by generating Cox models using maximum likelihood methodology.[24]

RESULTS

Glioma Pathology Review and Clinical Parameters

Table 1 lists the distributions of consensus histologic subtype and grade of the gliomas that were evaluated by FISH. Unanimous agreement among the three pathologists for histologic subtype classification was observed for 69% (36 of 52) of oligodendrogliomas, 13% (four of 31) of mixed oligoastrocytomas, and 76% (60 of 79) of astrocytomas. These findings are similar to those previously report by Smith et al.[3] Notably, when compared with primary examples, recurrent oligodendrogliomas and oligoastrocytomas were less often grade 2, and recurrent astrocytomas were less often grade 4. Grade 2 astrocytomas were rare with one primary and one recurrent case each. The clinical parameters for the 116 patients with primary glioma are listed in Table 2.

FISH Analysis of Chromosomes 1 and 19

Figure 1 illustrates representative histology for case number 62, a primary grade 2 oligodendroglioma. Also shown are FISH images and corresponding probe enumeration data demonstrating loss of the 1p and 19q common deletion regions for this case.

Figure 2 summarizes the FISH data for all gliomas analyzed and defines four distinct groups of gliomas based on alterations of chromosomes 1 and 19 (blue, green,

Table 3. 1p and 19q Losses Versus Glioma Subtype and Primary/Recurrent Status

Status	No. of Patients	Patients With Deletion of the Indicated Region of Common Loss							
		1p36			19q13.3			1p36 and 19q13.3	
		No.	%		No.	%		No.	%
Primary and recurrent									
Oligodendrogliomas	52	25	48		33	63		23	44
Mixed oligoastrocytomas	31	9	29		15	48		8	26
Astrocytomas	79	14	18		21	27		6	8
*P**	.0002			<.0001			<.0001		
Primary†									
Oligodendrogliomas	36	15	42		21	58		14	39
Mixed oligoastrocytomas	19	4	21		10	53		4	21
Astrocytomas	61	11	18		14	23		5	8
*P**	.011			.0009			.0004		
Recurrent‡									
Oligodendrogliomas	16	10	63		12	75		9	56
Mixed oligoastrocytomas	12	5	42		5	42		4	33
Astrocytomas	18	3	17		7	39		1	6
*P**	.012			.045			.002		

*X^2 test, astrocytomas v oligodendrogliomas, excluding mixed oligoastrocytomas.

†Specimens derived from initial surgical resection.

‡Specimens derived from a non-initial surgical resection.

brown, and red diamonds). Of the 162 gliomas analyzed, 75 (46%) had no apparent alterations of either chromosome 1 or chromosome 19, 11 (7%) had loss of 1p without loss of 19q, 32 (20%) had loss of 19q without loss of 1p, and 37 (23%) had loss of both 1p and 19q. Primary tumors with apparent loss of an entire copy of chromosome 1 or 19 were classified as having loss of 1p or loss of 19q, respectively, for comparisons of genotype with survival.

Associations of 1p and 19q Alterations With Histologic Subtype

Consistent with prior studies and as detailed in Table 3, loss of the 1p, 19q, and combined 1p and 19q common deletion regions were each highly associated with the oligodendroglial phenotype ($P = .0002$, $P < .0001$, and $P < .0001$, respectively). These associations were maintained when primary and recurrent tumors were evaluated separately. No statistically significant differences in the incidence of these alterations were noted between the primary and recurrent specimens (*P* values not shown). The frequencies of alterations in mixed oligoastrocytomas were intermediate between pure oligodendrogliomas and pure astrocytomas.

Survival Analyses

Combined loss of 1p and 19q is a statistically significant positive predictor of overall survival in oligodendroglioma patients. Table 4 lists the molecular and clinical data for each of the 36 primary oligodendroglioma patients. The incidence of recurrence did not differ significantly between those with combined alteration of 1p and 19q and those without this combined alteration (three of 14 *v* 11 of 22, respectively; $P = .16$). Notably, all of the patients with combined 1p and 19q loss were alive at last follow-up (median follow-up, 67.5 months), whereas six (27%) of those without these combined alterations have died. Figure 3 shows the Kaplan-Meier curves for these two subsets of oligodendroglioma patients, those with combined loss of 1p and 19q (n = 14) and those without combined loss of 1p and 19q (n = 22). As listed in Table 5, when all grades are analyzed together, the oligodendroglioma patients with combined loss of 1p and 19q demonstrate a significantly better probability of survival than do those without this combined alteration ($P = .03$).

Cox multivariate models were generated using all 36 primary oligodendroglioma patients analyzed. After adjusting for the effects of patient age and tumor grade, combined loss of 1p and 19q remained a statistically significant positive predictor of overall survival ($P < .01$).

Combined loss of 1p and 19q is not a statistically significant predictor of overall survival in grade 4 astrocytoma or in mixed oligoastrocytoma patients. Of the 53 grade 4 primary astrocytomas, five demonstrated combined loss of 1p and 19q. Though not statistically significant, these five patients exhibited a modest trend toward shorter overall survival (Table 6), with a median survival of 5.6 months compared with the 12-month median survival of the 48 patients without the combined alterations ($P = .17$). Com-

Table 4. Summary of Molecular and Clinical Data for Patients With Primary Oligodendroglioma

Patient No.	Primary Lesion				Recurrent Lesion			FU (months)	Status
	−1p/−19q	Dx	Chemo	RT (Gy)	Dx	Chemo	RT (Gy)		
62	Y/Y	O 2	None	63.2	M 2	None	None	115	Alive
77	Y/Y	O 2	None	None	NR	None	54.0	125	Alive
JH-197	Y/Y	O 2	None	Y-NA				28	Alive
231	Y/Y	O 2	None	50.4				82	Alive
JH-246	Y/Y	O 2	PCV	Y-NA				10	Alive
251	Y/Y	O 3	None	59.4	NR	PCV	None	47	Alive
259	Y/Y	O 2	None	Y-NA				90	Alive
278	Y/Y	O 2	None	57.6				89	Alive
308	Y/Y	O 3	None	60.0				87	Alive
427	Y/Y	O 3	PCV	64.0				72	Alive
467	Y/Y	O 2	None	None				64	Alive
502	Y/Y	O 2	None	Y-NA				23	Alive
603	Y/Y	O 2	None	54.0				16	Alive
611	Y/Y	O 3	PCV	56.0				61	Alive
JH-138	N/N	O 2	None	None	O 2		54.0	29	Alive
JH-142	N/N	O 2	None	None	A 2		Y-NA	25	Alive
JH-250	N/N	O 2	None	None				6	Alive
277	N/N	O 2	None	Y-NA	NA	NA	NA	64	Alive
300	N/Y	O 2	None	50.4				88	Alive
507	N/N	O 2	None	50.4	NR	None	None	9	Deceased
520	N/Y	O 2	None	60.0				62	Alive
555	N/N	O 2	None	55.8				65	Alive
584	N/N	O 2	BCNU	72.0	NR	None	None	24	Deceased
588	Y/N	O 3	PCV	60.0	NA	NA	NA	32	Deceased
626	N/N	O 2	None	55.8				60	Alive
638	N/N	O 2	None	None	NR	None	55.8	51	Alive
656	N/N	O 2	None	None	NR	PCV	None	39	Deceased
667	N/N	O 2	None	55.8	M 4	NA	None	49	Alive
678	N/Y	O 4	None	4.0				1	Deceased
690	N/Y	O 4	None	None	NR	None	None	45	Alive
755	N/N	O 2	None	None				28	Alive
760	N/N	O 4	PCV	59.4				30	Alive
806	N/Y	O 2	None	59.4				26	Alive
883	N/N	O 2	None	45.0				29	Alive
890	N/Y	O 3	None	55.8	A 4	CEC	None	26	Deceased
892	N/Y	O 4	None	55.8				37	Alive

Abbreviations: −1p/−19q, loss of one copy of the chromosome 1p arm/loss of one copy of the chromosome 19q arm; Y, yes; N, no; Dx, pathologic diagnosis; O, oligodendroglioma; M, mixed oligoastrocytoma; A, astrocytoma; 1-4, grade of lesion; chemo, chemotherapy; BCNU, carmustine; CEC, carboplatin, etoposide, and cytoxan combination therapy; Y-NA, treatment modality was administered but details are not available; RT, radiation therapy; NR, not resected though recurrence demonstrated by imaging; NA, information not available; FU, time of follow-up since initial surgical resection.

bined alteration of 1p and 19q was not a statistically significant predictor of survival in mixed oligoastrocytoma patients ($P = .98$, Table 6).

Loss of 1p or 19q, in isolation, was not a statistically significant predictor of overall survival in any of the glioma subtypes examined (Tables 5 and 6). Patients with oligodendroglioma did, however, demonstrate a trend toward better survival if their tumors exhibited loss of 1p ($P = .15$) or loss of 19q ($P = .15$). In contrast, grade 4 astrocytoma patients exhibited a trend toward shorter overall survival if their tumors demonstrated loss of 1p ($P = .12$) and a trend toward prolonged survival if their tumors had loss of 19q ($P = .18$).

DISCUSSION

Diffuse gliomas demonstrate considerable heterogeneity with regard to prognosis, both among and within the histologic subtypes. However, our current capacity to effectively stratify these lesions by histology is primarily limited to subjective differences in cellular morphology. Because no specific immunohistochemical markers are available, other diagnostic and prognostic aids are clearly needed.

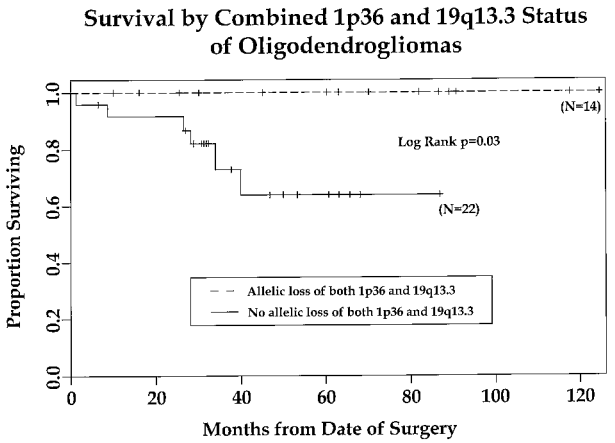

Fig 3. Kaplan-Meier curves showing the probability of survival among 36 patients with oligodendroglioma. Patients with combined loss of the 1p and 19q chromosome arms have a significantly better overall survival. Vertical lines represent length of evaluation for patients alive at the time of last follow-up.

Tumor genotyping offers the potential for further treatment stratification that minimizes patient morbidity and mortality and may also provide important clues to the underlying mechanisms of glioma tumorigenesis and malignant progression.

Allelic alterations of chromosomes 1 and 19 are frequent events in human gliomas, and in the present study we have used FISH analysis to examine the capacity of these alterations to serve as predictors of overall survival. Further, we have confirmed the strong association of these alterations with oligodendroglial morphology in a considerably larger collection of gliomas than has been previously reported. FISH was selected for the present studies because of its considerably higher resolution compared with CGH analysis and its higher sensitivity compared with LOH analysis. In general, FISH can detect a deletion when it is present in as few as 20% to 30% of the cells, whereas molecular techniques require much purer samples.[25] In this regard, FISH would be expected to have a considerably higher sensitivity for tumors with clonal heterogeneity or mixed populations.

Combined loss of 1p and 19q has been shown to be a statistically significant multivariate predictor of overall survival in anaplastic oligodendrogliomas.[10] Our results complement and extend these findings. We demonstrate that combined loss of the 1p and 19q common deletion regions is a statistically significant univariate and multivariate predictor of overall survival for patients with oligodendroglioma, independent of tumor grade. However, demonstrating this association using predominantly low-grade lesions is inherently more difficult because of the longer survival of these patients. Thus, it is important to appreciate these findings in light of the relatively few cases analyzed and the relatively few deaths among these patients.

As opposed to those with combined loss, patients with oligodendrogliomas exhibiting loss of 1p or 19q, in isolation, show a trend toward better survival, but these alterations, independently, do not reach statistical significance as predictors of overall survival. Previous reports regarding oligodendrogliomas have suggested that loss of 1p without concomitant loss of 19q is uncommon.[16,26] Indeed, the present study identified only two oligodendrogliomas (4%) with loss of 1p that lacked apparent alteration of 19q. There were, however, 10 oligodendrogliomas (19%) with loss of 19q that did not exhibit alteration of 1p, suggesting that loss of 19q precedes loss of 1p in oligodendroglioma development. Perhaps, loss of 19q predisposes these neoplasms to further loss of 1p, which, in turn, commits the tumor to a less aggressive biologic behavior. Alternatively, those tumors without deletion may have other alterations of 1p.

Table 5. Univariate Genetic Predictors of Risk of Death for Oligodendroglioma Patients

	Low-Grade Patients (grade 2)				High-Grade Patients (grades 3 and 4)				Total			
		Deaths				Deaths				Deaths		
Genetic Marker	No.	No.	%	*P**	No.	No.	%	*P**	No.	No.	%	*P**
Chromosome 1p												
Intact	16	3	19	.17	5	2	40	.36	21	5	24	.15
Hemizygous loss	10	0	0		5	1	20		15	1	7	
Chromosome 19q												
Intact	13	3	23	.07	2	1	50	.52	15	4	27	.15
Hemizygous loss	13	0	0		8	2	25		21	2	10	
Chromosomes 1p and 19q												
No hemizygous loss of both	16	3	19	.17	6	3	50	.10	22	6	27	.03
Hemizygous loss of both	10	0	0		4	0	0		14	0	0	

**P* values calculated using the log-rank test.

Table 6. Univariate Genetic Predictors of Risk of Death for Mixed Glioma and Astrocytoma Patients

Marker	Patients With Mixed Oligoastrocytomas*				Patients With Grade 4 Astrocytomas†			
		Deaths				Deaths		
	No.	No.	%	P‡	No.	No.	%	P‡
Chromosome 1p								
Intact	15	9	60	.98	43	37	86	.12
Hemizygous loss	4	2	50		10	9	90	
Chromosome 19q								
Intact	9	4	44	.98	39	35	90	.18
Hemizygous loss	10	7	70		14	11	79	
Chromosomes 1p and 19q								
No hemizygous loss of both	15	6	60	.98	48	42	88	.17
Hemizygous loss of both	4	2	50		5	4	80	

*Includes grades 2 to 4.

†Does not include grade 2 astrocytomas (one case: hemizygous deletion of 1p, intact 19q) or grade 3 astrocytomas (seven cases: all had intact 1p and intact 19q).

‡*P* values calculated using the log-rank test.

Consistent with previous reports,[27,28] the incidence of combined 1p and 19q loss in this set of astrocytic tumors was significantly low (six of 79 cases, 8%). Five of these cases were primary tumors, and the patients with these tumors demonstrated a trend toward shorter survival. This may hint of a rare alteration associated with poor prognosis or, more likely, may simply reflect overall genomic instability in a high-grade lesion. In contrast to the associations of combined 1p and 19q losses with the oligodendroglial phenotype, our prior FISH study demonstrated a strong association of *p16-RB-CDK4* alterations with the astrocytic phenotype.[29] Therefore, a FISH battery using combinations of these oligodendroglial and astrocytic markers could potentially provide diagnostically useful information for diffuse gliomas with equivocal morphologic features.

Loss of 1p or 19q, in isolation, was not a statistically significant predictor of overall survival in our astrocytomas. Further, the frequent association of 1p loss with 19q loss seen in oligodendrogliomas was not observed for astrocytomas, suggesting that these alterations are not interrelated in astrocytic tumors as they are in oligodendroglial tumors.

There was no association of 1p and 19q alterations, either separately or in combination, with overall survival of the patients with mixed oligoastrocytoma analyzed in this study. It should be noted, however, that this category of tumors is notoriously difficult to define, even among the most experienced neuropathologists, and consists of tumors with varying degrees of oligodendroglial and astrocytic differentiation. Although we did use consensus among three neuropathologists to define this category, in the present study there were no set percentages or criteria for the diagnosis of mixed oligoastrocytomas, and cases ranged from those with separate regions resembling oligodendroglial and astrocytic differentiation to those with intermediate nuclear and cellular features throughout the tumor. Although this represents the state of the art for mixed glioma diagnosis, these ambiguities complicate further histologic stratification of these lesions. Thus, further studies are needed to address whether the degree of oligodendroglial differentiation is prognostically relevant for these cases. Furthermore, only 19 primary mixed gliomas were studied in this series. Maintz et al[30] have demonstrated subsets of mixed gliomas with genotypes resembling either those of pure astrocytomas or oligodendrogliomas. Appropriately, our mixed gliomas demonstrated frequencies of 1p and 19q losses intermediate to the two pure subtypes. Likewise, our prior FISH study of *p16-RB-CDK4* alterations also demonstrated intermediate frequencies in this group of tumors.[29]

The high frequency of 1p and 19q loss in diffuse gliomas strongly suggests that these chromosome arms harbor tumor suppressor genes. Refined mapping and subsequent cloning of the putative 1p and 19q glioma tumor suppressor genes are important steps to further understand the significance of these alterations and to effectively translate this tool to the clinical setting. The importance of the 1p glioma tumor suppressor is underscored by the strong association between 1p loss and chemosensitivity in anaplastic oligodendrogliomas.[10] Furthermore, virtually all human malignancies, from solid cancers to leukemias and myeloproliferative disorders have demonstrated nonrandom involvement of chromosome 1, particularly the telomeric end of 1p.[31-34] *p73*, a recently identified homologue of *p53*, maps to the glioma 1p common deletion region[35] but has been eliminated as a candidate for the 1p glioma tumor suppressor gene based on mutational analysis of oligodendrogliomas with 1p loss.[36]

Alterations of 19q in human gliomas are of particular interest because they are the only known alterations shared

by all three major glioma subtypes.[11,13-15] Furthermore, most other common malignancies rarely demonstrate loss of 19q,[37] suggesting the presence of a glial-specific tumor suppressor gene. Considerable efforts have been made to map the deletions on this chromosome arm,[3,19,20] and several genes have been eliminated as candidate 19q glioma tumor suppressor genes based on mutational analysis.[38-40]

In summary, we have reaffirmed by FISH in a large set of gliomas that alterations of the 1p and 19q common deletion regions, both independently and in combination, are associated with the oligodendroglial phenotype. Further, we have shown that combined loss of 1p and 19q is a statistically significant predictor of longer overall survival in patients with oligodendroglioma, including low-grade examples. Our data suggests that FISH analysis could potentially provide useful diagnostic and prognostic information in selected patients with diffuse gliomas.

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