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Polysomy for chromosomes 1 and 19 predicts earlier recurrence in anaplastic oligodendrogliomas with concurrent 1p/19q loss

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Abstract

Purpose—Loss of chromosome arms 1p and 19q is a molecular feature of oligodendroglial tumors characterized by responsiveness to chemotherapy and a favorable prognosis. The purpose of this study was to evaluate the prognostic significance of polysomy of chromosomes 1 and 19 in the setting of 1p/19q co-deletion.

Experimental Design—We analyzed 64 anaplastic oligodendrogliomas with 1p/19q loss or maintenance diagnosed at Massachusetts General Hospital and Brigham & Women's Hospital from 1996 to 2005; fluorescence *in situ* hybridization (FISH) for 1p/19q and Ki-67 immunohistochemistry was performed. Polysomy was defined as >2 1q and 19p signals in >30% of the cells with concurrent 1p/19q deletion. Tumors were divided into groups based on their 1p/19q status and compared for progression free survival (PFS), overall survival (OS) and 5-year survival probabilities.

Results—Forty-six tumors (72%) in our cohort had 1p/19q loss and eighteen (28%) had 1p/19q maintenance. Of those with loss, 19 (41%) had concurrent polysomy and 27 (59%) lacked polysomy. In agreement with prior studies, the group of anaplastic oligodendrogliomas with 1p/19q loss had significantly better PFS and OS than anaplastic oligodendrogliomas with 1p/19q maintenance ($p=0.0009$ and $p<0.0003$, respectively). Among anaplastic oligodendrogliomas with 1p/19q loss, those with polysomy showed shorter PFS than those with 1p/19q loss without polysomy ($p=0.0048$). Overall survival was similar in tumors with and without polysomy. The Ki-67 labeling index was not associated with polysomy and did not have prognostic significance.

Conclusion—The presence of polysomy in anaplastic oligodendrogliomas with deletion of 1p/19q is a marker of earlier recurrence.

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Statement of Translational Relevance

1p/19q chromosomal loss is present in anaplastic oligodendrogliomas with improved response to chemotherapy and improved survival. We report that chromosomal polysomy in anaplastic oligodendrogliomas with 1p/19q loss identifies tumors with a high risk potential for recurrence. Polysomy does not correlate with Ki-67 staining, and thus appears independent of proliferation activity. Polysomy assessment, as a novel prognostic marker, can be rapidly introduced into the clinical practice since fluorescence *in situ* hybridization (FISH) is one of the most common molecular methods for 1p/19q status evaluation.

Keywords

anaplastic oligodendroglioma; polysomy; 1p/19q

Introduction

Oligodendrogliomas represent approximately 2-3% of adult primary CNS neoplasms and 7-10% of all gliomas (1-3). Loss of chromosomal arms 1p and 19q is commonly observed in glial tumors with an oligodendroglioma component, including approximately 80% of oligodendrogliomas, 50-60% of anaplastic oligodendrogliomas (AO) and 30-50% of oligoastrocytomas and anaplastic oligoastrocytomas (3-6). Numerous studies have shown an association between 1p/19q co-deletion and a favorable response to chemotherapy, including to procarbazine, lomustine and vincristine (PCV) (7-10) and to temozolomide (TMZ) (11, 12), as well as to radiotherapy (13). 1p/19q status thus represents a reliable marker of biologic behavior, and testing for 1p/19q loss is now considered standard of care (14,15). Nonetheless, despite the fact that most AO with 1p/19q co-deletion respond to therapy, the majority recurs and requires additional treatment. Therefore, markers to detect tumors with higher risk of early recurrence within the 1p/19q co-deleted population would be clinically useful. In this regard, molecular markers that can potentially identify higher risk tumors within subgroups defined by 1p/19q loss and maintenance have been proposed (10).

Although numerous studies have confirmed the significance of 1p/19q loss as a predictor of response to therapy in AO, the genes responsible for tumorigenesis and/or therapeutic sensitivity have not been identified (16-22). The exact mechanism of the loss has also been enigmatic, but recent studies suggest that loss of 1p and 19q is mediated by formation of a balanced whole arm translocation involving chromosomes 1 and 19, with subsequent loss of the derivative chromosome der(1;19)(p10;q10) and maintenance of the der(1;19)(q10;p10) (23,24). In clinical practice, polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH) are the most commonly used methods and have been considered to generally provide equivalent prognostic information. However, studies utilizing FISH have noted that in addition to the loss of 1p and 19q a subset of tumors possess additional copies of these chromosomes (5,25), and that this was more common in anaplastic and recurrent tumors. Our anecdotal observations of occasional cases of rapidly progressing AO with 1p/19q loss and extra copies of 1p and 19q using FISH, prompted us to explore polysomy in a larger cohort of AO.

In this study, we have quantitated 1p and 19q signals by FISH in a cohort of AO to detect polysomy of chromosomes 1 and 19. Progression-free and overall survival was determined for tumors with and without loss of 1p/19q; within the co-deleted group, we compared tumors with and without polysomy. To analyze if polysomy is a surrogate marker for increased mitotic activity, we also compared proliferation indices in 1p/19q co-deleted AO with and without polysomy.

Materials and Methods

Medical chart review, histopathological analysis and molecular studies were performed on patients with newly diagnosed AO, WHO Grade III, seen at the Massachusetts General Hospital and Brigham and Women's Hospital between 1996 and 2005, for whom clinical data were available. None of the patients had a history of a previous low grade tumor or chemoradiation. Of the 87 patients with primary AO in our database, 64 patients had either a loss or maintenance of both chromosome 1p and 19q by the report and further analyses were limited to this cohort while patients with loss of only 1p or of 19q or only polysomy were excluded due to a small

sample size of each subgroup. Clinical presentation, neuroradiologic imaging, extent of surgery, adjuvant therapy and follow-up were determined from medical records. Approval from the Dana-Farber/Harvard Cancer Center Institutional Review Board was obtained prior to the initiation of this study.

Progression was defined either radiologically by enlargement of the existing lesion or development of a new lesion, or by clinical deterioration attributed to the tumor. A new lesion in the brain consistent with tumor based on imaging and clinical symptoms was considered sufficient to make the diagnosis of progressive disease when biopsy or resection was not considered clinically indicated. Positive response to salvage therapy was defined as either stable disease or a positive clinical and/or radiographic response.

Progression free survival (PFS) was defined as the time from diagnosis to progression as defined above or the time to death if death occurred without progression. Overall survival (OS) was defined as the time from the initial diagnosis to death.

Histopathology and Immunohistochemistry

All studies were performed on formalin-fixed, paraffin-embedded tissues. Sections were stained with H&E and were reviewed, and diagnosis and grade were confirmed by two neuropathologists independently (MS, KLL) using WHO 2007 criteria (3). Low grade oligodendrogliomas and tumors with astrocytic components were excluded.

A representative paraffin block was selected for immunohistochemical studies. A rabbit monoclonal anti-Ki-67 IgG antibody (Clone 30-9; Ventana Medical Systems, Tucson, AZ), supplied and prediluted by the manufacturer, was used for the study. Immunohistochemistry was performed on BenchMark XT automated tissue staining systems (Ventana Medical Systems, Inc., Tucson, AZ) using validated protocols and tonsil as a positive control. Tissues were counter stained with hematoxylin. In the areas of the highest expression, 500 cells were counted (40× objective) and a labeling index was calculated as the percentage of positive tumor cells.

Fluorescence *In Situ* Hybridization (FISH)

FISH was performed for 1p and 19q loss as described previously (26). Briefly, 5-micron sections of formalin-fixed paraffin-embedded tumor material were prepared and an H&E section reviewed to select regions for hybridization that contain a majority of tumor cells. Two separate dual-color FISH assays were performed, one slide used Bacterial Artificial Chromosome probes RP11-558F24 (chromosome 1p, labeled Orange) and RP11-45903 (chromosome 1q, labeled green), and the second slide used RP11-75H6 (chromosome 19p, labeled green) and RP11-293G10 (chromosome 19q, labeled orange). Signal quantitation of 100 nuclei was used to generate a 1p/1q ratio and a 19q/19p ratio. A total number of signals was counted and a ratio of 1p:1q (or 19q:19p) of <0.75 was diagnosed as loss, and ≥ 0.75 as maintenance, which are the scoring parameters also used in our clinical practice. The tumor was considered to have polysomy if $\geq 30\%$ of nuclei showed 3 or more signals for both 1q and 19p.

Statistics

Based on FISH results, patients were divided into the following groups for survival analyses: (1) loss of 1p/19q with polysomy, (2) loss of 1p/19q without polysomy and (3) maintenance of 1p/19q. Groups were then compared for progression free survival (PFS), overall survival (OS), 5-year PFS, 5-year OS, Ki-67 labeling index, sex and tumor location.

Survival was estimated using the Kaplan-Meier method. The three group comparisons were as follows: (1) AO with 1p/19q loss vs AO with 1p/19q maintenance, (2) AO with 1p/19q loss and polysomy versus AO with 1p/19q loss without polysomy and (3) AO with 1p/19q loss and polysomy versus AO with 1p/19q maintenance. Bivariate Cox regression models were fit with 1p/19q loss and each known prognostic factor. Due to the small sample size, larger multivariate models were not considered.

For 5-year PFS and OS, the Kaplan-Meier survival probabilities at 60 months were compared between groups using a Z-test based on their approximate normality. The comparisons were conducted among the groups in the same manner as the above analysis. For subjects who progressed, survival from the time of progression was also compared between groups.

A Wilcoxon Rank Sum statistic was used to determine the difference in mitotically-active fraction (Ki-67 positive cells) between AO with 1p/19q loss with and without polysomy. Fisher's Exact tests were used to determine group differences in sex, and tumor location. Associations among prognostic variables were assessed using Fisher's exact test (two binary variables), Spearman's correlation coefficient (continuous variables), and the Wilcoxon rank sum test (one binary and one continuous variable).

A Bonferroni correction was applied to adjust for the three comparisons undertaken; p-values were multiplied by three and compared to a threshold of 0.05 for significance.

Results

Analysis of Polysomy in Anaplastic Oligodendrogliomas

FISH analysis of 64 AO revealed 1p/19q loss in 46 (72%) of tumors and 1p/19q maintenance in 18 (28%). Average 1q and 19p FISH signals per cell for those cases with 1p/19q loss were calculated, and divided into groups with and without polysomy. 1p and 19q signals were not used in the calculation due to their known deletion (polysomy of those chromosomal regions would be two or more copies, which is more difficult to enumerate). Of 46 AO with 1p/19q loss, 19 (41%) showed polysomy and 27 (59%) showed no polysomy. Representative images are shown in Figure 1A, 1B and 1C. Within the group of AO with 1p/19q loss and polysomy, 8 cases showed tetraploidy i.e. 2 signals for 1p/19q and 4 signals for 1q/19p in most of the nuclei with polysomy. However 11 cases showed high intratumor variability in the number of signals ranging from 3-6 signals for 1q/19p and 1-5 signals for 1p/19q. This high variability among tumors as well as within each tumor precluded further sub-stratification in a cohort of this size. Intratumor variability in the number of signals is demonstrated in Figure 1C.

For the 64 subjects with AO in this study, the median age at diagnosis was 44 years (range 17-78), and there was no difference in age between patients with maintenance or loss of 1p/19q, with or without polysomy. Thirty-five patients were male (55%) and 29 patients were female (45%). In the subgroup of patients with concurrent 1p/19q loss and polysomy, there was a male predominance (68% vs 32%), while the subgroup of patients with 1p/19q maintenance showed a slight female predominance (56% vs. 44%); however differences were not significant. In the subgroup of patients with 1p/19q loss without polysomy the male: female ratio was 1:1. Forty-five patients (70%) had Karnofsky performance score (KPS) 90-100 and there was no difference between AO with 1p/19q loss and with maintenance. Younger age was associated with better KPS (p-value 0.0147). There was a statistically significant difference in KPS between AO with 1p/19q loss with and without polysomy (p-value 0.0318) due to 30% of patients with AO and 1p/19q loss without polysomy having KPS 80 or lower, versus only 10% of those with polysomy. The clinical and pathological data are summarized in Tables 1 and 2.

Forty-eight tumors (75%) were centered in the frontal lobe, 11 (17%) in the temporal lobe, 3 (5%) in the parietal lobe and 2 (3%) in diencephalon; no tumors were in the occipital lobe. Tumors in the frontal lobe showed 1p/19q loss in 85% of cases while 73% of tumors in the temporal lobe had maintenance of 1p/19q. Both tumors in the diencephalon showed preservation of 1p/19q. There was a significant association between frontal lobe location and loss of 1p/19q and temporal lobe and maintenance of 1p/19q (p-value < 0.003), as previously reported (7,27).

The clinical management of patients in this cohort involved surgical gross total resection in 25 cases (39%), subtotal resection in 33 cases (52%) and biopsy only was performed in 6 cases (9%). Fifty patients (78%) received adjuvant radiation therapy and 55 patients (86%) received adjuvant chemotherapy. The first line chemotherapy was composed either of PCV (28 patients) or temozolomide (27 patients). Median PFS was 50 months (range 2-132 months) for the entire cohort, and median OS was 123 months (range 5-158).

In all cases, histopathological evaluation revealed cytological features of oligodendroglioma with round nuclei and perinuclear halos, but with high cell density, marked nuclear atypia and brisk mitotic activity. Branching capillary architecture was still recognizable, although all cases showed variable degree of microvascular proliferation. Tumor necrosis was not observed. Representative images are shown in Figures 1D and 1F.

Anaplastic oligodendrogliomas with 1p/19q loss have longer PFS and OS

We compared the survival of 46 patients with AO and 1p/19q loss (irrespective of the polysomy status) with 18 patients with AO with 1p/19q maintenance. Of the 46 AO with 1p/19q loss, 27 recurred (59%) with a median PFS of 81 months, while 16 out of 18 (89%) AO with 1p/19q maintenance recurred with a median PFS of 21 months. Twenty-two patients recurred and died during the time of the follow-up (34%), including 13/18 patients (72%) with maintenance of 1p/19q and 9/46 patients (20%) with deletion of 1p/19q. Patients with AO with 1p/19q loss had significantly better PFS and OS than patients with maintenance of 1p/19q (PFS 81 months vs. 21 months, p-value 0.0009; OS 158 months vs. 41 months, p-value < 0.0003, respectively) (Figure 2, Table 1).

Prognosis at five years was significantly better for patients with 1p/19q loss, both in terms of progression-free and overall survival. The 5-year overall survival probability for patients with loss of 1p/19q was 0.904 but only 0.389 for patients with preserved 1p/19q (p-value < 0.0003) and 5-year progression-free probability for patients with loss of 1p/19q was 0.573 but only 0.111 when 1p/19q was preserved (p-value < 0.0003) (Table 3).

Anaplastic oligodendrogliomas with concurrent 1p/19q loss and polysomy have earlier recurrence

During the follow up period, 15/19 (79%) patients with 1p/19q loss and polysomy progressed with PFS ranging between 5 to 96 months (median PFS: 47 months), while within the subgroup of the patients with 1p/19q loss without polysomy only 12/27 (44%) progressed, with PFS ranging between 16 to 132 months (median PFS: 87 months). This difference reached statistical significance (p-value = 0.0048). Although patients with 1p/19q deletion and polysomy had slightly better PFS than those with 1p/19q maintenance, this difference was not significant (p-value = 0.303), Figure 2. Similarly, the 5-year progression-free probability was significantly worse for patients who showed 1p/19q loss with polysomy (0.351) when compared to those with 1p/19q loss without polysomy (0.747) (p-value = 0.009) (Table 3).

Despite the shorter PFS seen in patients with 1p/19q deletion with polysomy compared to those with 1p/19q co-deletion but without polysomy, there was no significant difference in OS

between the two groups (101 months versus 158 months, respectively, p -value = 0.303). Interestingly, in a bivariate Cox model in which OS was adjusted for KPS, there was a decrease in hazard ratio (from 0.246 to 0.316) for AO with 1p/19q co-deletion and polysomy compared to AO with co-deletion without polysomy. Although the adjusted p -value was not significant, these results confirm the trend of worse OS for AO with co-deletion and polysomy and suggest that the apparent difference in OS might become statistically significant with a larger cohort size. OS for AO with maintenance of 1p/19q was significantly worse than OS for AO with 1p/19q loss and polysomy (p -value = 0.0172) (Figure 2).

The 5-year overall survival probability for patients with loss of 1p/19q and polysomy (0.892) was not significantly worse than for patients with 1p/19q loss and without polysomy (0.913) (p -value = 0.209) (Table 3).

Recurrent anaplastic oligodendrogliomas with 1p/19q loss with or without polysomy respond to the therapy

Treatment at the time of presentation is summarized in Tables 1 and 2. All 43 patients whose tumors recurred during the follow up period were treated with additional chemotherapy and/or radiation. Chemotherapy at the time of recurrence was predominantly temozolomide (32 patients); 2 patients received BCNU and 7 patients received a wide variety of experimental protocols including bevacizumab, rapamycin, thalidomide, etoposide and erlotinib without notable benefit. Five patients received radiation at the time of recurrence, either alone ($n=2$) or in combination with chemotherapy ($n=3$). The median survival from recurrence was 26 months.

In patients with 1p/19q loss, 18/27 had a positive response to salvage therapy. This response rate was similar for tumors with and without polysomy (10/15 and 8/12 patients, respectively) and there was no difference in survival from recurrence to death between both groups. In contrast, only 3/16 (19%) patients with recurrent AO with 1p/19q maintenance had a positive response to second-line therapy. Their survival from recurrence to death was significantly lower than of patients with AO with 1p/19q loss (p -value = 0.013, Table 1). The difference in survival from recurrence to death between AO with 1p/19q maintenance and 1p/19q loss and polysomy was marginally significant (p -value = 0.054).

Presence of polysomy does not correlate with Ki-67

The mean Ki-67 score for AO with 1p/19q loss and polysomy was 14.1% (ranging from 2% to 40%) and was not different from the proliferation activity of AO with 1p/19q loss and no polysomy (Ki-67 mean proliferation score 13.8%, ranging from 2% to 55%). There was no correlation between polysomy and proliferation activity measured by Ki-67 and there was no association between Ki-67 and survival (Table 2, and Figure 1E and 1G).

Discussion

Our results show that polysomy is a predictor of earlier recurrence in AO with 1p/19q loss. Although concurrent polysomy and 1p/19q loss has been noted before in oligodendrogliomas (5,25), its prognostic significance has not been evaluated. For example, Fallon et al. (5) observed polysomy in 14% of their 1p/19q deleted tumors and noted that it was more frequent in recurrent and high grade tumors. We limited our study to pure AO with either concurrent 1p/19q loss or maintenance of both 1p and 19q. Although some polysomic cases are purely tetraploid, many tumors show high intratumor variability in the number of copies, which precludes their further sub-stratification based on copy number at least within a cohort of this size. In addition, other less common patterns were seen in our practice and by others (25), such as isolated deletion of either 1p or 19q and/or isolated polysomy of chromosome 1 or 19. Due

to the limited number of cases, we were not able to evaluate significance of these less common variants.

The mechanism that leads to polysomy is not clear. We compared Ki-67 activity of AO with 1p/19q loss with and without polysomy to investigate if polysomy is reflective of higher proliferation activity. Multiple previous studies have evaluated the prognostic significance of Ki-67 activity in oligodendrogliomas and while there is a relative consensus that high proliferation activity measured by Ki-67 is an independent prognostic marker for low grade oligodendroglioma, Ki-67 does not seem to add additional prognostic information in AO (28-30). Our data confirm that there is no prognostic value of Ki-67 in AO, and as such suggest that polysomy is a marker that is independent of proliferative activity. However, since Ki-67 staining is variable among different laboratories and dependent on many variables including the quality of tissue fixation, there is still a possibility that polysomy as detected by FISH represents a more sensitive and reliable method for detecting mitotic activity. Another possible explanation for why polysomy cases show earlier recurrence is that these tumors are in fact glioblastomas with oligodendroglial component (GBM-O). This remains a formal possibility despite careful histologic review due to possible tumor sampling bias. Finally it is possible that polysomy is present in tumors with increased genomic instability, and that this instability then correlates with tumor progression.

In clinical practice, PCR-based loss of heterozygosity (LOH) studies and FISH are the most commonly used methods to detect 1p/19q loss, although FISH may be more frequently used (31). Both methods have advantages and disadvantages. LOH analysis is a PCR-based method that is technically straightforward, however requires normal DNA for comparison. The FISH method is also technically straightforward and commercial probe sets are available; however, scoring can be time consuming and requires experience. The results of our study suggest that evaluation by FISH provides additional information in the form of copy number assessment of polysomy, which is not possible with LOH analysis. Since polysomy predicts early recurrence, FISH might therefore hold better predictive value over LOH for clinical assessment of 1p/19q loss.

The prognostic value of 1p/19q loss in recurrent tumors and their response to salvage therapy is still debated. Van den Bent et al (32) showed a high response rate among recurrent 1p/19q deleted oligodendrogliomas to PCV chemotherapy and Brandes et al (12) showed good response of recurrent AO with 1p/19q loss to temozolomide, however others showed only limited response to second line alkylating agents even in 1p/19q co-deleted tumors (11). Our data indicate that salvage therapy still has a beneficial effect on AO with 1p/19q loss with and without polysomy and confirm the prognostic value of 1p/19q loss in recurrent AO. Since the current second line therapy is very similar to the first line therapy that might suggest that recurrent AO with 1p/19q loss do not develop resistance during the initial treatment. Although AO with loss of 1p/19q and polysomy had a worse OS and lower survival probability than cases with 1p/19q loss without polysomy, the difference did not reach significance. However, the trend toward shorter OS in the polysomy group might reach significance with a larger sample size. Therefore, results of this study need to be confirmed in a larger independent study.

In summary, we have found that AO with concurrent loss of 1p/19q and polysomy are associated with earlier recurrence than similar tumors lacking polysomy. Despite their early recurrence, however, such tumors can still respond to salvage therapy and their outcome remains better than that of patients with AO that have 1p/19q maintenance.

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Figure 1. Representative FISH figures for chromosome 1 probes illustrating 1p maintenance (A), 1p loss (B) and 1p loss with concurrent polysomy (C). H&E sections of anaplastic oligodendroglioma with 1p/19q loss only (D) and 1p/19q loss and polysomy (F). Ki-67 proliferation activity was similar in AO with 1p/19q loss only (E) and 1p/19q loss with polysomy (G). Scale bar represents 150 μ m.

Figure 2.

1- AO with 1p/19q maintenance; 2- 1p/19q loss, all cases; 3- 1p/19q loss and polysomy; 4- 1p/19q loss without polysomy; In the entire cohort of 64 patients, 1p/19q maintenance is associated with decreased progression free survival (1A; $p = 0.009$) as well as overall survival (1B; $p < 0.0003$) when compared to anaplastic oligodendrogliomas with 1p/19q loss. Anaplastic oligodendrogliomas with 1p/19q loss and polysomy have significantly worse progression free survival than AO with 1p/19q loss without polysomy (1C, $p = 0.0048$). Difference in PFS between AO with 1p/19q maintenance and 1p/19q loss with polysomy was not significant (1C; $p = 0.303$) while the overall survival of AO with 1p/19q loss and polysomy was significantly better than AO with 1p/19q maintenance (1D; $p = 0.0172$). The difference in OS between 1p/19q co-deleted AO with and without polysomy was not significant (1D; $p = 0.303$). All p-values are multiplied by 3 to adjust for multiple testing.

Table 1

Clinical Characteristics of patients with Anaplastic Oligodendrogliomas

	all patients		AO with 1p/19q loss		AO with 1p/19q maintenance		p-value*
	No.	%	No.	%	No.	%	
Number of Tumors (%)	64	100	46	72	18	28	-
Age, years							
Median	44		44		45		1.0
(range)	(17-78)		(27-78)		(17-72)		
Sex							
Male	35	55	27	59	8	44	1.0
Female	29	45	19	41	10	56	
Location							
Frontal	48	75	41	89	7	39	<0.003
Temporal	11	17	3	7	8	44	
Parietal	3	5	2	4	1	6	-
Diencephalon	2	3	0	0	2	11	-
Karnofsky Performance	90		90		85		
Score median (range)	(60-100)		(70-100)		(60-100)		0.2433
Distribution							
KPS = 60	1	2	0	0	1	6	
KPS = 70	3	5	1	2	2	11	
KPS = 80	15	23	9	20	6	33	
KPS = 90	29	45	24	52	5	28	
KPS = 100	16	25	12	26	4	22	
Initial surgery							
GTR	25	39	21	46	4	22	0.2646
STR	33	52	22	48	11	61	
Bx	6	9	3	6	3	17	
Initial Adjuvant Chemotherapy	55	86	40	87	15	83	-
Initial Adjuvant Radiotherapy	50	78	35	76	15	83	-
Progression	43	67	27	59	16	89	-
PFS, months							

	all patients		AO with 1p/19q loss		AO with 1p/19q maintenance		p-value*
	No.	%	No.	%	No.	%	
median (range)	50.0 (2-132)		81 (5-132)		21 (2-N/A)		0.0009
95% CI (L, U)	(36,84)		(49,94)		(7,36)		
Survival after Progression							
median (range)	26.0 (1-N/A)		N/A (5.0-N/A)		12.0 (1.0-N/A)		0.013
95% CI (L, U)	(18, N/A)		(22,N/A)		(3,43)		
Dead	22	34	9	20	13	72	-
OS, months							
median (range)	123.0 (5-158)		158 (11-158)		41 (5-N/A)		<0.0003
95 % CI (L, U)	(101,158)		(101,158)		(19,82)		

GTR: gross total resection; STR: subtotal resection; Bx: biopsy only; PFS: progression-free survival; OS: overall survival; 95% CI L, U: 95% confidence interval lower, upper; N/A: quantity not estimable with the current data;

* p-values are for comparisons between 1p/19q loss and 1p/19q maintenance groups, adjusted for additional multiple testing by multiplying by 3;
- : variable not tested.

Table 2

Characteristics of patients with Anaplastic Oligodendrogliomas with 1p/19q loss

	1p/19q loss with polysomy		1p/19q loss without polysomy		p-value *
	No.	%	No.	%	
Number of Tumors	19	41	27	59	-
Ki-67 score					
mean %		14.1 %		13.8 %	1.0
(range)		(2-40)		(2-55)	
Age, years					
median		47		44	1.0
(range)		(31-78)		(27-76)	
Sex					
Male	13	68	14	52	1.0
Female	6	32	13	48	
Location					
Frontal	16	84	25	93	1.0
Temporal	1	5	2	7	
Parietal	2	11	0	0	-
Karnofsky Performance Score					0.0318***
median (range)	90	(70-100)	90	(80-100)	
Distribution					
KPS = 60	0	0	0	0	
KPS = 70	1	5	0	0	
KPS = 80	1	5	8	30	
KPS = 90	8	42	16	59	
KPS = 100	9	47	3	11	
Initial surgery					
GTR	10	53	11	41	1.0
STR	9	47	13	48	
Bx	0	0	3	11	
Adjuvant Chemotherapy	17	89	23	85	-
Adjuvant Radiotherapy	16	84	19	70	-

	1p/19q loss with polysomy		1p/19q loss without polysomy		p-value*
	No.	%	No.	%	
Progression	15	79	12	44	-
PFS, months					
median (range)	47 (5-96)		87 (16-132)		0.0048
95% CI (L, U)	(18,79)		(81,132)		
Survival after Recurrence					
median (range)	N/A (5.0-N/A)		29.0 (9.0-N/A)		1.0
95% CI (L, U)	(20,N/A)		(26, N/A)		
Dead	5	26	5	15	-
OS, months					
median (range)	101 (11-N/A)		158 (27-158)		0.303
95 % CI (L, U)	(101,NA)		(123,158)		

GTTR: gross total resection, STR: subtotal resection, Bx: biopsy only, PFS: progression-free survival, OS: overall survival, 95% CI L, U: 95% confidence interval; lower, upper, N/A: Quantity not estimable with the current data;

* p-value adjusted for additional multiple testing by multiplying by 3,

** p-value reflects difference in KPS distribution not difference in median values,

- : variable not tested.

Table 3

5 Year Survival Probabilities

5-years Progression/Recurrence Free Survival			5-years Overall Survival		
Comparisons	Survival Probability	95%SCL L, U	p-value*	Comparisons	Survival Probability
1p/19q loss	0.573	0.406, 0.708	<0.0003	1p/19q loss	0.904
1p/19q maintenance	0.111	0.019, 0.298		1p/19q maintenance	0.389
1p/19q loss+polysomy	0.351	0.148, 0.563	0.009	1p/19q loss+polysomy	0.892
1p/19q loss only	0.747	0.517, 0.879		1p/19q loss only	0.913
1p/19q loss+polysomy	0.351	0.148, 0.563	0.114	1p/19q loss+polysomy	0.892
1p/19q maintenance	0.111	0.019, 0.298		1p/19q maintenance	0.389

95% SCL L, U: 95% confidence limits for the survivor function lower, upper

* p-value adjusted for multiple testing by multiplying by 3