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## The Importance of 10q Status in an Outcomes-Based Comparison Between 1p/19q Fluorescence In Situ Hybridization and PCR-Based Microsatellite Loss of Heterozygosity Analysis of Oligodendrogliomas

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### Abstract

1p/19q codeletion is a favorable prognostic marker of oligodendrogliomas. While fluorescence in situ hybridization (FISH) and microsatellite-based polymerase chain reaction (PCR) for loss of heterozygosity (LOH) are common methods to test for 1p/19q codeletion, it is unclear which test is better at prognostic stratification. This study analyzed outcomes of 111 oligodendrogliomas with both 1p/19q FISH and LOH done at the time of diagnosis. Overall concordance between the 2 assays was 81.1%. In grade III oligodendrogliomas, LOH was better than FISH at survival stratification ( $p < 0.0001$  for LOH vs.  $p = 0.02$  for FISH), although increasing the stringency of FISH interpretation criteria improved concordance and prognostic power. Oligodendrogliomas that were 1p/19q-codeleted by FISH but also had 10q LOH were negative for 1p/19q codeletion by PCR analysis in over 70% of cases, with very poor survival in the grade III subset. Thus, although PCR-based LOH is a better stratifier of 1p/19q status, FISH still has clinical and prognostic utility, especially if 10q data can be incorporated.

### Keywords

1p/19q; 10q; Epidermal growth factor receptor (EGFR); Fluorescence in situ hybridization (FISH); Loss of heterozygosity (LOH); Microsatellite; Oligodendroglioma

### INTRODUCTION

Codeletion of the short arm of chromosome 1 and the long arm of chromosome 19 (1p/19q) has long been known to be a prognostic marker of oligodendroglial tumors (1, 2), specifically correlating with improved response to adjuvant radiochemotherapy and longer survival (3–9). This codeletion is the result of an unbalanced translocation between chromosomes 1 and 19, with loss of the der(1;19)(p10;q10) (10, 11). 1p/19q codeletion is associated with a proneural expression profile, *MGMT* promoter methylation, and *IDH1/2* mutations (12–14), and is mutually exclusive with *EGFR* amplification (15). On a histologic level, the codeletion is more commonly seen in oligodendrogliomas with classic oligodendroglial morphology (e.g. round nuclei, delicate branching vasculature, and microcalcifications) compared to tumors with only some “oligo-like” features (16, 17).

Detection of 1p/19q codeletion can help clarify whether a hybrid oligoastrocytoma will behave more like an oligodendroglioma or an astrocytoma (18, 19), although not in the pediatric population in which even classic-appearing oligodendrogliomas rarely have this genetic feature (20, 21).

Because of its diagnostic and prognostic power, testing for 1p/19q codeletion has been a mainstay of the neuropathologic armamentarium for some time. Several methods have been described to detect this biomarker but 2 of the most commonly used are fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)-based microsatellite loss of heterozygosity (LOH). FISH is based on fluorophore-labeled DNA probes that selectively bind to regions of interest in the target nuclei. PCR-based LOH detects allelic loss via DNA repeats known as microsatellites. When 2 alleles have nearby microsatellites of varying lengths, that locus is said to be informative. This is because there would be 2 different sizes of PCR products if both alleles are intact but only one size if an allele is lost. Both FISH and PCR-based LOH can be performed on formalin-fixed, paraffin-embedded tissues and neither requires a large amount of tumor tissue; therefore, they are particularly well-suited to neurosurgical specimens.

FISH is more popular than the PCR technique because it can target specific areas of interest in biopsy material when there is a matched hematoxylin and eosin-stained slide available for comparison. However, the length of DNA that FISH probes can cover is limited, i.e. there is a risk of false-positive results if the tumor has partial/interstitial deletions rather than whole-arm losses. Prior studies suggested that 1p36.32 and 19q13.3 were minimally deleted regions in oligodendroglial tumors (22, 23), forming the rationale for selecting those areas to target by FISH.

LOH on 10q has long been known to correlate with higher World Health Organization (WHO) grade in gliomas (7, 24, 25). Several tumor suppressors have been identified on 10q, most notably PTEN, which normally serves as a brake on the oncogenic receptor tyrosine kinase pathways (26). Some studies have shown 10q deletion to be an adverse prognostic marker in oligodendrogliomas (7, 27, 28), but not all studies have shown this correlation (9). Recent work in particular has suggested that 10q LOH is more important than 1p/19q status insofar as oligodendrogliomas with codeletion still behave much worse if 10q is also deleted (28). It is possible, however, that some cases with 10q LOH may have only small losses on both chromosomal arms, as opposed to the extensive losses that correlate with better prognosis.

Prior studies have shown that there is generally good concordance between FISH and PCR-based LOH (23, 29–31), but outcome-based studies directly comparing these tests, using results generated and interpreted at the time of diagnosis, are rare. It is, therefore, of interest to know whether one test has greater prognostic power, i.e. which method is superior at identifying tumors with a better prognosis, and whether 10q status can help refine 1p/19q FISH results and prognosis in such tumors. Herein we describe outcomes from a cohort of 111 oligodendrogliomas, including 79 WHO grade II oligodendrogliomas and 32 grade III anaplastic oligodendrogliomas, in which all cases were tested for 1p/19q codeletion via both FISH and PCR-based LOH analysis at the time of initial biopsy and diagnosis.

## MATERIALS AND METHODS

### Cohort

Between 2002 and 2010, 111 oligodendrogliomas (WHO grades II–III) were prospectively analyzed for 1p/19q codeletion by FISH and LOH at the University of Pittsburgh (Table 1). Cases of recurrent and/or treated gliomas were excluded, as were pediatric cases (i.e. patient

age under 18 years). Diagnoses were made according to standard WHO criteria at the time of initial biopsy. The diagnosis “oligoastrocytoma” was used sparingly due to its well-known low interobserver reproducibility. Instead, only gliomas that had strong oligodendroglial morphology (e.g. round nuclei, little to no angulated nuclei, branching vasculature, and absence of pseudopalisading necrosis) were classified as oligodendrogliomas; all others were classified as astrocytomas, except 4 cases that had equally prominent oligodendroglial and astrocytic components and were excluded from analysis. Postsurgical follow-up data was obtained by the University of Pittsburgh Hillman Cancer Registry. All data collection and analyses were done in accordance with the University of Pittsburgh and the University of Kentucky committees on human subjects.

## FISH

Formalin-fixed paraffin-embedded blocks were analyzed via FISH using probes for 1p36 and 19q13 (Abbott Molecular, Des Plaines, IL), as previously described (32). For ploidy control, locus-specific probes were used for chromosomes 1 (1q25) and 19 (19p13) (Supplemental Fig. 1). At least 60 cells were analyzed in the targeted region per case. Deletion was scored for 1p36 and 19q13 if the target:ploidy control ratio was less than 0.87, with at least 20% of nuclei showing deletion. These cutoff points exceeded 3 standard deviations from the mean of 20 non-neoplastic autopsy brain tissue specimens.

## PCR-Based Microsatellite LOH Analysis

DNA from formalin-fixed paraffin-embedded tissue was probed as described previously (32) with 7 microsatellite markers on chromosome 1p22.2–36.32 (D1S1172, D1S226, D1S162, D1S1161, D1S199, D1S407, D1S171), 2 on 19q13.32 and 19q13.41 (D19S112 and D19S206, respectively), and 2 on 10q (D10S1173 and D10S520) (Supplemental Fig. 1). PCR was performed and the products were analyzed using capillary gel electrophoresis on GeneMapper ABI 3730 (Applied Biosystems, Foster City, CA). When available, patient-matched germline DNA from a peripheral blood sample was used as a control. When normal tissue was not available, peak height ratios falling outside of 2 standard deviations beyond the mean of previously validated normal values for each polymorphic allele pairing were assessed as showing LOH. LOH regions were defined by bracketing only with informative markers. Non-informative loci were mapped and included within preserved or lost regions. At least half of all informative microsatellite loci on both 1p and 19q had to show LOH in order to be designated as having 1p/19q codeletion. Any loss on 10q was counted as 10q LOH.

## Statistics

The Fisher exact test was used to compare relative risk of adverse outcome between 2 groups. Means were compared between multiple groups by Kruskal-Wallis test (nonparametric ANOVA and Dunn’s post hoc) or between 2 groups by Mann-Whitney test where appropriate. Univariate survival rates were compared via log rank tests on Kaplan-Meier curves; multivariate analyses were done via Cox proportional hazards survival regression. Statistical analyses were performed using GraphPad software (La Jolla, CA), Microsoft Excel (Redmond, WA), and <http://statpages.org/prophaz.html>. Differences were considered significant when  $p < 0.05$ .

## RESULTS

### Cohort Characteristics

A total of 111 oligodendrogliomas were prospectively analyzed for 1p/19q codeletion by both FISH and LOH techniques (Table 1; Supplemental Fig. 1). Median patient age was 42

years for grade II tumors and 49 years for grade III tumors. The majority of patients were male (56.8%). Of the 30 who had died at the time of analysis, median survival was 1.8 years, ranging from 47 days to 16.8 years. Of the 238 who were still alive at the time of analysis, median follow-up time was 2.8 years (range: 7.8 months to 19 years). Cases with radiotherapy all received external beam radiation; specific data on the types of chemotherapy used in each case was not retrievable.

### 1p/19q FISH Improves with More Stringent Cutoff Criteria

Overall, there was an 81.1% concordance between FISH and LOH analyses in all oligodendrogliomas (Table 1) (2-tailed  $p < 0.0001$  via Spearman rank correlation). Twenty cases were positive for 1p/19q codeletion by FISH but negative by LOH analysis, whereas only 1 case was codeleted by LOH but not by FISH.

As expected, grade III oligodendrogliomas had worse survival than grade II tumors (Fig. 1A,  $p < 0.0001$ ). Neither 1p/19q assay was able to stratify WHO grade II oligodendrogliomas by survival but the grade II curves generated by FISH were nearly identical whereas PCR-based analysis showed a trend toward improved survival when 1p/19q LOH was detected ( $p = 0.64$  for FISH vs.  $0.09$  for LOH) (Fig. 1B, C). Further survival splitting according to FISH-LOH concordance showed that there was a significant difference between cases that were codeleted by both FISH and LOH vs. those that were positive by just FISH but not LOH ( $p = 0.04$ ) (Fig. 1D).

The difference between the 2 tests was greater in grade III anaplastic oligodendrogliomas (Fig. 1B, C) because although FISH reached significance in its stratification of grade III oligodendrogliomas (Fig. 1B) ( $p = 0.02$ ), LOH analysis was more powerful (Fig. 1C) ( $p < 0.0001$ ). This improvement was achieved by “avoiding” tumors that ultimately behaved aggressively; of the 5 patients whose tumors were diagnosed as codeleted by FISH but not by LOH, median survival was only 1.1 years and none lived beyond 1.3 years after their diagnosis (Fig. 1E). This dismal survival curve was statistically indistinguishable from the cases that were 1p/19q-intact by both FISH and LOH, in which median survival was 1.2 years. In contrast, patients whose grade III oligodendrogliomas were codeleted by both FISH and LOH had a median survival of 5.3 years. In the single anaplastic oligodendroglioma where LOH showed codeletion but not FISH, the patient died 2.2 years after diagnosis (not graphed in Fig. 1E).

Original criteria for deletion of either 1p36 or 19q13 were derived from nonneoplastic autopsy control brain tissues (see Materials and Methods). Although 1p36/1q25 and 19q13/19p13 cutoff ratios of 0.87 were 3 standard deviations below control means, we sought to determine whether even more stringent cutoff criteria would improve the statistical power of 1p/19q FISH. Supporting this hypothesis was the finding that mean ratios for 1p36/1q25 and 19q13/19p13 were significantly higher in gliomas that were positive for 1p/19q codeletion by FISH but not by LOH ( $p < 0.001$ ) (Supplemental Fig. 2). Visual inspection of the ratio scatterplots suggested that lowering 1p36/1q25 and 19q13/19p13 cutoffs to 0.75 might improve specificity without excessive loss of sensitivity. Indeed, setting the cutoff ratios at 0.75 slightly increased overall FISH-LOH concordance to 82.9% and improved specificity and PPV relative to 1p/19q LOH (Table 2). Although some sensitivity and NPV was lost, this modification still produced a slightly improved Spearman rank correlation coefficient between FISH and LOH in all oligodendrogliomas (0.65 for modified FISH vs 0.63 with standard FISH cutoffs). Most importantly, the modification improved stratification power, as the modified FISH cutoff criteria were usually better at separating less aggressive from more aggressive anaplastic oligodendrogliomas (Fig. 1F) ( $p = 0.007$ ) compared to standard FISH criteria (Fig. 1B). Stratification of grade II

oligodendrogliomas remained non-significant even with the modified FISH criteria (Fig. 1F) ( $p = 0.92$ ).

### 10q Status Further Refines 1p/19q Data in Oligodendrogliomas

10q status had been determined at the time of diagnosis in 72 of 79 grade II oligodendrogliomas and in 26 of 32 grade III oligodendrogliomas; the remainder were either noninformative or were consult cases not subjected to 10q testing. 10q LOH was detected in 17 grade II and 9 grade III oligodendrogliomas (23.6% and 34.6%, respectively); this difference was not significant ( $p = 0.40$ ) (Fig. 2A). Of the 11 grade II oligodendrogliomas that had 1p/19q codeletion by original FISH criteria plus 10q LOH, only 3 were also codeleted by PCR-based LOH analysis. Likewise, of the 6 grade III oligodendrogliomas with 1p/19q codeletion and 10q LOH, only 1 was also codeleted by LOH analysis. Overall, oligodendrogliomas codeleted by FISH and intact for 10q were also codeleted for 1p/19q via PCR-based microsatellite analysis in 52 of 59 cases (88.1%) (Fig. 2B, Table 3). In contrast, oligodendrogliomas that were 1p/19q-codeleted by FISH but had 10q LOH also showed 1p/19q codeletion via PCR-based LOH analysis in only 4 of 17 cases (23.5%) ( $p < 0.0001$  via Fisher exact test). When FISH called an oligodendroglioma 1p/19q-intact, 10q status had no significant association with PCR-based LOH concordance, as the tumor was likely to be intact by microsatellite analysis regardless of 10q (Fig. 2C,  $p = 0.41$ ).

Re-interpretation of oligodendrogliomas by modified FISH criteria produced similar results (Table 3). In sum, if cases called 1p/19q-codeleted by original FISH criteria but with 10q LOH had been interpreted as 1p/19q-intact, concordance with PCR-based analysis would have improved from 75% to 88%. Likewise, 10q data improved modified FISH-LOH concordance from 81% to 87%.

From an outcomes-based perspective, the survival of 10q-deleted oligodendrogliomas was shorter compared to their grade-matched 10q-intact counterparts (Fig. 3A, B), although this was significant only in grade III tumors ( $p = 0.002$ ). 10q LOH suggested shorter overall survival in grade II oligodendrogliomas when 1p/19q was intact, though this difference was not significant either by FISH (Fig. 3C) or LOH (Fig. 3D). The only grade III oligodendrogliomas in which longer-term survival occurred was when 1p/19q was codeleted and 10q was intact (Fig. 3E,  $p = 0.03$ ). The same pattern was seen via 1p/19q LOH analysis (Fig. 3F). Because of the strong inverse relationship between 1p/19q LOH and 10q LOH (Fig. 2), subgroup statistical power became problematic. For example, when stratified by 1p/19q PCR-based LOH analysis, there were only 2 grade III oligodendrogliomas that were intact for both 1p/19q and 10q, and 2 other cases with concomitant 1p/19q LOH and 10q LOH. The survival of 1p/19q-intact grade III oligodendrogliomas was slightly but significantly longer when 10q was also intact ( $p = 0.03$  for 1p/19q LOH-negative 10q-intact vs. 1p/19q LOH-negative 10q LOH), though the subgroup sizes are small. Modified FISH criteria showed similar results (not shown).

### Treatment variables, 1p/19q, and 10q

Grade III oligodendrogliomas with either gross total resection or subtotal resection showed longer overall survival (OS) compared to those that were only biopsied ( $p = 0.03$ ; Supplemental Fig. 3A), as well as a trend for longer progression-free survival (PFS) ( $p = 0.08$ , not shown). In grade II oligodendrogliomas, surgery vs. biopsy showed a trend towards longer OS ( $p = 0.08$ ) but not PFS ( $p = 0.13$ ) (not shown). Treatment with radiation and/or chemotherapy showed a strong trend toward better OS in grade III oligodendrogliomas ( $p = 0.05$ ; Supplemental Fig. 3B), as well as significantly longer PFS ( $p = 0.009$ , not shown). However, in grade II oligodendrogliomas, adjuvant therapy showed no significant correlation with PFS ( $p = 0.82$ ) or OS ( $p = 0.63$ ) (not shown).

Focusing only on the 18 grade III oligodendrogliomas known to have received adjuvant radiation and/or chemotherapy, 1p/19q LOH data significantly stratified for OS ( $p < 0.0001$ ) and PFS ( $p = 0.0003$ ), whereas original 1p/19q FISH criteria did not (OS  $p = 0.23$ , PFS  $p = 0.39$ ). Modified FISH criteria improved PFS stratification ( $p = 0.04$ ) but still could not quite reach significance for OS ( $p = 0.14$ ). Grade III oligodendrogliomas subjected to adjuvant therapy still had worse PFS ( $p = 0.0007$ ) and OS ( $p = 0.001$ ) if 10q was deleted. None of the FISH criteria, 1p/19q LOH, or 10q LOH achieved statistical significance in adjuvant-treated grade II oligodendrogliomas (not shown).

### Multivariate Analyses

On multivariate analysis using original 1p/19q FISH criteria, the most significant variables for longer OS and PFS were patient ages  $<45$  years, WHO grade II, and intact 10q (Table 4). All 3 variables were also independent OS and PFS prognostic factors when applying the modified 1p/19q FISH criteria (not shown). Patient age and WHO grade showed the same power when using 1p/19q PCR-based LOH data instead of FISH, with 10q status weakening slightly to a trend toward significance. Neither resection vs. biopsy or adjuvant therapy were independent prognostic factors. Surprisingly, 1p/19q status also was not, unless 10q was excluded from the modeling, whereupon 1p/19q PCR microsatellite LOH reached significance for OS (0.04) and a trend toward improved PFS ( $p = 0.08$ ). However, even after excluding 10q data, 1p/19q FISH never approached independent prognostic significance by either original ( $p = 0.50$  and  $0.98$ ) or modified criteria ( $p = 0.61$  and  $0.37$ ) for PFS or OS, respectively. Our prior work showed that grade III oligodendrogliomas with moderate-to-strong expression of epidermal growth factor receptor (EGFR) by immunohistochemistry had better overall survival than cases in which EGFR immunohistochemistry was negative or weak (33). In 26 grade III oligodendrogliomas with known 1p/19q status, 10q status, polysomy status (according to the criteria established by Snuderl et al [34]) and EGFR expression, younger patient age, intact 10q, and moderate-to-strong EGFR staining were independent favorable prognostic variables for OS when incorporating either original or modified 1p/19q FISH criteria (Table 5). Modeling with 1p/19q PCR-based LOH data increased 1p/19q prognostic strength (though not to the point of significance) at the expense of all other variables. (Of note, there were not enough cases with these data plus treatment and progression-free survival information to expand multivariate modeling.)

## DISCUSSION

Despite its status as the most widely accepted biomarker in the workup of gliomas, 1p/19q codeletion has never been shown to be a truly predictive marker in the sense that HER2 is, wherein amplification predicts a specific response to trastuzumab in breast and upper gastrointestinal cancers. Thus, there has not been a broad-based initiative to standardize 1p/19q testing, which has resulted in remarkable interinstitutional heterogeneity of assays, probes, and cutoff criteria. For example, even when using FISH (specifically the commercially-available 1p36/1q25 and 19q13/19p13 probes from Vysis, Abbott Park, IL), many laboratories focus on 1p/1q and 19q/19p ratios in their evaluation whereas others rely solely on the percent of tumor nuclei showing relative 1p and 19q loss, with a cutoff generally around 40% (35, 36). Our original ratio cutoffs were established as 3 standard deviations beyond the ratios normally seen in non-neoplastic control tissues. To enhance the rigor of the assay, 20 non-neoplastic control samples were used. Applying hindsight, it is clear that increasing the number had the inevitable effect of decreasing standard deviations, i.e. the cutoffs were too inclusive, producing lower concordance with PCR-based LOH data and less prognostic power. Lowering the ratio cutoffs improved concordance with LOH and the prognostic power of FISH (Fig. 1).

From a biological perspective a more stringent pair of ratio cutoffs makes sense, because the unbalanced translocation that produces 1p/19q codeletion is believed to occur at a relatively early stage of gliomagenesis and should therefore be present in most if not all glioma cells. Thus, “true” 1p36/1q25 and 19q13/19p13 ratios ought to be closer to the hypothetical “pure” ratio of 0.5. In contrast, random interstitial deletions could occur at any point during tumor development and would thus be expected to show more cell-to-cell variation, with some cells in a tumor containing partial deletions but not others. It is therefore not surprising that 1p36/1q25 and 19q13/19p13 ratios tended to be higher in gliomas that had apparent codeletion by FISH but not by LOH vs. tumors with codeletion by both modalities (Supplemental Fig. 2).

There are no perfect criteria or cutoffs that produce 100% sensitivity and specificity for 1p/19q FISH (as in any diagnostic test). For example, applying the modified FISH criteria produced a better p value in survival stratification of grade III oligodendrogliomas because it avoided calling 2 cases as codeleted that were intact by LOH analysis; those patients died 2 months and 1.3 years after diagnosis. Yet it also excluded a case that was actually codeleted by original FISH criteria and by LOH analysis; that patient survived 5.3 years. Eschewing ratios altogether, instead using cutoffs of at least 40% tumor cells with relative 1p and 19q deletion, produced a comparable degree of prognostic stratification to the 0.75 ratio cutoffs in anaplastic oligodendrogliomas ( $p = 0.009$  for % deleted vs.  $0.007$  for ratios, data not shown). Furthermore, both the modified ratios and  $\geq 40\%$  relative deletion criteria had 95% concordance with each other in all oligodendrogliomas. Thus, it probably does not matter whether one employs ratios or % deletion because they produce essentially the same overall results. What seems more important is that these cutoffs have been determined in this study using prognostic stratification and whole-arm assays as the primary litmus tests rather than relying on non-neoplastic tissues.

Although in most cases there was good concordance between FISH and PCR-based LOH, in this cohort 1p/19q PCR-based LOH showed stronger prognostic value in grade III anaplastic oligodendrogliomas. That neither test was able to significantly stratify grade II oligodendrogliomas (Fig. 1B, C) is not surprising because 1p/19q codeletion appears to have less prognostic impact in low-grade gliomas and may only show significance with prolonged follow-up intervals (8, 37, 38). Indeed, an analysis of our grade II subset suggested insufficient power (0.67) to stratify by 1p/19q status given our cohort size, median follow-up interval and the relatively longer survival in this subgroup. On the other hand, because grade III tumors have shorter survival overall and a much sharper survival difference by 1p/19q (or 10q) status, the subset of 32 cases was adequately powered (0.91). Still, testing for codeletion at least has diagnostic value, particularly in gliomas in which morphologic features are equivocal between astrocytoma and oligodendroglioma (19).

Oligodendrogliomas with true 1p/19q codeletion but 10q LOH are rare to begin with and sometimes have fewer classic features of oligodendrogliomas, suggesting that the diagnosis of oligodendroglioma in the face of 10q deletion may be reconsidered (7, 25, 27, 39–42). Our present results support and extend these findings, specifically making the critical point that most cases called “codeleted” by FISH are false-positives if 10q is also deleted. Whereas rare gliomas do indeed have concomitant whole-arm 1p/19q codeletion and 10q LOH, many so-called 1p/19q-codeleted gliomas are likely not truly whole-arm codeleted when 10q is also lost (Fig. 2; Table 3).

Prior work showed that increased EGFR expression is paradoxically correlated with improved survival in anaplastic oligodendrogliomas (33, 43). The current data reconfirm this on multivariate analysis (Table 5). The reasons for this are not clear but it is not due to

nonrandom segregation of 1p/19q LOH ( $p = 0.46$ ), 10q LOH ( $p = 0.54$ ), or younger patient age ( $p = 0.41$ ).

Although PCR-based LOH is more powerful than FISH, and assays such as single nucleotide polymorphism, array comparative genomic hybridization, and multiplex ligation-dependent probe amplification show great promise (44–47), our results indicate that it is likely that FISH will remain a widely-used technique in the workup of gliomas. Standardized protocols for 1p/19q FISH have recently been recommended (36), which should be of great assistance to laboratories worldwide; however, universal standardization of 1p/19q FISH probe loci, interpretation criteria and reporting criteria are also needed. Based on our data, we suggest the following scoring and reporting parameters for 1p/19q FISH: 1) If using 1p36/1q25 and 19q13/19p13 ratios, they should each be  $<0.75$ . 2) If using relative deletion, both 1p36 and 19q13 should be deleted in at least 40% of cells. 3) 10q interrogation, either by PCR analysis or FISH (e.g. testing for PTEN deletion) should be done in cases where 1p/19q FISH is positive or equivocal for codeletion, and if 10q is deleted, the 1p/19q data should be interpreted with great caution; Indeed, it may even be advisable to test 10q status in oligodendrogliomas regardless of the 1p/19q results, as it may be an independent adverse prognostic factor (Tables 4, 5).

These are useful parameters to serve as a starting point for the development of universal scoring and reporting criteria for 1p/19q FISH. Once such standards are in place, they will enhance the quality of prognostic information provided to clinicians and patients. They will also improve inter-institutional reproducibility of glioma research, especially work that focuses on 1p/19q codeletion, 10q deletion, and their roles in glioma biology.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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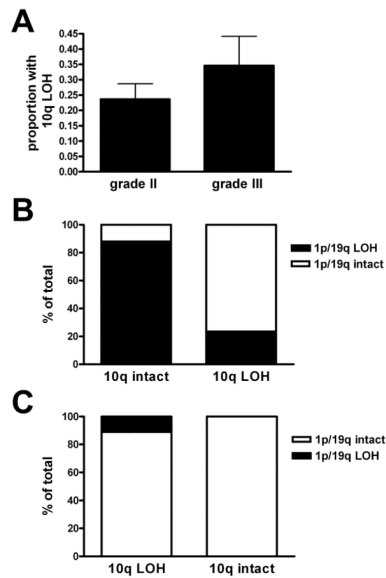
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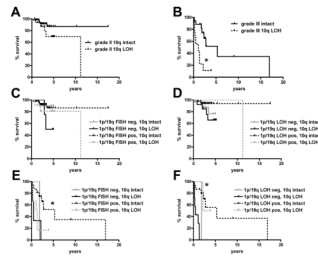
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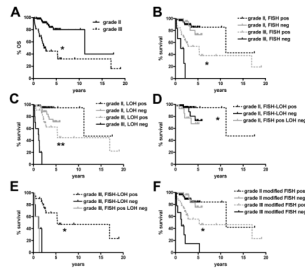
**Figure 1.**

Survival according to 1p/19q fluorescence in situ hybridization (FISH) and loss of heterozygosity (LOH) analyses. (A) Grade III oligodendrogliomas had worse survival than grade II tumors ( $*p < 0.0001$ ). (B, C) 1p/19q FISH (B) was weaker than PCR-based LOH (C) in differentiating longer-term from shorter-term survivors. This difference was most prominent in grade III anaplastic oligodendroglial tumors, in which prognostic stratification was better by LOH ( $**p < 0.0001$ ) than by FISH ( $*p = 0.02$ ). FISH could not stratify grade II oligodendrogliomas ( $p = 0.64$ ), survival differences trended toward significance by LOH ( $p = 0.09$ ). (D) Grade II oligodendrogliomas with 1p/19q codeletion by both modalities had longer survival than those that were positive by FISH but not by LOH ( $*p = 0.04$ ;  $p = 0.26$  vs. tumors that were negative by both tests). (E) Grade III anaplastic oligodendrogliomas that were codeleted by FISH and LOH showed longer survival compared to tumors that were negative by both tests ( $*p = 0.0006$ ), or positive only by FISH but not LOH ( $*p < 0.0001$ ). (F) Increasing the stringency of FISH codeletion criteria to a cutoff 1p36/1q25 and 19q13/19p13 ratio of 0.75 for each probe pair (from the original cutoff of 0.87) improved statistical power in stratifying grade III anaplastic oligodendrogliomas ( $*p = 0.007$ ).



**Figure 2.**

10q loss of heterozygosity (LOH) in gliomas. (A) 23.6% of grade II oligodendrogliomas had 10q LOH vs. 34.6% of grade III oligodendrogliomas ( $p = 0.40$  by Mann-Whitney test). (B) When either a grade II or grade III oligodendroglioma was called 1p/19q-codeleted by fluorescence in situ hybridization (FISH), concordance with PCR-based 1p/19q microsatellite LOH analysis was much less likely if 10q was also deleted ( $p < 0.0001$ ,  $n = 76$ ). (C) If an oligodendroglioma was 1p/19q-intact by FISH, 10q status had no bearing on whether the FISH result would be concordant with PCR-based 1p/19q microsatellite analysis ( $p = 0.41$ ,  $n = 22$ ).



**Figure 3.**

Impact of 10q loss of heterozygosity (LOH) on survival in oligodendrogliomas. **(A, B)** 10q LOH did not significantly change overall survival in grade II gliomas (**A**,  $p = 0.12$ ) though it did in grade III gliomas (**B**,  $*p = 0.002$ ). **(C, D)** Factoring in 1p/19q status, 10q LOH still did not significantly alter survival in grade II oligodendrogliomas as assessed by 1p/19q FISH (**C**,  $p = 0.39$ ) or PCR-based LOH (**D**,  $p = 0.27$ ), although the number of cases with 1p/19q codeletion and 10q LOH was very small ( $n = 3$ ). **(E)** In grade III oligodendrogliomas, 1p/19q-codeleted cases by fluorescence in situ hybridization (FISH) had better survival only if 10q was also intact ( $*p = 0.03$  vs. all other groups). **(F)** 10q status did not significantly refine survival when grade III oligodendrogliomas were 1p/19q-codeleted via PCR-based LOH ( $p = 0.86$ ). Survival of 1p/19q-intact grade III oligodendrogliomas was slightly but significantly longer when 10q was also intact ( $*p = 0.03$  for 1p/19q LOH-negative (neg.) 10q-intact vs. 1p/19q LOH-neg 10q LOH).

**Table 1**

## Clinical Characteristics of the Oligodendroglioma Cohort

Parameter	Grade II oligodendroglioma	Grade III oligodendroglioma
No. male (%)	41 (51.9)	22 (68.8)
Median age (range)	42 y (19—79 y)	49 y (25—80 y)
No. biopsy only (%)	21 (29.2)	7 (26.9)
No. GTR (%)	22 (30.6)	6 (23.1)
No. STR (%)	29 (40.3)	13 (50.0)
No. temporal lobe (%)	14 (23.7)	3 (15.8)
No. non-temporal lobe (%)	45 (76.3)	16 (84.2)
No. with no adjuvant therapy (%)	26 (36.6)	8 (30.8)
No. with radiation only (%)	13 (18.3)	4 (15.4)
No. with chemo only (%)	24 (33.8)	3 (11.5)
No. with radiochemotherapy (%)	8 (11.3)	11 (42.3)
Mean follow-up interval	3.4 y	3.4 y
Median follow-up interval	2.9 y	1.9 y
Follow-up range	0.3—17.5 y	0.1—18.9 y
No. died (%)	11 (15.5)	19 (59.4)
No. 1p/19q intact by FISH and LOH (%)	22 (27.8)	4 (12.5)
No. 1p/19q codeleted by FISH and LOH (%)	43 (54.4)	22 (68.8)
No. codeleted by FISH not LOH (%)	14 (17.7)	6 (18.8)
No. codeleted by LOH not FISH (%)	0 (0)	0 (0)
No. with 10q LOH (%)	17/72 (23.6)	9/26 (34.6)

Seventy-nine grade II oligodendrogliomas and 32 grade III oligodendrogliomas were subjected to prospective molecular testing at the time of diagnosis. The type of surgery was known in 72 grade II and 26 grade III tumors; postoperative treatment information was available for 71 grade II and 26 grade III tumors; precise tumor location was retrievable for 59 grade II and 19 grade III tumors. Overall 1p/19q concordance between FISH and LOH was 81.1%. FISH = fluorescence in situ hybridization; GTR = gross total resection; STR = subtotal resection; LOH = loss of heterozygosity.

**Table 2**

Comparison Between 2 Different Fluorescence In Situ Hybridization Interpretation Criteria and Loss of Heterozygosity Methods of 1p/19q Testing

1p/19q FISH criteria	1p/19q FISH relative to 1p/19q LOH			
	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
Original FISH criteria	98.5 (91.7—100.0)	56.5 (41.1—71.0)	76.2 (65.6—84.8)	96.3 (81.0—99.9)
Modified FISH criteria	93.9 (85.0—98.3)	67.4 (51.9—80.5)	80.3 (69.6—88.5)	88.6 (73.3—96.8)

One hundred eleven grade II and III oligodendrogliomas were analyzed for 1p/19q codeletion by both FISH and LOH assays. Relative to 1p/19q LOH testing, increasing the stringency of FISH ratio cutoff criteria from < 0.87 (Original) to < 0.75 (Modified) improved the specificity and positive predictive value (PPV) of FISH, at the cost of reduced sensitivity and negative predictive value (NPV). CI = confidence interval; FISH = fluorescence in situ hybridization; LOH = loss of heterozygosity.



Concordance Between 1p/19q Fluorescence In Situ Hybridization and PCR-Based Microsatellite Loss of Heterozygosity Data Is Further Refined by 10q Status

**Table 3**

1p/19q FISH	10q status	1p/19q PCR microsatellite-based LOH		% FISH-PCR concordance
		codeleted	Intact	
original criteria	codeleted	52	7	88.1
	10q LOH	4	13	23.5
	10q intact	0	13	100.0
	10q LOH	1	8	88.9
modified criteria	codeleted	49	5	90.7
	10q LOH	4	10	28.6
	10q intact	3	15	83.3
	10q LOH	1	11	91.7

Ninety-eight World Health Organization grade II and III oligodendrogliomas were tested for 1p/19q codeletion by FISH and PCR-based LOH analysis and for 10q deletion. Both by original and more stringent modified criteria, if a case was 1p/19q-codeleted and intact for 10q, PCR analysis also called the case codeleted about 90% of the time. If a case was called 1p/19q-codeleted by FISH yet had 10q LOH, PCR analysis called the same tumor negative for codeletion over 70% of the time. If a tumor was 1p/19q intact by either original or modified FISH criteria and also 10q intact, PCR analysis also called it 1p/19q intact 80–100% of the time; the presence of 10q LOH did not appreciably change that proportion (about 90%). FISH = fluorescence in situ hybridization; LOH = loss of heterozygosity; PCR = polymerase chain reaction.

**Table 4**  
Multivariate Analysis of Overall Survival and Progression-Free Survival in Oligodendrogliomas

Overall survival with 1p/19q FISH criteria			Progression-free survival with 1p/19q FISH criteria		
Variable	Relative Risk (95% CI)	p	Variable	Relative Risk (95% CI)	p
age < 45 y	0.12 (0.042–0.37)	0.0002	age < 45 y	0.25 (0.11–0.60)	0.0017
GTR or STR	0.51 (0.16–1.6)	0.24	GTR or STR	0.52 (0.17–1.5)	0.24
adjuvant Rx	0.62 (0.26–1.5)	0.29	adjuvant Rx	0.58 (0.26–1.3)	0.18
WHO grade II	0.16 (0.065–0.39)	0.0001	WHO grade II	0.14 (0.060–0.34)	0.00001
1p19q codeleted	1.68 (0.47–6.1)	0.42	1p19q codeleted	1.8 (0.58–5.8)	0.31
10q intact	0.36 (0.15–0.88)	0.026	10q intact	0.41 (0.18–0.95)	0.037
Overall survival with 1p/19q PCR-based LOH			Progression-free survival with 1p/19q PCR-based LOH		
Variable	Relative Risk (95% CI)	p	Variable	Relative Risk (95% CI)	p
age < 45 y	0.15 (0.048–0.44)	0.0007	age < 45 y	0.27 (0.11–0.63)	0.0025
GTR or STR	0.75 (0.30–1.9)	0.55	GTR or STR	0.79 (0.32–1.9)	0.61
adjuvant Rx	0.75 (0.31–1.8)	0.52	adjuvant Rx	0.69 (0.30–1.6)	0.39
WHO grade II	0.13 (0.048–0.34)	0.00001	WHO grade II	0.13 (0.050–0.32)	0.00001
1p19q LOH	0.44 (0.15–1.3)	0.13	1p19q LOH	0.59 (0.22–1.6)	0.30
10q intact	0.46 (0.19–1.1)	0.09	10q intact	0.51 (0.22–1.2)	0.12

Sixty-four grade II and 20 grade III oligodendrogliomas were modeled by Cox Proportional Hazards according to key clinical, histologic, and molecular variables. The most powerful factors for favorable progression-free and overall survival were age <45 years, WHO grade II histology, and intact 10q (underlined). When incorporating 10q status with the multivariate analyses, 1p/19q status was not an independently prognostic variable. CI = confidence interval; FISH = fluorescence in situ hybridization; GTR = gross total resection; LOH = loss of heterozygosity; STR = subtotal resection. (Relative to the 1p/19q FISH data, similar *p* values were seen when using the modified 1p/19q FISH criteria, not shown.)

**Table 5**  
Multivariate Analysis of Overall Survival Based on Age and Molecular Variables in Grade III Oligodendrogliomas

variable	original 1p/19q FISH criteria		modified 1p/19q FISH criteria		1p/19q PCR LOH	
	Relative Risk (95% CI)	p	Relative Risk (95% CI)	p	Relative Risk (95% CI)	p
age <45 years	0.07 (0.01–0.51)	0.009	0.06 (0.008–0.5)	0.009	0.12 (0.02–0.9)	0.04
10q intact	0.27 (0.08–0.85)	0.025	0.28 (0.09–0.9)	0.03	0.36 (0.1–1.3)	0.12
no polysomy	0.70 (0.19–2.5)	0.58	0.61 (0.16–2.3)	0.46	0.72 (0.2–2.2)	0.56
EGFR IHC moderate-to-strong	0.27 (0.08–0.88)	0.03	0.27 (0.08–0.9)	0.03	0.42 (0.12–1.4)	0.17
1p/19q deleted	0.72 (0.17–3.0)	0.65	0.53 (0.16–1.8)	0.31	0.30 (0.05–1.7)	0.18

Analysis of 26 grade III anaplastic oligodendrogliomas showed that younger patient age, intact 10q, and moderate-to-strong EGFR expression on IHC were independent favorable prognostic variables for overall survival when incorporating either original or modified 1p/19q FISH criteria. When using 1p/19q PCR-based LOH data instead, the only variable that retained significance was patient age. CI = confidence interval; EGFR = epidermal growth factor; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; LOH = loss of heterozygosity.