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# Frequency of false-positive FISH 1p/19q codeletion in adult diffuse astrocytic gliomas

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## Abstract

**Background.** Oligodendroglioma is genetically defined by concomitant IDH (*IDH1/IDH2*) mutation and whole-arm 1p/19q codeletion. Codeletion of 1p/19q traditionally evaluated by fluorescence in situ hybridization (FISH) cannot distinguish partial from whole-arm 1p/19q codeletion. Partial 1p/19q codeletion called positive by FISH is diagnostically a "false-positive" result. Chromosomal microarray (CMA) discriminates partial from whole-arm 1p/19q codeletion. Herein, we aimed to estimate the frequency of partial 1p/19q codeletion that would lead to a false-positive FISH result.

**Methods.** FISH 1p/19q codeletion test probe coordinates were mapped onto Oncoscan CMA data to determine the rate of partial 1p/19q codeletion predicted to be positive by FISH. Diffuse astrocytic gliomas with available CMA data (2015–2018) were evaluated and classified based on IDH1-R132H/ATRX/p53 immunohistochemistry, IDH/*TERT* promoter targeted sequencing, and/or CMA according to classification updates. Predicted false-positive cases were verified by FISH whenever possible.

**Results.** The overall estimated false-positive FISH 1p/19q codeletion rate was 3.6% (8/223). Predicted false positives were verified by FISH in 6 (of 8) cases. False-positive rates did not differ significantly ($P = .49$) between IDH-mutant (4.6%; 4/86) and IDH-wildtype (2.9%; 4/137) tumors. IDH-wildtype false positives were all WHO grade IV, whereas IDH-mutant false positives spanned WHO grades II-IV. Testing for 1p/19q codeletion would not have been indicated for most false positives based on current classification recommendations.

**Conclusion.** Selective 1p/19q codeletion testing and cautious interpretation for conflicting FISH and histopathological findings are recommended to avoid potential misdiagnosis.

## Key Points

- Partial 1p/19q codeletion called positive by FISH is diagnostically false positive.
- False-positive FISH 1p/19q codeletion rate is 3.6% in diffuse astrocytic gliomas.
- 1p/19q codeletion testing would not have been indicated for most false-positive FISH cases.

Molecular parameters including the presence of IDH (*IDH1/IDH2*) mutation and 1p/19q codeletion have been incorporated into the classification scheme of adult-type diffuse gliomas in the 2016 update of the WHO classification of central nervous system (CNS) tumors.[1] The updated diffuse glioma classification has been shown to more clearly delineate prognostically relevant groups than the prior histopathological-only classification scheme.[2,3] According to the 2016 WHO classification update, oligodendroglioma, which is associated with survival benefit and chemoradiation sensitivity,[4–7] now requires demonstration

## Importance of the Study

Despite current classification recommendations and known existence of false-positive FISH 1p/19q codeletion results in diffuse astrocytic gliomas, some clinical providers order upfront 1p/19q codeletion testing, often by FISH, in any adult diffuse glioma. We estimated a frequency of 3.6% partial 1p/19q codeletion leading to a false-positive FISH result in adult diffuse astrocytic gliomas. In light of integrated histological-molecular diagnoses, false-positive FISH 1p/19q codeletion rates did not differ significantly between IDH-mutant and IDH-wildtype tumors. Testing for 1p/19q codeletion would not have been indicated in most of the identified false-positive FISH cases based on current classification recommendations, underscoring the clinical value of these recommendations. Our estimated false-positive FISH 1p/19q codeletion rate may be used in clinical discussions regarding case and method selection for 1p/19q codeletion testing in adult diffuse gliomas to avoid potential confusing diagnostic scenarios and misdiagnosis.

of both IDH mutation and 1p/19q codeletion.[1] The 1p/19q codeletion characteristic of oligodendroglioma consists of combined whole-arm losses of 1p and 19q as a result of an unbalanced t(1;19)(q10;p10) translocation.[8,9] Therefore, only the loss of both 1p and 19q whole arms qualifies as the 1p/19q codeletion that is diagnostically and prognostically significant.

Historically, 1p/19q codeletion status has been largely determined by interphase fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)-based loss of heterozygosity (LOH) analysis.[6,10,11] FISH test probes cover chromosome regions 1p36 and 19q13 that were defined by minimal deletion studies[12–16] and encompass approximately only 0.4–1.5% of their respective chromosome arms.[17] As such, FISH cannot distinguish partial from whole-arm 1p/19q codeletion. Currently, there are alternative platforms able to discriminate partial from whole-arm 1p/19q codeletion which are becoming more broadly available and less cost-prohibitive, including chromosomal microarray (CMA), array comparative genomic hybridization (aCGH), genome-wide DNA methylation array, and next-generation sequencing (NGS).[16,18–20] At our institution, CMA has been widely utilized for neuro-oncology clinical testing as, in addition to distinguishing partial from whole-arm 1p/19q codeletion, CMA provides genome-wide copy number evaluation to assist in the integrated classification and prognostication of diffuse gliomas.

Partial combined losses of 1p and 19q (ie, partial 1p/19q codeletion) that may be called positive by FISH occur in diffuse astrocytic gliomas, with reported overall frequencies of 2–25%[6,12,17,21–25] and of 4–12% in glioblastoma[24,26–29] based on studies prior to the 2016 WHO classification update. Although partial 1p/19q codeletion consists of legitimate 1p and 19q interstitial or terminal deletions not due to inherent FISH technical flaws to be considered true false positives, the term "false positive" was chosen for clarity to indicate that a partial 1p/19q codeletion called positive by FISH is diagnostically a false-positive result that may lead to the incorrect conclusion regarding the presence of a whole-arm 1p/19q codeletion.[30] FISH 1p/19q codeletion testing of diffuse gliomas that are morphologically (ie, mixed oligoastrocytic or pure astrocytic) and/or immunohistochemically (ie, with ATRX loss of expression and/or p53 overexpression) not classic for an oligodendroglioma has been shown to result in a higher potential for false-positive results.[17,26] However, the integration of molecular parameters into the updated 2016 WHO classification of adult diffuse gliomas[1] seems to have encouraged many clinical providers to request upfront FISH 1p/19q codeletion testing in any adult diffuse glioma despite the subsequent recommendations from the cIMPACT-NOW (consortium to inform molecular and practical approaches to CNS tumor taxonomy) updates 2 and 3,[31–33] increasing the risk of obtaining a false-positive FISH result.

To the best of our knowledge, there are no reported estimates of the frequency of false-positive FISH 1p/19q codeletion in adult diffuse astrocytic gliomas after the 2016 WHO classification and cIMPACT-NOW updates. Herein, we estimate the frequency of partial 1p/19q codeletion that would lead to a false-positive FISH result in the context of integrated histological-molecular diagnoses and describe the morphological and immunohistochemical patterns that were associated with a false-positive FISH result.

## Materials and Methods

### Case Selection

Following institutional review board approval, we compiled a study group comprising in-house surgical and consultation cases diagnosed as a diffuse glioma within a 3-year period (2015–2018) in patients 18 years or older who had available CMA. Cases with whole-arm 1p/19q codeletion by CMA, including primarily oligodendroglioma and rare cases of dual-genotype/molecular hybrid oligoastrocytoma (reported separately),[34] were excluded since these cases would not be considered FISH 1p/19q false positives. Lower-grade IDH-wildtype diffuse astrocytic gliomas ($n = 22$; 2 WHO grade II and 20 WHO grade III) were reclassified as a "diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV" based on cIMPACT-NOW update 3 recommendations.[33]

We refer to our final study group as "diffuse astrocytic gliomas."

### Identification of False-Positive Cases

False-positive cases were identified by searching CMA clinical reports for copy number alterations involving the location of 1p36 and 19q13.3 FISH test probes [Chr1(GRCh37):g.3338313-3773313 and Chr19(GRCh37):g.48024005-48404005] and the 1q25 and 19p13 FISH control probes [Chr1(GRCh37):g.178787819-179405819 and Chr19(GRCh37):g.12301591-12803591] as given in Supplementary Table 1. Only CMA deletions or relative losses of 1p (vs 1q) and 19q (vs 19p) spanning both FISH test probe regions with at least one being partial arm were considered false positive, thereby simulating 1p/19q codeletion by FISH (Supplementary Table 2). See Supplementary Materials and Methods for additional details on the CMA testing and mapping of FISH probes to CMA.

### FISH Testing

Target tumor areas were delineated on a formalin-fixed paraffin-embedded hematoxylin and eosin (H&E)-stained tissue section to include at least 100 tumor cells. Using the H&E-stained slide as a reference, target tumor areas were etched with a diamond-tipped etcher. FISH testing was performed with dual-probe hybridization using a SpectrumOrange-labeled locus-specific probe for 1p and 19q and a SpectrumGreen-labeled control probe [1p36(TP73)/1q25(ABL2) and 19p13(D19S221)/19q13.3(EHD2); Abbott Molecular] as previously described.[6] Two technologists independently scored 50 nonoverlapping nuclei within the target tumor areas (100 total tumor cell nuclei) for each probe set. Results were reported as the ratio of the total number of red to green signals for each probe set (1p36:1q25 and 19q13.3:19p13 signals). Based on analytical validation studies,[6] interpretation for signal ratios and patterns was as follows: less than 0.8: Deletion; 0.8–0.89: Rescore sample; 0.9–1.29: Normal; more than 1.3: Gain. Aneusomy was called if more than 30% of nuclei exhibited at least 3 red and/or at least 3 green signals for one or both probe sets.

### IDH and *TERT* Promoter Targeted Sequencing

Targeted amplicon-based sequencing was performed by custom clinical pyrosequencing (cases prior to 2017) or NGS for IDH mutational hotspot codons (*IDH1* codon 132 and *IDH2* codon 172) and NGS for *TERT* promoter mutational hotspot region. Pyrosequencing was performed using the Qiagen PyroMark Q24 system to interrogate most of the IDH-reported mutations.[35] NGS library was prepared using a 2-stage PCR approach to generate PCR amplicons with gene-specific target regions [*IDH1* codons 113–138, *IDH2* codons 137–174 and *TERT* promoter region spanning Chr5(GRCh37):g.1295170-1295296]. Sequencing was performed in a MiSeq instrument (paired-end, 2 × 151; Illumina, Inc.) and data were processed by a custom bioinformatics pipeline. Variants with at least 5% variant allele frequency were evaluated and curated as previously described.[36]

### IDH1-R132H, ATRX, and p53 Immunohistochemical Studies

Immunohistochemical studies were performed according to validated protocols using IDH1-R132H (clone H09; 1:50; Dianova), ATRX (clone D-5; 1:1000; Santa Cruz), and p53 (clone DO-7; prediluted; Ventana) antibodies. For IDH1-R132H, strong cytoplasmic staining of any tumor cell was considered positive; for ATRX, the absence of nuclear staining was interpreted as a loss of protein expression only in the presence of internal positive control with nuclear staining of nonneoplastic cells; for p53, nuclear staining of over 50% of tumor cells was considered consistent with protein overexpression.

### Statistical Analysis

Categorical comparisons were made across IDH-wildtype and IDH-mutant groups using the Fisher's exact test. Numerical comparisons between the average copy number of CMA probes covering the FISH probe regions were made using *T*-test.

## Results

Results are detailed in Table 1. Our study group of diffuse astrocytic gliomas included 223 cases from 221 patients (2 recurrent tumors), with 137 (61%) IDH-wildtype and 86 (39%) IDH-mutant tumors. Among these 223 tumors, there were 24 (11%) WHO grade II (all IDH-mutant), 46 (21%) WHO grade III (9 IDH-wildtype and 37 IDH-mutant), and 153 (68%) WHO grade IV (128 IDH-wildtype and 25 IDH-mutant) tumors. A total of 8 (of 223, 3.6%) predicted

**Table 1.** Frequency of False-Positive FISH 1p/19q Codeletion in Adult Diffuse Astrocytic Gliomas

| WHO Grade | IDH Wildtype | IDH Mutant | Total |
|---|---|---|---|
| Diffuse astrocytoma (WHO grade II) | 0/0 (0%) | 1/24 (4.2%) | 1/24 (4.2%) |
| Anaplastic astrocytoma (WHO grade III) | 0/9 (0%) | 1/37 (2.7%) | 1/46 (2.2%) |
| Glioblastoma (WHO grade IV) | 4/128 (3.1%) | 2/25 (8.0%) | 6/153 (3.9%) |
| Diffuse astrocytic gliomas (WHO grades II–IV) | 4/137 (2.9%) | 4/86 (4.6%) | 8/223 (3.6%) |

false-positive FISH cases were identified: 4 IDH-wildtype (4/137, 2.9%) and 4 IDH-mutant (4/86, 4.6%). The rates of partial 1p/19q codeletion by CMA predicted to lead to a false-positive FISH result did not significantly differ between IDH-wildtype and IDH-mutant tumors ($P = .49$). IDH-mutant glioblastoma had the highest false-positive rate (8%, 2/25). Of the 8 predicted false-positive FISH cases, we were able to perform 1p/19q FISH testing in 6 (3 IDH-wildtype and 3 IDH-mutant) cases. All 6 tested cases had 1p/19q codeletion by FISH and verified our prediction approach. Morphologic and immunohistochemical patterns of the false-positive FISH cases are described below. The remaining 2 false-positive cases were not evaluated by FISH as there was no residual tissue available for testing.

## IDH-wildtype Cases

Four (of 137, 2.9%) IDH-wildtype diffuse astrocytic gliomas were predicted to render a false-positive FISH result. All 4 cases were WHO grade IV glioblastoma. Among IDH-wildtype glioblastoma, 3.1% (4/128) were predicted false-positive FISH cases (Table 1). Clinicopathologic features of the IDH-wildtype predicted false-positive FISH cases are detailed in Table 2. Morphologically, all cases had an astrocytic morphology and histological features (necrosis and/or microvascular proliferation) consistent with a WHO grade IV designation. Immunohistochemically, they were all negative for IDH1-R132H (and sequencing in 3 of the 4 cases) with retained ATRX and frequent p53 overexpression. Testing for 1p/19q codeletion would not be formally indicated in any of these 4 cases as they were all morphologically glioblastoma with sufficient evidence supporting an IDH-wildtype genotype.[37] In a single case (case 1), a right cerebellar glioblastoma in a 30-year-old man, upfront FISH 1p19q codeletion testing could have been performed in parallel to the IDH sequencing recommended due to the patient's age (Figure 1). The negative IDH sequencing result in this case would prompt follow-up studies to show that the positive FISH result was diagnostically false positive and course-correct away from misdiagnosing such tumor as an anaplastic oligodendroglioma. The remaining 3 cases were tumors from patients over 54 years of age with a negative IDH1-R132H immunostain. Following the 2016 WHO classification update, these glioblastomas would have a low likelihood of having a less common IDH mutation and could be diagnosed as a "glioblastoma, IDH-wildtype, WHO grade IV" without additional testing.[1] Representative results from case 2, a 56-year-old woman with a left cerebellar glioblastoma, are illustrated in Supplementary Figure 1. Of note, in case 3, a right frontoparietal glioblastoma in a 76-year-old woman, FISH 1p19q codeletion testing had been performed upon clinician/pathologist's request and CMA testing followed to resolve the apparently discordant FISH results.

## IDH-mutant Cases

Four (of 86, 4.6%) IDH-mutant cases were predicted to have a false-positive FISH result and included tumors of all WHO grades. Among WHO grade II, III, and IV IDH-mutant tumors, 4.2% (1/24), 2.7% (1/37), and 8% (2/25) were predicted false-positive FISH cases, respectively (Table 1). Clinicopathologic features of the IDH-mutant predicted false-positive FISH cases are detailed in Table 2. Most cases had an *IDH1* R132H mutation and a single case had an *IDH1* R132G mutation identified by sequencing. Three (of 4) tumors, one of each WHO grade, showed loss of ATRX immunohistochemical expression. Testing for 1p/19q codeletion would be unnecessary in all but one case (case 6). According to the cIMPACT-NOW update 2,[32] "in the setting of a diffuse astrocytic-appearing WHO grade II or grade III tumor that has IDH mutation, as well as, loss of ATRX nuclear expression and/or strong, diffuse p53 immunopositivity, a diagnosis of diffuse astrocytoma, IDH-mutant or anaplastic astrocytoma, IDH-mutant can be rendered in the absence of 1p/19q testing." Therefore, case 6, a low-grade diffuse glioma with mixed oligoastrocytic morphology and ATRX loss of expression without significant

**Table 2.** Clinicopathologic Features of False-Positive FISH 1p/19q Codeletion Adult Diffuse Astrocytic Gliomas

| Case | Age (years) | Sex | Tumor Location | Morphology | WHO Grade | IDH1-R132H IHC | IDH Seq | ATRX IHC | p53 IHC | FISH 1p/19q |
|---|---|---|---|---|---|---|---|---|---|---|
| IDH-wildtype | | | | | | | | | | |
| 1 | 30 | M | Right cerebellum | Astrocytic | IV | (–) | (–) | Retained | OE | (+) |
| 2 | 56 | F | Left cerebellum | Astrocytic | IV | (–) | ND | Retained | OE | (+) |
| 3 | 76 | F | Right frontoparietal | Astrocytic | IV | (–) | (–) | Retained | Not OE | (+) |
| 4 | 78 | M | Left occipital | Astrocytic | IV | (–) | (–) | Retained | OE | ND |
| IDH-mutant | | | | | | | | | | |
| 5 | 31 | F | Left parietal | Astrocytic | III | (–) | (+) | Loss | OE | ND |
| 6 | 40 | M | Right frontal | Mixed | II | (+) | (+) | Loss | Not OE | (+) |
| 7 | 46 | M | Right frontal | Astrocytic | IV | (+) | (+) | Loss | OE | (+) |
| 8 | 60 | M | Left frontal | Astrocytic | IV | (+) | ND | Retained | OE | (+) |

F, female; M, male; IHC, immunohistochemical testing; Seq, sequencing testing; (–), negative result; (+), positive result; ND, not done; OE, overexpressed.

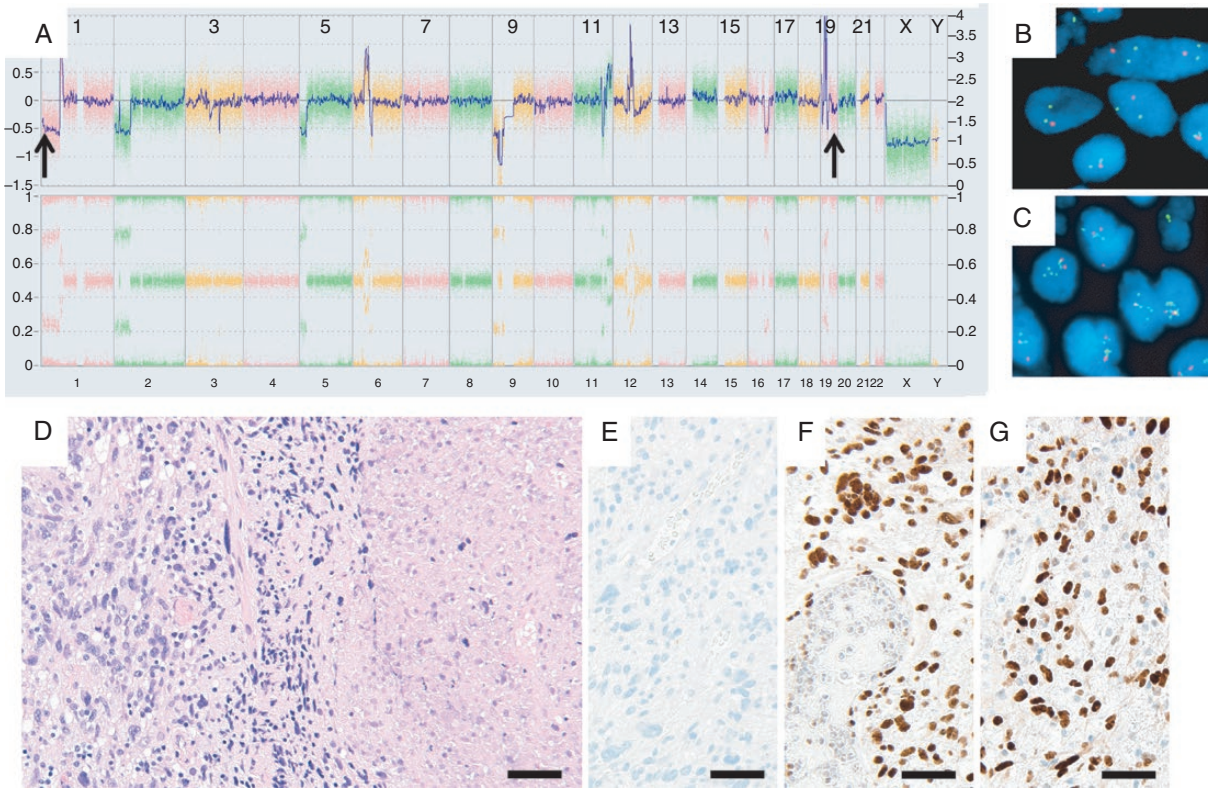


**Figure 1.** Case 1: False-positive IDH-wildtype glioblastoma. (A) Chromosomal microarray weighted log2 ratio and B-allele frequency traces show partial-arm deletion of 1p and 19q, which includes the areas spanning the FISH probes (indicated by arrows). (B and C) False-positive FISH results show a single red target signal and 2 green control signals for chromosome 1 and a relative loss of red to green signals for chromosome 19, indicating 1p deletion and relative 19q loss, respectively. (D) H&E showing astrocytic morphology with elongated nuclei and necrosis, consistent with a morphological diagnosis of glioblastoma. (E) IDH1-R132H immunostain is negative (sequencing for *IDH1/IDH2* was also negative), (F) ATRX immunostain shows retained protein expression, and (G) p53 immunostain is consistent with protein overexpression in tumor cells. Scale bars, 50 μm.

p53 overexpression by immunohistochemistry would require 1p/19q codeletion testing as it was not strictly a "diffuse astrocytic-appearing" tumor. Testing for 1p/19q codeletion in this case would have been positive if performed by FISH and potentially lead to a misdiagnosis of a low-grade oligodendroglioma as ATRX loss of expression has been reported in 2% of genetically defined oligodendroglioma[38] and could not have prompted follow-up studies to confirm the presence of a whole-arm 1p/19q codeletion. CMA, on the other hand, showed whole-arm deletion of 1p and partial-arm deletion of 19q in the context of an overall complex genomic copy number profile predominantly characterized by segmental and terminal chromosomal gains and losses, a pattern frequently observed in IDH-mutant astrocytomas (Figure 2). Case 5 was morphologically an anaplastic astrocytoma with immunohistochemical loss of ATRX expression and overexpression of p53 and therefore would not have required 1p/19q codeletion testing. The other 2 cases (cases 7 and 8) were morphologically glioblastoma with positive IDH1-R132H immunostain wherein additional genetic testing would not be formally indicated.[1] In case 8 (Supplementary Figure 2), however, the finding of retained ATRX expression by immunohistochemistry could have prompted 1p/19q codeletion testing despite the pure astrocytic morphology.[38] The finding of a positive FISH 1p/19q codeletion result in this case could have led to the misdiagnosis of anaplastic oligodendroglioma if the conflicting histological and molecular findings did not warrant follow-up testing.

## Discussion

Based on the 2016 WHO CNS tumor classification update, diffuse gliomas with an oligodendroglial morphology require demonstration of both an IDH mutation and 1p/19q codeletion in order to qualify as the "oligodendroglioma, IDH-mutant, 1p/19q codeleted" entity.[1] The testing platforms to detect these 2 hallmark diagnostic features for genetically defined oligodendroglioma are not mandated by the WHO, but it is recommended that 1p/19q codeletion testing be performed by assays able to detect whole-arm chromosomal losses.[1] FISH has been the traditionally used testing platform and is cost-effective when there is classic

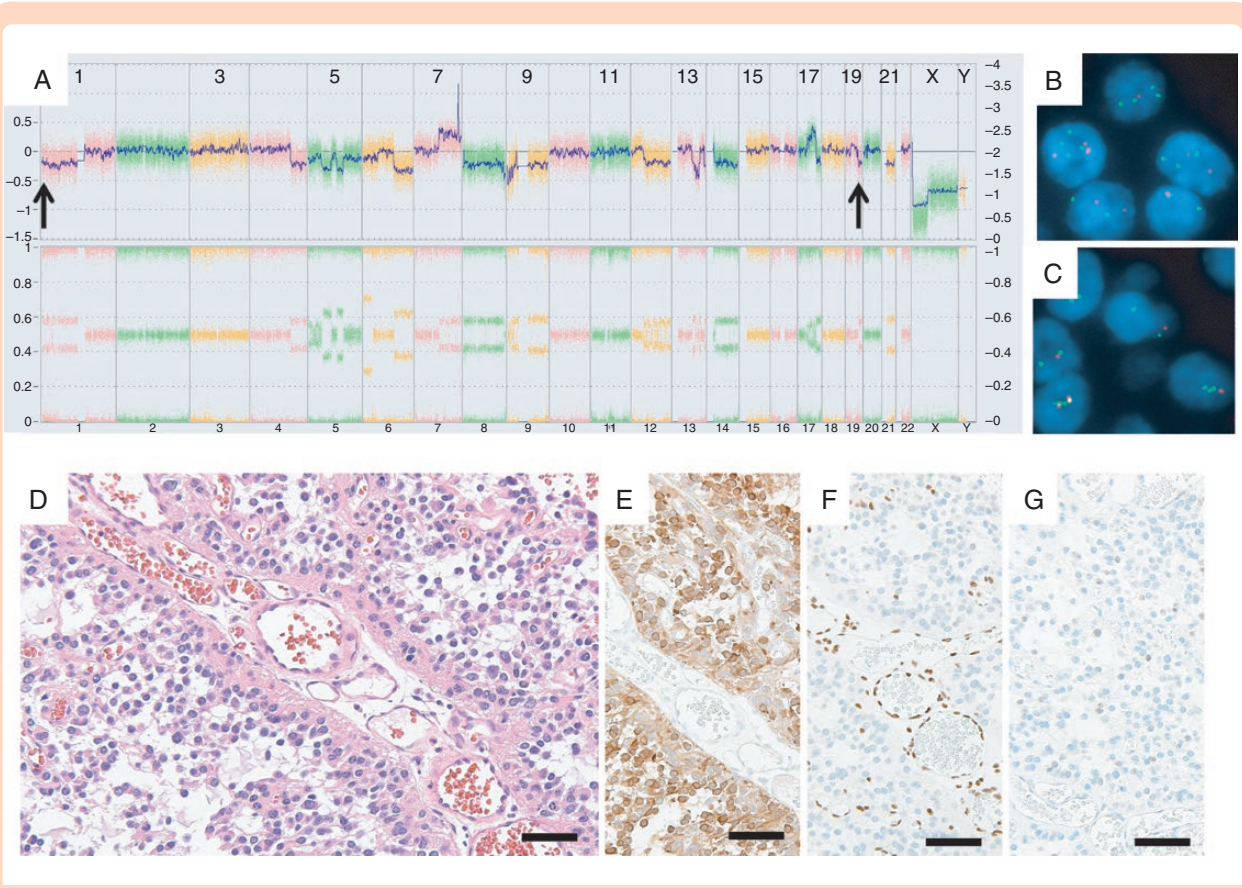


**Figure 2.** Case 6: False-positive IDH-mutant low-grade infiltrating glioma. (A) Chromosomal microarray weighted log2 ratio and B-allele frequency traces show whole-arm deletion of 1p and partial-arm deletion of 19q, which includes the areas spanning the FISH probes (indicated by arrows). (B and C) False-positive FISH results show a relative loss of red to green signals for chromosomes 1 and 19, indicating relative 1p/19q loss in the context of additional copies of chromosomes 1 and 19. (D) H&E showing tumor cells with round to oval nuclei, regular nuclear contours, and perinuclear halos, a morphology that was considered somewhat ambiguous and not definitive for a morphological diagnosis of astrocytoma or oligodendroglioma. (E) IDH1-R132H immunostain is positive, (F) ATRX immunostain shows loss of protein expression, and (G) p53 immunostain shows protein overexpression only in scattered tumor cells. Scale bars, 50 μm.

oligodendroglial morphology (which is highly associated with the presence of whole-arm 1p/19q codeletion[6,12,39,40]), limited available tissue/tumor percent, and/or desire for cytoarchitectural evaluation. For cases with lower pretest probability (ie, cases that may not represent an oligodendroglioma) such as tumors with pure or predominant astrocytic morphology and/or temporal lobe location,[41] however, testing platforms that evaluate the entire 1p and 19q chromosome arms are preferred due to the possibility of partial 1p/19q codeletion being called positive by FISH.

In this study, we reiterate that diffuse astrocytic gliomas show partial 1p and 19q losses that occasionally coexist leading to a false-positive 1p/19q codeletion result by FISH. Our overall estimate for the false-positive FISH 1p/19q codeletion rate in 223 diffuse astrocytic gliomas is 3.6%. A previous study by our group identified 8% (6/79) primary and recurrent diffuse astrocytic gliomas with a positive FISH 1p/19q testing result.[6] In a study using aCGH, 2% (9/266) of diffuse astrocytic gliomas were predicted to have a positive FISH result.[24] In another study comparing FISH to LOH in 491 histologically diagnosed glioblastomas, 4.3% (21 of 491) tumors had a positive result for FISH 1p/19q testing.[26] Lastly, a recent study reported a false-positive FISH 1p/19q codeletion rate in diffuse astrocytic gliomas of 3.1% (11/359; 10 IDH-wildtype and 1 IDH-mutant).[25] Our study differs from these prior studies in that we utilized final integrated histological-molecular diagnoses (and respective WHO grades) as recommended by the 2016 WHO classification update and cIMPACT-NOW.[1,32,33] This diagnostic approach dissolved the oligoastrocytoma diagnostic category, which was not accounted for in the aforementioned estimates, and significantly decreased the number of lower-grade IDH-wildtype diffuse astrocytic gliomas as most of these tumors were upgraded to a "diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV." The overall rates of 8% and 2% of false-positive FISH 1p/19q codeletion (vs 3.6% in the current study) may also be explained by the difference in sample size (79 and 266 vs 225 in the current study). In comparison with the third and fourth studies, which respectively evaluated only glioblastoma and all grades of diffuse astrocytic gliomas, our false-positive FISH 1p/19q codeletion rates are similar (4% vs 3.9% and 3.1% vs 3.6% in the current study, respectively), providing further evidence for an

estimated false-positive FISH 1p/19q codeletion rate of approximately 4% in glioblastoma and 3–3.5% in all grades of diffuse astrocytic gliomas regardless of the IDH status.

The overall false-positive FISH 1p/19q codeletion rate between IDH-mutant and IDH-wildtype tumors did not significantly differ (4.6% vs 2.9%; $P = .49$). As expected, IDH-mutant tumors had false positives in all grades whereas the IDH-wildtype cases were confined to glioblastoma, which is the predominant diagnostic category in this group of diffuse gliomas. Analysis of the clinicopathological features of the 8 cases predicted to have a false-positive FISH result revealed that in all IDH-wildtype cases, 1p/19q codeletion testing would not have been indicated whereas in one IDH-mutant case, 1p/19q codeletion testing would be formally recommended as part of the diagnostic workup based on current classification guidelines.[1,32,33,37] Importantly, a misdiagnosis could have happened in this single IDH-mutant case wherein 1p/19q testing was indicated if the apparent unusual pattern/discrepancy between histopathological findings and FISH 1p/19q codeletion testing result was unnoticed.

Although molecular findings trump morphological impression in many diagnostic conundrums, cautious interpretation for FISH 1p/19q testing results in adult diffuse gliomas is warranted. Follow-up testing using a platform able to distinguish partial from whole-arm 1p/19q codeletion is recommended in diagnostic scenarios wherein morphology and molecular findings appear unusual or discordant given the low but not insignificant rate of concomitant partial 1p/19q losses leading to a false-positive FISH result among diffuse astrocytic gliomas.

From a practice standpoint, our findings underscore the clinical value of the current classification recommendations[1,32,37] as 1p/19q codeletion testing was not formally indicated in most of our cases with partial 1p/19q codeletion predicted to have a false-positive FISH result. To decrease the risk of a false-positive FISH result and the need for follow-up testing to clarify discordant results in adult diffuse gliomas, we reinforce the recommendations for (1) IDH testing to generally precede 1p/19q codeletion testing and (2) 1p/19q codeletion testing be performed only after an IDH mutation has been identified in tumors that are not morphologically purely astrocytic with ATRX immunohistochemical loss of expression. In tumors with pure classic oligodendroglial morphology, which have high pretest probability for having a whole-arm 1p/19q codeletion, we believe that FISH is a cost-effective and reliable testing platform. Also, upfront IDH and 1p/19q codeletion testing is a reasonable approach when the tumor is immunohistochemically negative for IDH1-R132H (ie, likely harboring a less common *IDH1* mutation or an *IDH2* mutation) with retained ATRX expression and without significant p53 overexpression. In IDH-mutant tumors morphologically (eg, mixed oligoastrocytic) and/or immunohistochemically (eg, with ATRX loss of expression) not classic for an oligodendroglioma, and in cases with apparently discrepant FISH and histopathological findings, we strongly recommend 1p/19q codeletion testing using platforms able to discriminate partial and whole-arm 1p and 19q losses. Additional benefits of comprehensive platforms that evaluate genome-wide copy number changes such as CMA include assessment of the genome copy number pattern/variation and specific clinically relevant events (eg, *CDKN2A/B* status for IDH-mutant astrocytomas).[42,43] In cases with a limited amount of tumor tissue and/or low tumor percentage, however, testing using these comprehensive platforms may not be feasible and FISH remains an important alternative testing method. In these cases wherein evaluation of whole-arm 1p and 19q status is not possible, there are a couple of follow-up testing approaches if a conflicting diagnostic scenario occurs. One option would be FISH testing using probe sets outside the minimal deleted region that are more specific and strongly associated with whole-arm 1p/19q codeletion, with a rate of false positives of less than 1%.[41] Another option would be H3K27 trimethylation (H3K27me3) immunohistochemical testing using monoclonal C36B11 antibody. The presence of H3K27me3 retained expression in the context of a positive FISH 1p/19q codeletion result would be suggestive of a false-positive FISH 1p/19q codeletion result as genetically defined oligodendroglioma has been recently shown to be highly associated with loss of H3K27me3 whereas IDH-mutant astrocytomas typically retain H3K27me3 expression.[44–46]

Limitations of this study include the referral bias of our study group which was ascertained from our large neuropathology consultation and tertiary referral neurosurgery practices and may not represent the profile of adult diffuse gliomas encountered in smaller community practices. It would be important to determine the rate of false-positive FISH 1p/19q codeletion by a testing platform able to distinguish partial and whole-arm 1p/19q deletions at other institutions to better define the overall false-positive rate and understand the diagnostic scenarios wherein evaluation of 1p/19q status by FISH may be misleading. Additionally, although we verified most of our false-positive FISH cases, our estimated rate of false-positive FISH 1p/19q codeletion testing results was predicted from CMA data rather than directly extracted from actual FISH testing data.

In conclusion, routine 1p/19q codeletion testing by FISH in any adult diffuse glioma is discouraged as we estimate 3.6% of partial 1p/19q codeletion leading to a false-positive FISH 1p/19q codeletion result in diffuse astrocytic gliomas. Instead, selective 1p/19q codeletion testing following current classification recommendations and ideally using testing platforms that evaluate 1p and 19q whole-arm status in cases without classic oligodendroglioma features is recommended. It is hoped that our estimated false-positive FISH 1p/19q codeletion rate may be helpful in clinical discussions with physicians requesting upfront 1p/19q codeletion testing by FISH when 1p/19q codeletion testing is not indicated to avoid confusing diagnostic scenarios and prevent potential misdiagnoses.

## Supplementary Data

Supplementary data are available at *Neuro-Oncology Advances* online.

## Keywords

astrocytoma |chromosomal microarray | cIMPACT | oligodendroglioma | WHO

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