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Case Report

Recurrent oligodendroglioma with changed 1p/19q status

Valeria Barresi,[1] Andrea Mafficini,[1,2] Martina Calicchia,[2] Maria Liliana Piredda,[1] Angelo Musumeci,[3] Claudio Ghimenton[4] and Aldo Scarpa[1,2]

[1]Department of Diagnostics and Public Health, Section of Anatomic Pathology, University of Verona, [2]ARC-NET Research Centre, [4]Department of Pathology and Diagnostics, University and Hospital Trust of Verona and [3]Department of Neurosciences, Unit of Neurosurgery, Hospital Trust of Verona, Verona, Italy

We report a case of oligodendroglioma that had consistent histopathological features as well as a distinct change in 1p/19q status in the second recurrence, after temozolomide chemotherapy and radiotherapy. The first tumor recurrence had oligodendroglial morphology, *IDH1* R132H and *TERT* promoter mutations, and 1p/19q codeletion detected by fluorescent *in situ* hybridization (FISH). Copy number analysis, assessed by next-generation sequencing, confirmed 1p/19q codeletion, and disclosed loss of heterozygosity (LOH) of chromosomes 4 and 9 and chromosome 11 gain. The second recurrence featured not only oligodendroglial morphology but also the appearance of admixed multinucleated giant cells or neoplastic cells having oval nuclei and mitoses and showing microvascular proliferation; it maintained *IDH1* R132H and *TERT* promoter mutations, acquired *TP53* mutation, and showed 19q LOH, but disomic 1p, detected by FISH. Copy number analysis depicted LOH of chromosomes 3p, 13, and 19q, 1p partial deletion (1p chr1p34.2-p11), and gain of chromosomes 2p25.3-p24.1, 8q12.2-q24.3, and 11q13.3-q25. B-allele frequency analysis of polymorphic sites disclosed copy-neutral LOH at 1p36.33-p34.2, supporting the initial deletion of 1p, followed by reduplication of 1p36.33-p34.2 alone. These findings suggest that the two tumor recurrences might have originated from an initial neoplastic clone, featuring 1p/19q codeletion and *IDH1* and *TERT* promoter mutations, and have independently acquired other copy number alterations. The reduplication of chromosome 1p might be the result of temozolomide treatment, and gave rise to false negative 1p deletion detected by FISH. The possibility of 1p copy-neutral LOH should be considered in recurrent oligodendrogliomas with altered 1p/19q status detected by FISH.

Key words: H3K27me3, *IDH*, oligodendroglioma, tumor mutational burden, *TP53*.

INTRODUCTION

Diffuse gliomas are currently classified into two forms; one is the isocitrate dehydrogenase (IDH) gene (*IDH*) mutant, and the other is *IDH* wild-type.[1] *IDH* mutant gliomas with mutations in *IDH1* and *IDH2* include oligodendroglioma, which is defined by the presence of 1p/19q codeletion, and astrocytoma, which lacks 1p/19q codeletion and instead mostly shows mutations in the α-thalassemia mental retardation X-liked (ATRX) gene (*ATRX*) and the tumor protein p53 (*TP53*).[1] This classification has clinical relevance, as oligodendroglioma has a better prognosis and responds to chemotherapy with the regimen, including procarbazine, CCNU and vincristine (PVC).[2–4]

IDH mutation and 1p/19q codeletion are hypothesized to represent early genetic events in gliomagenesis as their status was reported to be unchanged in paired samples of primary and recurrent tumors, across independent studies.[5–7] However, the existence of dual-genotype gliomas, consisting of the admixture of tumor cells with astrocytic and oligodendroglial genotypes and the same *IDH* mutation,[1,8,9] contrasts with this hypothesis, and rather suggests that 1p/19q codeletion and the mutations in *ATRX* and *TP53* occur as subclonal events in *IDH* mutant glioma cells.

Herein, we report a rare case of diffuse glioma with distinct lineage conversion from oligodendrocytic cells to astrocytic cells in the recurrence. This case could help understanding the molecular pathogenesis of *IDH* mutant diffuse gliomas and the genetic modifications induced by postsurgical treatments.

Correspondence: Valeria Barresi, MD, PhD, Department of Pathology and Public Health, Policlinico G.B. Rossi, P.le L.A. Scuro, 10, 37134, Verona, Italy. Email: valeria.barresi@univr.it

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CLINICAL SUMMARY

A 34-year-old man underwent total resection of a grade II oligodendroglioma in the right frontal lobe, without adjuvant treatments.

Seventeen years later, he referred to a hospital because of generalized tonic–clonic seizures. Computed tomography (CT) and magnetic resonance imaging (MRI) disclosed a mass with subtle contrast enhancement at the site of a previous surgery **(**Supplementary Figure S1**)**. The recurrent tumor was completely resected. According to the World Health Organization (WHO) and the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) criteria,[1,10] the diagnosis was anaplastic oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted.

The patient received radiotherapy with temozolomide chemotherapy, and was followed-up on MRI every six months. Four years later, MRI disclosed a recurrent tumor with inhomogeneous contrast enhancement (Supplementary Figure S1). For this, the patient received six additional cycles of temozolomide chemotherapy. Due to a further increase in the lesion volume, he was re-operated on seven months later. The diagnosis was considered to be astrocytoma, *IDH*-mutant, grade 4, based on the results of fluorescent *in situ* hybridization (FISH);[11] however, next-generation sequencing (NGS) led the integrated diagnosis of anaplastic oligodendroglioma, *IDH*-mutant, 1p/19q-codeleted.

The patient underwent PVC therapy and is alive after a 12-month follow-up.

PATHOLOGICAL FINDINGS

Formalin-fixed, paraffin-embedded sections of each tumor were deparaffinized, rehdrated, and subjected to hematoxylin and eosin (HE) staining and immunohistochemical staining. The specimen obtained at the initial surgery was unavailable for histological revision and further analyses. The specimen obtained at the second surgery had morphological features consistent with anaplastic oligodendroglioma, being a diffuse glioma composed of cells with round, slightly atypical, nuclei and clear perinuclear halo **(**Fig. 1**)**, showing a brisk proliferative activity at five mitoses per 10 high-power fields (HPFs) and microvascular proliferation. On immunohistochemistry, the tumor cells were positively stained with monoclonal antibodies against glial fibrillary acidic protein (GFAP) (clone 6F2; Dako, Glostrup, Denmark; 1:250), oligodendrocyte transcription factor 2 (Olig2) (clone EPR2673; Abcam, Cambridge, UK; 1:100), and IDH1 R132H (clone H09; Dianova, New York, NY, USA; 1:40) (Fig. 1). They were uniformly positive with a mouse monoclonal anti-ATRX antibody (clone AX1; Dianova; 1:100), scatteredly positive with a mouse monoclonal anti-p53 antibody (clone D07; Monosan, Uden, The Netherlands; 1:100), and negative with a mouse monoclonal anti-histone 3 trimethylated lysine at codon 27 (H3K27me3) antibody (clone C36B11; Cell Signaling Technology, Danvers, MA, USA; 1:200) (Fig. 1). Nuclear immunohistochemical staining with mouse monoclonal antibodies against MutL homolog 1 (MLH1) (clone ES05; Dako; 1:30), MutS homoloh 2 (MSH2) (clone FE11; Dako; 1:30), MutS homolog 6 (MSH6) (clone EP49; 1:100; Dako; 1:100), and PMS1 homolog 2 (PMS2) (clone EP51; Dako; 1:100) was retained **(**Supplementary Table S1**)**.

The specimen obtained at the third surgery had areas with morphological features consistent with anaplastic oligodendroglioma composed of neoplastic cells having round nuclei and many mitotic figures, shoowing necrosis and microvascular proliferation, and admixed with pleomorphic or multinucleated giant cells (Fig. 2). On immunohistochemistry, tumor cells were positive for GFAP, Olig2, and IDH1 R132H, showed uniformly retained ATRX immunoreactivity, and were negative for H3K27me3 (Fig. 3) and p53, (Fig. 2). The tumor cells were negative for MLH1 and PMS2, and positive for MSH2 and MSH6 (Supplementary Figure S2).

GENETIC FINDINGS

The specimens of the first and second recurrent tumors were assessed for the following points: 1p/19q codeletion using FISH; microsatellite instability (MSI) using a fluorescent multiplex polymerase chain reaction (PCR) exploiting the five mononucleotide microsatellites BAT25, BAT26, NR21, NR22, and NR24;[12] the alterations of 174 cancer-related genes including mutations; copy number alterations, structural rearrangements, and tumor mutational burden (TMB) using NGS (details in Supplementary File S1).[13]

FISH for detecting 1p/19q codeletion was carried out by LSI 1p36/19q13 Dual-Color Probe Sets assay (Vysis/Abbott; Molecular Europe, Wiesbaden, Germany), according to the manufacturer's protocol. Slides on FISH were examined with a Olympus BX61 fluorescence microscope equipped with a 100× oil immersion objective and a triple band-pass filter for simultaneous detection of Spectrum Orange, Spectrum Green, and 4′,6-diamidino-2-phenylindole (DAPI) signals. Two-hundred non-overlapping nuclei containing a minimum of two reference probe signals were counted.

The first tumor recurrence was proved to have 1p/19q codeletion, based on the presence of > 70% tumor cells with two reference probe signals (1q and 19p) and one target (1p and 19q) probe signal (Fig. 1). The second tumor recurrence had disomic 1p (two target and two probe signals in > 80% neoplastic cells) and loss of heterozygosity

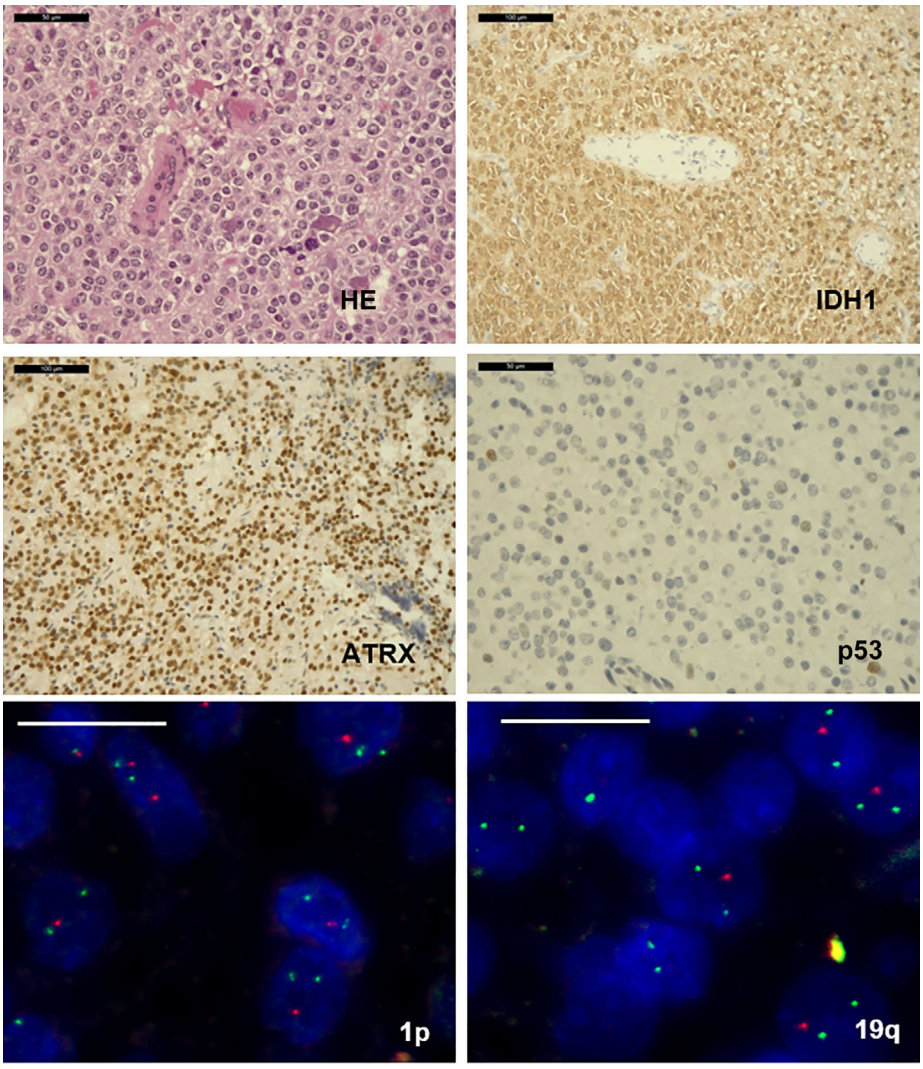

Fig 1 Findings of the first recurrent tumor on histology (HE), immunohistochemistry (IDH1, ATRX, and p53), and FISH (1p and 19q). The neoplastic cells have round, slightly atypical, nuclei and perinuclear clear halo and show brisk mitotic activity and microvascular proliferation. These histological features are consistent with anaplastic oligodendroglioma. The tumor cells are immunoreactive for IDH1 R132H, show retained TRX immunoreactivity, and are scatteredly immunoreactive for p53. FISH reveals 1p/19q codeletion. While green signals indicate 1q and 19, red signals indicate 1p and 19q. Scale bars: 100 μm (IDH1, ATRX), 50 μm (HE, p53, 1p, 19q).

(LOH) of 19q (> 80% tumor cells with two probes and one target signals (Fig. 2).

On PCR analysis, both the tumor recurrences were microsatellite-stable. The NGS findings are resumed in Supplementary Table S1.

The tumor obtained at the second surgery was microsatellite-stable (0.5 microsatellite insertion-deletions/Mbase), had a TMB of six mutations/Microbase, *IDH1* R132H mutation, the telomerase reverse transcriptase gene (*TERT*) promoter mutation (c. 124 C>T), and the phosphatidylinositol 3-kinase regulatory subunit α gene (*PIK3R1*) mutation. NGS confirmed 1p/19q codeletion, and revealed LOH of chromosomes 4 and 9 and chromosome 11 gain (Fig. 4). No deletions of the cyclin-dependent kinase inhibitor 2A/2B genes (*CDKN2A/B*) were found.

The tumor obtained at the third surgery was microsatellite-unstable (7 microsatellite insertion-deletions/Mbase), had a TMB of 88 mutations/Microbase, *IDH1* R132H mutation, *TERT* promoter mutation (c. 124 C>T), but not *PIK3R1* mutation. In addition, it harbored several additional mutations, including mutations in *TP53*, the retinoblastoma gene 1 (*RB1*), *MLH1*, and *CDKN1B* mutation coupled with the deletion of the other allele (Supplementary Table S1). No *CDKN2A/B* deletions were found.

Each tumor had the pattern of burden of mutations that are different based on pathogenic agents. These mutational signatures were computed with an MuSiCa software.[14] The two major contributing signatures were "alkylating agents" (signature 11, which exhibits a strand bias for C>T substitutions; contribution 83.3%) and "defective DNA MMR" (signatures 6 and 15, which are associated with high numbers of small insertions and deletions at mono/polynucleotide repeats; overall contribution 10.5%), indicating that the tumor's mutational landscape was mainly driven by temozolomide chemotherapy, and that MSI gave only a minor contribution. Copy number

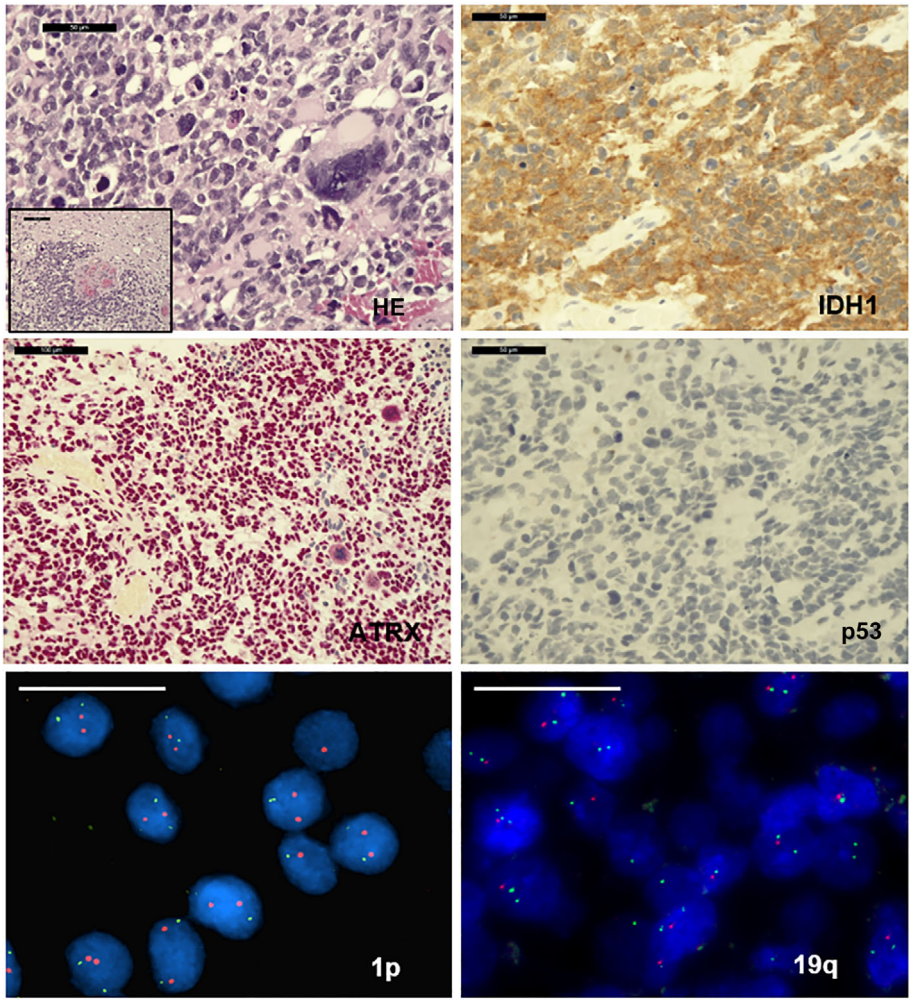

Fig 2 Findings of the second recurrent tumor on histology (HE), immunohistochemistry (IDH1, ATRX, and p53), and FISH (1p and 19q). The neoplastic cells have round or oval, markedly atypical nuclei show brisk mitotic activity and microvascular proliferation, and are intermingled with nultinucleated giant cells. These observations pose a differential diagnosis between astrocytoma and anaplastic oligodendroglioma. The tumor cells are immunoreactive for IDH1 R132H, show retained TRX immunoreactivity, and are negative for p53. FISH reveals disomic 1p and 19q loss of heterozygosity. While green signals indicate 1q and 19, red signals indicate 1p and 19q. Scale bars: 100 μm (insert, ATRX, p53), 50 μm (HE, IDH1, 1p, 19q).

analysis depicted partial 1p deletion, LOH of chromosomes 3p, 13 and 19q, and gain of chromosomes 2p25.3-p24.1, 8q12.2-q24.3, and 11q13.3-q25 (Fig. 3). To further clarify chromosome 1p status, we also performed B-allele frequency analysis of polymorphic sites available in the targeted regions of the panel and found that the second recurrence harbored copy-neutral LOH at 1p36.33-p34.2. This supported the initial deletion of 1p, followed by re-duplication of 1p36.33-p34.2 alone.

DISCUSSION

According to WHO, oligodendroglioma is defined by the co-occurrence of *IDH1/2* mutation and codeletion of whole chromosomal arms 1p and 19q,[1] which is mediated by a balanced whole-arm translocation of chromosomes 1 and 19 followed by the loss of one of the two derivative chromosomes composed of 1p and 19q.[15]

In the present study, we report a rare case of oligodendroglioma, which had distinct molecular conversion to astrocytic lineage in the second recurrence. The first recurrence still had oligodendroglial morphology and genotype, characterized by *IDH1* mutation, coupled with 1p/19q codeletion and *TERT* promoter mutation. The second recurrence had ambiguous morphology, and featured oligodendroglioma-like areas admixed pleomorphic and giant cells; it harbored the same *IDH1* and *TERT* promoter mutations as the first recurrence, and acquired *TP53* mutation. FISH revealed 19q, but not 1p, deletion, which was confirmed by copy number analysis that depicted only partial 1p deletion, consistent with an astrocytic genotype.[16] However, the partial 1p deletion resulted from re-duplication of 1p36.33-p34.2, which indeed displayed copy-neutral LOH.

We point to the possibility that both recurrences originated from a neoplasia featuring *IDH1* and *TERT* promoter mutations and 1p/19q codeletion and evolved independently. The minor component that later gave rise to the second recurrence could have been selected and further mutated by temozolomide treatment, acquiring *TP53* and *RB1* mutations.[17,18] The origin of the astrocytic recurrence from a minor, undetectable, clone is also

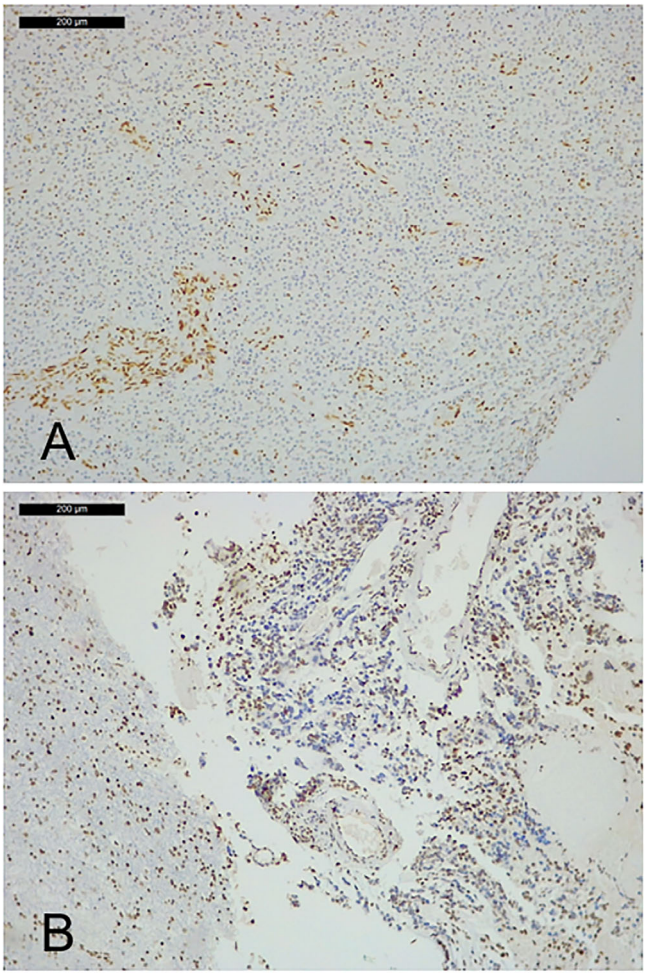

Fig 3 Immunohistochemical findings for H3K27me3 in the first (A) and second (B) recurrent tumors. In both the tumors, nuclear immunoreactivity is lost in tumor cells and in contrast retained in non-neoplastic glial cells and endothelial cells. Scale bars: 200 µm (A, B).

supported by the presence of chromosomal alterations, characterized by gains of chromosomes 2 and 8 and losses of chromosomes 3 and 13, lack of chromosome 11 gain, and losses of chromosomes 4 and 9. These findings were quite different from those seen in the preceding tumor. The NGS mutational signature, showing a major contribution of temozolomide, supports the hypothesis that the genetic alterations found in the second tumor recurrence were a consequence of the adjuvant therapy. As previously reported gliomas,[19] the prolonged treatment with temozolomide may have also produced an increase in TMB and the pathogenic *MLH1* mutation in the second tumor recurrence. The late and limited contribution of microsatellite instability (MSI) to the overall mutation burden may be responsible for negativity at MSI PCR in this case. Therefore, immunohistochemistry for mismatch repair (MMR) proteins may not be sufficient to assess the degree of MSI in brain tumors, and care should be taken whereas interpreting its clinical significance in these cases.[19–21]

The change in 1p/19q status in recurrent oligodendrogliomas was previously reported in four cases.[7,22,23] In all these, the change occurred after treatment with radiation, PVC or temozolomide chemotherapy.[7,22,23] In two cases, the astrocytic conversion, represented by a loss of 1p/19q codeletion in the recurrence, might be due to chemotherapy-related selection of a minor astrocytic component of a dual genotype glioma.[23] Indeed, the specimen preceding the conversion showed rare tumor cells lacking ATRX immunoreactivity, consistent with an astrocytic clone.[23] In line with this hypothesis, the recurrent tumors featured the loss of *TERT* promoter mutation and the acquisition of *ATRX* and *TP53* mutations.[23]

Similarly to what we found in the present case, the change in 1p/19q status was only distinct in the remaining two recurrent oligodendrogliomas.[7,22] In one of these, the recurrence had morphological features consistent with a gliosarcoma; however, the loss of 1p/19q codeletion found on FISH was due to 1p/19q copy-neutral LOH, as demonstrated using an single nucleotide pleomorphism (SNP) array.[22] In the other case, the recurrent oligodendroglioma maintained *TERT* promoter mutation, acquired *TP53* mutation, and featured 19q, but not 1p, LOH on FISH.[7] Although 1p copy-neutral LOH could not be demonstrated, it was classified as an oligodendroglioma using a methylation analysis.[7]

These two latter cases and the present one demonstrate that relapsed oligodendrogliomas can develop 1p/19q copy-neutral LOH, as a probable effect of post-surgical treatments. This genetic alteration cannot be detected by FISH, which reveals false 1p/19q disomy in these cases. As shown by Ono *et al.*, methylome analysis can be more effective in predicting tumor genotype.[7] In a recent study, we demonstrated that *IDH* mutant diffuse gliomas with retained ATRX and lost H3K27me3 expression are 1p/19q-codeleted oligodendrogliomas with a probability of 100%.[24] In agreement, the second recurrence of the present case featured retention of ATRX and loss of H3K27me3 on immunohistochemistry.

In conclusion, the present case shows that recurrent oligodendrogliomas can develop 1p/19q copy-neutral LOH, not being identifiable on FISH, can be interpreted as a change in 1p/19q codeletion status. The limited number of reported oligodendrogliomas with this genetic alteration does not allow any conclusions to be drawn on its clinical significance. In these cases, methylome analysis or immunohistochemical evaluation for H3K27me3 may predict 1p/19q status with a higher accuracy than FISH analysis.

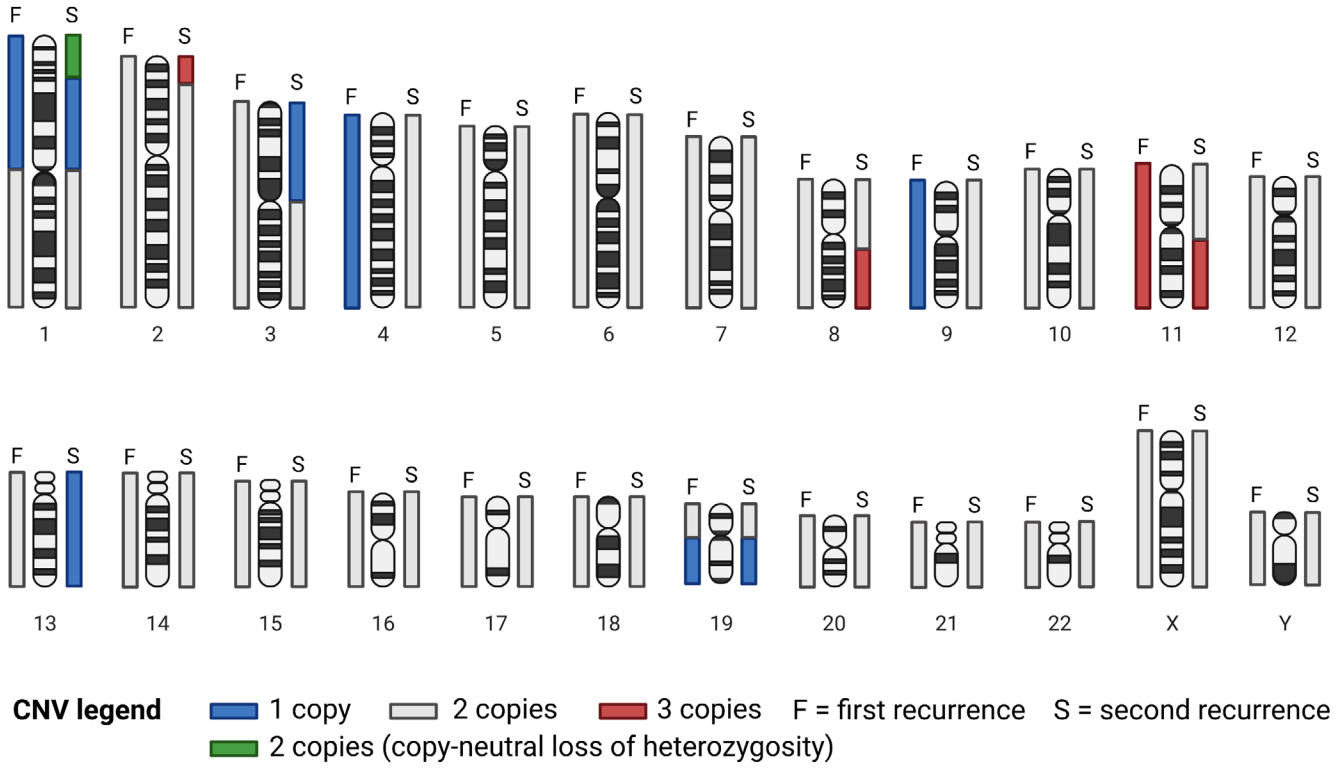

Fig 4 Chromosomal alterations detected by copy number variation (CNV) analysis in two oligodendroglioma recurrences. Chromosomal alterations are inferred from NGS. Targeted regions include coding exons of 174 genes and a whole-chromosome backbone at the resolution of 1 megabase.

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DISCLOSURE

Authors declare no conflict of interest for this article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website:

Fig. S1 Radiological imaging of the first (A) and second (B) recurrences. A. Computerized tomography showing a right fronto-temporal mass. B. MRI showing a recurrent tumor in the surgical cavity.

Fig. S2 Immunohistochemical expression of mismatch repair proteins in the second tumor recurrence. The tumor cells lose MLH1 and PMS2 immuno-expression, and retain MSH2 and MSH6 (scale bar: 100 μm).

File S1 Methods of genetic assay, list of genes included and types of alterations reported

Table S1 Morphological, immunohistochemical, genetic and chromosomal alterations in the first and second tumor recurrence