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Mitotic index, microvascular proliferation, and necrosis define 3 groups of 1p/19q codeleted anaplastic oligodendrogliomas associated with different genomic alterations

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Background. The aim of this study was to correlate histological features and molecular characteristics in anaplastic oligodendrogliomas (AOs).

Methods. The histological characteristics of 203 AO patients, enrolled in the French national network POLA, were analyzed. The genomic profiles of 191 cases were studied using genomic arrays. *IDH* mutational status was assessed by immunohistochemistry and direct sequencing.

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Results. 1p/19q codeletion was present in 79% of cases and was associated with alpha-internexin expression ($P < 10^{-4}$), *IDH1/2* mutation ($P < 10^{-4}$), chromosome 4 loss ($P < 10^{-3}$), and better overall survival ($P < 10^{-4}$). Based on mitotic index, microvascular proliferation (MVP), and necrosis, 3 groups of 1p/19q codeleted AOs were identified: (group 1) AO with more than 5 mitoses per 10-HPF, no MVP, and no necrosis; (group 2) AO with MVP and no necrosis; and (group 3) AO with MVP and necrosis. Compared with group 1, groups 2 and 3 AOs had a higher mean Ki-67 proliferation index and a higher rate of 9p and 9q losses. Compared with group 2, group 3 AOs had a higher number of chromosomal alterations including chromosome 4 loss. In the subgroup of 157 1p/19q codeleted AOs, chromosomal instability was associated with shorter progression-free survival ($P = .024$) and shorter overall survival ($P = .023$).

Conclusions. The present study shows that oligodendrogliomas with classic histological features remain a molecularly heterogeneous entity and should be stratified according to 1p/19q status because of its major prognostic relevance. Moreover, 1p/19q codeleted AOs are also heterogeneous. Interestingly, mitotic index, MVP, and necrosis help to classify them into 3 groups associated with distinct genomic alterations.

Keywords: anaplastic oligodendrogliomas, 1p/19q codeletion, mitoses, microvascular proliferation, necrosis.

Anaplastic oligodendrogliomas (AOs) are diffuse, infiltrative gliomas that occur mainly in adults and are classified as grade III according to the World Health Organization (WHO) classification.[1] Pathological diagnosis is made first on the assessment of the oligodendroglial nature of the tumor and then on the identification of histological features of anaplasia. Features typically considered classic for oligodendroglioma (CFO) include cellular monomorphism, round regular nuclei giving a honeycomb-like appearance, and chicken wire vasculature that is often associated with microcalcifications.[2] The definition of anaplasia is based on increased tumor cellularity, nuclear pleomorphism, increased mitotic activity and/or microvascular proliferation (MVP), and necrosis.[1] In the WHO system, a specific mitotic cutoff for anaplasia is not provided, although it has been suggested that at least 5 mitoses per 10 high power fields (HPF), even if local, predict a reduced survival time.[3] Necrosis can be recorded in AO, but its prognostic significance in these tumors remains controversial.[2,4] In contrast, anaplastic oligoastrocytomas containing necrosis should be classified as grade IV according to the WHO 2007 classification. However, histological classification of high-grade gliomas with an oligodendroglial component is often challenging in routine practice because most anaplastic gliomas might contain areas of rounded regular cells that mimic oligodendroglioma. Depending on how the pathologist classifies the tumor, pure oligodendroglioma or oligoastrocytoma, occurrence of necrosis changes the grade and subsequently the postoperative treatment. A distinctive feature of oligodendroglioma is combined deletion of chromosomal arms 1p and 19q as the result of an unbalanced t(1;19)(q10;p10) translocation.[5,6] However, the percentage of oligodendrogliomas exhibiting 1p/19q codeletion varies from one study to another depending on the application of "strict" or "flexible" criteria for diagnosing oligodendroglioma. For example, in RTOG trial 9402 (dedicated to anaplastic oligodendroglial tumors), histopathology reviewed by 5 neuropathologists showed 1p/19q codeletion in 78 of 94 (80%) tumors exhibiting CFO, whereas only 13 of 99 (13%) tumors with without classic features for oligodendroglioma were associated with 1p/19q codeletion.[2] Another relevant association is that all or almost all 1p/19q codeleted oligodendrogliomas harbor a mutation in *IDH1* or *IDH2* genes,[7] 2 master genes involved in adult gliomagenesis.[8]

The POLA network, a dedicated program for standardizing management of de novo adult AO, was established in France in 2008. One of the aims of the program is to provide centralized pathological review of the cases and centralized molecular analysis together with biobanking and recommendations for treatment.

Only cases showing pathological features of CFO were included during the first 4 years of the project (September 2008 to October 2012), providing a unique opportunity for the group to develop specific pathological criteria for AO and to correlate them with 1p/19q codeletion, *IDH* mutation and epidermal growth factor receptor (EGFR) status (expression and amplification), and other chromosomal abnormalities. Patient survival needs to be analyzed cautiously because of a short median follow-up, but we have already found that some promising pathological features and molecular data are associated with shorter overall survival (OS) and/or shorter progression-free survival (PFS) in the whole group and, to a lesser extent, in the subgroup of 1p/19q codeleted patients.

Material and Methods

Material

Two-hundred three patients with centrally reviewed confirmation of newly diagnosed AO were prospectively included in the present study. For all cases, formalin-fixed paraffin-embedded (FFPE) tumor tissue was available for pathological and immunohistochemical investigations. Frozen material was also available in up to 80% of cases.

Patients included prospectively in the POLA network have provided their written consent for clinical data collection and genetic analysis according to national and POLA network policies.

According to POLA network guidelines drawn up in 2008, it was strongly recommended that patients with 1p/19q codeletion receive radiotherapy only or be included in the CODEL EORTC trial, whereas those exhibiting non-1p/19q codeleted AO were treated by concurrent and adjuvant temozolomide radiochemotherapy[9] or were included in the CATNON EORTC trial (NCT00626990). However, the treatment recommendation for 1p/19q codeleted tumors was changed to radiotherapy followed by 6 cycles of adjuvant PCV (procarbazine, CCNU, and

vincristine) chemotherapy regimen after the long-term results of the RTOG and EORTC trials in AO were published in 2013.[10,11] Clinical characteristics of the cohort are summarized in Table 1. Preoperative Karnofsky Performance Status (KPS) was known in 133 of the 203 participants, and the extent of surgical removal was assessed by the local neurosurgeon and recorded in 166 of the 203 participants. Since the postoperative contrast-enhanced imaging was available for a minority of patients, 2 groups were established: biopsy ($n = 25$) versus surgery ($n = 141$). Regarding postsurgery treatment, 77 participants (74/77 1p/19q codeleted) received radiotherapy only, 14 participants (11/14 1p/19q codeleted) received chemotherapy only, 17 participants (14/17 1p/19q codeleted) were treated with adjuvant PCV radiochemotherapy, and 50 participants (26/50 1p/19q codeleted) were treated with adjuvant temozolomide radiochemotherapy. Treatment data were not available for 45 participants. Median follow-up was 24.3 months (range, 0–55.5 months). During this follow-up period, 73 participants relapsed, and 35 died.

Pathological Review

After initial diagnosis of AO by the local pathologist, cases were reviewed centrally by Prof. D. Figarella-Branger (or Dr. K Mokhtari for cases managed in the city of Marseille); they were included in the prospective POLA network if they met the pathological inclusion criteria of AO according to the WHO classification of brain tumors.[1] In addition, all cases were reviewed again by a panel of 4 pathologists (K. Mokhtari, E. Uro Coste, A. Jouvet, and D. Figarella-Branger). The pathologists were specifically looking for the presence or (or absence) of mitoses, marked atypia, areas of high cell density, microvascular proliferation, palisading necrosis, nonpalisading necrosis, branched vessels, honeycomb-like appearance, and calcification. The mitotic index (number of mitotic figures per 10 HPF) was also evaluated in all cases.

Immunohistochemistry

Automated immunohistochemtry (IHC) was performed on 4 μm thick FFPE sections with avidin-biotin peroxidase complex on Benchmark XT (Ventana Medical Systems) with the Ventana kit including diaminobenzidine reagent. The cases were screened for glial fibrillary acidic protein (polyclonal; 1:4000; Dako), Olig2 (clone AF2418 + biotine anti-goat;1:800; R&D Systems), Ki67 (clone Mib1; 1:100; Dako), internexin alpha (INA; clone 2E3; 1:50; Novus Biologicals), EGFR (clone EGFR.25; 1:100; Novocastra), p53 (clone DO.7; 1:200; Dako), and the monoclonal antibody IDH1 R132H (clone H09; 1:75; Dianova). Ki67 and p53 expression were scored as percentages by counting the immunostained nuclei of 400 cells in the most positive area. INA expression was scored as positive (if ≥10% of cells were positive with at least one cluster of positive cells) or negative.[12] EGFR expression was assessed by light microscopy using a x10 objective and scored as previously described[13] to generate a score for each case. Briefly, the percentage of positive tumor cells (0%–100%) was multiplied by the intensity of staining (from zero being negative to 4 being intense). Cases with a score of <200 were considered negative, whereas cases with a score of ≥200 were classified as positive.

DNA Extraction

Following the manufacturer's recommendations, tumor DNA was extracted from frozen tissue (if available) or FFPE samples using the iPrep ChargeSwitch Forensic Kit. Qualification and quantification of tumor DNA was conducted using a NanoVue spectrophotometer and gel electrophoresis, respectively.

Single Nucleotide Polymorphism Array and Agilent Comparative Genomic Hybridization Array Procedures

When DNA extracted from frozen tissue was available ($n = 168$), the genomic profile was determined using single nucleotide polymorphism (SNP) arrays. 1.5 μg of DNA was outsourced to Integragen Company for the SNP array experiments. Two types of platforms were used: HumanCNV370-Quad and Human610-Quad from Illumina.[14] When only DNA extracted from FFPE tissue was available ($n = 23$), the genomic profile was assessed using oligonucleotide-based comparative genomic hybridization (CGH) arrays (Agilent). Oligonucleotide-based CGH arrays were performed as previously described.[15] When the quantity of DNA was insufficient to perform SNP or CGH arrays ($n = 7$), microsatellite analysis was conducted, and loss of heterozygosity of chromosomes 1p and 19q was assessed using PCR techniques as described elsewhere.[16] Because the molecular abnormalities were included in the medical management of the participants (ie, non-1p/19q codeleted participants were included in the European Organization for Research and Treatment of Cancer [EORTC] 26053-22054 trial [NCT00626990] if they were eligible), the tumor DNA was run prospectively to obtain its genomic profile within 10 days after surgery.

1p/19q Codeletion

1p/19q codeletion status was determined in 198 out of 203 tumors based on SNP arrays ($n = 168$), CGH arrays ($n = 23$), or microsatellite marker analysis ($n = 7$). Tumors were considered as codeleted if they exhibited whole chromosome arm 1p deletion, whole chromosome arm 19q, chromosome 1 centromeric breakpoint, and chromosome 19 centromeric breakpoint. This pattern is highly suggestive of t(1;19)(q10;p10) translocation. Therefore, if these 4 criteria were not met concurrently, the tumor was classified as non-codeleted regardless of 1p and 19q status otherwise.

IDH1 and IDH2 Mutation Status

When IDH1R132H IHC was negative or unreliable, *IDH1* and *IDH2* mutational status was addressed by direct sequencing using the Sanger method and primers as previously described.[17]

Statistical Analysis

The SNP and CGH array analysis was performed as previously described.[14] For all arrays, genomic imbalances were classified as loss, gain, homozygous deletion, or amplification. The association of chromosomal arm imbalances with histological variables was estimated using either the Fisher' exact test (for factors) or Student' *t* test (for quantitative variables).

For other correlation analyses, the chi-square test (or Fischer' exact test) was used to compare variables when they scored as

Table 1. Comparison of 1p/19q codeleted and non-1p/19q codeleted anaplastic oligodendrogliomas

	All patients*	Non-1p/19q codeleted patients	1p/19q codeleted patients	*P* values
N	203	41	157	
Age at diagnosis (mean)	50.6	51.2	50.1	NS
Sex				NS
Male (%)	114 (56%)	23 (56%)	86 (55%)	
Female (%)	89 (44%)	18 (44%)	71 (45%)	
Sex ratio M:F	1:3	1:3	1:2	
Preoperative KPS				NS
≥80% (%)	111 (83%)	29 (85%)	82 (83%)	
<80% (%)	22 (17%)	5 (15%)	17 (17%)	
Extent of surgery				$P = .05$
Biopsy (%)	25 (15%)	9 (24%)	14 (11%)	
Surgery (%)	141 (85%)	29 (76%)	111 (89%)	
Postoperative treatment				$P < .0001$
Radiotherapy(%)	77 (49%)	3 (10%)	74 (59%)	
Chemotherapy (%)	14 (9%)	3 (10%)	11 (9%)	
Radiotherapy + PCV (%)	17 (11%)	2 (7%)	14 (11%)	
Temoradiation + temozolomide (%)	50 (31%)	22 (73%)	26 (21%)	
MVP (%)	169 (83%)	36 (88%)	129 (82%)	NS
Necrosis (%)	63 (31%)	18 (44%)	44 (28%)	$P = .06$
Palisading (%)	34 (17%)	13 (32%)	21 (13%)	$P = .01$
Nonpalisading (%)	50 (25%)	13 (32%)	36 (23%)	NS
Calcifications (%)	87 (43%)	14 (34%)	71 (45%)	NS
Number of mitoses (mean)	12	15.6	10.6	NS
KI67 expression (mean)	21	24	21	NS
INA positive expression (%)	152 (75%)	9 (22.5%)	139 (88.5%)	$P < .0001$
TP53 positive expression (%)	32 (16%)	12 (29%)	18 (12%)	$P = .01$
EGFR positive expression (%)	41 (20%)	8 (19.5%)	30 (19%)	NS
IDH R132H positive expression (%)	153 (75%)	12 (29%)	138 (88%)	$P < .0001$
IDH1/2 mutation status (%) *(Expression and DNA sequencing)*	173 (85%)	18 (44%)	152 (97%)	$P < .0001$
Amplifications (%)	16 (8%)	16 (41%)	0	$P < .0001$
EGFR (%)	5 (2.5%)	5 (13%)		$P = .0002$
PDGFRA (%)	4 (2%)	4 (10%)		$P = .001$
CDKN2A homozygous deletion (%)	11 (5.5%)	10 (24%)	1	$P < .0001$
Chr 1p partial loss (%)	11 (6%)	11 (29%)	0	NR
Chr 4 loss (%)	48 (25%)	1 (3%)	47 (31%)	$P = .0002$
Chr 7 gain (%)	33 (17%)	17 (45%)	16 (10%)	$P < .0001$
Chr 9p loss (%)	53 (28%)	12 (31%)	41 (27%)	NS
Chr 9q loss (%)	23 (12%)	0	23 (15%)	$P = .01$
Chr 10 loss (%)	23 (12%)	17 (44%)	6 (4%)	$P < .0001$
Chr 11q gain (%)	24 (12.5%)	0	24 (16%)	$P = .01$
Chr 17p loss	4 (4%)	6 (16%)	1	$P = .001$
Chr 19q loss (%)**	166 (82%)	9 (24%)	100%	NR
Mean number of chr arm alteration	5.1	7.1	4.7	$P = .003$

*1p/19q codeletion status was unavailable in 5 of the 203 patients for technical reasons.
**15% of non-1p/19q codeleted anaplastic oligodendrogliomas displayed both partial 1p loss and 19q loss.
Abbreviations: chr, chromosome; F, female; KPS, Karnofsky performance status; M, male; MVP, microvascular proliferation; NR, not relevant; NS, not significant; PCV, procarbazine, lomustine, and vincristine.

positive or negative. Continuous variables were compared using the Mann-Whitney U test. In order to identify pathological and/or genomic factors related to OS or PFS, survival curves were obtained according to the Kaplan-Meier method and compared using the log-rank test. The following variables were searched for prognostic significance in the whole group of 203 cases: age at diagnosis (cutoff = 50 years), sex, extent of surgical resection (biopsy and partial resection vs total and subtotal resection),

preoperative KPS (cutoff = 80%), microvascular proliferation, necrosis, number of mitoses (cutoff = 8, median), pathological groups, Ki67 labelling index (cutoff = 20%), p53 expression (cutoff = 10%), INA expression (cutoff = 10%), *IDH* mutation, 1p/19q codeletion, chromosome 4 loss, chromosome 9p or 9q loss, chromosome 11q gain, and the number of chromosomal arm alterations (cutoff = 4, median). In a second step, the same analysis was done in the subgroup of cases exhibiting 1p/19q codeletion. Age at diagnosis, sex, extent of surgical removal, postoperative treatment, and pathological subgroup were used to build the multivariate Cox proportional hazard backward models.

All statistical tests were 2-sided, and the threshold for statistical significance was $P = .05$. Analyses were conducted using PASW Statistics version 17.02 (IBM SPSS Inc).

Results

Pathological Review

Two-hundred three cases fulfilled the diagnosis of AO. Mitoses, nuclear atypias, areas of high cell density, and branched vessels associated with honeycomb appearance were recorded in all cases. Calcifications were observed in 87 cases (43%), MVP in 169 cases (83%), and necrosis in 63 cases (31%, including 34 of the 63 with palisading necrosis). (Table 1, Fig. 1)

Necrosis (palisading or not) was always associated with MVP. In the 34 cases lacking MVP, the mitotic index was always higher than 5 of 10 HPF, as is often recorded in nodules exhibiting higher cell density. According to the presence or absence of MVP and necrosis, 3 pathological groups were recorded. Group 1 (34 cases, 17%) was characterized by no MVP and no necrosis, group 2 by

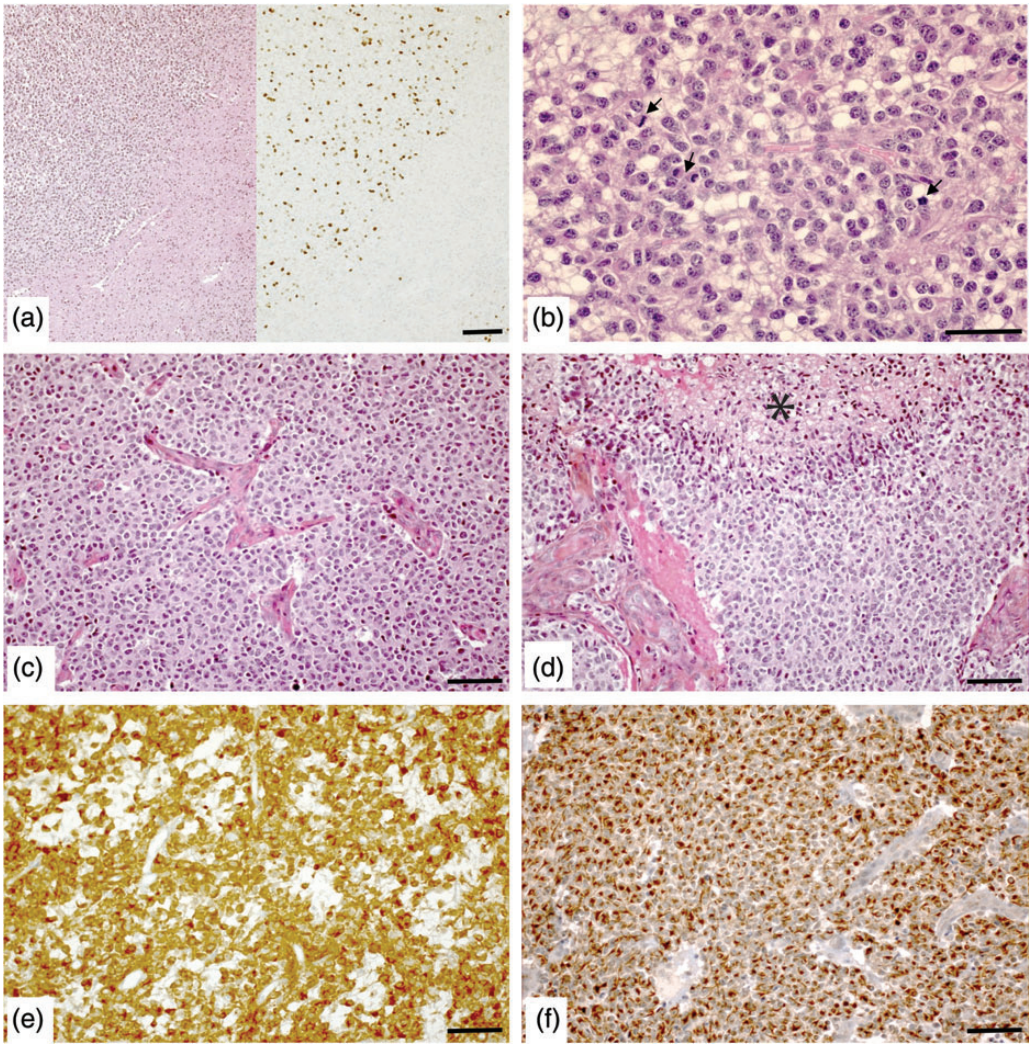

Fig. 1. Pathological features of the 3 subgroups of anaplastic oligodendrogliomas. (a–c) Anaplastic oligodendroglioma with high mitotic count but no microvascular proliferation (MVP) and no palisading necrosis (PN). (a) Nodule of high cell density (hematoxylin-eosin [HE], left) with a KI 67 labeling index of 12% (immunostaining, right). (b) Higher magnification showing 3 mitotic figures (arrows) within one HPF (HE). (c) Anaplastic oligodendroglioma characterized by MVP (HE). (d) Anaplastic oligodendroglioma characterized by MVP and PN (*, HE). (e and f) Characteristic pattern of IDH1R132H (e) and internexin alpha (f) immunostaining in the same case recorded in panel a and b. Scale bar = 50 μm.

MVP and no necrosis (106 cases, 52%), and group 3 by MVP and necrosis (63 cases, 31%).

Immunohistochemistry

Olig2 expression was recorded in all cases. GFAP expression was restricted in most cases to reactive astrocytes, and, when present, it stained minigemistocytes and gliofibrillar oligodendrocytes. Ki67 expression was at least 10% (median, 20%). INA expression was classified as positive in 152 of 202 cases (75%; it was not assessable in one case for technical reasons). P53 expression was scored as positive in 32 cases (16%). EGFR expression was classified as positive in 41 cases (20%). IDH1R132H was positive in 153 cases (75%). (Table 1)

Molecular Data

Genomic array data were available for 191 cases according to SNP array ($n = 168$) and CGH array ($n = 23$) results. In 7 additional cases, the 1p/19q status was determined using microsatellite analysis. A 1p/19q codeletion was present in 157 of 198 cases (79%) in which this alteration could be determined. According to immunohistochemical results and sequencing data, *IDH1/2* was mutated in 173 cases (85%). Beside the 1p/19q codeletion, the most frequent chromosomal alterations in the whole series were chromosome 4 loss (48/191 cases, 25%), chromosome 9p loss (53/191 cases, 28%), chromosome 7 gain (33/191 cases, 17%) chromosome 11q gain (24/191 cases, 12.5%), chromosome 9q loss and chromosome 10 loss (both 23/191 cases, 12%). *EGFR* amplification and *CDKN2A* homozygous deletion were only observed in 5 of 191 cases (2.5%) and 11 of 191 cases (5.5%), respectively. (Table 1)

Comparison of 1p/19q Codeleted and Non-1p/19q Codeleted Anaplastic Oligodendrogliomas

No significant difference was recorded between 1p/19q codeleted and non-codeleted AOs regarding age at diagnosis, sex ratio, and preoperative KPS. However, there were significant differences between the 2 subgroups ($P = .05$ and $P < 10^{-4}$, respectively) with regard to therapeutic care, extent of surgery, and postoperative treatment. Although the mean number of mitoses was higher in non-p/19q codeleted tumors, this did not reach statistical significance. The only histological characteristic that was significantly different between the 2 groups of AO was palisading necrosis, which was more frequent in non-codeleted tumors than in codeleted tumors (32% vs 13%; $P = .01$). Concerning immunohistochemical characteristics, IDH1R132H and INA were more frequently expressed in codeleted than in non-codeleted AOs (88% vs 29%; $P < 10^{-4}$ and 88.5% vs 22.5%, $P < 10^{-4}$), while p53 positivity was more frequent in non-codeleted AOs (29% vs 12%; $P = .01$). Beside the 1p/19q codeletion, the 2 groups of tumors exhibited different molecular characteristics. Codeleted tumors were characterized by a higher rate of *IDH* mutation (97% vs 44%; $P < 10^{-4}$), chromosome 4 loss (31% vs 3%; $P < .001$), chromosome 9q loss (15% vs 0%; $P = .01$), and chromosome 11q gain (16% vs 0%; $P = .01$), while non-codeleted tumors exhibited a higher rate of chromosome 7 gain (45% vs 10%; $P < 10^{-4}$), chromosome 10 loss (44% vs 4%; $P < 10^{-4}$), *CDKN2A* homozygous deletion (24% vs 0.6%; $P < 10^{-4}$), and chromosome 17p loss (16% vs 0%; $P = .01$). High-level gene amplifications (including *EGFR* and *PDGFRA* amplifications) were exclusively observed in non-codeleted tumors (41% vs 0%; $P < 10^{-4}$). Finally, non-codeleted AOs had a higher mean number of chromosomal arm alterations (7.1 vs 4.7; $P = .003$). (Table 1)

Association Between Pathological Features and Genomic Characteristics in 1p/19q Codeleted Anaplastic Oligodendrogliomas

Within the group of 157 1p/19q codeleted AOs characterized using genomic arrays, 28 cases were recorded in pathological group 1 (no MVP and no necrosis, 18%), 85 in group 2 (MVP only, 54%), and 44 in group 3 (MVP and necrosis, 28%). (Table 2) Compared with group 2 and group 3, group 1 AOs had a lower mean Ki67 proliferation index ($P = .002$) and a lower rate of chromosome 9p and 9q losses (7% vs 30%; $P = .008$ and 0% vs 20%; $P = .008$, respectively). Compared with group 2, group 3 AOs had a higher rate of chromosome 4 loss (45% vs

Table 2. Microvascular proliferation and necrosis define 3 groups of 1p/19q codeleted anaplastic oligodendrogliomas associated with distinct molecular alterations

	Group 1 *No MVP no necrosis*	Group 2 *MVP no necrosis*	Group 3 *MVP and necrosis*	Group 1 vs group 2 + 3	Group 3 vs group 1 + 2	Group 3 vs group 2
n (%)	28 (18%)	85 (54%)	44 (28%)			
Ki67 ≥20% (%)	6 (21%)	42 (49%)	31 (72%)	$P = .0007$	$P = .001$	NS
Ki67 expression (mean)	17	21	24	$P = .002$	$P = .06$	NS
Chr 4 loss (%)	7 (25%)	22 (26%)	18 (45%)	NS	$P = .01$	$P = .04$
Chr 9p loss (%)	2 (7%)	28 (33%)	11 (28%)	$P = .008$	NS	NS
Chr 9q loss	0	19 (22%)	7 (12.5%)	$P = .008$	NS	NS
Chr 11q gain	6 (21%)	5 (6%)	13 (32.5%)	NS	$P = .002$	$P = .0001$
Number of chr arm alteration (mean)	4	4.4	5.7	NS	$P = .01$	$P = .02$

Abbreviations: chr., chromosome; MVP, microvascular proliferation; NS, not significant.

26%, $P = .04$) and a higher mean number of chromosomal arm alterations (5.7 vs 4.4; $P = .02$). Chromosome 11q gain was more frequent in group 3 than in group 2 AO (32.5% vs 6%; $P = .0001$); however, no significant difference was observed when compared with group 1 only.

Correlation Between Clinical, Pathological Features, Molecular Data, and Follow-up

Despite the short follow-up, univariate analysis performed n the whole group of participants showed that among the clinical variables analyzed, preoperative KPS ≥80 was predictive of a longer PFS (36.5 months; 95% CI, 32.7–40.3 months vs 24 months; 95% CI, 17.6–30.3 months; $P = .015$). (Table 3) Moreover, postoperative treatment was predictive of OS ($P < 10^{-4}$) and PFS ($P = .001$), the longest OS or PFS being observed for participants being treated with radiotherapy and PCV (50.8 months for OS and 33.6 months; 95% CI, 29.2–38 months for PFS) or radiotherapy only (47.8 months; 95% CI, 45.2–450.4 months for OS and 39.4 months; 95% CI, 35.4–43.4 months for PFS) versus participants treated with chemotherapy only (35.8 months; 95% CI, 25.8–45.9 months for OS, and 23.8 months; 95% CI, 16.1–31.4 months for PFS), or participants receiving temoradiation and adjuvant temozolomide protocol (35.4 months; 95% CI, 29.4–41.3 months for OS and 26.3 months; 95% CI, 20.3–32.3 months for PFS). Among the pathological variables analyzed, pathological groups were predictive of PFS ($P = .045$), the longest PFS being observed in group 1 participants (41.6 months; 95% CI, 34.7–48.5 months vs 36.4 months; 95% CI, 32.5–40.3 months for group 2 and 30.4 months; 95% CI, 24.7–36 months for group3). Among the immunohistochemical variables tested, INA expression was predictive of a longer PFS (38.5 months; 95% CI, 35.1–41.9 months vs 27.7 months; 95% CI, 21.7–33.8 months; $P = .001$) and OS (49.9 months; 95% CI, 47.3–52.4 months vs 37.4 months; 95% CI, 31.2–43.5 months; $P < 10^{-4}$) (Fig. 2a). Regarding the molecular variables analyzed, occurrence of *IDH* mutation was correlated with a longer PFS (37.6 months; 95% CI, 34.4–40.8 months vs 25.3 months' 95% CI, 17.5–33.2 months; $P = .001$) and OS (48.7 months; 95% CI, 46.1–51.3 months vs 35.1 months; 95% CI, 26.7–43.5 months; $P < 10^{-4}$) (Fig. 2b), and 1p/19q codeletion was also predictive of longer PFS (39.3 months; 95% CI, 35.9–42.6 months vs 23.6 months; 95% CI, 18–29.2 months; $P < 10^{-4}$) and OS (50.5 months; 95% CI, 48.1–52.8 months vs 34.4 months; 95% CI, 27.9–40.8 months; $P < 10^{-4}$) (Fig. 2c). In addition, the number of chromosomal arm alterations >4 was associated with a shorter PFS (31.5 months; 95% CI, 26.6–36.4 months vs 39.8 months; 95% CI, 36.2–43.3 months; $P = .006$) and OS (40.7 months; 95% CI, 36–45.4 months vs 51.4 months; 95% CI, 48.9–53.9 months; $P < 10^{-4}$) (Fig. 3a).

On multivariate analysis, 1p/19q codeletion was predictive of longer PFS ($P = .03$; HR, 0.345; 95% CI, 0.173–0.690) and OS ($P = .010$; HR, 0.307; 95% CI, 0.125–0.754), and participants belonging to pathological group 3 had a tendency to display a shorter PFS ($P = .077$; HR, 1.461; 95% CI, 0.960–2.225).

In the group of 157 1p/19q codeleted participants, postoperative treatment was predictive of OS ($P = .01$). Surprisingly, the worst OS was observed for participants treated according to the protocol with temoradiation and adjuvant temozolomide (36.4 months; 95% CI, 28.6–44.1 months); very few events were recorded in the other groups. Chromosome 9q loss was predictive of a shorter PFS (32.7 months; 95% CI, 26.5–39 months vs 41.1 months; 95% CI, 37.7–44.5 months; $P = .048$), and the number of chromosomal arm alterations >4 was predictive of both shorter PFS (34.1 months; 95% CI, 28–40.2 months vs 41.9 months; 95% CI, 38.3–45.5 months; $P = .024$) and shorter OS (46 months; 95% CI, 40.6–51.2 months vs 52.4 months; 95% CI, 50–54.7 months, $P = .023$) (Fig. 3b).

Table 3. Clinical, pathological, immunohistochemical, and molecular markers predictive of prognosis in the 2 groups of anaplastic oligodendrogliomas according to 1p/19q status

	All patients ($n = 203$)		1p/19q codeleted patients ($n = 157$)	
	OS	PFS	OS	PFS
Age at diagnosis	0.558	0.202	0.391	0.087
Sex	0.370	0.977	0.557	0.789
Preoperative KPS	0.178	0.015	0.825	0.101
Extent of surgery	0.780	0.170	0.617	0.535
Postoperative treatment	<0.0001	0.001	0.01	0.23
MVP	0.207	0.103	0.070	0.172
Necrosis	0.054	0.023	0.213	0.174
Number of mitoses	0.095	0.126	0.097	0.165
Pathological subgroups	0.121	0.045	0.144	0.231
KI67 expression	0.020	0.465	0.224	0.928
P53 expression	0.125	0.022	0.559	0.286
INA expression	<0.0001	0.001	/	/
IDH mutation status	<0.0001	0.001	/	/
1p/19q codeletion status	<0.0001	<0.0001	/	/
Chr 4 loss	0.134	0.633	0.606	0.560
Chr 9p loss	0.352	0.496	0.929	0.286
Chr 9q loss	0.199	0.369	0.783	0.048
Chr 11q gain	0.851	0.990	0.201	0.418
Number of chr arm alteration	<0.0001	0.006	0.023	0.024

Abbreviations: chr, chromosome; KPS, Karnofsky performance status; MVP, microvascular proliferation;.OS, overall survival; PFS, progression-free survival

Discussion

In this study, we have reported the histomolecular features of a series of 203 AOs with classic CFOs. The number of codeleted 1p/19q tumors was 79%, which is within the same range (80%–90%) as other series that have focused on CFO tumors.[2,18,19] However, it remains that 10%–20% of CFO tumors do not exhibit 1p/19q codeletion. Importantly, 1p/19q status was unknown at the time of pathological diagnosis, including centralized review.

1p/19q codeleted CFO AOs were also characterized by higher frequency of *IDH* mutation, INA expression, chromosome 4 loss, and chromosome 11q gain, as previously reported.[7,12,14] Moreover, 1p/19q codeletion and *IDH* mutations were major prognostic markers and were associated with better OS and PFS in the

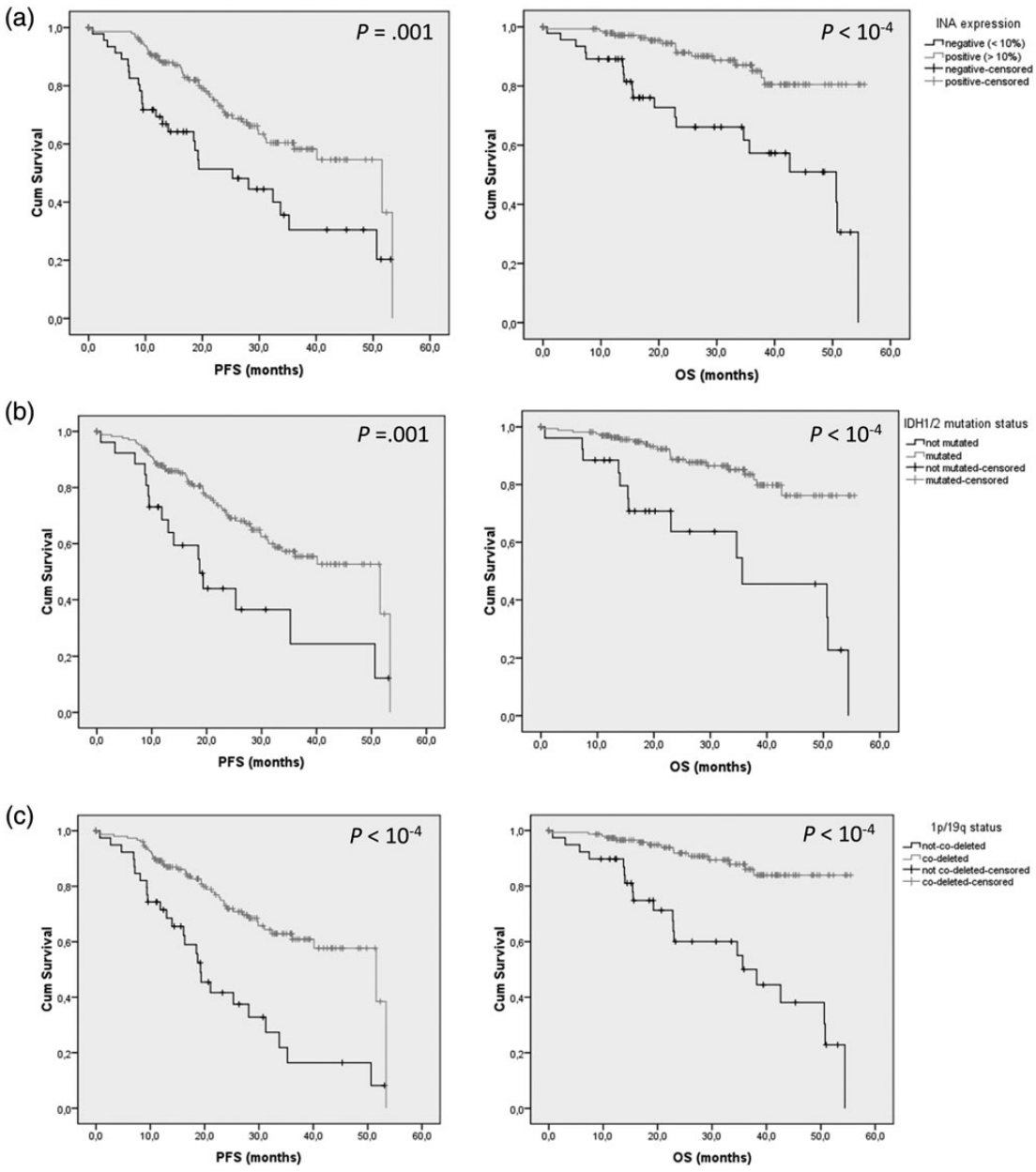

Fig. 2. (a) INA expression, (b) IDH mutation, and (c) 1p/19q status (c) are associated with longer progression-free survival and overall survival in anaplastic oligodendrogliomas.

whole cohort of patients, in keeping with previous studies.[2,7,18,19] Interestingly, INA expression was a relevant surrogate marker of 1p/19q codeletion and, in accordance, was also strongly predictive of better OS and PFS.[12] In contrast, CFO AOs lacking 1p/19q codeletion displayed molecular alterations that are frequently observed in primary glioblastomas, namely *EGFR* amplification, *CDKN2A* homozygous deletion, and chromosome 10 and chromosome 17p losses.[20] Therefore, in spite of careful pathological review, it is possible that some CFO AOs lacking 1p/19q codeletion were indeed true glioblastomas. It has been previously shown that small cell glioblastomas can mimic the histology of AOs.[21] Interestingly, glioblastomas usually lack IDH1 R132H mutation but, because of the recent discovery of this genetic alteration, *IDH* status is not included in the 2007 WHO classification. Besides, 1p/19q codeleted AOs may exhibit rare *IDH* mutations that are not recognized by the anti-IDH1R132H antibody; therefore, negative IDH1R132H staining does not exclude this diagnosis. Some MRI features are useful for preoperative diagnosis of 1p/19q codeleted AOs. These tumors usually demonstrate a preferential frontal lobe location, intratumoral signal heterogeneity, and a low rate of ring-like contrast enhancement.[22,23] Nevertheless, none of these MRI characteristics are specific, and up to 20% of 1p/19q codeleted AOs display a radiological aspect suggestive of a glioblastoma.

Taken together, diagnosis of AO remains difficult. Even in the group of CFO AOs, it is of utmost importance to distinguish 1p/19q codeleted tumors from the non-codeleted group because of their

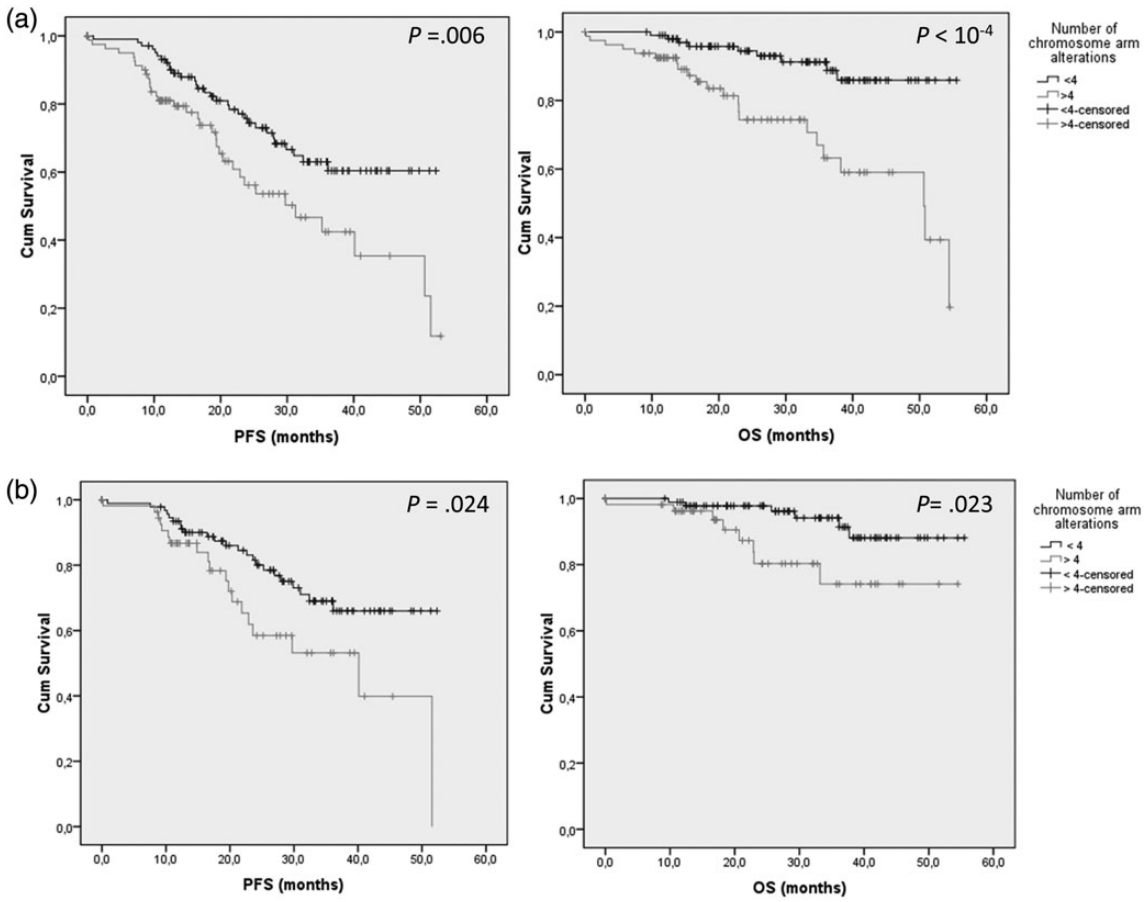

Fig. 3. Number of chromosomal arm alterations (>4) predicts shorter progression-free survival and overall survival in the whole group of anaplastic oligodendrogliomas (a) and in 1p/19q codeleted anaplastic oligodendrogliomas (b).

therapeutic implications and strong prognostic relevance.[10,11] Moreover, we observed in our series that temoradiation and adjuvant temozolomide protocol was associated with a shorter OS in codeleted AOs.

In this study, we also observed that 1p/19q codeleted AOs are heterogeneous with regard to some pathological features and additional genetic alterations. Moreover, we have shown that some genetic alterations are associated with peculiar pathological features. According to the WHO 2007 classification, diagnosis of anaplasia in CFO tumors relies on increased mitotic activity and/or MVP. Necrosis is not required. Some authors have further refined the criteria for an oligodendroglioma to be anaplastic: it has to contain at least 2 anaplastic features, one being frequent mitoses (> 5 mitoses per 10 HPF) or MVP.[2] An important observation is that necrosis (palisadic or not) arises only when MVP is present. Therefore, mitotic count, MVP, and necrosis can stratify AOs into 3 groups: group 1with > 5 mitoses per 10 HPF but no MVP and no necrosis; group 2 with MVP but no necrosis; and group 3 with MVP and necrosis. This pathological stratification was not predictive of prognosis (OS) probably because of limited follow-up. However, this pathological stratification was associated with distinct genetic alterations. MVP was associated with chromosome 9 alterations (either 9p or 9q loss). This result is consistent with previous studies showing that chromosome 9p loss and *CDKN2A* loss (located on 9q) are implicated in tumor progression[24] and development of MVP in 1p/19q codeleted oligodengrogliomas.[25] 9p loss has also been shown to be associated with larger tumor volume and with contrast enhancement in 1p/19q codeleted AOs.[26] We also observed that necrosis was associated with a higher number of chromosomal alterations including chromosome 4 loss, suggesting that chromosomal instability might contribute to the appearance of necrosis.

Whether the 3 pathological subgroups that we have identified represent 3 sequential steps of increased malignancy in 1p/19q codeleted oligodendrogliomas remains to be determined, but some findings are in keeping with that hypothesis: necrosis only occurs if MVP is present, Ki67 labeling index increases from group 1 to group 2, and the mean number of chromosomal arm alterations increases from group 2 to group 3.

In conclusion, the present study shows that oligodendrogliomas with classic histological features remain a molecularly heterogeneous entity. CFOs should be stratified according to 1p/19q status because of the major prognostic relevance of this genetic alteration and therapeutic implications. Moreover, 1p/19q codeleted AOs are also heterogeneous, and mitotic index, MVP, and necrosis help to classify them into 3 groups that are associated with distinct genomic alterations.

It is worth noting that some molecular alterations such as *ATRX* mutations, *FUBP1* mutations, *CIC* mutations, and *MGMT*

promoter methylation were not investigated in the present study. In addition, the short clinical follow-up of the cohort and the limited number of participants in each therapeutic group did not allow drawing definitive and robust conclusions in terms of prognostic analysis. Therefore, despite the limitations of the present study, which focus on histomolecular correlations, we are confident that further additional molecular studies, longer follow-up, and a larger number of participants will serve to refine our results.

POLA Network

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Conflict of interest statement. None declared.

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