Prevalence, clinico-pathological value, and cooccurrence of PDGFRA abnormalities in diffuse gliomas

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PDGFRA is a critical gene in glioma biology. Similar to EGFR, PDGFRA has been shown to be overexpressed, amplified, mutated, or truncated in gliomas, particularly glioblastomas. In addition, PDGFRA has been recently shown to be rearranged in glioblastoma. However, the frequency, cooccurrence, and clinical value of PDGFRA abnormalities in diffuse gliomas remain unclear. We investigated PDGFRA abnormalities and their clinical impact on 619 primary diffuse gliomas, including 167 grade II, 168 grade III, and 284 grade IV gliomas, with use of BAC-aCGH and validated our findings by quantitative polymerase chain reaction (PCR).

We studied PDGFRA expression using reverse-transcription quantitative PCR in 84 gliomas and 12 non-tumor samples. In 138 samples, we also screened PDGFRA point mutations in exons 5, 7, 8, 9, 10, 11, and 23; presence of KDR-PDGFRA fusion gene; and PDGFRA truncation. PDGFRA was amplified and gained in 5.2% and 1.9% of samples, respectively. In addition PDGFRA was point-mutated, rearranged, and truncated in 2.9%, 0%, and 0.7% of cases, respectively. PDGFRA point mutations were observed exclusively in grade IV gliomas and in 12.5% of PDGFRA-amplified tumors. High-level PDGFRA amplification was associated with PDGFRA overexpression, high malignancy grade, and older patient age. Of interest, high-level PDGFRA amplification has an independent negative prognostic value for progression-free survival and overall survival among patients with grade III tumors. PDGFRA is altered through various genetic mechanisms in a subset of high-grade gliomas in patients who might be ideal candidates for PDGFRA inhibitor treatment, and PDGFRA gene amplification could be used as a prognostic biomarker in anaplastic gliomas.

Keywords: amplification, glioma, mutation, PDGFRA, prognosis.

Gliomas are the most common primary brain tumors in adults. The WHO classifies diffuse gliomas based on the proliferating cell type (ie, astrocytoma, oligodendroglioma, or oligoastrocytoma) and the grade of malignancy (ie, from II to IV).

Although significant progress has been made in the treatment of patients with glioma, this disease remains incurable. Over the past several years, important advancements in the understanding of molecular glioma-genesis have been accomplished, leading to new therapeutic perspectives. Indeed, multiple growth factor receptors with tyrosine kinase activity have been shown to participate in glioma tumorigenesis and are currently targetable by innovative drugs (eg, EGFR, PDGFRA, or VEGFR).

Platelet-derived growth factor receptor A (PDGFRA) is the second most frequently mutated tyrosine kinase receptor, following EGFR, in glioblastomas (GBMs). PDGFRA is a transmembrane receptor with 5 immunoglobulin-like repeats in its extracellular domain and a tyrosine kinase (TK) in its intracellular domain. The binding of a ligand to the receptor activates pivotal downstream signal transduction pathways that...
promote oncogenesis, including MAP kinase, PI3K/AKT, JAK/STAT, and PLC-PKC. PDGFRA plays an important role in the normal development of the CNS by regulating normal glial cell proliferation and oligodendrocyte differentiation. PDGFRA has also been implicated in several cancers, including CNS malignancies. Indeed, several PDGFRA abnormalities have been detected in gliomas (e.g., amplification, overexpression, in-frame deletion, point mutation, and rearrangement). This led us to conduct the present study to assess PDGFRA status in diffuse gliomas, because these abnormalities are currently candidate targets for innovative molecular therapies in personalized medicine.

Materials and Methods

Patients

The following inclusion criteria were used for patients and tumors in the present study: age ≥18 years at pathological diagnosis, histological diagnosis of diffuse glioma, primary tumor with no history of brain tumor, detailed clinical information at diagnosis and during follow-up, availability of paired blood and tumor samples, consent form for molecular analysis provided by the patient, and available PDGFRA gene copy number status determined via BAC-array based comparative genomic hybridization (BAC-aCGH).

On the basis of the aforementioned inclusion criteria, 619 patients were enrolled in the present study: (1) 167 WHO grade II gliomas (88 oligodendrogliomas, 59 oligoastrocytomas, and 20 astrocytomas); (2) 168 WHO grade III gliomas (27 astrocytomas, 70 oligodendrogliomas, and 71 oligoastrocytomas); and (3) 284 WHO
grade IV gliomas (200 classic GBMs and 84 GBMs with an oligodendroglial component [GBMO]).

**DNA Extraction and BAC-aCGH**

DNA extraction was performed using the DNeasy Mini kit (Qiagen) according to the manufacturer’s recommendations. DNA concentration and quality were determined spectrophotometrically (NanoDrop).

BAC-aCGH experiments were conducted as previously described. Fluorescence levels (cyanine-5–labeled tumor DNA/cyanine-3–labeled control DNA) were measured and processed using GLAD pipeline to determine the genomic status of each BAC (amplification, gain, balanced, hemizygous deletion, or homozygous deletion). Extreme cyanine 5/cyanine 3 ratios of ≥ 3 and ≤ 0.6 were considered to be suggestive of amplifications and homozygous deletions, respectively (Fig. 1B).

**Validation of PDGFRA Copy Number Status Using Quantitative Polymerase Chain Reaction (qPCR)**

PDGFRA-amplified tumors detected on BAC-aCGH were further confirmed using real-time qPCR analysis. The PDGFRA primers amplified a genomic fragment overlapping *PDGFRA* intron 15 and exon 15 (TaqMan PDGFRA Copy Number Assays [FAM Dye], Assay ID Hs02749151_cn; Applied Biosystems). The reference primers amplified a genomic fragment from RNase P (TaqMan RNase P Detection Reagents [HEX Dye], no. 4316831; Applied Biosystems).

**RNA Extraction and PDGFRA Expression Using Reverse Transcription (RT) and qPCR**

Total RNAs were isolated using the RNasy Lipid Tissue Mini kit (Qiagen) according to the manufacturer’s recommendations. The RNA concentration was determined using spectrophotometer (NanoDrop). RNA was reverse transcribed using the SuperScript system (Invitrogen) according to the manufacturer’s protocol. RNA quality was assessed using Agilent RNA Nano 6000 LabChip kits and an Agilent 2100 Bioanalyzer (minimum RNA integrity number of 7).

RT-qPCR was performed using a Universal Probe Library (Roche Applied Science). The primers are reported in Table 1. cDNA was synthesized from 1 μg of total RNA and used for qPCR. Thermal cycling protocol was 10 min at 95°C, denaturation for 10 s at 95°C, annealing for 30 s at 60°C, and extension for 1 s at 72°C for 45 cycles, followed by 10 s at 40°C of cooling using the LightCycler 480 real-time PCR system. Data were expressed as ΔΔCt values (ΔΔCt = Ct of the target gene (PDGFRA) – Ct of the control gene (PPIA)]. The 2^ΔΔCt equation was used to determine the fold expression changes relative to nontumor tissues.

**PDGFRA mutations**

PDGFRA-amplified tumors were screened for point mutations in exons 5, 7, 8, 9, 10, 11, and 23, as described previously. The primers are reported in Table 2.

**Screening for PDGFRA Δ8,9 Mutants and for KDR-PDGFRA Fusions (KP Gene)**

RNA amplified from PDGFRA was reverse transcribed to search for truncated genes that lacked exons 8 and 9.

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**Table 1.** Primers used for qPCR testing PDGFRA mRNA expression level

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward or Reverse</th>
<th>Sequence</th>
<th>UPL probe</th>
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<tr>
<td>PDGFRA</td>
<td>F</td>
<td>AGGTGTTGACCTTCAATGG</td>
<td>#80</td>
</tr>
<tr>
<td>R</td>
<td>TTTGATTTCTTCCAGCATTGTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPIA</td>
<td>F</td>
<td>CCTAAAGCATACGGGTCCTG</td>
<td>#47</td>
</tr>
<tr>
<td>R</td>
<td>TTTCACTTTGCCAAACACC</td>
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**Table 2.** Primers used for PDGFRA Sanger sequencing

<table>
<thead>
<tr>
<th>Exon</th>
<th>F or R</th>
<th>Sequence</th>
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<tr>
<td>5</td>
<td>F</td>
<td>TGTTAACGACGGCCAGTCCACTGCTGAGGAATCGGTT</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>CAGGAAACACGCTATGACCCACCAAGGTGGGAAAGTCAAGG</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>TGTTAACGACGGCCAGTCCACTGCTGAGGAATCGGTT</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>CAGGAAACACGCTATGACCCACCAAGGTGGGAAAGTCAAGG</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
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</tr>
<tr>
<td>10</td>
<td>F</td>
<td>TGTTAACGACGGCCAGTCCACTGCTGAGGAATCGGTT</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>CAGGAAACACGCTATGACCCACCAAGGTGGGAAAGTCAAGG</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>TGTTAACGACGGCCAGTCCACTGCTGAGGAATCGGTT</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>CAGGAAACACGCTATGACCCACCAAGGTGGGAAAGTCAAGG</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>TGTTAACGACGGCCAGTCCACTGCTGAGGAATCGGTT</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>CAGGAAACACGCTATGACCCACCAAGGTGGGAAAGTCAAGG</td>
</tr>
</tbody>
</table>

**Note:** PDGFRA, platelet-derived growth factor receptor alpha; PPIA, peptidylprolyl isomerase A (cyclophilin A); F, forward; R, reverse; UPL, Universal Probe Library.
The same samples were screened for the *KP* fusion gene. All primers and PCR conditions were identical to those described previously.\(^\text{13,14}\) In brief, PCR amplification was performed using FastStart Taq DNA polymerase (Roche) in a thermocycling program consisting of 3 min at 94°C, followed by 30 cycles of denaturation for 45 s at 94°C, annealing for 30 s at 55°C, and extension for 90 s at 72°C, followed by a 10 min final extension. To validate these findings, the obtained cDNA was sequenced.

**IDH1 and IDH2 Mutational Status**

*IDH1* and *IDH2* mutations in the hotspot codons R132 and R172, respectively, were assessed by bidirectional cycle sequencing of PCR-amplified primers, as previously described.\(^\text{20}\)

**Chromosome Arm 1p/19q Codeletion Was Detected Using BAC-aCGH and Validated Using Microsatellite Analysis**

Chromosome arms 1p/19q status was assessed using BAC-aCGH and validated by microsatellites polymorphisms analysis using the following markers, as described previously: D1S450, D1S2667, D1S234, D1S890, D1S2841, D19S425, D19S219, D19S412, and D19S418.\(^\text{21}\)

**Statistical analysis**

For univariate analysis, χ² or Fisher’s exact, and Mann-Whitney U tests were used to compare categorical and continuous variables, respectively. *PDGFRA* expression level was compared among nontumor tissues, nonamplified tumors, and *PDGFRA*-amplified tumors with use of the nonparametric Kruskal-Wallis test. The Spearman’s rank order correlation test was used to analyze the relationship between *PDGFRA* amplification and other alterations (truncation, point mutation, and expression). A Spearman’s p value of −1 indicated perfect inverse/negative correlation, and a value of 1 indicated perfect/positive correlation. The agreement between BAC-aCGH and microsatellite analysis of 1p19q codeletion detection and between BAC-aCGH and qPCR for *PDGFRA* amplification was assessed using Fleiss κ statistics. A κ value of <0 indicated poor agreement, whereas a κ value of 0.81–1.00 indicated almost perfect agreement.\(^\text{22}\)

Overall survival (OS) and progression-free survival (PFS) were evaluated from diagnosis to death and to the first clinical and/or radiological documentation of progression, respectively. All patients without evidence of relapse at the time of the last clinical visit were censored. OS and PFS were estimated using Kaplan-Meier methodology and compared using the log-rank test. A Cox proportional hazards model was used for the multivariate analysis. Two-sided P values <.05 were interpreted as statistically significant. Analyses were performed in R, version 2.13.1 (http://www.R-project.org).

## Results

### Patient and Tumor Characteristics

From 2005 through 2009, 619 diffuse gliomas were characterized using BAC-aCGH (from 345 men and 274 women; sex ratio, 1.3). The median age of the study population was 49.5 years (interquartile range, 37.2–61.2 years). The clinical characteristics of patients with *PDGFRA*-amplified and nonamplified diffuse gliomas are shown in Table 3. All patients provided their written consent for molecular analysis.

**PDGFRA High-Level Amplification and Gain**

*PDGFRA* high-level amplification and gain were evaluated using BAC-aCGH validated by qPCR (κ = 0.89; 95% confidence interval [CI], 0.69–1.00); these events were detected in 32 (5.2%) of 619 and 12 (1.9%) of 619 cases, respectively (Fig. 1B).

**Correlation with Tumor Pathology.—**Both *PDGFRA* high-level amplification and gain were detected in 0 (0%) of 167 WHO grade II, 6 (3.6%) of 168 WHO grade III, and 26 (9.15%) of 284 WHO grade IV gliomas (Table 3). In addition, *PDGFRA* gain was detected in 1 (0.6%) of 167 WHO grade II, 2 (1.1%) of 168 WHO grade III, and 9 (3.1%) of 284 WHO grade IV gliomas.

*PDGFRA* high-level amplification was not associated with tumor phenotype (18 of 248, 3 of 127, and 11 of 230 of astrocytic, oligodendrogliotic, and oligoastrocytic gliomas, respectively; \(P = 0.1\)) (Table 3), even when GBMO were analyzed as oligodendrocytic tumors.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PDGFRA Amplified (%)</th>
<th>PDGFRA Non-amplified (%)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tumors (619)</td>
<td>32 (5.2)</td>
<td>587 (94.8)</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (years)</td>
<td>58.8</td>
<td>48.7</td>
<td>0.0002</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>51.2–68.6</td>
<td>37–60.9</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (274)</td>
<td>16 (5.8)</td>
<td>258 (94.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Male (345)</td>
<td>16 (4.6)</td>
<td>329 (95.4)</td>
<td></td>
</tr>
<tr>
<td>Tumor WHO grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II (167)</td>
<td>0</td>
<td>167 (100)</td>
<td>8.032e-06</td>
</tr>
<tr>
<td>Grade III (168)</td>
<td>6 (3.6)</td>
<td>162 (96.4)</td>
<td></td>
</tr>
<tr>
<td>Grade IV (284)</td>
<td>26 (9.1)</td>
<td>258 (90.9)</td>
<td></td>
</tr>
<tr>
<td>Tumor phenotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma (248)</td>
<td>18 (7.3)</td>
<td>230 (92.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Oligoastrocytoma (130)</td>
<td>3 (2.3)</td>
<td>127 (97.7)</td>
<td></td>
</tr>
<tr>
<td>Oligodendroglioma (including GBMO) (241)</td>
<td>11 (4.6)</td>
<td>230 (95.4)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** NS, not significant; WHO, World Health Organization; GBMO, glioblastoma with an oligodendrogial component.
*PDGFRA* high-level amplification was strongly associated with a high grade of malignancy (\(P = 8.032e-06\)) (Table 3). Likewise, *PDGFRA* gain was also associated with a high grade of malignancy (\(P = 6.793e-06\)).

**Correlation with Clinical Characteristics and Outcome of Patients.**—Patients with *PDGFRA*-amplified tumors were significantly older (59.8 years vs 48.7 years; \(P = .0002\)). No statistically significant association with patient sex was observed (Table 3).

In WHO grade III gliomas, *PDGFRA* amplification had a negative impact on OS and PFS (\(P = 5.18e-05\), Fig. 2A; \(P = .001\), Fig. 2B) and had an independent prognostic value in multivariate analysis that included chromosome arm 1p/19q status and *IDH* mutation (Tables 4 and 5). Both *IDH* mutation and 1p/19q codeletion were associated with longer PFS and OS in univariate and multivariate analysis (Tables 4 and 5). The agreement between BAC-aCGH and microsatellite analyses was very good (\(k = 0.91\); 95% CI, 0.78–1.00). Chromosome arms 1p/19q codeletion was observed in 22% of WHO grade III tumors.

In WHO grade IV tumors, *PDGFRA* amplification does not have any prognostic significance in terms of OS and PFS (Fig. 2C and D).

**PDGFRA Mutations**

Four of the 32 *PDGFRA*-amplified tumors (12.5%) exhibited somatic point mutations in *PDGFRA*. All tumors with point mutations were WHO grade IV. One silent mutation, a G > T transversion at codon G313 (GGT > GGG) (Fig. 1C, Panel 1), was detected. The other 3 were missense mutations: 1 at codon C235Y (TGT > TAT) (Fig. 1C, Panel 2), 1 at codon P443L (CCG > CTG) (Fig. 1C, Panel 3), and 1 at codon V536E (GTG > GAG) (Fig. 1C, Panel 4). Each point mutation occurred in different samples, and all have been reported previously.\(^5\) Of interest, among WHO grade IV tumors, *PDGFRA* mutation was observed more frequently in GBMO, although this difference in frequency did not reach statistical significance (75% vs 24%; \(P = 0.07\)).

**Screening for PDGFRA Truncation and KDR-PDGFRA Fusion in PDGFRA-Amplified Samples**

*PDGFRA* truncation (*PDGFRA\(\Delta^{8,9}\)*) was observed in 1 of the 12 *PDGFRA*-amplified tumors (Fig. 3A) and validated by direct sequencing. None of our *PDGFRA*-amplified tumors contained the fusion gene KDR-PDGFRA.\(^{14}\)

**PDGFRA mRNA Expression Analysis**

The expression level of *PDGFRA* mRNA in 16 *PDGFRA*-amplified tumors (all WHO grade IV) was compared with that in 68 nonamplified tumors (including 20 WHO grade IV, 26 WHO grade III, and 22 WHO grade II) and 12 nontumor brain tissues (epilepsy}

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**Fig. 2.** Prognostic value of *PDGFRA* abnormalities in gliomas (continuous line indicates *PDGFRA*- non amplified tumors and broken line indicates *PDGFRA* amplified tumors). (Panel A). OS in *PDGFRA* amplified vs nonamplified WHO III gliomas. (Panel B). PFS in *PDGFRA* amplified vs nonamplified tumors in WHO grade III gliomas. (Panel C). OS in *PDGFRA* amplified vs nonamplified WHO IV gliomas. Panel d. PFS in *PDGFRA* amplified vs nonamplified tumors in WHO grade IV gliomas.
samples). Of interest, tumors harboring PDGFRA amplification exhibited an increased level of PDGFRA mRNA expression compared with their nonamplified counterparts and with nontumor brain tissue ($P = 1.4e-6$ and $P = 1.5e-7$, respectively) (Fig. 3B).

### PDGFRA Molecular Abnormalities in PDGFRA Nonamplified Gliomas

We studied a cohort of 106 PDGFRA non-amplified glial tumors, including 32 low-grade gliomas (10 astrocytomas,
12 oligodendrogliomas, and 10 oligoastrocytomas); 32 anaplastic gliomas (6 astrocytomas, 18 oligodendrogliomas, and 8 oligoastrocytomas); and 42 GBMs. None of them was PDGFRAb-point-mutated, PDGFRAt-truncated, or KDR-PDGFRAr-rearranged.

Correlation Between PDGFRAb Amplification and PDGFRAb Mutation, Rearrangement, IDHb Mutation, and 1p/19q Codeletion

PDGFRAb amplification was strongly associated with the PDGFRAb expression level (Fig. 3B). In addition, an inverse correlation was observed between mutation and truncation. PDGFRAb amplification was about twice more frequent in the mutated samples than in the truncated samples. PDGFRAb amplification and IDHb mutation were inversely correlated; 12.9% of PDGFRAb-nonamplified tumors were IDHb mutated, compared with 2.6% of amplified tumors (P = 0.001). In addition, tumors featuring PDGFRAb locus gain were less frequently IDHb mutated, compared with tumors with normal PDGFRAb copy number status (16.7% vs 43.2%; P = .08). Moreover, the presence of 1p/19q codeletion and PDGFRAb amplification were mutually exclusive in WHO grade III tumors (0% of 1p/19q codeleted tumors were PDGFRAb-amplified vs 4.6% of non-codeleted; P = .03).

Discussion

PDGFRAb is a critical gene in neurogenesis and gliomagenesis. To our knowledge, this study represents the largest cohort of diffuse gliomas and the most comprehensive analysis of the prevalence, the clinicopathological significance, and the co-occurrence of the PDGFRAb abnormalities most frequently reported in gliomas. Of interest, PDGFRAb high-level amplification has an independent negative prognostic value in grade III tumors in terms of PFS and OS.

In our study, PDGFRAb amplification was restricted to high-grade gliomas and was not observed in diffuse low-grade gliomas. A previous study has described PDGFRAb amplification in low-grade gliomas (this study found amplification in 6 of 20 low-grade gliomas, but 83% of amplified tumors were astrocytomas).9 Our series included 20 WHO grade II astrocytomas, none of which were PDGFRAb amplified. These conflicting results require further investigation to specify the frequency of PDGFRAb amplification in low-grade gliomas (Table 6).

Previous studies have reported that PDGFRAb amplification occurs in 8.5%–26% of GBMs.4,5,9,14 This wide range might be attributed to distinct definitions of gene amplification. Taken together, the published data and our results suggest that PDGFRAb high-level amplification is observed in approximately 10% of GBMs (Table 6).4,14

PDGFRAb amplification is strongly correlated with deleterious effects in WHO III gliomas in terms of PFS and OS. These results are consistent with our previous study, which focused on anaplastic oligodendrogliomas.23 This prognostic value of PDGFRAb amplification is independent of age, KPS, type of surgery, IDH mutation, and 1p/19q codeletion. In our series of WHO grade III, tumor phenotype (ie, astrocytoma, oligoastrocytoma, and oligodendroglioma) does not impact significantly patients’ survival although a trend is observed. The prognostic value of phenotype in anaplastic
### Table 6. Frequency and prognostic significance of PDGFRA alterations in gliomas

<table>
<thead>
<tr>
<th>PDGFRA alteration</th>
<th>n</th>
<th>Histology and grade</th>
<th>Prognostic impact</th>
<th>References</th>
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<tr>
<td></td>
<td></td>
<td>AI</td>
<td>All</td>
<td>OII</td>
</tr>
<tr>
<td>Point Mutation</td>
<td>91</td>
<td>1/91</td>
<td>4/116</td>
<td>NP</td>
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<td></td>
<td>116</td>
<td>4/116</td>
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<td>NP</td>
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<tr>
<td></td>
<td>86†</td>
<td>NP</td>
<td>NP</td>
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<tr>
<td>Rearrangement (truncation)</td>
<td>212</td>
<td>6/212 (6/15*)</td>
<td>NP</td>
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</tr>
<tr>
<td>Rearrangement (fusion KP)</td>
<td>212</td>
<td>1/212</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Amplification</td>
<td>206</td>
<td>44/170</td>
<td>NP</td>
<td>NP</td>
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<tr>
<td></td>
<td>170</td>
<td>23/206</td>
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<td>87</td>
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</table>

Abbreviations: PDGFRA, platelet-derived growth factor receptor alpha; †histology and grade was not described, 4 silent mutations out of 86 gliomas; *PDGFRA amplified samples; **phenotype is not specified; KP, KDR-PDGFRA fusion gene; NP, not performed; A, astrocytoma; O, oligodendrogliaoma; M, mixed glioma or oligoastrocytoma; GBM, glioblastoma multiforme; I, WHO grade I; II, WHO grade II; III, WHO grade III; IV, WHO grade IV.
oligodendroglial tumors is debated, and some discrepancies might be at least partially explained by some variability in neuropathologic classification. Similarly, intra- and interobserver variability in pathologic diagnosis might induce some variability in the prevalence of 1p/19q codeletion in anaplastic oligodendroglial tumors. Our results are at the lower limit of the range of the prevalence reported in the literature.\(^{25,26}\)

Techniques used for 1p/19q codeletion testing in anaplastic oligodendroglial tumors might also explain variability in the prevalence of 1p/19q codeletion. Indeed, techniques testing a large number of genomic loci simultaneously (eg, comparative genomic hybridization or multiplex ligation-dependent probe amplification), compared with the techniques investigating a limited number of loci (eg, FISH or microsatellites analysis), allow a better distinction of whole chromosome arms 1p/19q codeletion vs partial 1p/19q codeletion.\(^{27,28}\) The former alteration corresponding to a reciprocal unbalanced translocation t(1;19)(q10;p10) is actually the favorable prognostic biomarker in anaplastic oligodendroglial tumors.\(^{29,30}\) Finally, the design of studies (eg, retrospective), because of biases issues, might also explain some variability in biomarkers assessment in oligodendroglial tumors.

No prognostic value for PDGFRA amplification was detected in WHO grade IV gliomas. Indeed, the prognostic significance of PDGFRA amplification has been debated in the literature (Table 6). Previous studies did not associate PDGFRA amplification with worse prognosis in any histological entity.\(^{31}\) However, Pupitti et al. showed that PDGFRA amplification was associated with worse prognosis in a univariate analysis in gliomas.\(^{32}\) Conversely, Nobusawa et al. studied 390 GBMs and did not find an association between the amplification of PDGFRA and survival.\(^{33}\) As is the case for EGFR amplification, PDGFRA amplification seems not to have a prognostic value in GBM (Table 6).\(^{34}\)

The prognostic value of PDGFRA mutations in gliomas has not been established previously (Table 6).\(^{9,31,35}\) Similarly, the KP-fusion gene has been described only recently,\(^{14}\) and there are no studies analyzing the clinical significance of these mutations. Unfortunately, we did not identify any sample with a KP-fusion gene; this seems to be a rare phenomenon in glioma.

Our series focused on PDGFRA-amplified gliomas because PDGFRA truncation and rearrangement has been described as unique to this group.\(^{14}\) Whether the percentage of PDGFRA mutations in nonamplified tumors is similar to that in amplified tumors remains unknown, with the exception of PDGFRA deletion,\(^{8,9}\) which has been detected exclusively in PDGFRA amplified gliomas.\(^{14}\) In a series of 106 PDGFRA nonamplified gliomas, we did not find any PDGFRA mutation or rearrangement. In our series, the percentage of gliomas with truncations is much lower than that previously reported (8.3% vs 40%).\(^{14}\)

We found a statistically significant association between PDGFRA amplification and the overexpression of PDGFRA mRNA (\(P = 0.045\)). In previous studies, the correlation between PDGFRA expression and copy number status was evaluated via immunohistochemistry.\(^{9,36–38}\) These studies found that PDGFRA was overexpressed in approximately 50% of malignant gliomas.\(^{9,36–38}\) In addition, the prognostic value of PDGFRA expression remains hotly debated.\(^{9,36–38}\)

PDGFRA is a critical gene in glioma and particularly in GBM. It is amplified in approximately 10% of GBMs, but PDGFRA amplification has poor independent prognostic value in anaplastic gliomas. Taken together, our data and those reported in previous studies highlight PDGFRA alterations as a potential prognostic biomarker and a therapeutic target. Indeed, multiple PDGFRA inhibitors are under development in academic and industrial laboratories.\(^{19,40}\) To date, some have been tested in humans with limited success, but the patients in these trials were not selected on the basis of PDGFRA status.\(^{41,42}\) PDGFRA truncation appears as a rare phenomenon in diffuse gliomas. Indeed, it is observed in 8%–40% of PDGFRA-amplified GBMs, which means in approximately 0.8%–4% of all GBMs. These results do not support the development of a vaccine therapy against PDGFRA\(^^\Delta\),\(^{8,5}\) similar to that developed for \(EGFRvIII\) observed in ~20% of all GBM. However, further studies are warranted to specify the exact prevalence of this abnormality in various normal tissues, diffuse gliomas, and other cancer types.\(^{43,44}\)

**Acknowledgments**

Institut Hospitalier Universitaire de Neurosciences Translationnelles de Paris.

**Conflict of interest statement.** None declared.

**Funding**

This work was supported by Obra Social la Caixa (to A.A.). This work is part of the national program Cartes d’Identité des Tumeurs (http://cit.ignite-cancer.net/), which is funded and developed by the Ligue Nationale Contre le Cancer.

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