An integrative radiological, histopathological and molecular analysis of pediatric pontine histone-wildtype glioma with MYCN amplification (HGG-MYCN)

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The 2016 WHO Classification of tumours of the central nervous system has introduced a new histomolecular entity, the midline diffuse glioma, H3K27 M-mutant [8]. This entity represents an infiltrative high-grade glioma (HGG) (grade IV) of the pons and the brainstem, which mainly effects children. It harbors K27 M mutations of H3F3A, HIST1H3B/C or HIST2H3A/C genes. In the pons, morphological differential diagnoses are numerous with a large spectrum of tumours ranging from benign such as pilocytic astrocytoma to malignant such as embryonal tumour with multilayered rosettes (ETMR). From the reclassification of CNS-PNETs in the study by Sturm et al., HGG-MYCN was described in 28/323 cases (9%), mainly located in the cerebral hemispheres. Moreover, in a recent series of pontine gliomas, three molecular subgroups were defined: H3K27 M-mutant, MYCN-amplified and silent [1]. The MYCN subgroup was the least frequent, with only 8% of cases (4/47) [1]. This corresponds to a very rare tumour and very few radiological, clinical and histopathological data are available in the literature. In our center, six cases of HGG-MYCN were diagnosed by whole exome sequencing (WES) based on the co-amplification of MYCN and ID2 (2 cases from BIOMEDE cohort) [3].

Herein, our aim is to describe the clinical, imaging, histopathological, immunohistochemical and molecular features of these cases to better characterize them.

Concordant with recent literature, the median age of our patients was 3.7 (1 to 7 years old) and concerned 4 girls (Cases 2, 3, 4 and 6) and 2 boys (Cases 1 and 5) [1]. Clinically, all our patients presented similarly to midline diffuse gliomas, H3K27 M-mutant, with a short clinical history (<6 months of symptom duration) and the presence of a pontine tumour infiltrating at least 50% of the pons. Radiologically, all tumours were centered in the pons, with variable involvement of the mesencephalon and the middle cerebellar peduncle. None was calcified nor hemorrhagic. HGG-MYCN displayed necrosis at first presentation, with annular enhancement, larger than in H3K27 M gliomas. Furthermore, diffusion was more restricted than classically reported in diffuse intrinsic pontine gliomas (n = 5, median Apparent Diffusion Coefficient: 570 μm²/s, IQR [543–734], versus 1504 μm²/s in Calmon et al.), and relative Cerebral Blood Flow was higher using arterial spin labeling (n = 4, median 2.1, IQR [1.7–2.8] versus 1.1 in Calmon et al.) Relative Cerebral Blood Volume using dynamic susceptibility contrast perfusion MRI was in the usual range (n = 3, values: 1.1, 1.8, 3.8) (Fig. 1) [2]. All patients died of their disease, two directly following a surgical biopsy, one (Case 3) after 1 month of chemotherapy (VP16 carboplatin) and three (Cases 4, 5 and 6) after radiotherapy and chemotherapy. The mean overall survival of our patients was 3.8 months and the median overall survival was 4.2 months, which is shorter than midline diffuse glioma, H3 K27 M-mutant [5, 6, 10].

Histopathologic examination showed a well circumscribed tumor from the parenchyma with only a few cells infiltrating the surrounding parenchyma (Fig. 2a). The multinodular, undifferentiated neoplasm presented with a subtle transition between spindle cells and nodules of epithelioid cells (Fig. 2a-e). In all cases, malignancy was obvious with high mitotic count, high proliferation index (mean MIB index of 51%), necrosis and microvascular proliferation (Fig. 2c and f). The neoplastic cells had round to oval nuclei, vesicular to coarse chromatin, prominent nucleoli and a scant to moderate
amount of eosinophilic cytoplasm (Fig. 2 c and e). There was no rhabdoid component or rosettes.

All cases of pontine HGG-MYCN did not express the H3K27 M mutated protein and presented a preserved expression of H3K27me3 (Fig. 2 g). In two cases (Cases 1 and 2), all other stains (GFAP, Olig2 - Fig. 2 h -, NFP70, synaptophysin, chromogranin A, NeuN, CD34, BRAFV600E, BCOR, NUT, Actin, Desmin, HMB45, Lin28A, EMA, CKAE1/AE3 and IDH1R132H) were negative. The four remaining cases expressed glial (Olig2 and GFAP) (Fig. 2i) and neuronal (NeuN and NFP70) markers (data not shown). All cases expressed CD56 and Vimentin. Moreover, case 3 presented a pluriphenotypic pattern with focal expression of epithelial marker (CKAE1/AE3) (Fig. 2j). In addition, the expression of INI1, BRG1 and ATRX were retained in all cases. Nuclear accumulation of p53 was present in 4/5 tested cases (Cases 2, 3, 5 and 6) (Fig. 2k) which was correlated with additional TP53 mutations found by WES. EGFR overexpression (Cases 3 and 6) and loss of PTEN (Cases 2 and 5) were mutually exclusive. No mutations of the hTERT promoter, IDH1, IDH2, H3F3A, HIST1H3B and BRAF were observed. The amplification of the MYCN gene was confirmed by FISH analysis ZytoLight SPEC MYCN/2q11 Dual Color Probe (Zytovision, Germany) in all cases (Fig. 2l). It should be noted that MYCN amplification is not a specific alteration of the HGG-MYCN entity. Indeed, it may be encountered in a small portion of pediatric glioblastoma-RTKI (Receptor of Tyrosine Kinase I) and –RTKII (Receptor of Tyrosine Kinase II) [6]. However, WES analysis revealed a co-amplification of the ID2 gene in all our cases which is not described in other types of glioblastomas. This co-amplification was described in the supra-tentorial and the pontine location of HGG-MYCN [1, 9]. ID2 encodes the protein ID2 (Inhibitor of DNA-binding 2) which is expressed by oligodendroglial precursor cells, a progenitor cell type implicated in the oncogenesis of gliomas [4, 7, 11]. Furthermore, a subset of diffuse midline gliomas with H3.3-K27 M mutations overexpress ID2 without amplification of this gene [1]. These data suggest a potential common ID2-related mechanism in pontine high-grade gliomas tumorigenesis.

We here extend the knowledge about the rare pontine HGG-MYCN representing a differential diagnosis of DIPG in young children. This tumoral entity presents with clinicoradiological and phenotypical features that clearly distinguishes it from classical midline diffuse glioma, H3K27 M-mutant and might allow for the
establishment of targeted molecular therapy in the future. In small biopsy of pontine tumors, the emergence of this HGG-MYCN subgroup reinforces the necessity for valuable immunohistochemistry and FISH analyses if molecular analysis are not feasible or non-contributory. Therefore, we strongly recommend adding MYCN FISH analysis in the diagnostic immunohistochemical/molecular panel of pediatric brainstem tumours, similarly to H3K27M, INI1/BRG1 and Lin28A antibodies for the differential diagnoses of DIPG, AT/RT and ETMR, respectively.

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ATE, MAD, DC, JG, SP, MS, KB, AG, VDR, EL, NB and PV participated in conception, design, collection and assembly of data, data analysis and interpretation, manuscript writing and approval. MAD and DC performed WES analyses. VDR and NB compiled radiological data. JG participated by providing study materials or patient information. All authors read and approved the final manuscript.

Competing interests
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