

of all tumors using IlluminaEPICarrays and compared it to the brain tumor classifier which allowed to generate the CNVs. RESULTS: Morphologically two cases were defined as anaplastic astrocytoma, two cases as glioblastoma. Based on the DMP, all cases were found to belong to the methylation class “glioblastoma, IDH wildtype, subclass midline”, hypermutants, with gain of chromosome 1q and loss of 1p. Two cases showed PDGFRA amplification. All patients were treated with Temozolomide combination therapy +/- Bevacizumab and radiation therapy. At progression three patients were treated with checkpoint inhibitors. CONCLUSIONS: The improvement of the precision medicine is fundamental in the therapeutic decision of brain tumors and even more in neoplasms secondary to antitlastic treatments. DMP and CNV have proven to be useful tools to complement the histological characterization of the reported cases.

### HGG-56. EXTENSIVE MOLECULAR HETEROGENEITY WITHIN H3-/IDH-WILDTYPE PEDIATRIC GLIOBLASTOMA

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About half of all pediatric high-grade gliomas (HGG) harbor mutations in histone 3 or IDH genes. The remaining HGG are currently broadly classified as H3-/IDH-wild-type. Since the introduction of a uniform approach to DNA methylation-based classification of CNS tumors in 2018, DNA methylation data from over 45,000 CNS tumor samples have been generated. From this large cohort, a number of smaller yet distinct subgroups start to emerge within H3-/IDH-wild-type HGG. Three such subgroups are enriched for focal gene amplifications and have been provisionally termed pedGBM\_MYCN, pedGBM\_RTK1 and pedGBM\_RTK2. Since a significant subset of samples in each subgroup is lacking characteristic alterations, we further investigated the molecular and transcriptional composition of H3-/IDH-wild-type HGG. We evaluated DNA methylation and copy-number profiles in >1000 tumors classified as H3-/IDH-wild-type HGG. Tumors classified pedGBM\_MYCN showed a focal MYCN amplification in 25%, with a similar fraction showing amplification of EGFR (8% of samples harbored both alterations) compared to 4% and 4% in pedGBM\_RTK1 and 14% and 22% in pedGBM\_RTK2. Deletion of CDKN2A/B was much more prevalent in the pedGBM\_RTK2 subgroup (~50% compared to 27% in pedGBM\_RTK1 and <10% in the pedGBM\_MYCN group). We defined a pedGBM\_MYCN transcriptional signature, which will be helpful in identifying subgroup-defining mechanisms and alterations. Initial results suggest an involvement of the sonic hedgehog pathway and genes controlling stem-cell pluripotency. Patient-derived xenograft models and murine neural stem cells are now being used for functional characterization and pre-clinical testing of potential drug targets in these molecularly defined subgroups.

### HGG-57. WHOLE-GENOME SEQUENCING, METHYLATION ANALYSIS, AND SINGLE-CELL RNA-SEQ DEFINE UNIQUE CHARACTERISTICS OF PEDIATRIC TREATMENT-INDUCED HIGH-GRADE GLIOMA AND SUGGEST ONCOGENIC MECHANISMS

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BACKGROUND: Pediatric treatment-induced high-grade glioma (TIHGG) is among the most severe late effects observed in childhood cancer survivors and is uniformly fatal. We previously showed that TIHGG are divergent from de novo pediatric high-grade glioma (pHGG) and cluster into two gene expression subgroups, one stemlike and the other inflammatory.

Here we systematically compared TIHGG molecular profiles to pHGG and evaluated expression and single cell sequencing profiles in order to identify oncogenic mechanisms and the cellular basis for the observed TIHGG gene expression subgroups. MATERIALS/METHODS: 450/850K methylation and mutational signature analysis was conducted in 36 TIHGG samples. Resultant data were analyzed for the presence of chromothripsis, distinct molecular alterations, and mutational signatures in a subset of 10 samples with whole genome sequencing data. Five TIHGGs underwent single-cell RNA-Seq analysis (scRNAseq). RESULTS: 26/36 TIHGG clustered with the pedRTK1 methylation class. TIHGG were characterized by an increased frequency of chromothripsis relative to pHGG (67% vs. 31%, p=0.036). FISH and WGS revealed frequent PDGFRA amplification secondary to enrichment in cDNA. TIHGG were enriched for COSMIC mutational signatures 5 and 19 (p=0.0003) relative to pHGG. scRNAseq data showed that TIHGG tumors are composed of stem-like, neuronal, and inflammatory cell populations which may contribute to the previously described dominant expression profiles. CONCLUSIONS: TIHGG represents a distinct molecular subtype of pHGG. Chromothripsis, leading to enriched expression of genes in extrachromosomal DNA, likely contribute to TIHGG oncogenesis. The dominant cell type (stem-like vs. inflammatory) may define the expression subgroup derived from bulk RNA-seq in heterogeneous tumors.

## IMAGING

### IMG-01. DWI RATIO OF HISTOLOGICAL MOLECULAR SUBTYPES OF PAEDIATRIC MEDULLOBLASTOMAS

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AIM: To evaluate if diffusion weighted imaging (DWI) ratio on MRI is able to distinguish between the histological molecular subtypes of paediatric medulloblastomas. MATERIALS AND METHODS: From 2002 to 2017, 38 cases of medulloblastoma with preoperative MRI available had histological subtyping performed with NanoString nCounter technology. The medulloblastomas were classified into 4 subtypes. There were 3 Sonic Hedgehog (SHH), 9 Wingless (WNT), 12 Group 3 and 14 Group 4 subtypes. Single operator manually outlined solid non-haemorrhagic component of the tumour on DWI images with largest axial tumour cross sectional diameter, correlating with the other MRI images (T1 pre and post contrast, SWI/GRE, FLAIR) to identify areas of haemorrhage. The same operator also drew region of interest to identify normal cerebellar tissue on the same axial images on which the tumour was outlined. All MRI images were obtained from the department's Radiological Information System Picture Archiving and Communicating System (RIS PACS). DWI ratio for each case was obtained by dividing the values obtained from tumour by normal cerebellar tissue seen on the same axial image. RESULTS: DWI ratio of all medulloblastomas is 1.34 +/- 0.18. DWI ratio of SHH subtype is 1.43 +/- 0.07. DWI ratio of WNT subtype is 1.40 +/- 0.07. DWI ratio of Group 3 subtype is 1.31 +/- 0.25. DWI ratio of Group 4 subtype is 1.30 +/- 0.17. There is no significant statistical differences in the DWI ratio between the various subtypes. CONCLUSION: DWI ratio of medulloblastoma is unable to distinguish between the 4 medulloblastoma subtypes.

### IMG-02. USEFUL DIAGNOSIS OF PEDIATRIC CYSTIC BRAIN TUMORS USING MULTIPLE POSITRON EMISSION TOMOGRAPHY STUDIES

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OBJECTIVE: Pediatric brain tumors are primarily diagnosed using MRI or CT examination; however, determining the correct diagnosis using only morphological MRI can sometimes be challenging. Positron emission tomography (PET) uses radiotracers for metabolic and molecular imaging. We examined the accumulation of multiple PET (FDG, MET, FLT, and FMISO) studies for diagnosing pediatric cystic brain tumors. METHODS: We performed PET scans for eight pediatric patients (five pilocytic astrocytoma, one pleomorphic xanthoastrocytoma, one diffuse astrocytoma with IDH1 mutation, one ganglioglioma) from April 2010 to December 2019. The resulting studies were compared by measuring the tumor-to-normal lesion (T/N) ratio of FDG, MET, and FLT and the tumor-to-blood value (T/B) ratio of FMISO between each pediatric cystic brain tumor. RESULTS: All pediatric brain tumors showed tumor uptake of FDG, MET, and FLT. We could not examine FMISO PET for one diffuse astrocytoma with IDH1 mutation. The T/N ratios of FDG, MET, and FLT and the T/B ratio of FMISO were 1.07, 2.76, 4.6, and 1.12 for pilocytic astrocytoma; 0.65, 4.6, 7.67, and 1.38 for pleomorphic xanthoastrocytoma; 0.61, 2.14, and 3.82 for diffuse astrocytoma with IDH1 mutation; and 0.79, 1.78, 5, and 1.49 for ganglioglioma, respectively. The T/N ratios of MET and FLT for pleo-