## S31-1

# Deciphering the histone code for pediatric gliomas

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Over the last five years next generation sequencing of pediatric high grade glioma has uncovered a plethora of information about the genetic underpinnings of this disease including the seminal discovery of novel histone H3 mutations, highlighting the importance of epigenetic alterations in this disease. Interestingly, H3.3 mutations show location- and age-dependent enrichment. H3.3G34R is exclusive to hemispheric high grade glioma, most frequently in adolescents and young adults, while H3.3K27M is exclusive to the midline and is most frequent in children. In this presentation I will discuss the diagnostic and prognostic implications of histone mutations in clinical practice as well as what is known about histones as oncogenes and their mechanistic role in cancer development.

## S31-2

# Whole chromosomal aberration signatures predict survival in standard-risk non-WNT/non-SHH medulloblastoma: Molecular analysis of the HIT-SIOP-PNET4 clinical trial

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【Introduction】 Most children with medulloblastoma fall within the 'standard-risk' clinical disease group (SR-MB; 50-60% of patients; 75-85% 5-year PFS), defined by absence of high-risk features (e.g. metastatic disease, large-cell/anaplastic histology, *MYC* amplification). Within SR-MB, a favourable prognosis is known for WNT-MB patients, but outcome prediction for the majority is imprecise. Novel prognostic biomarkers are urgently required to improve risk-adapted therapies, increase survival and reduce late-effects. 【Methods】 Comprehensive molecular analysis of tumour material from the pan-European SR-MB trial, HIT-SIOP-PNET4 (2001-2006; 4-21 years; n=136), was undertaken. WNT- and SHH-MB clinical behaviour was assessed, and novel independent prognostic markers identified for SR non-WNT/non-SHH patients (n=91); models were validated in an independent demographically-matched cohort (n=70). 【Results】 We discovered a novel whole chromosomal aberration (WCA) signature, associated with increased ploidy and multiple non-random whole chromosome aberrations, was common in non-WNT/non-SHH (42% of tumours). Importantly, WCA signature-associated biomarkers (≤2 of chr7 gain, chr8 loss and chr11 loss) predicted 100% and 98% 5-year progression-free survival within non-WNT/non-SHH patients from trial and validation cohorts, respectively. Remaining non-WNT/non-SHH tumours, SHH-TP53mut and older WNT (49%), conferred significantly higher disease-risk (5-year PFS, 63%). Derived survival models resolved SR-MB into favourable- and high-risk groups, outperforming current risk-stratification schemes. 【Conclusions】 Our molecular investigation of a contemporary SR-MB trial has identified a widespread WCA signature in non-WNT/non-SHH patients, which, together with WNT (<16 years) and SHH-TP53WT, define 51% of SR-MB patients with 100% 5-year PFS. WCA-signature positive and SHH-TP53WT patients should be urgently considered for therapy de-escalation in future biomarker-driven risk-adapted clinical trials.

## S31-3

# Challenges in modeling of pediatric brain tumors

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【Background】Pediatric brain tumors can be difficult to model. Challenges include limited access to tissue, slow growing tumors prone to senescence, and difficulties evaluating specific genetic drivers of neoplastic transformation and progression. We are using several approaches to address these issues.

【Methods】Conditional reprogramming cell (CRC) conditions, originally developed to facilitate growth of senescence-prone epithelial cells, are being used to culture a number of pediatric low-grade gliomas. We are also introducing specific genetic drivers into human and murine stem cells from defined sites in the brain and spinal cord in order to generate pediatric low and high-grade astrocytoma, ependymoma and embryonal tumors. The resulting models are being evaluated in vitro, as well as in vivo using immunocompromised mice and zebrafish.

【Results】CRC conditions allow long-term growth of a subset of previously hard to culture pediatric brain tumors, including several pilocytic astrocytoma and pleomorphic xanthoastrocytoma. These new lines have been used for preliminary preclinical testing of both established and experimental chemotherapeutic agents. Novel embryonal tumor models generated from human neural stem cells, and spinal glioma models from murine spinal progenitors, will also be described.

【Conclusion】Emerging techniques can facilitate the generation of new pediatric brain tumors models useful for preclinical therapeutic and mechanistic studies.

## S31-4

# Significance of molecular classification of ependymomas: C11orf95-RELA fusion-negative supratentorial ependymomas are a heterogeneous group of tumors

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【Introduction】Recent extensive molecular analyses of ependymomas unveiled that supratentorial ependymomas (ST-EPN) are characterized by the presence of C11orf95-RELA fusion. However, the pathogenesis of RELA fusion-negative ependymomas remains elusive. 【Materials and Methods】To validate the molecular classification of the ependymal tumors, we conducted thorough molecular analyses of 113 ependymal tumors from 107 patients within the framework of the Japan Pediatric Molecular Neuro-Oncology Group. In this presentation, we focus on the data from 38 locally diagnosed ST-EPNs.【Results】After central histopathological review, 9 ST-EPNs were re-classified as non-ependymomas. A combination of RT-PCR, FISH, RNA sequencing, or the DKFZ methylation classifier identified RELA fusion in 20 of 29 histologically verified ST-EPN (68.9%). Among the 9 RELA fusion-negative ST-EPN, single cases of YAP1 fusion or BCOR tandem duplication were identified. In addition, a novel EP300-BCORL1 fusion, or a FOXO1-STK24 fusion was detected in single cases. The methylation classification identified no consistent molecular class within the 9 RELA fusion-negative ST-EPN or 9 re-classified tumors. The presence of RELA fusion was not significantly associated with patients' survival among all histologically verified ST-EPN when a multivariate analysis using Cox regression was performed.【Conclusion】Our results indicated that RELA fusion-negative ST-EPNs are a heterogeneous group of tumors that are unlikely to form a single entity. Although they are histologically verified ependymomas according the WHO2016 Classification, whether those tumors belong to the same biological entity as RELA fusion-positive ST-EPN is questionable. Our results thus reinforce the significance of molecular classification in the diagnosis of ependymomas.

## S31-5

# Foxr2 promotes formation of CNS-embryonal tumors in a Trp53-deficient background

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Embryonal tumors in the CNS are primary, aggressive, and poorly differentiated pediatric brain tumors. We identified forkhead box R2 (*Foxr2*) as an oncogene for medulloblastoma through a transposon-based insertional mutagenesis screen. *FOXR2* translocation has been identified in a subset of human embryonal tumors of the CNS, designated as CNS neuroblastoma with FOXR2 activation (CNS NB-FOXR2); however, the *in vivo* functions of *FOXR2* remain elusive. In the present study, we analyzed the effect of Foxr2 overexpression in the mouse brain. Foxr2 and a deficiency of Trp53 promoted tumor formation in the olfactory bulb and brainstem. The tumors consisted of poorly differentiated cells that expressed neuronal and glial markers, consistent with CNS embryonal tumors. Importantly, all mice developed CNS embryonal tumors, albeit with a low incidence of medulloblastoma. The tumors were not continuous with the subventricular zone, and early proliferative lesions were observed inside the olfactory bulb and brainstem, suggesting that region-specific progenitors are involved in tumor initiation. Tumor-derived cells formed spheres *in vitro* and induced tumors that recapitulated the parental tumor upon transplantation, indicating the presence of tumor-initiating cells. Taken together, our data demonstrate that Foxr2 plays a causative role in the formation of CNS-embryonal tumors.