

#### EPEN-24. BIOLOGICAL MARKERS OF EPENDYMOMA IN CHILDREN AND ADOLESCENTS (BIOMECA): SYSTEMATIC COMPARISON OF METHODS FOR THE PRECISE EVALUATION OF BIOMARKERS FOR EPENDYMOMA DIAGNOSIS AND PROGNOSTICATION

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The identification and validation of prognostic and diagnostic biomarkers is a key element of The SIOP Ependymoma II trial, realised through the Biomarkers of Ependymoma in Children and Adolescents study (BIOMECA). BIOMECA aims to identify and validate biomarkers for prediction of outcome whilst enhancing stratification for the next generation of ependymoma trials. We outline our findings from the first 147 consecutive BIOMECA cases (posterior fossa, PF=111; supratentorial, ST=32; spinal, SP=4). We compared various methods for biomarker assessment, across six European laboratories to determine key analysis methods. Methods included: methylation-based classification (EPIC 850K DNA methylation array) (n=141); immunohistochemistry (IHC) for nuclear p65-RELA (n=32), H3K27me3 (n=115), and Tenascin-C (TNC) (n=147); copy number (CN) analysis by FISH, MLPA (1q, *CDKN2A*) (n=147), and MIP (molecular inversion probe) and DNA methylation array (1q, *CDKN2A*, 6q, 11q, 13q, 22q) (n=141); analysis of *ZFTA*- and *YAP1*-fusions by RT-PCR, sequencing, Nanostring assays and break-apart FISH (n=32). Using DNA methylation-based classification, 91% (n=101/111) of PF cases classified as PF ependymoma group A (PFA) and 69% (n=22/32) of ST cases as ST ependymoma, *ZFTA* fusion-positive (*ZFTA*). Most PFAs demonstrated inter-centre agreement for loss of H3K27me3, and were TNC positive, representing surrogate markers for PFA identification. Combinations of p65-RELA IHC, FISH analysis, and RNA-based methods were suitable to identify *ZFTA*- and *YAP1*- fused ST ependymomas. Predictive CN alterations were identified by high-resolution, quantitative MIP technology. The integration of histopathology assessment and molecular typing is now critical as the updated 2021 WHO CNS5 classification of ependymomas lists seven molecularly distinct entities. This study highlights the importance of evaluating different methods in a prospective trial cohort. Here, advanced molecular techniques represent powerful tools for the classification of ependymoma entities (DNA methylation array) and for the detection of CN alterations (MIP) and specific fusions, enabling the correct classification and identification of prognostic markers.

#### EPEN-25. A NOVEL SPONTANEOUS MODEL OF ZFTA-RELA FUSION EPENDYMOMA

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Ependymomas driven by the *ZFTA-RELA* fusion account for >70% of all supratentorial ependymomas. These tumours are now recognised in the WHO classification of CNS tumours and have been associated with a poor prognosis. Seven *ZFTA-RELA* fusion variants have been described: around two thirds of cases are fusion 1. No spontaneous genetically modified mouse models

(GEMMS) have been described and current models require invasive intracranial injection (of transduced cells or RCAS-TVA system). Here we describe the first spontaneous GEMM of *ZFTA-RELA* fusion-driven ependymoma. Nestin-Flx-STOP-Flx-*ZFTA-RELA* (Fusion 1) or E1alpha-Flx-STOP-Flx-*ZFTA-RELA* open reading frames were targeted together with luciferase to the Rosa-26 locus. Breeding these mice with Nestin-CreERT2 or Blnp-Cre lines that drive recombination in neural progenitor cells resulted in forebrain tumours that could be tracked with bioluminescence imaging from P20. Tumours displayed NF-κB and L1CAM expression and *ZFTA-RELA* protein was detected using a novel in-house antibody. Tumours display expression of a known *ZFTA-RELA* fusion ependymoma transcriptomic signature. *ZFTA-RELA* tumours can be grown as neurospheres and passaged as allografts in nude mice. We provide the first spontaneous GEMM of this important group of ependymomas. We are now characterising these tumours histologically and transcriptomically relative to the human disease and using these to understand the lineage origins of ependymoma and plan use of conventional and novel treatments.

#### EPEN-26. CHEMOKINE RECEPTOR BLOCKADE REVERSES CCL2 MEDIATED IMMUNOSUPPRESSION AND RESTORES CAR-T CELL FUNCTION IN POSTERIOR FOSSA EPENDYMOMA

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Trastuzumab-based HER2 CAR-T constructs have demonstrated preclinical efficacy in medulloblastoma and are being evaluated for use in children and young adults with recurrent or refractory CNS tumors. Preliminary results demonstrate immune activation but no objective tumor response in three patients, including two patients with posterior fossa (PF)-EPN. A key finding in the serum and CSF of all three patients was very high levels of the inflammatory chemokine CCL2 following treatment with CAR-T cells. Preclinical studies suggest that high levels of CCL2 may impede T cell mediated anti-tumor activity in CNS tumors. The role of CCL2 to enhance or diminish CAR-T cell efficacy for CNS tumors is unknown. We evaluated a second generation trastuzumab-based HER2 CAR construct with a 4-1BB co-stimulatory domain in two ultra-high-risk patient-derived xenograft (PDX) models that faithfully recapitulate PFA-EPN. In contrast to preclinical studies in other cancers, treatment with trastuzumab-based HER2 CAR-T cell alone causes only partial regression of tumors and robust infiltration of immunosuppressive monocytes in PFA-EPN PDX mouse models. We studied constitutive NF-κB activation because it is a hallmark of PFA-EPN that drives dysregulation of inflammatory genes and forms an immunosuppressive tumor microenvironment. Upon tumor recognition, CAR-T cells produce high amounts of the cytokine tumor necrosis factor-α, which is an extracellular stimulus that propagates NF-κB activation in PFA-EPN. We show that HER2 CAR-T cell treatment causes increased nuclear translocation of the RELA NF-κB subunit, which induces CCL2 gene transcription and chemokine release. This results in CCL2-CCR2 ligand/receptor mediated influx of inflammatory monocytes and regulatory T cells, impairing CAR-T cell effector function. Inhibition of CCR2 restores anti-tumor CAR-T cytotoxicity against bulky orthotopic tumors by decreasing the infiltration of inflammatory monocytes and regulatory T cells. Combinatorial strategies addressing tumor mediated immunosuppression should be evaluated in upcoming CAR-T cell trials for patients with high-risk CNS tumors.

#### EPEN-27. EPIGENETIC DISSECTION OF SPINAL EPENDYMOMAS (SP-EPN) SEPARATES TUMORS WITH AND WITHOUT NF2 MUTATION

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