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

Rebecca Chapman[1], David R Ghasemi[2,3], Pierre LeBlond[4], Hendrik Witt[5], Jacques Grill[6], John-Paul Kilday[7,8], Piergiorgio Modena[9], Pascale Varlet[10], Arnault Tauziede-Espariat[10], Christine Haberler[11], Francesca Buttarelli[12], Felice Giangaspero[12,13], Simon ML Paine[14], Thomas S Jacques[15,16], Ian Scott[14], Felipe Andreiuolo[17], Torsten Pietsch[17], Maura Massimino[18], Richard Grundy[1], Kristian W Pajtler[3,2], Timothy A Ritzmann[1]; [1]The Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, United Kingdom. [2]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Heidelberg, Germany. [3]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany. [4]Institute of Hematology and Pediatric Oncology (IHOPe), Leon Berard Comprehensive Cancer Center, Lyon, France. [5]Department of Paediatric Oncology, Heidelberg University Hospital, Heidelberg, Germany. [6]INSERM Unit [981] and Department of Pediatric and Adolescent Oncology, Gustave Roussy, Villejuif, France. [7]Children's Brain Tumour Research Network (CBTRN), Royal Manchester Children's Hospital, Manchester, United Kingdom. [8]The Centre for Paediatric, Teenage and Young Adult Cancer, Institute of Cancer Sciences, University of Manchester, Manchester, United Kingdom. [9]Genetics Unit, Pathology Department, Ospedale S. Anna, Como, Italy. [10]Departement de Neuropathologie, Hopital Sainte-Anne, Paris, France. [11]Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria. [12]Dept. of Radiological, Oncological and Anatomo-Pathological Sciences, Sapienza University of Rome, Rome, Italy. [13]IRCCS Neuromed, Pozzilli, Italy. [14]Department of Neuropathology, Nottingham University Hospital, Nottingham, United Kingdom. [15]Developmental Biology and Cancer Programme, UCL GOS Institute of Child Health, London, United Kingdom. [16]Department of Histopathology, Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom. [17]Department of Neuropathology, DGNN Brain Tumor Reference Center, University of Bonn, Bonn, Germany. [18]Paediatric Unit, Fondazione Istituto Di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale dei Tumori, Milan, Italy

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

Sigourney Bell, Claire King, Katherine Wickham Rahrmann, Amir Jassim, Jessica Taylor, Richard Gilbertson; CRUK Cambridge Institute, Cambridge, United Kingdom

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 Blbp-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-kB 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

Allison P. Cole[1], Andrew M. Donson[2], Angela M. Pierce[2], Enrique Grimaldo[2], Jacob D. Calhoun[2], Andrea M. Griesinger[2], Caroline Sublett[1], Rosandra N. Kaplan[1], Terry J. Fry[3], Nicholas K. Foreman[2], Anandani Nellan[1,2]; [1]Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD, USA. [2]Department of Pediatrics, University of Colorado School of Medicine, Morgan Adams Foundation Pediatric Brain Tumor Research Program, Aurora, CO, USA. [3]Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA

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-kB 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-alpha, which is an extracellular stimulus that propagates NF-kB activation in PFA-EPN. We show that HER2 CAR-T cell treatment causes increased nuclear translocation of the RELA NF-kB 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

Sina Neyazi[1,2], Erika Yamazawa[3,4], Catena Kresbach[2,5], Genta Nagae[4], Alicia Eckhardt[1,2], Takayoshi Umeda[6], Lara Pohl[1,2], Kenji Tatsuno[4], Ceren Saygi[7], Taijun Hana[8,9], Malik Alawi[7], Phyo Kim[10], Mario M. Dorostkar[11,12], Fumi Higuchi[10], Abigail K. Suwala[13,14], Toshihiro Takami[15], Annika Wefers[5], Yuta Nakanishi[16], Leonille Schweizer[17], Keisuke Takai[18], Lara Engertsberger[19], Takashi Komori[20], Theresa Mohme[21], Hirokazu Takami[3], Martin Mynarek[1], Masashi Nomura[3,22], Karin Lamszus[21], Akitake Mukasa[23], Lan Kluwe[24], Shunsaku Takayanagi[3], Andreas von Deimling[13], Kazuhiko Ishii[3], Martin Benesch[19], Hideaki Imai[25], Matija Snuderl[26], Stephan Frank[27], Koichi Ichimura[28], Christian Hagel[5], Viktor F. Mautner[29], Stefan Rutkowski[1], Shota Tanaka[3], Hiroyuki Aburatani[4], Saito Nobuhito[3], Ulrich Schüller[2,5]; [1]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [2]Research Institute Children's Cancer Center Hamburg, Hamburg, Germany. [3]Department of Neurosurgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan. [4]Genome Science and Medicine Laboratory, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan. [5]Institute of