inant negative form of *Gli2* indeed hampered tumorigenesis. Targeting GLI2 with arsenic trioxide caused extended survival of tumor-bearing animals, indicating GLI2 as a critical regulator of ZFTA fusion-positive tumorigenesis as well as a potential therapeutic vulnerability in these tumors.

EPEN-04. SIOP EPENDYMOMA I: FINAL RESULTS, LONG TERM FOLLOW-UP AND MOLECULAR ANALYSIS OF THE TRIAL COHORT: A BIOMECA CONSORTIUM STUDY

Timothy A. Ritzmann[1,2], Rebecca J. Chapman[1], Donald Macarthur[2], Conor Mallucci[3], John-Paul Kilday[4,5], Nicola Thorp[6], Piergiorgio Modena[7], Marzia Giagnacovo[7], Rob Dineen[1,2], Timothy Jaspan[2], Kristian W. Pajtler[8,9], Thomas S. Jacques[10,11], Simon M.L. Paine[2,1], David W. Ellison[12], Eric Bouffet[13], and Richard G. Grundy[1,2]; [1]The University of Nottingham, Nottingham, UK, [2]Nottingham University Hospitals NHS Trust, Nottingham, UK, [3]Alder Hey Children's NHS Foundation Trust, Liverpool, UK, [4]Royal Manchester Children's Hospital, Manchester, UK, [5]The University of Manchester, Manchester, UK, [6]The Clatterbridge Cancer Centre, Liverpool, UK, [7]ASST Lariana General Hospital, Como, Italy, [8]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany, [9]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany, [10]UCL GOS Institute of Child Health, London, UK, [11]Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK, [12]St. Jude Children's Research Hospital, Memphis, TN, USA, [13]The Hospital for Sick Children, Toronto, Canada

Introduction: Surgery and radiotherapy are established childhood ependymoma treatments. The efficacy of chemotherapy has been debated. We report final results of the SIOP Ependymoma I trial, with 12-year follow-up, in the context of a *post-hoc* analysis of more recently described biomarkers. Aims and Methods: The trial assessed event free (EFS) and overall survival (OS) of patients aged three to 21 years with non-metastatic intracranial ependymoma, treated with a staged management strategy targeting maximum local control. The study also assessed: the response rate (RR) of subtotally resected (STR) disease to vincristine, etoposide and cyclophosphamide (VEC); and surgical operability. Children with gross total resection (GTR) received radiotherapy of 54 Gy in 30 daily fractions over six weeks, whilst those with STR received VEC before radiotherapy. We retrospectively assessed methylation and 1q status alongside *hTERT*, RELA, Tenascin C, H3K27me3 and pAKT expression. Results: Between 1999 and 2007, 89 participants were enrolled, 15 were excluded with metastatic (n=4) or non-ependymoma tumours (n=11) leaving a final cohort of 74. Five- and ten-year EFS was 49.5% and 46.7%, OS was 69.3% and 60.5%. 1q gain was associated with poorer EFS (p=0.002, HR=3.00, 95%CI 1.49–6.10). *hTERT* expression was associated with worse five-year EFS (20.0% Vs 83.3%, p=0.014, HR=5.8). GTR was achieved in 33/74 (44.6%) and associated with improved EFS (p=0.006, HR=2.81, 95% confidence interval 1.35–5.84). There was an improvement in GTR rates in the latter half of the trial (1999-2002 32.4% versus 2003-2007 56.8%). Despite the protocol, 12 participants with STR did not receive chemotherapy. However, chemotherapy RR was 65.5% (19/29, 95%CI 45.7–82.1). Conclusions: VEC exceeded the pre-specified RR of 45% in children over three years with STR intracranial ependymoma. However, cases of inaccurate stratification at treating centres highlights the need for rapid central review. We also confirmed associations between 1q gain, *hTERT* expression and outcome.

EPEN-05. MUTATIONAL ANALYSIS OF THE C11ORF95 DOMAIN AND SINGLE-CELL RNA-SEQ PROFILE OF A MOUSE MODEL OF SUPRATENTORIAL EPENDYMOMA

Kevin Truong[1], James He[1], Gavin Birdsall[1], Ericka Randazzo[2], Jesse Dunnack[3], and Joseph LoTurco[1]; [1]University of Connecticut, Storrs, CT, USA, [2]Vanderbilt University, Nashville, TN, USA, [3]University of California, Berkeley, Berkeley, CA, USA

We used a recently developed mouse model to better understand the cellular and molecular determinants of tumors driven by the oncogenic fusion protein C11orf95-RELA. Our approach makes use of in utero electroporation and a binary transposase system to introduce human C11orf95-RELA sequence, wild type and mutant forms, into neural progenitors. We used single cell RNA-seq to profile the cellular constituents within the resulting tumors in mice. We find that approximately 70% of the cells in the tumors do not express the oncogene C11orf95-RELA and these non-oncogene expressing cells are a combination of different non-tumor cell cell-types, including significant numbers of T-cells, and macrophages. The C11orf95-RELA expressing tumor cells have a unique transcriptomic profile that includes both astrocytic and neural progenitor marker genes, and is distinct from glioblastoma transcriptomic profiles. Since C11orf95-RELA is believed to function through a combination of both activation of NF-κB response genes by constitutive activation of RELA, and genes not activated by NF-κB, we assessed the expression of NF-κB response genes across the populations of cells in the tumor. Interestingly, when tumor cells highly expressing C11orf95-RELA were analyzed further, the subclusters identified were distinguished by upregulation of non-NF-kB pathways involved in cell proliferation, cell fate determination, and immune activation. We hypothesized that the C11orf95 domain may function to bring RELA transcriptional activation to inappropriate non-NF-κB targets, and we therefore performed a point mutation analysis of the C11orf95 domain. We found that mutations in either of the cysteines or histidines that make up a possible zinc finger domain in C11orf95 eliminate the ability of the fusion to induce tumors. In cell lines, these loss-of-function point mutants still trafficked to nuclei, and activated NF-κB pathways. We are currently using RNAseq and CRISPR loss-of function to identify genes downstream of C11orf95-RELA that are required for tumorigenesis.

EPEN-06. CELL ECOSYSTEM AND SIGNALING PATHWAYS OF PRIMARY AND METASTATIC PEDIATRIC POSTERIOR FOSSA EPENDYMOMA

Rachael Aubin, Emma Troisi, Adam Alghalith, MacLean Nasrallah, Mariarita Santi, and Pablo Camara; University of Pennsylvania, Philadelphia, PA, USA

Childhood ependymoma is a cancer of the central nervous system with a chronic relapsing pattern. In children, 90% of ependymal tumors occur intracranially where prognosis is grim. Standard care for this disease includes surgical resection followed by radiation. Despite several clinical trials, adjuvant chemotherapies have yet to extend patient survival, highlighting a need for more effective treatment options. Ependymal tumors have been stratified into nine molecular subgroups based on their DNA methylation profile. The most prevalent and aggressive pediatric subgroup is known as posterior fossa ependymoma type A (PFA) which represents approximately 60% of pediatric cases and has a 5-year progression free survival rate of 30%. Whole genome sequencing studies have revealed that PFA tumors rarely harbor recurrent mutations. To inform the potential development of new treatment options for this disease, we sought to decipher the specific mechanisms leading to the tumorigenesis, progression, and metastasis of PFA tumors. By means of single-nuclei RNA-seq and an array of computational methods, we show that the expression profile of PFA tumor cells recapitulate the developmental lineages of radial glia in neurogenic niches, and is consistent with an origin in LGR+ stem cells and a pro-inflammatory environment. In addition, our analysis reveals the abundance of a mesenchymal cell population expressing TGF-β signaling, reactive gliosis, and hypoxia-related genes in distal metastases from PFA tumors. Taken together, our results uncover the cell ecosystem of pediatric posterior fossa ependymoma and identify WNT/β-catenin and TGF-β signaling as candidate drivers of tumorigenesis for this cancer.

EPEN-07. SINGLE-CELL RNA SEQUENCING IDENTIFIES A UNIQUE MYELOID SUBPOPULATION ASSOCIATED WITH MESENCHYMAL TUMOR SUBPOPULATION IN POOR OUTCOME PEDIATRIC EPENDYMOMA

Andrea Griesinger[1,2], Kent Riemondy[1], Andrew Donson[1,2], Nicholas Willard[1,2], Eric Prince[1,2], Faith Harris[1,2], Vladimir Amani[1,2], Enrique Grimaldo[1,2], Todd Hankinson[1,2], Richard Grundy[3], Andrew Jackson[3], Nicholas Foreman[1,2], and Timothy Ritzmann[3]; [1]CU Anschutz Medical Campus, Aurora, CO, USA, [2]Children's Hospital Colorado, Aurora, CO, USA, [3]University of Nottingham, Nottingham, UK

We have previously shown immune gene phenotype variations between posterior fossa ependymoma subgroups. PFA1 tumors chronically secrete IL-6, which induces secretion of myeloid cell IL-8 and pushes the infiltrating myeloid cells to an immune suppressive function. In contrast, PFA2 tumors have a more immune activated phenotype associated with a better prognosis. The objective of this study was to use single-cell(sc) RNAseq to descriptively characterize the infiltrating myeloid cells. We analyzed approximately 8500 cells from 21 PFA patient samples. Using advanced machine learning, we identified eight myeloid cell subpopulations with unique gene expression profiles. Interestingly, only one subpopulation was significantly enriched in PFA1 tumors. This subpopulation, denoted as the hypoxia myeloid subpopulation, was defined by genes associated with angiogenesis, response to hypoxia, wound healing, cell migration, neutrophil activation and response to oxygen levels. These myeloid cells also share similar gene expression profile to a mesenchymal tumor subpopulation (MEC) enriched in PFA1 and associated with poor outcome in EPN patients. This tumor subpopulation was the only population expressing IL-6. Using immunohistochemistry, we found the hypoxia myeloid located in regions of tumor necrosis and perivascular niches. The MEC cells were also more abundant in these regions. In an independent single-cell cytokine re-

lease assay, we identified eight subpopulations of functional myeloid cells. One subpopulation significantly secreted IL-8, which represented the hypoxia subpopulation based on IL-8 gene expression in the scRNAseq dataset. This data suggests the tumor necrosis resulting in the development of MEC tumor subpopulation is driving the immune suppressive myeloid phenotype in PFA1 tumors through polarization of myeloid cells to the hypoxia subpopulation. Further studies are needed to determine how these myeloid cells interact with the lymphocyte subpopulations and whether they contribute to the progression of PFA1 EPN.

EPEN-08. THE TREM1 POSITIVE HYPOXIC MYELOID SUBPOPULATION IN POSTERIOR FOSSA EPENDYMOMA

Nicholas Willard[1,2], Andrew Donson[1,3], Timothy Ritzmann[4], Richard Grundy[4], Andrew Jackson[4], Todd Hankinson[1,2], Andrea Griesinger[1,3], and Nicholas Foreman[1,1]; [1]Children's Hospital Colorado, Aurora, CO, USA, [2]University of Colorado Hospital, Aurora, CO, USA, [3]CU Anschutz Medical Campus, Aurora, CO, USA, [4]University of Nottingham, Nottingham, UK

We have previously shown the importance of immune factors in posterior fossa ependymoma (PF EPN). Recently, we found eight transcriptionally unique subpopulations of myeloid cells infiltrating PF EPN with one population particularly enriched in PFA1 tumors. This subpopulation, denoted as hypoxia myeloid subpopulation, is defined by genes associated with angiogenesis, hypoxia response, wound healing, cell migration, neutrophil activation, and response to oxygen levels. TREM1 (Triggering receptor expressed on myeloid cells 1) was found to be expressed almost exclusively within this hypoxia myeloid subpopulation. TREM1 encodes for a receptor belonging to the immunoglobulin superfamily that is expressed on myeloid cells, and stimulates neutrophil and monocyte inflammatory responses. However, single-cell RNAseq give little data suggesting location of cells within the tumor microenvironment. We performed immunohistochemistry (IHC) on our bank of ~90 FFPE PFA EPN samples using TREM1 to characterize and identify the location of the hypoxia myeloid cells. The TREM1 positive cells have an ambiguous cytomorphology reminiscent of a monocyte with modest cytoplasm and a mono-lobated nucleus. IHC also showed that TREM1+ myeloid cells are largely localized to the interface of necrosis and viable tissue, most frequently in a perivascular and intravascular distribution. The latter finding suggests that the TREM1+ cells are derived from the bone marrow and that they may be associated with the mesenchymal tumor population (MEC), which we have previously described as being enriched in PFA1 tumors and localizing to perinecrotic zones. This is supported by parallel IHC analysis of subpopulation-specific markers in the same cohort of PFA EPN which showed the highest TREM1 correlation was with CAIX, a marker of MEC. In PFA matched primary/recurrent pairs, the proportion of TREM1+ cells were increased at recurrence in the majority of cases, suggesting an evolving interaction between this TREM1+ hypoxia myeloid subpopulation and neoplastic cells over the disease course.

EPEN-09. SUPER ENHANCER GENES AS MOLECULAR TARGETS IN C11ORF95-RELA FUSION EPENDYMOMA

Kelsey Bertrand[1] and Stephen Mack[2]; [1]Texas Children's Hospital, Houston, TX, USA, [2]Baylor College of Medicine, Houston, TX, USA

Genomic sequencing has driven precision-based oncology therapy; however, genetic drivers remain unknown or non-targetable for many malignancies, demanding alternative approaches to identify therapeutic leads. Ependymomas comprise histologically similar tumor entities driven by distinct molecular mechanisms, such as fusion oncoproteins, genome-wide chromosomal instability, or disruption of DNA methylation patterns. Despite these differences, ependymomas resist chemotherapy and lack available targeted agents for clinical trial development. Based on our previous findings, we hypothesized that mapping chromatin landscapes and super enhancers (SE) could uncover transcriptional dependencies as targets for therapy (Mack, Pajtler, Chavez et al., Nature, 2018). To functionally test the requirement of these SE genes for ependymoma cellular growth, we designed a pooled RNA interference screen against 267 SE associated genes. Our screen was performed in one *C11ORF95-RELA*-fusion model and two PF-EPN-A models as controls in biological triplicates. As an indication that our screen was successful, positive controls scored among lead hits including *KIF11, BUB1B, PHF5A and MYC*. Importantly, we identified many subtype specific dependencies in both *C11ORF95-RELA*-fusion and PF-EPN-A models, thus revealing novel pathways that potentially govern subgroup-specific ependymoma cell growth. Further, several candidates detected across all ependymoma lines were also identified as pan-cancer dependencies or glioma/glioblastoma specific essential genes from the DepMap Cancer Dependency Gene Resource. Our findings reveal novel targets and pathways that are essential for ependymoma cell growth, which may provide new insight into therapeutic strategies against these aggressive brain tumors.

EPEN-10. UNRAVELLING THE TUMOR IMMUNE MICROENVIRONMENT OF POSTERIOR FOSSA A EPENDYMOMAS ON RNA AND PROTEIN EXPRESSION LEVELS

Fenna F. Feenstra[1], Friso Calkoen[1], Johan M. Kros[2], Lennart Kester[3], Mariëtte Kranendonk[3], Jasper van der Lugt[1], and Dana A. M. Mustafa[2]; [1]Department of Pediatric Neuro-oncology, Princess Maxima Medical Center, Utrecht, Netherlands, [2]Department of Pathology, the Tumor Immuno-Pathology Laboratory, Erasmus University Medical Center, Rotterdam, Netherlands, [3]Department of Pathology, Princess Maxima Medical Center, Utrecht, Netherlands

Background: Ependymomas account for 8–10% of pediatric brain tumors, and the standard therapy of surgery and radiation has not changed for the past two decades. Characterization of the tumor immune microenvironment (TIME) is of great importance in order to find better therapies. However, the TIME of ependymomas is still not defined. In this retrospective observational study we aimed to unravel the TIME of ependymomas at mRNA and protein expression levels. Methods: Ependymoma samples from two locations were selected: Posterior Fossa (PF-A, n=8), and supratentorial (ST, n=5). Targeted gene expression profile using the PanCancer immune profile panel of NanoString technology was performed. Data were analyzed using the nSolver software. In addition, 8 samples were subjected to RNA bulk sequencing, and the sequenced data were connected to the expression data of the same samples. To validate some of the findings, immunohistochemistry was performed. Results: Unsupervised hierarchical clustering showed that PF-A ependymomas can be divided into two groups based on the expression of their immune-related genes. PF-A that showed high immune-expression clustered closely to the ST ependymomas. Significant expressions of genes related to "antigen-processing" and "adhesion" pathways were found in the immune-active groups. On the contrary, the PF-A that had low expressions of immune-related genes showed a high expression of *BMI1* that has a prognostic and therapeutic value. Connecting gene expression to bulk sequence data validated the findings. In addition, immunohistochemical analysis confirmed that protein expression for some of the findings. Conclusion: The TIME varies in ependymomas based on the location of the tumor. Moreover, the immune-related expression profiles indicated that PF-A ependymomas can be divided into two groups: immune-active and immune-not active PF-A. The prognostic and therapeutic values of the immune activity of PF-A should be further studied.

EPEN-11. TUMOR DIFFERENTIATION IMPACTS THE BIOLOGY OF RECURRENCE IN CHILDHOOD POSTERIOR FOSSA EPENDYMOMA

Andrew Donson[1], Timothy Ritzmann[2], Nicholas Willard[1], Andrea Griesinger[1], Vladimir Amani[1], Faith Harris[1], Enrique Grimaldo[1], Bridget Sanford[1], Kent Riemondy[1], Todd Hankinson[1], Richard Grundy[2], and Nicholas Foreman[1]; [1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [2]University of Nottingham, Nottingham, Nottinghamshire, UK

Ependymoma (EPN) of childhood is curable in only 50% of cases, with recurrences in the remainder that are refractory to treatment. In recent years significant advances have been made in understanding the molecular and cellular biology of EPN. Recent studies show that PFA subgroup EPN are comprised of multiple neoplastic subpopulations that show undifferentiated, differentiated and mesenchymal characteristics. These studies focused on tumor at presentation, with recurrent EPN being less well understood. In the present longitudinal study we examine changes in neoplastic cell heterogeneity in serial presentations of PFA EPN using deconvolution (Cibersort) of bulk RNAseq data. Analysis of a cohort of 48 PFA EPN presenting at Children's Colorado showed survival and PFA1/PFA2 subtype assignment was associated with the proportion of individual neoplastic subpopulations as determined by deconvolution. Tumors that subsequently regrew had a significantly higher estimated proportion of undifferentiated EPN cells (UEC) at presentation, than those that were non-recurrent after 5 years follow-up. This outcome association potentially age related, as UEC proportions are significantly higher in PFA arising in children < 1 year old who have a particularly poor prognosis. Changes in PFA neoplastic subpopulations at recurrence was performed in two cohorts of patients from Children's Colorado (n=23) and Nottingham, UK (n=15). As a whole, no subpopulation proportion was significantly changed at recurrence. However, separation of PFA into subtypes PFA1 and PFA2 revealed an increase in the proportion of the cilia-differentiated EPN cell subpopulation is more frequent event in PFA1 (15/24), and rare in PFA2 (2/11). Changes in other neoplastic subpopulations at recurrence were smaller and only seen in PFA1, both UEC and mesenchymal subpopulations being lower at recurrence. In summary, only PFA1 showed dynamic changes in neoplastic subpopulation proportions at recurrence, with potential impacts on transcriptomic based-subgroup assignment, whereas PFA2 proportions remained largely stable.