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-