potent chemotherapeutic agents is feasible and safe; it validates stability of newly-designed NPs containing SN38 in the used vehicle, and it shows Rotarod testing to be an effective tool for studying transitory functional changes related to toxicity in the pons and cerebellum of rats. The lack of efficacy in treating HSJD-DIPG-007 tumors identifies important new starting points for further research.

EPENDYMOMA

EPND-01. THE ACTIVE REGULATORY LANDSCAPE OF EPENDYMAL TUMORS

Stephen Mack^{1,2}, Kristian Pajtler³, Lukas Chavez³, Stefan Pfister³, Marcel Kool³, Michael Taylor², and Jeremy Rich¹; ¹Cleveland Clinic, Cleveland, OH, USA, ²The Hospital for Sick Children, Toronto, ON, Canada, ³DKFZ, Heidelberg, Germany.

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 tumour entities driven by distinct molecular mechanisms, such as fusion oncoproteins, genome-wide chromosomal instability, or disruption of DNA methylation patterns. Despite these differences, ependymomas commonly resist chemotherapy and lack available targeted agents for clinical trial development. In the case of genomically balanced ependymomas, and those driven by fusion oncoproteins, we hypothesized that the chromatin landscapes could uncover oncogenes that would inform actionable targets for therapy and reveal specific transcriptional circuitries to identify the molecular origins of the disease. To this end, we mapped active chromatin landscapes in 42 primary ependymomas in discovery and validation cohorts using H3K27 acetylation ChIP-seq. Enhancer regions revealed novel oncogenes, molecular targets, and pathways, which when subjected to small molecule inhibitor or shRNA treatment, increased survival and halted proliferation in mouse and neurosphere patient-derived models of ependymomas. Reconstruction of enhancer networks permitted the identification of core transcription factors (TFs) that establish ependymoma cell state, and lineage-specifying TFs that dictate molecular subgroup identity. Lineage-associated TFs point to distinct spatio-temporal origins of ependymoma subgroups such as FOXJ1 TF activity and expression observed preferentially in subsets of hindbrain ependymomas. To translate our results into a potential clinical paradigm, we demonstrate cancer dependencies on super enhancer associated genes and lineage TFs, and establish the utility of chromatin landscape analysis to predict novel targets for cancer therapy.

EPND-02. GENOMIC COPY NUMBER ALTERATIONS IN EPENDYMOMA

Smadar Avigad^{1,2}, Yulia Margolin Miller^{1,2}, Suzana Fichman-Horn^{2,3}, Meora Feinmesser^{2,3}, Shalom Michowitz⁴, Hendrik Witt^{5,6}, Stefan M. Pfister^{5,6}, Marcel Kool^{5,7}, Kristian Pajtler^{5,7}, Isaac Yaniv^{1,2}, and Helen Toledano^{1,2}; ¹Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel, Petah Tikva, Israel, ²Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, ³Pathology Department, Rabin Medical Center, Petah Tikva, Israel, ⁴Department of Neurosurgery, Schneider Children's Medical Center of Israel, Petah Tikva, Israel, ⁵Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, ⁶Department of Pediatric Hematology Oncology, Heidelberg University Hospital, Heidelberg, Germany, ⁷German Cancer Consortium (DKTK), German cancer Research Center (DKFZ), Heidelberg, Germany.

Ependymoma is the third most common malignant pediatric tumor of the central nervous system. Poor outcome and unpredictable behavior of this tumor in children along with insufficient therapeutic approaches have created the need to better understand the biology of ependymoma. DNA copy number alterations (CNAs) are a hallmark of cancer that can drive the expression of oncogenes or delete tumor suppressor genes. CNAs were analysed in 34 primary ependymoma primary tumor samples and in 10 paired primary-relapse samples. Copy number alterations were identified in eighteen primary tumor samples (53%). The average number of CNAs was 3.9 alterations per tumor. The most common CNAs identified were: 1q gain (28%), 8 gain (33%) and 10p loss (28%). Progression Free Survival (PFS) of patients harboring chromosome 8 gain was 0% versus 31% for those with normal copy number ($p=0.016$). Using the cut off of under or equal/above 5 alterations per tumor, could identify a group of patients with an increased risk of 4.8 fold to progress ($p=0.005$). PFS of patients with ≥ 5 CNAs was 10% versus 32% for those with less than 5 CNAs ($p=0.026$). According to correlation between CNAs and clinical parameters, high number of CNAs is more common in high grade tumors ($p=0.015$) and in patients over 3 years

old ($p=0.017$). In the paired primary-relapse samples, number of CNAs in relapse tumor samples was higher than in primary tumors (6.5 vs. 3.9 alterations, respectively). Of the ten paired samples analyzed: three had no CNAs and were identical, two harbored the identical CNA changes between primary and relapse samples and in the remaining 5 clonal evolution was apparent. In two out of the five, the original clone acquired additional changes and in three, the relapse clone was completely different. We suggest CNAs as a new independent prognostic factor for relapse.

EPND-03. RNA-SEQ GENE EXPRESSION ANALYSIS OF 106 FORMALIN-FIXED PARAFFIN-EMBEDDED PAEDIATRIC EPENDYMOMAS

Timothy Ritzmann¹, Hazel Rogers¹, Andrew M Donson⁴, Rebecca Chapman¹, Lisa Storer¹, Jennifer Ward¹, Alex Virasami², Thomas S Jacques², Simon ML Paine^{3,1}, Nicholas Foreman⁴, and Richard G Grundy^{1,3}; ¹The Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, UK, ²UCL Great Ormond Street Institute of Child Health, London, UK, ³Nottingham University Hospitals NHS Trust, Nottingham, UK, ⁴Morgan Adams Foundation Pediatric Brain Tumor Research Program, Children's Hospital Colorado, Aurora, CO, USA.

BACKGROUND: Although ependymoma is the second most common malignant childhood brain tumour, obtaining frozen samples from extended cohorts is challenging. A large proportion of research tissue is stored in formalin-fixed, paraffin-embedded (FFPE) blocks, some many decades old. Use of these for RNA sequencing would facilitate biological studies of this enigmatic tumour. **AIMS:** 106 historical ependymomas were analysed to investigate the feasibility and challenges of using FFPE samples for RNA-seq and whether previously described gene expression patterns from frozen material would be replicated. **METHODS:** Ethics approval and consent were obtained. Pathologist confirmed ependymoma samples were collected from UK centres. 14 cases had matched frozen samples. RNA was extracted using the Qiagen FFPE Allprep kit and 1000ng per sample underwent whole transcriptome library preparation. Sequencing had a target depth of 50 million paired-end reads. Following quality control, samples were aligned using TopHat2. Differential expression analysis (DEA) was performed using EdgeR. **RESULTS:** Libraries were produced from all samples. The proportion of FFPE reads aligning to the genome was less than for frozen equivalents (54.2%) and showed higher inter-sample variation. Interestingly, samples with lower alignment to the genome had more reads aligning to bacterial sequences, suggesting potential contamination of the blocks. FFPE had more adapter read through when compared to the frozen samples. Clustering demonstrated 4 groups; poor quality samples, supratentorial tumours and two posterior fossa (PF) tumour groups. DEA of the PF groups showed good concordance with PFA and PFB gene expression patterns. **CONCLUSIONS:** RNA sequencing of historical FFPE ependymomas replicates subgroups. The benefit of investigating a wider range of samples has to be weighed against some compromise on quality. Researchers must be aware of the potential for bacterial contamination. Greater sequencing depth and more samples in addition to care over storage may help ameliorate quality issues.

EPND-04. INVESTIGATING THE BIOLOGICAL CONSEQUENCES OF PI3K PATHWAY INHIBITION ON PAEDIATRIC EPENDYMOMA TUMOURS

Jasper Estranero, Caitlyn Ascough, Holly Duane, Rebecca Chapman, Hazel Rogers, and Richard Grundy; Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, UK.

INTRODUCTION: Paediatric ependymoma has a relatively poor prognosis with current therapies yielding suboptimal outcomes due to these tumours' general chemoresistant nature. We have identified that the PI3K pathway, a cell survival signalling cascade implicated in many malignancies, was activated in 72% of primary ependymomas, suggesting it is a viable therapeutic target to treat these tumours. Pathway inhibition using a pan-PI3K enzyme inhibitor BKM120 reduced cell viability of ependymoma cell lines which supports its fundamental role in ependymoma pathogenesis. **METHODS:** This project will purposely expand on previous work to further elucidate the PI3K pathway's influence on ependymoma development. We treated a panel of ependymoma cell lines with BKM120 as a tool to inhibit canonical PI3K/AKT/mTOR signalling. Assays including western blot and cell death assays were performed to analyse the biological effects of PI3K signalling loss on ependymoma behaviour. Comparison of expression data of BKM120-treated and non-treated cells through microarray analysis will enable a global identification of molecular targets associated with disease progression. **RESULTS:** Our western blot data showed that BKM120 inhibited mTOR signalling, a known PI3K pathway target, as indicated by the decrease in pS6 levels. Apoptotic responses were absent based on the lack of cleaved caspase-3 whereas reduction in p62 expression suggests that autophagy was induced. Other cancer studies have shown that autophagy can be cytoprotective rather than cytotoxic. To determine the relevant effect in ependymoma, we inhibited BKM120-mediated

autophagy with Chloroquine (CQ). BKM120/CQ combination resulted in a cell viability increase suggesting that autophagy activation is detrimental to ependymoma survival. Gene expression analysis is ongoing to further elucidate downstream pathway targets in ependymoma cells. **CONCLUSION:** PI3K pathway inhibition triggers cellular mechanisms that oppose survival responses in ependymoma cells. The consequent adverse effects in these tumours implicates the pathway's oncogenic role, highlighting its therapeutic value for the clinical management of ependymoma.

EPND-05. LYSINE SPECIFIC DEMETHYLASE-1 (LSD-1) INHIBITOR SYC-836 IN COMBINATION WITH RADIATION PROLONGS ANIMAL SURVIVAL IN PATIENT-DERIVED ORTHOTOPIC XENOGRAFT (PDOX) MODEL OF POSTERIOR FOSSA EPENDYMOMA

Sibo Zhao, Huiyuan Zhang, Lin Qi, Holly Lindsay, Yuchen Du, Mari Kogiso, Frank Braun, Sarah Injac, Lazlo Perlaky, Donald W. Parsons, Murali Chintagumpala, Adekunle Adesina, Yongcheng Song, and Xiao-Nan Li; Baylor College of Medicine, Houston, TX, USA.

BACKGROUND: Recent molecular subgrouping of ependymoma (EPN) has identified one group, posterior fossa A (PFA), to have one of the worst prognosis and driven by epigenetic changes, suggesting targeting epigenetic changes in PFA EPN can potentially be effective. In this study, we examined the therapeutic efficacy of SYC-836, a novel LSD-1 inhibitor compound developed at Baylor College of Medicine, in PDOX models of posterior fossa EPN. **METHODS:** To examine *in vitro* anti-tumor activities, paired primary cultured cells (both as attached cells and neurospheres) from a posterior fossa EPN cell line (ICb-4423EPN) were subjected to SYC-836 at various concentrations (0-25uM). Cell viability and proliferation were measured over 14 days. To validate the drug's *in vivo* efficacy, two established posterior fossa EPN PDOX models, ICb-4423EPN and ICb-2002EPN, were utilized. 40 eight weeks old SCID mice per model were implanted with tumor cells and divided into 4 treatment groups each: 1) control, 2) radiation, 3) SYC-836 only, and 4) combination (radiation + SYC-836). Animal survival times were analyzed using log rank analysis. Changes of histone lysine methylation were examined through western hybridization. **RESULTS:** SYC-836 demonstrated effective cell killing *in vitro* in both time- and dose-dependent manner. *In vivo* experiment was completed in 1 of the 2 EPN PDOX models with the second model ongoing. There were no survival benefit with either XRT only ($P=0.205$) or SYC-836 only ($P=0.186$) when compared to the control group; however, when used in combination, the treatment strategy lead to significant improvement in animal survival ($P=0.004$). SYC-836 was well tolerated in mice. **CONCLUSION:** Our data showed that combining SYC-836 with current standard therapy of radiation synergistically prolongs animal survival significantly. SYC-836 may have a role in the clinical setting by either reducing radiation dosages, or be an adjuvant agent to other chemotherapy drugs in our treatment approach for ependymoma.

EPND-06. IDENTIFICATION OF DISTINCT MOLECULAR CHARACTERISTICS IN PEDIATRIC SPINAL EPENDYMOMA

Anbarasu Lourdasamy¹, Li Luo², Lisa Storer¹, Kenneth Cohen², Linda Resar², and Richard Grundy¹; ¹Children's Brain Tumour Research Centre, School of Medicine, University of Nottingham, Nottingham, UK, ²The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Pediatric spinal ependymomas (pSP) are exceedingly rare central nervous system (CNS) tumors. Children with these tumours are likely to experience a more aggressive disease course, with a higher rate of local failure following treatment, and a higher rate of CNS metastasis, compared to adults. Because these tumours are uncommon, the molecular basis of pSP is poorly characterized. Microarray analysis of 66 pediatric ependymomas showed distinct expression patterns and detected up-regulation of several genes involved in development-related processes for spinal tumours. In addition, multiple biological processes that are less known for their involvement in pSP, oxidative phosphorylation, cellular respiration, electron transport, and cofactor metabolic process were also detected. When we compared the expression profiles of pSP to adult spinal ependymomas (aSP), we found a very low number of differentially expressed genes (12.5%) shared between pSP and aSP. Analysis of microRNA (miRNA) expression arrays on an overlapping cohort of 49 patients showed that six miRNAs (miR-196a, miR-27b, miR-23b, miR-144*, and miR-10a and 10b) were up-regulated and four (miR-26a, and miR-885-5p, miR-124, and miR-153) were down-regulated in pSP. The integrative miRNA-mRNA network and predicted miRNA target analysis revealed that the miR-23-27 cluster (miR23b/miR27b) coordinately repressed expression of cell differentiation genes (ACVR1C, CBFA2T3, GPM1, and PKDCC), and the miR-10 family (miR10a/10b) that reside within the Hox clusters of developmental regulators, repressed expression of CAM2, HDAC4, IL1RAPL1, and MTSSL1. Furthermore, these miRNA-mRNA interactions were not detected in other cancers when we conducted the TCGA data analysis across ten different cancer types. Further investigation of gene expression and DNA methylation profiles in a larger cohort of

pediatric ependymomas is on going, and will establish specific molecular characteristics of pSP. The identification of these candidate genes/miRNAs provides an evidence-based rationale for functional studies that will help to interrogate the initiation and progression of pSP.

EPND-07. MOLECULAR HETEROGENEITY AMONG PEDIATRIC POSTERIOR FOSSA EPENDYMOMA

Kristian Pajtler¹, Ji Wen², Martin Sill¹, Tong Lin², Jens Hübner¹, Vijay Ramaswamy³, Chandanamali Punchihewa², David Jones¹, Hendrik Witt¹, Lukas Chavez¹, Ruth Tatevossian², Richard Grundy⁴, Thomas Merchant², Michael Taylor³, Stefan Pfister¹, Andrey Korshunov¹, Marcel Kool¹, and David Ellison²; ¹German Cancer Research Center, Heidelberg, Germany, ²St. Jude Children's Research Hospital, Memphis, TN, USA, ³Hospital for Sick Children, Toronto, ON, Canada, ⁴Queen's Medical Center, Nottingham, UK.

Previously, we have identified nine distinct molecular groups of ependymoma across all age groups, three in each major anatomical compartment of the CNS: spinal, posterior fossa, and supratentorial. These nine molecular groups are genetically, epigenetically, transcriptionally, demographically, and clinically distinct. Pediatric intracranial ependymomas are either affiliated with the RELA and YAP1 supratentorial groups or with the posterior fossa PFA group. RELA and YAP1 ependymomas harbor recurrent and distinct fusion genes that drive the disease, but PFA ependymomas lack significant recurrent mutations, and the specific oncogenic events underlying these tumors remain undefined. Observing distinct outcomes among children with PFA ependymomas, we tested the hypothesis that further molecular diversity with clinical utility, including possible novel genetic alterations, may exist among these tumors. Genome-wide DNA methylation profiles of 681 pediatric PFA ependymomas were analyzed by unsupervised consensus hierarchical clustering and t-distributed stochastic neighbor embedding (tsne), which identified not only three major distinct molecular subgroups of PFA ependymoma: PFA-1, PFA-2, and PFA-3, but additional small subgroups that segregate within PFA-1 and PFA-2. Subgroups were characterized by distinct clinical and genetic characteristics; most PFA-3 tumors harbored 1q gain and occurred in children significantly older than those with PFA-1 and PFA-2 tumors. Some of the small subgroups demonstrated distinctive copy number alterations or gene mutations, e.g. H3 K27M. Outcome data revealed considerable heterogeneity among the different subgroups, overall survival at 5 years was >90% for one subgroup, but <20% for two others. We conclude that further molecular refinement of pediatric PFA ependymomas has clinical utility and the potential for enhanced risk assessment or therapeutic stratification. In addition, identification of PFA subgroups and further molecular heterogeneity within these subgroups may help to identify the drivers of PFA ependymomas.

EPND-08. EPIGENETIC H3K27ME3 DEREGULATION IN PEDIATRIC POSTERIOR FOSSA EPENDYMOMAS

Sriram Veneti¹, Jill Bayliss¹, Piyali Mukherjee², Chao Lu⁹, Siddhant Jain³, Chan Chung¹, Pooja Panwalkar¹, Daniel Martinez^{4,7}, Benjamin Sabari⁹, Ashley Margol⁵, Melike Pekmezci⁶, Marcin Cieslik¹, Benita Tamrazi⁵, Benjamin Garcia⁷, Mariarita Santi^{4,7}, Peter Lewis³, Cynthia Hawkins⁸, David Allis⁹, Arul Chinnaiyan¹, and Alexander Judkins⁵; ¹University of Michigan, Ann Arbor, MI, USA, ²Weill Medical College of Cornell University, New York, NY, USA, ³University of Wisconsin, Madison, WI, USA, ⁴Children's Hospital of Philadelphia, Philadelphia, PA, USA, ⁵Children's Hospital Los Angeles, Los Angeles, CA, USA, ⁶University of California, San Francisco, San Francisco, CA, USA, ⁷University of Pennsylvania, Philadelphia, PA, USA, ⁸The Hospital for Sick Children, Toronto, ON, Canada, ⁹The Rockefeller University, New York, NY, USA.

Ependymomas in children occur most commonly in the posterior fossa of the brain and cause significant morbidity and mortality. The pathogenesis of these tumors remains obscure as recent whole genome and whole exome sequencing efforts have not yielded recurrent genetic mutations. These tumors exhibit abnormal CpG island methylation, suggesting that epigenetic alterations may be a significant driver. To gain insights into the epigenetics of childhood ependymomas, we performed mass spectroscopy for histone modifications to discover that H3K27 trimethylation was globally reduced in these tumors. Despite global reduction in H3K27me3, ChIP-sequencing for H3K27me3 revealed genomic enrichment at several loci important for neurodevelopment. In a cohort of 195 childhood posterior fossa ependymoma samples, reduction in H3K27me3 was associated with worse prognosis ($p<0.0001$). Comparison of DNA methylation and genome-wide H3K27me3 enrichment from both H3K27M mutant diffuse intrinsic pontine gliomas and childhood ependymomas patient samples showed many similarities and pointed to factors that regulate radial glial (neural stem cells) development. Human radial glial cells during development exhibit low H3K27me3. Ependymomas with reduced H3K27me3 corresponded to younger children ($p<0.0001$), arose mainly from the roof of the fourth ventricle, exhibited increased evidence of radiologic and histopathologic invasion