EPEN-20. PDL1 EXPRESSION AND TUMOR-INFILTRATING IMMUNE CELL SUBPOPULATIONS: THE ROLE OF IMMUNE-CHECKPOINT MOLECULES IN THE PROGNOSIS OF PATIENTS WITH SUPRATENTORIAL EPENDYMOMA AND ITS CORRELATION TO SURVIVAL

RAFAT MALIK, AGRIMA SHARMA, AMANDEEP KUMAR, MANMOHAN SINGH, VAISHALI SURI, MEHAR CHAND SHARMA; AIIMS, NEW DELHI, DELHI, India

Immune checkpoint blockade of the programmed cell death ligand-1 (PD-L1)/programmed death-1 (PD-1) axis has shifted the way advanced cancers are treated. The 'ST-RELA fusion positive' and 'PF-A' molecular subgroups of ependymomas (EPN) have a dismal prognosis. AIMS: This study is undertaken to look for immunological microenvironment in ependymomas. Based on L1CAM protein expression and/or the presence of RELA/YAP1 fusion transcripts by RT-PCR, all supratentorial (ST) Grade-II/III EPNs were classified as ST-RELA, ST-YAP, and ST-not otherwise specified (NOS). Based on H3K27me3 protein expression and chromosome 1q gain, all posterior fossa (PF) EPNs were classified as PF-A or PF-B. PD-L1 (SP263, Ventana) and CD8 immunohistochemistry was performed. The enrichment of RelA protein at the PDL1 promoter site was investigated using chromatin immunoprecipitation-qPCR (ChIP-qPCR) in three ST-RELA samples. A total of 83 intracranial EPNs were included in our preliminary data, and we discovered that PD-L1 expression correlates with increasing cytotoxic T-lymphocyte infiltrates in intracranial ependymomas using PD-L1 and CD8 IHC. The majority of PD-L1-positive intracranial EPNs are members of the ST-RELA subset, which also has the highest CTL density. Our findings suggest that ST-RELA EPNs could be used in immunotherapy to target immune checkpoint modulators. To further this research, we are looking into the presence of tumour infiltrating cells such as CD3, CD8, CD163, FOXP3, and others, which can aid in characterising the tumour microenvironment in cell types expressing PD-L1.This study will also be used to predict the prognostic significance of PD-L1/PD1 and other tumour cell infiltrating biomarkers in ependymomas and correlate it with survival outcomes.

EPEN-21. DEVELOPING A SENSITIVE METHOD FOR DETECTION OF MINIMAL RESIDUAL DISEASE IN EPENDYMOMA USING METABOLOMIC ANALYSIS OF CEREBROSPINAL FLUID

Alison Woodward[1], Laudina Amugi[2], Kishan Patel[1], Charlotte Kilpatrick[1], Dong-hyun Kim[2], Madhumita Dandapani[1]; [1]Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, United Kingdom. [2]Centre for Analytical Bioscience, School of Pharmacy, University of Nottingham, Nottingham, United Kingdom

INTRODUCTION: Ependymoma (EPN) is the second most common malignant paediatric brain tumour with poor survival and significant neuro-cognitive impairment from current treatments (surgery and radiotherapy). Relapse occurs in 50% of patients within 2 years, despite no evidence of tumour on MRI. This suggests that they have minimal residual disease (MRD) at the end of treatment. Developing an accurate MRD detection method could help select patients who would benefit from further continuation chemotherapy, thereby improving survival. There is also an unmet need for an accurate test to diagnose relapse early when the disease could be more treatable. METHODS: Pilot untargeted liquid chromatography-mass spectrometry (LC-MS) analysis was carried out in cerebrospinal fluid (CSF) samples from patients with ependymoma. CSF from patients in remission from leukemia were used as controls. RESULTS: Pilot data from analysis of CSF using LC-MS demonstrates that this is a feasible approach to characterise CSF metabolomic profile. Also, EPN CSF profile is significantly different from control CSF, with significant elevation of few key metabolites (Vitamin D derivatives and betaine) in EPN CSF compared to control CSF. Immunohistochemical analysis of EPN tumour tissue microarrays confirms the expression of betaine / one-carbon pathway enzymes such as methionine synthase and betaine—homocysteine S-methyltransferase. Further validation of CSF profile with tumour metabolomic profile and serial CSF sample profiling is currently underway. Subgroup-specific differences and targeted analysis to develop a panel of biomarkers is also being explored. CONCLUSION: Early results suggest that CSF-based metabolite profiling using LC-MS is feasible and could help detect minimal residual disease in ependymoma. Further validation is required to analyse subgroup-specific differences and correlate quantitative changes in metabolites with changing disease burden.

EPEN-22. MOLECULARLY AGGRESSIVE EPENDYMOMAS TREATED WITH IMAGE GUIDED PENCIL BEAM PROTON THERAPY: CONSECUTIVE PATIENT INDIAN EXPERIENCE

Rishan Thimma Sudarsan, Srinivas Chilukuri, Noufal MP, Adhithyan R, Ramya Uppuluri, Pankaj Kumar Panda, Sushma Patil, Dayananda Sharma, Roopesh Kumar V R, Rakesh Jalali; Apollo Proton Cancer Centre, Chennai, Tamilnadu, India

AIM: To assess the toxicities and early clinical outcomes in patients of primary and recurrent ependymomas treated with image guided pencil beam scanning proton beam therapy (PBS-PBT). MATERIALS AND METHODS: Between January 2019- December 2021, we analyzed consecutive patients of ependymomas treated with image guided PBS-PBT. They were also analyzed molecularly, and all recurrent/re-irradiated patients were considered for craniospinal irradiation (CSI). Acute toxicities were assessed based on NCI CTC v5.0, local control and radiological response by 3-monthly MRI post therapy. RESULTS: Seventeen consecutive patients with ependymoma (13 Grade III and 4 Grade II) (median age-9 years) were analyzed. 9 patients were primary, 7 were treated at first recurrence and 1 at second recurrence. Majority had posterior fossa (PF) tumors (12) followed by supratentorial (ST) (3) and spine (2). Among PF ependymomas, 10 had global loss of H3K27me3, whereas amongst ST, 1 had YAP1 fusion, 1 had L1CAM positivity and other, negative L1CAM and p65/RELA on immunohistochemistry. Gross/near-total resection was achieved in 87% patients. Primary ependymomas were treated with focal radiotherapy; and among recurrent cases, 7 received CSI followed by primary site boost to a total median dose of 55CGE (50.2-55.8CGE). Grade 2 dermatitis, grade 2 and 3 hematological toxicities (CSI) were noted in 3,2 and 2 patients respectively. Grade 2 fatigue noted in 53% of all and 71% receiving CSI. With a median follow-up of 10 months (2-24 months), local control (LC) and disease-free survival (DFS) was 87.5% (primary-100%, recurrent-75%) and overall survival (OS) was 94% (primary-100%, recurrent-88%). CONCLUSION: Our experience of treating patients of ependymoma with complete resection followed by PBS-PBT including CSI is encouraging with low acute toxicities. Recurrent/molecularly aggressive cases may be considered for CSI.

Keywords: PT, proton beam therapy, ependymomas, primary and recurrent

EPEN-23. INTERACTION OF EPIGENETIC REGULATION AND TELOMERASE RE-ACTIVATION IN HIGH-RISK EPENDYMOMA

Carola N. Jaunecker[1], Dominik Kirchhofer[1], Sibylle Madlener[2], Anna Laemmerer[1,2], Lisa Gabler[1,3], Christine Pirker[1], Martin Piontek[1,2], Kendra Maaß[4], Konstanitin Okonechnikov[4], Bernhard Englinger[5,6], Li Jiang[5,6], Lisa Mayr[2], Daniel Senfter[2], Sabine Spiegl-Kreinecker[7], Natalia Stepien[2], Christian Dorfer[3], Mariella Filbin[5,6], Marcel Kool[4,8], Johannes Gojo[2], Walter Berger[1], Daniela Loetsch-Gojo[3]; [1]Center for Cancer Research, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria, Austria. [2]Department of Pediatrics and Adolescent Medicine, Comprehensive Cancer Center and Comprehensive Center for Pediatrics, Medical University of Vienna, Vienna, Austria, Austria. [3]Department of Neurosurgery, Medical University of Vienna, Vienna, Austria, Austria. [4]Hopp Children's Cancer Center (KiTZ) and Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Heidelberg, Germany, Germany. [5]Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. [6]Department of Pediatric Oncology, Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, Massachusetts, USA. [7]Department of Neurosurgery, Kepler University Hospital GmbH, Johannes Kepler University, Linz, Austria, Austria. [8]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands, Netherlands

Ependymomas (EPN) account for 10% of pediatric CNS tumors. Among the ten subgroups characterized by DNA methylation profiling, tumors located in the supratentorial region that harbor *ZFTA* fusions (e.g. ZFTA-RELA), and tumors in the posterior fossa region group A (PF-A) represent the most aggressive entities. As currently therapy success relies on the extent of tumor resection and druggable targets are so far widely missing, new therapeutic approaches are urgently needed. Epigenetic dysfunction, resulting in aberrant histone modifications as well as altered DNA methylation patterns, majorly contributes to the aggressiveness of high-risk EPN. In earlier studies, we discovered that high-risk EPN is composed of a cellular hierarchy initiating from stem-cell like populations, frequently showing telomerase re-activation. Considering that epigenetic mechanisms regulate stemness maintenance and telomerase reverse transcriptase (TERT), we studied the impact of epigenetically active drugs on differentiation and telomerase re-activation in these tumors. Accordingly, we first investigated the basal expression levels of TERT and EZH2 in a panel of patient-derived high-risk EPN cell models of different subtypes (n=7). Interestingly, both, TERT and EZH2, were highly expressed predominantly in ZFTA-RELA cell models. Corroboratively, increased sensitivity of ZFTA-RELA cells towards the EZH2 inhibitor DZNep was observed in cell viability and clonogenic assays. While HDAC inhibitors were similarly active across high-risk EPN cell models, the BET inhibitor JQ1 more efficiently reduced survival of ZFTA-RELA cells. Treatment with DZNep resulted in a loss of H3K27me3 histone marks accompanied by decreased ubiquitination of H2AK119 in the investigated ZFTA-RELA cell models, and induced apoptosis indicated by PARP cleavage. Currently, impacts of direct or pharmacological EZH2 blockade on *TERT* promoter methylation, induction of senescence and differentiation are analyzed. Summarizing, we proof varying efficacy of epigenetically active drugs in high-risk EPN subgroups, in particular EZH2 inhibition in ZFTA-RELA cell models.