MEDB-24. TUMOUR SECRETED EXTRACELLULAR MATRIX PREDICTS SURVIVAL AND INFLUENCES MIGRATION AND CELL DEATH IN SHH MEDULLOBLASTOMA 3D MODELS

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Medulloblastoma is the most common malignant paediatric brain tumour. Four molecular sub-groups exist (WNT, Sonic hedgehog (SHH), Group 3 and Group 4), each associated with different patterns of metastasis and chemoresistance. We have shown that within these sub-groups further clinically relevant sub-types exist, characterised by differential expression of extracellular matrix (ECM) proteins. For example, good overall survival in two-thirds of SHH sub-group patients is associated with the expression of specific ECM proteins. Our aim here is to further characterise these ECM components in SHH medulloblastoma and to determine how they confer better overall survival in this sub-group. Using a combination of 3D OrbiSIMs and immunohistochemical staining we have identified, that when grown in 3D hyaluronic acid hydrogels or as spheroids, SHH medulloblastoma cell lines form an ECM shell-like structure composed of laminin, collagen and lumican. In addition, scRNAseq of SHH hydrogel nodules revealed sub-group specific clusters of cells with high levels of ECM interaction and adhesion. We therefore hypothesise that ECM interaction restricts SHH tumour invasion and metastasis through this shell-like structure. To understand how this ECM shell-like structure potentiates better survival in SHH medulloblastoma we have created a CRISPRcas9 laminin knockout SHH medulloblastoma cell line. 3D culture of the laminin knockout cell line demonstrated that laminin is essential for the formation of 3D cell structures as well as migration in SHH medulloblastoma. Furthermore, we have identified that apoptosis is increased in the laminin knockout SHH cell line, suggesting that apoptosis-targeted therapeutics may represent a beneficial treatment option for SHH patients whose tumours exhibit this ECM shell. In summary, tumour-secreted ECM plays a major role in SHH medulloblastoma progression. Expression of laminin, collagen or lumican can be used to classify SHH patients into low and high-risk groups with different therapeutic outcomes and treatment options.

MEDB-25. DO EXTRACELLULAR VESICLES TRANSFER A MULTIDRUG RESISTANT PHENOTYPE VIA ABC TRANSPORTERS IN MEDULLOBLASTOMA?

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INTRODUCTION: Medulloblastoma is the most common malignant paediatric brain tumour accounting for 20% of all childhood tumours; approximately one-third of patients present with metastatic disease at diagnosis and the outcome for these patients remains very poor. The high frequency of recurrence and metastatic relapse in medulloblastoma supports the idea of intrinsic drug resistance within cells. This work looks at the ATP-binding cassette (ABC) transporters, known to be upregulated in several cancers, and we hypothesise that extracellular vesicles may transfer ABC transporters to surrounding cells, promoting multidrug resistance within tumours. METHODS: The Cavalli dataset, made up of 763 patient samples, was used to assess the gene expression of a number of ABC transporters across medulloblastoma subgroups. ABC transporter gene and protein expression was then further assessed in medulloblastoma cell lines, including drug-tolerant lines, using qPCR and western blot. Cell viability analysis was used to assess changes in drug response. Matched cell and EV protein samples were used for proteomic analysis. RESULTS: Patient gene expression data showed that high expression of two ABC transporters correlated with reduced patient survival in high-risk subgroups. qPCR analysis of medulloblastoma cell lines showed differential subgroup expression patterns. Additionally, qPCR analysis of drug-tolerant cell lines showed significant increases in the expression of specific drug transporters across the subgroups. RNA-seq confirmed the presence of ABC transporter mRNA in exosomes isolated from high-risk medulloblastoma cell lines. Functional studies of EV transference of ABC transporters are ongoing. CONCLUSIONS: Data to date supports the hypothesis that multidrug transporter carrying extracellular vesicles may transfer their multidrug resistant phenotype to surrounding cells in medulloblastoma, promoting drug resistance. Future work will test this hypothesis by knocking down candidate ABC transporters and assessing the effect on transference of drug resistance by extracellular vesicles.

MEDB-26. OUTCOMES OF CHILDREN WITH STANDARD-RISK AND HIGH-RISK MEDULLOBLASTOMA TREATED WITH PRE-IRRADIATION CHEMOTHERAPY AND RISK-ADAPTED CRANIOSPINAL IRRADIATION: A REPORT ON PATIENTS FROM THE POLISH PEDIATRIC NEURO-ONCOLOGY GROUP

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BACKGROUND: The last two decades have witnessed several efforts to minimize the adverse sequelae of craniospinal irradiation (CSI), a standard of care treatment modality in medulloblastoma. This has been accomplished by adding chemotherapy to the treatment backbone. The use of pre-irradiation chemotherapy has also been previously reported. In one of the largest studies to date, we analyze treatment outcomes in children with standard and high-risk medulloblastoma treated with pre-irradiation chemotherapy followed by reduced-dose radiotherapy in SR and maintenance chemotherapy. METHODS: Data from the Polish Pediatric Neuro-oncology Group (PPNG) was analyzed in patients greater than 3 years of age with newly-diagnosed medulloblastoma. RESULTS : Among 138 patients, median age at diagnosis was 7.9 years and median follow-up was 5.5 years. Comprehensive molecular subgrouping was not available for all patients at the time of data collection. Of 60 standard-risk patients, there was pre-irradiation disease recurrence in one patient. One patient expired prior to radiation due to metastatic disease. Of 78 high-risk patients, one had pre-irradiation recurrence. Overall survival (OS) for high-risk patients at 3 and 5 years (± standard error) was 89.2 ± 4.0% and 81.3 ± 5.8%, respectively. OS for standard-risk patients at 3 and 5 years was 92.5 ± 3.8% and 88.2 ± 5.1%, respectively. Among high-risk patients, event-free survival (EFS) at 3 and 5 years was 82.5 ± 5.3% and 81.0 ± 5.6%. Among standard-risk patients, 3-year EFS was 89.2 ± 4.6% and 5-year EFS was 86.8 ± 5.3%. CONCLUSION : This study demonstrates promising survival outcomes in pediatric medulloblastoma patients treated with pre-irradiation chemotherapy followed by reduced-dose CSI and adjuvant chemotherapy. Such an approach may be helpful if delays in starting radiotherapy are expected, which is usually the case in many institutions around the globe.

MEDB-27. CLINICO-RADIOLOGICAL OUTCOMES IN WNT-PATHWAY MEDULLOBLASTOMA: RETROSPECTIVE SINGLE INSTITUTIONAL AUDIT

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BACKGROUND: Medulloblastoma (MB) is a heterogeneous disease comprising 4 molecular subgroups - wingless (WNT), sonic hedgehog, Group 3, and Group 4 tumors - with distinct developmental origins, diverse clinico-demographic characteristics, unique transcriptional profiles, and widely varying outcomes. WNT-MB is associated with the best outcomes (5-year survival >90%) prompting attempts at treatment de-escalation to reduce late toxicity. We undertook a clinical audit of WNT-MB patients treated at our tertiary-care comprehensive cancer centre. METHODS: Patients with molecularly confirmed WNT-MB treated with maximal safe resection followed by post-operative standard-of-care risk-stratified adjuvant radio(chemo)therapy were identified retrospectively via electronic search of the neuro-oncology database. Data regarding clinico-demographic characteristics, histo-molecular features, treatment details, patterns of failure, and survival outcomes was retrieved from electronic medical records and/or hospital case files. Time-to-event outcomes were analyzed using Kaplan-Meier methods and compared with the log-rank test. RESULTS: Between 2004 to 2018, a total of 65 patients of WNT-MB were registered at our institute. Five patients treated on a prospective clinical trial of therapy de-intensification were excluded leaving 60 patients that constitute the present study cohort. Median age at presentation was 12 years (inter-quartile range 9-18 years) with male preponderance (2:1). Six patients (1 post-operative mortality

and 5 without adequate details of treatment or outcomes) were excluded from the survival analysis which was restricted to 54 patients. At a median follow-up of 66 months, Kaplan-Meier estimates of 5-year progression-free survival and overall survival were 87.9% and 92.8% respectively. Traditional high-risk features such as age, residual tumor (>1.5cm^2) and leptomeningeal metastases (M+) did not emerge as significant prognostic factors for survival in this molecularly-characterized WNT-MB cohort. CONCLUSION: WNT-MB patients have excellent survival outcomes irrespective of traditional high-risk features suggesting the need for more tailored and refined risk-stratification with potential de-intensification of therapy. ACKNOWLEDGEMENTS: Brain Tumor Foundation (BTF) of India

MEDB-28. CDK9 IS A DRUGGABLE MEDIATOR SUSTAINING MYC-DRIVEN CIRCUITRY IN MEDULLOBLASTOMA

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BACKGROUND: Though long recognized as a master regulator of cell proliferation across a wide range of cancers, Myc has proven elusive to direct therapeutic targeting. The CDK9-containing PTEFb, complexed with either BRD4 or SEC, facilitates Myc-driven transcriptional programs and is necessary for sustaining expression of Myc itself. Advances in development of clinical-grade CDK9 inhibitors creates an opportunity to examine this as a rational therapy for Myc-driven medulloblastoma. METHODS: We used both RNAi depletion and a panel of pharmacologic agents to characterize the mechanistic and functional consequences of CDK9 inhibition in Myc-driven medulloblastoma. We used a combination of clonogenic assays and live cell imaging to assess the cytotoxic effects of CDK9 activity loss. We then performed a combination of CUT&RUN and RNA-seq to evaluate alterations to Myc binding and downstream Myc-driven transcriptional programs. Finally, we employed orthotopic xenograft models of medulloblastoma to assess CNS penetration, tolerability, and anti-tumor efficacy of lead CDK9i candidate compounds. RESULTS: Genetic or pharmacologic inhibition of CDK9 leads to a loss of Myc expression and downregulation of hallmark Myc-driven transcriptional programs. This corresponds to a loss of cell fitness, as measured by decreased proliferation and clonogenic potential. Clinically relevant CDK9 inhibitors show variable efficacy in vivo, but the CNS-penetrant zotiraciclib achieved a significant prolongation in xenograft survival. CONCLUSION: CDK9 catalytic activity represents a druggable vulnerability underpinning Myc-driven transcriptional programs. The development of CNS-penetrant CDK9 inhibitors may open new avenues for rational therapy in these high-risk medulloblastomas.

MEDB-29. APPLICATION OF ROTTERDAM POST-OPERATIVE CEREBELLAR MUTISM SYNDROME PREDICTION MODEL TO PATIENTS OPERATED FOR MEDULLOBLASTOMA IN A SINGLE INSTITUTION

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BACKGROUND: Post-operative cerebellar mutism syndrome (CMS) develops in up to 30% of children. The Rotterdam model (RM) predicts a 66% risk of CMS in patients with a score ≥100. However, our findings suggested that surgical experience contributes to CMS risk. The aim of this study was to retrospectively apply the RM and report incidence of CMS in high-risk patients from our institution. METHODS: Participants had to have first tumor resection at our institution and be enrolled on SJMB12 protocol (NCT01878617). All participants got structured serial neurologic evaluations. CMS, when present, was categorized into type 1 (complete mutism) and type 2 (paucity of speech with an inability to string 3-word sentence). Rotterdam score is calculated based on pre-operative imaging parameters and study neurologist (RBK) obtained it while blinded to CMS status. RESULTS: Of the 40 (14 female, 26 male) study participants, 4 (10%) had CMS (3 CMS1, 1 CMS2). Median age at tumor resection was 11.7 years (range 3.5-17.8). Tumor location was midline in 30 (75%), right lateral 6 (15%) and left lateral 4 (10%). Median Evans index was 0.3 (0.2-0.4) and 34 (85%) were ≥0.3 (indicative of hydrocephalus); 5 participants needed ventricular shunt. Median tumor volume was 50 cm3 (2-180.6). Gross total resection was achieved in 35 (87.5%), near total in 4 (10%) and subtotal in 1. Twelve tumors were SHH, 7 WNT, and 29 NWNS. Median RM score was 90 (25 – 145). Eighteen participants had a score of ≥100 and 16.7% of these (n=3) had CMS. Scores for the 4 with CMS were 85, 125, 145 and 145. CONCLUSION: At our institution, the incidence of CMS in those that had RM of ≥100 was much lower than reported risk of 66%. This data supports our hypothesis that neurosurgical experience remains a significant risk factor in the development of CMS.

MEDB-30. SUBCLASSIFICATION OF GROUP 3/4 MEDULLOBLASTOMA AS A POTENTIAL PROGNOSTIC BIOMARKER TO REDUCE THE DOSE OF CRANIOSPINAL IRRADIATION IN PATIENTS WITH METASTATIC TUMORS: A JAPANESE PEDIATRIC MOLECULAR NEURO-ONCOLOGY GROUP STUDY

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BACKGROUND: In patients with medulloblastoma, one of the most significant challenges is to reduce the dose of craniospinal irradiation (CSI) to minimize neurological sequelae in survivors. Molecular characterization of patients treated using lower-dose CSI rather than standard therapy is important for further reducing the treatment burden. METHODS: We conducted DNA methylation analysis using an Illumina Methylation EPIC array to investigate molecular prognostic markers in 38 patients with medulloblastoma who were registered in the Japan Pediatric Molecular Neuro-Oncology Group and were treated using lower-dose CSI rather than standard-dose radiation therapy. RESULTS: Among the patients, 23 were classified as having a "standard-risk" and 15 as having a "high-risk" according to the classic classification based on tumor resection rate and presence of metastasis, respectively. The median follow-up period was 71.5 months. The median CSI dose was 18 Gy in both groups, and 10 patients in the "high-risk" group received a CSI dose of 23.4 Gy or 24 Gy. Molecular subgrouping revealed the "standard-risk" cohort included 5 WNT, 2 SHH, and 16 Group 3/4 cases; all 15 patients in the "high-risk" cohort had Group 3/4 medulloblastoma. Among the patients with Group 3/4 medulloblastoma, 13 of the 16 "standard-risk" patients were subclassified as subtypes I, IV, VI, and VII, which were associated with a good prognosis according to the novel sub-subclassification among Group 3/4 medulloblastomas. However, only 6 of the 15 "high-risk" patients were included in the subtypes. The good prognostic subtype cases among "high-risk" cohort were all survived without recurrence, in contrast to a worse prognosis (5-year progression free survival=33.3%; p=0.01) of the other cases. CONCLUSION: Although these findings require validation in a larger cohort, the present findings suggest that the novel sub-subclassification of Group 3/4 medulloblastoma may be a promising prognostic biomarker for reducing the dose of CSI in patients with metastatic medulloblastoma.

MEDB-31. THE CLINICAL SIGNIFICANCE OF EXTENT OF RESECTION IN MEDULLOBLASTOMA

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Medulloblastoma (MB) patients determined to have a sub-total resection (STR), defined by >1.5cm^2 post-surgical tumour residuum, receive intensified treatment regimes, but recently the designation of STR as a high risk feature is being questioned. We aimed to assess the clinical correlates of ex-