Title
MEDULLOBLASTOMA

Journal
Neuro-oncology, 16(Suppl 1)

ISSN
1522-8517

Authors
Morfouace, Marie
Shelat, Anang
Megan, Jacus
et al.

Publication Date
2014-06-01

Peer reviewed

Neuro-Oncology 16:i71–i96, 2014.
doi:10.1093/neuonc/nou074

NEURO-ONCOLOGY

Abstracts

MEDULLOBLASTOMA

MB-001. PRE-CLINICAL EFFICACY STUDY OF INTRA-THECAL (IT) ^{177}LU-DOTA-TATE (SOMATOSTATIN ANALOGUE) IN ATHYMIC RATS WITH LEPTOMENINGEAL DISEASE (LMD) FROM MEDULLOBLASTOMA (MBL)

Ganesan Vaidyanathan, Sri Gururangan, Darell Bigner, and Michael Zalutsky; Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, NC, USA

BACKGROUND: LMD in children with recurrent medulloblastoma and other PNETs carries a poor prognosis and novel therapies are urgently needed to improve disease control. Somatostatin receptor-2 (SSR-2)is over-expressed in medulloblastoma and other central PNETs and can serve as a target for radionuclide tagged somatostatin analogues like ^{177}Lu-DOTA-TATE that has shown considerable efficacy in adults with SSR-2 positive neuro-endocrine tumors. As a preliminary step prior to testing this agent in children with LMD, we performed an efficacy study of i.t. ^{177}Lu-DOTA-TATE in athymic rats bearing LMD from MBL. METHODS: The subarachnoid space was accessed through the animal's cervical spine and a catheter was threaded along the dorsal aspect of spinal cord to the lumbar region and injected with 1 x 10^7 D341 human MBL cells and treatment initiated 3 days later. Groups of 10 animals received a single i.t. dose of 2, 3, or 5 mCi of ^{177}Lu- DOTA-TATE or saline control. Animals were followed 300 days for survival. RESULTS: Treatment with 2 mCi resulted in an increase in median survival of 58.3% compared with saline control ($p < 0.001$). Treatment with 5.0 mCi of ^{177}Lu-DOTA-TATE increased median survival by 75.0% compared with the saline control group while a single dose of 3.0 mCi ^{177}Lu-DOTA-TATE increased median survival compared with saline controls by 519.4%. Long-term survivors were seen in 0 of 10 animals treated with saline, 4 of 11 treated with 3 mCi, and 3 of 12 treated with 5.0 mCi. CONCLUSION: Intrathecal ^{177}Lu-DOTA TATE is efficacious in controlling LMD from medulloblastoma in athymic rats. A phase I trial of this agent is being planned in children with LMD from recurrent MBL and other CNS PNETs.

MB-002. PEMETREXED AND GEMCITABINE: TWO NEW DRUGS FOR THE TREATMENT OF GROUP3 MEDULLOBLASTOMA (G3 MB)

Marie Morfouace[1], Anang Shelat[1], Jacus Megan[1], Burgess B Freeman[1], Sarah Robinson[1], Stacy Throm[1], James M Olson[3], Xiao-Nan Li[2], Kip R Guy[1], Giles Robinson[1], Clinton Stewart[1], Amar Gajjar[1], and Martine Roussel[1]; [1]St Jude Children's Research Hospital, Memphis, TN, USA; [2]Baylor College of Medicine, Houston, TX, USA; [3]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Medulloblastoma (MB), a cerebellar tumor, is the most frequent malignant pediatric brain tumor. MBs are molecularly divided into four major subgroups: Sonic Hedgehog (SHH), WNT, Group3 (G3) and G4. G3 tumors overexpressing MYC are the least curable form of medulloblastoma harboring a large cell anaplastic pathology. Despite aggressive therapies, these tumors metastasize and kill most patients. We generated a mouse model that faithfully recapitulates human G3 MB (Kawauchi et al, Cancer Cell, 2012) by overexpressing Myc in Tpr53-null granule neural progenitors. These tumors grow as neurospheres in culture, can be passaged continuously, and can form tumors, after transplantation into the brain of naïve recipient animals, retaining the same molecular profile as the tumor of origin. Using G3 tumor spheres, we screened a library of 840 FDA-approved drugs. Two drugs, pemetrexed and gemcitabine, were active against tumor spheres derived from mouse G3 MBs as well as human xenografts of G3 MB with MYC amplification or overexpression, with EC_{50} (half maximal effective concentration) lower than 100 nM. Pharmacokinetic studies of these two drugs in mouse G3 MB-bearing mice revealed tumor drug exposures to be greater than those required for in vitro cytotoxicity (Jacus et al., Eur J Pharm Sci, 2013). Administrated as single agents or together in vivo, pemetrexed and gemcitabine increased survival in mouse or human G3 MB-bearing mice. Most importantly, when administered in combination with two chemotherapeutic drugs currently in clinical use, cisplatin and cyclophosphamide, both drugs increased the survival of mice bearing mouse and human G3 MBs, but not mouse SHH MB. These data suggest that pemetrexed and gemcitabine can be added to currently used chemotherapy, with enhanced effect and little additional myelosuppressive toxicity (Morfouace et al., Cancer Cell, in press).

MB-003. OUTCOMES OF NEWLY DIAGNOSED HIGH RISK MEDULLOBLASTOMA/PNET TREATED WITH CARBOPLATIN, VINCRISTINE, CYCLOPHOSPHAMIDE, AND ETOPOSIDE

Nongnuch Sirachainan[1], Samart Pakakasama[1], Usanarat Anurathapan[1], Ake Hansasuta[2], Mantana Dhanachai[3], Chaiyos Khongkhatithum[1], and Suradej Hongeng[1]; [1]Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University,, Bangkok, Thailand; [3]Department of Radiology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University,, Bangkok, Thailand

INTRODUCTION: Medulloblastoma/PNET is the most common malignant brain tumor in children. For children older than 3 years, the treatment of high risk group includes surgery, craniospinal (CSI) radiation therapy (30-36 Gy) plus local boost radiotherapy (54-56 Gy) and adjuvant chemotherapy, such as cisplatinum, carboplatin, lomustine, cyclophosphamide, and vincristine. The results have demonstrated 5-year overall survival (OS) of 40-60%. This study aimed to evaluate the outcomes of high risk medulloblastoma/PNET patients who were treated with radiation and adjuvant chemotherapy. METHODS: Patients were diagnosed with high risk medulloblastoma/PNET according to the histopathology, medulloblastoma risk classification by an evidence of metastasis or the residual tumor more than 1.5 cm^2 and evidence of residual tumor after surgery in PNET. Treatment protocol was CSI RT 36 Gy with local boost at tumor 54-56 Gy. Two to four weeks after RT, patients received 8 courses of chemotherapy consisting of cyclophosphamide 800 mg/m^2, day 1-3 and vincristine 2 mg/m^2, week 1-3, alternated with carboplatin 200 mg/m^2, day 1-3 and etoposide 150 mg/m^2, day 1-3. RESULTS: Total of 25 patients, male: female of 2.6:1 and mean $\pm$ SD for age of 9.7 $\pm$ 3.0 years, were enrolled. The 5-year progression free survival and OS were 41.6 $\pm$ 11.7% and 61.5 $\pm$ 12.9%,respectively. The age and sex did not determine the difference in outcomes. The hematotoxic side effect, according to the National Cancer Institute's Common Terminology Criteria, were grade 4 leucopenia 60%, grade 4 neutropenia 60%, grade 4 anemia 20%, grade 4 thrombocytopenia 16%, grade 3 leucopenia 20%, grade 3 neutropenia 20%, grade 3 anemia 40%, and grade 3 thrombocytopenia 36%. Febrile neutropenia was found in 11 patients (44%). CONCLUSION: The present study demonstrated the similar outcomes of high risk medulloblastoma/PNET with the previous studies. Although, the grade 3 and 4 hematologic toxicity was high, no treatment related death was found.

MB-004. mTORC2/Akt SIGNALING IS MODULATED BY NONCANONICAL MITOCHONDRIAL Notch1/PINK1 INTERACTION IN myc-AMPLIFIED MEDULLOBLASTOMA TUMORIGENESIS

Abdullah Feroze[2], Kyu-Sun Lee[3], Sharareh Gholamin[2], Zhihao Wu[3], Bingwei Lu[3], Siddhartha Mitra[1], and Samuel Cheshier[2]; [1]Institute of Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA; [2]Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA; [3]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

Medulloblastoma is known to be the most malignant pediatric brain tumor. Given the limited therapies, a budding focus on the role of mitochondrial dysregulation in the tumorigenesis of such pathologies merits consideration. Mitochondria are known to play fundamental roles in multiple processes conserved across eukaryotic species. Aside from their key role in energy production through oxidative phosphorylation, the organelles also serve as the sites of essential metabolic pathways, redox regulation, calcium homeostasis, apoptosis, and cell fate determination and differentiation. Recently, we documented the role of noncanonical Notch signaling and mitochondrial involvement in adult glioblastoma brain tumor-initiating cells (GenesDev Lee et al., 2013). . Although the canonical Notch pathway and is generally well-characterized, the role of a second noncanonical pathway, where Notch1 can function independently of ligand binding remains to be fully elucidated. The regulatory self-renewal versus differentiation choice of Drosophila and mammalian human neural stem cells requires Notch signaling, and in our work, we found noncanonical Notch pathway interaction with the PTEN-induced kinase 1 (PINK1) to influence mitochondrial function, activating mTORC2/Akt signaling. siRNA-induced knockdown preferentially impaired the maintenance of Drosophila and human

glioblastoma cancer stem cell-like tumor-forming cells to a far greater degree than normal stem cell counterparts. We have elucidated similar findings in medulloblastoma. In medulloblastoma sub group 3 patient derived primary neurosphere lines we observed an increased Notch1-Pink1 interaction in the mitochondria as compared to normal human cells. additionally siRNA knockdown of PINK1 expression strongly co-related with decreased mTORC2 activity and decreased cellular proliferation. We are currently investigating the role of this interaction in medulloblastoma tumoregenesis in an orthotopic mouse xenograft model. Such results underscore the importance of mitochondria in both normal and cancer stem cell biology, a highly conserved mechanism across species, with exciting implications for the treatment of pediatric central nervous system malignancies.

MB-005. STRUCTURAL VARIANTS SHUFFLE CHROMATIN TO ACTIVATE GFI1 FAMILY ONCOGENES IN MEDULLOBLASTOMA

Paul Northcott[1], Catherine Lee[2], Thomas Zichner[3], Peter Lichter[1], Jan Korbel[3], Robert Wechsler-Reya[2], Stefan Pfister[1], and ICGC PedBrain Tumor Project[1]; [1]German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Sanford Burnham Medical Research Institute, La Jolla, CA, USA; [3]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Medulloblastoma is a highly malignant pediatric brain tumour currently treated with a combination of surgery, radiation, and non-specific chemotherapy, posing a significant threat to the developing child. Genomics has illuminated extensive intertumoural heterogeneity and identified at least four distinct molecular subgroups of the disease. Group 3 and Group 4 subgroup medulloblastomas account for the majority of pediatric cases, yet, oncogenic drivers for these subtypes remain poorly understood. Exome and genome sequencing studies have confirmed a paucity of recurrent gene-level mutations in Group 3 and Group 4, suggesting that alternative oncogenic mechanisms must account for the large fraction of cases that currently remain unexplained. Analysis of whole-genome sequencing data from 128 primary Group 3 and Group 4 medulloblastomas facilitated a systematic, high-resolution screen for chromosomal breakpoints recurrently targeting novel drivers by structural variation. This approach identified highly disparate genomic structural rearrangements, restricted to Groups 3 and 4, resulting in specific and mutually exclusive activation of the growth factor independent 1 family proto-oncogenes, GFI1 & GFI1B. Diverse mechanisms of structural variation, including duplications, deletions, inversions, translocations, and other complex genomic variants were observed in nearly all GFI1/1B-activated cases. Comprehensive characterization of these structural variants and integration with enhancer histone mark ChIP-sequencing data established that GFI1/GFI1B expression becomes activated through relocation of their coding sequences to genomic regions of transcriptionally active chromatin. Functional analyses performed in mice confirmed the oncogenicity of Gfi1/Gfi1b in the context of medulloblastoma and demonstrated apparent synergy between both of these candidates and the c-Myc oncogene. These studies establish GFI1 and GFI1B as novel, highly prevalent medulloblastoma oncogenes specifically active in Group 3 and Group 4. Given their high frequencies of activation, GFI1 and GFI1B represent excellent candidates for prioritization of molecularly targeted therapy aimed at treatment of a significant proportion of Group 3 and Group 4 medulloblastoma patients.

MB-006. miR-106b IS OVEREXPRESSED IN MEDULLOBLASTOMAS AND TARGETS PTEN

Kay Ka-Wai Li[1], Tian Xia[1], Fanny Man Ting Ma[1], Rong Zhang[3], Liangfu Zhou[3], Kin-Mang Lau[1], and Ho-Keung Ng[1]; [1]Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, Shatin, N.T., Hong Kong; [2]Shenzhen Research Institute, The Chinese University of Hong Kong, ShenZhen, China; [3]Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai, China

MicroRNAs (miRNAs) are an abundant group of small non-coding RNAs that have been implicated in tumorigenesis. They regulate expression of target genes by complementary base pairing. In this study, we analyzed expression of miR-106b in 32 medulloblastoma (MB) samples by quantitative RT-PCR. We applied gain- and loss-of-function strategies to delineate the functional roles of miR-106b in MB. Luciferase reporter assay was conducted to confirm target gene of miR-106b. Expression of miR-106b was overexpressed in MB, and was significantly associated with its host gene MCM7 ($p = 0.020$). Kaplan-Meier survival analysis revealed that high expression of miR-106b was associated with significantly shorter progression-free survival ($p = 0.016$) and overall survival ($p = 0.011$). Transfection of miR-106b inhibitor in MB cell lines markedly reduced cell proliferation, migration and invasion potential, and tumor sphere formation. Cell cycle analysis indicated that miR-106b inhibition induced G1 arrest and apoptosis. The cell cycle regulators, p21 and cyclin D1, and apoptotic marker cleaved PARP were differentially expressed in miR-106b inhibitor-transfected cells. The tumor suppressor PTEN was identified as a direct target gene of miR-106b. Luciferase reporter assay confirmed miR-106b directly interacted with the 3'UTR of PTEN. We found miR-106b directly targeted PTEN at transcriptional and translational levels. Immunohistochemistry revealed a trend between PTEN and miR-106b in MB tumors ($p = 0.07$). These data suggested the upregulation of miR-106b in MB and the involvement of miR-106b in MB biology.

MB-007. LONG TERM FOLLOW UP OF INFANTS WITH MEDULLOBLASTOMA TREATED WITH SEQUENTIAL HIGH DOSE CHEMOTHERAPY

Lucie Lafay-Cousin[1], Susan Chi[2], Jennifer Madden[3], Amy Smith[4], Elisabeth Wells[5], Emily Owens[4], Douglas Strother[1], Nicholas Foreman[3], Roger Packer[5], and Eric Bouffet[6]; [1]Alberta Children Hospital, Calgary, AB, Canada; [2]Dana Farber Cancer Institute, Boston, MA, USA; [3]Children's Hospital Colorado, Denver, CO, USA; [4]Arnold Palmer Hospital for Children, Orlando, CA, USA; [5]Children's National Medical Center, Washington, DC, USA; [6]Hospital for Sick Children, Toronto, ON, Canada

BACKGROUND: High dose chemotherapy strategies were developed to avoid the use of craniospinal irradiation and prevent unacceptable neurotoxicity in infants and young children. However, long-term outcome, including neurocognitive outcome, of this approach has not been widely described. METHODS: This retrospective study collected data from 6 institutions, on young children with medulloblastoma who received high-dose Carboplatin, Thiotepa according to the protocol CCG99703 between 1998-2012. Data on chemotherapeutic management, adjuvant radiation, ototoxicity and neurognitive evaluations and survival were collected. RESULTS: There were 47(25 males) patients diagnosed at a median age of 24.5 months(2.9-63.2). Nineteen(39.6%) had metastatic disease and 30(62.5%) underwent gross total resection(GTR). Fifteen(31.3%) had nodular desmoplastic(ND) subtype. Three patients received intrathecal chemotherapy, 6 received HD MTX during induction and 7 underwent maintenance chemotherapy post HDC. Fifteen patients received radiation, including 9(18.7%) in an adjuvant setting. Complete continuous remission(CCR) rates after induction and after consolidation were respectively 66.7% and 75 %. Two patients died of treatment related toxicity. Thirty seven patients are alive at a median follow-up of 3.7 years from diagnosis with a projected 5-year PFS and OS of respectively 68.4%($\pm$7.5)and 76.4%($\pm$6.6). GTR, M0&M1 stage, ND histology, and CCR were significantly associated better PFS, but only CCR post consolidation and M0&M1 stage remained significant for better OS. Non irradiated children had a PFS compare to those who received radiation(5y PFS 82.3% versus 45%$p = 0.017$). Severe ototoxicity ($\geq$ Brock grade 3) was present in 23.3% of 30 evaluable patients. Nine required hearing support. Neurocognitive assessements were available in 19 patients (51%). Mean FSIQ for the cohort was 91 (range 67-119). CONCLUSION: Young children with MB treated with this strategy have an encouraging OS (76.4 %). Less than 20% of the patients received adjuvant radiation. Although the ototoxicity of this regimen was significant, neurocognitive profile of the survivors appears to be within normal range.

MB-008. CHROMOSOME ENGINEERING AND STEM CELL DIFFERENTIATION TECHNOLOGIES TO MODEL SOMATIC COPY NUMBER ABERRATIONS IN MEDULLOBLASTOMA

Takafumi Wataya[2], John Peacock[1], and Michael D. Taylor[1]; [1]The Hospital for Sick Children, Toronto, ON, Canada; [2]Shizuoka Children's Hospital, Shizuoka, Japan; [3]Faculty of Medicine, Kyoto University, Kyoto, Japan

OBJECTIVE: Medulloblastoma is the most common childhood malignant brain tumor and a major cause of oncologic death in children. Recent progress in genetic analysis has revealed a number of chromosome abnormalities in medulloblastoma. Chromosome 17 aberrations including loss of 17p, gain of 17q and isochromosome i17q, are seen across medulloblastoma subgroups. A chromosome "17p deletion" knock-out mouse model has been developed, however, this model is embryonic lethal at an early stage of development. It is unclear if neuronal precursors can be generated when mouse 17p equivalent region is deleted. Mouse embryonic stem cells cultured with serum-free floating culture of embryoid body-like aggregates (SFEBq) methods can be differentiated into many cell types of the brain including cerebellar cells. We aimed to create in vitro assay system as a model of medulloblastoma with chromosome 17 aberrations. METHODS: To make specific cerebellar cells, the SFEBq method was optimized for use with chromosome engineered mouse embryonic stem cells harboring an 18 million bp deletion equivalent to human 17p deletion. Cultured spheroids were immunostained with cerebellar specific markers to determine differentiation efficiency. Ki67 staining was used to analyze proliferative status. RESULTS: Mouse ES cells

with 17p equivalent deletion were successfully differentiated into cerebellar precursors in vitro. Immunoflorescence staining of SFEBq-cultured cells with Math1, Neph3 and L7 demonstrates "17p deletion" ES cells give rise to granule cell precursor, purkinje cells and other GABAergic neurons. 17p deletion cells were more proliferative in Ki67 staining comparing to the parental floxed cells without 17p deletion. CONCLUSION: SFEBq allows us to generate cell types of the cerebellum chromosome engineered with a mouse equivalent 17p deletion. These cells are more proliferative but insufficient to cause medulloblastoma. The addition of oncogene overexpression or gene supression will allow us to model the events that transform cerebellar progenitors into medulloblastoma.

MB-009. A NOVEL, THREE-DIMENSIONAL (3D) SPHEROID CO-CULTURE MODEL OF MEDULLOBLASTOMA AND HUMAN FOETAL BRAIN STEM CELLS, SUITABLE FOR HIGH-THROUGHPUT SCREENING OF NEW DRUG DELIVERY SYSTEMS FOR PRE-CLINICAL ASSESSMENT

Delyan Ivanov[1], Martin Garnett[1], Terry Parker[1], Cameron Alexander[1], Lisethe Meijer[1], Richard Grundy[1], Paul Gellert[2], Marianne Ashford[2], and David Walker[1]; [1]University of Nottingham, Nottingham, UK; [2]AstraZeneca, Macclesfield, UK

INTRODUCTION: Local interstitial or intra-cerebrospinal fluid (intra-CSF) delivery bypasses the blood-brain barrier, minimising systemic exposure and potentially reducing radiotherapy-induced neurotoxicity. Preclinical testing of enhanced local drug delivery systems can help refine these strategies maximising anti-tumour effect while minimising toxicity. AIMS: To develop a pre-clinical 3D co-culture system of medulloblastoma and human foetal stem cells in order to establish a therapeutic window of etoposide exposure during local drug delivery through high-throughput testing of efficacy and neurotoxicity. METHODS: We have developed a bio-representative 3D human spheroid co-culture in-vitro model of medulloblastoma using the cell line UW228-3 and human foetal brain stem cells marked with two supra-vital fluorescent dyes, cultured together in ultra-low attachment 96-well plates, forming reproducible single co-culture spheroids ($d = 500\ \mu m$, $CV\% = 10\%$). Spheroids were exposed to concentrations of etoposide (0.3-100 μM), spanning CSF concentrations for systemic versus local delivery, based upon human pharmacokinetic data. Upon spheroid dissociation, flow cytometry quantified the absolute numbers of each cell population and determined their viability, separately. Multi-photon fluorescence microscopy was used to make qualitative assessment of tumour and foetal cell viability and distribution. RESULTS: Both the UW 228-3 tumour cells and normal foetal brain stem cells were easily distinguished permitting assessment of their numbers and viability. A concentration of etoposide of ~ 10 μM was shown to maximise tumour cytotoxicity (5% viability), while sustaining a four-fold higher viability of human foetal brain cells. This concentration exceeds that achievable, clinically, by systemic administration by ~10 fold and the AUC(-0-48) has been selected in the calculation of a ceiling concentration for a phase 1 trial of infusional intra-CSF etoposide, seeking to assess feasibility and safety of sustained intra-CSF drug delivery to leptomeningeal metastases. CONCLUSIONS: This proof-of-concept study offers the opportunity to perform relevant, pre-clinical assessment of novel drug delivery systems targeting brain tumours, with high clinical relevance.

MB-010. TREATMENT RELATED TOXICITIES IN CHILDREN WITH MEDULLOBLASTOMA

Julie Brent, Fathima Zumla Cader, Daniel Ford, Andrew Kay, Richard Walsh, Guirish Solanki, Andrew Peet, and Martin English; Birmingham Children's Hospital, Birmingham, UK

BACKGROUND: Medulloblastoma is the commonest malignant brain tumour in children. Current standard treatment for children >3 years involves surgical excision, radiotherapy then adjuvant chemotherapy with cisplatin, lomustine, and vincristine (PCV) for 8 cycles. Survival has improved, but with significant acute and chronic toxicity. We reviewed patients treated in our unit to quantify this. METHODS: Children diagnosed with medulloblastoma over an 11 year period between 2001 and 2011 treated at our hospital were identified. Records of patients >3 years old who had received PCV chemotherapy were reviewed. Chang Stage M1 +, residual disease $>1.5\ cm^2$, and anaplastic or large cell histology were considered high-risk factors. RESULTS: Sixty-four patients were identified. Twenty-two fulfilled the above criteria (11 high risk, 11 standard risk). Their overall 5-year survival was 60%. Patients received either conventional (13/22) or hyperfractionated accelerated (9/22) cranio-spinal radiotherapy. Acute toxicities from cisplatin occurred in 16/21 patients (data unavailable for 1) and required carboplatin substitution. Oto-toxicity was seen in 11/16 and nephrotoxicity in 5/16. Seven proceeded to have all platinum omitted from further treatment. Vincristine and lomustine toxicity occurred in 13/21 and 7/21 respectively, requiring omission or dose reduction. All 22 patients had late effects; hearing loss (20/22 with two requiring hearing aids), hypothyroidism (8/22), growth hormone deficiency (7/22), panhypopituitarism (5/22), cataracts (2/22), learning difficulties (2/22), focal motor difficulty (2/22), cerebellar dysfunction (2/22), and posterior fossa syndrome (1/22). RELEVANCE: Children treated for medulloblastoma with surgical resection, radiotherapy and adjuvant PCV chemotherapy develop significant late effects. Future challenges will be continuing to improve survival whilst reducing these long-term consequences of treatment.

MB-011. INTRACELLULAR AND EXTRACELLULAR microRNAs IN MEDULLOBLASTOMA

Tarek Shalaby, Giulio Fiaschetti, Sylvain Baulande, Nicolas Gerber, Martin Baumgartner, and Michael Grotzer; University Children's Hospital, Zurich, Switzerland

Although the majority of microRNAs exist within cells, a number of microRNAs have been found extracellular in body fluids including CSF. The role of both intracellular and extracellular microRNAs has been established in preclinical models of human brain cancers; however their contribution to medulloblastoma biology remains poorly understood. Using qRT-PCR analysis, we examined in medulloblastoma the expression levels of the intracellular miR-21 and miR-9 that typify tumor promoter and suppressor microRNAs successively. MiR-9 expression was found to be downregulated in 22/29 medulloblastoma tissues and in all six medulloblastoma cell lines tested. MiR-9 suppression was reversed through treatment with DNA demethylating agent indicating a causal relationship between miR-9 methylation and its low expression in medulloblastoma cells. Importantly MiR-9 expression significantly correlated with unfavorable histopathological variants and with poor clinical outcome. On the other hand miR21 was found to be up-regulated in 29/29 medulloblastoma samples and 6/6 medulloblastoma cell lines. miR-21 inhibition decreased protein expression of the invasion mediator MAP4K1 and increased expression of the metastasis suppressor PDCD4. Remarkably miR-21 suppression decreased the motility of medulloblastoma cells and reduced their migration. To disclose the identity of key circulating miRNA in the CSF of MB patients, we used hybridization arrays of 1719 miRNAs to compare their expression profiles to control subjects. Circulating miRNA profiling identified 86 microRNAs that were differentially expressed in the CSF of medulloblastoma patients compared to control. 15 /86 mRNAs are known to be highly expressed in medulloblastoma tissues. In summary our research highlighted the importance of intracellular miR-9 and miR-21 in medulloblastoma biology and drew attention to the value of investigating extracellular microRNAs inS the CSF of medulloblastoma patients which could be useful in finding a tumor at an early stage or in monitoring metastasis, specially as drawing CSF postoperatively is a routine procedure in the medulloblastoma staging process.

MB-012. A CASE OF MEDULLOEPITHELIOMA TREATED BY HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT)

Tomomi Hayase[1], Yuta Kawahara[1], Masaki Yagi[1], Takaomi Minami[1], Nobuyuki Kanai[3], Takehiko Yamaguchi[3], Akira Gomi[2], and Akira Morimoto[1]; [1]Department of Pediatrics, Jichi Medical University, Shimotsuke, Tochigi, Japan; [2]Department of Pediatric Neurosurgery, Jichi Children's Medical Center Tochigi, Shimotsuke, Tochigi, Japan; [3]Department of Pathology, Jichi Medical University, Shimotsuke, Tochigi, Japan

INTRODUCTION: Medulloepithelioma of the central nervous system (CNS) is a rare primitive neuroectodermal tumor occurring in early childhood. It is characterized by highly malignant behavior. Due to its rarity, the optimal management is still unknown. We report the case of CNS medulloepithelioma successfully treated by high-dose chemotherapy with autologous peripheral blood stem cell transplantation (auto-PBSCT) after gross total resection (GTR). CASE REPORT: A 2-years-old boy presented with a right hemiparesis, vomiting and drowsiness. Brain MRI revealed a huge partly cystic heterogeneous unenhanced mass in the left frontal/parietal lobe without signs of dissemination. Emergent craniotomy was performed to resolve brain herniation. Gross total resection was confirmed by postoperative MRI. Pathological diagnosis was medulloepithelioma. High-dose chemotherapy (Busulfan 4.8 mg/kg / melphalan 140 mg/m^2) with auto-PBSCT was performed after 5 courses of combination chemotherapy. Engrafment was obtained on +day9. He was discharged 1.5 months after the PBSCT. At last follow up 1.2 year after PBSCT, he is alive with no sign of relapse. DISCUSSION: Medulloepithelioma has been reported only

about 40 cases. 3- and 5-year overall survival rate is about 30%. Factors predicting poor prognosis is onset before 4-years of age and non-GTR. Although radiotherapy has been applied in the majority of cases and seems to be effective, we chose the treatment strategy including high-dose chemotherapy to avoid radiotherapy-related late effects considering his age. CONCLUSION: Radiotherapy could be replaced by high-dose chemotherapy for infants with medulloepithelioma who obtained GTR.

MB-013. MYC AND TP53 DEFECTS EMERGE AND INTERACT AT MEDULLOBLASTOMA RELAPSE, DEFINE RAPIDLY PROGRESSIVE DISEASE AND CAN BE TARGETED THERAPEUTICALLY

Rebecca Hill[1], Sanne Kuijper[2], Janet Lindsey[1], Ed Schwalbe[1], Karen Barker[2], Jessica Boult[2], Daniel Williamson[1], Zai Ahmad[2], Albert Hallsworth[2], Sarra Ryan[1], Evon Poon[2], Simon Robinson[2], Ruth Ruddle[2], Florence Raynaud[2], Louise Howell[2], Colin Kwok[2], Abhijit Joshi[3], Sarah Leigh Nicholson[1], Stephen Crosier[1], Stephen Wharton[4], Keith Robson[5], Antony Michalski[6], Darren Hargrave[6], Thomas Jacques[7], Barry Pizer[8], Simon Bailey[1], Fredrik Swartling[9], Kevin Petrie[2], William Weiss[10], Louis Chesler[2], and Steven Clifford[1]; [1]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK; [2]The Institute of Cancer Research, Sutton, UK; [3]Department of Cellular Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, UK; [4]Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK; [5]Children's Brain Tumour Research Centre, Queen's Medical Centre, University of Nottingham, Nottingham, UK; [6]Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK; [7]Neural Development Unit, UCL Institute of Child Health/Department of Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK; [8]Oncology Unit, Alder Hey Children's Hospital, Liverpool, UK; [9]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden; [10]Departments of Pediatrics, Neurology and Neurosurgery, University of California, San Francisco, CA, USA

Relapse following multi-modal therapy is the most adverse event in medulloblastoma. Currently >90% of relapsing patients die, accounting for ~10% of childhood cancer deaths. Medulloblastoma is heterogeneous at diagnosis, comprising four molecular subgroups with distinct clinicopathological and molecular features. The relevance of these features at relapse is unknown, making characterisation, modelling and targeted therapy of relapse biology essential to improve outcomes. We undertook a comprehensive investigation of the molecular and clinicopathological features of 29 relapsed medulloblastomas and paired tumour samples taken at diagnosis, including the assessment of features with established significance at diagnosis (e.g. TP53 pathway status, MYC/MYCN amplification and molecular subgroup status). Molecular subgroup was unchanged, however evidence of alteration of all other features examined was found at relapse, with the majority of changes (30/44) representing acquired high-risk events. Most notably, MYC gene family amplifications and TP53 pathway defects commonly emerged in combination at relapse following conventional multimodal treatment ($P = 0.02$, 7/22, 32%) and predicted rapid progression to death ($P = 0.016$). Spontaneous development of Trp53 inactivating mutations was similarly common in a transgenic model of MYCN driven medulloblastoma (GTML;Glt1-tTA/TRE-MYCN-Luc). Direct modelling of this interaction in GTML/Trp53$^{KI/KI}$ mice enhanced medulloblastoma formation (100%, 43/43 vs. 6%, 3/50 in GTML; $P < 0.0001$), mimicked clinicopathological characteristics of TP53-MYC relapsed human tumours, and validated the role of TP53 in potentiating the growth of MYCN-driven medulloblastoma. Therapeutic inhibition of Aurora-A kinase using MLN8237 in these tumours, and in derived neurospheres in vitro, promoted degradation of MYCN, reduced tumour growth and prolonged survival. In summary, medulloblastomas display altered molecular, pathological and clinical features at relapse and the emergence of combined TP53-MYC gene family defects is common following conventional therapy. Their association with rapid demise, and their validation as driving and therapeutically exploitable events in a novel mouse model, strongly support routine biopsy at relapse to drive future therapeutic strategies.

MB-014. FAMILIAL MEDULLOBLASTOMA IN THREE SIBLINGS

Lidija Kitanovski[1], Tomaž Prelog[1], Barbara Faganel Kotnik[1], and Maruša Debeljak[2]; [1]University Medical Center Ljubljana, Dpt. of Paediatrics, Div. of Haematooncology, Ljubljana, Slovenia; [2]University Medical Center Ljubljana, Dpt. of Paediatrics, Unit for Special Laboratory Diagnostics, Ljubljana, Slovenia

Most medulloblastomas are sporadic, but rare familial forms have been described. To our knowledge, 12 case reports of familial MB with not more than two affected siblings in one family have been published so far. Here, we present a family of gipsy origin with six siblings, three of whom were diagnosed with medulloblastoma in their first two years of life. All three brothers were diagnosed with a localized, beta-cathenin negative, classical type MB. The first child was diagnosed at the age of sixteen months. After complete tumor removal and combined chemotherapy he progressed locally and craniospinal irradiation was used at the age of 22 months. He is in complete remission for ten years already. The second brother was diagnosed with tumour accompanied by severe cerebral oedema and severe hydrocephalus at the age of 24 months. Urgent surgery led to partial tumour resection, but the child died the day after. The last brother was diagnosed with medulloblastoma at the age of fourteen months. The tumour was completely removed. He was treated with combined chemotherapy (HIT 2000 protocol) for 6 months, followed by posterior fossa irradiation. One year later MRI revealed a tumour temporoparietally and the boy was reoperated. Tumour was completely removed, histology revealed MB. Craniospinal irradiation was given, followed by maintenance treatment with cisplatin, vincristine and CCNU, which is ongoing. Due to familial appearance the last patient was tested for germline P53 and PTCH1 mutation and found to be negative for both mutations. Further searching for possible germline mutation is warranted.

MB-015. NOTCH LIGANDS JAG1 AND JAG2 CONTROL MEDULLOBLASTOMA CELL SURVIVAL AND REPRESENT POTENTIAL PROGNOSTIC MARKERS AND THERAPEUTIC TARGETS

Giulio Fiaschetti, Tarek Shalaby, Martin Baumgartner, and Michael A. Grotzer; University Children's Hospital Zurich, Zurich, Switzerland

Medulloblastoma (MB) is the most common pediatric malignant brain cancer, arising as a pathological result of deregulated developmental pathways, such as NOTCH. To better understand the molecular mechanisms controlling aberrant NOTCH in MB, our study focused on NOTCH ligands, whose functions in MB are largely unexplored. We demonstrated that, compared to normal cerebellum, MB tumors harbor aberrant levels of NOTCH ligands: JAG1 is broadly overexpressed, whereas high JAG2 expression is restricted to MYC-driven MBs. JAG1 mediates pro-proliferative signals via activation of NOTCH2 receptor and induction of HES1 expression, a NOTCH target gene associated with poor clinical outcome. Thus JAG1 represents a novel potential therapeutic target for blocking NOTCH cascade in the majority of MB tumors. Furthermore, we identified JAG2 as novel MYC target in MB and showed that high-MYC MB cells acquire induced expression of JAG2 through MYC-induced transcriptional activation. We demonstrated that the association of JAG2 and MYC levels is specific for Group 3 MB tumors and that JAG2 mediates some oncogenic properties of MYC by participating in the regulation of the proliferation/apoptosis status of high MYC MB cells. The positive and specific correlation of MYC and JAG2 with LCA tumors and highly metastatic MB stages further strengthened the notion of a functional interaction between the two proteins, suggesting that high JAG2 expression may be useful as additional prognostic marker to identify aggressive MBs. In conclusion, this study examined for the first time the pathological roles of NOTCH ligands in MB and demonstrated that interfering with the activation mechanisms of NOTCH pathway through targeting the ligands may represent a new direction for the development of specific therapeutic strategies against MB.

MB-016. RESULTS OF HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF PEDIATRIC BRAIN TUMORS

Asmik Gevorgian[1], Elena Morozova[1], Ilya Kazantsev[1], Tatiana Iukhta[2], Svetlana Safonova[2], Ella Kumirova[3], Yury Punanov[2], Boris Afanasyev[1], and Olga Zheludkova[3]; [1]R.M. Gorbacheva Memorial Institute of Children Hematology, Oncology and Transplantation, St. Petersburg Pavlov State Medical University, Saint-Petersburg, Russia; [2]Federal State Institution N.N. Petrov Research Institute of Oncology, Saint-Petersburg, Russia; [3]Federal Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russia

AIM: Central nervous system (CNS) tumors are the second most common pediatric malignancies with an about 30% 5-year overall survival rate in high-risk group. The aim of this study was to assess the effectiveness of single or tandem high-dose chemotherapy (HDCT) with autologous hematopoietic stem-cell transplantation (auto-HSCT) in this patient group. METHODS: From February 2006 to February 2014, 40 pediatric patients with high-risk or relapsed medulloblastoma ($N = 23$), germ cell tumors ($N = 5$), and other embrional tumors ($N = 12$) received HDCT with auto-HSCT after induction chemotherapy, radiotherapy and surgical treatment. At the moment of HDCT 16 patients were in complete remission

(CR), 17 patients were in partial remission (PR) and 7 patients had stable disease (SD). RESULTS: The median follow-up after auto-HSCT is 15 months (range, 1–96), the median time to engraftment was day +15 (range, 12–30). The following therapy toxicity was observed: liver toxicity grade 3-4 (N = 16), skin toxicity grade 3-4 (N = 9), severe mucositis grade 3-4 (N = 23), nausea/vomiting grade 3-4 (N = 11), infectious complications grade 3-4 (N = 24). Four patients died of toxicity. Overall survival (OS) in all groups was 52% and disease free survival (DFS) was 49%. DFS was significantly better among high-risk patients in 1st CR compared to patients in 2nd or following CR: 65% and 36%, accordingly (p = 0,02). Patients in CR or PR at the moment of HDCT had better DFS rate than patients in SD: 61%, 53% and 25% (p = 0,00), respectively. Medulloblastoma and germ cell tumors had better survival rate (DFS 63% and 60%, respectively) in compared to other embrional tumors (DFS 45%, p = 0,05). CONCLUSIONS: HDCT with auto-HSCT in pediatric patients with high-risk CNS tumors may be a feasible option for patients in CR or PR after induction chemotherapy. It is ineffective as a salvage therapy in refractory patients.

MB-017. RETICULIN-POSITIVE DESMOPLASTIC/NODULAR TUMOURS ARE ASSOCIATED EXCLUSIVELY WITH SHH GROUP OF MEDULLOBLASTOMA

Wieslawa Grajkowska, Maciej Pronicki, Bozena Cukrowska, Bozenna Dembowska-Baginska, and Maria Lastowska; The Children's Memorial Health Institute, Warsaw, Poland

OBJECTIVE: Recent investigations revealed an association between transcriptional subtypes and morphological features in medulloblastoma. Since both characteristics are of prognostic significance, a precise correlation between them should be well established. Therefore we re-examined paediatric nodular medulloblastoma tumours for correlation with molecular subtypes of disease. METHODS: Paediatric patients with previously diagnosed desmoplastic/nodular (D/N) or medulloblastoma with extensive nodularity (MBEN) histopathology were re-analysed by two neuropathologists. In addition to H&E-stained slides, reticulin preparations were simultaneously analysed from the same FFPE blocks. For identification of transcriptional subtypes of tumours immunohistopathological analyses were performed using a panel of representative antibodies. MYCC amplification was detected by FISH. RESULTS: Altogether 28 tumours with original MBEN or D/N diagnosis where molecular subtypes could be determined were identified. All tumours with MBEN histology belonged to SHH group and displayed distinctive reticulin-positive internodular reaction. However, only ~60% of tumours with original D/N diagnosis were reticulin-positive. They belonged to SHH type, were mainly infantile and patients are still alive. Among reticulin-negative tumours only two were of the SHH type and were subsequently reclassified as classic and anaplastic tumours with pseudonodules. Importantly, all remaining reticulin-negative tumours with a presence of nodules in H&E staining belonged to the non-WNT,SHH type. Therefore the original diagnosis was again reclassified as classic or anaplastic tumours with pseudonodules. Patients from this group were only males, with median age 14 years old, one had MYCC amplification and two of them died because of disease. Therefore, non-WNT,SHH tumours did not display typical desmoplastic/nodular histology accompanied by reticulin positive reaction as opposite to truly D/N tumours being typical for SHH molecular group ($p < 0.001$). CONCLUSION: Reticulin staining is necessary to distinguish two different biologically and clinically group of nodular tumours which appear morphologically similar under H&E staining alone.

MB-018. EMBRYONAL TUMORS WITH ABUNDANT NEUROPIL AND TRUE ROSETTES: CLINICOPATHOLOGICAL REPORT OF TWO CASES AND LITERATURE REVIEW

Ayako Murase[1], Sumihito Nobusawa[2], Yuki Gemma[1], Fumito Yamazaki[1], Aki Masuzawa[1], Teruaki Uno[1], Tomoo Osumi[1], Yoko Shioda[1], Chikako Kiyotani[1], Tetsuya Mori[1], Kimikazu Matsumoto[1], Hideki Ogiwara[1], Nobuhito Morota[1], Junko Hirato[2], Atsuko Nakazawa[1], and Keita Terashima[1]; [1]National Center for Child Health and Development, Tokyo, Japan; [2]Gunma University, Maebashi, Gunma, Japan

Embryonal tumors with abundant neuropil and true rosettes (ETANTR) are very rare childhood embryonal brain tumors with distinct histopathological features. Standard therapy for other embryonal brain tumors is ineffective and current prognosis is dismal. Recently, novel fusion of TTYH1 with microRNA cluster C19MC was identified as common genetic aberration in this tumor. This fusion drives expression of microRNAs and subsequent deregulation of brain-specific DNMT3B isoform. We present clinical course and analyze molecular characteristics including DNMT3B expression in two cases of ETANTR. We also review the published report of 76 cases of ETANTR and describe the clinical characteristics. CASE 1: Three-month-old girl presented with increasing head circumference. MRI showed a tumor in the fourth-ventricle, which was sub-totally resected. Complete resolution of the tumor on images was achieved by multi-agent chemotherapy and focal proton therapy. The tumor recurred 17 months after diagnosis. Despite sub-total resection followed by high-dose chemotherapy with hematopoietic stem cell rescue, the tumor progressed and the patient died 2 years after diagnosis. CASE 2: One-year-old girl presented with vomiting and CT showed a tumor in right cerebellar hemisphere. The tumor was subtotally resected. The tumor recurred during multi-agent chemotherapy. Craniospinal irradiation was started, however the tumor progressed rapidly and patient died 5 months after diagnosis. Microscopic morphology was typical for ETANTR and immunohistochemical staining showed significant LIN28A expression in both cases. Amplification of 19q13.42 was confirmed by fluorescent in situ hybridization in case 2. DNMT3B were expressed in nuclei of ETANTR cells in both cases. Literature review of 76 cases identified 6 patients who survived without tumor progression for longer than one year. All 6 cases underwent some degree of tumor resection and systemic chemotherapy and 3 of them had radiation therapy as well.

MB-019. LONG TERM NEUROPSYCHOLOGICAL FOLLOW-UP OF YOUNG CHILDREN WITH MEDULLOBLASTOMA TREATED ACCORDING TO THE CCG 99703 REGIMEN

Taryn Fay-McClymont[1], Karin Walsh[3], Donald Mabbott[2], Amy Smith[4], Elizabeth Wells[3], Jennifer Madden[5], Susan Chi[6], Emily Owens[4], Douglas Strother[1], Roger Packer[3], Nicholas Foreman[5], Eric Bouffet[2], and Lucie Lafay-Cousin[1]; [1]Alberta Children's Hospital, Calgary, AB, Canada; [2]Hospital for Sick Children, Toronto, ON, Canada; [3]Children's National Medical Center, Washington, DC, USA; [4]Arnold Palmer Hospital for Children, Orlando, FL, USA; [5]Children's Hospital of Colorado, Denver, CO, USA; [6]Dana Farber Cancer Institute, Boston, MA, USA

RATIONALE: High dose chemotherapy (HDC) strategies were developed in infant brain tumor protocols to prevent neuropsychological (NP) impairments associated with radiation. However comprehensive NP evaluations of young children treated with such strategies remain scant. Our aim was to examine long term NP outcomes in young children with medulloblastoma treated with sequential HDC according to protocol CCG 99703. METHODS: This retrospective study included young children diagnosed with medulloblastoma from 1998-2011 at 6 North American institutions. Neurocognitive data were extracted from institutional NP reports on children old enough to be tested. RESULTS: The initial cohort included 48 patients. Nineteen of the 37 survivors (40%) had at least one NP assessment. This sample was 53% male (n = 10) and mean age at diagnosis was 29.3 months (SD = 14.3). Posterior fossa syndrome (PFs) was reported in five patients (26%). Seven (36.8%) received radiotherapy (3 Focal, 4 CSI), 1 received IT chemotherapy and 2 received HD MTX during induction. On average, children were assessed 3.3 years (SD = 1.6) post-diagnosis, at 5.7 years of age (SD = 1.8). FSIQ ranged from 67-119 (=94.3; SD = 16.5). The majority of children had low average to average NP functioning (76%). Four children (3 received RT) accounted for the majority (61%) of the impaired scores (<10th percentile). While sample size limited power, patients treated with radiotherapy had lower working memory scores (WMI; =85 vs. 100; p = .08); patients with PFs had lower perceptual reasoning scores (PRI; =85 vs. 101; p = .06); and patients with hearing support had lower verbal comprehension scores (VCI; 85 vs. 101; p = .09). NP outcomes did not statistically differ by age at diagnosis, gender, extent of resection, metastasis, or histology. CONCLUSION: NP data obtained from this sample of survivors of infant medulloblastoma treated according to the 99703 regimen are encouraging and indicate average neurocognitive functioning.

MB-020. GENOME-WIDE PROFILING ALLOWS MOLECULAR (RE)CLASSIFICATION OF CNS-PRIMITIVE NEUROECTODERMAL TUMORS

Dominik Sturm[1], Paul A Northcott[1], David T.W. Jones[1], Andrey Korshunov[2], Peter Lichter[3], Stefan M Pfister[1], and Marcel Kool[1]; [1]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Department of Neuropathology, University Hospital Heidelberg, Heidelberg, Germany; [3]Division of Molecular Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany

According to the WHO classification of CNS tumors, childhood CNS primitive neuro-ectodermal tumors (CNS-PNETs; WHO °IV) are poorly differentiated embryonal tumors with early onset and aggressive clinical behavior. Histological diagnosis is complicated by morphological heterogeneity and divergent differentiation. Recent studies suggest the existence of molecular subgroups of CNS-PNETs sharing biological characteristics with other childhood CNS tumors. A collection of 211 fresh-frozen or paraffin-embedded tumor samples with an institutional diagnosis

"CNS-PNET" was profiled for genome-wide DNA methylation patterns and copy-number alterations, complemented by transcriptomic profiling of a subset (n = 71). The (epi-)genetic profiles were compared to those of >3.000 other pediatric brain tumor entities. We screened selected groups of CNS-PNETs for recurrent mutations and expression of established molecular markers. Analysis of DNA methylation and gene expression patterns clearly segregated pediatric brain tumors by histological entities and molecular subgroups. CNS-PNET profiles showed a significant overlap with various entities, including AT/RT, ETMR, high-grade glioma, medulloblastoma, and ependymoma. Hallmark genetic alterations of these entities, such as amplification of 19q13.42, mutations in IDH1 or H3F3A, or alterations of the SMARCB1 locus, were frequently detected in CNS-PNETs with overlapping profiles. Differential expression of protein markers, such as INI-1, LIN28A, and OLIG2, confirmed the re-classification of CNS-PNETs. A subset (~25%) of CNS-PNETs segregated into 3-4 distinct subgroups with characteristic molecular profiles. Currently, whole genome and RNA-sequencing of these subgroups is ongoing to reveal their underlying genetics. The correct classification of CNS-PNETs remains challenging. Based on detection of recurrent genetic aberrations, many cases can be re-classified, indicating that a significant proportion of CNS-PNETs may comprise a variety of other tumor subtypes. The use of molecular markers is needed to assist the histopathological evaluation of CNS-PNETs. In addition, we have identified a number of novel CNS-PNET subtypes and are currently analyzing them in detail to further elucidate their molecular biology.

MB-021. GENE EXPRESSION ANALYSES OF THE SPATIO-TEMPORAL ORIGINS OF MEDULLOBLASTOMA

Cornelia Hooper[1], Susan Hawes[2], Ursula Kees[1], Nicholas Gottardo[3], and Peter Dallas[1]; [1]Telethon Institute for Child Health Research, Subiaco, Western Australia, Australia; [2]Monash Institute of Medical Research, Clayton, Victoria, Australia; [3]Princess Margaret Hospital for Children, Subiaco, Western Australia, Australia

Medulloblastoma is the most common type of malignant paediatric brain tumour and is the leading cause of childhood cancer related mortality. The four molecular subgroups of medulloblastoma that have been identified – Wingless, Sonic Hedgehog, Group 3 and Group 4 - have molecular characteristics suggestive of different cells of origin. Definitive identification of the cell(s) of origin of the medulloblastoma subgroups, particularly the poorer prognosis Group 3 and Group 4 medulloblastoma, is critical to understand the pathogenesis of the disease, and ultimately for the development of more effective treatment options. To address this issue, the gene expression profiles of normal human neural tissues and cell types, representing a broad neuro-developmental continuum spanning human CD133+ embryonic stem cell derived neural stem cells to adult frontal cortex, were compared to those of two independent cohorts of primary human medulloblastoma specimens. Clustering, co-expression network, and gene expression analyses revealed that Wingless and Sonic Hedgehog medulloblastoma may be derived from distinct neural stem cell populations during early embryonic development, while the transcriptional profiles of Group 3 and Group 4 medulloblastoma resemble cerebellar granule neuron precursors at weeks 10-15 and 20-30 of embryogenesis, respectively. Our data indicate that Group 3 medulloblastoma may arise through abnormal granule neuron differentiation, whereas deregulation of synaptic pruning-associated apoptosis may be driving Group 4 tumorigenesis. Overall, these data provide significant new insight into the spatio-temporal origins and molecular pathogenesis of the human medulloblastoma subgroups, and provide an important framework for the development of more targeted therapies.

MB-022. DESMOPLASTIC/NODULAR MEDULLOBLASTOMA AND HIT-SKK TREATMENT. CLINICOPATHOLOGICAL DATA FROM A FRENCH SERIES

Aurore Siegfried[1], Anne Isabelle Bertozzi[2], Annick Sevely[3], Najat Loukh[1], Caroline Munzer[2], Catherine Miquel[4], Franck Bourdeaut[5], Torsten Pietsch[6], Christelle Dufour[7], and Marie Bernadette Delisle[8]; [1]Department of Pathology, Rangueil University Hospital, Toulouse, France; [2]Department of Haematology-Oncology, Children University Hospital, Toulouse, France; [3]Department of Neuro-Radiology, University Hospital, Toulouse, France; [4]Department of Neuropathology, Sait-Anne Hospital, Descartes Paris V University, Paris, France; [5]Department of Pediatric Oncology, Inserm U 830, Curie Institute, Paris, France; [6]Department of Neuropathology, Unversity of Bonn, Bonn, Germany; [7]Department of Pediatry, Gustave Roussy Institut, Villejuif, France; [8]Department of Pathology, Rangueil University Hospital, INSERM U 1037, Toulouse, France

PURPOSE: To identify the clinical and pathologic features involved in the prognosis of patients with desmoplastic/nodular medulloblastoma (DNMB) treated with HIT-SKK protocol. PATIENTS AND METHODS: Between 2009 and 2012, 17 children with newly diagnosed DNMB, <5 years of age treated with HIT-SKK protocol were evaluated. Retrospective central radiological review, pathological and immuno-histochemical study, array-CGH and SUFU analysis were performed. RESULTS: The median age at diagnosis was 26 months (6-59). One patient had spinal metastasis. The radiological features were heterogeneous. The surgery was complete for 13 children at diagnosis and 4 patients had postoperative residual tumor. Chemotherapy began after initial surgery with a median time of 23 days (14-119). The diagnosis of DNMB was confirmed by central review in 15 cases. Pathology showed nodularity and desmoplasia in varying degrees. In one relapsing case, diagnosis of classic medulloblastoma with nodules and without desmoplasia was retained. Immuno-histochemistry was homogenous with SHH pathway markers (GAB1, FilaminA, YAP1) and p75NTR) present in 15 DNMB cases. No impact of MIB1 index was noticed regarding follow-up. Molecular biology showed heterogeneous abnormalities. No amplification of C-MYC was detected, N-MYC and MYC-L amplification was observed in one case. Mutation of SUFU was found in the tumor in one case. At 2 years, the event free survival and the overall survival were 64 +/- 16 % and 92 +/- 8% respectively. Recurrence occurred in 4 patients (median age 38.5 months (6-43), 3 patients was in RC2 and one patient is dead). CONCLUSIONS: The survival rate was worse compared to the results published with HIT-SKK protocol. Regarding DNMB, we found no correlation between the outcome and the radiological and immunohistochemical features. Keywords: Desmoplasic/Nodular Medulloblastoma, Pediatric, HIT-SKK, Treatment.

MB-023. IN VIVO ELECTROPORATION-BASED SPONTANEOUS MOUSE MODEL OF MYC-DRIVEN MEDULLOBLASTOMA

Daisuke Kawauchi[1], Jerold Rehg[2], David Finkelstein[2], Frederique Zindy[2], Timothy Phoenix[2], Richard Gilbertson[2], Stefan Pfister[1], and Martine Roussel[2]; [1]German Cancer Research Center, Heidelberg, Baden-Württemberg, Germany; [2]St Jude Children's Research Hospital, Memphis, TN, USA

Medulloblastoma (MB) is the most common malignant brain tumor in children. Recent genomics and gene expression studies from a large cohort of patients revealed at least 4 distinct molecular subgroups of MBs (WNT, SHH, Group3 (G3) and G4). Patients with G3 MBs have the worst prognosis requiring more effective therapies. Most of G3 MBs are associated with amplification or overexpression of the MYC oncogene. A better understanding of the etiology of this subgroup will lead to the identification of novel diagnostic markers and targets for therapy. Animal models that recapitulate human diseases represent a powerful tool to tackle this issue. We previously established an orthotopic mouse model of G3 MB by overexpression of Myc in purified Trp53-null cerebellar neural progenitors (Kawauchi et al., Cancer Cell, 2012). We now report the development of a spontaneous tumor model of G3 MB using electroporation-based in vivo gene transfer. Transduction of a plasmid carrying Myc-IRES-Luciferase and the gene encoding a dominant-negative form of Trp53 in embryonic neural progenitors induced large cell anaplastic MBs with a G3 gene signature within 50 days after birth. Luciferase expression enabled the tracking of tumor growth by luminescence in real time and in live mice. Furthermore, analysis of early stages of tumor development identified hyperplasia of Pax6-positive cells that could be the cell of origin of G3 MB. Thus, our in vivo gene transduction approach induced spontaneous G3 MB, which will be helpful for the etiology of the disease and preclinical studies with candidate drugs.

MB-024. PROGNOSTIC VALUE OF *TP53* MUTATIONS IN SHH TYPE OF PEDIATRIC MEDULLOBLASTOMA

Joanna Trubicka[1], Maria Borucka-Mankiewicz[1], Elzbieta Ciara[1], Krystyna Chrzanowska[1], Marta Perek-Polnik[3], Dorota Abramczuk-Piekutowska[1], Wieslawa Grajkowska[2], Dorota Jurkiewicz[1], Sylwia Luczak[1], Pawel Kowalski[1], Malgorzata Krajewska-Walasek[1], and Maria Lastowska[2]; [1]Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland; [2]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland; [3]Department of Oncology, The Children's Memorial Health Institute, Warsaw, Poland

Recently, multiple molecular prognostic factors have been tested in attempt to better determine the clinical risk stratification and treatment options in medulloblastoma. One of them, TP53 is the most frequently inactivated gene in human cancers, pointing to important role of this gene in tumorigenesis. Up to now several reports describing the impact of TP53 mutations on survival in medulloblastoma presented conflicting conclusions, however recent study has demonstrated that the patients with TP53 somatic

mutation in SHH molecular type of tumours have profoundly worse outcome (Zhukova 2013). PATIENTS AND METHODS: In our study 118 pediatric medulloblastoma tumours have been investigated to determine the TP53 mutation status by direct sequencing of selected TP53 exons. Identification of transcriptional types of tumours was performed using immunohistopathological analyses. In addition, for identification of the WNT type a presence of chromosome 6 monosomy and CTNNB1 mutations were investigated. RESULTS: *TP53* mutations were found predominantly in SHH type of medulloblastoma (4 /14, 28%). In contrast, TP53 mutation were observed in only one of 8 WNT type (12%) and in two of 63 non-WNT/SHH type (3,7%) tumors. All identified mutations were reported previously as deactivating the p53 protein which occurred in the crucial DNA-binding domain. Within SHH group of tumors children with TP53 mutation had shorter progression-free survival and overall survival than children without mutation ($p = 0.007$ and 0.003 respectively). The majority of TP53 positive tumours in group SHH had anaplastic histology (75%). Most of the patients with TP53 positive tumors were above 3 years of age (71%). CONCLUSION: The TP53 mutation status is an important risk factor for the SHH subtype of medulloblastoma hence should be routinely employed as part of diagnostic procedure.

MB-025. PERSONALIZING THE TREATMENT OF PEDIATRIC MEDULLOBLASTOMA: POLO-LIKE KINASE 1 AS A MOLECULAR TARGET IN HIGH-RISK CHILDREN

Cathy Sheila[1], Singh Lee[1], Colleen Foster[1], Branavan Manoranjan[2], Mary Pambit[1], Rachel Berns[1], Abbas Fotovati[1], Chitra Venugopal[2], Katherine O'Halloran[1], Arul Narendran[3], Cynthia Hawkins[4], Vijay Ramaswamy[4], Eric Bouffet[4], Michael Taylor[4], Ash Singhal[1], Juilette Hukin[1], Rod Rassekh[1], Stephen Yip[5], Paul Northcott[6], Sheila Singh[2], Chris Duhman[1], and Sandra Dunn[1]; [1]University of British Columbia, Vancouver, BC, Canada; [2]McMasters University, Hamilton, ON, Canada; [3]University of Calgary, Calgary, AB, Canada; [4]University of Toronto, Toronto, ON, Canada; [5]Vancouver General Hospital, Vancouver, BC, Canada; [6]DKFZ, Heidelberg, Germany

Medulloblastoma (MB) is the most common malignant brain tumor in children. This disease is heterogeneous and it is comprised of four subtypes of MB (WNT, SHH, Group 3, and Group 4). While the subtypes are informative in regard to patient prognostication they have not yet provided a clear rationale for targeted therapies yet. An immediate goal is to identify novel molecular targets for the most aggressive forms of MB. Polo-like kinase 1 (PLK1) is an oncogenic kinase that controls cell cycle and proliferation making it a strong candidate for MB treatment. In this study, pediatric MBs were subtyped in two patient cohorts (Discovery cohort; $n = 63$ patients from the British Columbia Children's Hospital, and a Validation cohort; $n = 57$ patients from The Hospital for SickKids) using NanoString nCounter platform and PLK1 mRNA was assessed. The SHH and Group 3 subtypes were independently associated with poor outcomes in children using Cox regression analyses. Moreover, as PLK1 further define high-risk patients and its expression spanned all molecular subtypes. A library of 129 compounds in clinical trials was screened using a model of pediatric MB and we determined that PLK1 inhibitors were the most promising class of agents against the growth of MB. In patient-derived primary MB isolates, BI-2536 suppressed the self-renewal of PLK1-high but not PLK1-low expressing cells. PLK1 inhibition prevented MB cell proliferation, self-renewal, cell cycle progression, and induced apoptosis. In contrast, the growth of normal neural stem cells was unaffected by BI-2536. Finally, BI-2536 extended survival in MB-bearing mice with efficacy comparable to Headstart, a standard-of-care chemotherapy regime. We conclude that patients with MB expressing high levels of PLK1 are at elevated risk. These pre-clinical studies pave the way for improving the treatment of MB through PLK1 inhibition.

MB-026. MEDULLOBLASTOMA IN A CHILD WITH TROYER SYNDROME: A CASE REPORT

Tsulee Chen and Sarah Rush; Akron Children's Hospital, Akron, OH, USA

Troyer syndrome, a form of hereditary spastic paraplegia (HSP) described in the Old Order Amish population is a rare autosomal recessive disease associated with distal amyotrophy, dysarthria, developmental delay, cerebellar signs, and skeletal abnormalities. We present the first case reported in the literature of a patient diagnosed with medulloblastoma in the setting of Troyer Syndrome. Our 10 year old patient originially presented to clinicians with short stature and developmental delay. She was documented to have growth hormone deficiency and was started on growth hormone. Several months after starting therapy, she developed headaches, progressive clumsiness, and worsening balance. Ophthalmalogic exam revealed papilledema. Imaging studies obtained demonstrated a large, midline, posterior fossa tumor and hydrocephalus. She underwent gross total resection of the tumor. Surgical pathology confirmed classical histology for a WHO grade IV medulloblastoma with a Ki-67 index of 15-20%. There was no amplification of N-myc or C-myc genes. She did not have evidence of metastatic disease. Synaptophysin was diffusely positive. She was also found to have a mutation in the SPG20 gene associated with Troyer Syndrome. Of the known chromosomal loci and genes related to HSP, none are located near the gene mutations linked to medulloblastoma. Post operatively she suffered from neurologic delay and weakness that was unsubstantiated. Nerve conduction study showed evidence of mixed motor/sensory demyelinative neuropathy. CONCLUSIONS: While a diagnosis of Troyer syndrome is limited to a subset Amish population mostly found in Ohio, a combination of both this rare syndrome and a medulloblastoma has not been reported in the literature prior to now. There is no noted molecular connection between these two syndromes.

MB-027. CLINICAL OUTCOMES OF PATIENTS WITH MEDULLOBLASTOMA TREATED BY PROTON BEAM THERAPY: THREE-YEAR FOLLOW-UP DATA

Hiroshi Fuji, Yuji Ishida, Tsuyoshi Onoe, Touru Kanda, Yuki Kase, Haruo Yamashita, Shigeyuki Murayama, and Yoko Nakasu; Shizuoka Cancer Center Hospital, Nagaizumi, Shizuoka-pref., Japan

INTRODUCTION: The advantages of proton beam therapy (PBT) in the treatment of medulloblastoma lie in a decrease in toxicity without a compromise on tumor control. However, few studies report the clinical outcomes of PBT over a sufficient follow-up time to confirm its efficacy and feasibility. This report describes our experiences with PBT for medulloblastoma in patients who were followed up for more than 3 years. METHODS: The clinical records of patients who underwent PBT for medulloblastoma between January 2006 and April 2009 were reviewed. Disease control and adverse events were evaluated with special interests in event predominantly developed by 36 months after treatment. RESULTS: During the study period, seven patients with medulloblastoma were treated with PBT, including three adults. Five patients were classified as being at average risk and the others at high risk. During the median follow-up time of 54 months, all patients were alive and free of disease. The prescribed doses ranged from 18 to 36 Gy for craniospinal irradiation and from 18 to 36 Gy for boost irradiation of the tumor bed. Mean doses to the thyroid gland, pituitary gland, hypothalamus, and cochlear were 2, 26, 28, and 29 Gy, respectively. None of the patients developed primary hypothyroidism or hypopituitarism. One patient developed bilateral high-frequency hearing impairment, and another unilateral high- and low-frequency hearing impairment. CONCLUSION: Despite the small number of studied cases, our early experience with PBT indicated that it was effective in terms of disease control, with results equivalent to those with conventional photon therapy. PBT presented an advantage in reduced risks of late adverse event with decreased doses for normal tissue in medulloblastoma.

MB-028. RELATIONSHIP BETWEEN CLINICAL OUTCOME AND EXPRESSION OF O^6-METHYLGUANINE-DNA METHYLTRANSFERASE (MGMT) IN MEDULLOBLASTOMA

Tomoko Kurimoto[1], Akihide Kondo[2], Sachi Sakaguchi[1], Junya Fujimura[1], Masahiro Saito[1], Takashi Arakawa[3], Hajime Arai[2], and Toshiaki Shimizu[1]; [1]Department of Pediatrics and Adolescent Medicine,Juntendo University School of Medicine, Tokyo, Japan; [2]Department of Neurosurgery,Juntendo University School of Medicine, Tokyo, Japan; [3]Department of Human Pathology,Juntendo University School of Medicine, Tokyo, Japan

BACKGROUND: Medulloblastomas are highly malignant brain tumors that predominately affect children. Alkylating chemotherapeutic drugs are effective agents in the treatment of medulloblastoma patients. O^6-methylguanine-DNA methyltransferase(MGMT) is a DNA repair enzyme that plays an important role in tumor resistance to alkylating agents. Low MGMT expression or MGMT promoter methylation is associated with favorable outcomes in malignant glioma patients treated with alkylating agents such as temozolomide. However,the relationship between MGMT status and clinical outcomes for medulloblastoma patients has not been fully evaluated. OBJECTIVE: The objective of this study was to investigate the association between MGMT status and clinical outcomes for pediatric patients with medulloblastoma. METHODS: Medulloblastoma patients who were treated at our institution between 1995 and 2012 were reviewed retrospectively. Relevant clinical information including current disease status and tumor response to chemotherapy was obtained from hospital charts. Tumor tissue was obtained from formalin-fixed paraffin-embedded tumor samples. MGMT status was evaluated by

immunostaining with a MGMT antibody as well as bisulfite sequencing analysis to determine MGMT promoter methylation. RESULTS: Tumor tissue and detailed clinical information were available for 22 patients. Of these patients, 13 were living(11 in complete remission), 7 had died of disease, and 2 were lost to follow-up. Five patients had tumor dissemination at diagnosis. We succeeded in evaluating the MGMT status of tumors using both immunohistochemistry and sequencing analysis; the results of our analyses will be presented at the meeting. CONCLUSIONS: Our study provides information on the relationship between MGMT status and the clinical outcome of children with medulloblastoma.

MB-029. TRANSCRIPTIONAL PROFILES OF MEDULLOBLASTOMA TUMOURS CORRELATE WITH THE LOCATION OF TUMOUR

Maria Lastowska, Elzbieta Jurkiewicz, Pawel Daszkiewicz, Monika Drogosiewicz, Joanna Trubicka, Wieslawa Grajkowska, and Maciej Pronicki; The Children's Memorial Health Institute, Warsaw, Poland

OBJECTIVE: Biological heterogeneity of medulloblastoma is of clinical relevance including associations with patients age, survival and presence of metastases. Here, molecular subtypes of tumours were tested for further correlations, especially with the site of disease. METHODS: Transcriptional subtypes of tumours were identified by immunohistochemistry using a panel of representative antibodies in a series of >150 paediatric medulloblastoma patients. In addition, for the WNT type detection chromosome 6 monosomy and CTNNB1 mutation were investigated. A subset of fifty tumours has been previously analysed by application of expression microarrays and NanoString technologies and the results were exploited for groups C and D discrimination. The site of tumour was assessed using MR images on diagnosis in 60 patients. Postoperative MR images (additional 50 tumours) supplemented by intra-operative surgical reports were also analysed. RESULTS: Cerebellar hemispheric location was strongly associated with the SHH type of tumours ($p < 0.0001$) and ~60% of them had desmoplastic/nodular pathology. By contrast, tumours from the WNT type were located exclusively within the IV ventricle and attached to the brain stem. Similarly, tumours from the non-WNT/SHH group had also midline location and only around 5% showed atypical site, including hemispheric region. They were all of classic or anaplastic histology. Among cases with atypical location some tumours displayed marker identified previously in a subset of PNET tumours (LIN28), raising the possibility that they may be biologically distinct from the rest of analysed non-WNT/SHH medulloblastomas. In the subset of tumours with known C or D group category, there was no difference in terms of tumour location between those two groups. CONCLUSION: Our analysis confirmed that the site of tumour is associated with molecular and histopathological features and should be taken into account for better diagnosis of the disease. In case of atypical tumour location an application of further diagnostic markers is recommended.

MB-030. UNRAVELLING THE BIOLOGY OF AGGRESSIVE AND THERAPY-RESISTANT EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES (ETMR)

Marcel Kool[1], Dominik Sturm[1], David T.W. Jones[1], Volker Hovestadt[1], Ivo Buchhalter[1], Natalie Natalie Jäger[1], Adrian Stuetz[2], Pascal Johann[1], Christin Schmidt[1], Marina Ryzhova[3], Pablo Landgraf[4], Martin Hasselblatt[5], Ulrich Schüller[6], Marie-Laure Yaspo[7], Andreas von Deimling[8], Jan Korbel[2], Roland Eils[1], Peter Lichter[1], Andrey Korshunov[8], and Stefan Pfister[1]; [1]German Cancer Research Center DKFZ, Heidelberg, Germany; [2]European Molecular Biology Laboratory, Heidelberg, Germany; [3]NN Burdenko Neurosurgical Institute, Moscow, Russia; [4]Heinrich Heine University Düsseldorf, Düsseldorf, Germany; [5]University Hospital Münster, Münster, Germany; [6]Ludwig-Maximilians-Universität, München, Germany; [7]Max Planck Institute for Molecular Genetics, Berlin, Germany; [8]University Hospital Heidelberg, Heidelberg, Germany

Embryonal tumor with multilayered rosettes (ETMR) is a highly aggressive embryonal CNS tumor predominantly affecting young children with reported overall survival times ranging from 5–30 months. As these tumors have often been misdiagnosed as medulloblastoma or CNS-PNETs it was thought that ETMR is a very rare tumor. However, now molecular tools are available to detect ETMR and distinguish them from other brain tumors it has become clear that it is actually one of the most common brain tumors among infants. Amplification of a miRNA cluster at 19q13.42 and high expression of LIN28A have been identified as molecular hallmarks of ETMR, affecting 95-100% of samples tested and are considered unifying molecular diagnostic markers to detect them and distinguish from other brain tumors. A comprehensive clinical, pathological, and molecular analysis of 97 cases of these fatal brain neoplasms identified uniform molecular signatures in all tumors irrespective of histological patterns, indicating that the three histological variants ETANTR, EBL, and MEPL comprise a single biological entity. In order to better understand the biology of these highly aggressive pediatric CNS malignancies, we performed whole genome DNA sequencing of 18 tumor-normal pairs plus 3 recurrences, complemented by (mi)RNA sequencing of tumor RNA. Mutations detected included the recurrent fusion of TTYH1 with the C19 miRNA cluster as well as gene mutations in TP53, CTNNB1, and mutations affecting the miRNA processing pathway. Chromothripsis was detected in several cases and in all cases affecting chromosome 19q. Finally, as DNA sequencing identified only very few somatic mutations per tumor, we next studied the epigenome of these tumors by performing whole genome bisulfite sequencing and ChIP-seq for various activating and inactivating histone marks. Integrating these high throughput genomic analyses may now lead to alternative treatment strategies for these highly aggressive and therapy-resistant tumors.

MB-031. PNMT: A NOVEL TARGET FOR MEDULLOBLASTOMA IMMUNOTHERAPY

Arunkumar Modi, Monaben Patel, Michael Berk, Li-xin Wang, and Gregory Plautz; Cleveland Clinic, Cleveland, OH, USA

Multiple mutations have been found to be associated with Medulloblastoma including ERBB2. Copy number amplification is a common genetic event in Medulloblastoma. PNMT gene is located in close proximity to ERBB2. ERBB2 locus CNA on 17q12-21 is a frequent genomic event present in 10-15% of breast cancers. We hypothesized that a subset of amplicon genes with minimal expression in normal tissue could serve as candidates for shared tumor-specific antigens. We eliminated from consideration other amplicon genes with high expression in specific tissues or broad expression in normal tissues due to potential for autoimmune toxicity. We tested several peptide epitopes with predicted high affinity for HLA-A2 and confirmed that these could stabilize MHC expression on T2 cells. In vitro sensitization of normal donor lymphocytes with the predicted epitopes led to production of IFN-g. Moreover, Dendritic cells pulsed with lysate from SKBR3 (ERBB2 amplified) but not MCF7 breast cancer cells could sensitize autologous lymphocytes to epitopes. This suggests that natural abundance of the amplicon proteins in ERBB2 positive tumors is sufficient to provide cross-priming. To test whether the amplicon proteins could serve as tumor-rejection antigens we used the murine TM15 breast cancer cell line, with amplification of syntenic region around ERBB2. qPCR showed 50-fold overexpression of PNMT in TM15 cells. Mice vaccinated with recombinant murine or human PNMT protein, but not ovalbumin, rejected challenge with TM15 tumor without any overt toxicity and developed memory against delayed re-challenge. These data indicates that PNMT protein is abnormally expressed at high levels in the ERBB2+ breast tumors. Analysis of Medulloblastoma microarray databases shows overexpression of PNMT in tumor samples. T-lymphocytes normally have tolerance to non-mutated host proteins however tolerance can be reversed with proper sensitization. Antigen overexpression in tumors might allow effecter T-cells to surpass an activation threshold while preserving tolerance for normal tissue.

MB-032. NEUROPSYCHOLOGICAL OUTCOME OF CHILDREN TREATED FOR STANDARD RISK MEDULLOBLASTOMA IN THE HIT-SIOP PNET4 EUROPEAN RANDOMISED CONTROLLED TRIAL OF HYPERFRACTIONATED (HFRT) VERSUS STANDARD RADIOTHERAPY (STRT) AND MAINTENANCE CHEMOTHERAPY

Hugo Câmara-Costa[1], Anika Resch[2], Clémence Lalande[3], Virginie Kieffer[4], Geraldina Poggi[5], Colin Kennedy[6], Kim Bull[6], Gabriele Calaminus[7], Jacques Grill[3], François Doz[8], Stefan Rutkowski[2], Maura Massimino[9], Rolf-Dieter Kortmann[10], Brigitta Lannering[11], Georges Dellatolas[12], and Mathilde Chevignard[13]; [1]University Paris Descartes, Sorbonne Paris Cité and INSERM U669, Paris, France; [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [3]Gustave Roussy, Villejuif, France; [4]Saint Maurice Hospitals, Saint Maurice, France; [5]Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy; [6]University of Southampton Faculty of Medicine and University Hospital Southampton NHS Foundation Trust, Southampton, UK; [7]University of Muenster, Paediatric Oncology, Muenster, Germany; [8]Institut Curie and University Paris Descartes, Sorbonne Paris Cité, Paris, France; [9]Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; [10]University of Leipzig, Department of Radiation Therapy, Leipzig, Germany; [11]University of Gothenburg, Paediatric Oncology, Gothenburg, Sweden; [12]INSERM U669, Paris, France; [13]ER6, Pierre et Marie Curie University - Paris 6 and Saint Maurice Hospitals, Paris, France

BACKGROUND: In the PNET4 European randomised controlled trial, children with standard risk medulloblastoma were allocated to HFRT or to STRT. All received maintenance chemotherapy. Event-free survival was similar between the two treatment arms. HFRT was associated with worse growth and better questionnaire-based executive function 6.1 years post-diagnosis, especially in children aged <8 years at diagnosis (Kennedy et al., IJROBP, 2014). Therefore the aim of this study was to compare cognitive outcomes between treatment arms. METHODS: Neuropsychological data was collected prospectively in 137 patients from Germany, France, Italy and Sweden. Using results of the Wechsler Intelligence Scales, Kaufman Assessment Battery for Children, and Raven's progressive matrices, we generated: Full scale IQ (FSIQ), Verbal IQ (VIQ), Performance IQ (PIQ), working memory index (WMI) and speed of processing index (PSI). RESULTS: Among the 137 participants, [n = 71 HFRT, n = 66 STRT, 63.5% males; mean (SD, range) age at diagnosis 9.3 years (3.2, 4 to 17.6), 40.9% aged <8 years at diagnosis; age at assessment 15.6 years (3.7, 8.3 to 25). Mean (SD, range) FSIQ in all participants was 88 (19, 40-137); mean intergroup difference [95% CIs] (3.88, [-2.66 to 10.41], p = .24). No difference was found in children aged > 8 years at diagnosis. In children aged <8 at diagnosis, VIQ was significantly higher in children receiving HFRT compared to STRT; a similar trend was found for PSI but not for PIQ,WMI and FSIQ (mean inter-group differences [95% CIs]): VIQ 12.02 [2.37 to 21.67], p = 0.02; PIQ (2.73 [-7.89 to 13.35], p > .10); WMI (5.20 [-2.07 to 12.47], p > .10), PSI (10.91 [-1.54 to 23.36], p = .08; FSIQ (5.28 [-4.23 to 14.79], p > .10). CONCLUSION: HFRT was associated with higher verbal IQ in children aged <8 years at diagnosis, consistent with the previous report using questionnaire-based data. Effect sizes were small, albeit with 10 point inter-group differences within their 95% CIs.

MB-033. CROSS-SPECIES EPIGENETICS IDENTIFIES A CRITICAL ROLE FOR VAV1 IN THE MAINTENANCE OF SONIC HEDGEHOG SUBGROUP MEDULLOBLASTOMAS

Janet Lindsey[1], Daisuke Kawauchi[2], Ed Schwalbe[1], David Solecki[2], Peter McKinnon[2], Jim Olson[3], James Hayden[1], Richard Grundy[4], David Ellison[2], Dan Williamson[1], Simon Bailey[1], Martine Roussel[2], and Steven Clifford[1]; [1]Newcastle University, Newcastle upon Tyne, UK; [2]St Jude Children's Research Hospital, Memphis, TN, USA; [3]University of Washington, Seattle, WA, USA; [4]University of Nottingham, Nottingham, UK

The identification of key tumorigenic 'driver' events in sonic hedgehog subgroup medulloblastomas (MB_{SHH}) will be essential for the development of individualised therapies and improved outcomes. However, beyond confirmation of characteristic SHH-pathway mutations, recent genome-wide sequencing studies have not revealed additional commonly-mutated targets with widespread therapeutic relevance. We therefore examined any role for epigenetic DNA methylation events in MB_{SHH} using a cross-species approach to candidate identification, prioritisation and validation. MB_{SHH}–associated DNA methylation events were first identified in >200 human tumors, and their conservation assessed in tumors from four independent Shh-medulloblastoma murine models, alongside any role in tumorigenesis using functional assessments in mouse and human models. This strategy identified regional CpG hypo-methylation of VAV1, leading to its elevated expression, as a conserved aberrant epigenetic event which characterises the majority of MB_{SHH} tumors in both species, and which is associated with a poor outcome in MB_{SHH} patients. Moreover, we show VAV1 plays a critical role in tumor maintenance in our models and its abrogation markedly reduces tumor growth. Further, our initial data implicate Vav1 in the regulation of early cerebellar development. These findings establish VAV1 hypo-methylation as a frequent and critical epigenetic 'driver' in MB_{SHH}, and support its development and explotation as a prognostic biomarker and therapeutic target.

MB-034. WIP1 AUGMENTS *SONIC HEDGEHOG* (SHH) SIGNALING AND IS A TARGET IN SHH MEDULLOBLASTOMAS

Meghan Buss[1], Marc Remke[4], Juhyun Lee[1], Tamara Caspary[3], Michael Taylor[4], and Robert Castellino[2]; [1]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA; [2]Aflac Cancer & Blood Disorders Center, Children's Healthcare of Atlanta, and Emory University School of Medicine, Atlanta, GA, USA; [3]Department of Human Genetics, Emory University, Atlanta, GA, USA; [4]Division of Neurosurgery, Arthur and Sonia Labatt Brain Tumour Research Center, and Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Sonic Hedgehog (SHH) signaling is present in up to 30% of childhood medulloblastomas. Retrospective analyses suggest that survival of patients with SHH medulloblastoma is intermediate between that of the near 100% survival of WNT –activated and the 30-40% survival of patients with Group 3 medulloblastoma. Dysregulation of p53 signaling is especially prognostic for poor survival in SHH medulloblastomas. But, p53 is mutated in < 10% of medulloblastomas. Large-scale genomic analyses have demonstrated high-level amplification of PPM1D (WIP1) in SHH medulloblastomas, which is independent of p53 mutation. WIP1 functions as an oncogene, in part by inactivating p53. We hypothesized that, similar to deletion of mouse Trp53, overexpression of WIP1 augments Shh-driven medulloblastoma tumorigenesis and is an important target in the treatment of SHH-active medulloblastomas. WIP1 overexpression increased proliferation of granule neuron precursor (GNP) cells as well as medulloblastoma cells derived from SmoM2 transgenic mice, which express a mutant, constitutively-active Smoothened (Smo) gene, SmoM2. This was associated with increased expression of downstream targets of Shh signaling, Ptch1 and Gli1. Although WIP1 transgenic mice, which express WIP1 from the Neurod2 promoter, did not exhibit increased tumor incidence, SmoM2; WIP1 double transgenic mice exhibited an increased incidence of medulloblastoma, larger tumors at diagnosis, and reduced survival, compared to SmoM2 mice. Conversely, Wip1 knock out significantly suppressed medulloblastoma formation in SmoM2 as well as Tamoxifen-treated Math1-cre^{ER}; Ptc1 fl/fl mice. Combined treatment with a small molecule inhibitor of SMO and shRNAs against WIP1 or with the small molecule WIP1 inhibitor, CCT007093, suppressed growth of medulloblastoma cells derived from SmoM2 or Tamoxifen-treated Math1-cre^{ER}; Ptc1 fl/fl mice to a greater extent than treatment with a SMO inhibitor alone. This suggests an important cross-talk between SHH and WIP1 signaling, that accelerates medulloblastoma tumorigenesis. Our findings also suggest an important role for WIP1 inhibition in the treatment of SHH-active medulloblastomas.

MB-035. PATTERNS AND TREATMENT OF RELAPSE AFTER STANDARD RISK MEDULLOBLASTOMA: A REPORT FROM THE HIT-SIOP PNET 4 TRIAL

Birgitta Lannering[1], Magnus Sabel[1], Goran Gustafsson[2], Gudrun Fleischhack[3], Martin Benesch[4], Francois Doz[5], Rolf-Dieter Kortmann[6], Maura Massimino[7], Aurora Navajas[8], Roel Reddingius[9], and Stefan Rutkowski[1]; [1]University of Gothenburg, Inst of Clinical Sciences, Göteborg, Sweden; [2]Karolinska Institute, Stockholm, Sweden; [3]University Hospital of Essen, Pediatrics III,, Essen, Germany; [4]Division of Pediatric Hematology and Oncology, Medical University Graz, Dept of Pediatrics, Graz, Austria; [5]Institut Cure and University Paris Descartes, Paris, France; [6]University of leipzig, Radiation Oncology, Leipzig, Germany; [7]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; [8]University of Basque Country, Cruces Hospital, Bilbao, Spain; [9]Erasmus Medical Center, Rotterdam, The Netherlands; [10]University of Hamburg-Eppendorff, Pediatric Oncology, Hamburg, Germany; [11]Univerisity of Liverpool, Alder Hey Childreńs Hospital,, Liverpool, UK

INTRODUCTION: The multicenter PNET 4 trial for standard risk medulloblastoma (MB) included 338 patients between January 2001 and December 2006. Event-free and overall survival (OS) at 10 years were 76 ± 2% and 78 ± 2%, respectively. Relapse and treatment of relapse for trial patients was studied in detail. PATIENTS AND METHODS: Data was extracted either from the relapsed MB database of the HIT study group or by a specific case report form in other patients. Results were up-dated as of June 30, 2013. RESULTS: After a median follow-up of 7.5 years since first diagnosis seventy-four patients experienced relapse, of whom 11 are alive. Twelve relapses (16%) occurred solely in the posterior fossa (PF), 62 (84%) were solitary or multiple craniospinal (metastatic) relapses with or without PF disease. Solitary relapses in PF versus all other relapses occurred at a mean/median of 38/33 and 30/26 months after first diagnosis, respectively (p = 0.24). Late relapses (>5 years from diagnosis) occurred in 7 patients (9.5%). Expression of ß-catenin, MYC status and 17pq imbalances in the primary tumor did not correlate with survival after relapse. In 70 patients with full information on relapse treatment 20% had surgery, 20% local radiotherapy and 21% high dose chemotherapy with stem cell rescue (HDSCR). Temozolomide was the most commonly used drug. Mean/median OS after relapse was 21/18 months; 5-year OS was 6.3 ± 3.4%. In multivariate analysis, time in continuous complete remission, solitary relapse in PF and surgery were significantly associated with prolonged survival. Treatment with radiotherapy and HDSCR had no impact on survival. CONCLUSIONS: Survival after relapse was very poor in the PNET 4 study. This is despite several patients receiving aggressive treatment at relapse. Patients with the longest survival after relapse had local late relapse and surgery.

MB-036. DIVERGENCE AND CONVERGENCE OF MORPHOLOGY, IMMUNOHISTOCHEMISTRY AND MOLECULAR PROFILING TO DETERMINE SHH TYPE MEDULLOBLASTOMA IN CHILDREN < 5 YEARS

Catherine Miquel[2], Marie-Bernadette Delisle[3], Christelle Dufour[4], Delphine Lafon[5], Nicolas Sevenet[5], Gaelle Pierron[1], Olivier Delattre[1], and Franck Bourdeaut[1]; [1]Institut Curie, Paris, France; [2]Hopital Sainte-Anne, Paris, France; [3]CHU Toulouse, Toulouse, France; [4]Institut Gustave Roussy, Villejuif, France; [5]Institut Bergognie, Bordeaux, France

Before 3 years of age, a strong concordance is found between the nodular desmoplastic (DNMB) and extensive nodularity (MBEN) histotypes and Sonic Hedgehog (SHH) subgroup. This might underlie the favourable outcome of DNMB/MBEN and justify a specific treatment strategy. However, infants protocols also include children between 3 and 5 years. Therefore, identifying truly SHH-MB among DNMB/MBEN might be critical for appropriate treatment. MATERIAL AND METHODS: We centrally reviewed medulloblastomas occurring before 5 years with a diagnosis of "desmoplastic medulloblastomas" referred to our institutions from 2007 to 2013. Morphology was centrally reviewed. Reticulin, and immunohistochemistry (IHC) with YAP, GAB1, FilaminA and NRL were analysed. DNMB/MBEN, classical and anaplastic MB were distinguished according to OMS 2007 classification. IHC deciphered "SHH" from "non SHH" tumours. Whenever possible, array-CGH, and SUFU and PTCH1 sequencings were performed. RESULTS: 29 medulloblastomas were reviewed (median age: 2.1years). After central review, 21 were confirmed to be DNMB/MBEN, 3 were reclassified as "classic with nodules without desmoplasia" and 3 "classic medulloblastomas"; one was anaplastic, one not specified. Immunohistochemistry classified 21 medulloblastomas as SHH (7 MBEN, 12 DNMB, 1 classical, 1 NOS; median age: 2.3 years). Among the 8 non SHH medulloblastomas (median age: 2years), 2 were truly DNMB (1 and 3.1 years), none were MBEN. Array-CGH evidenced 9q deletion exclusively in SHH MB, with 8/8 PATCH1 biallelic alteration; 6 SUFU mutations were detected, with 10q deletion in only 1 case. Gains of chromosome 7 were seen in 4 cases, all non SHH. Finally, flat profiles were found in all categories. CONCLUSION: Central review is critical but truly non SHH DNMB may still be distinguished. Sequencing is useful if it can be applied in a real-time frame. Array-CGH gives specific insights and IHC seems reliable. Methylome-arrays or expression profiling may also be implemented to clinical practice.

MB-037. TARGETING CLASS I HISTONE DEACETYLASES IN HIGH RISK MEDULLOBLASTOMA - ANALYSIS OF MOLECULAR MECHANISMS AND TRANSLATIONAL IMPLICATIONS

Jonas Ecker[1], Ina Oehme[1], Ralph Mazitschek[2], Andrey Korshunov[3], Marcel Kool[4], Marco Lodrini[1], Hedwig E. Deubzer[1], Andreas von Deimling [3], Andreas E. Kulozik[5], Stefan M. Pfister[4], Olaf Witt[1], and Till Milde[1]; [1]Clinical Cooperation Unit Pediatric Oncology (G340), German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Center for Systems Biology, Massachusetts General Hospital, Boston, MA, USA; [3]Department of Neuropathology, University Hospital Heidelberg, Heidelberg, Germany; [4]Division of Pediatric Neurooncology (B062), German Cancer Research Center (DKFZ), Heidelberg, Germany; [5]Section of Pediatric Brain Tumors, Department of Pediatric Oncology, Hematology and Immunology, University Hospital Heidelberg, Heidelberg, Germany

INTRODUCTION: Medulloblastoma (MB) is the most frequent malignant brain tumor in children. Four molecular subgroups with distinct genetic and epigenetic backgrounds as well as clinical characteristics have been identified. Overall survival remains poor especially in patients with Group 3 tumors, despite aggressive multimodal treatment. As previously shown, targeting MB tumors with histone deacetylase inhibitors (HDACis) is a promising strategy. We here explore the molecular mechanisms and translational implications of class I HDAC inhibition in high-risk Medulloblastoma subgroups. METHODS: Expression of HDACs in MB subgroups was compared to normal brain tissue analyzing gene expression profiles and tissue microarrays. For subgroup specific analysis a panel of established MB cell lines was used for cell culture experiments, including those with MYC-amplification (typical for Group 3 tumors). Cells were treated with inhibitors selectively targeting class I or IIa HDACs and RNAi-mediated depletion of class I HDAC isozymes HDAC1 and HDAC2 was performed. Readouts included analysis of viability, metabolic activity, caspase activity, cell cycle progression, RNA and protein expression. In co-localization studies, Co-IPs of HDAC1, HDAC2 and c-MYC were performed. RESULTS: Both HDACs 1 and 2 are constitutively overexpressed in two of the four MB subgroups (SHH and Group 3). Class I HDAC inhibition as well as knockdown of HDAC1 or 2 reduces metabolic activity, induces apoptosis and leads to hyperacetylation of H4. Differential response of MYC-amplified versus non-amplified cell lines to HDACis preferentially inhibiting class I HDACs, shows increased susceptibility of MYC-amplified cells, with cell death occurring at clinically relevant inhibitor concentrations. HDAC1 and 2 are found to be co-localized in a protein complex in MYC-amplified cell lines. CONCLUSION: Our results suggest that high-risk patients with MYC-amplified Group 3 MB tumors may benefit from class I HDACi treatment. Analyses are ongoing in order to elucidate the direct interaction of HDAC1 and 2 and c-MYC.

MB-038. MEDULLOBLASTOMA SUBTYPES SPECIFY INTER-TUMORAL VASCULAR HETEROGENEITY

Timothy Phoenix, Deanna Patmore, Nidal Boulos, Karen Wright, Scott Boop, and Richard Gilbertson; St. Jude Children's Research Hospital, Memphis, TN, USA

Pediatric brain tumors that were previously treated as single diseases are now known to comprise molecular subgroups with distinct clinical behaviors. Understanding how subgroup-specific, genetic alterations dictate treatment response is critical to advancing treatments for all patients. The central nervous system contains a unique vascular system that tightly regulates exchange between the blood and brain, termed the blood brain barrier (BBB). How brain tumors specify newly formed blood vessels and if this dictates tumor behavior remains unanswered. Using a series of human tumors and mouse models of medulloblastoma subtypes, we show that human WNT-subtype medulloblastomas establish a highly-aberrant, non-CNS vasculature that lacks typical endothelial markers associated with CNS barriergenesis. Concordant with these observations, we show that mouse WNT-subtype tumors also produce abnormal vessels, and are extremely permeable to systemically delivered fluorescent tracers. This aberrant vasculature results from the production of secreted WNT-inhibitors by WNT-medulloblastoma that blocks normal WNT-signaling and angiogenesis in recruited endothelial cells. Microarray analysis of WNT-subtype endothelial enriched samples show a global downregulation in BBB associated genes, and upregulation of peripheral specific endothelial genes. Importantly, this phenotype can be reversed in vivo by overexpressing Wnt7A in tumor cells, turning back on BBB endothelial markers, and decreasing vascular permeability. In stark contrast, SHH-subtype tumors maintain strong expression of CNS endothelial markers, and retain a mainly intact, impermeable BBB in vivo. This study is the first to show that brain tumor vasculature is specified by the surrounding tumor microenvironment and may explain why WNT-subtype medulloblastomas are so sensitive to current therapies.

MB-039. LONG-TERM SURVIVAL (APPROACHING 20 YEARS), COMPLETE RESPONSE AND NORMAL CHILDHOOD DEVELOPMENT IN MEDULLOBLASTOMA (PNET) WITHOUT RECURRENCE: A CASE REPORT

Tomasz Janicki, Stanislaw Burzynski, Gregory Burzynski, and Ania Marszalek; Burzynski Clinic, Houston, Texas, USA

Medulloblastoma is the most common brain tumor in age group of 0-4 years with the survival 55% at 10 years and no survival data at 20 years. The authors describe the case of a successful treatment followed by normal childhood development of patient with medulloblastoma who is approaching 20 years survival without recurrence. A 32-months old male with newly-diagnosed brain tumor 4 x 4 cm of the cerebellum and brainstem with massive hydrocephalus underwent a craniotomy with subtotal resection of the tumor in February 1994. Pathology confirmed medulloblastoma. Patient did not have radiation or chemotherapy. Six weeks later, he was presented to Burzynski Clinic and was admitted for treatment with antineoplastons A10 and AS2-1 (ANP). He received IV dosage of 4.0 g/kg/d of A10, reduced to 2.9 g/kg/d after 12 months, and 0.4 g/kg/d of AS2-1, divided into multiple infusions via portable pump (protocol CAN-1). The post-operative/pretreatment MRI showed contrast enhancing lesion 1.8 x .8 cm which was resolved after six weeks of treatment. Follow-up MRIs at 4, 8, 12, 16, 20, 24 and 28 months, confirmed complete response per central reviewer. Patient is asymptomatic and free of enhanced and non-enhanced leasion as confirmed by MRI on 10/09/2007. Intravenous ANP was given for 3 years and the maintenance treatment with A10 and AS2-1 capsules (0.12 g/kg/d each) continued for additional 2 years. Reversible toxicity possibly treatment related included hypernatremia, grade 3 and anemia, grade 4. The patient is approaching 20 years of normal life (April 2014). This

report indicates that it is possible to obtain normal quality of life in long-term survival of medulloblastoma (PNET) without recurrence with a currently available investigational treatment.

MB-040. POLO-LIKE KINASE 1 (PLK1) ACTIVATES TRANSLATIONALLY CONTROLLED TUMOR PROTEIN (TCTP) PROMOTING THE DEVELOPMENT OF REFRACTORY MEDULLOBLASTOMA

Joanna Triscott[1], Marcus Green[2], Colleen Foster[1], Abbas Fotovati[1], Rachel Berns[1], Katrina O'Halloran[1], Ash Singhal[1], Juliette Hukin[1], S. Rod Rassekh[1], Stephen Yip[3], Brian Toyota[3], Christopher Dunham[1], and Sandra E. Dunn[1]; [1]University of British Columbia, Vancouver, Canada; [2]Exeter Medical School, Exeter, UK; [3]British Columbia Cancer Agency, Vancouver, Canada

Medulloblastoma (MB) is the most common malignant brain tumor in children. Our group has demonstrated polo-like kinase 1 (PLK1) mRNA expression is also associated with elevated risk of relapse and death in young children (Triscott, 2013). This oncogenic kinase controls proliferation and cell cycle through phosphorylation of substrates such as translationally controlled tumor protein (TCTP). TCTP has been suggested to inactivate p53 in cancer cells thereby escaping the initiation of the DNA damage response. We have reported that silencing PLK1 in MB led to reduced levels of P-TCTP^{S46} and question whether TCTP mediates tumor cell growth and response to chemotherapeutics. To better understand the role of this PLK1 substrate we asked whether P-TCTP^{Ser46} correlates with PLK1 levels in patients. We therefore validated a P-TCTP^{Ser46} antibody for immunohistochemical staining of tissue microarrays (TMA) with clinical follow up (young adult MB, n = 23; pediatric MB, n = 74). TMAs will be stained for both PLK1 and P-TCTP using serial sectioning. Preliminary immunofluorescent detection of P-TCTP^{Ser46} demonstrated it co-localizes with PLK1 in the nuclei of proliferative MB cells. Further, TCTP siRNA silencing reduced the proliferation of MB cell lines and enhanced sensitivity to Vincristine. Currently we are characterizing primary MB cultures with low PLK1 expression (BT027). These cells respond poorly to PLK1 inhibitor, BI6727, and have a moderate growth response to siTCTP. Similarly, P-TCTP^{Ser46} was near undetectable in BT027 by western blot. Our future work entails the treatment of BT027 cells with PLK1 overexpression vectors. Also, we will compare the tumorigenicity of PLK1 high and low MB by intracranially injecting cells into mice. Cells have been labeled with pcDNA3.1(+)/Luc2 = tdT which will allow in vivo detection with an IVIS imaging system. These studies aim to demonstrate TCTP as a biologically relevant substrate of PLK1 that acts a reliable biomarker for measuring PLK1 activity in MB.

MB-041. HIGH-THROUGHPUT DRUG SCREENING IDENTIFIES HDAC INHIBITORS AS CANDIDATE THERAPEUTICS FOR MYC-DRIVEN MEDULLOBLASTOMA

Kun-Wei (Ken) Liu, Yanxin Pei, and Robert Wechsler-Reya; Sanford-Burnham Medical Research Institute, La Jolla, CA, USA

Medulloblastoma (MB) is the most common malignant brain tumor children. Despite aggressive multimodal therapy, many patients succumb to the disease, and survivors experience severe long-term side effects from the treatment. Patients whose tumors express high levels of the MYC oncogene have a particularly poor prognosis, and would benefit from more effective therapies. To identify such therapies, we used a recently developed animal model of MYC-driven MB as a platform for high-throughput drug screening. Among the most effective compounds identified in our screen were histone deacetylase inhibitors (HDACi). In vitro, HDACi showed potent activity against cells from murine and human MYC-driven MB, with minimal toxicity to normal cerebellar cells. Gene expression analysis suggested that a major target of HDACI is Foxo1, a transcription factor that antagonizes MYC-mediated transformation. Since PI3K inhibitors (PI3Ki) have also been shown to activate Foxo1 by promoting its nuclear localization, we tested the combination of HDACi and PI3K inhibitors on MB cells. HDACi and PI3Ki synergized in vitro, and potently inhibited growth of murine and human MYC-driven MB in vivo. These findings highlight the power of high-throughput drug screening for identifying novel cancer therapies, and point to HDACi/PI3Ki combination therapy as a promising avenue for treatment of aggressive MB.

MB-042. MOUSE FUNCTIONAL GENOMICS TO PREDICT NOVEL DRUG TARGETS FOR ALL SUBGROUPS OF MEDULLOBLASTOMA

Laura Genovesi[1], Pengxiang Ji[1], Melissa Davis[2], Ching Ging Ng[3], Marc Remke[4], Michael Taylor[4], Yoon-Jae Cho[5], Nancy Jenkins[6], Neil Copeland[6], and Brandon Wainwright[1]; [1]The University of Queensland, Institute for Molecular Bioscience, St Lucia, QLD, Australia; [2]National Information Communications Technology Australia, The University of Melbourne, Parkville, VIC, Australia; [3]Cancer Genetics Group, Institute for Molecular and Cell Biology, Singapore, Singapore; [4]Division of Neurosurgery, Arthur and Sonia Labatt Brain Tumor Research Centre and Program in Developmental and Stem Cell Biology, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [5]Stanford University School of Medicine and Lucile Packard Children's Hospital, Stanford, CA, USA; [6]Cancer Research Program, The Methodist Hospital Research Institute, Houston, TX, USA

Medulloblastoma (MB) is the most common malignant paediatric brain tumour and a leading cause of cancer-related mortality and morbidity. Existing treatment protocols are aggressive in nature, resulting in significant neurological, intellectual and physical disabilities. Clearly, there is an urgent need for less invasive and improved, more targeted therapies that minimise harmful side effects. In this study, we sought to identify novel drug candidates by exploiting the protein regulatory networks common to all four subgroups of human MB, identified from our previously published transposon mutagenesis mouse MB model. Network components were mapped to human orthologs and significantly up-regulated genes common to all subgroups of MB were identified on the basis of expression data from two independent sets of previously described human MB samples. Using these genes, we created a drug-target network using the DrugBank database, overlaying drugs and their known targets with significantly up-regulated genes in MB. The druggable network contains several novel up-regulated genes that interact with previously known MB genes, such as ZEB1, TP53, CREBBP and the SMARCA family of genes. Additionally, top-ranked up-regulated genes within our drug-target network, including AURKA and BRD4, were recently validated as promising therapeutic targets for certain subgroups of MB. Combined, these findings validate this strategy as a powerful approach for the discovery of novel therapeutic candidates relevant to MB pathogenesis. Several additional candidate genes from the drug-target network have been investigated in the laboratory and the data presented here functionally define the therapeutic potential of these genes relevant to all subgroups of MB.

MB-043. OVERCOMING ACQUIRED AND A PRIORI RESISTANCE TO SMO ANTAGONISTS THROUGH EPIGENETIC REGULATION OF HEDGEHOG PATHWAY TRANSCRIPTIONAL OUTPUT

Yujie Tang[1], Simone Schubert[1], Brian Nguyen[1], Sabran Masoud[1], Sharareh Gholamin[1], Alex Lee[1], Minde Willardson[3], Pratiti Bandopadhayay[2], Guillame Bergthold[2], Scott Atwood[1], Ramon Whitson[1], Samuel Cheshier[1], Jun Qi[2], Rameen Beroukhim[2], Jean Tang[1], Rob Wechsler-Reya[4], Anthony Oro[1], Brian Link[3], James Bradner[2], and Yoon-Jae Cho[1]; [1]Stanford University, Stanford, CA, USA; [2]Dana-Farber Cancer Insitute, Boston, MA, USA; [3]Medical College of Wisconsin, Milwaukee, WI, USA; [4]Sanford-Burnham Medical Research Institute, La Jolla, CA, USA

Aberrant activation of Hedgehog signaling drives oncogenesis in several types of cancer, including medulloblastoma. As a result, there has been significant interest in developing therapeutic strategies targeting this pathway, most notably through inhibition of Smoothened. Though Smoothened inhibitors have shown efficacy in several clinical trials, the initial enthusiasm for these drugs has been tempered by emergence of resistance and a priori resistance, often via mutation of Smoothened itself or through dysregulation of downstream components of the Hedgehog signaling axis. Here we reveal a strategy that overcomes these resistance mechanisms by targeting the far downstream transcriptional mediators of Hedgehog signaling through inhibition of the BET bromodomain protein, BRD4. We show that knockdown of BRD4 or treatment with the BET bromodomain inhibitor, JQ1, dramatically inhibits transcription of GLI and other Hedgehog target genes upon ligand-mediated or genetic activation of the Hedgehog pathway. We confirm the inhibitory effect of JQ1 occurs downstream of SMO and SUFU and verify by chromatin immunoprecipitation that BRD4 directly occupies the GLI1 and GLI2 promoters, with a substantial decrease in the engagement of these genomic sites upon treatment with JQ1. Correspondingly, genes associated with medulloblastoma-specific GLI binding sites are downregulated upon exposure to JQ1, confirming the direct regulation of GLI by BET bromodomain proteins. Finally, using patient- and GEMM-derived cells from various Hedgehog-driven cancers (basal cell carcinoma, medulloblastoma and ATRT), we show that JQ1 decreases Hh pathway output and proliferation, in vitro and in vivo, even in cells resistant to Smoothened antagonists. In sum,

our results expand the role of BET bromodomain inhibitors to targeting Hedgehog-driven cancers and highlight a strategy that overcomes the limitation of Hedgehog pathway inhibitors currently in clinical use.

MB-044. HART-BASED THERAPY FOR THE TREATMENT OF HIGH-RISK MEDULLOBLASTOMA: A REPORT OF 21 PATIENTS FROM 2 ITALIAN CENTERS

Stefano Gabriele Vallero[1], Daniele Bertin[1], Maria Eleonora Basso[1], Claudia Milanaccio[2], Paola Peretta[3], Armando Cama[4], Anna Mussano[5], Salvina Barra[6], Giovanni Morana[7], Isabella Morra[8], Paolo Nozza[9], Franca Fagioli[1], and Maria Luisa Garrè[2]; [1]Pediatric Oncohematology, Stem Cell Transplantation and Cell Therapy Division, A.O.U. Città della Salute e della Scienza – Ospedale Infantile "Regina Margherita", Torino, Italy; [2]Neuroncology Unit, Giannina Gaslini Institute – IRCSS, Genova, Italy; [3]Neurosurgery Division, A.O.U Città della Salute e della Scienza – Ospedale Infantile "Regina Margherita", Torino, Italy; [4]Neurosurgery Dept., Giannina Gaslini Institute – IRCSS, Genova, Italy; [5]Radiotherapy Unit, A.O.U Città della Salute e della Scienza – Ospedale Infantile "Regina Margherita", Torino, Italy; [6]Pediatric Radiation Therapy Unit, IST-San Martino IRCSS, Genova, Italy; [7]Neuroradiology Dept., Giannina Gaslini Institute – IRCSS, Genova, Italy; [8]Pathology Unit, A.O.U. Città della Salute e della Scienza – Ospedale Infantile "Regina Margherita", Torino, Italy; [9]Pathology Dept., Giannina Gaslini Institute – IRCSS, Genova, Italy

Medulloblastoma (MB) is the most frequent malignant brain tumor in children. High-risk patients need intensive chemotherapy and radiotherapy. The Milan strategy (Gandola et al., JCO 2009) encompasses sequential chemotherapy (methotrexate, etoposide, cyclophosphamide, carboplatin) and hyperfractionated accelerated radiotherapy (HART) with a maximum of 39 Gy to the neuraxis and 60 Gy to the posterior fossa (2 fractions/day). Patients with persistent disease before radiotherapy undergo two high-dose chemotherapy (HDCT) courses with autologous peripheral stem cells rescue, while patient who are disease-free before radiotherapy receive maintenance with lomustine (every 9 weeks, total 6 doses) and vincristine (every 3 weeks, total 18 doses). Twenty-one patients, diagnosed with high-risk MB between 2008 and 2013, were treated either in Genoa or in Turin (Italy) according to such protocol. Ten patients (48%) had anaplastic MB (anaplasia being the only high-risk feature in 2/10), 17 patients (81%) had metastases (Chang M1 in 2 cases) and 10 patients (48%) had residual disease $>1.5\ cm^2$. The median follow-up time was 28.7 months. After HART, 13 patients underwent HDCT and 6 patients received maintenance therapy. Overall, 8 patients had disease progression (3 during treatment) and 6 died (2 before HART). Progression-free survival was 85.7% at 6 months (SE 7.4%), 76.2% at 12 months (SE 9%) and 76.2% at 24 months (SE 10%). Overall survival (OS) was 90.5% at 6 months (SE 6.2%), 81% at 12 months (SE 8.3%) and 75.6% at 24 months (SE 10%). None of the patients abandoned therapy or died due to treatment-related toxicity. In our experience, HART-based treatment was feasible and tolerable. The observed OS is favorable in comparison to older protocols and seems in line with the most successful intensive schedules recently published. Nonetheless, we are eager to reach a longer observation time in order to provide more reliable and precise outcome measures for our cohort.

MB-045. INCREASED NUMBERS OF HYPOXIA INDUCING FACTOR-2 ALPHA (HIF-2α) PRODUCING CELLS IN MEDULLOBLASTOMAS

Anna Darabi[1], Emma Sandén[1], Edward Visse[1], Nils Ståhl[2], and Peter Siesjö[2]; [1]Clinical Sciences, Neurosurgery, Lund University, Lund, Sweden; [2]Clinical Sciences, Neurosurgery, Skåne University Hospital, Lund, Sweden

BACKGROUND: Primary brain tumors, including medulloblastomas, high-grade astrocytomas, atypical rabdoid tumors and ependymomas, are the most common solid tumors in children, Less than 60% survive 5 years after diagnosis and the survivors display a substantial morbidity from long-term cognitive and neurological sequels after radio- and chemotherapy. The immune system plays a dual role in cancer - it can either destroy tumor cells or promote tumor growth. Hypoxia in tumors has been shown to redirect the immune system from tumor elimination to tumor immune escape. Tumor promoting and immune suppressive effects are mediated by both cell-to-cell contacts but also by soluble molecules such as hypoxia-inducible-factor (HIF). PRELIMINARY RESULTS: We have detected significantly higher amounts of hypoxic cells in sections of high-malignant medulloblastomas compared to low malignant pediatric brain tumors (pilocytic astrocytomas). HIF-2α was found in the nuclei of both $CD45^+$ and $CD45^-$ immune cells inside or in close proximity to vessels. Hypoxic cells were often located in small clusters surrounding one or more $CD45^+CD15^+CD24^+$ cell with a nuclear morphology of a granulocyte. Macrophages ($CD68^+$), either $HIF\text{-}2\alpha^+$ or $HIF\text{-}2^-$, were closely associated with these clusters. CONCLUSIONS: We hypothesize that granulocytes and macrophages might induce HIF-2α expression in tumor cells and that this represents a tumor promoting nische.

MB-046. IDENTIFYING VULNERABILITIES IN GROUP 3/MYC-AMPLIFIED MEDULLOBLASTOMA VIA INTEGRATED HIGH-THROUGHPUT GENETIC AND CHEMICAL SCREENING

Yoon-Jae Cho[1], Dedeepya Vaka[1], Simone Schubert[1], Francisca Vasquez[2], Barbara Weir[2], Glenn Cowley[2], Charles Keller[3], and William Hahn[2]; [1]Stanford University, Stanford, CA, USA; [2]Broad Institute, Cambridge, MA, USA; [3]Oregon Health & Sciences University, Portland, OR, USA

Medulloblastomas are the most common malignant brain tumors in children. Despite continued refinement of neurosurgery, chemotherapy and radiotherapy, durable remission is achieved in only 60% of patients diagnosed with this disease. Our group and others have established that most deaths from medulloblastoma occur within a defined molecular subtype, termed "Group3" and characterized by high level MYC-amplification. Importantly, many patients with MYC-amplified tumors receive the maximally tolerated doses of chemotherapy and radiation and still succumb to their disease, highlighting the inherent resistance of these tumors to conventional treatments and the tremendous need for more effective strategies focused on treating these aggressive cancers. Although large-scale genomic analysis of medulloblastoma have identified distinct gene expression signatures suggesting the involvement of various biological pathways in driving/maintaining MYC-amplified tumors, the true biological and clinical consequence of targeting these genes and pathways in medulloblastoma cannot be clearly elicited from these studies. In order to translate our genomic findings into realistic therapies for patients, a systematic functional assessment of each gene and pathway is necessary. Here, we have assembled a functional annotation of the MYC-amplified medulloblastoma genome by systematically knocking down each gene in the genome of patient-derived MYC-amplified medulloblastoma cell lines and identifying the genes essential for medulloblastoma cell survival. Furthermore, we have integrated these genetic screens with chemical library screens to identify concordant targets and biological pathways that represent therapeutic vulnerabilities in these highly lethal tumors. Results from these studies should help guide the next generation of clinical trials for patients with Group 3/MYC-amplified medulloblastoma.

MB-047. UNRAVELING THE TALE OF MEDULLOBLASTOMA: IMPACT OF MOLECULAR SUB-TYPE AND CRANIOSPINAL IRRADIATION (CSI) DOSE ON RELAPSE

Iris C. Gibbs[1], Sonia Partap[1], Kristen Yeom[1], Mesha Martinez[3], Hannes Vogel[1], Sarah S. Donaldson[1], Paul Fisher[1], Sébastien Perreault[2], and Yoon-Jae Cho[1]; [1]Stanford University/Lucile Packard Children's Hospital, Stanford, CA, USA; [2]University of Montreal, Montreal, Canada; [3]Temple University, Philadelphia, PA, USA

PURPOSE: To explore the potential pitfalls of medulloblastoma managed based on clinic-pathological factors by assessing the impact of craniospinal irradiation (CSI) dose and molecular sub-type on relapse, METHODS: Among 115 patients with medulloblastoma evaluated at our institution, 36 had complete clinical, imaging, histopathology (24 classic, 7 large cell/anaplastic (LC/A), 3 desmoplastic, 1 extensive nodularity, and 1 other) and molecular sub-typing data by nanostring assay (WNT, SHH, and non-WNT/non-SHH (NWNS)). Patients received CSI doses from 18 Gy to ≥36 Gy determined on clinic-pathological factors. Relapse rates were assessed and CSI dose was categorized as either divergent or similar to a proposed CSI schema based on prognostic implications of molecular subtype. Accordingly, the proposed CSI dose: ≥36 Gy for M+ and Group 3; ≤18 Gy for non-disseminated WNT; 23.4 Gy all others. Three representative cases are illustrated. RESULTS: CSI dose was 18 Gy(11), 23.4 Gy(9), and ≥36 Gy(16). CSI dose was divergent from the proposed dose schema in 18 cases (14-lower dose, 4-higher dose) and non-divergent in 18 tumors. In this cohort, there were 14 relapses (8- classic, 6-LC/A,1-EN). 50% of tumors treated to a lower dose relapsed (1 SHH; 6 NWNS); only 1 (NWNS) of 4 patients with higher divergent dose relapsed. Six of the 18 patients treated to similar dose relapsed, all were NWNS (group 3); 4 of 6 had M3 disease. The 3 illustrative cases 1) non-disseminated WNT tumor treated to 36 Gy; 2) non-disseminated NWNS treated to 18 Gy; 3) non-disseminated SHH treated to 18 Gy. CONCLUSION: We confirm that histological subtypes may still hold some predictive value to guide treatment. Molecular typing may help to avoid under-treating NWNS tumors, but innovative strategies for metastatic NWNS tumors are needed. De-escalation

<18 Gy for non-metastatic WNT tumors should be explored as no relapse occurred in WNT tumors

MB-048. BENEFITS AND RISKS OF NEOADJUVANT CHEMOTHERAPY IN METASTATIC MEDULLOBLASTOMA: A COMPARATIVE STUDY IN 71 PATIENTS

Léa Guerrini-Rousseau[1], Christelle Dufour[1], Stéphanie Pujet[2], Virginie Kieffer-Renaux[1], Marie Anne Raquin[1], Pascale Varlet[3], Audrey Longaud[1], Christian Sainte-Rose[2], Dominique Valteau-Couanet[1], and Jacques Grill[1]; [1]Institut de Cancérologie Gustave Roussy, Villejuif, France; [2]Hôpital Necker Enfants Malades, Paris, France; [3]Hôpital St Anne, Paris, France

Preoperative chemotherapy is often used in pediatric oncology to treat metastatic disease and facilitate the surgery of the primary tumor, but not for brain tumors. A first pilot study showed in 2004 the feasibility and effectiveness of preoperative chemotherapy in treating high-risk medulloblastomas. Seventy-one patients were treated for a metastatic medulloblastoma between 2002 and 2010 at Gustave Roussy. Two strategies were compared in intention to treat. Forty-two children were operated at the time of the diagnosis (group A) and the 29 others children were treated with 2 courses of carboplatin and etoposide (group B) after histological diagnosis (biopsy) and before a delayed surgery. Children of group A received the two courses of carboplatin and etoposide afterwards. The rest of the protocol (high-dose chemotherapy and cranio-spinal irradiation) was similar in the both groups. Complete neuropsychological testing, including intelligence quotient measurements, was scheduled before surgery, before radiotherapy and every year thereafter. MRI performed after neo-adjuvant chemotherapy showed an objective response in 24 patients (83%) and a stable disease in 4 patients (14%). Complete excision rate was significantly higher in group B (100% versus 64%, $p = 0.00248$) without any difference in postoperative complication rate. Medulloblastoma cells could still be evidenced despite preoperative chemotherapy. The median Total Intelligence Quotient (TIQ) was 81 and 90 in group A and B respectively ($p = 0.02543$). This difference was even more significant in young children (77 vs 91 points for groupe A and B, respectively). Hydrocephalus and brain radiotherapy dose were not associated with TIQ. Neo-adjuvant chemotherapy had no negative impact on local disease control (79 versus 83%). Event-free survival and overall survival at 3 years were 54 versus 62% and 58 versus 68% respectively ($p = NS$). Neo-adjuvant chemotherapy in metastatic medulloblastoma is safe and could have a positive impact on neuropsychological outcome and completeness of surgery.

MB-049. TOWARD A QUANTITATIVE PROTEOMIC ANALYSIS OF MEDULLOBLASTOMA SUBGROUPS

Jerome Staal[1], Ling San Lau[1], Huizhen Zhang[1], Wendy J. Ingram[2], Yoon Jae Cho[3], Yetrib Hathout[1], Kristy Brown[1], and Brian R. Rood[1]; [1]Children's National Medical Center, Washington, DC, USA; [2]Queensland Children's Medical Research Institute, Brisbane, Australia; [3]Stanford School of Medicine, Stanford, CA, USA

BACKGROUND: Medulloblastoma biology is largely viewed as bounded by genomic subgroups. Missing from this paradigm is an analysis of the contribution of the proteome toward subgroup specific functional biology. METHODS: Proteomics has historically contributed less to the field of cancer biology due to technical limitations in the ability to accurately quantitate large numbers of proteins in a discovery environment. We have used stable isotope labeling of amino acids in cell culture (SILAC) to construct a Labeled Atlas of Medulloblastoma Proteins (LAMP) which contains a complete complement of mass shifted proteins elaborated by 4 medulloblastoma cell lines. The LAMP was then spiked into cell lysates of primary cell lines representing the 4 medulloblastoma subgroups and an LC-Orbitrap mass spectrometer was used to accurately quantitate protein relative to the LAMP. RESULTS: Accurate quantitation was achieved for an average of 1215 proteins per primary cell line. Technical replicates resulted in r^2 values between 0.91 and 0.98. 94.2-98.6% of sample proteins were found within a 5 fold dynamic range relative to the reference atlas. The majority of proteins (588) were found to be common to all lines with a subset of proteins (8-11%) being unique to the cell lines from each subgroup. Group 3 and Group 4 tumors exhibited the greatest overlap between any 2 groups (53 proteins). Unique proteins were subjected to pathway analysis to determine upstream regulators. In addition, metastatic and non-metastatic Group 3 lines were compared to identify proteins with potential functional significance. CONCLUSIONS: SILAC reference atlases can be used to develop mass spectrometry platforms for relative proteomic quantitation between biological tumor groups. Given that proteins represent the functional compartment of the cell, proteomics has the potential to augment the genomic understanding of tumor biology and identify biologically significant proteins that could serve as drug targets or translational biomarkers.

MB-050. PERIPHERAL BLOOD CYTOKINE PROFILES IN PEDIATRIC BRAIN TUMOR PATIENTS REFLECT TUMOR MALIGNANCY

Emma Sandén[1], Edward Visse[1], Nils Ståhl[2], Peter Siesjö[2], and Anna Darabi[1]; [1]Clinical Sciences, Neurosurgery, Lund University, Lund, Sweden; [2]Clinical Sciences, Skåne University Hospital, Lund, Sweden

BACKGROUND: Pediatric brain tumors represent a heterogeneous group of tumors, and the genomic era has revealed even further heterogeneity within distinct tumor types. A common hallmark and important step in the progression of all tumors is however the acquisition of immune evading properties to avoid destruction by immune cells. We have previously developed immunotherapeutic treatment strategies modulating these pathways in adult malignant brain tumors, and increasing evidence suggests that children may also benefit from this treatment approach. The present study was conducted in order to characterize inflammatory cytokines in blood samples from pediatric brain tumor patients. Such an inflammatory profile could be utilized as a biomarker of tumor progression, to identify novel targets of immunotherapy, and eventually aid in monitoring the therapeutic response in patients. METHODS: Blood samples from 38 pediatric and 2 adult brain tumor patients were collected at the time of surgery. Tumor types included medulloblastoma (MB, adult $n = 2$, pediatric $n = 13$), primitive neuroectodermal tumor (PNET, $n = 1$), glioblastoma (GBM, $n = 2$), anaplastic ependymoma (AEP, $n = 2$), anaplastic astrocytoma (AA, $n = 2$), ependymoma (EP, $n = 2$), pilomyxoid astrocytoma (PMA, $n = 2$), pilocytic astrocytoma (PA, $n = 12$), meningioma (MN, $n = 1$) and sarcoma (SC, $n = 1$). Plasma was isolated and analyzed with high-sensitivity cytokine multiplex assays (Meso Scale Discovery). RESULTS: Preliminary results show a distinct cytokine phenotype of grade IV tumors, including high levels of interleukin (IL)-7 and low levels of tumor necrosis factor (TNF)-ß. In normal developing human brain it is suggested that IL-7 influences neural differentiation of progenitor cells, however very little is known of the role of IL-7 in brain tumors. CONCLUSIONS: A peripheral cytokine profile holds great promise as a marker for tumor malignancy. Future functional analyses on isolated blood lymphocytes and cultured brain tumor cells will elucidate the origin of blood cytokines, and their role in tumor-related inflammation.

MB-051. DISSEMINATED LEPTOMENINGEAL PNET WITHOUT A PRIMARY TUMOR: 5 CASES

Michael Handler[1], Todd Hankinson[1], Jenny Madden[1], B. K. Kleinschmidt-Demasters[2], and Nicholas Foreman[1]; [1]Children's Hospital Colorado, Aurora, CO, USA; [2]University of Colorado School of Medicine, Aurora, CO, USA

INTRODUCTION: While medulloblastomas commonly are metastatic at presentation, disseminated tumor without a primary site is extremely rare. We reviewed a single institution experience with this manner of presentation. METHODS: Patients were identified from records of the tumor program, spanning 19 years. Demographics, details of presentation, histology, and clinical course were reviewed. RESULTS: 5 patients were identified, 3 males and 2 females. The mean age was 8 years at presentation for medical treatment. 2 had "typical" presentation of headaches and papilledema, with hydrocephalus and diffuse leptomeningeal enhancement in brain and spine, without a definable mass. One presented with repeated subarachnoid hemorrhage, the diagnosis made after multiple large volume lumbar punctures over 3 months, and the development of disseminated disease on MRI. One presented with intermittent hip pain over 6 months, who underwent a bone biopsy demonstrating metastatic (non-Ewing) PNET before spine imaging demonstrated extensive leptomeningeal disease. One presented with progressively worsening headaches, ataxia, diplopia, and urinary incontinence with extensive cranial leptomeningeal enhancement, interpreted as inflammatory. Eventually, craniotomy demonstrated the tumor. All had a histopathological diagnosis of PNET after open procedures, at which only limited material could be collected. One patient had extensive glial as well as neuronal differentiation. Four required ventriculoperitoneal shunting. All were treated with craniospinal radiation. Response to radiation was poorer that usually seen in classic PNET, and all received high dose chemotherapy with autologous stem cell transplantation. Three died, one within 6 months, one each at 1.5 and 2.5 years, and one has survived 2 years; the last is newly diagnosed. CONCLUSION: These are extremely unusual presentations of PNET, which seem to behave more aggressively. This may correlate to differences in biology which have, as yet, remained obscure.

MB-052. ROLE OF THE LIM DOMAIN BINDING PROTEIN LDB1 AND THE LIM ONLY FACTOR LMO4 IN SHH SUBGROUP MEDULLOBLASTOMA

Sonja Hutter[1], Paul A. Northcott[1], Marcel Kool[1], Stefan Pfister[1], Daisuke Kawauchi[1], and David T. Jones[1]; [1]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Department of Pediatric Oncology, Hematology & Immunology, Heidelberg, Germany

Recent genomic analyses of medulloblastoma, a malignant embryonal tumor comprising about 15% of pediatric brain cancers, have identified distinct somatic alterations occurring in specific molecular subgroups of the disease. These new findings have shed light on new pathways which may be involved in these subgroups. For example, large-scale sequencing and copy number studies have identified recurrent inactivating mutations of LIM domain binding 1 (LDB1) and amplifications of the LDB1 interacting protein, LIM-only 4 (LMO4) in 7% and 1.1% of SHH-subgroup medulloblastoma (SHH-MB), respectively. LMO4 is also highly expressed in SHH-MB versus other subgroups. LDB1 is a ubiquitously expressed cofactor that participates in the assembly of multi-protein complexes (e.g. the N-CoR complex) involving LIM domain transcription factors and other partners. LMO4 belongs to the LIM only family of zinc finger proteins that are implicated in the onset or the progression of several cancers, including T cell leukemia, breast cancer and neuroblastoma. We therefore investigated the functional role of LDB1 and LMO4 in SHH-MB. Cerebellar granule neuron precursors (CGNPs) are thought to be the cell-of-origin of the SHH-dependent subtype of medulloblastoma and therefore represent the ideal cell type to study novel candidate genes. To this end, we manipulated gene expression in CGNPs purified from normal C57BL/6 or Ptch1$^{+/-}$ mice by either retroviral expression of Lmo4 or shRNA-mediated knockdown of Ldb1. Forced expression of Lmo4 was found to stimulate CGNP proliferation. Subsequently, these manipulated CGNPs have been transplanted into cerebella of immunocompromised recipient mice to assay for tumorigenicity in vivo. Overexpression of the Mycn oncogene (amplified in a subset of SHH-MB) was used as a positive control for all in vitro and in vivo experiments. Tumors arising from Mycn-transduced CGNPs, as well as any arising from Lmo4 or Ldb1 manipulation will be further characterized in terms of downstream pathway activity.

MB-053. RETROSPECTIVE ANALYSIS OF RECURRENT PATTERNS AND CLINICAL OUTCOME BY REDUSED-DOSE IRRADIATION PLUS ADJUVANT CHEMOTHERAPY IN MOLECULAR SUBGROUPING OF MEDULLOBLASTOMAS

Naoki Kagawa[1], Ryuichi Hirayama[1], Noriyuki Kijima[2], Yasuyoshi Chiba[1], Manabu Kinoshita[3], Koji Takano[1], Daisuke Eino[1], Shogo Fukuya[1], Fukuko Yamamoto[4], Katsuhiko Nakanishi[1], Naoya Hashimoto[1], Yoshiko Hashii[5], Jyunichi Hara[6], Michael D Taylor[2], and Toshiki Yoshimine[1]; [1]Department of Neurosurgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Division of Neurosurgery, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [3]Department of Neurosurgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; [4]Department of Neurosurgery, Suita Manucipal Hospital, Suita, Osaka, Japan; [5]Department of Developmental Medicine, Osaka Univeristy Graduate School of Medicine, Suita, Osaka, Japan; [6]Department of Pediatric Hematology/Oncology, Osaka City General Hospital, Osaka, Japan

BACKGROUND: Therapeutic challenges against recurrence of medulloblastomas have difficult problems, although prognosis of medulloblastomas has been improved by craniospinal irradiation and adjuvant chemotherapy. We retrospectively analysed recurrent patterns and differences of clinical outcome based on molecular subgrouping for medulloblastomas treated by redused-dose irradiation and high-dose chemotherapy. Patients and METHODS: Twenty-one patients with medulloblastomas treated in our institution from 1994 to 2013 were classified into four subgroup by nanoStrings assay using frozen specimens. Age distribution was one to twenty-two years old. The subgroup distribution was four SHH, five group 3 and eleven group 4 without WNT case. In all cases older than 3 years old, redused-dose craniospinal or cranial irradiation (18 Gy) plus adjuvant chemotherapy was done after tumor removal. High-dose chemotherapy was performed in high-risk group. RESULTS: No recurrence was seen in SHH. Of five group 3 cases, four had recurrent medulloblastomas and the period between initial treatment and recurrence was within 16 months. 5 year progression-free survival (5y-PFS) was 20.0%. Recurrent cells were rapidly and extensively disseminated and progressive despite of many therapeutic chanllenges. The period between recurrence and death was 4 to 7 months and 5 year overall survival (5y-OS) was 26.6%. Of eleven group 4, slow-growing and asymptomatic recurrences were shown in four cases. The period between initial treatment and recurrence was 18 to 70 months. Both 5y-PFS and 5y-OS were 70%. Although high-dose chemotherapy or intrathecal injection of chemotherapeutic agents had little effect, the conditions of partial response or stable disease were maintained by stereotactic radiotherapies and metronomic chemotherapies using oral etoposide and temozolomide. CONCLUSION: By therapeutic regimen including redused-dose irradiation, 5y-PFS, 5y-OS and survival time after recurrence in group 4 are significant longer than those in group 3. Molecular subgrouping may predict recurrent patterns, response against treatment and prognosis in each group.

MB-054. A 13 YEARS CLINICAL EXPERIENCE OF MULTIDISCIPLINARY TREATMENT FOR MEDULLOBLASTOMA: ANALYSIS OF 104 CASES

Jian Wang, Chengcheng Guo, Qunying Yang, and Zhongping Chen; Sun Yat-Sen University Cancer Center, Guangzhou, China

BACKGROUND: Medulloblastoma is primary pediatric brain tumors that requires multidisciplinary therapies. We summarized and reported the management and the therapeutic efficacies of medulloblastomas in our cancer center. METHODS: We evaluated 104 pts newly diagnosed as medulloblastoma from Feb 2000 to Apr 2013 retrospectively. Mean patient age was 9.6 years (range, 16months to 16 years), including 94.2% (98/104) pts who were $\geq$ 3years old. There were 69.2% (72/104) with brainstem compression, 67.3% (70/104) with moderate and severe hydrocephalus, 4.8% (5/104) spinal dissemination. After gross total resection, pts $\geq$ 3years old underwent craniospinal radiation 24 Gy with boosting primary brain site to 56Gy,followed by 6 cycles of chemotherapies including VCR,CCNU and DDP; pts $<$3 years old only underwent alternated chemotherapy including IFO, VP16,CTX, VCR etc. RESULTS: At a median follow-up of 6.3 years, the 2-year OS and 5-year OS were 87.9% and 63.6%, respectively. 5-year OS of he heightened risk and average risk were 42.9% and 66.7% respectively. There were 26 cases died from tumor relapsed with 23 cases of primary site relapse and 3 of spinal dissemination. And there was no significant relationship between the brainstem compression and the prognosis. CONCLUSION: Combination of radiation and chemotherapy following gross total resection is an effective and tolerable treatment for pediatric medulloblastoma. The prognosis is poorer in heightened risk pts. Searching the tumor markers combined with molecular diagnostics mode may improve the survival of pediatric medulloblastoma.

MB-055. PROTOCOL VIOLATIONS AND ITS IMPACT ON SURVIVAL IN NON-WNT/SHH TYPE OF MEDULLOBLASTOMA IN CHILDREN OVER 3 YEARS OF AGE TREATED ACCORDING TO POLISH PEDIATRIC NEUROONCOLOGY GROUP (PPNG) PROTOCOL

Marta Perek-Polnik, Maria Lastowska, Monika Drogosiewicz, Bozenna Dembowska-Baginska, Wieslawa Grajkowska, Iwona Filipek, Ewa Swieszkowska, Magdalena Tarasinska, and Danuta Perek; The Children's Memorial Health Institute, Warsaw, Poland

AIM: Analysis of protocol violations and its impact on survival in patients with non-WNT/SHH type of Medulloblastoma, treated according to PPNG protocol. MATERIAL AND METHOD: Between 1997 and 2007 103 Medulloblastoma patients older than 3 years of age were treated according to PPNG protocol (pre-irradiation chemotherapy, craniospinal radiotherapy, maintenance chemotherapy). Biological non-WNT/SHH group of tumours was identified by immunohistochemistry using a panel of representative antibodies. Clinical factors analyzed included: age, gender, stage of disease, protocol violations (reduction of drug dose administered, delays in chemotherapy, radiotherapy in days) and its impact on EFS. RESULTS: After exclusion of the WNT, SHH, and MYCC amplified tumours 93 patients were identified (66 boys and 27 girls) aged from 3,14 to 17,33 (median 9,5). Chemotherapy was given in doses from 85 to100% (mean 99,6%, median 100%) for pre-irradiation and from 50-100% (mean 86%, median 89%) for maintenance therapy. Number of administered chemotherapy courses ranged from 2 to11 (mean 7,5; median 8) and single VCR courses from 0 to19 (mean 9,5; median 9). Treatment delays ranged from 0 to125 days (mean 34, median 23). Mean values were considered as the cut-off-points. For the whole group of patients 5 years EFS was 69,6%. Reducing the number of chemotherapy courses as well as single VCR courses statistically influenced survival (EFS 46,7 vs. 78%, $p = 0{,}016$ and 54,3 vs. 78%, $p = 0{,}006$ respectively), while dose reductions and delays did not (60 vs. 74,2, $p = 0{,}09$ and 44,4 vs. 72,29 $p = 0{,}16$, respectively). CONCLUSIONS: Our data based on analysis of patients with biologically relevant molecular group suggest that the number of chemotherapy courses, including VCR, are of importance for survival rate and should not be modified. Less stringent rules may apply with regard to dose reductions and chemotherapy delays in a case of chemotherapy toxicity.

MB-056. PINEALOBLASTOMAS

Rejin Kebudi[1], Basak Koc[1], Omer Gorgun[1], Fulya Yaman Agaoglu[2], Johannes Wolff[3], and Emin Darendeliler[2]; [1]Istanbul University, Cerrahpasa Medical Faculty and Oncology Institute, Pediatric Hematology-Oncology, Istanbul, Turkey; [2]Istanbul University, Oncology Institute, Radiation Oncology, Istanbul, Turkey; [3]Cleveland Clinic, Lerner College of Medicine, Pediatric Hematology-Oncology, Cleveland, OH, USA

Pinealoblastomas (PBL) are rare tumors of the central nervous system and more common in children. The objective of this study is to evaluate the demographic data and outcome of children with PBL in a single center. During 1990-2012, 6 children (3 male, 3 female) with a median age of 6 years (2 years- 16 years), were diagnosed with pinealoblastoma, in the Istanbul University, Oncology Institute. At the same time interval 494 patients <19 years old were diagnosed with malignant CNS tumors in the same center, thus pinealoblastomas constituted 1. 2 % of all Pediatric CNS tumors. Three had subotal resection and three underwent a biopsy. At diagnosis, one had spinal seeding both in MRI and CSF cytology. All recieved craniospinal radiotherapy and chemotherapy, the patient < 3 years old recieved neoadjuvant chemotherapy first, one patient recieved treatment abroad and is followed up in this center. The median follow up is 5 years (1-9 years). Two patients are alive for 5 and 9 years. All others have died at a median of 2.7 (1-8) years due to relapse/ progressive disease. In conclusion, PBL are aggressive tumors necessitating intensive treatment strategies.

MB-057. SMARCA4 AND THE CHROMATIN REMODELING COMPLEX SWI/SNF IN BRAIN DEVELOPMENT AND DISEASE

Christin Schmidt[1], Kornelius Kerl[2], Jan Gronych[1], Daisuke Kawauchi[1], Peter Lichter[1], Ulrich Schüller[3], Stefan Pfister[1], and Marcel Kool[1]; [1]German Cancer Research Center, Heidelberg, Germany; [2]Westfalian-Wilhelms-University, Münster, Germany; [3]Ludwigs-Maximilian-University, Munich, Germany

The chromatin remodeling complex SWI/SNF is an important regulator of embryogenesis and development. Whole genome and exome sequencing of medulloblastoma, one of the most common malignant brain tumors in childhood, identified somatic mutations in SWI/SNF complex members in 12% of all cases. SMARCA4 was the most commonly affected member with mutations in 6% of all patients and were identified in all four molecular subgroups. Heterozygous SMARCA4 mutations were found in the bromodomain, the helicase domain and in the ATPase domain, presumably all leading to reduced activity of SMARCA4 and the SWI/SNF complex. In order to understand the role of SMARCA4 in brain development and medulloblastoma tumorigenesis, we are generating three different mouse models of Smarca4 knockout in the CNS. Using the cre-loxP recombination system, we are targeting distinct neuronal precursor cell populations to generate cell-specific knockout mice. In our Math1-cre::Smarca4$^{Fl/Fl}$ mouse model with Smarca4 knockout in neuronal granular precursor cells, heterozygous mice looked healthy with no signs of brain abnormality or tumor growth. Homozygous mice showed light symptoms of tremor starting at the age of approximately 14 days without further worsening of symptoms. However, no tumors were found thus far. Histological analyses of brains at postnatal day 7, 22 and 45 showed an overall reduced size of the cerebellum and severe developmental defects of the rostral part of the cerebellum. Within the rostral region of the cerebellum, disturbed cell layering and an almost complete absence of the purkinje cell layer was apparent. Additional immunohistochemical analyses of Math1-cre::Smarca4$^{Fl/Fl}$ cerebelli and in vitro analyses of Smarca4-deficient mouse primary granular precursor cells have been performed to further understand the role of Smarca4 during cerebellar development. Other models, currently being generated include a Prom1-creERT2::Smarca4$^{Fl/Fl}$ mouse model for knockout in neuronal stem cells and a Blbp-cre::Smarca4$^{Fl/Fl}$ mouse model for knockout in cerebellar radial glia cells.

MB-058. EVALUATION OF A NOVEL COMBINATORIAL CHEMOTHERAPY IN PAEDIATRIC BRAIN TUMOUR MODELS

Jacqueline McGlade[1], Raelene Endersby[1], Hilary Hii[1], Terrance Johns[2], and Nicholas Gottardo[3]; [1]Telethon Institute for Child Health Research, University of Western Australia, Western Australia, Australia; [2]Monash Institute of Medical Research, Victoria, Australia; [3]Princess Margaret Hospital, Western Australia, Australia

Medulloblastoma and pineoblastoma are malignant childhood brain tumours where many patients don't respond to existing therapy, highlighting the need for new therapeutic strategies. To evaluate the efficacy of potential new drugs, as single agents and in combination with conventional chemotherapeutics, we have developed in vitro models and multiple in vivo systems of these diseases. Overexpression of the ERBB family of tyrosine kinases is associated with poor prognosis in medulloblastoma and thus form potential targets for therapy. The expression and activity of EGFR and ERBB2 were evaluated by immunoblot in two paediatric brain tumour cell lines, PER-452 (pineoblastoma) and DAOY (medulloblastoma) which had been modified to express firefly luciferase. The pan-ERBB receptor inhibitor dacomitinib effectively blocked receptor activity and downstream signalling in vitro. Moreover the drug had potent anti-proliferative effects in each cell line and was observed to synergistically interact with cyclophosphamide (CPA), a drug commonly used in treatment of these cancers, to inhibit tumour cell proliferation. Therefore dacomitinib and/or combination treatment was further evaluated in vivo in animals that had been intracranially implanted with each cell line. Using bioluminescence, tumours were monitored until well established, then animals were treated with vehicle, dacomitinib, CPA, or both. Immunohistochemical analyses of treated tumours confirmed inhibition of ERBB receptors and staining for markers of proliferation and apoptosis revealed in vivo anti-tumour activity. Despite these positive results, no overall improvement in survival was observed in medulloblastoma-bearing animals treated with these chemotherapeutics. This study illustrates a unique and thorough drug evaluation pipeline, in which the efficacy of new drugs for paediatric brain tumours can be determined, enabling the exclusion of potentially ineffective treatments and prioritisation of truly beneficial novel treatments for clinical trial.

MB-059. LONG TERM FOLLOW UP OF SURVIVORS OF MEDULLOBLASTOMA (MBL) AND PNETS (PRIMITIVE NEURO ECTODERMAL TUMOURS): SCOTTISH SINGLE CENTRE EXPERIENCE

Jairam Sastry[1], Dermot Murphy[1], Milind Ronghe[1], Corrie Cunningham[1], Fiona Cowie[2], and Richard Jones[2]; [1]Royal Hospital for Sick Children, Glasgow, UK; [2]The Beatson West of Scotland Cancer Centre, Glasgow, UK

METHODS: A retrospective audit of long term neurologic, endocrine, sensory, renal and audiological outcomes for children treated at our centre. Cases were identified via the unit data base over a 10 year period (2000-2009). A standardised proforma was used to collect information from database, case notes, computerised hospital records and correspondences. RESULTS: 41 patients were identified of whom 25 fitted the inclusion criteria (those who were still under follow up and at least 1 year off treatment) were for analysis. Age at diagnosis varied from 11months to 16 years with an average of 6.77 years and the length of follow up varied from just over one year to 11 years. 4 had Standard Risk MBL, 16 had High Risk MBL, 4 had Supratentorial PNET and one had spinal cord PNET. Therapy included maximal surgical resection, followed by Cranio Spinal Irradiation (30-36Gy) and boost to primary (19-25 Gy) followed by chemotherapy. 15 children received chemo according to Packer regimen, 2 were treated with Headstart III with peripheral blood stem cell transplant and 7 were treated with other protocols. One child did not receive any RT. Long term effects were significant and included; Audiological 60%, Neuropsychological defect 44%, Endocrine 32%,Sensory 26%,, and Renal impairment 26%. CONCLUSIONS: Significant predictable long term sequelae were noted in the survivors, impacting on survival quality. Future research should aim to decrease long term effects, while maintaining good survival rates. A structured follow up program should be in place to anticipate and address long term effects enabling survivors to attain their maximum potential.

MB-060. SECONDARY SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMOUR (PNET): TWO CASE REPORTS FROM A SCOTTISH PAEDIATRIC NEURO-ONCOLOGY UNIT

Jairam Sastry, Amedo Calisto, Meharpal Sangra, Calan Mathieson, and Jennifer Brown; Royal Hospital for Sick Children, Glasgow, UK

INTRODUCTION: Secondary cancer in the brain in children are rare(0.8%), Supratentorial PNET being even rarer. Radiotherapy and chemotherapeutic agents, including alkylating agents and topoisomerase II inhibitors, have been reported to result in secondary malignancies. We report two cases of secondary supratentorial PNETs. CASE 1: A 16-year-old Caucasian male was diagnosed with a supratentorial PNET seven years following the diagnosis of nonmetastatic osteosarcoma of the right distal femurs. The patient had been treated with standard chemotherapy (MAP-Methotrexate, Adriamycin and Cisplatin) and limb salvage resection for osteosarcoma. Almost 6 and half years after the completion of osteosarcoma treatment, the patient was diagnosed with a supratentorial PNET. CASE 2: A 14 year old girl Arabic girl was diagnosed with a Supratentorial PNET, four years following the diagnosis of a supra sellar

Intra cranial Malignant Germ cell tumor. The diagnosis of Malignant GCT was based on elevated tumor markers from CSF and Blood, without biopsy of the primary tumor. She recieved Chemotherapy (PEI-Cisplatin, Etoposide and Ifosfamide) and focal radiotherapy. she developed the secondary supratentorial PNET 3 and half year after completing treatment. DISCUSSION: Secondary intra cranial tumours are well recognized in children, although it is rare. The most common histological types reported are malignant gliomas, meningiomas and schwannomas. Pathology should be carefully interpreted, so as not to miss the recurrence of the original tumour, transformation into another type or radiation induced necrosis. The interval between primary and secondary tumours in case 2 is very short and hence it may be possible that this may be a transformation of the previous tumour. The reason for the occurrence of second tumour is difficult to be certain, although it is often reported as secondary to previous radiotherapy and or chemotherapy. Other underlying genetic predispositions may co-exist and should be investigating. Prognosis is generally regarded as very poor for secondary PNETs.

MB-061. BIFOCAL PINEAL AND SUPRASELLAR TUMOR AS A RARE PRESENTATION OF SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMOR: CASE REPORT

Kamon Phuakpet[1], Valerie Larouche[2], Cynthia Hawkins[1], Ute Bartels[1], and Eric Bouffet[1]; [1]The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [2]Centre Hospitalier Universitaire de Quebec, QC, Canada

BACKGROUND: Bifocal pineal/suprasellar tumor (BPST) is an entity of brain tumor involving both pineal and suprasellar regions without any continuity between both lesions. Diabetes insipidus (DI) is the most common presentating symptom of BPST. For many authors, BPST is pathognomonic of CNS germ cell tumor (GCT). Since BPST has never been reported in other pediatric brain tumors, some physicians have questioned the relevance of biopsying these lesions. Therefore, in many situations, BPST will be treated as CNS GCT based on radiological findings only. CASE REPORTS: We report 2 cases of BPST who presented without DI. In both patients, tumor markers for GCT (AFP, β-HCG) were negative. Tumor biopsy revealed in each case the diagnosis of supratentorial primitive neuroectoderlal tumor (sPNET). As of our knowledge, these are the first pediatric reports of BPST outside the context of CNS GCT. The first patient is a 3-year-old girl who presented with headache, lethargy, bilateral hemianopia, papilledema and parinaud syndrome. She also has enhancing lesion at the thoracic spinal cord and subarachnoid space between both frontal lobes. She was treated with craniospinal irradiation (CSI) followed by high dose chemotherapy and stem cell rescue. She is alive and in remission 5 years after the diagnosis. The second patient is a 14-year-old boy who presented with headache, bilateral visual loss and bilateral papilledema. He was treated with CSI and conventional chemotherapy. He developed diffuse leptomenigeal relapse after has been remission for 3 years. He is currently receiving salvage treatment. CONCLUSION: Although the majority of BSPT is CNS GCT in origin, it should be kept in mind that there is other possibility especially when typical clinical manifestation such as DI and other endocrine abnormalities are missing. Tumor biopsy is highly recommended in this situation to provide a correct diagnosis and an appropriate treatment.

MB-062. HIGH DOSE TOPOTECAN, MELPHALAN, AND CYCLOPHOSPHAMIDE (TMC) WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT FOR ADVANCED MEDULLOBLASTOMA, SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMORS, AND PINEOBLASTOMA

Toshiaki Ishida[1], Daiichiro Hasegawa[1], Kenji Miyata[1], Satoshi Ochi[1], Atsuro Saito[1], Aiko Kozaki[1], Tomoko Yanai[1], Keiichiro Kawasaki[1], Kazuki Yamamoto[2], Atsufumi Kawamura[2], Tatsuya Nagashima[2], Yoshinobu Akasaka[3], Toshinori Soejima[3], Makiko Yoshida[4], and Yoshiyuki Kosaka[1]; [1]Department of Hematology and Oncology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan; [2]Department of Neurosurgery, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan; [3]Department of Radiology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan; [4]Department of Pathology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan

BACKGROUND: Despite recent advances in the treatment of pediatric brain tumors, the prognosis of children with advanced brain tumors is still poor. High-dose chemotherapy (HDCT) with autologous peripheral blood stem cell transplant (PBSCT) has been used for these children. Here we present the efficacy and toxicity of the new HDCT regimen consisting of topotecan, melphalan, and cyclophosphamide (TMC) in children with advanced medulloblastoma, supratentorial primitive neuroectodermal tumors (sPNETs), and pineoblastoma. METHODS: Between January 2011 and July 2013, 12 children aged 1 to 10 years (median; 7 years) were enrolled. Nine patients had medulloblastoma, two had sPNETs, and one had pineoblastoma. Five patients were in complete remission, six in partial response, and 1 in progressive disease at HDCT. Ten patients had primary high risk disease and two were first relapse. Patients were treated with topotecan 3.5 mg/m^2/d on days -7 to -3, melphalan 70 mg/m^2/d on days -4 and -3, and cyclophosphamide 1000 mg/m^2/d on days -7 to -5, followed by PBSCT on day 0. The adverse events of the regimen were evaluated in accordance with Common Terminology Criteria for Adverse Events v4.0, and compared with the former regimen consisting of thiotepa and melpharan. RESULTS: Eleven of 12 patients were event free survived at a median of 17 months post HDCT (range, 4 to 32 months). No patients died of treatment related toxicity. One patient died of primary disease. The 18-month event-free survival was estimated to be 92% ± 8% (95%CI: 54%, 99%). Common nonhematological grade 3 or greater adverse events included grade 3 oral mucositis, grade 3 diarrhea, and grade 3 febrile neutropenia. These adverse events were less toxic than historical control. CONCLUSION: The new HDCT regimen, TMC regimen followed by PBSCT appears to have substantial effect and acceptable toxicity in children with advanced medulloblastoma, sPNETs, and pineoblastoma.

MB-063. PROGNOSTIC IMPACT OF HISTOPATHOLOGY, WNT ACTIVATION AND MYCC / MYCN GENE AMPLIFICATION IN A HOMOGENOUSLY TREATED METASTATIC MEDULLOBLASTOMA HIT2000 COHORT

Stefan Rutkowski[1], André von Bueren[2], Tobias Goschzik[3], Rolf Kortmann[4], Katja von Hoff[1], Carsten Friedrich[1], Anja zur Muehlen[3], Nicolas Gerber[5], Monika Warmuth-Metz[6], Niels Soerensen[6], Frank Deinlein[6], Martin Benesch[7], Isabella Zwiener[8], Andreas Faldum[9], Joachim Kuehl[6], and Torsten Pietsch[3]; [1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University Hospital Goettingen, Goettingen, Germany; [3]University Hospital Bonn, Bonn, Germany; [4]University Hospital Leipzig, Leipzig, Germany; [5]University Hospital Zuerich, Zuerich, Switzerland; [6]University Hospital Wuerzburg, Wuerzburg, Germany; [7]University Hospital Graz, Graz, Austria; [8]IMBEI, University of Mainz, Mainz, Germany; [9]IBKF, University of Muenster, Muenster, Germany

BACKGROUND: We prospectively evaluated histopathological findings and molecular variables, which have shown to impact prognosis of medulloblastoma patients, for outcome prediction in homogenously treated metastatic medulloblastoma patients. METHODS: One hundred twenty-three patients aged 4-21 years, diagnosed from 2001 to 2007, received systemic induction chemotherapy and intraventricular methotrexate, followed by hyperfractionated radiotherapy, and maintenance chemotherapy. From 86 patients paraffin material was available for immunohistochemical analysis to determine the activation status of the wingless (WNT) pathway, by immunostaining for β-catenin and sequencing of CTNNB1. In 81 of those 86 patients, material was available for gene amplification assessment of MYCC and MYCN by multiplex ligation-dependent probe amplification. RESULTS: Tumors with nuclear β-catenin immunopositivity showed excellent outcome (n = 4, all classic histology, 3 / 4 with CTNNB1 mutation; 5-year event-free (EFS) and overall survival (OS): 100%) compared to patients with tumors lacking nuclear β-catenin staining (n = 82; 5-year rates: 63% and 72%, respectively; p = 0.134 for EFS, p = 0.213 for OS). Patients with tumors characterized by MYC amplification (n = 5; MYCC, n = 2; MYCN, n = 3; all classic histology despite one case with MYCC amplification showing large cell histology) had poor outcome (20% for EFS and OS, respectively) when compared to patients with non-MYCC/MYCN amplified tumors (n = 76; 5-year EFS and OS: 65% and 75%, respectively; p = 0.011 for EFS, p < 0.001 for OS). Based on histopathological and molecular findings, we identified three risk groups: Favorable- (β-catenin nucleopositive and/or desmoplastic histology (n = 7; one death of disease), n = 11; 5-year EFS and OS 89 ± 11%), poor- (MYCC/MYCN amplification and/or anaplastic or large cell histology, n = 6; 5-year EFS and OS 33 ± 19%), and intermediate-risk group (n = 64; 5-year EFS and OS 61 ± 7%, and 72 ± 6%, respectively; p = 0.020 for EFS, and p = 0.002 for OS). CONCLUSION: Histopathological subtyping together with molecular features (WNT pathway and MYCC/MYCN amplification status) differentiate three risk groups in homogenously treated metastatic medulloblastoma patients. Supported by Deutsche Kinderkrebsstiftung.

MB-064. HIGH RISK AND RECURRENT MEDULLOBLASTOMA (MB): LESSONS LEARNED USING RADIOIMMUNOTHERAPY

KIM KRAMER, NEETA PANDIT -Taskar, Pat Zanzonico, John L. Humm, Suzanne L. Wolden, and Nai-Kong V. Cheung; Memorial Sloan-Kettering Cancer Center, New York, NY, USA

BACKGROUND: Recurrent and high risk MB is challenging to cure. Intraventricular compartmental radioimmunotherapy (cRIT) using ^{131}I-3F8 has been used to eradicate cells in cerebrospinal fluid (CSF) space. We present an updated series incorporating cRIT for this challenging scenario. PATIENTS: 36 patients with high risk (n = 5) or recurrent MB (n = 31) were treated on MSKCC IRB-approved protocol. Patients had <72 Gy prior radiation (brain parenchyma) and 45 Gy (spinal cord). MR brain and spine, and CSF cytology were obtained before and after treatment. Patients received 2 mCi ^{131}I-3F8 followed by 2-4 weekly injections (10 mCi ^{131}I-3F8/injection), maximum CSF dose 2400 cGy, determined by CSF samplings and region of interest analyses on whole-body scintigraphy. Response rate, PFS and OS at 6 months were analyzed. RESULTS: 30 patients received >2 therapeutic injections. 32/36 (89%) remained alive > 6 months after cRIT. Long term survivors in CR included 10 of 19 (53%) patients treated with ^{131}I-3F8 as consolidative therapy, mean f/u 49.6 mon (6 mon - 6 years); 4 of 17 (24%) with radiographic evidence of relapsed or refractory MB remain alive, 2 in CR 3.5 yrs and 5.5 yrs since relapse. Four of 5 patients with high risk MB remain in CR, mean f/u 54.4 months; 1 patient died of secondary glioma 5.3 years after initial diagnosis. Mean total absorbed CSF dose was 1250 cGy (237 - 2239) by sampling; average CSF dose was 64.1 cGy/mCi and blood 2.9 cGy/mCi. Toxicities included self-limited headache, fever, and vomiting. No long term side effects directly attributable to ^{131}I-3F8 have been observed. CONCLUSIONS: Patients who benefit most from cRIT: 1) non-progressive high risk disease upfront and 2) relapsed disease incorporating ^{131}I-3F8 as consolidation. Therapeutic injections of 10 mCi intra-Ommaya ^{131}I-3F8 are well tolerated, and as such, >4 weekly injections should be considered.

MB-065. INHIBITION OF BRD4 ATTENUATES TUMOR CELL SELF-RENEWAL AND PROMOTES SENESCENCE IN MYC AMPLIFIED MEDULLOBLASTOMA

Sujatha Venkataraman[1], Irina Alimova[1], Peter Harris[1], Diane Birks[1], Ilango Balakrishnan[1], Andrea Griesinger[1], Marc Remke[2], Michael D Taylor[2], Michael Handler[3], Nicholas K Foreman[1], and Rajeev Vibhakar[1]; [1]University of Colorado, Denver, CO, USA; [2]Hospital for Sick Children, Toronto, ON, Canada; [3]Children's Hospital Colorado, Denver, CO, USA

Patients with Myc amplified medulloblastoma have poor prognosis and have not shared in the improvement in outcome seen in other medulloblastoma subgroups. Myc, however, is a very difficult target to drug since it lacks clear ligand binding sites. Recent studies have shown that inhibiting the binding of bromodomain (BRD4) and extraterminal (BET) proteins to chromatin will target Myc dependence in cancer cells. BRD4 associates with acetylated chromatin and facilitates transcriptional activation and inhibiting this binding to chromatin, which is necessary for Myc function, should inhibit growth in Myc dependent cancer cells. We pursued the rationale that targeting BRD4 would attenuate Myc medulloblastoma cell growth. BRD4 inhibition by RNAi or JQ1, a novel BRD4 targeting small molecule drug, suppresses medulloblastoma cell growth in vitro. To understand the mechanism of growth inhibition by JQ1 and to provide rationale for possible clinical trials with BRD4 inhibition, several assays were carried out with high myc expressing medulloblastoma cells. Our results show that JQ1 treatment diminishes tumor cell self-renewal capacity and decreases expression of stem cell factors such as Nestin and SOX2. In parallel, JQ1 treatment induces senescence by decreasing E2F1-Rb signaling and curbing Myc driven transcription. Finally JQ1 inhibits growth of medulloblastoma tumors in vivo. Moreover, in clinical samples we found that transcriptional programs suppressed by JQ1 are associated with adverse risk in medulloblastoma patients. These data suggest that inhibition of BRD4 is a new therapeutic strategy for treating Myc amplified medulloblastoma and that development of the inhibitor, JQ1, may represent a potential of achieving this inhibition once this agent is available for trials.

MB-066. SIGNIFICANCE OF TUMOR ASSOCIATED MACROPHAGES IN MEDULLOBLASTOMAS

Ashley Margol[1], Nathan Robison[1], Janahan Gnanachandran[1], Long Hung[1], Rebekah Kennedy[1], Marzieh Vali[1], Girish Dhall[1], Jonathan Finlay[1], Anat Erdrich-Epstein[1], Mark Krieger[1], Rachid Drissi[2], Maryam Fouladi[2], Floyd Gilles[1], Alex Judkins[1], Richard Sposto[1], and Shahab Asgharzadeh[1]; [1]Children's Hospital Los Angeles, Los Angeles, CA, USA; [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

PURPOSE: Children with medulloblastoma can be subgrouped into at least four molecular categories, offering the potential for targeted therapeutic approaches to reduce treatment related morbidities. Little is known about the role of tumor microenvironment in medulloblastoma or its contribution to these molecular subgroups. Tumor microenvironment has been shown to be an important source for therapeutic targets in both adult and pediatric neoplasms. In this study, we investigated the hypothesis that expression of genes related to tumor-associated macrophages (TAMs) correlate with the medulloblastoma molecular subgroups and contribute to a diagnostic signature. METHODS: Gene expression profiling using Human Exon Array (n = 168) was analyzed to identify medulloblastoma molecular subgroups and expression of inflammation-related genes. Expression of 45 tumor-related and inflammation-related genes was analyzed using a custom-built TaqMan Low Density Array (TLDA) card in 83 medulloblastoma samples to build a gene signature predictive of molecular subgroups. TAMs in medulloblastomas (n = 54) comprising the four molecular subgroups were assessed by immunohistochemistry (IHC). RESULTS: A 31-gene medulloblastoma subgroup classification score inclusive of TAM-related genes (CD163, CSF1R) was developed with a misclassification rate of 2%. Tumors in the Sonic Hedgehog (SHH) subgroup had increased expression of inflammation-related genes and significantly higher infiltration of TAMs than tumors in the Group 3 or Group 4 subgroups ($p < 0.001$ and $p < 0.001$, respectively). IHC data revealed a strong association between location of TAMs and proliferating tumor cells. CONCLUSIONS: Our study reports the first evidence of the presence of TAMs in medulloblastomas and provides a novel 31-gene TLDA signature that accurately determines medulloblastoma molecular subgroups. These data suggest that SHH tumors have a unique tumor microenvironment and interactions of TAMs and SHH tumor cells may contribute to their pathogenesis revealing TAMs as a potential therapeutic target.

MB-067. A METRONOMIC AND TARGETED ANTIANGIOGENESIS THERAPY APPEARS TO BE PROMISING IN RECURRENT MEDULLOBLASTOMA – EXPERIENCE IN 15 PATIENTS

Andreas Peyrl[1], Monika Chocholous[1], Stefan Holm[2], Pernilla Grillner[2], Klas Blomgren[2], Amedeo Azizi[1], Thomas Czech[3], Bengt Gustafsson[4], Karin Dieckmann[5], Ulrike Leiss[1], and Irene Slavc[1]; [1]Medical University of Vienna, Department of Pediatrics, Vienna, Austria; [2]Karolinska Institute, Department of Women's and Children's Health, Stockholm, Sweden; [3]Medical University of Vienna, Department of Neurosurgery, Vienna, Austria; [4]Karolinska Institute, Department of Clinical Neuroscience, Stockholm, Sweden; [5]Medical University of Vienna, Department of Radiotherapy, Vienna, Austria

INTRODUCTION: Patients with recurrent medulloblastoma have a poor prognosis, irrespective of salvage therapy used. An evolving alternative approach to conventional chemotherapy is to target neovascularisation by interfering with tumor angiogenesis at various levels. We report on 15 patients from two institutions with recurrent medulloblastomas treated with an antiangiogenic combination therapy. PATIENTS AND METHODS: From 11/2006 to 12/2013, 15 patients were diagnosed with a recurrent medulloblastoma (10 first, 5 multiple recurrences). Median age at primary diagnosis was 8 years (range 4-12) and at start of antiangiogenic therapy 14 years (range 7-24) For their current relapse patients received an antiangiogenic combination therapy consisting of bevacizumab, thalidomide, celecoxib, fenofibrate, and etoposide, alternating with cyclophosphamide and augmented with intraventricular therapy (etoposide and liposomal cytarabine). RESULTS: As of 02/2014, 10/15 patients are alive at a median of 32 (3 to 66) months after their last recurrence. 8/10 surviving patients are currently in CR for 66, 62, 62, 36, 34, 14, 9, and 4 months, five of them off therapy for 48, 31, 27, 14, and 6 months. One patient is alive with disease for 30 months and another is on therapy for 1.5 months and too early to be evaluated. Four patients died of tumor progression 63, 27, 10, and 3 months after their last recurrence. One patient died of an accident without signs of tumor progression on MRI 23 months after initiation of antiangiogenic therapy. OS was $84.4 \pm 10.2\%$ after 1 year, and 65.7 ± 14.1 after 5 years. EFS was $77.0 \pm 11.8\%$ after one year, and $44.0 \pm 16.7\%$ after 5 years. Therapy was generally well tolerated and toxicities were manageable. CONCLUSION: Our results suggest that antiangiogenic metronomic chemotherapy has clinical activity in recurrent

medulloblastoma. Further investigation with a formal phase II study is in progress (MEMMAT; ClinicalTrials.gov Identifier: NCT01356290).

MB-068. HIGH–DOSE CHEMOTHERAPY WITH HEMATOPOIETIC STEM CELL RESCUE IN CHILDREN OLDER THAN 3 YEAR WITH MEDULLOBLASTOMA: RESULTS OF SINGLE INSTITUTION TRIAL

Stepan Babelyan[1], Igor Dolgopolov[1], Roman Pimenov[1], Georgy Mentkevich[1], Sergey Gorelishev[2], and Mikhail Laskov[1]; [1]Institute of Pediatric Oncology and Haematology N. N. Blokhin Cancer Research Centre, Moscow, Russia; [2]N.N. Burdenko Research Institute of Neurosurgery, Moscow, Russia

BACKGROUND: Medulloblastoma (MB) is the most common malignant brain tumor in children, comprises about 20% of all pediatric brain tumors. Among average risk MB, survival rate has been reached over 80%. However, in high-risk patients it remains poor. In our trial, we aimed to investigate effectiveness of risk-adapted radiotherapy followed by dose-intense chemotherapy with PBSCs rescue. METHODS: 30 patients, 14 with AR disease and 16 with HR were enrolled onto our study from 2007 to 2013. After tumor resection, all patients received risk-adapted craniospinal radiotherapy (23.4 Gy for AR MB and 36 Gy for HR MB, with local boost 54 Gy), followed by 4 cycles of high-dose chemotherapy with PBSC rescue. Each cycle consisted of cyclophosphamide (4000 mg/m^2 per cycle), cisplaitn (75 mg/m^2 per cycle), and vincristine (2 mg /m2 per cycle). Support with PBSCs was administered after each cycle of chemotherapy. The dose of CD34+ cells was $1.27*10^6$/kg per cycle (range $0.2–2.7*10^6$/kg). RESULTS: 28 of the 30 patients completed all 4 cycles of chemotherapy. There were two (6.7%) toxic related deaths during treatment. Both patients have died for bacterial sepsis. All patients had neutropenia after each cycle of chemotherapy; however, febrile neutropenia was observed in 83% of patients. Most of the toxicities that occurred during high-dose chemotherapy was anticipated. All of the 30 patients required platelet and RBCs transfusion. In our study, 3-year PFS for the 14 AR patients was 77.4 ± 11.5%, for HR patients was 66.1 ± 11.4% with median follow up 56.8 ± 5.6 and 39 ± 4.7 months respectively. DISCUSSION: The results showed that dose-intensive chemotherapy improves PFS in patients with HR MB, but it still worse than in AR MB. Based on our results we can say that future trials for MB treatment should consider molecular and biological features of MB.

MB-069. PRIMITIVE NEUROECTODERMAL TUMORS OF THE BRAINSTEM: CLINICAL FINDINGS OF A RARE DISEASE WITH A VERY UNFAVORABLE DIAGNOSIS

Carsten Friedrich[1], Monika Warmuth-Metz[3], André O. von Bueren[1], Johannes Nowak[3], Katja von Hoff[1], Torsten Pietsch[4], Rolf D. Kortmann[5], and Stefan Rutkowski[1]; [1]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, University Medical Center Goettingen, Goettingen, Germany; [3]Department of Neuroradiology, University of Wuerzburg, Wuerzburg, Germany; [4]Department of Neuropathology, University of Bonn, Bonn, Germany; [5]Department of Radiation Oncology, University of Leipzig, Leipzig, Germany

BACKGROUND: Primitive neuroectodermal tumors of the central nervous system (CNS-PNET) arising in the brainstem are extremely rare and knowledge is limited. The few existing case series report about a fatal outcome. METHODS: Between September 1992 and November 2011, 6 eligible children with histologically proven brainstem CNS-PNET not otherwise specified and 2 children with brainstem ependymoblastomas, median age 3.3 (range, 1.2-10.6) years, were treated according to consecutive multimodal HIT protocols for CNS-PNET/medulloblastoma. Postoperative treatment consisted of maintenance chemotherapy protocols (3, craniospinal irradiation (CSI) followed by maintenance chemotherapy), sandwich chemotherapy protocols (2, neoadjuvant chemotherapy, CSI plus boost RT, maintenance chemotherapy) or a therapy protocol for young children aged < 4 years (3, postoperative chemotherapy followed by CSI). RESULTS: The median duration of prediagnostic symptoms, predominantly cranial nerve deficits ($n = 7$), pyramidal tract signs ($n = 5$) or ataxia ($n = 5$), was 5 (range, 1-13) weeks. The primary tumors were all located in the pons, one patient had additional tumor dissemination. Most primaries involved more than half of the pontine axial diameter and were sharply marginated. Two tumors were classified as focal and four as diffuse on central neuroradiologic review of the available initial MRI from six patients. All tumors were incompletely resected (3, partial resection; 3, subtotal resection; 2, biopsy). With one exception all tumors progressed early during treatment at a median time of 3.9 (range, 2.5-10.4) months. The only surviving patient had a partially resected localized CNS-PNET diagnosed at 4.4 years of age. He received a sandwich chemotherapy protocol with irradiation (36 Gy craniospinal, 60 Gy tumor region), and is relapse-free 14 months after diagnosis. CONCLUSIONS: CNS-PNET is a rare but important differential diagnosis in childhood brainstem tumors. So far, efficient therapies are lacking. Collaborative sampling of tumor material for improved biological understanding and identification of new therapeutic targets is important.

MB-070. ADDITION OF LOCAL RADIOTHERAPY TO CHEMOTHERAPY DOES NOT IMPROVE SURVIVAL IN CHILDREN <4 YEARS WITH NON-METASTATIC NON-DESMOPLASTIC/NODULAR MEDULLOBLASTOMA: PRELIMINARY RESULTS OF A NON-RANDOMIZED PROSPECTIVE PHASE II CLINICAL TRIAL

Martin Mynarek[1], Katja von Hoff[1], Klaus Müller[2], Carsten Friedrich[1], André O. von Bueren[3], Nicolas U. Gerber[4], Martin Benesch[5], Torsten Pietsch[6], Monika Warmuth-Metz[7], Holger Ottensmeier[7], Robert Kwiecien[8], Andreas Faldum[8], Joachim Kuehl[7], Rolf D. Kortmann[2], and Stefan Rutkowski[1]; [1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University of Leipzig, Leipzig, Germany; [3]University Medical Center Goettingen, Goettingen, Germany; [4]University Children's Hospital, Zurich, Switzerland; [5]Medical University of Graz, Graz, Austria; [6]University of Bonn Medical Center, Bonn, Germany; [7]University of Wuerzburg, Wuerzburg, Germany; [8]University of Münster, Münster, Germany

INTRODUCTION: Addition of local radiotherapy to postoperative chemotherapy protocols has been suggested in children <4 years with non-metastatic non-desmoplastic/nodular medulloblastoma as a compromise between treatment intensity and omission of craniospinal irradiation. The non-randomized prospective phase II arm "HIT2000-BIS4" of the multicentric trial HIT2000 tested the hypothesis that addition of local radiotherapy improves outcomes in non-desmoplastic medulloblastoma. METHODS: All patients with localised, non-desmoplastic/nodular medulloblastoma received 3 cycles of SKK chemotherapy. From 2001 to 2005, patients in complete remission (CR) subsequently received 2 additional modified SKK cycles, patients who did not achieve CR received craniospinal radiotherapy (regimen A). From 2006 to 2011, all patients received local radiotherapy (54Gy) after 3 SKK cycles (regimen B). RESULTS: Fourty-seven patients with centrally confirmed non-desmoplastic/nodular medulloblastoma were registered by 31 different treatment centres. 26 of them received treatment according to regimen A, with 5 of them failing to achieve CR during initial chemotherapy. Twenty-one patients received therapy according to regimen B. Median follow-up for the surviving patients was 4.5 years (regimen A: 5.3 years, regimen B: 2.5years). Fourteen patients had postoperative residual tumour (A: 38%, B: 19%, $p = 0.205$). Both event-free survival (EFS) and overall survival (OS) did not differ between treatment groups. 3-year-EFS was 35 ± 8% (A: 37 ± 10%; B: 29 ± 12%, $p = 0.823$) and 3-year-OS was 67 ± 7% (A: 64 ± 10%; B: 70 ± 12%; $p = 0.675$). Relapse pattern shifted from predominantly local relapses in regimen A (12/17 relapses were isolated local relapses) to distant in regimen B (13/13 were distant or combined relapses). CONCLUSION: Addition of local radiotherapy after postoperative chemotherapy did not improve survival compared to postoperative chemotherapy alone and shifted the relapse pattern from local to distant failure. Moreover, salvage craniospinal irradiation is hampered after local radiotherapy to the posterior fossa. Supported by Deutsche Kinderkrebsstiftung

MB-071. INTENSIFICATION OF INDUCTION CHEMOTHERAPY IMPROVES OUTCOMES IN PATIENTS WITH METASTATIC MEDULLOBLASTOMA BELOW 4 YEARS OF AGE: RESULTS OF A PROSPECTIVE, NON-RANDOMIZED PHASE II CLINICAL TRIAL

Martin Mynarek[1], Katja von Hoff[1], Klaus Müller[2], Carsten Friedrich[1], André O. von Bueren[3], Nicolas U. Gerber[4], Martin Benesch[5], Torsten Pietsch[6], Monika Warmuth-Metz[7], Holger Ottensmeier[7], Robert Kwiecien[8], Andreas Faldum[8], Joachim Kuehl[7], Rolf D. Kortmann[2], and Stefan Rutkowski[1]; [1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University of Leipzig, Leipzig, Germany; [3]University Medical Center Goettingen, Goettingen, Germany; [4]University Children's Hospital, Zurich, Switzerland; [5]Medical University of Graz, Graz, Austria; [6]University of Bonn Medical Center, Bonn, Germany; [7]University of Wuerzburg, Wuerzburg, Germany; [8]University of Münster, Münster, Germany

BACKGROUND: We tested the hypothesis that intensification of pre-radiotherapy induction chemotherapy improves response- and survival-rates in patients <4 years with metastatic medulloblastoma. METHODS: Patients were to receive postoperative induction chemotherapy, followed by response-adjusted consolidation with either tandem high-dose chemotherapy

(HDCT) ± radiotherapy or craniospinal radiotherapy alone. In 2001-2005, induction chemotherapy consisted of 2-3 cycles of 96h-carboplatin/etoposide plus intraventricular methotrexate (regimen A). In 2006-2011, induction chemotherapy was intensified to a modified Head-Start induction plus intraventricular methotrexate (regimen B). RESULTS: 50 fully eligible patients with centrally reviewed staging were treated in 32 centres according to regimen A (n = 20) or regimen B (n = 30). Two patients died of toxicity during induction (regimen A, n = 1; regimen B n = 1). Of the remaining 48 patients, 70% had objective response (A: 58%, B: 86%, p = 0.041). Event-free survival (EFS) was higher in patients treated with regimen B (3-year-EFS 56 ± 9% vs. A: 29 ± 10%, p = 0.019). A difference in overall survival (OS) was not observed (A: 3-year-OS 44 ± 11% vs B: 54 ± 10%; p = 0.410). Eight of 10 patients with desmoplastic/nodular histology are alive; both deaths were due to toxicity (3-year-EFS 70 ± 15%, 3-year-OS 80 ± 13%). Of the 40 patients with non-desmoplastic histology, one died of toxicity during induction, 9 of 17 patients (52%) responded to induction therapy in regimen A and 18 of 22 in regimen B (82%; p = 0.082). Regimen B trended to translate into a better EFS (3-year-EFS A: 28 ± 11% vs. B: 47 ± 11%; p = 0,103), but not OS (3-year-OS A:40 ± 12% vs. B:48 ± 11%; p = 0.555). Craniospinal irradiation could only be omitted in 3/16 survivors with non-desmoplastic/nodular histology after HDCT. CONCLUSION: Since intensification of induction chemotherapy led to improved response- and EFS-rates, it should be further considered especially in patients with metastatic non-desmoplastic/nodular medulloblastoma. Patients with metastatic desmoplastic/nodular medulloblastoma might benefit from less aggressive therapy regimens. Supported by Deutsche Kinderkrebsstiftung

MB-072. A PILOT STUDY OF INDUCTION CHEMOTHERAPY FOLLOWED BY FOCAL IRRADIATION WITH CONCURRENT HIGH DOSE METHOTREXATE IN YOUNG CHILDREN WITH INCOMPLETELY RESECTED AND METASTATIC MEDULLOBLASTOMA

Maxim Yankelevich[1], Mikhail Laskov[2], Vasily Boyarshinov[2], Igor Glekov[2], Roman Pimenov[2], Sergey Ozerov[3], Sergey Gorelyshev[3], Alexander Popa[2], Igor Dolgopolov[2], Natalia Subbotina[2], and George Mentkevich[2]; [1]Children's Hospital of Michigan, Detroit, MI, USA; [2]NN Blokhin Russian Cancer Research Center, Moscow, Russia; [3]Burdenk Institute for Neurosurgery, Moscow, Russia

High dose chemotherapy (HDC) improved survival in young medulloblastoma (MB) patients with M0 stage and gross total resection, but failed in the patients with metastases and residual tumors. With a goal to improve results in this high-risk group, we conducted a pilot trial of chemotherapy followed by focal radiation therapy (RT) with concurrent high dose methotrexate (HDMTX) as a radiosensitizer and a substitute for craniospinal irradiation. Patients received 2 cycles of chemotherapy (vincristine, cisplatin, etoposide, and cyclophosphamide (OPEC)) followed by focal RT. Two biweekly cycles of HDMTX (250 mg/kg) were given concurrently with RT. Consolidation consisted of 2 OPEC and HD MTX cycles followed by tandem HDC. Nine patients (median age of 28 months) were treated between 2007 and 2009. Seven patients had residual tumors and 4 patients had metastases (M3a in 2 and M3b in 2 cases). Histological subtypes were classic in 6, anaplastic in 2, and desmoplastic in one patient. Seven/8 patients with measurable disease responded after 2 cycles of chemotherapy (CR-3, PR-4, and SD-1). All PRs converted to CRs after RT. The tolerability of RT with 2 doses of concurrent HD MTX was excellent. Six patients developed recurrence at a median time of 11 months. One of these patients rescued with gamma knife RT, bevacizumab, isotretinoin, and dendritic cell vaccine is alive at 66+ months post recurrence. There were no local recurrences. Three patients are alive in continuous CR at 69 + , 72 + , and 78+ months following surgery. All of the patients with anaplastic histology (2/2) or metastatic disease (4/4) recurred. The 6-year event-free survival (EFS) and overall survival (OS) was 33 ± 15% and 44 ± 16%, respectively. In conclusion, the combination of intensive chemotherapy and focal RT with concurrent HDMTX was well tolerated and resulted in good outcomes in M0R1 patients, but failed in metastatic patients.

MB-073. DIFFERENTIAL EXPRESSION OF PD-L1 ON HEMATOPOIETIC VERSUS TUMOR CELLS IN MEDULLOBLASTOMA

Allison M Martin[1], Christopher Nirschl[1], Magda Polanczyk[1], Robert Bell[1], Daniel Martinez[2], Lisa M Sullivan[2], Mariarita Santi[2], Peter C Burger[1], Janis M Taube[1], Charles G Drake[1], Drew M Pardoll[1], and Michael Lim[1]; [1]Johns Hopkins University, Baltimore, MD, USA; [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA

Medulloblastoma (MB) was recently classified into 4 molecular subgroups with diverse clinical outcomes. Molecular oncogenes, such as Sonic Hedgehog (SHH) and c-MYC, are active in multiple other tumor types; however little is known about how they might interact with the immune system. Brain tumors have been shown to develop complex mechanisms to avoid immune detection such as down regulating major histocompatibility complexes and secreting immunosuppressive cytokines. Recent studies have shown that blocking antibodies against immune checkpoints can induce objective responses in a variety of tumor types. Programmed Death Ligand 1 (PD-L1), is one such checkpoint molecule that is expressed on activated myeloid cells and the cell surface of some solid tumors. By interacting with its co-receptor, PD-1, on the surface of lymphocytes, PD-L1 blocks immune-mediated anti-tumor effector function, allowing tumors to escape immune surveillance. Our data utilizing human medulloblastoma cell lines suggests that this tumor expresses the primary ligand of PD-1, PD-L1, in a subgroup dependent manner. That is, cell lines with SHH pathway activation show both constitutive and inducible expression of PD-L1 consistent with both innate and adaptive resistance, while those that are c-MYC amplified show only inducible expression of PD-L1 consistent with a purely adaptive resistance mechanism. Adaptive resistance mechanisms are typical of melanoma and the presence of this pattern in MB suggests that targeting the PD-1 pathway could be a successful treatment strategy. The significance of constitutive expression is unknown but could indicate that SHH subgroups will be especially sensitive to PD-1 blockade. Interestingly, in a sampling of formalin fixed paraffin embedded (FFPE) human medulloblastoma tumors, the majority of cells expressing PD-L1 appear to be tumor associated macrophages and microglia suggesting that complex interactions between the tumor and cells of the immune microenvironment may play an important role in the pathogenesis of MB.

MB-074. PEROXIREDOXIN 1 (PRDX1) AS A RADIO-SENSITIZATION TARGET IN GROUP 3 MEDULLOBLASTOMAS

Lin Li[1], Wei-Guang Wang[2], Jian-Xin Pu[2], Han-Dong Sun[2], Marc Remke[3], Michael D Taylor[3], Rosamaria Ruggieri[4], Marc H Symons[4], and Magimairajan Issai Vanan[1]; [1]Manitoba Institute of Cell Biology (MICB), University of Manitoba, Winnipeg, MB, Canada; [2]Kunming Institute of Botany, Kunming, Yunnan, China; [3]Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [4]Feinstein Institute for Medical Research, Manhasset, NY, USA

PURPOSE/OBJECTIVE: Medulloblastoma (MBL) is the most common malignant brain tumor in children. Group 3 MBLs have metastatic dissemination at time of diagnosis and respond poorly to radiation and chemotherapy. Peroxiredoxin 1(PRDX1) is a member of the 2-Cys subfamily of thiol-dependent antioxidant PRDX family of enzymes that is over-expressed in multiple cancers. PRDX1 is also a cancer biomarker with over expression indicating advanced disease, increased angiogenesis, metastasis and poor outcome in several cancers. Down regulation of PRDX1 has been shown to have radio-sensitizing effects in lung cancer and several other tumors. We investigated the role of PRDX1 as a radio-sensitization target in group 3 Medulloblastomas. METHODS: Gene expression analysis was done to find out the expression levels of PRDX1 in group-3 MBL. We used siRNA transfection approach to knock down PRDX1 expression in primary Group-3 MBL cell lines (D341-MED and IMB-226). A small molecule inhibitor (Adenanthin) was used to inhibit the activity of PRDX1. An apoptotic FACS assay and an MTS assay were used to evaluate cell survival and radio-resistance after irradiation. The protein expressions were analyzed with western blots. DCFDA-probed FACS was used to measure intra cellular H2O2. RESULTS: PRDX1 is over expressed in group-3 MBL cells compared to other subgroups. This was confirmed by both gene expression profiling and protein levels. Down regulation of PRDX1 by RNA interference sensitizes MBL cells to irradiation. Adenanthin inhibition of PRDX1 also caused similar radio-sensitization comparable to siRNA knockdown. Inhibition of PRDX1 increased intracellular H2O2 levels in group-3 MBL cells. Preliminary data suggests that the MAPK-pathway might be involved downstream in radio-sensitization. CONCLUSIONS: PRDX1 is over expressed and plays a role in radio-resistance in group-3 MBL tumors. Adenanthin is a selective PRDX1 inhibitor. Further in vitro/ in vivo studies are required to validate PRDX1 as a potential radio-sensitization target in group-3 MBL tumors.

MB-075. MEDULLOBLASTOMA MODELS WHICH HARBOR AMPLIFICATIONS OF MYC FAMILY MEMBERS ARE SENSITIVE TO BET-BROMODOMAIN INHIBITION

Pratiti Bandopadhayay[1], Guillaume Bergthold[1], Brian Nguyen[2], Simone Schubert[2], Sharareh Gholamin[2], Yujie Tang[2], Sara Bolin[3], Steven Schumacher[4], Rhamy Zeid[4], Sabran Masoud[2], Furong Yu[2], Nujsaubnusi Vue[2], William Gibson[4], Brenton Paolella[4], Siddhartha Mitra[2], Samuel Cheshier[2], Jun Qi[4], Kun-Wei Liu[5], Robert Wechsler-Reya[5], William Weiss[6], Fredrik J Swartling[3], Mark W Kieran[1], James E Bradner[4], Rameen Beroukhim[4], and Yoon-Jae Cho[2]; [1]Dana-Farber Cancer Institute, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA; [2]Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA; [3]Uppsala University, Uppsala, Sweden; [4]Dana-Farber Cancer Institute, Harvard Medical School and The Broad Institute of MIT and Harvard., Boston, MA, USA; [5]Sanford-Burnham Medical Research Institute, CA, USA; [6]Depts of Neurology, Pediatrics and Neurosurgery, University of California, San Francisco, CA, USA

PURPOSE: MYC-amplified medulloblastomas are highly lethal tumors. BET-bromodomain inhibition has recently been shown to suppress MYC-associated transcriptional activity in other cancers(1). The compound JQ1 inhibits BET bromodomain-containing proteins, including BRD4. Here we investigate BET bromodomain targeting for the treatment of MYC-amplified medulloblastoma. EXPERIMENTAL DESIGN: We evaluated the effects of genetic and pharmacological inhibition of BET-bromodomains on proliferation, cell-cycle, and apoptosis in established and newly generated patient- and GEMM-derived medulloblastoma cell lines and xenografts that harbored amplifications of MYC or MYCN. We also assessed the effect of JQ1 on MYC expression and global MYC-associated transcriptional activity and other transcriptional pathways. We assessed in vivo efficacy of JQ1 in orthotopic xenografts established in immunocompromised mice. RESULTS: Treatment of MYC-amplified medulloblastoma cells with JQ1 decreased cell viability associated with arrest at G1 and apoptosis, with an IC50 of all cell lines in the nM range. In contrast, cell lines that did not harbor amplification of MYC isoforms demonstrated relative resistance. We observed down-regulation of MYC expression and confirmed inhibition of MYC-associated transcriptional targets. We also observed significant transcriptional inhibition of E2F associated gene. Exogenous expression of MYC reduced the effect of JQ1 on cell viability, suggesting that attenuated levels of MYC contribute to the functional effects of JQ1. JQ1 significantly prolonged survival of orthotopic xenograft models of MYC-amplified medulloblastoma ($p < 0.001$). We confirmed MYC transcriptional pathway down-regulation in xenografts harvested from mice after five doses of JQ1 which had reduced expression of MYC mRNA and a reduced proliferative index. CONCLUSION: JQ1 suppresses MYC expression and MYC-associated transcriptional activity in medulloblastomas, resulting in an overall decrease in medulloblastoma cell viability. These preclinical findings highlight the promise of BET bromodomain inhibitors as novel agents for MYC-amplified medulloblastoma and we are currently in protocol development for a phase 1 clinical trial for a derivative of JQ1.

MB-076. METACHRONOUS MEDULLOBASTOMA IN A CHILD WITH METASTATIC NEUROBLASTOMA: CASE REPORT

Ossama Maher, Soumen Khatua, Nidale Tarek, and Wafik Zaky; MD Anderson Cancer Center, Houston, TX, USA

BACKGROUND: A number of different second neoplasms have been reported in patients with neuroblastoma including brain tumors. None of these second cancers have occurred with sufficient frequency to indicate a specific relationship between neuroblastoma and any other neoplasm except in rare cancer syndromes. OBJECTIVE: We report a case of medulloblastoma in a child 5 months after successful treatment of metastatic neuroblastoma. DESIGN AND METHOD: A MEDLINE search was conducted for queries including "Children", "medulloblastoma" and 'neuroblastoma". Relevant papers were selected for literature review. RESULT: A 10 months old female presented with 2-month history of right eye proptosis. CT scan of the abdomen showed a right adrenal mass with liver metastasis. Brain MRI revealed disease at the right orbital fossa extends to middle cranial fossa with no intracranial disease. Pathology of the right adrenal mass confirmed neuroblastoma with non amplified N myc. The patient was classified as intermediate risk disease and started on chemotherapy consisted of vincristine, cyclophosphamide, cisplatin, dacarbazine, ifosphamide and Adriamycin followed by 5 cycles of Accutane. Surveillance MIBG revealed no evidence of residual disease. Five months later, she had a new onset of trouble walking. CT and MRI of the brain showed a mass in the right cerebellar hemisphere with hydrocephalus. Complete Surgical resection of the mass was done and pathology revealed anaplastic medullobastoma. The patient initiated chemoradiation treatment for medulloblastoma. Her treatment was stopped because of disseminated fungal infection. She is currently receiving metronomic therapy with cyclophosphamide and topotecan and has been clinically stable. Molecular genetic tests are pending for familial cancer syndromes. CONCLUSION: We report a case of metachronous medullobastoma after treatment of metastatic neuroblastoma in a two year-old child. The occurrence of metachronous neoplasms is rare and challenging to treat. Cancer familial syndromes like neurofibromatosis type 1 and Simpson-Golabi-Behmel syndrome need to be excluded.

MB-077. PROSPECTIVE LONGITUDINAL ASSESSMENT OF SENSORI-NEURAL HEARING LOSS WITH HYPERFRACTIONATED RADIATION THERAPY ALONE IN PATIENTS WITH AVERAGE-RISK MEDULLOBLASTOMA

Tejpal Gupta[1], Sarthak Mohanty[2], Sadhana Kannan[1], and Rakesh Jalali[2]; [1]ACTREC, Tata Memorial Centre, Maharashtra, India; [2]Tata Memorial Hospital, Maharashtra, India

BACKGROUND: To report on sensori-neural hearing loss (SNHL) in a cohort of patients treated with hyperfractionated radiation therapy (HFRT) without upfront platinum-based chemotherapy in average-risk medulloblastoma. METHODS: Hearing thresholds were assessed by ear-specific pure-tone audiograms at stimulus frequencies of 0.25, 0.5, 1, 2, 4, and 8 kilohertz (KHz). Audiometric assessments were done serially longitudinally at baseline, between 6-12 months after HFRT, and annually thereafter. Pure-tone audiograms were analyzed and graded according Brock's pediatric ototxicity grading criteria. RESULTS: Five of 20 (25%) children had communicatively and developmentally significant SNHL (Brock's grade 2 or worse) at baseline even before starting radiotherapy. On follow-up, new onset Brock's grade 2 or worse ototoxicity was documented in 6 previously normal ears. Eleven patients had preserved hearing in both ears on last-audiometric follow-up. Compared to baseline testing, post-HFRT audiometry at 2-3 years showed modest decline in hearing threshold across all frequencies. Age at diagnosis and gender were not significantly associated with hearing impairment. Tumors that extended more towards one side expectedly showed significant worsening in the ipsilateral ear. There was a differential impact of treatment on the right and left ears with the right ear (and not the left ear) showing significantly worse hearing thresholds in the low-to-intermediate speech frequency range over time. CONCLUSION: The use of HFRT for craniospinal irradiation and conformal tumor bed boost without upfront platinum-based chemotherapy in children with average-risk medulloblastoma results in preserved hearing in a large proportion of patients in the audible speech range.

MB-078. MEDULLOBLASTOMA OF INFANCY: HISTOLOGY REFLECTS GENETICS AND BIOLOGY

Elena Kapitza[1], Dorota Denkhaus[1], Anja zur Mühlen[1], Stefan Rutkowski[2], and Torsten Pietsch[1]; [1]Department of Neuropathology, University of Bonn, Bonn, Germany; [2]Department of Pediatric Hematology/Oncology, University of Hamburg, Hamburg, Germany

The identification of desmoplastic/nodular histology as an independent favourable prognostic marker in medulloblastoma (MB) of infancy has allowed the reduction of therapy in these patients. We have previously demonstrated different origins and pathway activation of desmoplastic versus non-desmoplastic MB. So far, genomics and pathway activation have not been exactly related to the different histological MB subtypes in this age group. 168 MBs of children younger than 5 years were diagnosed according to the WHO 2007 classification, and FFPE material was analysed in detail by immunohistochemical techniques (including staining of hedgehog targets YAP-1 and p75-NGFR) and by molecular inversion profiling (MIP) to assess the genomic landscape of infant MB. GISTIC analysis was performed to identify significant copy number alterations in MB subtypes. MB with extensive nodularity (MBEN, $n = 22$) and desmoplastic MB (DMB, $n = 42$) were both characterized by LOH of chromosomal arms 9q or 10q (MBEN, 77% of cases; DMB, 74%), whereas classic MB (CMB, $n = 90$) and large cell or anaplastic MBs (LCA-MB, $n = 14$) frequently showed specific gains of chromosomal arm 17q (CMB, 67 %; LCA-MB, 79%), partially occurring as isochromosome 17q (CMB, 30%); LCA-MB, 57%), and gains of *OTX2* (CMB, 73%; LCA-MB, 79%). Moreover, 10/14 (71%) of LCA-MB carried *MYCC* or *MYCN* ($n = 1$) amplifications. The overall frequency of chromosomal copy number aberrations was significantly lower in the desmoplastic/nodular subtypes compared to non-desmoplastic subtypes. Importantly, there was a high correlation between desmoplastic/nodular histology (MBEN and DMB) versus non-desmoplastic subtypes with hedgehog pathway activation shown by the expression of YAP-1 and p75-NGFR (concordance rate, >98%). In summary, histology of infant MB is associated with specific recurrent chromosomal alterations and reflects their biology determined by their cellular origin and developmental pathway activation. Immunohistological assessment of pathway markers and genomic profiling by MIP can help to classify infant MBs in individual cases.

MB-079. PROGNOSTIC FACTORS IN CHILDHOOD CNS-PNET AND PINEOBLASTOMA: AN INTERNATIONAL META-ANALYSIS OF ORIGINAL CLINICAL DATA

Katja von Hoff[1], Barry Pizer[2], Christelle Dufour[3], Dannis G. van Vuurden[4], Miklos Garami[5], Maura Massimino[6], Jason Fangusaro[7], Tom B. Davidson[8], Maria Joao Gil da Costa[9], Jaroslav Sterba[10], Martin Benesch[11], Nicolas U. Gerber[12], Martin Mynarek[1], Robert Kwiecien[13], Steven C. Clifford[14], Marcel Kool[15], Torsten Pietsch[16], Jonathan L. Finlay[17], and Stefan Rutkowski[1]; [1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Alder Hey Children's Hospital, Liverpool, UK; [3]Institut Gustave Roussy, Villejuif, France; [4]VU University Medical Center, Amsterdam, The Netherlands; [5]Semmelweis University, Budapest, Hungary; [6]Instituto Nazionale dei Tumori, Milano, Italy; [7]Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [8]University of California, Los Angeles, Los Angeles, CA, USA; [9]University Hospital S.João, Porto, Portugal; [10]University Hospital Brno, Brno, Czech Republic; [11]Medical University of Graz, Graz, Austria; [12]University Children's Hospital Zurich, Zurich, Switzerland; [13]University of Münster, Muenster, Germany; [14]Northern Institute for Cancer Research, Newcastle upon Tyne, UK; [15]German Cancer Research Center, Heidelberg, Germany; [16]University of Bonn Medical Center, Bonn, Germany; [17]Children's Hospital, Los Angeles, Los Angeles, CA, USA

INTRODUCTION: CNS-PNET and pineoblastoma are rare heterogeneous malignancies often treated with the same, unstratified multimodal regimen and only limited progress has been achieved to identify clinical prognostic factors and improve treatment. METHODS: Clinical data sets from patients with CNS-PNET or pineoblastoma were collected from 11 national groups. Inclusion criteria for analysis were: existing pathology review or FFPE material available for re-evaluation, age at diagnosis below 21, diagnosis after 1987. RESULTS: Of 495 evaluable patients, histological diagnoses were: CNS-PNET not other specified (nos.), 311; pineoblastoma, 131; ependymoblastoma/ETANTR, 24; CNS (Ganglio-) neuroblastoma, 16; medulloepithelioma, 13. Histological review was available for 340. A glial component was present in 41 of 110 evaluated patients. Five-year PFS and OS were $36 \pm 2\%$, and $41 \pm 2\%$. Relapse location was predominantly local in CNS-PNETnos., and distant/combined in pineoblastoma. Independent prognostic factors for PFS were age at diagnosis (age <4 vs.$>=4$years: HR 0.17; 95% CI [0.10-0.29], $p = <0.0001$) for pineoblastoma, and initial staging (M0R+ vs. M0R0: HR 1.38; 95% CI [1.00-1.91], $p = 0.053$; M+ vs. M0R0: HR 1.7; 95% CI [1.16-2.50], $p = 0.006$) for CNS-PNETnos. Best response was available for 407 CNS-PNETnos. and Pineoblastoma patients. 93 had early progressive disease to frontline treatment (7/93 survived after salvage radiotherapy). In the remaining 314 patients (best response: SD/PR/CR), application of radiotherapy, administration of HDCT and best response were independent prognostic factors for PFS in a multivariable model. Five-year OS for patients with CR as best response was $60 \pm 4\%$. CONCLUSION: Outcome of pineoblastoma is mainly influenced by age and the application of radiotherapy. In CNS-PNET, local disease control seems to be important. Application of HDCT may be beneficial. A high rate of patients showed early progression. Together with a better molecular classification and characterization of this very heterogeneous disease, our findings may lead to an improved stratification to risk adapted treatment concepts. Supported by Deutsche Kinderkrebsstiftung

MB-080. PROGNOSTIC SIGNIFICANCE OF CLINICAL, HISTOPATHOLOGICAL, AND MOLECULAR CHARACTERISTICS OF MEDULLOBLASTOMAS IN THE PROSPECTIVE HIT2000 MULTICENTER CLINICAL TRIAL COHORT

Torsten Pietsch[1], Rene Schmidt[2], Marc Remke[3], Andrey Korshunov[5], Volker Hovestadt[6], David TW Jones[3], Joerg Felsberg[7], Tobias Goschzik[1], Marcel Kool[3], Paul A Northcott[3], Katja von Hoff[8], Andre von Bueren[8], Heiko Skladny[9], Michael Taylor[4], Friedrich Cremer[9], Peter Lichter[6], Andreas Faldum[2], Guido Reifenberger[7], Stefan Rutkowski[8], and Stefan Pfister[3]; [1]Department of Neuropathology, University of Bonn, Bonn, Germany; [2]Institute of Biostatistics and Clinical Research, WW University of Muenster, Muenster, Germany; [3]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [4]The Hospital for Sick Children, Toronto, Canada; [5]Department of Neuropathology, University of Heidelberg, Heidelberg, Germany; [6]Division of Molecular Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany; [7]Department of Neuropathology, Heinrich Heine University Duesseldorf, Duesseldorf, Germany; [8]Department of Pediatric Haematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [9]Centre for Human Genetics, Mannheim, Germany

This study aimed to prospectively evaluate clinical, histopathological and molecular variables for outcome prediction in medulloblastoma patients. Patients from the HIT2000 cooperative clinical trial were prospectively enrolled based on the availability of sufficient tumor material and complete clinical information. This revealed a cohort of 184 patients (median age 7.6 years), which was randomly split at a 2:1 ratio into a training ($n = 127$), and a test ($n = 57$) dataset in order to build and test a risk score for this population. Independent validation was performed in a non-overlapping cohort ($n = 83$). All samples were subjected to thorough histopathological investigation, CTNNB1 mutation analysis, quantitative PCR, MLPA and FISH analyses for cytogenetic variables, and methylome analysis. By univariable analysis, clinical factors (M-stage), histopathological variables (large cell component, endothelial proliferation, synaptophysin pattern), and molecular features (chromosome 6q status, MYC amplification, subgrouping) were found to be prognostic. Molecular consensus subgrouping (WNT, SHH, Group 3, Group 4) was validated as an independent feature to stratify patients into different risk groups. When comparing methods for the identification of WNT-driven medulloblastoma, this study identified CTNNB1 sequencing and methylation profiling to most reliably identify these patients. After removing patients with particularly favorable (CTNNB1 mutation, extensive nodularity) or unfavorable (MYC amplification) markers, a risk score for the remaining "intermediate molecular risk" population dependent on age, M-stage, pattern of synaptophysin expression, and MYCN copy-number status was identified, with speckled synaptophysin expression indicating worse outcome. Test and independent validation of the score confirmed significant discrimination of patients by risk profile. Methylation subgrouping and CTNNB1 mutation status represent robust tools for the risk-stratification of medulloblastoma. A simple clinico-pathological risk score was identified, which was confirmed in a test set and by independent clinical validation.

MB-081. MicroRNAs AS MARKERS FOR MOLECULAR CLASSIFICATION AND RISK STRATIFICATION OF MEDULLOBLASTOMAS

Ratika Kunder[1], Rakesh Jalali[1], Epari Sridhar[1], Ali Asgar Moiyadi[1], Atul Goel[2], Naina Goel[2], and Neelam Shirsat[1]; [1]Advanced Centre for Treatment, Research and Education in Cancer, Navi Mumbai, India; [2]Seth G. S. Medical College and K.E.M. Hospital, Mumbai, India

Medulloblastoma is a common malignant brain tumor in children. Improvements in surgical and radiation techniques has improved five year survival rate to about 80% for average risk patients and about 55-76% for high risk patients. Accurate risk stratification is necessary to avoid aggressive treatment that results in long term side effects as well as to improve survival of high risk patients. Genome wide expression profiling studies including our study has demonstrated that medulloblastoma is comprised of 4 core molecular subgroups. The 4 subgroups viz WNT, SHH, Group 3 and Group 4 are not only distinct in their underlying biology but also vary in clinical characteristics like age related incidence, presence of metastasis and overall survival.In addition to the clinical parameters molecular classification is now necessary for better risk assessment and management of the disease.microRNAs are 18-24 nucleotide long non-coding RNAs that regulate expression of protein coding genes. MiRNA expression was found to be deregulated in medulloblastomas as compared to normal cerebellar tissues. Further the four subgroups were found to differ in their miRNA expression profiles, with the WNT subgroup having the most distinctive miRNA profile. The differential miRNA expression was validated in a set of 103 medulloblastomas. Based on this differential miRNA eexpression a real time PCR based was developed for molecular classification. The assay has an accuracy of 97% and is particularly useful for FFPE tumor tissues. Non-WNT, non-SHH medulloblastomas underexpressing miR-592 were found to have poor survival rates indicating usefulness of this miRNA for risk stratification.

MB-082. ABCB1 IS ASSOCIATED WITH MEDULLOBLASTOMA CELL INVASION AND METASTASIS

Ramadhan Othman[1], Lisa Storer[1], Andrey Korshunov[2], Stefan M. Pfister[2], Ian Kerr[1], and Beth Coyle[1]; [1]University of Nottingham, Nottingham, UK; [2]UniversitatsKlinkum, Heidelberg, Germany

Medulloblastoma (MB) is the most common malignant paediatric brain tumour. Metastasis of disease occurs in 30% of patients, which is associated with poor survival outcome. Additionally, most cases of recurrent disease are metastatic for which there is no standard treatment and metastasis is fatal in most cases. Little is known about the molecular biology of metastatic MBs. Therefore, investigations are needed to identify cells, genes and processes involved in metastases in primary disease. Here we hypothesise that multidrug transporter ABCB1 expressing cells could be involved in metastasis. Expression of ABCB1 was assessed by immunohistochemistry (IHC) on a

patient tissue microarray, mouse orthotopic xenografts and matched patient samples. A 96-well kinetic cell migration assay was used to investigate the effect of ABCB1 inhibition on MB cells migration in vitro. ABCB1 expression was significantly associated with increased risk of metastasis (P= 0.04). Inhibition of ABCB1 function in vitro, using vardenafil or verapamil resulted in a significant inhibition of MB cell migration. MED1 xenograft mouse models showed local invasion of ABCB1 expressing cells into surrounding mouse brain and migration down the spine. Our data demonstrate that ABCB1 is associated with metastatic MB. Hence targeting resistant (ABCB1 positive) cells in the primary disease could reduce the chance of metastasis. A combination of chemotherapy and the phosphodiesterase 5 inhibitor vardenafil may represent a valid therapeutic approach in patients with ABCB1 positive metastatic disease and non-metastatic ABCB1 positive primary disease to prevent recurrence and metastasis.

MB-083. EXECUTIVE FUNCTION IN PEDIATRIC MEDULLOBLASTOMA

Nicole Law[1], Mary Lou Smith[2], Mark Greenberg[1], Eric Bouffet[1], Michael D. Taylor[1], Suzanne Laughlin[1], David Malkin[1], Fang Liu[1], Iska Moxon-Emre[1], Nadia Scantlebury[1], and Donald Mabbott[1]; [1]Hospital for Sick Children, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada; [3]Pediatric Oncology Group of Ontario, Toronto, ON, Canada

INTRODUCTION: In the years following treatment for pediatric medulloblastoma (MB), patients are at risk for neurocognitive and behavioural deficits. These deficits may reflect a reduced ability to obtain novel information from the environment and a slower rate of processing. Executive Functions (EF) play an important role in the acquisition of novel information and serve to guide goal-directed behaviour (e.g. behavioural regulation). It is possible that multiple components of EF are impaired in MB impacting overall neurocognitive/behavioural outcome. We examined the impact of treatment for MB on EF. METHODS: Twenty-four children treated for MB and twenty healthy control (HC) children were seen for neurocognitive testing at the Hospital for Sick Children. Subtests of the Delis-Kaplan Executive Function System (D-KEFS), the Working Memory Test Battery for Children (WMTB-C), and the Childhood Emotion Regulation Questionnaire (CERQ) were administered to obtain a broad spectrum of EF. A Principal Components Analysis (PCA) was performed to distill EF measures into components and compared between MB and HC. RESULTS: PCA revealed six components (C1-C6) of EF. C1 reflected a "cognitive efficiency" construct; C2 reflected a "planning, and problem solving" component; C3 reflected a "positive cognitive emotion regulation" factor; C4 revealed an "attention, working memory, and memory span" construct; C5 reflected a "negative cognitive emotion regulation" dimension; and C6 represented a "mixed cognitive emotion regulation" component. Multivariate analyses revealed group differences for C1, C2, C3, and C4; the MB group had scores significantly below that of the HC group ($p < .01$), reflecting poorer performance on tasks of EF and less use of positive cognitive strategies for emotion regulation. CONCLUSION: Core EF are impaired in MB relative to age-matched peers including executive control, cognitive flexibility, problem solving, planning, attention and memory span, and cognitive emotion regulation. We are currently evaluating whether these group differences are related to cerebrocerebellar pathway microstructure.

MB-084. THREE-DIMENSIONAL (3D) GROWTH OF MEDULLOBLASTOMA CELLS CONFIRMS A ROLE FOR THE EPITHELIAL-MESENCHYMAL TRANSITION FACTOR Twist1 IN METASTASIS

Aishah Nasir[1], Ramadhan Othman[1], Lisa Storer[1], David Onion[2], Anbarasu Lourdusamy[1], Anna Grabowska[2], and Beth Coyle[1]; [1]Children's Brain Tumour Research Centre (CBTRC), School of Medicine, University of Nottingham, Nottingham, UK; [2]Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, Nottingham, UK

Medulloblastoma is an aggressive malignant embryonal tumour of the cerebellum that is frequently metastatic (one-third of primary and almost all recurrences) resulting in poor outcome. Epithelial-Mesenchymal Transition (EMT) occurs during the early stages of metastasis where cells gain migratory and invasive abilities by altering phenotypically. By using a 3D *in vitro* culture system to better represent the *in vivo* microenvironment this study aims to investigate metastatic markers in medulloblastoma. Myc-immortalised cerebellar progenitor cells (C17.2), metastatic group 4 (MED1) and non-metastatic WNT subtype (MED6, C17.2 WNT) medulloblastoma cells were suspended in basement membrane extract to provide a 3D culture system. Expression of a panel of EMT genes was assessed by quantitative real time polymerase chain reaction on RNA extracted from 3D (on days 3, 6 and 8) and 2D monolayers. Twist1 expression was assessed in the MED1 primary tumour and a panel of 27 patient medulloblastomas by immunohistochemistry. Further markers were identified from published gene expression profiles of 62 medulloblastomas analysed according to metastatic status. 3D-culture of metastatic MED1 formed metabolically active branched interconnecting aggregates whilst MED6, C17.2 WNT and non-tumourigenic C17.2 cells showed low metabolic activity and differentiation by day 3; supporting a non-metastatic phenotype. Expression of the Twist1 transcription factor doubled in MED1 cells grown in 3D compared to MED1 2D monolayers with no increase in expression observed in other cell types. The MED1 primary tumour showed focally high Twist1 positivity. Twist1 expression significantly correlated with metastasis in patient samples ($p < 0.02$). Four further markers differentially expressed in metastatic medulloblastoma have also been identified and are undergoing analysis. This data suggests that the EMT transcription factor Twist1 plays a role in metastatic medulloblastoma and supports the use of this 3D culture system to screen further metastatic markers.

MB-085. INVESTIGATING THE MECHANISM OF MEDULLOBLASTOMA CELL DEATH IN RESPONSE TO TREATMENT WITH THE N-3 PROPARGYL ANALOGUE OF TEMOZOLOMIDE

Yanhua Cai[1], Ramadhan Othman[1], Tracey Bradshaw[2], and Beth Coyle[1]; [1]CBTRC, School of Medicine,University of Nottingham, Nottingham, UK; [2]Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, UK

Medulloblastoma is the most common malignant paediatric brain tumour. Recurrence and progression of disease occurs in 15-20% of standard risk and 30-40% of high risk patients. Prognosis for these patients is poor despite treatment with more intensive regimes usually involving the alkylating agent temozolomide (TMZ). Activity of TMZ depends on the absence of direct repair by O6-methylguanine-DNA-methyltransferase (MGMT) and proficient mismatch repair (MMR). N-3 Propargyl is a novel imidazotetrazine TMZ analogue previously demonstrated to be able to overcome MGMT resistance in glioma and medulloblastoma in a single agent approach. The cytotoxicity of propargyl was assessed in MGMT-positive MED1 and MGMT-negative UW228-3 cells using clonogenic and cell viability assays (MTT and sulforhodamine B (SRB)) and was compared to TMZ. MGMT, MLH1 and MSH6 expression were measured by Western blotting. γH2AX and cell cycle analysis using flow cytometry were used to interrogate the cell death mechanism. We found that unlike TMZ the dose-dependent cytotoxicity of propargyl was irrespective of MGMT expression. In MTT/SRB assays, resistance was observed after MED1 cells were continuously treated with TMZ. Analysis of mismatch repair demonstrated that whilst both cell lines expressed high levels of MSH6, MLH1 expression was restricted to cancer stem cells in MED1 cells. Flow cytometry analyses indicated that propargyl may induce more rapid cell death than TMZ. In conclusion, our data suggest propargyl is a promising pre-clinical lead candidate and may be an alternative treatment to TMZ in the future.

MB-086. THE AVAILABILITY OF HISTOPATHOLOGICAL AND BIOMARKER ANALYSIS AS PROGNOSTIC FACTORS IN PATIENTS WITH MEDULLOBLASTOMA IN BRAZIL

Raphael Salles S de Medeiros, Annick Beaugrand, Simone Soares, and Sidnei Epelman; Santa Marcelina Hospital, Sao Paulo, Brazil

INTRODUCTION: Recent discoveries demonstrated that meduloblastoma is a heterogeneous disease with distinct clinical, morphological and molecular profiles. Set a feasible algorithm classification to be used in clinical practice based on subgroup-specific molecular biomarkers can improve patient prognostication. METHODS: Clinical data were retrieved retrospectively. All cases were classified according to WHO criteria and immunohistochemistry was performed for Ki-67. Clinical and pathological factors related to PFS or OS were calculated according to the Kaplan- Meier method and compared using the log-rank test. Combined risk stratification models were designed based on clinical and biomarkers identified by multivariable Cox proportional hazards analyses. RESULTS: 66 patients were analyzed which 39 samples were reviewed for histopathological clasification and Ki67 expression. The mean age at diagnosis was 8.9 years (range 0.6-23 years). Median and mean follow-up were 2.63 and 3.9 years, respectively (range 0.1-12 years). 23 (59%) patients were diagnosed as classical type, 10 (26%) as desmoplastic/nodular type and 6 (15%) as anaplasic/large cell type. The mean of proliferative index was 33.5% (SD + - 27.3%). The anaplastic/large cell type was shown higher index (58.3% versus 33.1 and 19.6% for classical and desmoplastic/nodular type, respectively, $P = 0.03$) and lower age (6.2 years versus 9.5 and 13 years for classical and desmoplastic/nodular type, respectively, $p = 0.06$). On univariate analysis, histological

classification and proliferative index were predictive of OS and PFS. On multivariate analysis, only proliferative index was marginally independent predictor of OS but not of PFS. CONCLUSION: Histopathological classification and Ki67 expression demonstrated as two important prognostic factors. These first results are promising. An expanded panel including immunohistochemical and cytogenetic analysis for molecular classification in Wnt, SHH, group 3 and group 4 has been applied in a central review for all patients in Brasil. Supported by TUCCA (www.tucca.org.br).

MB-087. DECODING THE REGULATORY LANDSCAPE OF MEDULLOBLASTOMA USING DNA METHYLATION PROFILING

David T.W. Jones[1], Volker Hovestadt[1], Wei Wang[1], Paul A. Northcott[1], Marcel Kool[1], Marc Sultan[2], Pablo Landgraf[3], Guido Reifenberger[3], Roland Eils[1], Marie-Laure Yaspo[2], Robert J. Wechsler-Reya[4], Andrey Korshunov[5], Marc Zapatka[1], Bernhard Radlwimmer[1], Stefan M. Pfister[1], and Peter Lichter[1]; [1]German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Max Planck Institute for Molecular Genetics, Berlin, Germany; [3]Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; [4]Sanford Burnham Medical Research Institute, La Jolla, CA, USA; [5]University Hospital Heidelberg, Heidelberg, Germany

Several recent genomics studies in medulloblastoma (MB), the most frequent embryonal brain tumor, have made great progress in dissecting the genomic complexity underlying this disease. Four core subgroups, defined according to divergent gene expression and methylation profiles, display distinct clinical, biological and genetic features. A significant subset of tumors, however, still lacks a clear genetic driver event, suggesting that mechanisms such as epigenetic alteration may represent alternative regulators of MB tumorigenesis. We therefore performed an integrative analysis including whole-genome bisulphite sequencing (WGBS) of 42 samples, combined with whole-genome, mRNA and miRNA sequencing as well as DNA methylation and gene expression microarrays. WGBS data of mouse MB and matched precursor cells was also generated, together with WGBS and ChIP-seq data (H3K4me3, H3K9me3, H3K27me3, H3K36me3) for 2 MB cell lines. This allowed for a comprehensive investigation of the downstream effects of differential methylation on gene regulation. Multiple patterns of differential methylation were observed. Most striking were highly prevalent regions of hypomethylation extending downstream of transcription start sites, which tightly correlated with increased gene expression. Focal lowly-methylated regions, marking transcription factor binding sites, revealed differential transcriptional networks between tumor subgroups, while increased methylation in DNA methylation valleys was positively correlated with gene expression through re-normalisation of repressed chromatin. Large, partially methylated domains displaying increased mutation rates and reduced expression were medulloblastoma subgroup-specific, suggesting non-random heterochromatisation of up to one-third of the tumor genome. Epigenetic alterations also affected novel medulloblastoma candidate genes (e.g. LIN28B), resulting in alternative promoter usage and/or differential mRNA/miRNA expression. Whole-genome analysis of mouse medulloblastoma and precursor cell methylation demonstrated a somatic origin for many of these changes. Our data provide fundamental new insights into epigenetic mechanisms of transcriptional regulation in medulloblastoma pathogenesis that are likely also of importance in a wider context of development and disease.

MB-088. RESPONSE TO INTRAVENTRICULAR TOPOTECAN (ITV), ORAL TEMOZOLAMIDE AND ETOPOSIDE IN CHILDREN WITH RELAPSED MEDULLOBLASTOMA: A MONO-INSTITUTIONAL EXPERIENCE

Daniel Alderete, Lorena Baroni, Fabiana Lubinieki, Federico Auad, Maria Laura Gonzalez, Waldimir Puya, and Patricia Pacheco; Garrahan Hospital, Buenos Aires, Argentina

PURPOSE: Patients with relapsed medulloblastoma are unlikely to be cured. New effective treatment strategies are needed. METHODS: We retrospectively reviewed 9 cases of relapsed medulloblastoma between Jun 2011 - Oct 2013, treated with palliative care criteria with IVT 0.4mg/dose, temozolamide 160mg/m2/day orally d1-5/28d and etoposide 50mg/m2/day orally d1-14/28 d. Ommaya reservoir was implanted. IVT was administrated twice a week for 3m; weekly for others 3m and twice a month since that. Surgery was performed when only one site was affected or to improve symptoms. Response was assessed by MRI every 3m. At initial diagnosis the median age was 76m (31-108). Six were standard-risk and 2p were high risk. They received Craniospinal radiotherapy 2340/3600cGy respectively and 5400/5580cGy respectively in posterior fossa. It was followed by adjuvant chemotherapy using Cisplatin, Vincristine, Lomustine and/or ciclophosphamide (adaptive COG strategy). One was younger than 36m and was treated with baby protocol including posterior fossa radiotherapy. At relapse the median age was 117m (82-131). Median time from diagnosis to relapse was 23m (18-47). Five had isolated intraventricular relapse, 2 had leptomeningeal dissemination and 2 had extra-axial supratentorial meningeal metastasis. RESULTS: Four underwent surgical resection (2p gross resection and 2p subtotal resection). Evaluable tumor was seen in 7p. Responses to chemotherapy were seen in all cases, 1p complete remission, 5p with partial response and 1p stable disease with a median follow up of 11m (4-27). Eight remain alive: 6 without progression. Two showed MRI progression (12m and 13m). One relapsed and died, 13m and 27m respectively. The treatment was well-tolerated. Grade 3 thrombocytopenia was observed in 2p, leading to 25% decrease of temozolamide. CONCLUSIONS: The combination of ITV, oral Temozolamide and Etoposide produces objective responses with minimal toxicity in children with relapsed medulloblastoma. This strategy allows our patients to live as normal as possible their residual life.

MB-089. FOLLOW UP OF SPANISH CHILDHOOD MEDULLOBLASTOMA

Olaia Aurtenetxe[1], Ayman Gaffar[2], Luis Gros[3], Ofelia Cruz[4], Carlota Calvo[5], and Aurora Navajas[1]; [1]Pediatric Oncology of Hospital Universitario Cruces, Bilbao, Spain; [2]Pathology Department of Hospital Universitario Cruces, Bilbao, Spain; [3]Pediatric Oncology of Hospital UNiversitario Vall d'Hebron, Barcelona, Spain; [4]Pediatric Oncology of Hospital Sant Joan de Deu, Baecelona, Spain; [5]Pediatric Ocncology of Hospital Miguel Servet, Zaragoza, Spain

PURPOSE: The participation of Spain in SIOP collaborative brain tumor protocols, gave us the possibility of centralize biological studies in childhood medulloblastoma (MB). Since the PNET 4 protocol, we have studied retrospectively and prospectively a non-uniformly treated cohort using combined clinical, pathologic and molecular variables in this tumor. METHOD: Paraffin-embedded (FFPE) tissue samples from 100 pediatric patients submitted from 17 Spanish hospitals were referred to Hospital Universitario Cruces and analyzed using fluorescent in situ hybridization (FISH) to study the expression of MYCC and MYCN using Vysis specific probes and immunohistochemistry with a SIOP-E BTG operative protocol standarized for β-catenin. Detailed clinical data were available for all these patients. RESULTS: Mean age at diagnosis was 6,5 years. The MB histology was distributed in Classic 60% of all tumors, followed by Nodular/Desmoplastic (ND) 31% of the cohort and finally, Large cell/Anaplastic (LCA) 9%. In this study the combination of metastatic disease and Classic or LCA phenotype were the clinicopathologic variables associated with poorer overall survival. All MYCC amplified tumors, within classic and LCA histological subtypes were metastatic tumors and were associated to poor outcome, except 1 patient with localized LCA that remains alive. MYCN and MYCC amplification was not found in ND tumors. Global survival and at 3 years of followed up were for ND group was 71/59%, for classic type 72/64% and for LCA tumors 56/17%.β-catenin nuclear expression only was found in two localized tumors. CONCLUSION: High Risk disease has been defined by clinical findings (M+ disease), pathological subtypes (LCA variant) and in recent years molecular markers (MYCC and MYCN amplification). Our results in a cohort of medulloblastomas support these criteria, although we were not able to validate the prognostic role of β-catenin nuclear expression in this study that could be different with the increased number of samples.

MB-090. A PATIENT WITH MEDULLOBLASTOMA IN ITS EARLY DEVELOPMENTAL STAGE

Naoki Shinojima, Hideo Nakamura, and Jun-ichi Kuratsu; Department of Neurosurgery, Kumamoto University Hospital, Kumamoto, Japan

Medulloblastoma is the most frequent malignant brain tumor of the posterior fossa in children. It has been suggested that medulloblastomas be categorized into distinct genetic subgroups, i.e. Wnt (Dkk1), Shh (SFRP1), group 3 (NPR3), or group 4 (KCNA1), since each subgroup is individual and there is no overlap among subgroups. We report a 13-year-old boy with medulloblastoma. He presented with sudden-onset nausea and vomiting due to intratumoral hemorrhage. We thought that the medulloblastoma was in an early developmental stage because the tumor volume was extremely small. Immunohistochemically the tumor was mainly composed of Dkk1- and NPR3-positive areas. The expression of Dkk1 and NPR3 was mutually exclusive in the tumor. Samples obtained by laser microdissection of individual areas and subjected to mass spectrometry confirmed the exclusive expression patterns of proteins. Our findings indicate that early-stage medulloblastoma is comprised of distinct entities and hint at the origin and development of medulloblastomas.

MB-091. TARGETING GLUTAMINE METABOLISM AS A THERAPEUTIC STRATEGY IN MYC-DRIVEN MEDULLOBLASTOMA

Allison Hanaford[1], Charles Eberhart[1], Tenley Archer[3], Pablo Tamayo[4], Scott Pomeroy[3], and Eric Raabe[2]; [1]Johns Hopkins School of Medicine, Baltimore, MD, USA; [2]Johns Hopkins Hospital, Baltimore, MD, USA; [3]Boston Children's Hospital, Cambrdige, MA, USA; [4]Eli and Edythe Broad Institute of MIT and Harvard University, Cambrdige, MA, USA

Group 3 medulloblastoma with elevated c-MYC has the worst prognoses of the four molecular subgroups. To generate a genetically defined model of this disease, we transformed human fetal cerebellar stem cells with MYC along with hTERT, dominant negative p53 and constitutively active AKT. The resulting xenografts showed morphological similarities to large cell/anaplastic medulloblastoma, leptomeningeal and spinal dissemination, and had a gene expression profile closely aligned with Group 3 primary tumors. Because MYC over-expression leads to increased glutamine metabolism and glutamine addiction in other cancers, we hypothesized that MYC-driven MB would exhibit increased glutamine metabolism and be sensitive inhibitors of glutamine metabolism. In both our human stem cell derived and established medulloblastoma cell lines, MYC expression positively correlates with increased expression of glutaminolytic enzymes. Inhibition of glutamine metabolism was achieved using two glutamine analogs, acivicin and 6-diazo-5-L-norleucine (DON). In our human stem cell models, MYC-transformed cells experienced a significant decrease in proliferation and an increase in apoptosis after acivicin treatment ($p < 0.005$), while SV40-transformed cells were unaffected. Both compounds also significantly decreased growth and increased apoptosis of the high-MYC MB cell lines D425Med and D283Med. In xenograft experiments using D283Med, fewer mice receiving acivicin developed flank tumors, and the resulting tumors were significantly smaller (80.9 mm^3 treated vs 273.8 mm^3 untreated). Glutaminase knockdown using short hairpins also significantly decreased growth of D425Med and D283Med. In summary, we present a novel human cerebellar stem cell based model of Group 3 medulloblastoma, and data suggesting that glutamine metabolism may be a therapeutic target in MYC-driven MB.

MB-092. TOLERABILITY AND EFFICACY OF CHEMOTHERAPY DURING AND AFTER RADIATION THERAPY (RT) IN ADULTS WITH CNS EMBRYONAL TUMORS

Kevin De Braganca, Stephen Gilheeney, Yasmin Khakoo, Kim Kramer, Suzanne Wolden, and Ira Dunkel; Memorial Sloan Kettering Cancer Center, New York, NY, USA

BACKGROUND: No standard regimen for chemotherapy in adults with CNS embryonal tumors exists, although adjuvant chemotherapy is routinely recommended for patients with high-risk disease. METHODS: Adult patients treated with high-risk medulloblastoma and pineoblastoma treated as per COG ACNS-0332 were retrospectively analyzed. RESULTS: 7 patients (4 males) between the ages of 24-44 were treated. 5 patients had medulloblastoma. 6 patients had M+ disease. 5 patients were treated per RegimenD with isotretinoin; 2 patients per RegimenB. All patients completed RT/carboplatin/vincristine on schedule. RT dose was 3600cGy craniospinal irradiation (CSI) with primary tumor site boost [1800-1980cGy]. Side effects included Grade 3 dermatitis [n = 1], transient myelosuppression requiring PRBC and/or platelets [n = 3] and prolonged myelosuppression delaying post-RT chemotherapy initiation [n = 1]. Vincristine was discontinued in all patients due to sensory neuropathy. Patients received a median of 7 vincristine doses [range 5-10]. 4 patients received all 6 planned doses of cisplatin, although dose reductions or discontinuation due to ototoxicity were necessitated in 3 patients and 1 patient, respectively. 3 patients completed all courses of isotretinoin. 3 patients developed dry skin (Grade 2), one of whom discontinued isotretinoin. 6 patients required blood product transfusions; median PRBC units = 11 [0-23], median platelet units = 8 [0-22]. 6 patients had 10 hospitalizations due to febrile neutropenia. 9 were uncomplicated; 1 was associated with septic shock. 3 patients discontinued treatment before completion of all 6 planned cycles due to: myelosuppression [n = 1], ototoxicity [n = 1] and Guillain-Barre Syndrome-variant [n = 1]. Event free survival is 100% with a median follow-up of 23 months [range 11-28 months]. CONCLUSIONS: Ototoxicity and myelosuppression in adult patients treated with CSI and cisplatin-based chemotherapy are to be anticipated in most patients. Concurrent RT-vincristine causes intolerable neuropathy in all patients. Carboplatin is well-tolerated. Carboplatin during RT and post-RT isotretinoin may be useful agents for adults with medulloblastoma. Vincristine dose-reduction or schedule change is warranted.

MB-093. microRNA EXPRESSION ANALYSIS REVEALS FOUR DISTINCT GROUPS OF PEDIATRIC MEDULLOBLASTOMA

Rishi R. Lulla[1], Joseph Laskowski[1], Jason Fangusaro[1], Stewart Goldman[1], and Vidya Gopalakrishnan[2]; [1]Northwestern University Feinberg School of Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA; [2]University of Texas MD Anderson Cancer Center, Houston, TX, USA

BACKGROUND: Recent progress in medulloblastoma biology has confirmed the presence of 4 clinically and molecularly distinct subgroups. MicroRNAs are dysregulated in cancer, including pediatric central nervous system tumors. We aim to determine if microRNA expression analysis can aid in the molecular classification of medulloblastoma. METHODS: RNA purification from 55 formalin-fixed paraffin embedded medulloblastoma samples was performed using RecoverAll™ RNA isolation kit and used for cDNA synthesis. TaqMan® miRNA assays were used to quantify the levels of 762 mature miRNAs from each sample using the Applied Biosystems 7900HT Fast Real-Time PCR system in 384-well low density arrays (TLDAs). Validation of molecular subgrouping using selected gene sets is performed using the NanoString® platform. Clinical, pathologic and outcome data was collected on all patients. RESULTS: Unsupervised hierarchical clustering revealed 4 statistically distinct subgroups (arbitrarily termed groups A, B, C and D for this study) based entirely on microRNA expression patterns and independent of tumor histology. Group A had a lower median age at diagnosis (2.7 years) compared to the other groups. Presence of metastatic disease was more likely group D (36%) as compared to the other groups. Three samples from the same patient at different time points clustered together in Group D suggesting that microRNA expression patterns are conserved from diagnosis to recurrence. Validation of microRNA subgroups with previously reported molecular subgrouping by NanoString® as well correlations of individual microRNA expression with patient outcomes is underway and will be presented. CONCLUSIONS: Our results suggest that microRNA expression patterns in pediatric medulloblastoma may distinguish clinically and molecularly distinct subgroups that correlate with genotype subgrouping.

MB-094. DURATION OF THE PRE-DIAGNOSTIC INTERVAL IN MEDULLOBLASTOMA IS SUBGROUP-SPECIFIC

Vijay Ramaswamy[1], Marc Remke[1], David Shih[1], Xin Wang[1], Paul Northcott[2], Claudia Faria[1], Charles Raybaud[1], Uri Tabori[1], Cynthia Hawkins[1], James Rutka[1], Michael Taylor[1], and Eric Bouffet[1]; [1]Hospital for Sick Children, Toronto, ON, Canada; [2]German Cancer Research Centre, Heidelberg, Germany

BACKGROUND: Children presenting with medulloblastoma have a wide range of initial presenting symptoms. However, the influence of underlying tumor biology on the initial presentation of medulloblastoma is currently unknown. In light of the recent discovery of distinct medulloblastoma subgroups, we sought to define the initial presentation of childhood medulloblastoma in a subgroup specific manner. METHODS: We assembled a cohort of 126 medulloblastoma cases at the Hospital for Sick Children between 1994-2012 and determined subgroup affiliation using nanoString. Clinical details pertaining to the initial presentation were determined through a retrospective chart review. The pre-diagnostic interval was defined as the time of initial symptom onset as reported by the patient/parents to the time of confirmatory neuroimaging. RESULTS: The median pre-diagnostic interval across all medulloblastoma cases was 4 weeks (IQR: 4-12 weeks). Strikingly, when the pre-diagnostic interval was then determined in a subgroup specific manner, cases with WNT and Group 4 tumors showed a significantly longer median pre-diagnostic intervals of 8 weeks compared to 2 weeks for SHH and 4 weeks for Group 3 ($p = 0.0001$). Very long pre-diagnostic intervals of over 12 weeks were almost exclusively WNT and Group 3 patients.($p < 0.001$). Younger age was significantly associated with a prolonged pre-diagnostic interval, however this association was only significant in Group 4 when stratifying by subgroup ($p = 0.02$ for all, $p = 0.04$ for Group 4). Improved survival was significantly associated with a longer pre-diagnostic interval ($p = 0.02$), however is no longer significant when controlling for subgroup ($p = 0.07$). CONCLUSIONS: The pre-diagnostic interval in childhood medulloblastoma is highly subgroup dependent, further highlighting the clinical heterogeneity and biological relevance of the four principle subgroups of medulloblastoma. Although we continue to advocate for early and rapid diagnosis, our findings suggest that delays in diagnosis are more related to the molecular biology of the underlying tumor than either patient or physician delay.

MB-095. SECOND TUMORS IN CHILDREN WITH MEDULLOBLASTOMA TREATED BETWEEN 1964 AND 2007: A SINGLE CENTER RETROSPECTIVE STUDY

Sandra Jacobs[1], Florent De Vathaire[2], Ibrahima Diallo[2], Damien Llanas[2], Cristina Verez[2], Fara Diop[2], Amar Kahlouche[2], Jacques Grill[2], Stéphanie Puget[3], Dominique Valteau-Couanet[2], and Christelle Dufour[2]; [1]UZLeuven, Leuven, Belgium; [2]Gustave Roussy Institute, Villejuif, France; [3]Necker-Sick Children Hospital, Paris, France

BACKGROUND: Brain tumor survivors have increased risk of developing second tumors. As patients affected with medulloblastoma undergo prophylactic neuraxis irradiation, they are particularly at risk to develop second tumors. METHODS: We performed a retrospective study, identifying patients treated for a medulloblastoma. Inclusion criteria for this study were: (1) children < 18 years at diagnosis with (2) a medulloblastoma who (3) survived at least 5 years, and were (4) treated at Gustave Roussy Institute, (5) between 1964 and 2007. Original data were collected from all medical records. From these data the incidence of secondary tumors was identified and risk factors for the development of second tumors will be stratified. RESULTS: Among 303 patients evaluable, 47 patients developed a second tumor four to thirty-four years from initial diagnosis. Most common second tumors were skin carcinoma (10 patients) and meningioma (10 patients), followed by glioblastoma (5 patients), sarcoma (5 patients), thyroid adenocarcinoma (4 patients), osteosarcoma (3 patients), cavernous angioma (3 patients), thyroid adenoma (3 patients), lymphoma (1 patient), AML (1 patient), melanoma (1 patient), and benign sex cord tumor (1 patient). Thirty-seven tumors were located within the radiation field, mostly in the CNS (19 patients) and in the skin (10 patients). Cancer predisposition was evidenced in 7 patients (5 Gorlin, 1 Li-Fraumeni syndrome, 1 SUFU mutation). We will further analyze the data to identify the impact of the development of second tumors on the overall survival and stratify risk factors for the development of second tumors within patients treated for medulloblastoma.

MB-096. VALUE OF EXTENT OF RESECTION ACROSS MEDULLOBLASTOMA SUBGROUPS (A MEDULLOBLASTOMA ADVANCED GENOMICS INTERNATIONAL CONSORTIUM (MAGIC) STUDY)

Vijay Ramaswamy, Eric Thompson, and Michael Taylor; Hospital for Sick Children, Toronto, ON, Canada

BACKGROUND: Extent of resection is an important prognostic marker in medulloblastoma. Children over the age of 3 with subtotal resections are considered a high-risk group and are treated with 36Gy of craniospinal irradiation. However, the identification of 4 distinct molecular subgroups of medulloblastoma has fundamentally changed our understanding of medulloblsatoma. We sought to determine if extent of resection remains prognostic when reanalysed in a subgroup specific manner. METHODS: Medulloblastoma subgroup affiliation was determined using nanosString profiling. Extent of resection was classified as gross total (GTR), near total (NTR- $< 1.5cm^2$), or subtotal (STR- $\geq 1.5cm^2$) based on post-operative contrast-enhanced imaging. Metastatic status was determined at initial staging. RESULTS: Across a cohort of 644 medulloblastoma cases where extent of resection and survival were available, the frequency of near total resection (0-$1.5cm^2$ residual disease) was 15% and subtotal resection ($>1.5cm^2$ residual disease) was 12.9%. The distribution of near total and subtotal resections across the 4 subgroups was not significantly different ($p = 0.16$). Extent of resection is a marker of poor overall survival across all medulloblastoma ($p = 0.006$). However, when when the analysis is repeated in a subgroup-specific manner, it is no longer significant with the possible exception of SHH patients ($p = 0.001$). In the excellent prognosis WNT patients and the poor prognosis Group 3 patients, there are no survival differences whatsoever between the three levels of extent of resection, independent of metastases. CONCLUSIONS: The prognostic value of an incomplete resection is significantly reduced when re-analysed in a subgroup specific manner. In those patients with near totally resected disease, an aggressive surgery to achieve gross total resection at the cost of significant neurological sequelae is unlikely to improve outcome. Intraoperative subgrouping has the potential to reduce neurological sequelae in those patients unlikely to benefit from a more aggressive resection.

MB-097. IDENTIFYING SIGNALING PATHWAYS ASSOCIATED WITH MEDULLOBLASTOMA SUBTYPES FROM "OMIC" DATA

Scott Pomeroy[1], Tenley Archer[1], Paul Northcott[2], and Pablo Tamayo[3]; [1]Boston Children's Hospital, Boston, MA, USA; [2]German Cancer Research Center, Heidelberg, Germany; [3]Broad Institute of MIT and Harvard, Cambridge, MA, USA

Medulloblastoma, the most common malignant pediatric brain tumor, comprises multiple subtypes classified by their distinct molecular signatures. In order to develop patient-specific targeted therapies we need to understand the underlying molecular events that drive tumor growth in each patient. We have previously shown that medulloblastoma subtypes have very different mutations and genomic alterations. We hypothesize that this diversity in reality targets a small set of pathways. To better understand the causes of these transcriptional changes, we integrate data sets in medulloblastoma tumors and cell lines that profile the transcriptome, genome and epigenome. Our approach integrates transcriptional profiles measured by Affymetrix plus2 microarray, genomic alterations (mutations and copy number variations) using whole exome sequencing and epigenomic signatures using Illumina 450K arrays.

MB-098. TARGETING CHK1 TO POTENTIATE CISPLATIN THERAPY IN MEDULLOBLASTOMA

Eric Prince, Vlad Amani, Andrea Griesinger, Nicholas Foreman, and Rajeev Vibhakar; Neuro-Oncology, Section of Hematology/Oncology, Department of Pediatrics, University of Colorado Denver, Denver, CO, USA

BACKGROUND: Medulloblastoma is the most common childhood malignant intracranial tumor that is inherent with a significant amount of therapy-related morbidity. We recently showed that multiple cell cycle associated kinases are dysregulated in medulloblastoma including Chk1. Checkpoint 1 (CHK1) kinase is an integral component of the cell cycle and the DNA damage response (DDR) pathway, which is responsible for maintaining genomic integrity. Previous work has shown the effectiveness of inhibiting CHK1 with small molecule inhibitors in epithelial tumors, but its role in medulloblastoma is unknown. METHODS: Low dose administration of a Chk1 inhibitor, AZD-7762 and cisplatin, the standard chemotherapy agent for medulloblastoma, were used both separately and together to assess the effect of CHK1 inhibition on medulloblastoma cell lines. Colony focus assays were performed to assess the effect of AZD-7762, with and without cisplatin, on cell proliferation. To further investigate whether cell proliferation results were caused by AZD-7762, cell cycle analyses were performed using a flow cytometer. RESULTS: Colony formation assay results revealed that low dose AZD-7762 combined with low dose cisplatin proved to be a more effective therapeutic approach than either of the two treatments alone. Additionally, results demonstrated that low dose AZD-7762 treatment decreased the percentage of cells actively in the cell cycle. Furthermore, dual-treatment of AZD-7762 with cisplatin yielded a synergistic effect with even less cells in cycle than either AZD-7762 or cisplatin alone. CONCLUSION: Overall, results demonstrate that small molecule inhibition of CHK1 alongside the ubiquitous chemotherapeutic, cisplatin, is more advantageous than either treatment alone, and therefore this combined therapeutic approach serves as an avenue for further investigation.

MB-099. DELINEATING TRANSFORMING MECHANISMS AND THERAPEUTIC TARGETS OF THE C19MC OncoMiR CLUSTER

Patrick Sin-Chan[1], Mei Lu[1], Claudia Kleinman[2], Tara Spence[1], Daniel Picard[1], King Ching Ho[1], Jennifer Chan[3], Cynthia Hawkins[1], Jacek Majewski[2], Nada Jabado[2], Peter Dirks[1], and Annie Huang[1]; [1]Arthur and Sonia Labatt Brain Tumor Research Centre, The Hospital for Sick Children, Toronto, ON, Canada; [2]Department of Human Genetics, McGill University, Montreal, QC, Canada; [3]Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada

Central nervous system-primitive neuro-ectodermal tumors (CNS-PNETs) represent a distinctly aggressive and clinically heterogeneous class of embryonal brain tumors with poorly understood biology. We have recently shown that CNS-PNETs with C19MC amplification and/or high LIN28 expression comprise a single molecular entity that spans various histologic categories and anatomic compartments. These tumors, classified as 'Group 1', are distinguished by highly primitive neural gene signatures. Taken together with our prior observations that C19MC miRNAs alters human neural stem cell (hNSC) phenotypes, these findings suggest C19MC OncoMiRs may critically modulate cell differentiation and growth pathways to promote tumorigenesis. To elucidate molecular mechanisms of C19MC-mediated tumorigenesis, we combined miRNA target prediction algorithms with comparative gene expression analyses of primary C19MC amplified tumors and hNSCs with stable expression of 5-oncogenic C19MC miRNAs. These analyses revealed multiple cell cycle regulatory tumor suppressors as candidate C19MC gene targets including p21, p27 and p130 (RBL2), which displayed highly conserved C19MC binding sites. We observed that p21, p27 and RBL2 were directly and synergistically targeted by C19MC miRNAs in cell lines with stable 5-C19MC OncomiRs expression. Significantly, miRNA in-situ hybridization and immuno-histochemical analyses confirmed p21, p27 and RBL2 as bonafide gene targets in primary C19MC-amplified human tumor cells. We observed stable 5-C19MC miRNA expression

conferred a proliferative phenotype in hNSCs, thus suggesting C19MC OncomiRs may synergize to activate pro-growth pathways. Recently, we identified gene fusions of C19MC and TTYH1 and demonstrated that the distinct methylation landscape of Group 1 CNS-PNETs correlated with C19MC targeting of RBL2 with consequent up-regulation of DNMT3B. Consistent with these observations, growth of primary Group 1 CNS-PNET cells was robustly inhibited by 5-azacytidine and Vorinostat treatment. These studies collectively suggest that C19MC OncoMiRs promote tumorigenesis by targeting cell cycle regulators to modulate the epigenome and provides one of the first rational therapeutics for these devastating tumors.

MB-100. GOOD OUTCOME IN MULTIPLY RELAPSED METASTATIC MEDULLOBLASTOMA ASSOCIATED WITH IMMUNE REACTION

Jennifer R. Madden, Nicholas K. Foreman, Andrew M. Donson, and David M. Mirsky; Children's Hospital Colorado; University of Colorado, Aurora, CO, USA

BACKGROUND: Multiply relapsed medulloblastoma has a very poor prognosis. Immune modulation may have tumor effect and improve survival. OBSERVATION: An eleven year old boy, who relapsed twice after standard therapy and subsequent bone marrow transplant, is now maintained 32 months from last relapse possibly by immune response induced by cetuximab. The immune response was difficult to control and required cyclosporine. The immune reaction was characterized by clinical, radiologic, and laboratory changes. CONCLUSIONS: Although cetuximab is approved for high grade gliomas, it is not reported to have effect on medulloblastomas. Provoked immune response may be promising treatment for progressive medulloblastomas, although further clarification of the underlying immunological mechanism is warranted.

MB-101. MAINTENANCE OF MOLECULAR SUBGROUP AFFILIATION IN METASTATIC MEDULLOBLASTOMA

Xin Wang[1], Adrian Dubuc[1], Andrey Korshunov[2], Vijay Ramaswamy[1], Marc Remke[1], Stephen Mack[1], Deena Gendoo[1], John Peacock[1], Betty Luu[1], Yoon-Jae Cho[3], Charles Eberhart[4], Tobey MacDonald[5], Xiao-Nan Li[6], Timothy Van Meter[7], Paul Northcott[8], Sidney Croul[9], Eric Bouffet[1], Stefan Pfister[8], and Michael Taylor[1]; [1]Hospital for Sick Children, Toronto, ON, Canada; [2]Clinical Cooperation Unit Neuropathology, German Cancer Research Center, Heidelberg, Germany; [3]Department of Neurology, Stanford University, Palo Alto, CA, USA; [4]Johns Hopkins University, Baltimore, MD, USA; [5]Emory Children's Pediatric Research Center, Atlanta, GA, USA; [6]Texas Children's Cancer Center, Houston, TX, USA; [7]Virginia Commonwealth University, Richmond, VA, USA; [8]Division of Pediatric Neurooncology, German Cancer Research Center, Heidelberg, Germany; [9]University Health Network Pathology, Toronto, ON, Canada

BACKGROUND: Previous genomic and molecular analyses have revealed that medulloblastoma comprises four distinct molecular variants with distinct genetics, transcriptomes, and outcomes. Subgroup affiliation has been previously shown to remain stable at the time of recurrence, which likely reflects their distinct cells of origin. However, an important question that remains unanswered is subgroup stability in the metastatic compartment. METHODS/RESULTS: We assembled a cohort of 12-paired primary-metastatic tumors collected in the MAGIC consortium, and established their molecular subgroup affiliation by performing integrative gene expression and methylation analysis. Frozen tissues were collected and profiled using Affymetrix gene expression arrays and Illumina methylation arrays. Class prediction and hierarchical clustering were performed using existing published datasets. Our molecular analysis establishes the unequivocal maintenance of molecular subgroup affiliation in metastatic medulloblastoma. We further validated these findings by interrogating a non-overlapping cohort of 19-pairs of primary-metastatic tumors from the Burdenko Neurosurgical Institute using an orthogonal technique of immunohistochemical staining. We confirm the perfect concordance, identified using integrative molecular analysis, between molecular subgroup affiliation at both the primary site and metastatic lesions on the basis of immunohistochemical staining. CONCLUSIONS: This investigation represents the largest reported primary-metastatic paired cohort profiled to-date and provides a unique opportunity to evaluate subgroup-specific molecular aberrations within the metastatic compartment. Although previous studies have shown the existence of clonal evolution of the metastatic compartment from its matching primary tumor, the maintenance of subgroup affiliation presents a treatment opportunity to target subgroup-specific events. Our findings further support the notion that medulloblastoma subgroups arise from distinct cells of origin, which are carried forward from ontogeny to oncology.

MB-102. ADOPTIVE NATURAL KILLER CELL IMMUNOTHERAPY FOR MEDULLOBLASTOMA AND ATRT

Alvaro Laureano[3], William Brugmann[1], Cecele Denman[1], Harjeet Singh[1], Helen Huls[1], Judy Moyes[1], Soumen Khatua[1], David Sandberg[2], Lucia Silla[3], Laurence Cooper[1], Dean Lee[1], and Vidya Gopalakrishnan[1]; [1]University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]University of Texas Health Science Center, Houston, TX, USA; [3]Hospital de Clinicas de Porto Allegro, Porte Allegro, RS, Brazil

Medulloblastomas (MBs) and Atypical teratoid/rhabdoid tumors (ATRTs) are malignant pediatric brain tumors. Although survival has improved, recurrence and metastasis are frequent. Unfortunately, there are few therapeutic options for these children. Immunotherapy is an alternative to traditional therapies that may circumvent the potential toxicities associated with traditional chemotherapy and radiation approaches. Many immune based therapies rely on the presence of tumor associated antigens (TAAs) and/or antigen processing and class I human leukocyte antigen (HLA I) expression. However TAAs for MB or ATRT are not well defined and neuronal tissues have very low HLA I expression. Natural killer (NK) cells, do not rely on TAA for cytolysis and therefore have potential for the treatment of these diseases. The current barriers to clinical application of NK therapy are quantitative and qualitative. To overcome quantitative barriers, we have developed a platform technology for the ex vivo expansion of NK cells through co-culture of peripheral blood mononuclear cells with artificial antigen presenting cells expressing membrane-bound IL-21 (mbIL21) to promote a 30,000-fold expansion of NK cells. We also demonstrate prolonged life-span of ex vivo expanded NK cells and persistance for up to 3 weeks post-infusion in the murine brain. These cells express high levels of immune stimulatory and anti-tumor cytokines interferon gamma and TNF-alpha following activation. Finally we demonstrate NK cytolytic activity in vitro against a panel of primary and established ATRT and MB cells and in vivo following locoregional delivery in a mouse orthotopic model of MB. Our data provide the first pre-clinical evidence supporting the use of mbIL21 expanded NK cells against pediatric brain tumors. Based on this data we have initiated a novel phase I clinical trial to assess the safety and efficacy of locoregional delivery of mbIL21 expanded NK cells for the treatment of posterior fossa pediatric brain tumors.