INTRODUCTION: Ginsenoside Rg3 is a natural active ingredient that is extracted from Korean red ginseng root. It elevates therapeutic effect of radiotherapy and chemotherapy, but the study found that the application of Rg3 is heavily limited by its low bioavailability and poor absorption via oral administration. METHOD: Rg3-loaded PEG-PLGA NPs (Rg3-NPs) were prepared by the modified spontaneous emulsification solvent diffusion (SESD) method, and the physicochemical characteristics of Rg3-NPs were investigated in our study. We treated primary glioblastoma with 50 μM Rg3-NPs for 48h. We then used gene expression arrays (Illumina) for genome-wide expression analysis and validated the results for genes of interest by means of Real-Time PCR. Functional annotations were then performed using the DAVID and KEGG online tools. RESULTS: MTI showed that the growth of cells can be significantly inhibited by Rg3-NPs in a dose-dependent manner. FCX test shows Rg3-NPs can be released from the conjugate nanoparticle and react with the genes in the cell nuclei causing changes in the gene molecules. We also found that cancer cells treated with Rg3-NPs undergo cell-cycle arrest at different checkpoints. This arrest was associated with a decrease in the mRNA levels of core regulatory genes as determined by microarray-analysis and verified by Real-Time PCR. Furthermore, Rg3-NPs induced the expression of apoptotic and anti-migratory proteins p53 in cell lines. CONCLUSIONS: The results of the present study, together with the results of earlier studies show that Rg3-NPs targets genes involved in the progression of the M-phase of the cell cycle. It is associated with several important pathways, which include apoptosis (p53). Rg3-NPs may be a potent cell-cycle regulation drug targeting the M-phase in glioblastoma cell lines.

0004. INDUCTION OF THE ASTROCYTIC LINEAGE PATHWAY SELECTIVELY ENHANCES THE CHEMOTHERAPEUTIC POTENTIAL OF TEMOZOLOMIDE AND COMBINATION IN STEM-CELL ENRICHED PEDIATRIC GLIOBLASTOMA

INTRODUCTION: The treatment of glioblastomas remains a considerable therapeutic challenge due to their heterogenous nature and resistance to current treatment regiments. The cancer stem cell (CSC) population is emerging as a critical target for successful chemotherapeutic treatments as this small subset of cells show a high degree of resistance to radio- and chemotherapy agents. The induction of astrocytic differentiation plus sequential mitochondrial-mediated (Combination) or DNA-alkylating (Temozolomide) therapy is an attractive method which may render such CSCs more susceptible to treatment. METHOD: Using a patient-derived early passage paediatric glioblastoma, expressing unprecedented levels of CD133 under normoxic (5% CO2, 25% O2) and hypoxic conditions (5% CO2, 1% O2), we have depleted the CSC population and further induced cell-cycle arrest using a modified spontaneous emulsification solvent diffusion (SESD) method, and the physicochemical characteristics of Rg3-NPs were investigated in our study. We treated primary glioblastoma with 50 μM Rg3-NPs for 48h. We then used gene expression arrays (Illumina) for genome-wide expression analysis and validated the results for genes of interest by means of Real-Time PCR. Functional annotations were then performed using the DAVID and KEGG online tools. RESULTS: MTI showed that the growth of cells can be significantly inhibited by Rg3-NPs in a dose-dependent manner. FCX test shows Rg3-NPs can be released from the conjugate nanoparticle and react with the genes in the cell nuclei causing changes in the gene molecules. We also found that cancer cells treated with Rg3-NPs undergo cell-cycle arrest at different checkpoints. This arrest was associated with a decrease in the mRNA levels of core regulatory genes as determined by microarray-analysis and verified by Real-Time PCR. Furthermore, Rg3-NPs induced the expression of apoptotic and anti-migratory proteins p53 in cell lines. CONCLUSIONS: The results of the present study, together with the results of earlier studies show that Rg3-NPs targets genes involved in the progression of the M-phase of the cell cycle. It is associated with several important pathways, which include apoptosis (p53). Rg3-NPs may be a potent cell-cycle regulation drug targeting the M-phase in glioblastoma cell lines.

0006. SIMULTANEOUSLY ACTIVATED Shh And Wnt SIGNALING IN NEURAL PRECURSORS IS SUFFICIENT TO INDUCE SUPRATENTORIAL PRIMITIVE NEOECTODERMAL TUMORS (sPNET)

INTRODUCTION: Supratentorial primitive neuroectodermal tumors (sPNET) are malignant forebrain tumors typically arising in early childhood. The biology of sPNETs is largely unknown, and targeted therapies do not exist, mainly due to the lack of mouse models that may be used for preclinical studies. Recently, global gene expression patterns identified three different molecular subgroups of sPNET with one of them displaying a concurrent upregulation of Wnt- and Sonic hedgehog (Shh) target genes, also referred to as Embryonal tumor with multilayered rosettes (ETMR). METHOD: In order to generate a genetically tailored mouse model, we used the Cre-loxP system and conditionally activated both Shh and Wnt signaling in sGAP-positive precursor cells of the CNS. Brains from such mice were thoroughly investigated by standard histology, immunohistochemistry and global gene expression analysis. RESULTS: Constitutive activation of both Shh and Wnt signaling in neural CNS precursor cells resulted in the formation of forebrain tumors that displayed remarkable similarities to human sPNETs with respect to localization, morphology and global gene expression patterns. The investigation of early embryonic stages revealed that the development of such tumors may be initiated in Pax6 positive precursor cells within the ventricular zone. Finally, ex vivo cultures of murine sPNET cells were characterized by rapid tumor cell proliferation as well as sustained elevation of Wnt and Shh target genes and therefore perfectly reflected the in vivo growth properties of the tumor. CONCLUSIONS: We report on the first genetic mouse model for human sPNETs, which represents a promising tool for the development of targeted therapies against such tumors.

0007. RESULTS OF HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF PEDIATRIC BRAIN TUMORS

INTRODUCTION: Central nervous system (CNS) tumors are the second most common pediatric malignancies with an about 30% 5-year overall survival (OS) rate in relapsed 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. METHOD: For 6 years, 31 patients with high-risk or relapsed medulloblastoma (N = 16), supratentorial PNET (N = 5), germinoma (N = 4), atypical teratoid/rhabdoid tumor (N = 3), and atypical teratoid rhabdoid tumor (N = 3) received HSCT with auto-HSCT after induction chemotherapy, radiotherapy, surgical treatment. At the moment of HDCT 13 patients were in complete remission (CR), 15 patients were in partial remission (PR) and 3 patients had stable disease (SD). 18 Patients received single auto-HSCT, 4 patients received tandem auto-HSCT and 9 patients received only the first of tandem. The conditioning regimens included intraventricular/intrathecal metotrexat. The mean transplanted CD34+ cell dose was 5.27 ± 10^6/kg (1.0–8.9 x 10^6/kg). RESULTS: The median follow-up is 12 months (1–82). All patients with SD at the moment of auto-HSCT died of disease progression. Eight of 28 patients with CR or PR relapsed after HDCT, the other 20 patients are currently in CR. The following therapy toxicity included liver (N = 21), skin (N = 6), severe mucositis (N = 15), nausea, vomiting (N = 10), infectious (N = 18). OS in all groups was 44% and DFS was 35%. OS was significantly better among high-risk patients in 1st CR compared to patients in 2nd or following CR: 69% and 30%, respectively (p = 0.05). The same correlation was observed in DFS: 46% and 26%, according to WIP1 and auto-HSCT with medulloblastoma 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.

**0008. THE WPI1 ONCOGENE DRIVES METASTASIS IN GROUP 3 MEDULLOBLASTOMA**

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**INTRODUCTION:** Recent studies have suggested that medulloblastoma, the most common malignant brain tumor of childhood, comprises actually four distinct variants. We have previously demonstrated overexpression of the WPI1 oncogene in Group 3 tumors, the medulloblastoma subgroup with the most aggressive clinical behavior. We have recently discovered an important interaction between WIP1 and signaling through the G protein-coupled receptor CXCR4 that promotes invasion and metastasis, which may explain the aggressive clinical behavior of WPI1 high-expressing medulloblastomas. **METHOD:** We examined the correlation of WIP1 expression to clinical characteristics, including survival, in a cohort of 62 patients diagnosed with medulloblastoma. Knock-down of WIP1 in vitro and in an orthotopic, xenografted mouse model. **RESULTS:** WIP1 was noted to be increased in metastatic medulloblastomas, along with poor progression-free and overall survival. Microarrays identified up-regulation of metastasis genes, including CXCR4, in WIP1 high-expressing medulloblastomas. The CXCR4 ligand, SDF1a, activated PI3-kinase signaling and promoted growth and invasion in vitro and in an orthotopic, xenografted medulloblastoma model. Knock-down of WIP1 or CXCR4 inhibiting SDF1a effects and improved survival of xenografted mice. WIP1 knock-down inhibited CXCR4 surface localization by suppressing GRK5. Restoration of GRK5 promoted Ser339 CXCR4 phosphorylation and inhibited growth of WIP1-sustained medulloblastomas. **CONCLUSIONS:** Our results demonstrate an important cross-talk among WIP1, CXCR4, and GRK5, which may be a key determinant of the aggressive and metastatic phenotype of Group 3 medulloblastoma in children.

**0009. CONGENITAL POSTERIOR FOSSA POLAR SPONGIOSASTOMA**

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**INTRODUCTION:** ‘Polar spongiosablastoma’ is a historical microscopic description of a tumor pattern that can be observed in a variety of high-grade pediatric and adult CNS neoplasms. It is characterized by bipolar neoplastic cell clusters arranged in distinctive palisades. It is not recognized as a diagnostic entity in the current WHO CNS tumor classification, though some argue it is indeed a unique tumor entity. **METHOD:** Following IRB approval, the medical record of an infant classified as having congenital posterior fossa ‘polar spongiosablastoma’ was abstracted for demographic information, diagnostic details, tumor histopathology and overall clinical course. The medical literature pertaining to congenital CNS tumors, young-age medulloblastoma and ‘polar spongiosablastoma’ was surveyed. **RESULTS:** A 3 month-old presented with progressive irritability, back arching and poor feeding since birth. Scans revealed a large rim-enhancing posterior fossa mass. Gross total resection was performed. There were no metastases. The tumor was comprised nearly exclusively of palisading columns of bipolar cells with a brisk MB-1 index (10%). There were no morphologic features of medulloblastoma or glioma. Atypical teratoid/rhabdoid tumor was excluded by strong INI-1 immunostaining. There was patchy weak synaptophysin (neuronal) and focal GFAP (glial) staining. A diagnosis of malignant ‘polar spongiosablastoma’ was assigned based on the distinctive histologic appearance. **CONCLUSIONS:** Whether ‘polar spongiosablastoma’ is a unique tumor entity, rather than a description of tumor cell palisading that can be commonly seen in a variety of high-grade CNS tumors, is controversial. We suggest that the unique feature of virtual complete cellular palisading, in the absence of diagnostic features which would otherwise categorize the tumor into a known neuroepithelial tumor class (e.g., medulloblastoma), should prompt ongoing discussions about the nomenclature for this rare condition. We suggest ongoing reporting of this condition.

**0011. ABERRANT Otx2 EXPRESSION ENHANCES MIGRATION AND INDUCES ECTOPTIC PROLIFERATION OF HINDBRAIN NEURONAL PROGENITOR CELLS**

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**INTRODUCTION:** Dysregulation of Otx2 is a hallmark of the pediatric brain tumor medulloblastoma, yet its functional significance in the establishment of these tumors is unknown. METHOD: Here we have sought to determine the functional consequences of Otx2 overexpression in the mouse hindbrain to characterize its potential role in medulloblastoma tumorigenesis and assign the cell types responsive to this lineage-specific oncogene. **RESULTS:** Expression of Otx2 broadly in the mouse hindbrain resulted in the accumulation of proliferative clusters of cells in the cerebellar white matter and dorsal brainstem of postnatal mice. We found that brainstem extemoceptive neurons derived from neuronal progenitors of the rhombic lip and that cerebellar ectopia were derived from granule neuron precursors (GNPs) that had migrated inwards from the external granular layer (EGL). These hyperplasias exhibited various characteristics of medulloblastoma precursor cells identified in animal models of SHH or Wnt group tumors, including aberrant localization and altered spatiotemporal control of proliferation. **CONCLUSIONS:** These studies implicate a role for Otx2 in altering the dynamics of neuronal progenitor cell proliferation.

**0012. PERSONALIZING THE TREATMENT FOR MEDULLOBLASTOMA: POLO-LIKE KINASE 1 (PLK1) AS A MOLECULAR TARGET FOR THE SONIC HEDGEHOG (SHH) SUBTYPE**

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**INTRODUCTION:** Medulloblastoma (MB) is the most common malignant brain tumor in children. There are four different molecular subtypes of MB (WHO, 2007 Group 3, and Group 4) that are used to stratify risk related to patient outcome and guide the development of targeted therapies. We recently published that Polo-Like Kinase 1 (PLK1) is a promising molecular target for glioblastoma and therefore questioned whether it too could be beneficial for MB. PLK1 is an oncogenic kinase that controls mitosis, cell cycle and proliferation making it a strong candidate for treatment of MB. **METHOD:** Using NanoString nCounter analysis, we subtyped 74 primary pediatric MB and measured levels of PLK1 expression. As well, a library of small molecule inhibitors of SHH signaling and PLK1 were analyzed for synergistic effects and improved survival of xenografted mice. WIP1 knock-down inhibited CXCR4 surface localization by suppressing GRK5. Restoration of GRK5 promoted Ser339 CXCR4 phosphorylation and inhibited growth of WIP1-sustained medulloblastomas. **CONCLUSIONS:** Our results demonstrate an important cross-talk among WIP1, CXCR4, and GRK5, which may be a key determinant of the aggressive and metastatic phenotype of Group 3 medulloblastoma in children.
used to examine the efficacy of BI2536 and comparisons were made to the standard-of-care chemotherapy protocol Headstart. RESULTS: Patients belonging to the SHH subtype had the poorest prognosis. PLK1 inhibitors represented 6/16 most potent inhibitors tested in the SHH MB screen. As well, both PLK1 mRNA and protein were prognostic for relapse and overall survival. PLK1 was the highest in SHH tumors and overexpressed in MB compared to normal cerebellum. We show that BI2536 has efficacy against self-renewal and PLK1 high but non-PLK1-low MB cell. In contrast, human neural stem cell growth was unaffected by BI2536. Lastly, as a single agent BI2536 extended survival in MB-bearing mice and it was comparable to the Headstart chemotherapy cocktail. CONCLUSIONS: In conclusion, patients who had tumors expressing very high levels of PLK1 are considered to be at elevated risk for relapse and death. We anticipate that PLK1 inhibitors may have fewer detrimental side-effects as it is not expressed at high levels in normal brain tissue. Therefore, it could be a great improvement to many of the chemotherapies currently being used that can often cause long-term adverse effects. These pre-clinical studies pave the way for improving the treatment of SHH MB through PLK1 inhibition.

INTRODUCTION: Embryonal tumours with multilayered rosettes (ETMR) demonstrate a unique amplification of the C19q13.42 genomic locus, leading to overexpression of the related microRNA (miR) cluster, including the functionally important miR-520g. ETMRs exhibit significant vascular pathology including intravascular thrombosis and angiogenesis, which are often a consequence of oncogenic deregulation of tissue factor (TF). TF acts as an essential receptor for coagulation factor VIIa and thereby mediates both clotting and proangiogenic functions in the ETMR. TF is methylated in ETMR the amplified miR-520g may be a part of the oncogenic circuitry that controls TF resulting in perturbations in vascular homeostasis and increased disease aggressiveness. METHOD: TF immunopositivity was evaluated for ETMR amplifications with the C19q 13.42 amplification. To mimic this effect, multiloblastoma (MB) and globlastoma (GBM) cell lines were transected to overexpress miR-520g (DAOY, UW228, U373 MB). The cells were also treated with 5-AZA-2’ deoxycytidine to mask endogenous miR-520g. TF mRNA and protein levels were assessed in the cellular and extracellular vesicle (EV) fraction under different conditions. Bioinformatics, LNA miR inhibitors, an luciferase reporter plasmids were used to assess the regulation of TF by miR-520g. Biological implications were studied by assessing TF procoagulant activity and levels of TF target genes (plasminogen activator inhibitor-1, PAI-1). RESULTS: TF is expressed in pediatric embryonal brain tumours, but expression is lower in C19q13.42-amplified ETMR. Moreover, miR-520g is methylated in MB and GBM cells expressing high levels of TF. Forced expression of miR-520g reduces cellular and EV-associated TF levels in vitro and in DAOY tumour xenografts. Luciferase assays indicate that this effect is mediated by miR-520g binding sites in the TF-3’UTR sequence, and is reversible by the respective anti-miR. Growth factors (TGFs) attenuate miR-520g effects. MiR-520g lowers the TF-dependent procoagulant activity of MB cells and their EV, in agreement with our results. In vitro and in vivo, the absence of clinically manifest thrombosis, pediatric brain tumours are able to activate the coagulation pathway by overexpression of TF. Ours is the first study that examines this as a function of miRs and shows TF downregulation by miR-520g in ETMR. The significance of this change for ETMR pathogenesis is presently unclear. Since miR-520g is methylated in other types of brain tumour cells (MB, GBM) where TF levels are normally high, we suggest that this silencing may be indicative of a tumour suppressive activity. We propose that oncomirs may control tumour coagulome.

INTRODUCTION: Supratentorial ependymoma with histologic progression: CASE REPORT

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INTRODUCTION: Supratentorial ependymoma is a rare malignancy with poor long-term prognosis due to local and systemic disease recurrences. Outcomes are best when gross total resection is followed by radiation. We report the case of a expansive tumor recurrence following an 8 year disease free interval. METHOD: A 3 year old girl presented with seizures and left hemiparesis, and large local tumor recurrence. On reoperation, histology was WHO grade III anaplastic ependymoma. Four months later, biopsy of a small area of new enhancement at the margin of the resection cavity revealed no malignancy. She has been disease free for two years. RESULTS: Outcomes studies in patients with ependymoma show similar overall
survival and progression-free survival at 5 and 10 years following treatment. Median time for progression or relapse has been found to be about 2 years. Prognosis is similar between WHO grade II and grade III histologies, worse with younger age at initial presentation, and worse with shorter time to relapse. Disease progression or recurrence first seen at 5 to 10 years after initial treatment is very rare. Factors which could lead to delayed recurrence after gross total resection are unknown. CONCLUSIONS: Tumor surveillance following optimal treatment for supratentorial ependymoma should be continued for at least 10 years. Further study is needed to identify patients who may be at risk for delayed recurrence despite gross total resection.

0019. EXPLORING THE FUNCTIONAL ROLES AND THERAPEUTIC POTENTIAL OF TARGETING POTASSIUM CHANNEL EAG2 IN MEDULLOBLASTOMA GROWTH AND METASTASIS

INTRODUCTION: Medulloblastoma (MB) is the most common malignant pediatric CNS tumor, and is characterized by rapid progression and tendency to spread along the leptomeninges of the brain and spinal cord. Standard-of-care treatment with surgery, radiation and chemotherapy typically results in serious cognitive and neuroendocrine deficits that substantially impact quality of life. It is therefore critically important to identify novel targets that drive MB cell growth and tumor progression. The contribution of ion channels towards MB tumorigenesis and progression is essentially unexplored. Here we have investigated the functional roles and therapeutic potential of targeting potassium channel Eag2 in MB growth and metastasis.

METHOD: We conducted a genome-wide gene expression microarray survey of mouse Shh-MBs and found that the expression of voltage-gated potassium channel Eag2 was consistently elevated. Using qPCR, IHC and biochemical approaches we confirmed Eag2 overexpression in a subset of human MBs and have evaluated molecular and histological subtype-specific roles of Eag2 using electrophysiology, molecular biology, cell biology, and xenograft approaches following specific RNAi knockdown of Eag2 expression. We also crossed Eag2 knockout mice with transgenic mice that develop spontaneous MBs and determined changes in tumor progression. Finally, we studied the evolutionarily conserved role of eag channel in Drosophila melanogaster. RESULTS: We determined that Eag2 expression is elevated across different molecular and histological subtypes of MB. Eag2 knockdown reduces tumor growth in vitro and in vivo. Mechanistically, we show that Eag2 controls mitotic entry and tumor growth by regulating cell volume dynamics. Intriguingly, we found that Eag2 also promotes MB cell migration and tumor metastatic potential. In addition, we determined that Eag2 deficiency or pharmacological inhibition reduces MB cell proliferation and migration via effect on the p38 MAPK pathway. Importantly, we found that the brain tumor-specific function of eag potassium channel is evolutionarily conserved across distant organisms. CONCLUSIONS: Our study establishes the functional significance of Eag2 in promoting MB tumor progression via regulating cell volume dynamics, the perturbation of which acts through the p38 MAPK pathway, and establishes clinical relevance for targeting this ion channel in human MBs.

0020. DELAYED FUNCTIONAL PATENCY OF ENDOSCOPIC THIRD VENTRICULOSTOMY WITH RADIATION-INDUCED ALTERATION OF MASS EFFECT: CASE ILLUSTRATION

INTRODUCTION: Endoscopic third ventriculostomy (E3V) is an established option for treatment of obstructive hydrocephalus and is often performed concurrent with biopsy of an obstructing mass lesion. Delayed
failure is a known complication. We report the case of a patient whose E3V became functionally patent in a delayed fashion, three weeks postoperatively, when mass effect on the brainstem had been adequately relieved. METHOD: A 13 year-old boy underwent anterior, posterior, and right temporal parietal decompressive craniectomy for intractable hydrocephalus from a pineal region tumor. He underwent endoscopic biopsy, E3V, and external ventricular drain (EVD) placement. Pathology was consistent with pure germinoma. The patient returned to surgery four days post E3V for a right E3V. He remained atropin-dependent, and was treated with radiation therapy on postoperative day #7. Placement of a ventriculoperitoneal shunt was deferred while the patient was still receiving radiation. RESULTS: EVD output declined from 300 cc/day to 100 cc/day by the 11th day of radiation therapy. On postoperative day #22 the EVD was clamped, and the patient remained asymptomatic. A new brain MRI the following day showed significantly decreased size of the germinoma along with flow artifact across the floor of the third ventricle, and his EVD was removed. There has been no tumor progression and the patient’s hydrocephalus has not recurred over the following two years. CONCLUSIONS: Resolving mass effect from a rapidly shrinking pineal region tumor can delay relief of hydrocephalus from E3V, but has the potential to ultimately result in shunt independence.

0023. INTRACRANIAL XENOGRAFTING OF EXTRANEURAL DISSEMINATED ANAPLASTIC MEDULLOBLASTOMA IN IMMUNOCOMPROMISED MICE AND SUBSEQUENT IN VIVO BIOLUMINESCENCE IMAGING

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INTRODUCTION: Medulloblastoma (MB) represents the most common malignant brain tumor in children. Although survival has significantly improved over the last decade, the result for high-risk patients remains poor. Criteria associated with poor clinical outcome are: age ≤ 3 years, metastasis, subtotal resection, anaplastic histology and c-myc amplification. We isolated MB cells from the pleura effusion of a 2 year old boy suffering from a c-myc positive anaplastic MB that also showed dissemination via the cerebrospinal fluid. To further investigate the biology of these “high risk” cells, we established a xenogenic mouse model with the ability for bioluminescence imaging (BLI). METHOD: MB cells, transduced with a lentiviral vector coding for enhanced green fluorescent protein (eGFP) and firefly luciferase (FLuc), were evaluated by fluorescence microscopy, flow cytometry and in vivo BLI. For intracranial grafting transduced cells were injected i) infratentorially and ii) supratentorially into the brains of immunocompromised mice. Tumor growth was evaluated by clinical surveillance and kinetic in vivo BLI. Tumors were histologically analyzed. RESULTS: Survival after lentiviral transduction, expression of eGFP could be easily detected by fluorescence microscopy and flow cytometry. Analyzing FLuc activity by limited dilution assay and BLI revealed emission of 483 photons/second/cell. When implanting the cells into the brains of immunocompromised mice, there was a 100 % penetration. As few as 500 cells were capable to form tumors. BLI showed a continuously increasing signal after supra- as well as infratentorial grafting. Histology and immunohistochemistry revealed that tumors were identical to the primary tumor. They were anaplastic MBs and showed a high tendency to invade and spread through the cerebrospinal fluid. CONCLUSIONS: We established a highly reproducible anaplastic MB model that closely resembles the morphological (i.e. anaplasia) and biological (i.e. dissemination) features of the human tumor. Transplantation of MB cells in vivo cell culture is now possible thus providing an ideal tool for monitoring tumor biology or treatment effects.

0025. RADIODIAGNOSIS OF BRAIN WHITE MATTER BY THE CATALYTIC MnSOD MIMIC / ANTIOXIDANT, BMX-001

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INTRODUCTION: Cranial irradiation is a proven therapy for primary and metastatic brain tumors that increases cancer control. However, radiation-induced oxidative stress in normal brain tissue is thought to contribute to neurocognitive decline. METHOD: We evaluated the effectiveness of a novel catalytic mimic of Mn superoxide dismutase (MnSOD), BMX-001 (MnTnBuOE-2-Pep+)1, to provide neuroprotection following 8 Gray of cranial irradiation. C57BL/6j mice were pretreated with BMX-001 by twice-daily injections of 1.5 mg/kg for one week prior to and one month following irradiation. The BMX-001 dose was then reduced to 0.5 mg/kg/day for another month and then discontinued for a third month post-irradiation. Mouse brains were evaluated by MRI (radial diffusion, susceptibility and functional anisotropy) using a 9.4 T Oxford Instruments vertical bore magnet at 3 months post RT. RESULTS: Concentrations of drug in the brain averaged 25nM, which is within the therapeutic dose range, based on other studies in stroke and spinal cord injury. Mice treated with RT + BMX-001 exhibited strong MRI evidence for myelin preservation in the corpus callosum and anterior commissures compared with controls that received saline + RT. The saline + RT groups
0026. BLOCKING THE ANTI PHAGOCYTIC SIGNAL PROVIDES A COMMON AND EFFICACIOUS TREATMENT MODALITY FOR PEDIATRIC BRAIN TUMORS

INTRODUCTION: Extensive molecular characterization of Brain tumors has proven valuable for classification, risk stratification and outcome prediction for current treatment. However the standard of care has not improved prognosis and there is an urgent need of risk of cognitive in a characteristic feature of tumor progression and recurrence is its ability to evade the immune system. We hypothesized that by disrupting the interaction between the cell surface antigen CD47 and its binding partner Sirpa we can induce the innate immune system to attack and remove the tumor by enhancing the ability of macrophages to phagocytose brain tumor cells. METHOD: We looked at CD47 expression in freshly isolated patient and postmortem samples from 3 different tumor types; Diffused Intracranial Pontine Glioma, Medulloblastoma, and PNETs. Using radiobinding assays we hypothesized that blocking CD47-Sirpa interaction induced phagocytosis in an in-vitro phagocytosis assay. Finally we established orthotopic xenografts models from primary patients samples in immune compromised mice and treated them with anti-CD47 humanized antibody, which is currently being developed for clinical trials in hematopoietic and non-CNS malignancies. RESULTS: CD47 expression was upregulated in all tumor types and was present in >90% of the cells in high grade tumor. Increased CD47 expression was observed in CD15+ and CD133+ putative cancer stem cell population. Blocking the CD47-Sirpa interaction increases tumor phagocytosis by macrophages in-vitro. Systemic treatment with anti-CD47 antibody significantly reduced tumor burden in an orthotopic xenograft setting. CONCLUSIONS: Anti-CD47 therapy is a viable and effective treatment modality for pediatric high grade brain tumors.

0027. GENETIC AND HISTOPATHOLOGICAL SPECTRUM OF PAEDIATRIC DIFFUSE INTRINSIC PONTINE GLIOMAS

INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is a devastat- ing malignancy with poor prognosis, no effective therapy and a median survival of ~10 months. In the majority of cases, the diagnosis is established on clinical and imaging grounds only, based exclusively on MRI findings, resulting in a scarcity of pre-treatment specimens available to study. Focal radiation is currently the standard of care with many chemotherapeutic agents showing little success thus far. To try to improve our ability to treat these patients, an ongoing project has developed an experimental protocol to investi- gate the biology of DIPG and correlate this with histologic and clinical features. METHOD: Here we report the histologic spectrum of seventy-one DIPG and its relation to genetic events including mutations, structural variants and copy-number alterations. All cases were reviewed for histologic diagnosis and classified according to WHO criteria. Sixty patients had high-grade-astrocytomas (grade-III/IV), eight had grade-II-astrocytomas, and three had features of primitive neuroectodermal tumour (PNET). To iden- tify genetic alterations underlying DIPG we performed deep sequencing of 33-tumour normal pairs (20 whole genomes and 15 whole exomes) and integrated this data with SNP copy number for 40 DIPGs. RESULTS: There was no correlation between histologic grade and survival. Approximately 1/3 of pa- tients had leptomeningeal spread of their tumour. Diffuse invasion of the brainstem, spinal cord and đámus was common with some cases showing spread as distant as the frontal lobes. Sixty-eight percent and patients had K27M-H3 mutations. Recurrent copy number alterations and structural var- iants corresponding to histone mutational status were identified including those involving PDGFRα and MYCN. An associated in higher age of diagnosis and ALT (alternative lengthening of telomeres) was observed (ALT: 11.25 ± 3.27 vs non-ALT 5.91 ± 2.40; p = 0.0001). ATRX muta- tions were present in 1/3 of ALT positive patients. CONCLUSIONS: DIPG tumour heterogeneity is very heterogeneous with many showing focal GBs (GBs) intermixed with areas of grade-II or grade-III histology, which has potential implications for biopsy. Histone mutations were more homogenously present and were found in low grade tumours with poor clinical outcome. The frequency of leptomeningeal disease in this patient group suggests that focal radiation may be inadequate for some cases. These findings suggest that new therapeutic approaches need to incorporate both histopathological and molecular data, including histone mutational status at biopsy in order to achieve maximum benefit for DIPG patients.

0028. TRANSCRIPTOSOMIC PROFILING AT IGF-2 PROMOTERS THROUGH BIOTINYLATED-DNA ‘FISHING’; ANALYSIS

INTRODUCTION: Medulloblastomas have four molecularly distinct sub- classes. Tumors of the subclass marked by over-expression and amplification of Sonic hedgehog (Shh) pathway components and targets also display high levels of insulin-like growth factor-2 (IGF2). In mouse models for Shh-medulloblastomas, IGF2 is required for tumor formation, growth, and metastasis. We showed that YAP over-expression induces IGF2 expression as a result of YAP’s radiation-resistant properties for childhood brain tumors (1) and in tumorigenesis in mice. IGF2 is also amplified in medulloblastomas, and in cerebellar granule neuron precursors (CGNPs). IGF2 and its regulatory program may represent a therapeutic target in medulloblastoma, but the mechanism of IGF2 induction downstream of YAP is not well understood. METHOD: The anomalous loss of IGF2 imprinting in the human fetal brain is intriguing and exemplifies the complexity of the IGF2 gene’s regulation. Although CTCF mediates allele-specific expression at the IGF2/ H19-imprinted locus in both mice and humans, subsequent evidence suggests that CTCF imprinting may not be a prerequisite for IGF2 regulation. We hypothesized that blocking CD47–Sirpa interaction induced phagocytosis in an in-vitro phagocytosis assay. Finally we established orthotopic xenograft models from primary patients samples in immune compromised mice and treated them with anti-CD47 humanized antibody, which is currently being developed for clinical trials in hematopoietic and non-CNS malignancies. RESULTS: CD47 expression was upregulated in all tumor types and was present in >90% of the cells in high grade tumor. Increased CD47 expression was observed in CD15+ and CD133+ putative cancer stem cell population. Blocking the CD47-Sirpa interaction increases tumor phagocytosis by macrophages in-vitro. Systemic treatment with anti-CD47 antibody significantly reduced tumor burden in an orthotopic xenograft setting. CONCLUSIONS: Anti-CD47 therapy is a viable and effective treatment modality for pediatric high grade brain tumors.

0029. VALIDATION OF THE HGG-IMMUNO RPA MODEL IN A NEW COHORT OF PATIENTS WITH RELAPSED MALIGNANT GLIOMA TREATED BY ADJUVANT POSTOPERATIVE DECEPTIVE CAR T CELL VACCINATION

INTRODUCTION: Immunotherapy with autologous mature dendritic cells loaded with autologous tumor lysate (DCm-HGG-L) is an innovative treatment for patients with high grade glioma (HGG). We run the HGG-IMMUNO-2003 cohort comparison study to treat children and adults with relapsed HGG with immunotherapy after new (sub)total resec- tion of the relapsed tumor, confirmed with postoperative MRI, central pa- thology confirmation and quick tapering off of corticosteroids. Tumors were conserved sterile, dry and frozen at -80°C for preparing the lysate with snap freeze/thaw cycles and subsequent 60 Gy irradiation. Dendritic cells (DC) were differentiated out of monocytes, obtained with elutriation of the leuka- pheresis product, in tissue bags in the presence of GM-CSF/IL-4 for 7 days. DCs were loaded with lysate (HGG-L) and matured with IL-1b and TNF-a for 24 hours. They were injected intralesionally in immunocompetent hosts with one injection at weeks 1/2/3/4/8/12/16 and then each 3 months. RESULTS: HGG-IMMUNO RPA data were available for 92 adults aged 17-75y (median 49). Patients were treated postoperatively with a median of 6 vaccines (1-23). Up to 4 weekly induction vaccines with DCm-HGG-L, boost vac- cines consist of only HGG-L (weeks 8/12/6/ + 12). Median PFS and OS were
3.5 and 11 months with a 2-y OS of 17%. Patients were classified in HGG-IMMUNO RPA classes I (n = 8), II (n = 23), III (n = 45) and IV (n = 16). Median PFS and OS were respectively 3.7, 2.9, 4.1, 3.3 and 16.3, 14.7, 10.4 and 9.4 months (log-rank: p = 0.048). CONCLUSIONS: Although long-term follow up of the E cohort is still short, the HGG-IMMUNO RPA model could already significantly distinguish the median OS for patient subgroups with relapsed HGG treated in the E cohort. These data together with the published data strongly support the HGG-IMMUNO RPA model to describe patient groups treated with immunotherapy, in order to appropriately evaluate the effects of immunotherapy. The model allows a more accurate interpretation of immunotherapy treatment results amongst different research groups and opens the possibility for appropriate stratification of randomized clinical trials in future. Similar modelling is urgently needed for pediatric patients with HGG.

0030. TELOMERE INHIBITION INDUCES GROWTH ARREST IN PAEDIATRIC EPENDYMOMA

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INTRODUCTION: Ependymomas represent the third most common paediatric brain tumour, yet effective therapeutic strategies are lacking and 5-year survival rates remain poor at approximately 50%. Previous studies have shown that over 50% of paediatric ependymomas possess active telomerase, an enzyme that permits a limitless growth potential through the prevention of telomere erosion and subsequent senescence. Since telomerase is present in the majority of ependymomomas and absent in the majority of somatic cells, telomerase inhibition represents an ideal therapeutic strategy for telomerase-positive paediatric ependymomas. We hypothesize that inhibiting telomerase will induce growth arrest in paediatric ependymoma cell and animal models. METHOD: Paediatric ependymoma cell lines (R254, BXD-1425EPN) and tumour initiating cells (TICs) (E520) were treated with the telomerase inhibitor Imetelstat and tumour initiating cell (TICs) (E520) were treated with the telomerase inhibitor Imetelstat in parallel with untreated and mismatch control until growth arrest was observed. Throughout treatment, a number of parameters were assessed including senescence (β-galactosidase), apoptosis (TUNEL), telomerase activity (telomere repeat amplification protocol) and telomere length (telomere restriction fragment assay). To study telomerase inhibition in vivo, subcutaneous injections of 5.0x10^6 E520 cells were performed and mice were treated with either PBS, mismatch control or Imetelstat (3x/week) for 4 weeks (N = 6 mice/group). Tumour growth was measured with calipers and tumours were weighed upon sacrifice. RESULTS: Imetelstat treated R254 cells showed a reduced proliferative rate following 6 weeks of treatment and total growth arrest following 15 weeks of treatment. This observed growth arrest was associated with a marked inhibition of telomerase activity, shortened telomeres, an 80% increase in senescence and 20% increase in apoptosis. BXD-1425EPN cells treated with Imetelstat exhibited drastically reduced growth with around 40% loss of telomere inhibition and a 50% increase in senescence, while E520 TICs have thus far shown a reduced growth rate.

FOR TARGETED PEDIATRIC BRAIN TUMOR THERAPY

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INTRODUCTION: More than 4,200 new cases of pediatric brain tumors are diagnosed every year in the US. Brain tumors are genetically heterogeneous and are often difficult to treat with a single therapeutic agent. Hence, combination therapies targeting multiple tumor-associated antigens are being widely investigated. D2C7, a murine monoclonal antibody (mAb), recognizes both the EGFRwt and the tumor-specific EGFRVIII receptors, two antigens that are highly expressed in glioblastoma multiforme (GBM). The tumor antigen chondroitin sulfate proteoglycan (CSPG) is overexpressed in gliomas and is recognized by the mAb Mel-14. METHODOLOGY: The reactivity of D2C7 and Mel-14 antibodies were tested in cells isolated from pediatric GBM xenografts, D212MG, D456MG, D2135MG, D2322MG, H2224MG, and N225MG by flow cytometry. Results: Immunoreactivity of recombinant, trispecific single-chain antigen tandem variable region fragment (scFv), D2C7-Mel-14, was cloned from the D2C7 and Mel-14 mAbs and fused to Pseudomonas exotoxin A, carrying a C-terminal KDEL peptide (D2C7-Mel-14-PE38KDEL). In vitro cytotoxicity was measured as apoptosis. The D2C7-Mel-14-PE38KDEL with cytotoxically against CSPG-expressing H2224MG cells, EGFRwt-overexpressing 43 GBM xenograft cells, and an EGFRVIII-transfected N66M cell line. RESULTS: FACS analysis revealed Mel-14 and D2C7 mAb reactivity to be 27–99% and 12–99%, respectively, in the pediatric GBM cell line D2135MG. The D2C7-Mel-14-PE38KDEL was cytotoxic against CSPG-expressing H2224MG cells, with an IC50 in the range of 6.8 ng/mL, which was 12-fold lower than that of the monospecific IT, Mel-14-PE38KDEL (IC50 85 ng/mL). The IC50 of D2C7-Mel-14-PE38KDEL was 4-fold higher than that of the monospecific IT dsiD2C7- PE38KDEL (IC50 4.1 ng/mL) and EGFRwt-transfected N66M cell line. Again, the cytotoxicity of D2C7-Mel-14-PE38KDEL was 4-fold higher than that of dsiD2C7-PE38KDEL against EGFRwt-overexpressing 43 GBM xenograft cells (IC50 9.0 vs 2.2 ng/mL). CONCLUSIONS: The D2C7-Mel-14-PE38KDEL is a novel, trispecific IT exhibiting significant cytotoxicity against all three brain tumor antigens, EGFRwt, EGFRVIII and CSPG. We are in the process of testing the in vitro and in vivo efficacy of the D2C7-Mel-14-PE38KDEL against EGFRwt, EGFRVIII, and CSPG-expressing pediatric brain tumor xenograft models.

0032. MUTANT HISTONE H3.3 INDUCES CHANGES IN KEY EPIGENETIC MODIFICATIONS IN PEDIATRIC GLIOBLASTOMA

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INTRODUCTION: Chromatin structure is tightly regulated by a diverse set of mechanisms including covalent modification of histone tails and incorporation of non-canonical histone variants. Deregulated epigenetic modifications and abnormal chromatin configuration have been associated with tumor-specific and nonspecific development. Besides numerous mutations targeting the enzymatic machinery of histone modifications in multiple cancer types, it has been shown that pediatric glioblastomas (pedGBMs) harbor mutations within the histone variant H3.3. These recurrent somatic mutations lead to the substitution of amino acids at lysine 27 (K27M) or glycine 34 (G34R/V) of histone H3.3 - at or near sites of key epigenetic modifications. METHOD: In order to identify the complex pattern of alterations to posttranslational histone modifications (PTMs) in primary H3.3-mutated pedGBM samples, we performed immunohistochemistry on our tissue microarray containing 128 tumor cores. In addition, histone extracts of mutant H3.3-overexpressing cell lines were analyzed in more detail by mass spectrometry. Subsequent validation of single histone PTMs was performed both in cells and primary tumors by western blot analysis. RESULTS: In addition to previously identified alterations in gene expression and DNA methylation, we now show that pedGBMs expressing mutant H3.3 display distinct patterns of histone modifications. We demonstrate that the expression of both H3.3 mutants (K27M and G34R/V) induces complex changes in the crosstalk of PTMs. A very striking dominant-negative effect is observed in pedGBMs expressing the K27M mutant, with tumors displaying global downregulation of the key epigenetic mark H3K27me3. Moreover, several PTMs were found to be deregulated in the tail of G34R-mutant H3.3. CONCLUSIONS: Our findings provide novel insights into the complex alterations of the epigenetic code induced by mutant histone H3.3 in pedGBM. We demonstrate global K27M-induced downregulation of the key histone mark H3K27me3, which might result in aberrant gene expression due to altered chromatin structure. In line with this, our results also provide evidence that arginine 34 at the tail of G34R-mutated H3.3 interferes with critical histone marks, leading to aberrant genome output. Taken together, our data provides evidence that at least some of the genome-wide (epi)genetic and transcriptional alterations found in H3.3-mutant pedGBMs are likely explained by dysregulated histone modifications.

0033. DEVELOPMENT OF A NOVEL SMALL MOLECULE INHIBITOR OF SMOOTHENED FOR MEDULLOBLASTOMA TREATMENT

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INTRODUCTION: Hedgehog signaling pathway plays a key role for embryo development and adult homeostasis. However, it also plays a deleterious role in tumorigenesis. Mutations in the HEDGEHOG (HH) gene family, including the hedgehog signal transducer GLI2 and GLI3, are consistently observed in many cancers, including medulloblastoma (MB). A recent study identified the small molecule SM04631 (SMA010) as a GLI2/3 inhibitor and showed anticancer activity in preclinical models of MB and other cancers, via inhibition of downstream GLI transcriptional activities. While SMA010 was shown to induce apoptosis and inhibit glioma cell growth both in vitro and in vivo, the mechanism of action is not well understood, and the clinical utility of this agent needs to be validated.

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INTRODUCTION: Brain tumors are one of the deadly forms of human malignancies with a high degree of morbidity and mortality. Pediatric brain tumors are also the most common form of solid tumor in children accounting for about 20-25% of all pediatric cancers. Chemotherapy options for brain tumor treatment are very much limited because of the blood brain barrier and emergence of drug resistance in brain tumor cells. Combining nutraceuticals or botanical drugs with cancer drugs is one of the ways to improve the efficiency of chemotherapy and quality of life in an integrative oncology setting.

METHOD: Cytotoxicity of anticancer drugs [Etoposide-ETP, Temozolomide-TMZ] and supercritical-ethanolic (SE) extracts of Curcuma xanthorrhiza/GFAP-Cre mouse model. RESULTS: We identified a potent Smo inhibitor ZM that directly competed with cyclopamine for binding Smo receptors. As a consequence, the Gli-reporter activity and Gli mRNA expression in PZp53 medulloblastoma cells as well as Hedgehog-dependent proliferation of granule cell precursor cells were significantly inhibited by ZM compound treatment. Interestingly, ZM-mediated inhibitory effect on hedgehog signaling was not affected by the presence of a-acid glycoprotein (AAG), whereas AAG was reported to reduce the efficacy of GDC-0049 in inhibiting Smo activity in vivo. More importantly, oral dosing of ZM led to prolonged survival of PChlon/Cre-GFAP-Cre medulloblastoma mice. CONCLUSIONS: Taken together, our study identified ZM compound as a potent and effective Smo antagonist that can be further developed as a drug candidate for medulloblastoma treatment.

INTRODUCTION: Measles virus (MV) is effective at treating medulloblastoma in murine xenograft models of intracerebral and CSF-disseminated tumor. Treatment is not always, but not always, curative. To increase the efficacy of the virus, we have manufactured two MV that contain either a transgene for human endostatin-angiostatin (hEA) or murine endostatin-angiostatin (mEA). We showed that biologically active EA is secreted by cells infected with either virus. We have tested the oncolytic activity of each virus separately and in combination in our intracerebral xenograft model. We find that the combination of MV-hEA and MV-mEA is more efficacious at prolonging survival compared to either virus alone. METHOD: The biologically active EA had significantly better cytotoxic efficacy when administered between the viral H and L genes. Production of EA by infected cells was measured by ELISA. Supernatents from infected cells were assayed for EA activity by assessing their effect on HUVEC cells treated with VEGF. Intracerebral tumors were established by stereotactic injection of 106 D283luc cells. Tumors were treated with one intratumoral injection of 2×106 TCID50 of virus 10 days later. Tumor progression was measured by MR-based dynamic contrast enhancement (DCE), changes in tumor gene expression were investigated by dot blot, and survival benefit from treatment was determined by the Kaplan-Meyer method. RESULTS: MV-hEA and MV-mEA were effective at killing medulloblastoma cells in vitro. EA secreted by infected cells inhibited proliferation, migration, and tubule formation of VEGF treated HUVEC cells. Unmodified MV treated tumor-bearing animals survived longer than untreated animals, but the addition of hEA or mEA to the virus did not further increase survival. Interestingly, combination treatment with both MV-hEA and MV-mEA (1×10⁶ of each) resulted in the longest survival (p = 0.001). Changes in tumordiagnostic serum and tumor gene expression was investigated. Administration of MV-hEA alters expression of angiogenesis-related genes. Paradoxically, little improvement in survival was seen when animals were treated with MV-hEA or MV-mEA compared to unmodified virus. Surprisingly, treatment with the combination of MV-hEA and MV-mEA resulted in better survival than that seen with either EA virus alone. This result suggests that the endothelium in this xenograft model may be derived both from the tumor and from the host.

INTRODUCTION: Radiation therapy (RT) is an integral component of the treatment for medulloblastoma (MB) and the only effective adjuvant therapy for ependymoma (EP). Survival is frequently accompanied by one or more radiation-induced adverse developmental and psychosocial sequelae, as MB and EP most frequently occur in children less than 10 years old. These considerations emphasize the need to develop new strategies to enhance the tumoricidal action of RT while sparing adjacent normal tissue. The multifunctional DNA repair protein Ape1 interacts with RT. Our goal is to suppress Ape1 activity through nanoparticle mediated anti-Ape1 siRNA delivery. METHODS: Iron oxide nanoparticle (NPs) were coated with various combinations of polymers including chitosan, PEG, and PEI, and tested for DNA binding and protection using size, zeta potential, and electrophoresis analyses. Delivery of green fluorescent protein (GFP) encoding plasmid DNA was tested in vitro and in vivo. RESULTS: An NP containing a siRNA encoding anti-GFP was delivered in vitro and in vivo to GFP expressing C6 cells. Chlorotoxin targeted NP delivery to the tumors was verified through magnetic resonance imaging (MRI). GFP fluorescence was monitored to assess successful DNA or siRNA delivery using flow cytometry and fluorescence imaging. RESULTS: NPs coated with a copolymer comprising chitosan, PEG, and PEI (namely, NC-P-PEI) provided the best combination of high transfection efficiency and low toxicity. NC-P-PEI provided similar transfection efficiencies as commercially available agents in vitro and were able to transfect brain tumor cells in vivo. Chlorotoxin targeted NPs were able to transfect a higher number of brain tumor cells throughout the tumor in vivo even though the degree of tumor uptake was not affected by targeting as determined by MRI. NPs loaded with siRNA were able to knockdown transgene expression in vivo and in vivo in orthotopic brain tumors. CONCLUSIONS: NC-P-PEI were shown to be effective at delivering DNA and siRNA both in vitro and in vivo indicating the NPs are able to bypass physiological and cellular barriers to delivery functional DNA and siRNA. Notably, the addition of the targeting agent chlorotoxin to the NPs did not increase the binding to the tumor, but did increase the distribution of the NP throughout the tumor to transfect a larger proportion of cells. Therefore, these NPs should be effective at delivering anti-Ape1 siRNA specifically to pediatric brain tumors to increase sensitivity to RT.
INTRODUCTION: Myeloid/lymphoid or mixed-lineage leukemia (MLL) family genes encode histone lysine methyltransferases that play important roles in epigenetic regulation of gene transcription. MLL genes are frequently mutated in human cancers. In particular, the MLL2 pathway genes, including MLL2, MLL3, and Utx, have been found to have frequent driver mutations in pediatric medulloblastoma. Unlike MLL1, MLL2 (also known as ALR/MLL4) and its homologue MLL3 are not well understood. Specifically, little is known regarding the extent of global MLL2 involvement in the regulation of gene expression and the mechanism underlying its alterations in driving tumorigenesis.

METHOD: Here we used an innovative somatic gene editing-based assay and profiled the global loci targeted by MLL2, and performed a comprehensive molecular characterization of a targeted therapy for therapy-resistant iGCTs. This finding may lead to the development of a novel physical interaction between C-Myc and Bcl11b.

RESULTS: We identified direct transcriptional target genes and revealed the connection of MLL2 to multiple cellular signaling pathways, including the p53 pathway, c-Myc-mediated oncogenesis, and cholesterol signaling. In particular, we demonstrated that MLL2 participates in retinoic acid receptor signaling by promoting retinoic acid-responsive gene transcription. CONCLUSIONS: Our results present the first genome-wide integrative analysis of the MLL2 complex and suggest potential mechanisms underlying the tumorigenesis driven by MLL2 alterations.

INTRODUCTION: Medulloblastoma are a heterogeneous group of highly malignant brain tumors that most often affect children. Outcomes among children vary widely and C-MYC amplification is the single most important molecular indicator of poor prognosis. Here we analyzed gene expression in high C-MYC expressing tumors and compared gene expression to low C-MYC expressing controls to identify new therapeutic targets for high C-MYC expressing medulloblastoma. METHODOLOGY: We analyzed whole genome mRNA expression in 5 primary tumor samples with high C-MYC expression and an in vitro model system compared to that in 14 low C-MYC expressing tumors. We performed a detailed histologic study to identify the leukemia associated zinc-finger protein Bcl11b as upregulated in high C-MYC expressing anaplastic tumors. This observation was externally verified in a dataset of over 103 primary medulloblastoma tumors, as well as in a genomewide study to examine the impact of BCL11B on cell growth, proliferation and tumor self renewal in vitro. Finally, we utilized immunoprecipitation to identify physical interactions between Bcl11b and C-Myc. RESULTS: Here we show that BCL11B is overexpressed in high C-MYC expressing medulloblastoma tumors (p < 0.037, n = 7). We also demonstrate a novel physical interaction between C-Myc and Bcl11b. CONCLUSIONS: The negative prognostic value of C-Myc amplification in medulloblastoma has been well established clinically. These results indicate that the leukemia associated zinc finger protein Bcl11b may be an important player in C-Myc driven oncogenesis. Moreover these results demonstrate a physical interaction between C-Myc and Bcl11b that, to our knowledge, has not been previously described. The specific mechanisms of this interaction remain to be elucidated; however, it seems clear that BCL11B expression is necessary for elevated C-Myc protein levels. Thus, BCL11B or its downstream effectors may represent novel therapeutic targets in high C-MYC expressing medulloblastoma.

INTRODUCTION: In Japan, intracranial germ cell tumors (iGCTs) are the second most common central nervous system (CNS) tumors in patients under the age of 14. The majority of germnomas respond well to combined chemo- and radiotherapy, however some show resistance to therapy and may have a poor clinical outcome. Despite their clinical significance, the biology of iGCTs is mostly unknown. The aim of the study is to elucidate molecular pathogenesis of iGCTs through a comprehensive genomic analysis.

METHOD: In order to achieve the molecular classification of iGCT, we first performed whole exome sequencing (WES) and array-comparative genomic hybridization (aCGH) in a selected set of tumours. Statistically significantly mutated genes identified by WES are validated in an independent tumour cohort using the IonTorrent PGM system. RESULTS: A total of 31 centres have so far joined the iGCT Consortium, through which 89 iGCTs (38 germnomas and 51 non-germnomas) have been collected. Of these, 33 tumours were subjected to WES and 56 to aCGH. On average, 29 non-synonymous somatic mutations were observed in each tumour. Mutually exclusive mutations of c-kit and RAS, or amplification of c-kit, were the most common abnormalities, predominantly found in germnomas. In the tumours that do not have c-kit mutations, MTOR, NF1 and EGFR were among the genes mutated. CONCLUSIONS: iGCTs are heterogeneous tumors driven by alterations in several the components of the retinoic acid and MAPK signal transduction pathways play a critical role in the pathogenesis of iGCTs, particularly in germnomas. This finding may lead to the development of a targeted therapy for therapy-resistant iGCTs.
INTRODUCTION: Smoothened (SMO)-inhibitors have recently entered clinical trials for SHH-driven medulloblastoma (SHH-MB). Early evidence suggests that even within this molecularly defined patient subgroup response to therapy is highly variable. To better understand the mechanism(s) of primary resistance to conventional SMO-inhibitors and identify other pathways coopting aberrant SHH signaling, we conducted a comprehensive genome-wide replication study of 125 SHH medulloblastomas obtained from patients between 0 and 49 years of age. METHOD: Tumor and blood DNA of 57 SHH-MBs (31 pediatric [16–16 years of age] and 26 adult [≥16]) were subjected to whole-genome or whole-exome sequencing. Two independent non-overlapping replication cohorts (41 pediatric and 27 adult) were sequenced to whole-genome or whole-exome sequencing. Two independent non-overlapping replication cohorts (41 pediatric and 27 adult) were sequenced for at least ~400 prioritized candidate genes. Gene expression (n = 112), DNA copy number (n = 266), and DNA methylation (n = 53) data complemented the genomic profiles. RESULTS: Three age-related subgroups exhibiting highly discriminate genomic profiles were identified. Mutations in the SHH-pathway involved generation of 125 SHH medulloblastomas obtained from patients between 0 and 49 years of age. METHOD: Tumor and blood DNA of 57 SHH-MBs (31 pediatric [16–16 years of age] and 26 adult [≥16]) were subjected to whole-genome or whole-exome sequencing. Two independent non-overlapping replication cohorts (41 pediatric and 27 adult) were sequenced for at least ~400 prioritized candidate genes. Gene expression (n = 112), DNA copy number (n = 266), and DNA methylation (n = 53) data complemented the genomic profiles. RESULTS: Three age-related subgroups exhibiting highly discriminate genomic profiles were identified. Mutations in the SHH-pathway involved
commonly activated pathways in cancer with a number of drugs targeting the pathway in current clinical trials. PI3Ks transduce signals from growth factors and cytokines resulting in the phosphorylation and activation of AKT which in turn induces changes in cell growth, proliferation and apoptosis. METHOD: PI3K pathway status was analyzed in ependymoma using gene expression data and immunohistochemical analysis of phosphorylated AKT (p-AKT). The effect of the PI3K pathway on cell proliferation was investigated by immunohistochemical analysis of cyclin D1 and Ki67 in vitro functional analysis. To identify a potential mechanism of PI3K pathway activation, PTEN protein expression and the mutation status of PIK3CA was investigated. RESULTS: Genes in the pathway displayed significantly higher expression in supratentorial compared to posterior fossa and spinal ependymomas. p-AKT protein expression, indicating pathway activation, was seen in 72% of tumors (n = 169) and p-AKT expression was found to be an independent marker of a poorer progression free survival. A significant association between PI3K pathway activation and cell proliferation was identified suggesting pathway activation was influencing this process. CONCLUSIONS: p-AKT protein loss was not associated with p-AKT with ablation of tumor suppressor, neurofibromin (Nf1). The Nf1-/astrocytes were further challenged by losing p53 function through overexpressing a dominant-negative p53 (DNp53) molecule. These Nf1-/;DNp53 astrocytes were targeted against the PI3K and insulin growth factor (IGF) in vitro, or implanted into immunodeficient mice. In addition, Illumina microarray assays were performed to compare the gene expression profiles in male and female astrocytes in each step of the malignant transformation. RESULTS: EGFR treatment results in in vitro transformation of Nf1-/;DNp53 astrocytes but only in male cells, and this result was further supported by limiting dilution analyses. Consistent with the in vitro data, subcutaneous implants of Nf1-/;DNp53 cells into nude mice led to a sexually dimorphic tumor formation, increased cell death, inhibited proliferation, and induced mitochondrial depolarization. Additional new studies on the mechanism of the tumor-suppressor-like function in medulloblastomas, glioblastomas and ATRTs.

**0045. CELL-INTRINSIC SEX DIFFERENCES UNDERLIE THE MALE PREVALENCE IN BRAIN TUMOR RATES**

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INTRODUCTION: Clinical studies have shown that brain cancers including glioblastoma multiforme (GBM) occur more frequently in males than in females worldwide, while the reason for this sexual disparity is poorly understood. More interestingly, the sex difference in the brain tumor rates also exists in pediatric population, in which the brain tumor incidence peaks at about 4 years of age, suggesting that besides the effects of sex hormones, cell-intrinsic sex differences may also play a role in determining the sexual dimorphism in the occurrence of brain tumors. The goal of the current study is to examine the potential role of sex in glomagenses. METHOD: To model the impact of sex on the occurrence of glioblastoma, we established a step-wise transformation of primary astrocytes and glioblastoma cell lines. RESULTS: Analysis of genome-wide gene expression and mutation status of PIK3CA was investigated. RESULTS: When compared to the mouse model of glomages, there is a higher percentage of ependymoma patients. In medulloblastomas higher PID1 mRNA correlated with longer overall survival (OS) and longer radiation-free PFS. Higher PID1 mRNA also correlated with longer OS in gliomas. Ectopic expression of PID1 decreased colony formation, increased cell death, inhibited proliferation, and induced mitochondrial depolarization. Additional new studies on the mechanism of the biology of the brain-tumor inhibitory action of PID1 will be presented. CONCLUSIONS: These data are the first to link PID1 to cancer in general and to pediatric and adult brain tumors specifically, and suggest that PID1 has a tumor-inhibitory/tumor-suppressor-like function in medulloblastomas, glioblastomas and ATRTs.

**0046. PID1 (NYGGF4), A NEW TUMOR SUPPRESSOR-LIKE GENE IN PEDIATRIC AND ADULT BRAIN TUMORS**

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INTRODUCTION: Medulloblastomas are the most common malignant brain tumors in children, glioblastomas are the most common malignant primary brain tumors in adults, and atypical teratoid rhabdoid tumors (ATRT) are a highly malignant brain tumor in children. PID1 (Phosphotyrosine Interaction Domain containing 1; NYGGF4) is a recently-identified gene that acts as a modulator of insulin signaling in adipocytes and muscle cells, and to date, has only been reported in the context of obesity, diabetes and Alzheimer’s Disease. Expression and roles of PID1 in cancer and in brain tumors are still unknown, and are a central topic in our laboratory. METHOD: PID1 mRNA expression in medulloblastoma tumors was analyzed using microarray analysis of RT-PCR in the CHLA cohort and from expression microarrays of three published and unpublished independent medulloblastoma datasets (total n = 821). PID1 mRNA level in gliomas was obtained through analysis of online data from REMBRANDT (344 cases) and TCGA (424 cases). RESULTS: PID1 was the most differentially expressed gene in our medulloblastoma dataset. Expression of PID1 was found to be associated with longer overall survival and with metastatic disease at diagnosis. Furthermore, in glioblastomas, PID1 expression was found to be associated with longer overall survival. CONCLUSIONS: PID1 is a novel tumor suppressor gene that may play a role in determining the sexual dimorphism in the occurrence of brain tumors. The PID1 mRNA level in gliomas was obtained through analysis of online data from REMBRANDT (344 cases) and TCGA (424 cases). RESULTS: PID1 was the most differentially expressed gene in our medulloblastoma dataset. Expression of PID1 was found to be associated with longer overall survival and with metastatic disease at diagnosis. Furthermore, in glioblastomas, PID1 expression was found to be associated with longer overall survival. CONCLUSIONS: PID1 is a novel tumor suppressor gene that may play a role in determining the sexual dimorphism in the occurrence of brain tumors.
INTRODUCTION: The epithelioid and rhabdoid variants of glioblastoma are rare. Although similar in standard histological preparations, they are distinguished by their immunophenotypes. In particular, the rhabdoid glioblastoma (r-GB) contains foci of cells that are immunopositive for multiple diverse proteins and immunonegative for INI1, with the additional phenotype of an atypical teratoid/rhabdoid tumor (AT/RT). In contrast, cells with an epithelioid or rhabdoid morphology in the epithelioid glioblastoma (e-GB) express INI1 and lack the diverse immunophenotype of the r-GB. Here, we report a series of seven pediatric e-GBs describing their clinical, pathological, and genetic characteristics.

METHOD: We retrospectively reviewed the clinical characteristics of all patients with epithelioid glioblastoma seen at our institution between 1999 and 2017. Central pathologic and radiologic review of all cases was performed. Molecular genetic and cytogenetic analyses were undertaken, including evaluation of INI1 status and sequencing of mutational hotspots in H3F3A, HIST1H3B, BRAF, IDH1, and IDH2. RESULTS: Seven patients were identified. Median age at diagnosis was 10.2 years. Tumor sites were diencephalon (n = 4) and cerebral cortex (n = 3). Three patients presented with acute, symptomatic intra-tumoral and intra-ventricular hemorrhage. Three patients had leptomeningeal tumor spread at diagnosis. Despite the use of multi-modality therapy, all patients experienced rapid disease progression. First tumor progression for five patients was metastatic, either in leptomeninges (n = 3) or at extra-axial sites (n = 2). All patients eventually experienced distant tumor progression. Diffuse extra-axial spread was documented at autopsy in two additional cases. Median survival was 5 months (range, 1.4 to 9.7 months). Pathologically, tumors had a dominant epithelioid morphology, and their cells expressed INI1, but not the diverse range of proteins characteristic of AT/RT. One thalamic tumor had an H3F3A:p.K27M mutation. Four tumors (57%; 2 thalamic and 2 cortical) harbored a BRAF V600E mutation. Two tumors showed homonymous loss of CDKN2A, and one tumor harbored EGFR amplification. CONCLUSIONS: We report the first pediatric series of the rare e-GB, which is a particularly aggressive variant with a high frequency of BRAF:p.V600E mutation.

0049. MEMBRANE DEPOLARIZATION IS A MEDULLOBLASTOMA TUMOR SUPPRESSOR
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INTRODUCTION: Membrane depolarization is a particularly aggressive and neuronal membrane homeostasis. We performed pharmacological and optogenetic modulation of membrane potential to functionally assess its role in medulloblastoma. RESULTS: We identified neuronal development as a frequent target of gene silencing. Genes involved in synaptic biology, ion channels and neurotransmitters are also frequently and locally deleted in medulloblastomas. Sonic Hedgehog medulloblastomas arise from cerebellar progenitor cells that give rise to mature glutamatergic neurons. We treated medulloblastoma cultures with glutamate agonists finding a decrease in cellular proliferation and increased differentiation. Using a new optogenetic mouse model of medulloblastoma, we depolarized MB cells using a blue laser. Our optogenetic results clearly demonstrate that membrane depolarization per se is tumor suppressive as depolarized cells are unable to transplant the disease in immunocompromised mice. CONCLUSION: Membrane depolarization is targeted by recurrent, highly convergent somatic genetic and epigenetic events in medulloblastoma. Our optogenetic approach provides high level evidence that medulloblastoma cells evade differentiation in response to membrane depolarization through selection of clones with somatic genetic or epigenetic events affecting genes involved in synaptic biology. This selection for cells impaired in synaptic function attenuates membrane depolarization in response to local neurotransmitters, maintains an undifferentiated state, and contributes to tumor malignancy; therefore membrane depolarization in medulloblastoma is a non-genic tumor suppressor and represents an attractive therapeutic avenue for the treatment of children with malignant brain tumors.

0050. SONIC HEDGEHOG ACTIVATION MODULATES CXCR7 EXPRESSION AND PROMOTES CXCR4 SIGNALING IN MEDULLOBLASTOMA
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INTRODUCTION: Stratification of medulloblastoma into molecular subtypes elevates hopes that subtype-specific therapy can be more efficacious and less toxic. To realize this goal, the molecular targets that distinguish each subtype (more efficacious and best discriminate between benign and tumor cells; less toxic) must be fully defined. We published that Sonic hedgehog (Shh) subtype medulloblastoma can be further divided by CXCR4 expression into clinically important subgroups and that CXCR4 inhibition has potent anti-tumor effects. Here we present data that Shh regulates CXCR4 activity through CXCL12, whose expression is downregulated in medulloblastomas. Cells and medulloblastoma cells make its function an ideal therapeutic target. METHOD: Cells were isolated from P6 mouse cerebellum or from primary mouse cerebellar tumors (SmoA1). Single-cell suspensions were plated on poly-D-lysine coated dishes with or without: 1) Shh and 2) cyclopamine, followed by treatment with CXCL12. SmoA1 brain tumors were dissociated into single cells and transplanted into nude recipient mice, and after growth to 100mm3 were treated with vehicle, AMD3100, GDC-0449, or both. Tumors were measured by digital caliper and harvested when one dimension reached 20mm or at the conclusion of treatment. RESULTS: Using primary cultures of Shh-driven medulloblastoma we found that Shh activation enhances CXCR4 surface localization and promotes CXCL12-induced G Prote in signaling. This was similar to effects of Shh on cerebellar granule precursor cells, the normal developmental counterpart of Shh-medulloblastoma. We evaluated expression of CXCR4 internalization regulators and found that Shh activation altered the abundance of a subset of GRKs, arrestins and CXCR7. Among these, CXCR7 expression exhibited the greatest differential response to Shh pathway activation between normal granule cells and primary medulloblastoma samples: CXCR7 expression was highly expressed in normal cells despite Shh effects, but almost completely absent in tumor cells. CONCLUSIONS: We conclude that activation of the Shh pathway in medulloblastoma promotes CXCR4 surface localization through inhibition of internalization mediators, which promotes prolonged CXCR4 signaling. This cross-talk is also evident in developing GPCs but with one notable difference: CXCR7 expression is high in young, but decreases in aged neuronal and glioblastoma populations. This distinction suggests that CXCR7 antagonists, which are moving towards clinical trials in a number of cancers, may be detrimental to normal cerebellar development and inactive against medulloblastoma. We propose that restoring CXCR7 activity would have negligible effects on granule cells and potentially block CXCR4 function in medulloblastoma.
INTRODUCTION: Medulloblastoma (MB) is the most common paediatric malignant brain tumor. By the way of optimal surgery, radiation, and chemotherapy, medulloblastoma can be treated in a good fraction of children but despite the best therapy the disease recurs in 40% of the cases. Many factors composing the tumor niche can also influence radiosensitivity. For this reason we developed a protocol to study the genetic differences between primary and recurrent (radioreistant) tumors in vivo, using our transposon mutagenesis driven mouse model. METHOD: We developed a novel murine model of metastatic MB, which is highly penetrant, has a short latency, and involves random secondary genetic events. The model is based on mobilizing the Sleeping Beauty transposon in the cerebella of Pichh/+/ mice. We used this model to identify the genes important for recurrence of MB in vivo. We performed surgical removal of the murine tumors, and then treated the mice by multi-fraction CT-guided cranial irradiation. By the way of next generation sequencing followed by gCIS prediction or convergence analysis we identified driver genes in the primary tumors as compared to the recurrences. RESULTS: 70% of the mice treated with surgery and CSI recurred locally, a smaller fraction (30%) recurred distally with recurrent disease on the spinal cord. Recurrences are highly genetic divergent from their matched primary tumors. We sequenced the first set of recurrent murine tumors, primary, local and distal recurrences identifying several potential synthetic lethal genes in the primary tumors, and relapse drivers, which are only found mutated in the recurrences. We selected actionable targets and performed in vitro radiosensitization assays with small molecules inhibitors of the predicted driver genes, showing a reversal of radiation resistance. CONCLUSIONS: Recurrent medulloblastoma can be modelled in mice by the way of surgery followed by CSI irradiation. Murine primary MBs are highly genetically different from the recurrences, urging the scientific community to develop different therapeutic approaches to efficiently target primary and recurrent human tumors. As our mouse model shows the same rate and pattern of recurrence observed in human patients is an extremely valuable translational platform to design new strategy against recurrent MB. Highly targetable events in genes known to play a role in cell-cycle, apoptosis and proliferation, like Trp53 and ALK, are potential drivers of local and distal MB recurrence.

LONGITUDINAL ASSESSMENT OF CIRCULATING microRNAs As Biomarkers In Pediatric Central Nervous System Tumors

INTRODUCTION: Altered expression of miRNAs contributes to the development of cancer, including pediatric central nervous system (CNS) tumors. MicroRNAs have tissue specific expression patterns and are stable molecules making them ideal cancer biomarker candidates. Circulating miRNAs have been previously identified in plasma and cerebrospinal fluid (CSF) of pediatric patients with CNS tumors. We determined miRNA expression levels in corresponding tumor, plasma, urine and CSF of newly diagnosed patients with CNS tumors at the time of diagnosis and at routine intervals during their treatment. METHOD: Tumor tissue and body fluids were prospectively collected at specified time points. RNA purification from cell-free body fluids was performed with the mirVana miRNA Isolation Kit. The purified total RNA was then used for cDNA synthesis. TaqMan™ miRNA assays were used to quantify the levels of 76 mature miRNAs from each sample using the Applied Biosystems 7900HT Fast Real-Time PCR system in 384-well low density arrays (TLDAs). Cycle threshold (Ct) values under 33 were considered positive for the presence of target miRNAs. ExpressionSuite® software was used for data analysis. RESULTS: To date, 25 patients are enrolled on the study with longitudinal specimens available for 14 patients. Profiling on 16 patients has been completed. In all cases, a number of miRNAs are present in the tumor and body fluids at the time of diagnosis. For patients with longitudinal samples, several candidate biomarker miRNAs have been identified in patient samples at diagnosis which are no longer detectable in subsequent specimens following initial treatment. For example, in patients with embryonal tumors, miR-30a-5p and miR-661 are candidate biomarkers in the CSF. Profiling of the remaining samples is ongoing and will be presented. CONCLUSIONS: Our results establish that circulating miRNAs in plasma, CSF and urine are candidate biomarkers in pediatric CNS tumors. Individual miRNAs of interest vary based upon the histotypic subtype of the tumor and expression levels decrease after surgery and/or other tumor directed therapy. Ongoing studies include continued surveillance of body fluids of patients enrolled on this study and comparison of miRNA expression of affected patients to those without CNS tumors. We hypothesize that circulating miRNAs may represent a novel diagnostic tool for patients with CNS tumors and could be a valuable biomarker for therapy and early indication of relapse.

LABEL FREE PROTEOME PROFILING OF CEREBROSPINAL FLUID IDENTIFIES PROFILIN-1 AS A PUTATIVE BIOMARKER OF MEDULLOBLASTOMA WITH A PRO-MIGRATORY FUNCTION

INTRODUCTION: A major challenge to the treatment of medulloblastoma is the toxicity of cranio-spinal radiation to a child’s developing brain. Current efforts to risk stratify medulloblastoma therapy to minimize craniospinal radiation and its sequelae would greatly benefit from the ability to assess disease status using serially accessible biomarkers. Unlike brain tumor tissue, cerebrospinal fluid (CSF) represents a reservoir of brain-associated proteins that can be serially sampled. We have identified profilin-1 as a candidate protein biomarker with an important functional role in medulloblastoma biology. METHOD: We used mass spectrometry based label-free protein quantitation (MS-LFPQ) to compare the CSF proteome of 12 children newly diagnosed with medulloblastoma to 12 tumor-free, age-matched controls. Cell lysates and conditioned media from medulloblastoma cell lines were also analyzed by MS-LFPQ. Pathway analysis revealed a significant set of proteins involved in cell motility, including profilin-1, an actin-binding protein implicated in the migration of breast cancer and bladder cancer cells. Profilin-1 expression was validated by ELISA and immunohistochemistry. Migration of medulloblastoma cell lines DAOY and D556 was assessed by transwell migration and wound healing assays following siRNA knockdown of profilin-1 gene expression. RESULTS: We identified 39 proteins that were differentially abundant in CSF from children newly diagnosed with medulloblastoma compared to controls, 10 fold, p < 0.03, 13 of which were significantly upregulated. Profilin-1 was 11.7 fold elevated in tumor samples (p < 0.03) and detected in 7 out of 12 medulloblastoma samples. We increased profilin-1 in medulloblastoma-associated CSF using ELISA. We also found increased profilin-1 in tumor supernatants of medulloblastoma cell lines and present in 59% (15/22) of medulloblastoma cases by immunohistochemistry. In vitro migration assays demonstrated significantly decreased migration in medulloblastoma cell lines following siRNA knockdown of profilin-1. CONCLUSIONS: Our proteomic investigation of CSF from children with medulloblastoma has identified profilin-1 as a promising putative biomarker for medulloblastoma that is detectable in CSF and robustly expressed by tumor tissue and cell lines. We also demonstrate that profilin-1 is involved in the migration of medulloblastoma cells; an important role in tumor biology related to tumor progression and metastasis. Biomarkers with the ability to reflect the biologic characteristics of individual tumors would offer an additional capability to not only assess the presence of disease but provide real-time evidence of in situ tumor biology.

STUDY OF HISTONE METHYL TRANSFERASE G9A INHIBITION IN ATRT AND MEDULLOBLASTOMA

INTRODUCTION: Medulloblastoma and atypical teratoid rhabdoid tumor (ATRT) are malignant pediatric brain tumors. The survival rate for children with AT/RT who are below the age of three is around 10%, whereas that for medulloblastoma patients is approximately 75–80%. Survivors face increased risk for recurrence and current therapeutics are ineffective against
recurrent tumors. Thus, there is a dire need for novel therapies. Abrupt epigenetic silencing of gene expression has been increasingly implicated in cancer development. One such repressive epigenetic modification is histone H3 lysine (K)-9 methylation, which is catalyzed by histone methyl transferases (HMTs), called G9a and G9a-like protein (GLP). METHOD: A panel of established and patient derived human medulloblastoma cell lines ATRT cell lines were used to measure G9a expression across by q-RT-PCR analyses. Cell growth in response to drug treatment was measured by (MTT) assay. Cell cycle analysis was performed by flow cytometry. Western blot analysis was done to observe global changes in H3K9me2 following G9a inhibition. In vivo tumorigenic potential of drug treated cells was measured in mouse orthotopic models. Tumors were assessed by H&E staining and immunohistochemistry. RESULTS: We observed elevated G9a expression in ATRT and human medulloblastoma samples compared to normal cerebellum. Ablation of G9a activity by treatment with small molecule inhibitors of histone methyl transferases decreased ATRT and medulloblastoma cell growth in vitro and in vivo. HMT inhibitor-loss of cell growth inhibition in vitro was accompanied by a significant decrease in global histone H3K9 methylation. In vivo, HMT inhibitors decreased the tumorigenic potential of ATRT and medulloblastoma cell lines and surprisingly supported the formation of circumscibed and less invasive tumors. CONCLUSIONS: Treatment of ATRT and advanced stage/relapsed medulloblastoma continues to remain a challenge. This calls for the development of novel therapeutic approaches based on a better understanding of tumor biology. Here, we provide the first demonstration of aberrant expression of G9a in human medulloblastoma and ATRT. Our data show that pharmacological inhibition of G9a activity blocked tumor cell growth in vitro and in vivo, suggesting that G9a inhibition may have future therapeutic application for pediatric brain tumors.

INTRODUCTION: Medulloblastomas, the most common malignant pediatric brain tumors, are molecularly divided into 4 major groups: SHH, WNT, Group 3 and Group 4. Group 3 tumors, which overexpress and frequently amplify MYC, are the most aggressive and least curable. Our recently developed mouse models recapitulates Group 3 medulloblastoma features, including Myc in Tg(ip53-null) granule neural progenitors. Myc-induced medulloblastoma cells can be grown as neurospheres and recapitulate primary tumors after transplantation into the brain of nude recipient mice. These properties provide an ideal platform on which to conduct high throughput screens and then test the “hits” in the live model system. METHOD: A library of 830 FDA-approved drugs was screened against Myc-induced mouse medulloblastoma neurospheres (hereon Myc-neurospheres) by measuring ATP consumption. Drugs active against Myc-neurospheres (primary screen) or single point (10 μM) were moved to a secondary screen (dose response from 1 nm to 10 μM) in order to assess their selectivity. Wash out experiments were performed on 21 compounds. Drugs that efficiently inhibited Myc-neurosphere proliferation and that were selective (i.e., inactive against Tg(ip53-null) granule neural progenitors) were moved forward to pharmacokinetics and in vitro studies. RESULTS: Pemetrexed (an inhibitor of the folate pathway) and gemcitabine (an inhibitor of nucleotide synthesis), were active and selective against the Myc-neurospheres. Pharmacokinetic modeling of these compounds demonstrated they were detectable in tumor extracellular fluid at concentrations that exceeded the in vitro IC50 values for 6 hours. When administered as single agents to mice orthotopically transplanted with Myc-neurospheres, each increased the survival by 6 and 10 days, respectively. However, when administered in combination survival doubled from 13 to 30 days. Importantly, they also inhibited the proliferation of several primary neurosphere lines derived from non-SHH/non-WNT human medulloblastomas. CONCLUSIONS: Group 3 medulloblastomas grow robustly as neurospheres allowing for the generation of high throughputs of vast numbers of different compounds. Using a library of FDA-approved drugs and preclinical studies we have identified pemetrexed and gemcitabine as candidate drugs for the treatment of patients with this most aggressive form of medulloblastoma. This work is funded by the Office of the V-Foundation CA-096832 (MFR), a Core Grant CA-021765 and the American Lebanese-Syrian Associated Charities (ALSAC) of St. Jude Children’s Research Hospital.

INTRODUCTION: New targeted therapies are needed for pediatric patients with high grade gliomas, and adoptive T-cell therapy has the potential to fulfill this need. One attractive target antigen for T-cell immunotherapy is IL13Rα2, which is expressed in >50% of pediatric high grade gliomas. While two groups have reported the construction of IL13Rα2-specific antigen receptor (IL13Rα2-CARs) using IL13, at present it is unclear, which IL13 mutein is optimal for CAR generation. The goal of this project was to evaluate different IL13 mutemers for CAR generation and determine the effector function of IL13Rα2-CAR expressing T cells. We constructed four SFG retroviral vectors encoding IL13Rα2-CARs. While the IL13Rα2-CARs had identical IgG1-CH2CH3 spacers, CD28 transmembrane domains, and signaling domains derived from CD28 and the CD3-ζ chain, their extracellular binding domain contained IL13 mutemers, with increased affinity to IL13Rα2. Two had a single amino acid substitution at position 105 (K105R), CAR-expressing T cells were generated by retroviral transduction, and the phenotype, specificity, and antitumor activity of CAR T cells was determined using standard immunological assays. RESULTS: All four IL13Rα2-CARs were expressed at >80% on the cell surface of transduced T cells. IL13Rα2-CAR T cells were activated in the presence of IL13Rα1 or IL13Rα2 protein in contrast to control protein (IL4α) as judged by cytokine production. In cytotoxicity assays IL13Rα2-CAR T cells killed L13Rα1+ and/or IL13Rα2+ target cells in contrast to target cells that were negative for IL13Rα1 and IL13Rα2. While we observed no significant differences between the four constructed IL13Rα2-CARs ex vivo, only T cells expressing CARs with E13K and E13K, E13K, E13K, E13K, K105R induced expression of global gliomas in the U373 ortophot xenograft model. CONCLUSIONS: T cells expressing IL13Rα2-CARs with IL13.E13K mutemers have significant antitumor activity in vitro. However, IL13Rα2-mutemergenerated CARs all displayed low expression and will limit the use of IL13Rα2-based CAR T cells to local applications. Our results provide the rationale to develop IL13Rα2-specific CARs that use an IL13-specific single variable fragment as an antigen recognition domain for IL13Rα2 to prevent cross reactivity to IL13Rα1.

INTRODUCTION: In a subset of pediatric malignant astrocytoma (MA), activating BRAFV600E mutation leads to expression of hyper-activated BRAFV600E kinase, this frequently occurs alongside deletion of the cell cycle inhibitor Cldn2a (Cancer Res. 70:512-9; 2010). BRAFV600E-targeted therapies retard the growth of intracranial BRAFV600E MA xenografts (Clin. Cancer Res. 17:2959-64; 2011). Stem cell-like glioma cell subpopulations have been shown to exhibit differential therapy responses, and consequently contribute in distinct manners to therapy resistance (Cancer Cell. 18:669-82; 2010). The potentially divergent effects of BRAFV600E-targeted therapies on heterogeneous MA cell subpopulations have not been previously explored. METHOD: Athymic mice were orthotopically implanted with AM38, a human BRAFV600E and Cldn2a null MA cell line, modified with lentivirus-luciferase. Tumor-bearing mice were treated with BRAFV600E-specific inhibitor PLX4720, or left untreated. Whole brains were harvested and subjected to immunohisto pathological analyses; tumor cells were isolated and analyzed by flow cytometry. Stem (CD133) and progenitor (NG2) cell markers, co-labeling with BrDU, were used to identify and quantify proliferation rates of asymmetric divisions of distinct cancer stem-like cell subpopulations. Tumor tissue and AM38 cells at drug-responsive and un-responsive stages were comparatively analyzed. RESULTS: AM38 cells injected into athymic mouse brain produce astrocytoma-like tumors, with animal induction (My succumbing to tumor burden. Treatment with PLX4720 retarded tumor growth and extends survival. Untreated and PLX4720-treated tumors harbor distinct proportions of MA subpopulations marked by CD133+ and NG2+ expression, and having distinct proliferation capacities in situ. Rates of asymmetric divisions of CD133+ and NG2+ cell were determined. Results from ongoing work for further analysis of treatment effects on tumor stem and progenitor subpopulation composition in situ, as well as on subpopulation proliferation and asymmetric division, in vitro, will be presented at the meeting. Our studies show multiple cell subpopulations in AM38 cultures and orthotopic xenografts. PLX4720 retards AM38 orthotopic xenograft growth, and in so doing may alter tumor subcell population composition. Ongoing work will explain the impact of this BRAFV600E inhibitor effect, especially with respect to CD133.
INTRODUCTION: Schwannomatosis is characterized by the onset of multiple schwannomas, without involvement of the vestibular nerve, which is diagnostic of neurofibromatosis type 2. Genetic analysis permitted the localization of mutations in the tumor suppressor gene SMARCB1/Snf5/Ini1 in patients with familial or sporadic schwannomatosis. Specific inactivating mutations of the SMARCB1/Snf5/Ini1 gene are also the hallmark of malignant rhabdoid tumors (MRT), aggressive pediatric cancers, and also lead to a familial cancer predisposition syndrome. The mechanism by which SMARCB1/Snf5/Ini1 germline mutations predispose to schwannomatous versus MRT are still unknown. METHOD: Schwannomas are benign neoplasms of the peripheral nerve sheath and are believed to have their origin in embryonic neural crest cells. We used conditional mutagenesis to investigate the role of Smarcb1 biallelic inactivation in mouse neural crest and Schwann cell lineage. In this model, Cre recombinase expression was under the control of the protein zero (P0) gene promoter. RESULTS: P0Cre;Smarcb1flox/flox mice showed reduced viability and at 5 months of age most of the mice presented tumors arising from different cranial nerves (olfactory, trigeminal, vestibulocochlear). Analysis of the tumor spectrum showed a continuum from schwannoma to MPNST, to MRT presenting one or combinations of histological features characteristic of these different tumor types. Infiltration cells were found in 90% of tumors emerging from the fronto-nasal region. Histologically these tumors appeared malignant and poorly differentiated, without areas of low grade tumor or better differentiation. Molecular analysis of tumors showed derepression of cyclin D1 as found in mouse and human MRT. CONCLUSIONS: Altogether, our data not only establish a new model of Smarcb1-related tumorigenesis, but also provide important insight into the cell of origin of MRT.

INTRODUCTION: Diffuse intrinsic pontine gliomas (DIPGs) carry a dismal prognosis despite the use of aggressive multi-modality treatment. No significant advances in the survival of DIPG patients have been made over the last few decades, and new therapeutic approaches are desperately needed. Recent results have identified a somatic mutation in the H3F3A gene, resulting in replacement of lysine 27 by methionine in its encoded histone H3.3 protein (H3.3-K27M) that occurs primarily in DIPGs. We hypothesize that the expression of this mutant protein in DIPG provides a unique therapeutic opportunity for treating this cancer. METHOD: H3.3-K27M mutant DIPG models were established by direct sequenc- ing of two pediatric DIPGs and one pediatric GBM tumor, using DNA from cell lines established from surgical biopsies. Histone H3.3 lysine 27 (H3K27) methylation status was evaluated by western blotting with antibodies specific for mono-, di-, and trimethylated H3K27. Cell proliferation assays were performed on these cell lines, and additional glioma cell lines lacking the H3.3 mutation, in order to measure the response to pharmacological inhibition with GSK-J4, a selective inhibitor of H3K27 demethylase JMJD3. GSK-J4 inhibits JMJD3 induced H3K27 demethylation, resulting in an increase of H3K27 methylation. RESULTS: The H3.3-K27M mutation was identified in the two DIPG cell lines, but not in the pediatric GBM cells, nor in other adult glioma cells. H3.3-K27M mutant DIPG cells showed rapid growth in vitro, with doubling time of approximately 30 hours. In contrast, a pediatric GBM cell line with wild-type H3.3 grew much more slowly, with a doubling time of 72 hours. H3.3-K27M mutant DIPG cells showed elevated H3K27 methylation in comparison to H3.3 wild-type glioma cells. GSK-J4 induced a marked dose-dependent inhibition of growth in H3.3-K27M mutant DIPG cells while H3.3 wild-type glioma cells showed no response to GSK-J4. CONCLUSIONS: Our findings support altered histone H3.3 K27 methylation and dysregulation of the H3.3-K27M mutant DIPG model, which could lead to increased tumor cell growth in vitro, and is potentially associated with selective tumor cell sensitivity to GSK-J4.

INTRODUCTION: The superparamagnetic iron oxide nanoparticles (SPIONS) prepared by co-precipitation from Fe2+, Fe3+ salt solutions have the ability to function as theranostic agents. SPIONS act as powerful contrast agents for MRI due to their high magnetic moment, and both can be used as delivery vehicles for anti-cancer agents and thermotherapy. For increasing the therapeutic activity of SPIONS we conjugated the latter with heat shock protein Hsp70 that is well known for its immunomodulatory anti-tumor activity. The possibility for the targeted delivery of the conjugate was analyzed in series of in vitro and in vivo experiments. METHOD: Synthesized magnetic Hsp70 conjugates were analyzed by spectrophotometry, ELISA assays. Proton magnetic relaxation times T1 and T2 were measured with the help of the NMR-spectrometer (CXP-300, Bruker) in magnetic field 7.1 T. The in vitro binding and uptake of SPIONS and its Hsp70 conjugates was assessed on the C6 glioma cell culture by confocal and electron microscopy, flow cytometry assay. The in vivo traffic was analyzed in the model of intracranial C6 glioma. MR images (gradient echo (FLASH), T1- and T2-weighted, multi-sc and multi-echo (MSME)) of rat glioma were obtained by Bruker Avance II NMR spectrometer 11 T. RESULTS: The parameters of SPIONS measured relaxivity corresponded to properties of negative contrast agents with a hypointensive change of resonance signal in MR imaging. According to the data from in vitro studies SPIONS were incorporated into C6 cells mostly by endocytosis pathway. Intriguingly, the conjugation of protein Hsp70 to the SPIONS increased the internalization of nanoparticles as was demonstrated by confocal microscopy and flow cytometry assay. In vivo study confirmed the targeting ability of Hsp70 conjugate uptake and tumor contrast enhancement in comparison to non-coated nanoparticles. CONCLUSIONS: Magnetic nanoparticles based on the iron oxide represent a promising nanomaterial for the targeted therapy and imaging of malignant brain tumors. Covalent attachment of heat shock protein Hsp70 to the magnetic nanoparticles increased the uptake of SPIONS by C6 glioma cells and, what is more important, – provided the selectivity of tumor targeting in in vivo conditions. Thus, conjugation of SPIONS with the chaperone Hsp70 represents the attractive approach in the targeted therapy of malignant brain tumors.

INTRODUCTION: Improvement in therapy for relapsed and refractory medulloblastoma represents an urgent unmet clinical need. Most tumors with this phenotype are in Group C, driven by MYC amplification. MYC amplification leads to a state of deregulated growth and increased metabolism. It is associated with an aggressive/invasive phenotype in medulloblastoma and other human cancers. PD-L1 is an immune regulatory molecule expressed on the cell surface of some solid tumors. Our group and others have shown that PD-L1 expression correlates strongly with outcome. However the interaction of MYC with immune regulatory molecules like PD-L1 has not been previously explored. METHOD: The expression of human PD-L1 and PD-L2 was evaluated via flow cytometry in human medulloblastoma cell lines with low stem like-cell and NG2 progenitor-like cell subpopulations. Understanding why current treatment strategies only work in certain tumor cell subpopulations will allow intelligent design of chemotherapeutic strategies with anti-tumor effects that are greater than those of conventional therapies.

INTRODUCTION: In this model, Cre recombinase expression was under the gate the role of Ini1 in embryonic neural crest cells. We used conditional mutagenesis to investigate why current treatment strategies only work in certain tumor cell subpopulations will allow intelligent design of chemotherapeutic strategies with anti-tumor effects that are greater than those of conventional therapies.
MYC amplification, DAOY and UW228, and those with high MYC amplification, D283 and D425. Expression patterns of these molecules were recorded both at rest and after stimulation with 100units/ml of recombinant human interferon gamma. The same low MYC lines, DAOY and UW228, were transfected with a MYC overexpression plasmid and treated the same way. Finally DAOY was sorted using a FACS Aria into two populations based on PD-L1 expression, high versus low, and implanted into the flank of nude mice. RESULTS: Data from the first two populations that harbor medulloblastoma cell lines express the immune regulatory ligands of PD-1, PD-L1 and PD-L2, both at baseline and in response to cytokine stimulation. The pattern of ligand expression correlates strongly with MYC amplification status. Tumors with constitutive ligand expression are generally negative for MYC amplification while those with inducible ligand expression are positive, for MYC amplification. Artificially over-expressing MYC blunts the expression of both PD-L1 and PD-L2, changing the immune phenotype of the cell line. Tumors selected for high PD-L1 expression exhibited a growth disadvantage when compared to low expressers in vivo. CONCLUSIONS: These findings indicate that medulloblastoma has a unique immune phenotype corresponding with MYC expression patterns, and that MYC amplification may directly influence this tumor's ability to evade the host immune system. Expression of PD-L1 may confer a growth disadvantage, suggesting a novel role for the ligands of PD-1 in tumor growth and metabolism and lending a possible explanation for their decreased expression in the presence of MYC. Understanding the interaction between MYC and the host immune system could lead to the development of therapies for MYC driven medulloblastoma that may have broader implications for the general role of MYC amplification in cancer.

INTRODUCTION: Since the first successful use of radiation therapy (RT) for medulloblastoma in the 1950’s, RT has been integral to medulloblastoma treatment. The molecular mechanisms that promote or deter recurrence of medulloblastoma to RT remain unknown. Identifying why medulloblastomas typically respond to RT may yield critical insight into how to enhance tumor response and to predict treatment failure. METHOD: We have previously demonstrated that medulloblastomas have an inherent tendency to undergo programmed cell death that depends on the pro-apoptotic Bcl-2 family protein Bax. To test the role of Bax-dependent apoptosis in medulloblastoma response to RT, we administered 10Gy external beam radiation focused on the posterior fossa of SmoM2 mice with primary medulloblastoma with and without simultaneous deletion of Bax. We then examined the histologic response to treatment and the effect of treatment on animal survival. RESULTS: All Math-1cre;SmoM2 mice developed medulloblastoma by 12 days of age and tumors in Bax-deficient SmoM2 mice were equally aggressive. Medulloblastomas in Math-1cre;SmoM2 mice were markedly sensitive to RT. Within 4 hours of treatment, almost all tumor cells demonstrated activated caspase 3 and by 5 days after treatment tumors had largely regressed. 40% of mice were long-term survivors in both Bax-proficient and -deficient SmoM2 medulloblastomas were thoroughly radiation-resistant. Rather than undergoing apoptosis en masse, Bax-deficient tumor resolved within days into 2 distinct populations, as cells either differentiated as neurons or continued to proliferate. No SmoM2 mice with Bax deletion survived long-term. CONCLUSIONS: Our data highlight the absolute importance of intact apoptotic pathways to medulloblastoma treatment response. We show that the radiation sensitivity of medulloblastoma is not simply an effect of high proliferation rate. Importantly, we did not detect cell death through Bax-independent mechanisms such as mitotic collapse. Inability to initiate cell death programs may identify human patients who will not respond to standard therapy, while enhancing the tendency of cytotoxic therapies to trigger Bax-dependent cell death may increase therapeutic efficacy.

INTRODUCTION: Amplification of MYCN is associated with high-risk SHH-driven and group 4 medulloblastoma, while mis-expression and amplification of MYCN is associated with aggressive group 3 tumors. The biology of these tumors, particularly the group 3/4 tumors, is poorly understood, with no effective targeted therapies. Published data by others shows that translational control downstream of the mammalian target of rapamycin (mTOR) is required for MYC-driven oncogenesis. mTOR signals through two primary outputs, rp56 kinase (S6K) and the translation initiation factor eIF4E. A new class of clinical mTOR ATP-competitive inhibitors disrupts signaling through both effectors, whereas the allosteric binder rapamycin disrupts only S6K. METHODS: We have developed a model of group 4 medulloblastoma in which high levels of mutationally-stabilized MYCN protein drive oncogenesis. Transplantation of MYCN(-/-)-transduced cerebellar neuropheres into nude mice generates tumors with high penetrance. Here, we incorporate S6K, and rp56-driven mouse model of mTOR signaling with rapamycin or 4E-BP, the latter of which is involved in regulating downstream eff4E activity. Using these strains, we will clarify the individual importance of signaling through S6K and 4E-BP in MYCN(-/-)-driven tumor formation. Furthermore, we use both cell-based assays and mouse models to determine whether MYC- and MYCN-expressing, SHH-independent medulloblastoma is sensitive to mTOR kinase inhibition. RESULTS: Transduction of MYCN(-/-), but not wild-type MYC or GFP control, into neuropheres derived from cerebellum, forebrain, or brainstem generated tumors when orthotopically transplanted into nude mice, and those generated from postnatal (P0) cerebellar neuropheres are SHH-independent. Transduction of MYCN(-/-) increases proliferation of P0 cerebellar neuropheres compared to transduction of either MYCN WT or GFP. Immunoablating of NMYC WT-transduced P0 neuropheres verifies that rapamycin decreases phosphorylation of rp56, whereas the mTOR active site inhibitors PP242 and INK128 disrupt phosphorylation of both rp56 and 4E-BP. Measurement of cell number shows that PP242 but not rapamycin inhibits proliferation of NMYC WT-transduced P0 neuropheres. The mechanistically distinct activities of mTOR allotsteric vs. active site inhibitors have enormous therapeutic implications, as in our medulloblastoma model, active site inhibitors of mTOR, but not rapamycin, demonstrate efficacy. Taken together, this suggests that S6K is dispensable, whereas eIF4E is required for MYC-driven oncogenesis.

INTRODUCTION: Low-grade gliomas (LGGs), the most common pediatric brain tumors, constitute a broad and heterogeneous group. Histologic diagnosis is often controversial due to the lack of reproducibility and the absence of correlation to clinical outcome. Although the recent identification of recurrent BRAF duplications and V600E point mutations in subsets of pediatric LGGs has spurred research into key drivers of these tumors, little is known about their molecular characteristics. The aim of this study was to describe the expression patterns of a large cohort of pediatric LGGs and to compare these patterns to patterns of expression in normal brains. METHOD: We performed gene expression profiling on 163 paraffin-embedded pediatric and 20 adult LGGs across 6100 selected genes known to be dysregulated in cancer. Unsupervised and supervised clustering methods were applied to distinguish specific molecular features associated with age, location, histologic subtype and BRAF genomic status of the tumors. We also performed multivariate analyses to specify differences associated with each clinical feature, and compared those differences with a large cohort of normal brain samples matched for location and age (Brainspan database). RESULTS: NMF (Non-negative Matrix Factorization) clustering on the 163 pediatric and 20 adult LGGs distinguished three molecular groups, associated with location, age, BRAF genomic status, and to a lesser extent histologic subtype. Differences between tumors with these clinical features were also showed using principal component analysis. Pediatric BRAF+ LGGs and tumors did not exhibit a distinct molecular pattern compared to BRAF WT tumors. After controlling for tumor location, we found that 75% of the genes differentially expressed between adolescent and adult LGGs were similarly differentially expressed between normal adolescent and adult brains (p< 0.0001). CONCLUSIONS: We report the largest cohort of expression profiles of pediatric LGGs and identify differences associated with age, location, histologic subtype and BRAF genomic status. Most of the molecular differences between adolescent and adult LGGs are reflected in normal brain development, suggesting that normal brain development from adolescence to adulthood may influence tumor behavior.
0065. EXPRESSION OF miR-203 IS ASSOCIATED WITH AGGRESSIVE AN PHENOTYPE IN PEDIATRIC MEDULLOBLASTOMA
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INTRODUCTION: MicroRNAs (miRNAs) are small non-coding RNAs that play various roles in biological processes including proliferation, differentiation and apoptosis. Altered expression of miRNAs contributes to the development and maintenance of medulloblastoma. Additionally, some miRNAs are over-expressed in medulloblastoma. In both prostate and breast cancer, miR-203 has been shown to be responsible for cell invasiveness. From our own previous experience, expression of miR-203 in pediatric ependymoma was identified as predictor of early recurrence. Therefore, we investigated the association of miR-203 expression with clinical outcomes in a pediatric medulloblastoma.

METHOD: Primary tumors and corresponding clinico-pathologic data were collected on 48 patients with medulloblastoma. RNA purification of tumors was done with RecoverAll™ RNA isolation kit. The purified total RNA was used for cDNA synthesis with primers for miR-203 and mamm-u6 as a control. TaqMan™ miRNA assays were done in triplicate to quantify the levels of each target using the Applied Biosystems 7900HT Fast Real-Time PCR system in 96-well plates. Cycle threshold (Ct) values under 35 were considered positive for the presence of target miRNAs. Expression of miR-203 across samples was compared using delta-delta Ct. Expression of the ΔΔCt software was used for data analysis.

RESULTS: Levels of miR-203 varied considerably in our cohort, with a median delta-Ct of 10.87. Among tumors with large cell/anaplastic histology (n = 12), 9 had a high relative expression of miR-203 with a delta-Ct value below the median. Similarly, high miR-203 levels were observed in 8 of 11 patients with metastatic disease, regardless of histologic subtype. No correlation between miR-203 expression levels and patient age or overall survival was observed. Medulloblastoma subgrouping for this cohort is underway as is determination of miR-203 expression in normal cerebellum for comparison and will be presented.

CONCLUSIONS: Our preliminary results suggest that miR-203 is associated with an aggressive phenotype in medulloblastoma. Tumors with large cell/anaplastic histology and those with invasion into other intracranial structures had the highest expression of miR-203 in our cohort. Future studies include: identification and validation of downstream targets of miR-203 as well as determination of medulloblastoma subgroup specific miR-203 expression levels. We hypothesize that miR-203 may be implicated in invasion and migration in medulloblastoma as it is in other tumors.

0066. IDENTIFICATION OF MOLECULARLY AND CLINICALLY DISTINCT SUBGROUPS OF CHOROID PLEXUS CARCINOMAS
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INTRODUCTION: Choroid plexus tumors are a group of rare intraventricular tumors arising in young children. While choroid plexus papillomas (CPPs) and atypical choroid plexus papillomas (aCPP) exhibit favourable outcomes, choroid plexus carcinomas (CPCs) exhibit variable outcomes; tumor progression and relapse is observed in ~60% of patients, and most survivors experience significant long-term developmental and cognitive deficits. We studied a large cohort of choroid plexus tumors using an integrative high-throughput molecular approach to characterize the molecular profile and refine the current classification of these poorly understood tumors.

METHOD: We hybridized 35 choroid plexus tumors with known TP53 status to Affymetrix Human Exon 1.0 ST expression, and SNP6.0 genotyping microarrays. We analyzed gene expression profiles and copy number aberrations, and distinguished between molecularly distinct tumor subclasses. Molecular and clinical data were correlated to uncover relationships between newly defined subgroups and clinical outcomes. RESULTS: Copy number alterations expanded across choroid plexus carcinomas, suggesting the prevalence of aneuploidy in most tumor samples. Chromosome-wide copy number and gene expression profiles segregated CPCs from the benign CPPs and aCPPs. The molecular profile of CPCs was further refined into subgroups harboring a mutant vs. wildtype TP53. In mutant TP53 CPCs, chromosome-wide copy number profiles distinguished between two subgroups with significantly distinct clinical outcomes. Hyperdiploid mutant TP53 CPCs exhibited a less favourable long-term overall survival compared to the hypodiploid subgroup (median OS: 0% vs. 83% in hypodiploid mutant TP53 CPCs, p = 0.005). CONCLUSIONS: Our integrative study has characterized the recurrent chromosome-wide alterations and gene expression profiles found in choroid plexus tumors, and further identified novel molecular subgroups in CPCs with significantly distinct survival outcomes. Our findings suggest that CPCs can be further classified into clinically relevant subgroups to improve the clinical management of these malignant tumors.

0067. TARGETING Wee1 FOR THE TREATMENT OF PEDIATRIC HIGH-GRADE GLIOMAS
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INTRODUCTION: Wee1 is a key cell cycle regulatory kinase. In this study we evaluated Wee1 expression in pediatric gliomas to assess its relevance as a therapeutic target when used in combination with radiation therapy. Based on our expression results, which showed increasing Wee1 expression in association with increasing glioma malignancy grade, we investigated whether Wee1 inhibitor MK-1775 was efficacious in pediatric high-grade gliomas (HGG). MK-1775 expression was performed in 38 primary pediatric gliomas (3 grade 3, 10 grade 2, 11 grade 3, 14 grade 4) and 8 normal brain samples using the Agilent 4x4K array. Clonogenic survival assays were carried out in HGG cell lines (n = 6). DNA repair capacity was evaluated by measuring levels of γH2AX, a marker of double strand DNA breaks.

RESULTS: Wee1 was overexpressed in pediatric HGGs, especially in DIPG, with positive correlation between increasing Wee1 expression and higher malignancy grade (p = 0.007 for grade 3 vs. 4 x 1 + 2). Combination treatment with MK-1775 and radiation, in combination with decreasing clonogenicity and increased expression of γH2AX in HGG cells to a greater extent than achieved by radiation alone. Finally, combined MK-1775 and radiation conferred greater survival benefit to mice bearing engrafted, orthotopic HGG and DIPG tumors, than was achieved by radiation with radiation alone (BR at 100 mg/kg, allograft model p = 0.0061 and DIPG brain xenograft model p = 0.0163). CONCLUSIONS: Our results highlight MK-1775 as a promising new therapeutic agent for use in combination with radiation for the treatment of pediatric HGGs, including DIPG. To our knowledge this is the first investigation that reports on the expression of Wee1 in all grades of pediatric gliomas, including DIPG, and the development of specific models to assess the effect of MK-1775 in combination to radiation in vivo. Our results have motivated a soon-to-be-initiated phase 1 clinical trial for newly diagnosed DIPG.

0068. FLUORESCENCE-GUIDED SURGERY WITH 5-AMINOULEVULINIC ACID FOR RESECTION OF BRAIN TUMORS IN CHILDREN
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INTRODUCTION: After exogenous administration, 5-aminolevulinic acid (5-ALA) is metabolized to tumors cells, where its major metabolite accumulates. Irradiation with light of a specific wavelength induces fluorescence. This mechanism can be exploited to differentiate between normal and neoplastic tissue during surgery. A RTC established the impact of 5-ALA on gross total resection and prognosis in adult glioblastoma. As the impact of radical resection on outcome of pediatric brain tumors is evident, 5-ALA could be beneficial. Two case reports of fluorescence-guided surgery in children were published. We report our experience with 5-ALA in children harboring WHO grade I, II, III, IV brain tumors. METHOD: 10 patients with a mean age of 11 years (range 4–15 years) harboring brain tumors with infiltrative progression with preoperative MRI received 5-ALA according to the previously published adult protocol. Informed consent of parents was obtained on the basis of individual treatment attempts. Patients orally received 20 mg/kg body weight 5-ALA 2–4 hours before induction of anaesthesia. During surgery, the haematoxylin channels repeatedly switched the microscope (OPMI Pentero, Carl Zeiss, Germany) into fluorescence mode to inspect the resection cavity for positive fluorescence.
fluorescence. Routine blood samples were taken and clinical monitoring for adverse events was performed. The local ethics committee approved this retrospective analysis. RESULTS: Histology revealed pilocytic astrocytoma WHO grade I (n = 6), classical medulloblastoma WHO grade IV (n = 3) and anaplastic ependymoma WHO grade III (n = 1). Positive fluorescence of tumor tissue was observed in none of these cases. Significant increases were registered for alanine aminotransferase (14.9 U/l vs. 37.7 U/l, p < 0.0001) and gamma glutamyl transpeptidase (12.7 U/l vs. 39.3 U/l, p < 0.0001). Renal and hematological parameters remained stable. No adverse reactions were evident on clinical examination. CONCLUSIONS: Positive fluorescence was not observed in this pediatric series. Adverse reactions were similar to those reported for adults. The value of fluorescence-guided surgery appears ambiguous. The treatment of pediatric brain tumors, which might be considered for negative findings. Further studies are required to elucidate pharmacokinetic and pharmacodynamic particularities of 5-ALA in children and to assess its role in the resection of pediatric brain tumors.

0069. EXPERIMENTAL COMBINATION THERAPY: HISTONE DEACETYLASE AND DNA METHYLTRANSFERASE INHIBITORS IN BRAIN TUMOR CELL MODEL

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INTRODUCTION: Gene expression is controlled by several epigenetic mechanisms; one involves DNA methylation which is regulated by distinct but related methyltransferases. Another mechanism involves the acetylation and deacetylation of histone and non-histone proteins that control the transcription and regulation of genes involved in proliferation, cell cycle, control, survival, DNA repair and differentiation. Investigation shows that aberrant epigenetic alterations can be reverted with the use of epigenetic modification. Several clinical studies of combination therapy in hematological malignancies have improve the response rates of patients. Therefore the use of DNMT inhibitors and HDAC inhibitors for the treatment of solid tumors seems like a very promising strategy. METHOD: We have combined DNMT inhibitor 5-aza-2′-deoxycytidine and sodium +Phenybutyrate, using medulloblastoma cell models, and have noticed that the combination with imatinib and sorafenib results in significant cell killing at very low dosages. We have checked for caspase 3, and 7 activity using a luciferase based assay. HDAC and DNMT activity has been checked using an enzyme based assay. Expression of HDAC and DNMT by western blotting. DNA methylation before and after treatment using an enzyme system. Acetylation by Elisa and Western Blotting. RESULTS: We observed an increased activity of caspasas 3, and 7 after triple treatment, and a decrease in cell viability. The activity levels of HDACs differ according to to therapy. The activity of DNA methyltransferases also changes and we are currently checking on the expression of DNMT1, DNMT3A, and DNMT3B. We have checked for changes in DNA global methylation and observed a change towards hypomethylation and increased expression was that imatinib and sorafenib gave a change in acetylation. Currently we are checking for changes in acetylation after triple combination. We are also currently conducting some in vitro radiation studies after triple treatment. CONCLUSIONS: Our results suggest that these drugs act together to enhance cell killing most likely by apoptosis. Although this was an in vitro study our results suggest that by reverting methylation and acetylation we are enhancing cell death and overcoming resistance to target therapy. We suggest that these drug combination and therefore should be further tested for their precise molecular activity in preclinical models of medulloblastoma.

0070. PROTEOGLYCANS IN PEDIATRIC ASTROCYTOMA

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INTRODUCTION: Proteoglycans, components of the cell microenvironment, regulate cell signaling and migration by interacting with growth factor receptors, extracellular ligands, the extracellular matrix, and intracellular structural proteins. Inverse correlation between proteoglycan expression and brain cancer, proteoglycans have been shown to regulate multiple oncogenic pathways and promote tumor growth, invasion, inflammation and angiogenesis. As proteoglycans and their enzymatic modification may be important in disease, and may represent novel therapeutic targets and tumor biomarkers, we examine the expression of proteoglycan core proteins and their related biosynthetic and modifying enzymes in pediatric astrocytoma. METHOD: Gene expression data was obtained from a total of 26 astrocytomas at UCSF as described previously using Agilent 4x44 arrays (G4214F): pilocytic astrocytoma (n = 6), anaplastic astrocytoma (AA) (n = 8) and glioblastoma (GBM) (n = 12) (Engler J. et al., 2012). Non-neoplastic brain samples rejected for epilepsy were derived from pediatric (n = 2) and adult (n = 6) patients. Expression data is displayed as mean log2(tumor/normal) and compared to data in adult GBM (Wade A. et al., 2013). Genes of interest were analyzed using unpaired Mann Whitney U test or Kruskal-Wallis ANOVA followed by Dunn’s post hoc comparisons test. RESULTS: Proteoglycan core proteins and their enzymatic modifications were identified with a marked increase in expression of the proteoglycan core protein genes ACAN and PTPRZ1 (ANOVA p = 0.0028, 0.0044 respectively). In contrast, in high grade astrocytomas, AA and GBM, the gene expression of CD44 was significantly increased when in GBM the expression ofGPC3 was significantly decreased. In GBM and AA ANOVA p = 0.003, 0.001 respectively and results in pediatric gliomas. Expression of core gene proteoglycan alterations between pediatric pilocytic astrocytoma, pediatric high-grade astrocytoma, and adult GBM, reveals multiple similarities including increased expression of the extracellular heparan sulfate (HS) endosulfase gene SULF1 and SULF2, which act to change the sulfation status and binding properties of HS. CONCLUSIONS: Pediatric astrocytomas exhibit multiple alterations in the expression of proteoglycan core proteins and proteoglycan synthetic, modifying and degrading enzymes. In some cases, the alterations in pediatric tumors mirrored alterations previously identified in adult GBM, such as increased expression of the extracellular endosulfatases, increased expression of the 35 HS sulfotransferases, and decreased expression of the 65 HS sulfatases, suggesting potential roles for HS sulfation in disease. Proteoglycans and their modifying enzymes represent potential extra-cellular therapeutic targets and tumor biomarkers in pediatric glioma. Ongoing studies in the laboratory are investigating these possibilities and examining proteoglycan expression at the protein level.

0071. IDENTIFICATION OF TRANSCRIPTOMIC AND EPIGENETIC SUBGROUPS OF SUPRATENTORIAL EPENDYMOMAS

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INTRODUCTION: Recently, ependymomas were classified into molecular subgroups based on transcriptomic alterations. Focusing on tumors localized within the posterior fossa, two distinct entities of ependymoma were delineated by several studies, which also show striking differences in genetic characteristics and clinical outcome. So far, subgrouping approaches for supratentorial ependymomas have been lacking clinical and prognostic data, and have been limited to small cohorts. To develop an ependymoma consensus of clinically relevant molecular subgroups analogous to medulloblastoma, a broadly accepted molecular classification scheme is desperately needed. METHOD: We studied genome-wide mRNA expression profiling and RNA sequencing and DNA methylation changes (Illumina, 450k) in 65 primary supratentorial ependymomas. When performing unsupervised clustering, we identified three major clusters, which displayed significant overlap when comparing transcriptome-based and methylation-based subgrouping. We integrated these findings with previously detected cytogenetic aberrations. Three independent expression profiling data sets from previously published ependymoma studies (Johnson et al.; Wani et al.; Witt et al. etc) were being used as control and validation cohorts. For validation, a single molecular data set is presented, the data set is a compliment to the initial set of 120 primary supratentorial ependymomas was available on tissue microarrays. RESULTS: Supratentorial ependymomas can be classified into three principle molecular subgroups, while whole transcriptome or whole methylation arrays, the subgroup 1 consists of medulloblastoma-like tumors, subgroup 2 of these patients died, and high-risk aberrations such as gain of chromosome 1q and deletions of CDKN2A were absent. Patients belonging to Subgroups 2 and 3 have a much worse outcome, and comprise predominantly children and infants. These tumors often show stable genomes, with some harboring high-

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risk cytogenetic aberrations including gain of chromosome 1q and deletion of CDKN2A. CONCLUSIONS: In summary, we could decipher molecular subgroups of supratentorial ependymoma using genome-wide transcriptome and methylome approaches. These subgroups show a close overlap with recently published transcriptome-based analyses across all locations. Our results suggest that robust molecular subgroups of ependymoma with very distinct demographics and clinical course can be readily distinguished, and should therefore be considered in a consensus molecular classification of ependymoma.

0072. USP37 IS A NOVEL DEUBIQUITYLASE WITH TUMOR SUPPRESSIVE FUNCTION IN MEDULLOBLASTOMA


INTRODUCTION: Expression of the REST-Silencing Transcription Factor (REST), a repressor of neurogenesis, is elevated in the pediatric brain tumor, medulloblastoma. Elevated REST expression is associated with diminished tumor differentiation and poor overall/event-free survival in patients. REST loss prevents tumor formation in mouse xenograft models, whose in vivo constitutive expression in neural progenitors contributes to tumor development. Here, we demonstrate a novel role for REST in the control of cell proliferation. We have identified an unexpected link between REST and a deubiquitylase called USP37, in the proteasome-dependent destabilization of the cyclin-dependent-kinase-inhibitor (CDK1) p27 and deregulation of cell proliferation and medulloblastoma development. METHOD: REST knockdown and constitutive USP37 expression was achieved by transient transfection of human medulloblastoma cells and analyzed by qRT-PCR and Western blotting. REST binding to target genes was assessed by chromatin immunoprecipitation assays. Interaction between USP37 and p27 was measured by co-immunoprecipitation and in vitro deubiquitylation assays. USP37 dependent tumor suppression was measured using mouse orthotopic models and tumors analyzed by H&E staining and immunohistochemistry. Human tumor samples and mouse cerebellum were stained and REST, USP37 and p27 levels determined by immunofluorescence assay. RESULTS: REST loss in human medulloblastoma cell lines and in tumor xenografts decreased and increased cell proliferation respectively. REST knockdown increased p27 protein levels and concomitantly depressed USP37 transcription through loss of REST binding to the cognate promoter. Constitutive wildtype (WT) USP37 but not mutant USP37 expression rescued REST-associated p27 destabilization, blocked cell proliferation and promoted neurogenesis. Constitutive expression of WT USP37 in tumor cells also decreased their tumorigenic potential in the murine cerebellum compared to vector or mutant USP37 expressing tumor cells. Significantly, genetic and pharmacological targeting of REST-associated epigenetic activity similarly upregulated USP37 expression and blocked tumorigenesis. CONCLUSIONS: Our work has identified a novel role for the repressor of neurogenesis REST, in the control of medulloblastoma cell proliferation. We have shown that REST represses the transcriptome in a previously unknown specific deubiquitylase called USP37, which results in p27 destabilization and sustained tumor cell proliferation in vitro and tumorigenesis in vivo. Genetic or pharmacological upregulation of USP37 blocks tumor growth suggesting that USP37 is a novel medulloblastoma tumor suppressor. Consistent with this, human tumor samples exhibit reciprocal of REST and USP37 expression and blocked tumorigenesis. CONCLUSIONS: Our work has identified an unexpected link between REST and a deubiquitylase called USP37, in the proteasome-dependent destabilization of the cyclin-dependent-kinase-inhibitor (CDK1) p27 and deregulation of cell proliferation and medulloblastoma development.

0074. PLACENTAL GROWTH FACTOR/Neuropilin 1 SIGNALING IS A THERAPEUTIC TARGET IN PEDIATRIC MEDULLOBLASTOMA

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INTRODUCTION: Medulloblastoma is the most common malignant pediatric brain tumor and constitutes 20% of all brain tumors in children. The standard of care includes initial surgery followed by radiation and chemotherapy. Current regimens have demonstrated good survival rates even in high-risk populations. However, the treatment entails devastating morbidity, including cerebral mutism syndrome, decline in cognition and intellect, secondary malignancies and infertility and growth problems. Therefore, there is an urgent need for new therapies in children with medulloblastoma that provide not only increased survival in non-responders but also reduce the significant morbidity associated with current treatments. METHOD: We analyzed 72 clinical samples of medulloblastoma for expression of Placental Growth Factor (PlGF) and its receptors Nrp1 and VEGFR1. Samples were classified based on histology and molecular criteria using the immunohistochemical panel (IHC), array comparative genomic hybridization, and deep gene sequencing. We used 2 orthotopic human medulloblastoma models, D283-MED and transgenic spontaneous Smo+/- medulloblastoma model and inhibited PI3K and Nrp1 using genetic inhibition by shRNA and species specific antibodies (aPIGF, aNrp1) in vivo. We followed tumor growth and survival, circulating biomarkers and analyzed underlying molecular changes at the end of the study. RESULTS: Approximately 90% of medulloblastomas expressed PI3K across all WHO subtypes and all main molecular subtypes of medulloblastoma. PI3K was produced by stromal cells in response to stimulation by tumor PlGF. Inhibition of PI3K signaling using PI3K and Nrp1 signaling axes killed the medulloblastoma cells via MAPK pathway. aPIGF treated mice survived significantly longer both in the D283-MED (p < 0.0001) and D341-MED (p < 0.0001) models. aPIGF treated Smo+/- mice had significantly smaller tumors and preserved body weight (p = 0.047 and p = 0.0044, respectively). aNrp1 therapy led to tumor regression and improved survival compared to...
0075. THE MODULATION OF mTOR/HIF-1 PATHWAY IS EFFICIENTLY INHIBITING PEDIATRIC MALIGNANT BRAIN TUMOR CELL LINES
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INTRODUCTION: Intratumoral hypoxia plays a fundamental role in tumor progression and resistance to therapies. Tumor cell adaptation to hypoxic environment is regulated partially by the Hypoxia Inducible Factor-1 (HIF-1), which is involved in gene expression of the cell energy metabolism, tumor angiogenesis, and metastasis. Inhibitors of HIF-1 activity are under development because HIF-1 activity is driven in particular by mTOR pathway activation. Recently, studies demonstrated that concomitant inhibition of HIF-1 plus mTOR is completely suppressing HIF-1 accumulation. Interestingly, these targets have been identified in both pediatric and adult brain tumors. Therefore, preclinical studies were launched to understand the efficacy of such inhibition in brain tumor cell lines. METHOD: For this study, 3 high-grade glioma (HGG) and 2 diffuse intrinsic pontine glioma (DIPG) patient-derived cell lines were developed. RESULTS: The xenografted tumors were also treated and, to understand the effects, alone or in combination, with clonogenic and proliferation evaluation, and Western blotting were used to assay the effects of Aurora B inhibition. METHOD: The MYC mRNA expression in current glioblastoma were recently initiated at the Preston Robert Brain Tumor Center at Duke (NCT01491893). Administration is by convection-enhanced intracerebral/intratumoral infusion. Five patients have been treated on this protocol with rapid dose escalation to the max. feasible dose (10-10 TCID50). In parallel, mechanistic studies of poliovirus oncolytic activity and the resulting host inflammatory response have been conducted in vitro and in syngeneic, immune-competent mouse models for glioma. RESULTS: Early indications from our clinical trial are that PVS-RIPO is exceedingly well tolerated in this patient population, without any evidence for signs of clinical improvement and radiological responses in multiple patients (> 9 months post infusion). Mechanistic studies in vitro and in animal tumor models indicate that initial, direct viral tumor cell killing is critical, but host response to tumor infection and killing may constitute the main mechanism of clinical efficacy. Our early clinical results are consistent with these empirical findings. CONCLUSIONS: Our early clinical observations suggest that oncolytic virotherapy with PVS-RIPO may be promising treatment option in pediatric and in adults. Major aspects of safety, efficacy and mechanisms of action, which were established in basic mechanistic studies in vitro, in animal model systems, and in non-human primates, appear to be corroborated in our clinical studies. Of central importance for future endeavors is the identification of the immunologic response that results from PVS-RIPO mediated tumor infection and tumor cell killing in patients.

0076. MECHANISM OF ACTION AND THERAPEUTIC APPLICATION OF AURORA KINASE B INHIBITION IN c-MYC OVEREXPRESSING MEDULLOBLASTOMA
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INTRODUCTION: Aurora kinase B regulates multiple steps in mitosis including the completion of cytokinesis. Aurora B inhibition triggers G2/M and post-mitotic checkpoints and has been demonstrated to elicit apoptosis in multiple cancer cell types. Since cells that overexpress c-MYC are uniquely sensitive to agents that trigger G2/M checkpoints, we hypothesized that medulloblastoma cells which overexpress c-MYC would be sensitive to the effects of Aurora B inhibition. METHOD: The MYC mRNA expression in sub-grouped medulloblastoma samples (n = 117) was compared to normal cerebellum. Specific inhibition of Aurora B with AZD1152-HQPA was determined by Western blotting. Cell counts, microscopy, FACS DNA content analysis, and Western blotting were used to assay the effects of Aurora B inhibition in c-MYC overexpressing medulloblastoma cells versus isogenic controls. The transcriptional response to Aurora B inhibition was assessed by mRNA microarray and analyzed using bioinformatics process gene ontology (GSEA) and gene enrichment mapping. D458 (MYC amplified medulloblastoma) cerebellar xenografts were monitored using bioluminescence imaging. Animals received AZD1152-HQPA (50 mg/kg/day) or vehicle subcutaneously for 21 days.

RESULTS: MYC mRNA is highest in Wnt and Group 3 medulloblastoma when compared to adult or fetal cerebellum. Medulloblastoma cells overexpressing c-MYC undergo apoptosis after 48 hours of Aurora B inhibition and show downregulation of a DNA binding genes compared to isogenic control. Change in tumor photon flux from day 7 to day 14 was 3687 ± 913.3 SEM% in control versus 729.3 ± 93.68 SEM% with drug treatment (N = 11/group, P = 0.009). The mean survival time was 18 days (17 – 18 days, 95% CI) for control and 34 days (27 – 40 days, 95% CI) for AZD1152-HQPA treated animals (N = 5/group, P < 0.003). CONCLUSIONS: Aurora kinase B inhibition with AZD1152-HQPA triggers a unique transcriptional response in c-Myc overexpressing medulloblastoma cells. The growth of medulloblastoma endogenously expressing high levels of c-Myc is impaired by Aurora B inhibition in vivo resulting in prolonged survival. This suggests the potential for use of Aurora B inhibitors as subgroup specific chemotherapeutic agents in patients with Group 3 medulloblastoma.

0077. ONCOLYTIC POLIOVIRUS IMMUNOTHERAPY TO THE REGULATION AND MOLECULAR SPECIFICITIES OF THE MEDULLOBLASTOMA SUBGROUPS
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INTRODUCTION: The poliovirus receptor Nect5/CD155, an onco-fetal cell membrane molecule widely expressed in malignant brain tumor cells, is co-expressed with pediatric medulloblastomas/gliomas and adult glialomas. Therefore, primary explant tumor cells from patients are infected and killed by poliovirus. The main deterrents to clinical use of poliovirus are its grim neuropathogenic profile and its inherent genetic instability. We solved these hindrances through genetic recombination with human rhinoviruses. The resulting recombinant, PVS-RIPO, is neuron-incompetent, due to translation restrictions in the normal CNS, but fully replication-capable in CNS tumor cells, due to continuously active MAPK signals to translation machinery. METHOD: PVS-RIPO was cGMP manufactured by NCI, tested in extensive IND-directed toxicity studies in macaques, FDA approved (IND no. 14,735) and IRB approved; Phase I/II clinical trials in adult patients with recurrent glioblastoma were recently initiated at the Preston Robert Brain Tumor Center at Duke (NCT01491893). Administration is by convection-enhanced intracerebral/intratumoral infusion. Five patients have been treated on this protocol with rapid dose escalation to the max. feasible dose (10-10 TCID50). In parallel, mechanistic studies of poliovirus oncolytic activity and the resulting host inflammatory response have been conducted in vitro and in syngeneic, immune-competent mouse models for glioma. RESULTS: Early indications from our clinical trial are that PVS-RIPO is exceedingly well tolerated in this patient population, without any evidence for signs of clinical improvement and radiological responses in multiple patients (>9 months post infusion). Mechanistic studies in vitro and in animal tumor models indicate that initial, direct viral tumor cell killing is critical, but host response to tumor infection and killing may constitute the main mechanism of clinical efficacy. Our early clinical results are consistent with these empirical findings. CONCLUSIONS: Our early clinical observations suggest that oncolytic virotherapy with PVS-RIPO may be promising treatment option in pediatric medulloblastoma and in non-human primates, appear to be corroborated in our clinical studies. Of central importance for future endeavors is the identification of the immunologic response that results from PVS-RIPO mediated tumor infection and tumor cell killing in patients.
0079. REGULATION OF MEDULLOBLASTOMA STEM CELL SELF-RENEWAL THROUGH THE DEVELOPMENTALLY CONSERVED FoxO1-Bmi1-p21 AXIS

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INTRODUCTION: Brain tumors represent the leading cause of childhood cancer mortality, of which medulloblastoma (MB) is the most frequent malignan

tic pediatric brain tumor. By merging cancer genomics and developmental biology, recent studies have demonstrated the presence of several MB molecular

subgroups, each distinct in terms of prognosis and predicted therapeutic re-

sponse. Subgroups 1 and 2 are characterized by a relatively good clinical outcome and activation of the Wnt and Shh signaling pathways, respectively. In contrast, subgroups 3 and 4 ("non-Shh/Wnt subgroups") are distinguished by metastatic disease, poor patient outcome, and the current deficiency of a

promising therapeutic target. In this study we will evaluate current mouse models in the context of the recent characterization of MB molecular subgroups.

METHOD: We have previously developed a novel mouse model that recapitulates the genetic changes and clinical correlates of human MB. We will use this model to evaluate the influence of the Wnt and Shh signaling pathways on MB self-renewal.

RESULTS: Intracranial implantation of MB cells results in a significant increase in MB tumor burden in the first 3 days post-implantation. This increase is largely due to an increase in MB self-renewal, as measured by the number of MB stem cells that are capable of giving rise to new MB tumors. However, the increase in MB self-renewal is not due to an increase in the number of MB stem cells, but rather to an increase in the stemness of the MB stem cell population. This increase in stemness is accompanied by an increase in the expression of key MB stem cell markers, such as Sox2 and Oct4. These results are consistent with the recent characterization of MB molecular subgroups, and suggest that the Wnt and Shh signaling pathways are important regulators of MB self-renewal.

CONCLUSIONS: Our results support the hypothesis that the Wnt and Shh signaling pathways are important regulators of MB self-renewal. This suggests that targeting these pathways may be a promising therapeutic strategy for MB.

0080. GROSS TOTAL RESECTION AND SUPRATENTORIAL LOCATION SIGNIFICANTLY IMPROVE OVERALL SURVIVAL IN PEDIATRIC PATIENTS WITH HIGH GRADE GLIOMA

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INTRODUCTION: Although 50% of pediatric CNS tumors are gliomas, higher WHO grade astrocytomas are less common within this group (<10% of CNS tumors). Survival, duration, and prognostic factors in adults have been comprehensively analyzed, but less is known about factors contributing to overall survival (OS) and progression free survival (PFS) in pediatric high grade glioma. METHOD: We reviewed databases from Memorial-Sloan Kettering Cancer Center between 1988 and 2010 including patients if they were 21 years or less at time of diagnosis, had a diagnosis of glo-

blasta, anaplastic astrocytoma, high grade astrocytoma, or high grade ol-

garocytoma. Brainstem gliomas were also included in the analysis if they had undergone biopsy suggestive of a lower grade. Kaplan-Meier curves and log-rank statistics were used to compare groups univariately. Multivariate analyses were completed using Cox proportional hazards regres-

sion models. RESULTS: 102 patients were identified with median age of 11 years. Median OS was 1.9 years, and median PFS was 278 days. Location was significant (p < .0001): children with supratentorial, brainstem, thalac-

m, or cerebellar tumors had median overall survivals of 2.7, 1.0, 1.3, and 1.9 years, respectively. Patients with GTR had median OS of 4 years vs. 1.6 years for STR and 1.3 years for biopsy patients (P < .0009). PFS varied by age subgroups with < 3 years and those 13 and older having signifi-

rantly shorter PFS (P = .002). The majority of patients were treated with chemotherapy and radiation, thus analysis could not be performed to determine if certain subgroups benefited from these treat-

ments. Retrospective molecular analysis will hopefully further clarify sub-

groups of patients within this difficult to treat population.

0081. TARGETING MITOCHONDRIA AND METABOLISM AS A NOVEL THERAPEUTIC APPROACH IN THE TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMA

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INTRODUCTION: Diffuse Intrinsic Pontine Gliomas (DIPGs) are the most devastating of all brain tumours. They mostly affect young children and, as there are no effective treatments, almost all will die of their tumour within 12 months. Mitochondria are attractive cancer drug targets as they play a fundamental role in energy production through glucose and lipid metabolism as well as cell growth and apoptosis. One of the key proteins responsible for main-

taining proper mitochondrial function is adenine nucleotide translocase (ANT). A recently developed ANT inhibitor, PENAO has been shown to inhibit mitochondrial function and glucose metabolism. METHOD: To eval-

uate the cytotoxic effectiveness of PENAO in DIPG neurospheres a number of methodologies were employed. These included, alamar blue assays, Annexin/ PI and caspase 3/7 activities. To assess loss of mitochondrial integrity we per-

formed flow cytometric analysis of mitochondrial superoxide production (deby-

droethidium stain) and membrane depolarization (JC-1 stain) in PENAO-treated DIPG neurospheres. Inhibition of glucose metabolism was examined by a lactate assay. To assess whether the cytotoxic ability of PENAO can be further enhanced in DIPG neurospheres we performed combi-

nation treatments of PENAO/Reversar (MRP1 transmembrane inhibitor) and PENAO/3BP (glycology inhibitor) and examined cell death by alamar blue and Annexin/PI staining. RESULTS: Treatment of DIPG neurospheres with PENAO profoundly inhibited cellular proliferation with IC50 concentrations ranging 0.1-0.5uM. Sensitivity increased in stem self-renewal and tumorigenicity by inhibition of p21. Through the application of stem cell data gathered from genomic platforms, we have performed functional BTIC assays to discover novel BTIC self-renewal mechanisms amenable to therapeutic targeting. We further demonstrate the presence of developmentally conserved pathways such as the FoxG1-Bmi1-p21 axis in tumor-initiating cells, indicating brain tumorigenesis as development gone awry. BTIC-specific targeted therapies involving these regulatory networks may assist in improving the overall survivorship of non-Shh/Wnt subgroup MB patients.
0082. MYXOPAPILLARY SPINAL EPENDYMOMAS DISPLAY A HIFI-ALPHA DRIVEN ‘WARBURG’ PHENOTYPE

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INTRODUCTION: Spinal ependymomas are rare slow-growing tumours found in both adults and children. Myxopapillary ependymomas are a distinct histological variant arising predominantly in the conus medullaris, cauda equina, or filum terminale. Despite an overall favorable prognosis, distant metastases, subarachnoid dissemination, and late recurrences have been reported. Currently the only effective treatment for myxopapillary ependymoma is gross-total resection. We characterized the genomic and transcriptional landscape of spinal ependymomas in an effort to delineate the genetic basis of this disease and identify new leads for therapy. METHOD: Gene expression profiling was performed on 35 spinal ependymomas (Affymetrix Gene 1.1ST), and copy number profiling on an overlapping cohort of 38 spinal ependymomas (Affymetrix SNP6.0). GSTC2.0 was used to identify significant broad and focal copy number events. Consensus hierarchical clustering and negative matrix factorization were used to establish subgroup assignments. Pathway analysis was performed using gene set enrichment analysis and visualized with Cytoscape: Enrichment map. Western blot analysis was used to confirm gene expression values. Functional validation experiments were performed on tumour lysate consisting of assays measuring pyruvate kinase M1 activity (PKM1), hexokinase activity (HK), and lactate production. RESULTS: At a gene expression level, we demonstrate that classic and myxopapillary spinal ependymomas are transcriptionally and biologically distinct. These findings are supported by specific copy number alterations occurring in each histological variant. Pathway analysis revealed that myxopapillary ependymomas are characterized by increased cellular metabolism, associated with up-regulation of HK1 and PKM1 and its transcriptional targets. These findings were validated by western blot analysis demonstrating increased protein expression of HK-1, HK2, PDK1, and phosphorylation of PDHE1a. Functional assays were performed on myxopapillary tumour lysates to demonstrate decreased PCKm activity and increased lactate production. CONCLUSIONS: Our findings suggest that myxopapillary ependymoma may be driven by a Warburg metabolic phenotype, mediated by the HIF1a transcriptional network. The key enzymes promoting the Warburg phenotype: HK1, PKM2, and PDK are targetable by next-generation small molecule inhibitors/activators that inhibit aerobic glycolysis, and which should be tested in pre-clinical studies as therapy for myxopapillary ependymoma.

0083. EFFECTIVE EPITHELIC THERAPY FOR CIMP+ POSTERIOR FOSSA EPENDYMOMA

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INTRODUCTION: Ependymoma is the third most common pediatric brain tumor and remains incurable in 45% of patients. We previously reported the identification of two molecularly and clinically distinct subgroups of posterior fossa (PF) ependymoma, which we named Group A and B. While patients with Group B tumors harbor a large number of gross chromosomal alterations and have variable outcomes, patients with Group A tumors have balanced genomic profiles with poor clinical outcomes. Given the rarity of large genomic alterations, we hypothesized that SNVs or epigenetic alterations may be key determinants of this heterogeneity and we are investigating these determinants in the design of new therapeutic strategies that target these factors and for an improved understanding of the pathways that drive this behavior in the diffuse ependymoma. Interestingly, tumors can have diverse patterns of intraparenchymal invasion, including perivascular invasion and diffuse, single cell invasion. To identify the molecular mechanisms driving these patterns, we conducted gene expression profiling on and overlapping cohort of 38 spinal ependymomas (Affymetrix Gene 1.1ST), and transcriptionally profiled 38 spinal ependymomas (Affymetrix SNP6.0). GSTC2.0 was used to identify significant broad and focal copy number events. Consensus hierarchical clustering and negative matrix factorization were used to establish subgroup assignments. Pathway analysis was performed using gene set enrichment analysis and visualized with Cytoscape: Enrichment map. Western blot analysis demonstrating increased protein expression of HIF-1α, HK2, PDK1, and phosphorylation of PDHE1α. Functional assays were performed on tumour lysate consisting of assays measuring pyruvate kinase M1 activity (PKM1), hexokinase activity (HK), and lactate production. RESULTS: At a gene expression level, we demonstrate that classic and myxopapillary spinal ependymomas are transcriptionally and biologically distinct. These findings are supported by specific copy number alterations occurring in each histological variant. Pathway analysis revealed that myxopapillary ependymomas are characterized by increased cellular metabolism, associated with up-regulation of HK1 and PKM1 and its transcriptional targets. These findings were validated by western blot analysis demonstrating increased protein expression of HK-1, HK2, PDK1, and phosphorylation of PDHE1α. Functional assays were performed on myxopapillary tumour lysates to demonstrate decreased PCKm activity and increased lactate production. CONCLUSIONS: Our findings suggest that myxopapillary ependymoma may be driven by a Warburg metabolic phenotype, mediated by the HIF1a transcriptional network. The key enzymes promoting the Warburg phenotype: HK1, PKM2, and PDK are targetable by next-generation small molecule inhibitors/activators that inhibit aerobic glycolysis, and which should be tested in pre-clinical studies as therapy for myxopapillary ependymoma.

0084. DIVERGENT GROWTH AND INVASIVE PATTERNS IN A NEURAL PROGENITOR CELL-BASED MODEL FOR GLIOMA

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INTRODUCTION: Pediatric high-grade astrocytoma (HGA) is highly invasive, driving malignant tumor cells deep within the adjacent brain. Identification of the determinants of tumor cell invasion is critical for the design of new therapeutic strategies that target these factors and for an improved understanding of the pathways that drive this behavior in the diffuse ependymoma. Interestingly, tumors can have diverse patterns of intraparenchymal invasion, including perivascular invasion and diffuse, single cell invasion. To identify the molecular mechanisms driving these patterns, we have established two murine gliomas with distinct growth and invasive phenotypes. METHOD: To model pediatric disease we isolated neural progenitor cells from the postnatal day 5 brain of Ink4a-Arf−/− mice, and transduced with HGF/EGF. While less frequent than in the adult, EGFR alterations are present in pediatric HGA, including the constitutively active vIII variant. Intracerebral transplantation of tumor-prone neurospheres generated two tumor lines with divergent and stable phenotypes: large bulky tumors with perivascular invasion (PV) versus smaller diffuse tumors with single cell invasion (DI). RESULTS: PV tumors were found to overexpress genes related to DNA replication & repair and the cell cycle process (>2-fold, FDR < 1%), consistent with their higher proliferative rate in vitro (average doubling time: 20hrs versus 38hrs, p < 0.001) and shorter median survival (21d vs. 30d DI tumors, p = 0.085). Interestingly, even at equivalent median survival time points (21d and 28d, respectively) tumor cell number was greater for PV than for DI (average: 3x107 cells versus 3.5x106 cells, p = 0.001). In contrast, DI tumors had significantly higher expression of genes associated with interactions with the extracellular environment, including cell adhesion and integrin signaling and sulfate biosynthesis. CONCLUSIONS: We have generated a neural progenitor cell-based murine model for HGA that recapitulates some of the heterogeneity of the human disease, including divergent invasive phenotype and survival. PV tumors appear to use a perivascular invasion mechanism while DI tumors show increased extracellular interactions. We are currently investigating downstream signaling pathways and key transcription factors to understand the determinants of this heterogeneity and we are investigating these determinants in human pediatric HGA. As both response to therapy and therapeutic approach may be influenced by tumor cell invasion, it is critical that we better understand this fundamental process.

0085. SUBGROUP SPECIFIC PATTERNS OF RECURRENT IN MEDULLOBLASTOMA

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INTRODUCTION: Recurrent medulloblastoma remains an enormous treatment challenge, and is almost uniformly fatal. Recent studies confirmed that medulloblastoma comprises four distinct subgroups. We sought to...
delineate subgroup-specific differences in medulloblastoma recurrence patterns. METHOD: We identified a screening cohort of all recurrent medulloblastomas at the Hospital for Sick Children between 1994-2012, and sub-grouped the cases from FFPE tissues using a nanoString-based gene expression class prediction algorithm. Our findings were confirmed through analysis of two independent non-overlapping FFPE validation cohorts. RESULTS: A screening cohort of 30 recurrent medulloblastoma was assembled; 9 local tumor bed recurrences, 21 metastatic recurrences. SHH tumors recurred more frequently in the tumor bed (8/11, 73%), Group 3 and Group 4 recur more exclusively with metastases (16/18, 89%; p < 0.001). Latency to death post recurrence was longer in Group 4 patients (p = 0.03). Tailor-made-approach location of recurrence was confirmed in a multicenter cohort (p = 0.02, n = 40), and an independent cohort (SHH 21/24 local recurrences, Group 3/4 69/72 metastatic relapses, n = 96, p < 0.001). Significantly, in all 40 cases where matched primary and recurrent pairs were available, subgroup affiliation remained stable at recurrence. CONCLUSIONS: Medulloblastoma does not change subgroup at the time of recurrence. Significant differences in the pattern of recurrence exist across medulloblastoma subgroups, further highlighting the clinical differences between the four principle subgroups. Intensified local (posterior fossa) therapy should be considered upon initial treatment for SHH patients. 81% of the SHH subgroup exhibit c-MET that crosses the blood-brain barrier, shows antitumor effect and promotes growth, migration and invasion of medulloblastoma cell lines in a dose-dependent manner. We also determined that foretinib crosses the blood-brain barrier and was able to reduce tumor growth and metastasis and to increase survival when used as a single agent. We confirmed that foretinib enhances the antitumor efficacy of the MEK inhibitor cobimetinib. CONCLUSIONS: SHH tumors exhibit high c-MET expression and dependence on c-MET signaling pathway in the pathogenesis of medulloblastoma. High expression of c-MET is found most often in conjunction with the sonic hedgehog (SHH) and some Group 3 medulloblastomas. We hypothesized that subgroup-specific targeting of the c-MET receptor in medulloblastoma. METHOD: We performed mRNA expression analysis of c-MET in a cohort of 103 medulloblastomas and confirmed the results using a non-overlapping validation cohort of 439 medulloblastomas. Immunostaining of clinically annotated medulloblastoma tissue microarrays with c-MET and phospho-c-MET antibodies was performed, and the expression levels were correlated with survival. Foretinib, an orally available multi-kinase inhibitor of c-MET, was used for all in vitro and in vivo experiments. Two cell lines representative of SHH and Group 3 tumors were used. To determine the ability of foretinib to penetrate the blood-brain barrier we used high-performance liquid chromatography with mass spectrometry detection. RESULTS: We determined that c-MET is highly expressed, both at the transcriptomic and at the protein level, in SHH medulloblastomas (81%) and some Group 3 tumors (33%). In the SHH subgroup, high expression of c-MET and phospho-c-MET (the activated receptor) correlates with a shorter progression free survival. We showed that foretinib inhibits proliferation and adhesion of medulloblastoma cell lines in a dose-dependent manner. We also determined that foretinib crosses the blood-brain barrier and can be quantified in the mouse brain. In medulloblastoma xenografts, foretinib was able to reduce tumor growth and metastasis and to increase survival when compared to controls. CONCLUSIONS: The HGF/c-MET pathway is a targetable pathway in medulloblastoma. Foretinib is an orally available inhibitor of c-MET that crosses the blood-brain barrier, shows antitumor effect and prolongs survival in preclinical models of medulloblastoma. Our findings suggest that a subgroup of patients harboring high HGF/c-MET medulloblastomas may benefit from targeted therapies with c-MET inhibitors.
mice. Though preliminary, our experience to date supports the feasibility of developing a panel of DIPG cell lines and xenografts, though such an initiative would likely benefit from a multi-institutional approach that would take advantage of an increased number of opportunities for establishing DIPG resources.

0089. MOLECULAR PROFILING REVEALS CNS-PNETS OVERLAP WITH VARIOUS WELL-DEFINED ENTITIES
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INTRODUCTION: Childhood CNS primitive neuro-ectodermal tumors (CNS-PNETs; WHO IV) are poorly classified embryonal tumors with early onset and aggressive clinical behavior. Histological diagnosis is contentious and complicated by divergent differentiation along neuronal, astrocytic, or other lines and is based on absence of a clear morphological pattern. In recent profiling studies, we identified molecular subgroups of CNS-PNETs with biological characteristics suggestive of overlap with other childhood CNS tumors. Here, we aimed at a comprehensive molecular characterization of CNS-PNET in relation to a large cohort of other classes of childhood brain tumors to further define/clarify the molecular nature of tumors diagnosed as CNS-PNETs. METHOD: A large cohort of 147 fresh frozen or paraffin embedded tumor samples with the histological diagnosis "CNS-PNET" was investigated for genome-wide DNA methylation patterns and copy-number aberrations using Illumina Infinium HumanMethylation450K Arrays. Transcriptomic profiling on Affymetrix HumanExon 1.0 ST arrays was performed for a subset of cases (n = 45). Molecular profiles of selected cases were complemented by targeted sequencing of H3F3A and IDH1, immunostaining for INI1, LIN28A, and OLIG2, and Fluorescence In Situ Hybridization for 19q13.42. RESULTS: Genes were compared to molecular classes of other tumor subtypes. Deeper molecular analysis, central histopathological examination of primary gliomas, express markers of tumor-derived vascular channels (processes not observed in 2D) and exhibit slower proliferation rates (11-fold for Res196, 1.25-fold for BXD1425). Res196 and BXD1425 were sensitive to vorinostat (IC50 1.5uM, 8.0uM respectively) and entinostat (IC50 3.0uM, 4.0uM respectively) upon exposure in 2D culture; however, sensitivity was 6-10-fold reduced in 3D culture. siRNA targeting of HR23B resulted in >90% knockdown, allowing us to determine whether HR23B is associated with HDACi sensitivity. However HR23B protein expression was not prognostic of either overall or progression-free survival in a prospective ependymoma cohort. CONCLUSIONS: Sensitivity to HDACi is significantly reduced in RCCS 3D culture compared to conventional 2D cultures, likely reflecting impaired drug penetration into tumor aggregates and drug resistance induced by the tumor microenvironment. We propose that 3D culture provides a better approximation of in vivo and clinical responses. Studies to determine whether telomerase inhibition is a mechanism of HDACi, whether HR23B is required for sensitivity to HDACi and whether ependymoma cells are sensitive to the sodium valproate will be discussed.

0091. THE GENOMIC LANDSCAPE OF TREATMENT-NAÍVE DIPG BIOPSIES SAMPLES
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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) have a universally dismal prognosis (median 9-12 months), with neither chemotherapeutic nor targeted agents showing any substantial survival benefit in clinical trials in children with this disease. Recent high-throughput sequencing approaches have revealed a striking viability of K27M mutations in the genes encoding the histone methyltransferases H3F3A and IDH1 or IDH2. On the other hand, no data has been reported of the secondary mutations accompanying these changes, nor of structural or non-coding variants. In addition, clinical practice has necessitated the study of samples retrieved from autopsies, and it is unclear whether the mutational spectrum is secondary to treatment-related changes. METHOD: We have carried out whole genome sequencing of 20 DIPG samples taken at diagnosis by stereotactic biopsy at The Necker Hospital, Paris, which were confirmed histologically as high grade astrocytomas. Raw sequence reads from tumour and matched constitutive DNA were aligned to the reference genome and analysed using a somatic analysis pipeline to identify single nucleotide variants (SNV), small insertions and deletions (Indels) and large structural variants (SV). A median of 30Gb of sequence was generated for each genome representing a median depth of >40x corresponding to a coverage of greater than 10x across 95% of known genes. RESULTS: Interim analysis has shown the number of somatic coding variants in DIPG biopsies to range from 8-22 (mean = 16) per sample, representing a frequency of 0.5-1.7 mutations/million bases. This is substantially lower than that observed in support regions (3-31, mean = 15) but significantly lower than adult GBM (17-85, mean = 47), as well as a small series of DIPG autopsy samples (6-40, mean = 24, mutation frequency = 1.3-4.6/ Mb). Although the transition/transversion ratios were higher in biopsies compared to the latter group, the Conway-Gardner model, with C > T/G > A transitions more likely to be preceded by a C and succeeded by a G in untreated than treated samples. CONCLUSIONS: K27M mutations were observed in all cases, usually in conjunction with TP53 (75%), and occasionally AKT1/4/3, and NRAS oncogenes. Secondary somatic alterations were detected in approximately 20% of DIPG cases. The increased number of mutations found in the latter may involve changes associated with clonal evolution. Study of the DIPG genome at biopsy provide a unique opportunity to identify targets for novel intervention in the upfront setting.
0092. VEGF/FGF-DEPENDENT VASCULOCENIC MIMICRY AND TUMOR-DERIVED ANGIOGENIC RESPONSE IN HIGH GRADE GLIOMA
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INTRODUCTION: The assumption of a genetically stable endothelial phenotype has been challenged with evidence of tumor-derived endothelial cells (TDEC) in gliomas. TDEC are phenotypically diverse from recruited endothelial cells and can form tumor vessels that derive from an embryonic-like process intrinsic to the tumor cell, termed ‘vasculogenic mimicry’ (VM). Whether glioma cells merely mimic the vessel function or whether glioma stem-like cells differentiate into endothelial cells is unclear. Here we investigate whether VM and the tumor-derived endothelial cell response can be modeled and manipulated in a dynamic 3-dimensional (3D) brain tumor culture by virtue of response to micro-environmental stimuli such as hypoxia. METHOD: We cultured glioblastoma, ependymoma, medulloblastoma, PNET, and untransformed neural cells as 3D aggregates using the Rotary Cell Culture System (RCCS) in addition to brain tumor endothelial cell cultures. Tumor-derived angiogenic response was analyzed by array qRT-PCR of 84 angiogenesis-related genes and endothelial protein expression analyzed by immunohistochemistry/immunofluorescence. Data was compared to angiogenic expression in primary glioma explants cultured in 3D, primary high grade glioma tissue and 2D cultures. Mouse flank xenografts were used to confirm the prevalence of TDEC in vivo. Small molecule competitive inhibitors of VEGF/FGF receptors were used in attempting to abrogate angiogenic response. 3D-induced microenvironment is sufficient to promote expression of the endothelial markers CD105, CD31 and vWF. CD31 and vWF were expressed in proportion of glioma high grade glioma, while in the glioblastoma and medulloblastoma, PNET cells, but not in untransformed neural cells. Co-culture experiments suggested that tumor-derived endothelial cells demonstrated comparable CD105 expression, whereas CD31 expression was absent. Glioma xenografts reveal CD105/CD31 positive vessel-like structures near necrotic areas and TDEC CD105/CD31 expression was widespread in primary glioma tissue. Many pro-angiogenic genes were upregulated in glioma xenografts and primary glioma explants. Inhibition of either VEGF or FGF signaling was sufficient to impair VM and downregulate the tumor-derived angiogenic response. CONCLUSIONS: The RCCS permits VM modeling, allowing manipulation of this potential anti-angiogenic therapy resistance mechanism. Our findings support studies indicating that hypoxia promotes the microvascular niche whereby TDEC may derive from a stem-like cell. The prevalence of TDEC expression and VM structures in xenografts and primary glioma tissue indicates that these are distinct biological processes contributing to neo-angiogenesis in primary gliomas. Co-culture experiments suggest that in the presence of sufficient pre-existing endothelial cells there is less selective pressure for TDEC to form. VEGF and FGF inhibition using small molecule inhibitors are promising strategies for reducing angiogenic response and inhibiting VM.

0093. HISTONE H3.3 MUTATIONS DRIVE PAEDIATRIC GLiOBlastoma THROUGH UPRgULATION OF MYCN
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INTRODUCTION: Chromosomal rearrangements resulting in novel fusion genes are strongly associated with cancer and numerous examples exist in both adult and childhood malignancies. Structural variants frequently result in chimeric proteins targetable by novel therapeutic approaches, an outcome desperately needed in paediatric high grade glioma (pHGG). These fusions are increasingly understood to be driven by specific mutations in the genes encoding the histone H3.3 variant (H3F3A) and the chaperones ATRX/DAXX, however to date there have been no reports of structural variation. We sought to address this by carrying out integrated analysis of whole genome and transcriptome sequencing of three pHGG cell lines. METHOD: We fully sequenced the genomes and transcriptomes of the pHGG cell lines KNS42 (H3F3A G34V, TP53 R158L), SF188 (TP53 G266E) and UW479 (TP53 R158L, DAXX 5683Y) using the IlluminaHiSeq2000 platform at 37-40× (DNA) and generating 74-92 million reads (RNA), respectively. Structural variants were detected using an integration of computed copy number and BreakDancer and ChimeraScan pipelines, and validated in the reference cells by PCR/Sanger sequencing. Break-apart and/or fusion FISH probes were generated for validated expressed fusion transcripts and screened on tissue microarrays containing 130 pHGG samples to look for recurrence in clinical specimens, and the functions of these fusions assessed. RESULTS: 305 DNA-level structural variants involving one gene at either end were nominated in the three pHGG cell lines, many involving genes that are co-amplified. Seventy genome breakpoints junctions were shown to be highly recurrent in the three pHGG cell lines, many involving genes that are co-amplified. Seventy genome breakpoints junctions were shown to be highly recurrent in the three pHGG cell lines, many involving genes that are co-amplified. Several genome breakpoints junctions showed microhomology and BreakDancer overlap of genome with transcriptome expression. Overlap of genome with transcriptome expression identified 26 expressed fusion transcripts associated with DNA-level breakpoints. These included TULIP4/RPTOR (16:17 - SF188), GORASP2/CDA1 (2:13 - KNS42) and AKAPE6/RP102 (4:14 - UW479). CONCLUSIONS: Numerous expressed fusion transcripts have been identified in pHGG cell lines which confer dysregulation of a variety of known actionable targets in cancer-related signalling pathways. Although the prevalence of fusions in clinical pHGG is not suggestive of structural variation during tumorigenesis of this disease, the genetic basis of these fusions may be a complementary mechanism to pathway dysregulation (such as PI3K/mTOR). Integration with functional screening data may help to identify novel drug targets, such as the protein kinase CDA1, knockdown of which selectively kills pHGG cells in vitro.

0094. INTEGRATED WHOLE GENOME AND RNA SEQUENCING IDENTIFIES NOVEL EXPRESSED FUSION TRANSCRIPTS IN PAEDIATRIC HIGH GRADE GLIOMA
Diana Carvalho, Maria Vinci1, Ilirjana Bajrami1, Imelda McGonnell2, Chris Lord1, Rui Reis4, Ruman Rahman; University of Nottingham, Nottingham, UK

INTRODUCTION: Paediatric high grade gliomas (pHGG) are the commonest solid tumour type, extrinsically associated with the histone H3.3 variant (H3F3A), occurring either at or close to key residues marked by methylation for regulation of transcription - K27 and K36. H3F3A mutations are associated with childhood glioma, however to date there have been no reports of structural variation. We sought to address this by carrying out integrated analysis of whole genome and transcriptome sequencing of three pHGG cell lines. METHOD: We fully sequenced the genomes and transcriptomes of the pHGG cell lines KNS42 (H3F3A G34V, TP53 R158L), SF188 (TP53 G266E) and UW479 (TP53 R158L, DAXX 5683Y) using the IlluminaHiSeq2000 platform at 37-40× (DNA) and generating 74-92 million reads (RNA), respectively. Structural variants were detected using an integration of computed copy number and BreakDancer and ChimeraScan pipelines, and validated in the reference cells by PCR/Sanger sequencing. Break-apart and/or fusion FISH probes were generated for validated expressed fusion transcripts and screened on tissue microarrays containing 130 pHGG samples to look for recurrence in clinical specimens, and the functions of these fusions assessed. RESULTS: 305 DNA-level structural variants involving one gene at either end were nominated in the three pHGG cell lines, many involving genes that are co-amplified. Seventy genome breakpoints junctions were shown to be highly recurrent in the three pHGG cell lines, many involving genes that are co-amplified. Seventy genome breakpoints junctions showed microhomology and BreakDancer overlap of genome with transcriptome expression. Overlap of genome with transcriptome expression identified 26 expressed fusion transcripts associated with DNA-level breakpoints. These included TULIP4/RPTOR (16:17 - SF188), GORASP2/CDA1 (2:13 - KNS42) and AKAPE6/RP102 (4:14 - UW479). CONCLUSIONS: Numerous expressed fusion transcripts have been identified in pHGG cell lines which confer dysregulation of a variety of known actionable targets in cancer-related signalling pathways. Although the prevalence of fusions in clinical pHGG is not suggestive of structural variation during tumorigenesis of this disease, the genetic basis of these fusions may be a complementary mechanism to pathway dysregulation (such as PI3K/mTOR). Integration with functional screening data may help to identify novel drug targets, such as the protein kinase CDA1, knockdown of which selectively kills pHGG cells in vitro.

0095. ENHANCING AUTOPHAGY AS STRATEGY TO TARGET HIGH GRADE GLIOMAS AND DIFFUSE INTRINSIC PONTINE GLIOMAS
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INTRODUCTION: The histone H3.3 variant (H3F3A) and the chaperones ATRX/DAXX are the only actionable targets in gliomas. H3F3A mutations are identified in 20% of high grade gliomas (pGBM), occurring either at or close to key residues marked by methylation for regulation of transcription - K27 and K36. Although the recurrent base changes may be predicted to cause profound effects on gene expression, the mechanism by which the mutations effect these changes and promote tumorigenesis is unknown. METHOD: Gene expression profiles of pGBM samples from independent studies were analysed on the basis of their H3F3A status. We carried out Western blot analysis and chromatin immunoprecipitation linked to whole genome sequencing (ChIP-Seq) for the histone H3K36 trimethylation marker (H3K36me3) in a panel of pGBM cell lines including the G4V mutant KNS42 cells, sirRNA screening was also carried out in this panel using a library directed against the human kinesin, with hits selectively targeting the G4V mutant cells validated by individual oligonucleotides and small molecule inhibition in vitro. RESULTS: We observed no difference in total H3K36me3 levels in G4V mutant versus wild-type cells, however ChIP-Seq identified 156 differentially bound and expressed genes. The transcriptional program induced reciprocates that of the developing forebrain, and involves numerous markers of stem cell maintenance, cell fate decisions and self-renewal. Critically, G4V mutations cause profound upregulation of MYCN, a potent oncogene which is causative of globlastomas when expressed in the correct developmental context. A synthetic lethality siRNA screen revealed this driving aberration to be selectively targetable in this patient population by inhibiting kinases responsible for stabilisation of the protein such as AURKA and CHK1. CONCLUSIONS: The H3F3A G4V mutation is specific to tumours of the cerebral hemispheres and is associated with a distinct age of incidence and gene expression signature compared to K27 and wild-type tumours. Our data thus tie together our previous explanation for how the G4V mutation acts to deliver MYCN, a potent tumorigenic initiator, into a stem cell compartment of the developing forebrain, selectively giving rise to cerebral hemispheric globlastoma. Employing synthetic lethal approaches to these mutant tumour cells provides a rational way to develop novel and highly selective treatment strategies.
INTRODUCTION: Pediatric high-grade gliomas (pHGG) including diffuse intrinsic pontine gliomas (DIPG) are one of the most formidable challenges faced by pediatric oncologist. Recently the use of molecular genetic has allowed to gain a deeper understanding of the genomic make up of these tumors. IGF1R, a receptor tyrosine kinase, has been found to be amplified. IGF1R, a receptor tyrosine kinase, has been found to be amplified. High grade gliomas, especially those bearing the IDH1-R132H mutation, are known to be responsible for chromosomal changes within the NBN gene with the classic type of medulloblastoma. In one patient we detected monosomy of chromosome 6 associated with low-risk medulloblastoma. In three patients with c.657_661del5) in the NBN gene with the classic type of medulloblastoma. In three patients with c.657_661del5) in the NBN gene with the classic type of medulloblastoma.

METHOD: To identify the presence of NBN mutations direct sequencing of selected exons was performed using a 3130 Genetic Analyzer (Applied Biosystems). The analyzed sequence fragments were compared with the NBN DNA (GenBank RefSeq NC.000008.9). To determine the chromosomal aberrations we analyzed five NBN positive medulloblastoma tumor samples using a whole-genome oligonucleotide CGH microarray (NimbleGen HG18, CGX Cyrogenetic 6x315K array). Feature extraction and primary data analysis were performed using Roche NimbleGen’s DEVA v.1.1 software. Detailed analysis and data visualization were performed using Genoglyphix genome browser software. RESULTS: All identified in our study chromosomal aberrations have been frequently detected in medulloblastomas. In three patients with c.657_661del5) in the NBN gene with the classic type of medulloblastoma. In three patients with c.657_661del5) in the NBN gene with the classic type of medulloblastoma.

CONCLUSIONS: This was the first study that evaluated the pattern of chromosomal aberrations in germ-line NBN mutation carriers with medulloblastoma.

0097. THE NATURE OF CHROMOSOMAL ABERRATIONS DETECTED IN PEDIATRIC PATIENTS WITH PETERODYGOUS GERMLINE-GENE MUTATIONS IN THE NBN GENE AND CLASSIC TYPE DIPG

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INTRODUCTION: Medulloblastoma is the most common malignant brain tumor in children. At present, multiple molecular dysfunctions are known to be responsible for medulloblastoma formation. Based on the comprehensive analysis four molecular subtypes of this type of cancer are identified. Nonetheless, the recent studies evaluating the role of mutations in DNA repair genes in the development of medulloblastomas indicate that changes within NBN seem to be particularly important. The aim of our study was to evaluate the nature of the chromosomal aberrations in pediatric patients with heterozygous germ-line mutations (c.111A > G and c.657_661del5) in the NBN gene with the classic type of medulloblastoma.

METHOD: To identify the presence of NBN mutations direct sequencing of selected exons was performed using a 3130 Genetic Analyzer (Applied Biosystems). The analyzed sequence fragments were compared with the NBN DNA (GenBank RefSeq NC.000008.9). To determine the chromosomal aberrations we analyzed five NBN positive medulloblastoma tumor samples using a whole-genome oligonucleotide CGH microarray (NimbleGen HG18, CGX Cyrogenetic 6x315K array). Feature extraction and primary data analysis were performed using Roche NimbleGen’s DEVA v.1.1 software. Detailed analysis and data visualization were performed using Genoglyphix genome browser software. RESULTS: All identified in our study chromosomal aberrations have been frequently detected in medulloblastomas. In three patients with c.657_661del5) in the NBN gene with the classic type of medulloblastoma. In three patients with c.657_661del5) in the NBN gene with the classic type of medulloblastoma.

CONCLUSIONS: This was the first study that evaluated the pattern of chromosomal aberrations in germ-line NBN mutation carriers with medulloblastoma. The most of the identified changes were frequently altered in intermediate or poor-risk medulloblastoma (subtype A/WNT). Our results were consistent with the patients clinical data. A comparison of clinical features between medulloblastoma patients with and without NBN mutations indicated a more aggressive course of disease in NBN heterozygotes. The study was financed by the National Science Centre, project no. 6917/B/P01/2011/40 and the Internal Project of The Children’s Memorial Health Institute no S11/2009.

0099. CLINICAL AND BIOLOGIC CHARACTERISTICS OF CLEAR CELL EPENDYMOMA

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INTRODUCTION: Clear cell ependymoma (CCE) is an uncommon histologic variant of ependymoma. Additionally, CCE is clinically treated with a tendency to recur and metastatize despite standard therapy. No data is available to describe the biologic differences between CCE and other ependymomas. MicroRNAs (miRNAs) are small non-coding RNA molecules that play a role in a variety of biological processes. Altered expression of miRNAs contributes to the development pediatric brain tumors including ependymomas. We aim to describe the clinical features of patients with CCE at our institution as well as identify biologic differences in miRNA expression in CCE compared to other ependymoma subtypes. METHOD: Tumor specimens and clinico-pathologic information for patients with CCE or ependymoma with clear cell features was obtained. RNA purification from tumor samples will be performed using RecoverAll. Total Nuclear Acid Isolation system. Tumor miRNA analyses will be used to quantify the level of approximately 762 mature miRNAs from each sample using the Applied Biosystems

0096. TSP-1 MODULATES PI3K SIGNALING TO ALTER METASTASIS PHENOTYPES AND CHEMO- AND RADIOTHERAPY-SENSTIVITY IN MEDULLOBLASTOMA

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INTRODUCTION: Survival of localized medulloblastoma (MB) has improved significantly with intensified chemotherapeutic regimens in recent years, however, treatment and/or prevention of craniospinal metastases remains a major obstacle in clinical management of MB. In prior studies, we generated MYC driven xenograft models of metastatic MB and identified thrombospondin-1 (TSP-1), a potent tumour suppressor and angiogenesis inhibitor, as a candidate effector and therapeutic target in metastatic MB. In this study we sought to determine whether expression of TSP-1 or TSP-1 peptidomimetics alters metastatic behavior and chemoradiotherapeutic response of MB in vitro and in vivo. METHOD: TSP-1 effects on MB cell growth in vitro was assessed using orthotopic xenograft assays in nu/nu mice; full brain and spine histological exam was performed to evaluate for tumor invasion and metastasis. For in vitro phenotypic assays, cell lines with stable TSP-1 expression or cells treated with a peptidomimetic to the structural homology repeat domain type 1 of TSP-1, were characterized using MTT assay. Cell Death and Body chamber assay. For cell to cell contact assays, cell lines were pre-treated with TSP-1 mimetics prior to cellular and western blot assays to determine TSP-1 associated signaling pathways. RESULTS: Stable TSP-1 expression significantly impaired tumour growth and metastases in orthotopic xeno-graft assays; mice with TSP-1 expressing xenograft had significantly longer survival; (p < 0.02) and diminished frequency of metastases (r < 0.05). TSP-1 stable expression or mimetics treatment (50-100uM) both potently inhibited migration (decrease by up to 80%; p < 0.05) of MB lines with eotopic (UW426-Myc, DAOY-Myc) and high endogenous (D341, D4S8) Myc expression. TSP-1 mimetics treatment alone has been shown to reduce MB cell proliferation, decrease ErbB2/HER2 expression and radiation induced MB cell death was significantly increased with TSP-1 mimetic treatment. Interestingly, TSP-1 induced phenotypic changes correlated with diminished phospho-AKT but not phospho-Erk expression. CONCLUSIONS: TSP-1 expression potently inhibits phospho-Erk expression and reduces ErbB2/HER2 activity, and, induces potent effects on invasive and metastatic phenotypes in MB via inhibition of PI3K signaling. TSP-1 effects in vitro are strikingly re-capitated by a peptide-mimetic of TSP-1 thus highlighting such mimetics as important new therapeutics for MYC-associated MB, one of the most lethal of MB variants.


9709H0T Fast Real-Time PCR system in 384-well low density arrays (TLDA). Cycle threshold (Ct) values under 35 will be considered positive for the presence of target miRNAs. Data from miRNA profiling of CCE will be compared to other ependymoma subtypes using the RealTime StatMiner® software.

RESULTS: Eleven patients with CCE or ependymoma with clear cell features were identified from a total of 166 ependymoma cases over a 20 year period. Median age at diagnosis of CCE was 7 years (range 2-16). Six patients were female (54%) and a majority of tumors were supratentorial (72%). Tumor recurrence occurred in 7 patients (63%) and 2 patients (18%) developed extra-neural metastasis. Median time to recurrence was 8 months (range 7-48 months). Three patients (27%) died of their disease at the time of data censoring. MicroRNA profiling of CCE specimens underway and will be presented. CONCLUSIONS: Our results confirm that CCE is an aggressive variant of ependymoma characterized by frequent tumor recurrence and extraneural metastases. We hypothesize that CCE is a biologically distinct disease and that the miRNA profiles of CCE patients will differ from that of other subtypes of ependymoma. Future studies include identifying changes in miRNA expression profiles over time in patients with CCE and multiple recurrences.

100. CLONAL EVOLUTION OF MEDULLOBLASTOMA BRAIN TUMOUR-INITIATING CELLS (BTICS) IN RESPONSE TO THERAPY: DISCOVERING THE REFRACTORY BTIC POPULATION

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INTRODUCTION: The most clinically compelling component of the Cancer Stem Cell (CSC) hypothesis holds that CSCs evade current therapy, and are responsible for disease recurrence. Although there is strong evidence that tumor-initiating cell (TIC) populations are chemo- and radio-resistant, no studies to date have demonstrated these treatment-resistant TICs to be exclusively causative in solid tumor relapse and recurrence. In the present study, we aim to identify the treatment-refractory brain tumor-initiating cell (BTIC) in medulloblastoma (MB), the most frequent malignant childhood brain tumor. MB TICs were cultured as tumourpheres, which were treated with chemotherapy and radiation, and subjected to flow analysis, and in vitro stem cell assays for self-renewal, proliferation and differentiation. We developed a mouse-adapted MB therapy model using our human-mouse BTIC xenograft, in which Daxx-GFP+ or Med18a-GFP+ MB cells were intracranially transplanted into NOD-SCID mice that were then treated with radiation, and chemotherapeutic drugs Vincristine, Cisplatin and Cyclophosphamide. Brains of mice sacrificed at experimental time points were harvested for immunohistochemistry and stem cell culture. Flow cytometric analysis of tumour cells from cultured brains, in vitro stem cell assays and flow characterization were performed. RESULTS: Initial experiments revealed that drug-treated cells showed an increase in self-renewal post-treatment, and an enrichment of CD15+ and CD133+ BTICs by flow analysis. A further study to compare the response of MB BTICs in vivo and in vitro showed that MB BTICs harvested from tumour bearing mice treated with radiation and chemotherapy were enriched for stem cell markers CD153 and Sox2. The treated cells also displayed an enhanced self-renewal capacity. Previous studies confirm the presence of a treatment-refractory population with stem cell properties. CONCLUSIONS: Comparative BTIC profiles of surviving and relapsed mice will identify BTICs that respond to or evade specific therapy. Profiling genomic changes in “treatment-responsive” tumors against those that fail therapy will generate a differential profile of the refractory BTIC, which may guide future therapeutic approaches targeting this cell, and will serve as a model for targeting such CSCs in other TIC-driven solid tumors.

1001. RAF INHIBITOR RESISTANCE AND PARADOXICAL ACTIVATION OF KIAA1549-BRAF FUSIONS PREDICT PEDIATRIC LOW-GRADE ASTROCYTOMAS

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INTRODUCTION: Astrocytomas are the most common type of brain tumor found in children. Activated BRAF mutations are unifying feature of this heterogeneous group of tumors with KIAA1549-BRAF fusion genes typifying low-grade astrocytomas and V600E BRAF alterations characterizing higher-grade tumors. BRAF targeted therapies such as vemurafenib have proven oncogenic BRAF signaling can be inhibited in V600E-dependent melanomas. Like the canonical V600E BRAF mutant, BRAF fusions activate MAPK signaling and are sufficient for malignant transformation. However, here we characterize the distinct mechanisms of action of KIAA1549-BRAF and its differential responsiveness to PLX4720, a first-generation BRAF inhibitor. METHODS: A novel MPG-expressing pediatric low-grade astrocytoma cell lines that harbor the BRAF fusion; therefore, we generated stably expressing BRAF fusion cell lines in Ba/F3, NIH/3T3, and murine neurosphere cells. Mutant BRAF constructs were subcloned into a Gateway compatible pMXs-Puro Retroviral Vector. Cells were infected with retroviruses and selected for stable expression of the fusion protein. BRAF inhibition studies were performed in the presence of increasing concentrations of PLX4720, a first generation BRAF-specific inhibitor or PLX-PB-3, a second generation “paradox-breaker” inhibitor (Flexikon Inc). BRAF dimerization and KR assay performed for BRAF fusion constructs. RESULTS: We find that in cells expressing KIAA1549-BRAF, the fusion kinase functions as a homodimer that is resistant to PLX4720 and accordingly is associated with CRAF-independent paradoxical activation of MAPK signaling. Mutagenesis studies demonstrate KIAA1549-BRAF fusion mediated signaling is diminished with the disruption of the BRAF kinase dimer interface. Additionally, the KIAA1549-BRAF fusion displays increased binding affinity to KR, a RAF relative recently demonstrated to facilitate MEK phosphorylation by BRAF. Despite its resistance to PLX4720, the KIAA1549-BRAF fusion is responsive to a second-generation selective BRAF inhibitor that, unlike vemurafenib, does not induce activation of wild-type BRAF. CONCLUSIONS: BRAF fusions, typically found in low-grade astrocytomas, can be resistant to first-generation BRAF inhibitors designed to target V600E BRAF mutant cancers. Instead, first-generation BRAF inhibitors can induce paradoxical activation resulting in increased tumor growth in cells harboring BRAF fusions. We believe this is due to key cell signaling characteristics associated with BRAF fusion proteins. Second-generation selective BRAF inhibitors offer promise that direct targeting of BRAF fusions is still possible. Our data support an approach in which pediatric astrocytoma therapies must be tailored to the specific mutational context and the distinct mechanisms of action of the mutant kinase.

0102. TARGETING DNA DAMAGE RESPONSE PATHWAYS TO OVERCOME ALKYLATING AGENT RESISTANCE IN PEDIATRIC GLIOBLASTOMA

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INTRODUCTION: Pediatric glioblastoma (pGBM) is among the most lethal primary brain tumors in children. Current therapy includes a combination of surgery, radiosurgery, chemotherapy, typically an alkylating agent temozolomide (TMZ). However, TMZ resistance is common and in many cases cannot be attributed to O-6-methylguanine-DNA methyltransferase (MGMT) expression. We hypothesized that other DNA damage response (DDR) pathways may account for the treatment resistance seen in pGBM. Here we report that the DNA repair proteins N-methylpurine-DNA glycosylase (MPG), involved in base excision repair (BER), and Ataxia telangiectasia mutated (ATM), a master regulator of the DDR pathway, contribute towards alkylating agent resistance in pGBM. METHODS: Primary pGBM cultures were grown with or without basal media with EGF, FGF and B27 growth factors. pGBM cell lines (SJG2 and KNS42) were maintained in DMEM-F12 and 10% FBS. Stable knockdown of MPG or ATM or both were achieved by retroviral transduction of pMB2-hCAG-SMRT retroviral constructs. Cell lines were transfected using aptopous assays (Cleaved Caspase 3/7), cell viability (trypan blue exclusion, cell count), and DNA damage (Comet Tail assay) in the presence or absence of temozolomide/BCNU. In vivo work and MRI imaging of mice harbouring SJG2 xenografts treated with vehicle or temozolomide in vivo. RESULTS: MPG expression in TMZ-resistant pGBM cell lines and primary cultures enhanced TMZ resistance, while exogenously expressing MPG in TMZ-sensitive lines conferred resistance. Surprisingly, we identified a novel non-MPG phosphorylation site regulated by ATM kinase. Loss of phospho-MPG (serine 172) reduced MPG’s ability to protect cancer cells against temozolomide and other alkylating agents. Dual targeting of ATM and MPG led to a synergistic increase in survival against TMZ in vivo. CONCLUSIONS: Targeting MPG through inhibition of BER may lead to promising new therapeutic strategies for the treatment of pGBM. In support of this, high nuclear MPG expression...
was observed in pGBM (60/80 samples) and not surrounding normal brain. Nuclear MPG also correlated with poorer overall survival in pGBM in one of our datasets. Evidence to date suggests that MGMT, another key determinant of TMZ resistance cannot accurately predict overall survival or response to TMZ in pGBM. We hypothesize that the BER pathway, mediated through an ATM-MPG axis, maybe a key promoter of alkylating agent resistance in pGBM.

**0103. SILENCING OF THE miR-17 ~ 92 CLUSTER FAMILY BY SEED-TARGETING 8-mer anti-miR INHIBITS MEDULLOBLASTOMA PROGRESSION IN THE BRAIN**
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INTRODUCTION: Medulloblastoma (MB), originating in the cerebellum, is the most common malignant brain tumor in children. MB consists of four major groups with different histological and molecular characteristics. Of these, the SHH subgroup (SHH) signaling pathway is a hallmark of one group. Mouse and human SHH MBs exhibit increased expression of microRNAs (miRNAs) encoded by the miR-17 ~ 92 and miR-106b ~ 25 clusters compared to granule progenitors or post-mitotic granule neurons. Here, we assessed the therapeutic potential of 8-mer seed-targeting LNA-antimiR oligonucleotides, termed tiny LNAs, that inhibit miRNA seed families expressed by the miR-17 ~ 92 and miR-106b ~ 25 clusters in a mouse model of SHH MB. METHOD: MiR-17 ~ 92 levels were assessed in spontaneously arising MBs from [Ptch1 + /;Cdkn2c−/−] and [Ptch1 + /;Trp53−/−] mice. MBs were cultured and treated in vitro with tiny LNAs. The percentage of cells incorporating FAM-labeled tiny LNAs, Ki-67 immunoreactivity and BrdU incorporation were determined after tiny LNA treatment. Pre-treated cells were transplanted into the flanks of CD-1 nude mice to test the effect of tiny LNAs on tumor growth. Lastly, tiny LNA naive tumor cells were transplanted into the flanks and cortices of recipient mice and treated intravenously with saline formulated tiny LNAs to assess whether systemic delivery of tiny LNAs could suppress MB growth. RESULTS: We found miR-19a and miR-17-1~20a to be the highest expressed miRNAs from the miR-17 ~ 92 cluster within MBs from [Ptch1 + /;Cdkn2c−/−] and [Ptch1 + /;Trp53−/−] mice. When treated in culture with tiny LNAs, SHH tumor cells passively and effectively took up tiny LNAs, and specifically inhibited targeted miRNA seed-sharing family members. Inhibition of miR-17/20a/106b/93 and miR-19a/19b by an anti-miR-17 and anti-miR-19, respectively, resulted in diminished tumor cell proliferation in vitro. When tiny LNA pre-treated tumor cells were transplanted into the flanks of recipient mice, tumor growth was suppressed. Importantly, systemic delivery of tiny LNAs into flank or brain allograft-bearing mice suppressed SHH-subgroup MB progression. CONCLUSIONS: We investigated the role of miR-17/20a/106b/93 and miR-19a/b in MB proliferation by inhibiting their function using 8-mer LNA-modified anti-miRs directed against their seed sequences, designated as anti-miR-17 and anti-miR-19. Results from this study imply that 8-mer LNA-antimiRs directed against their seed sequences, designated as anti-miR-17 and anti-miR-19, have the potential to be used in the treatment of human SHH MBs; thus, warranting consideration in the treatment of these challenging cancers. This work is now being extended to the field of MB research, but also the cancer field as a whole by demonstrating the utility of systemically delivered tiny LNAs for the treatment of brain tumors.

**0104. A RETROVIRAL GENE-TRAP SCREEN IDENTIFIES PTEN INDUCED KINASE 1 (PINK1) AS A NEGATIVE REGULATOR OF AEROBIC GLYCOLYSIS IN PEDIATRIC AND ADULT GlioBLASTOMA**
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INTRODUCTION: Pediatric glioblastoma (pGBM) comprises of 8-12% of primary CNS tumors in children and adult Glioblastoma (GBM) is the most common and lethal of all gliomas. Mutations to both is tumor aggressiveness and poor survival despite surgery, radiation and chemotherapy. Using a retroviral gene-trap screen on astrocytes cultures isolated from newborn transgenic mice predisposed to developing gloma we identified several novel genes not previously implicated in GBM and pGBM. Of great interest was PTEN induced kinase 1, PINK1, an at least 12 known functions that we demonstrate to be lost in GBM and pGBM and functions to oppose tumour metabolism.

**0105. CONCLUSIONS: We investigated the role of miR-17 and miR-19a**

**0106. PEDIATRIC GANGLIOGLIOMAS INCLUDING THOSE IN THE BRAINSTEM SHOW BRAF V600E MUTATION IN A HIGH PERCENTAGE OF CASES**
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INTRODUCTION: Standard treatment for ganglioglioma (GG) currently consists of optimal tumor resection, which is curative in the majority of cases. While ability to surgically remove the tumor is critical, little is known about other genetic changes in GG that may affect progression. We sought to characterize BRAF mutations in GG.

METHOD: We evaluated a subset of 18 BRAF wild-type and 43 pGBM with BRAF V600E mutations and 25 adult GBM with BRAF mut ations. Total DNA was extracted from FFPE samples and BRAF was amplified using targeted bisulfite sequencing. BRAF mutations were then analyzed using Sanger sequencing.

RESULTS: There were no mutations in BRAF in the two subsets of pGBM and adult GBM. Of the 25 pediatric brainstem GGs screened for BRAF mutations, 8 (32%) were positive for mutant BRAF, including 5 (20%) with BRAF V600E. Of these 8 samples, 6 (75%) had BRAF exon 15 mutations, consistent with the findings of past studies. Our results validate that BRAF mutation is a genetic hallmark of GG that arises in the brainstem and is present in a significant fraction of tumors including those in the brainstem.

CONCLUSIONS: BRAF mutations in GG, including those in the brainstem, are frequent and likely occur early in the developmental process. Further studies are needed to determine the implications of BRAF mutations in GG and their role in tumor biology.

**0107. IN VIVO AND FUNCTIONAL ANALYSIS OF NOVEL MAPK PATHWAY ACTIVATING MUTATIONS IN CHILDHOOD ASTROCYTOMA**
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INTRODUCTION: Pilocytic astrocytoma (PA), the most common childhood brain tumor, can occur throughout the central nervous system. These...
tumors harbor oncogenic alterations of the MAPK/ERK pathway. While most of the cerebellar tumors mainly show alterations of BRAF, the majority of cases without identified MAPK alterations arise outside the cerebellum. Although survival rates are excellent, there is an unmet need for complete surgical resection resulting in multiple recurrences and a considerably reduced quality of life. Using deep sequencing techniques we have identified alternative MAPK alterations in these tumors, which are now subject to further validation in vitro and in vivo. METHOD: To test the downstream signaling of the identified mutations on a protein level, we have assessed their potential to activate MAPK signaling and possible synergistic combinations of alterations using in vitro models. Furthermore, since a new BRAF mutation we have identified suggests a novel mechanism of enhanced dimerization of the mutant leading to constitutive activation, we looked at protein-protein binding using co-immunoprecipitation. For in vivo analysis, the mutant genes will be delivered to nestin-positive cells of the neonatal mouse cerebellum using the RCAS/TV-a technique testing them for their tumorigenic potential. RESULTS: We have identified point mutations which have not been described in PAs so far. The most striking alterations were two hot spot mutations of the FGFR1 tyrosine kinase with co-occurring V600E model for PA. In the case of successful tumor induction, the established causal role in PA pathogenesis, these mutations are currently being tested for enhanced dimerization which we verified by co-immunoprecipitation. Structural data suggested that this could confer constitutive activity through two hot spot mutations of the FGFR1 tyrosine kinase with co-occurring have not been described in PAs so far. The most striking alterations were MEDULLOBLASTOMA

**INTRODUCTION:** Much has recently been discovered with respect to genomic and transcriptomic alterations underlying medulloblastoma, the most common embryonal brain tumor. One of the most important insights from recent studies is that medulloblastoma is not a single disease, but rather comprises four core molecular subgroup. While the WNT and SHH subgroups are relatively well characterized in terms of key pathways and alterations involved in tumorigenesis, Group 3 and Group 4 tumors remain poorly understood despite extensive genomic screens. We therefore sought to characterize global genomic alterations occurring in medulloblastomas as part of the International Cancer Genome Project (ICCG) PedBrain Tumor project. METHOD: In order to get a global, base-resolution profile of the medulloblastoma genome, we performed high-throughput whole-genome bisulfite sequencing on a total of 34 primary tumors and 8 normal cerebellar samples. In-house algorithms were designed in order to optimize mapping and downstream analysis of bisulfite-converted DNA reads. To supplement this, we also conducted genome-wide methylation analysis on over 300 primary medulloblastoma samples using the Illumina Infinium HumanMethylation450 bead array. Matched transcriptome data, either from Affymetrix U133 Plus2 arrays or RNA sequencing, as well as miRNA sequencing data, was available for over 100 tumors, allowing correlation of gene expression with methylation. Results: We identified 235 microRNAs that were upregulated and 22 microRNAs decreased in the invasive GBM cells in at least 5 of the 7 GBM models. While some of the microRNAs have been previously associated with tumor invasion (such as miR-126 and miR-138) or deregulated in multiple human tumors (such as miR-18a and miR-383), most of the identified microRNAs have not been associated with GBM invasion. We investigated whether dasatinib, an MDR substrate, would have significantly higher anti-tumor activity in a DIPG mouse model deficient for ABCG2 and MDRa. METHOD: We infected the brainstem of neonatal mice with a human medulloblastoma cell line that expresses the RCAS/TV-a technique testing them for their tumorigenic potential. RESULTS: We have identified point mutations which have not been described in PAs so far. The most striking alterations were two hot spot mutations of the FGFR1 tyrosine kinase with co-occurring

**INTRODUCTION:** Diffuse invasion into normal brain tissue is one of the most important biologic features that make GBM particularly difficult to treat. Efforts of developing new targeted therapies against GBM invasion are hampered by our poor understanding of the invasion process, primarily because the invasive GBM cells are rarely available for comparative analysis. MicroRNAs have been implicated in regulating diverse cellular pathways; their role in mediating glioma invasion has not been fully explored. We hypothesize that direct comparison of the matched invasive and tumor core GBM cells would facilitate the discovery of key genetic changes driving GBM invasion. METHOD: To isolate matched pairs of invasive and tumor core GBM cells, whole mouse brains from a panel of 7(1 adult and 6 pediatric) patient-tumor derived orthotopic xenograft mouse models were cut into 1 mm slices, followed by microscopic dissection of tumor mass from the mouse brains from the invasive GBM cells were isolated. MicroRNA expression was profiled with Taqman MicroRNA array (768 microRNAs); and whole genome gene expression with Affymetrix chips. To identify the affected target genes, we correlated the mRNA levels of the predicted microRNA targets with the changes of the dysregulated microRNAs. RESULTS: We identified 23 microRNAs that were upregulated and 22 microRNAs decreased in the invasive GBM cells in at least 5 of the 7 GBM models. While some of the microRNAs have been previously associated with tumor invasion (such as miR-126 and miR-138) or deregulated in multiple human tumors (such as miR-18a and miR-383), most of the identified microRNAs have not been associated with GBM invasion. When the mRNA expression levels of the predicted genes were examined, the microRNAs with elevated expression in the invasive front appear to have better reverse correlation than those microRNAs that were down regulated. CONCLUSIONS: In conclusion, we have established a novel **in vivo** model system that allows for isolation of paired and biologically accurate invasive and tumor core GBM cells in **in vivo** mouse brains. Using this system, we have identified a novel subset of microRNAs that are significantly altered in the invasive cells. This group of microRNAs warrants further evaluation as potential therapeutic targets.

**INTRODUCTION:** DIPG is an incurable tumor that arises in the brainstem of children. Our lab evaluates novel inhibitors in a DIPG mouse model to help progress the translation of novel treatments. The DIPG mouse model is generated by expressing PDGF-B and Cre in nestin progenitors of the neonatal brainstem of nestin tv-a; p53 floxed mice. Dastatinib, an inhibitor of PDGFR-A and SRC activation, is currently being evaluated in clinical trials for children with DIPG. We investigated whether dasatinib, an MDR and ABCG2 substrate, would have significantly higher anti-tumor activity in a DIPG mouse model deficient for ABCG2 and MDRa. METHOD: We infected the brainstem of neonatal...
0111. IN VIVO CYTOTOXICITY OF TEMOZOLOMIDE AGAINST DAOY CELLS MAY BE POTENTIATED BY ENZYMATIC DEPLETION OF ASPARAGINE/GLUTAMINE
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INTRODUCTION: Enzymatic depletion of amino acids for therapeutic purposes is understudied in brain tumors. Asparaginase (ASase) effectively contributes in acute lymphoblastic leukemia by decreasing asparagine levels in serum and CSF without crossing the blood-brain barrier. Cancer cells may become ASase resistant by up-regulation of asparaginase synthetase (ASNS), higher expression of which has been linked to biological aggressiveness of various tumors, and perhaps including gliomas (probesetanalyzer.thermofisher.com). We tested if depletion of asparagine and glutamine may augment brain tumor cell death in vitro or in vivo. METHOD: First, in vitro enzymatic treatment with ASase (or inhibition of glutamine synthetase by methionine sulfoximine) demonstrated activity against brain tumor cell lines (MTS assay), which can be reversed by glutamine supplementation. ASNS expression (by PCR) is likely to be responsible for variable sensitivity patterns observed in our cell lines. Given that our initial in vitro data suggested possible synergistic effect of ASNase and cytotoxic agents, we have utilized heterotopic subcutaneous DAOY xenografts (1x10^5 cells/animal) in SCID mice to test Temozolomide and ASNase combination. Over three weeks, transplanted animals (n = 5 in each group) were treated with IP ASNase and/or temozolomide. Tumor growth kinetics were compared and included untreated controls (serum asparaginase depletion in ASNase treated animals was confirmed with HPLC previously). In Vivo data showed no effect with ASNase mono-therapy; Tumor growth kinetics were compared and included untreated controls with at least 4 mice per group (ABC KO-drug, ABC KO-vehicle, ABC WT-drug, ABC WT-vehicle). Treated mice were sacrificed 24 hours later and brains were extracted and placed in formalin. FFPE sections were stained for cleaved caspase-3 (CC3) and phospho-histone H3 (pH3) by immunohistochemistry.

RESULTS: There was no significant survival difference between DIPG-bearng mice in ABC KO and ABC WT mice. One dose of dasatinib to DIPG-bearing ABC KO mice resulted in a significant increase in CC3 levels relative to control (p = 0.03) which was reversed with the addition of ASase to DIPG-bearing ABC WT mice did not result in a significant increase in CC3 levels relative to vehicle (p = 0.30). Treatment with dasatinib resulted in 20-fold higher levels of CC3 levels in DIPGs of ABC KO mice relative to DIPGs of ABC WT mice (p = 0.002). pH3 levels did not change significantly in response to dasatinib treatment alone. CONCLUSIONS: This study demonstrates the potential role of amino acid depletion in brain tumor therapeutics. Additional studies with parallel pharmacodynamic measurements to further elucidate potential mechanisms are ongoing.

0112. A PATIENT-TUMOR-DERIVED ORTHOTOPIC NEUROECTODERMAL TUMOR OF THE BRAIN OF CHILDHOOD: A CASE REPORT
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INTRODUCTION: Supratentorial primitive neuroectodermal tumor (pNET) is a highly malignant brain tumor with poor prognosis. New models that replicate the molecular subtypes and maintains the cancer stem cells (CSCs) of this deadly disease is highly desired. Here, we describe the establishment of a novel transplantable orthotopic xenograft mouse model through direct injection of fresh surgical specimen. We treated a pediatric brain tumor of childhood, namely medulloblastoma, and generated long-term survivors. Our ability to treat these patients and spare normal tissues do not align completely. METHOD: We are generating iPSC-cell based models of medulloblastoma, which enable us to model SCNVs. First, we are differentiating human iPSC to two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs.

RESULTS: Testing multiple differentiation protocols, we have successfully generated NSCs from our human iPSCs, as evidenced by positive staining for NSC markers Nestin, Sox2, and Pax6, and negative staining for the pluripotency marker Oct4. In addition, we successfully generated GNPC derived from human iPSCs, evidenced by positive staining for GNPC markers Math1, En1, Gbx2 and HoxA2. However, modeling SCNVs is challenging, as human and mouse chromosomes do not align completely. METHOD: We are generating iPSC-cell based models of medulloblastoma, which enable us to model SCNVs. First, we are differentiating human iPSCs to two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs. We are differentiating human iPSCs toward two distinct and presumed cells of origin of medulloblastoma: neural stem cells (NSC) and granule neuron precursor cells (GNPC). Separately, we are developing Transcription Activator-Like Effector Nucleases (TALEN) to knockout TP53 and PCDH11 in NSCs.
INTRODUCTION: Medulloblastoma (MB) is the most common paediatric malignant brain tumor. By the way of optimal surgery, radiation, and chemotherapy, the majority of patients show favorable outcomes. However, a small number of these children will eventually relapse, and their treatment remains a significant challenge.

MATERIALS AND METHODS: We developed a novel murine model of metastatic MB, which is highly penetrant, has a short latency, and involves random secondary genetic events. We investigated the genetic landscape of primary and leptomeningeal metastatic MB tumors through whole-genome sequencing and targeted sequencing of key genes. The results were compared with primary MB tumors from the same patient.

RESULTS: We observed two new mutations off the hotspots at IDH1 and IDH2 gene. One A-I sample showed R172 mutation, one A-II sample showed R132 mutation, one A-I sample showed R172 mutation and one A-II sample showed R132 mutation. Two mutations were found off the previously described hotspots, one in MB and one in leptomeningeal metastases. One A-I sample showed R172 mutation, one A-II sample showed R132 mutation, one A-I sample showed R172 mutation and one A-II sample showed R132 mutation.

CONCLUSIONS: We demonstrated that the expected mutations may not necessarily be in the exon 4, but also in other regions of the gene. We observed a smaller frequency of mutations in IDHs than previously described and there is growing evidence for widespread presence of these mutations in different cancers, we assume that the expected mutations may not necessarily be in exon 4, but also in other regions of the gene.
xenografts. This activation was associated with an increase in AKT activity and may also contribute to MAPK recovery from inhibitor treatment of BRAF-mutant gliomas. Addition of an EGFR kinase inhibitor to either BRAF or MEK inhibitor prevents EGFR/AKT activation, resulting in increased anti-tumor effect against BRAF-V600E cell lines and xenografts. CONCLUSIONS: BRAF or MEK inhibition results in EGFR activation in glioma cell lines with BRAF-V600E mutation. This appears to be a consequence of reactivation of a negative feedback loop downstream of MEK. Combined EGFR and MEK (or BRAF) inhibition prevents EGFR activation, resulting in improved efficacy from combination treatment, both in vitro and in vivo.

0118. NOVEL SMALL MOLECULE AND ENZYME REGULATORS OF HDACs: NEW EPGENIC TARGETS

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INTRODUCTION: The role of epigenetics in pediatric brain tumor development has been highlighted in a number of recent studies. A more complete understanding of epigenetic mechanisms is needed to fully elucidate the pathogenicity of these tumors and potential therapeutic targets. To that end, we identify a novel regulator of class I histone deacetylase (HDAC) activity, inositol polyphosphate multikinase (IPMK). IPMK is required for the generation of higher-order insoluble polyphosphates, an under-characterized class of second messengers involved in numerous cell-signaling processes. This finding opens the door for the development of more targeted HDAC inhibitors and insights into important mechanisms of epigenetic regulation. METHOD: Protein-protein interaction screens were performed with over-expressed IPMK and HDACs 1 through 8 in HEK 293T cells with further analysis in mouse embryonic fibroblasts (MEFs) to confirm endogenous interaction. To further investigate the role of IPMK in histone acetylation, a CRE-inducible IPMK knock-out model was utilized in MEFs and the role for inositol kinase activity was assessed with and without expression of IPMK constructs. Cell-based assays of HDAC activity were performed in this knock-out model using a reporter-based assay, followed by in vitro activity assays designed to elucidate the mechanism(s) by which IPMK regulation of HDACs occurs. RESULTS: Protein-protein interaction studies demonstrated that IPMK strongly interacts with HDAC1, HDAC2, and HDAC3. CRE-mediated loss of IPMK lead to a decrease in the acetylation of numerous histone lysine residues, while return/ rescue of kinase-active IPMK restored acetylation levels. In vivo, cell-based reporter analyses of HDAC activity identified IPMK as a negative regulator of HDAC3. Additional studies elucidated the mechanism by which IPMK affects active complex formation of HDAC3 with its co-repressor SMRT. This mechanism likely depends upon the recently characterized presence of an inositol polyphosphate positioned in the interaction surface of the HDAC3/SMRT complex. CONCLUSIONS: The role of epigenetics and epigenetic dysregulation in pediatric brain tumors has been highlighted in a number of studies that identify genetic alterations in epigenetic elements. These findings emphasize the need for more research on this important and rapidly evolving area of biology. To that end, we have identified IPMK as a novel regulator of class I HDACs, particularly HDAC3. These findings provide the opportunity for new pharmacologic targets and will direct future studies into the complex regulation of HDACs. They also help to better define the diverse roles of IPKs and their enzymatic products.

0119. GENE NETWORKS IMPLICATED IN PEDIATRIC BRAIN TUMORS PROVIDE THE OPPORTUNITY FOR NEW PHARMACOLOGIC TARGETS AND WILL DIRECT THE PATHWAY TO THERAPEUTIC DEVELOPMENT

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INTRODUCTION: MicroRNAs (miRNAs) are involved in tumorigenesis and tumor progression by regulating post-transcriptional gene expression. However, the miRNA-mRNA regulatory network is far from being fully understood. The objective of this study is to identify the ependymoma specific miRNAs and their target mRNAs using an integrated approach. METHOD: We performed microRNA as well as mRNA expression analysis of 64 ependymoma samples: 23 Posterior fossa (PF); 29 Supratentorial (ST); and, Spinal (SP) tumors by means of microarrays. RESULTS: We identified 170 miRNAs and 1911 genes that are differentially expressed between different tumor types. Expressions of 52% miRNAs are decreased in ST compared to PF and SP. The integrated analysis of miRNA and mRNA expression pointed out regulatory networks including HADC4, MTSS1L, SDC1, and EPHA2 as potential targets of miR-10a. Notably, a family of several miRNAs including miR-23, miR-27, and miR-30 alters the expression of EPHB2 whereas the expression of LAMA2 is altered by miR-29. Genes involved in neurogenesis (P = 1.31 x 10^-25) and cytoskeletal protein binding (P = 5.83 x 10^-30) are enriched in significant miRNA-mRNA anti-correlations. CONCLUSIONS: This study provides a comprehensive dataset as well as methods and systems-level results that jointly form a basis for further work on understanding the role of miRNA in ependymoma.

0120. INTEGRATED ANALYSIS OF microRNA AND mRNA EXPRESSION PROFILES HIGHLIGHTS ALTERATIONS IN EPENDYMOMA

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INTRODUCTION: MicroRNAs (miRNAs) are involved in tumorigenesis and tumor progression by regulating post-transcriptional gene expression. However, the miRNA-mRNA regulatory network is far from being fully understood. The objective of this study is to identify the ependymoma specific miRNAs and their target mRNAs using an integrated approach. METHOD: We performed microRNA as well as mRNA expression analysis of 64 ependymoma samples: 23 Posterior fossa (PF); 29 Supratentorial (ST); and, Spinal (SP) tumors by means of microarrays. RESULTS: We identified 170 miRNAs and 1911 genes that are differentially expressed between different tumor types. Expressions of 52% miRNAs are decreased in ST compared to PF and SP. The integrated analysis of miRNA and mRNA expression pointed out regulatory networks including HADC4, MTSS1L, SDC1, and EPHA2 as potential targets of miR-10a. Notably, a family of several miRNAs including miR-23, miR-27, and miR-30 alters the expression of EPHB2 whereas the expression of LAMA2 is altered by miR-29. Genes involved in neurogenesis (P = 1.31 x 10^-25) and cytoskeletal protein binding (P = 5.83 x 10^-30) are enriched in significant miRNA-mRNA anti-correlations. CONCLUSIONS: This study provides a comprehensive dataset as well as methods and systems-level results that jointly form a basis for further work on understanding the role of miRNA in ependymoma.

0121. PHASE II STUDY OF SORAFENIB IN CHILDREN WITH RECURRENT/PROGRESSIVE LOW-GRADE ASTROCYTOMA

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INTRODUCTION: Activation of the RAS-RAF-MEK-ERK signaling pathway, most commonly through loss of NF1 or KIAA1549-BRAF tandem duplication, is thought to be a driver of pediatric low-grade astrocytoma (PLGA) growth. Sorafenib is a multi-kinase inhibitor targeting BRAF, VEGFR, PDGFR and c-kit. This multi-center phase II study was performed to determine the response rate to sorafenib in children with recurrent or progressive PLGA. We are reporting clinical study results, along with complementary preclinical data using sorafenib. METHOD: Twelve patients, including three with neurofibromatosis type 1 (NF1), were enrolled on this trial. Key eligibility criteria included age ≥ 2 years and at least one prior standard chemotherapy treatment. Histological confirmation was required, except for optic pathway gliomas. Sorafenib was administered twice daily at 200mg/m2 dose (maximum of 400mg/day). Magnetic resonance imaging, including three-dimensional volumetric tumor analysis, was performed after every third 28-day cycles, or earlier if clinically indicated. Radiologic response to sorafenib was determined by analyzing tumor progression by regulating post-transcriptional gene expression. However, the miRNA-mRNA regulatory network is far from being fully understood. The objective of this study is to identify the ependymoma specific miRNAs and their target mRNAs using an integrated approach. METHOD: We performed microRNA as well as mRNA expression analysis of 64 ependymoma samples: 23 Posterior fossa (PF); 29 Supratentorial (ST); and, Spinal (SP) tumors by means of microarrays. RESULTS: We identified 170 miRNAs and 1911 genes that are differentially expressed between different tumor types. Expressions of 52% miRNAs are decreased in ST compared to PF and SP. The integrated analysis of miRNA and mRNA expression pointed out regulatory networks including HADC4, MTSS1L, SDC1, and EPHA2 as potential targets of miR-10a. Notably, a family of several miRNAs including miR-23, miR-27, and miR-30 alters the expression of EPHB2 whereas the expression of LAMA2 is altered by miR-29. Genes involved in neurogenesis (P = 1.31 x 10^-25) and cytoskeletal protein binding (P = 5.83 x 10^-30) are enriched in significant miRNA-mRNA anti-correlations. CONCLUSIONS: This study provides a comprehensive dataset as well as methods and systems-level results that jointly form a basis for further work on understanding the role of miRNA in ependymoma.
introduces tumor growth as well as migration and invasion. This suggests that NLR can act as an oncogene in MB and could be potentially involved in the metastatic properties of subgroup 3. Our results based on shRNA mediated loss of function support a crucial role of NLR for MB progression. CONCLUSIONS: We demonstrate that NLR is required for cell cycle progression and protection from apoptosis in MB. Accordingly, NLR knock-down increases survival in orthotopic grafting experiments. We identify two NLR target genes by ChIP, the expression of which is correlated with NLR in human MBs that are likely involved in these two activities. The current study demonstrates for the first time an oncogenic role of the photoreceptor transcription factor NRL and that the photoreceptor program participates actively in tumor progression of group 3 MB.

0124. GLIOMA-ASSOCIATED SOMATIC MUTATIONS DRIVE A RAPID CONVERSION OF NEURAL STEM CELLS INTO TUMORIGENIC OLIGODENDROGLIAL PROGENITORS

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INTRODUCTION: Recent findings regarding the cell (or cells) of origin in brain tumors have yielded conflicting results. Specifically, several studies have indicated that glioma stem cells show remarkable similarity to neural stem cells (NSCs). Alternatively, a large body of evidence by multiple groups has shown that in many cases the tumor cell of origin is a rapidly proliferating oligodendrogial progenitor cell (OPC)-like population. METHOD: We have created a novel, autochthonous, in vivo model of grade high oligodendroglioma which allows for exquisite control over the genetic determinants as well as the temporal and spatial genesis of glioma, permitting for greater insight into the cellular dynamics of tumor initiation. Somatic mutations of the RTK/Ras pathway are introduced by neonatal electroporation of plasmid DNA in combination with transposon technology. RESULTS: Within 40 hours, transgenes are expressed at a high level with coincident fluorescent protein labeling. Notably, starting at these early time points and proceeding through the first week, radial glial stem cells transform into antigenev grassroots oligodendroglial-like progenitor cells, prematurely depleting the neural stem cell population. CONCLUSIONS: Thus, we conclude that naturally-occurring somatic mutations drive a rapid and virtually complete conversion of NSCs into an OPC phenotype in a manner that would be difficult to observe in other models, thus unifying the previously disparate findings.

0125. DEVELOPMENT OF A FIVE-GENE HEDGEHOG SIGNATURE AS A PATIENT PRESELECTION TOOL FOR HEDGEHOG PATHWAY-TARGETED THERAPY IN MEDULLOBLASTOMA

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INTRODUCTION: Medulloblastoma (MB) is the most common malignant brain tumor in children. This tumor arises in the cerebellum and is highly invasive. Recent transcriptomic studies identified four MB subgroups: WNT, HH, 3 and 4. Group 3 is of poorer prognosis, frequently metastatic and refractory to current therapies. Despite these clinical features, it remains poorly characterized. It expresses genes of the "photoreceptor" a differentiation program. It is not infrequent that cancer cells express aberrant terminal differentiation programs unrelated to the tissue of origin. These aberrant differentiation programs are not thought to participate directly and actively to cancer progression. METHOD: The photoreceptor differentiation program, normally expressed in retina, has long been identified in MB but still remains an enigma. This program found in group 3 includes the photoreceptor specific transcription factor Neural Retina Leucine zipper (NRL). NRL expression is highly specific of photoreceptors in the retina, where it controls their terminal differentiation. Although it belongs to the MAP oncogene oncogenic, its oncogenic activity has not been assessed. MAP proteins have been demonstrated to be atypical oncogenes involved in terminal differentiation during normal development, but potent oncogenes in cancer. RESULTS: We are currently investigating the involvement of this key player of the photoreceptor program NRL in the MB metastatic subgroup 3, using in vitro and in vivo approaches including xenografts and orthotopic grafting. We show that overexpression of NRL in MB cell lines
Cis-retinoic acid (RA) was used to promote neurite outgrowth and/or depolymerize the actin cytoskeleton or with colchicine to disrupt microtubules. Malignant glioma cells were treated with cytochalasin D to depolymerize actin. RESULTS: The five-gene signature assay for determination of Hh activation status demonstrated a perfect concordance with the status determined by Affymetrix profiling in 25 independent MB samples. Analysis of MB samples from 37 patients treated with LDE225 showed a correlation between Hh activation status and tumor response. All six patients evaluated who responded (partial or complete response by RECIST 1.0) to LDE225 treatment were predicted to have Hh pathway-activated tumors using the five-gene signature assay. Of the 31 remaining patients, 29 were predicted to have Hh pathway-nonactivated tumors and two (with stable disease) were predicted to have Hh pathway-activated tumors. CONCLUSIONS: A five-gene Hh signature assay, based on RT-PCR, was developed and validated for use as a patient preselection tool for Hh inhibitor therapy. A strong correlation between Hh activation status, as predicted by the five-gene signature assay and response to LDE225 treatment was observed. The predictive value of the assay for treatment benefit in patients with recurrent MB is under further evaluation in an ongoing phase I/II trial of LDE225. In addition, the five-gene Hh assay will be used to determine eligibility in a randomized phase III trial of LDE225 versus temozolomide in patients with Hh pathway-activated relapsed MB.

0128. UNCOVERING CLONAL EVOLUTION PATTERNS IN MEDULLOBLASTOMA METASTASES USING WHOLE GENOME SEQUENCING

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INTRODUCTION: Medulloblastoma is the most common malignant brain tumor in children. Although MB arises in the cerebellum, 40% of patients have leptomeningeal dissemination at diagnosis, a strong marker of poor prognosis. Despite surgery, whole brain and spinal cord radiation, and aggressive chemotherapy, patients with disseminated disease often have a poor outcome. Response to therapy can differ between the primary tumor and the metastases. The aims of our current study are (1) to understand the clonal evolution of disseminated disease from primary tumors, and (2) to identify metastasis-specific driver events that may provide targets for novel therapies. METHOD: We generated whole genome sequencing data from 12 children with matched primary and metastatic disease. Somatic aberrations were derived from this data, and 192 events (SNVs, indels) were selected from each patient for verification using deep amplicon sequencing. Selected variants included those detected in either the primary or metastatic tumor and events displaying significant allelic frequency changes between primary tumor and metastasis, and all damaging mutations that could be identified. Deep sequencing at these loci (~20,000X coverage) provides the necessary sampling depth to robustly identify changes in the clonal composition of the primary and metastatic tumors. RESULTS: The WGS data reveals shared mutations between patient cohorts with low or high grade glioma. Furthermore, with the availability of a GMF8 knockout mouse, the role of GMF8 in glioma tumor invasion and signaling pathways can be addressed in vivo.
the primary and metastatic tumors, confirming their clonal relationship. In the first patient studied, deep-sequencing identified metastasis-specific mutations confirmed to be absent in the primary tumor genome, indicating these arose de novo post-dispersal. Another subset of mutations, initially observed as metastasis-restricted in the WGS data, were identified at subclonal levels in the primary tumor. Together, these data support a model of dispersal in which a restricted clone in the primary tumor seeds metastases, and is under- goes further selection of existing and novel mutations. Mutations specific to each grade of damaging mutations observed are enriched either in the primary or the metastatic compartments. Deep-sequencing data confirms that active clonal evolution is evident and affects the spectrum of damaging mutations in these disease compartments. This underscores the importance of studying metastatic disease, and the relevance of such an approach to the design of rationally applied targeted therapies.

0129. PHASE II STUDY OF RAD001 IN CHILDREN AND ADULTS WITH NEUROFIBROMATOSIS TYPE 2 AND PROGRESSIVE VESTIBULAR SCHWANNOMAS

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INTRODUCTION: Activation of the mammalian target of rapamycin (mTOR) signaling pathway is thought to be a key driver of tumor growth in Merlin (NF2) deficient tumors. Recent preclinical data indicate that differential activation of mTOR complex 1 (mTORC1) and mTORC2 may be cell-type dependent in NF2-deficient tumors and correlate with response to specific classes of mTOR inhibitors. RAD001 (everolimus) is an oral mTORC1-only inhibitor with anti-tumor activity in a variety of cancers. We conducted a single institution, prospective, open-label, two-stage phase II study to estimate the response rate to RAD001 in neurofibromatosis type 2 (NF2) patients with progressive vestibular schwannomas (VS). METHOD: Ten eligible patients were enrolled. RAD001 was administered at a daily dose of 10 mg (adults) or 5 mg/m²/day (children ≤ 18 years) PO in continuous 28-day cycles, for up to 12 cycles. Response was assessed every 3 months with MRI using 3-D volumetric tumor analysis, and audiograms. Nine patients were evaluable for the primary endpoint (one patient came off study after 3 weeks for personal reasons), defined as a ≥15% decrease in VS volume. Hearing response, defined as statistically significant increase in word recognition scores, was evaluable as a secondary endpoint in eight hearing patients. RESULTS: Three patients came off trial due to tumor progression after 3, 6 and 9 cycles, respectively. Three other patients discontinued treatment after 3, 6 and 6 cycles, respectively, because of lack of volumetric and/or hearing response. One patient completed 12 cycles, at which time he met criteria for hearing progression. One patient with stable disease is receiving his 11th cycle of treatment. One patient came off study after 3 cycles due to toxicity, i.e. pneumonia and decreased pulmonary function. All other observed toxicities were minor and expected. No objective imaging or hearing responses were observed and accrual was halted. CONCLUSIONS: None of the 9 patients with evaluable disease experienced an MRI or hearing response. Therefore, we conclude that RAD001 is ineffective for the treatment of progressive VS in NF2 patients. We are currently conducting a pharmacodynamic/pharmacokinetic (“Phase 0”) study of RAD001 including pre-surgical VS patients to elucidate the biological basis for treatment resistance to mTORC1 inhibition in these tumors.

0130. DOMINANT NEGATIVE Olig2 IS INSUFFICIENT TO PREVENT GLIOMA INITIATION AND PROGRESSION

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INTRODUCTION: Recent findings have suggested that Olig genes are essential for the proliferation of tumor cells and progression of glioma. Specifically, it has been documented that Olig genes are important for tumor cell proliferation and resistance to p53 responses to genotoxicity. Moreover, Olig2 knockdown has created a novel model of high grade Oligodendroglioma, which displays ubiquitous expression of Olig2 and other oligodendrocyte markers. This model allows for co-expression of any plasmid. Employing this advantage, we employed a dominant negative form of Olig2 to assess the contribution of Olig2 repressor function to glioma initiation and propagation. RESULTS: Notably, expression of this DN-Olig2 failed to prevent tumor proliferation and invasion. Instead, the tumor cells converted from oligodendroglia-type progenitors into immature astroglial lineage tumor progenitors. CONCLUSIONS: These findings suggest that targeting Olig genes to treat tumors may be ineffective as gliomas can utilize Olig2-negative tumor pathways for progression by changing lineages. However, more investigation needs to be done regarding the possible function of Olig2 as a transcriptional activator in this context.

0131. IDENTIFICATION OF RESISTANCE MECHANISMS TO SMO INHIBITION IN MEDULLOBLASTOMA

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INTRODUCTION: The sonic hedgehog pathway (Shh) has been implicated in one-fourth of all medulloblastoma cases and thus provides a promising therapeutic target for these tumors. Most known inhibitors of the Shh pathway have undertaken an investigation of allele-frequency data from matched primary and terminal metastatic tumors to establish the evolutionary history patterns of medulloblastoma. This disease is characterized by a low number of MSH2 mutations, but notably, even at 30X WGS coverage, the majority of damaging mutations observed are enriched either in the primary or the metastatic compartments. Deep-sequencing data confirms that active clonal evolution is evident and affects the spectrum of damaging mutations in these disease compartments. This underscores the importance of studying metastatic disease, and the relevance of such an approach to the design of rationally applied targeted therapies.

0132. A PIGGYBAC TOOLKIT FOR RAPID SOMATIC MUTATION AND CELL/TUMOR IMAGING IN THE CONTEXT OF IN VIVO PEDIATRIC GLIOMA

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INTRODUCTION: We have created a novel method for studying glioma initiation and progression in vivo-postnatal electroporation combined with piggyBac transposition. Unlike engineered mouse models or virally-derived glioma, electroporation allows for rapid addition of transgenes, simply by the inclusion of plasmid DNA in the initial injection. METHOD: Thus, we have created a toolkit for interrogating the resulting tumor cells in vivo. Specifically, we have created piggyBac plasmids expressing a spectrum of fluorescent proteins for labeling these cells in situ. Moreover, the latest in non-invasive genetic markers, including infrared proteins, luciferase, and a marker for use with magnetic resonance imaging has been cloned for this system. Finally, we are investigating the use of even more precise methods of genetic control, including inducible and reversible transgenic systems. RESULTS: These various imaging modalities have allowed for robust and flexible imaging of tumor cells, in particular, membrane-targeted EGFR has allowed us to observe the rapid depletion of neural stem cells in our oligodendroglioma model. In addition, MRI imaging allows for precise non-invasive
analysis of tumor progression. CONCLUSIONS: Here we discuss these methodologies and their use in the context of our novel pediatric glioma models.

0133. LONG-TERM OUTCOMES FROM A PROSPECTIVE PHASE II STUDY OF MULTI-AGENT SYSTEMIC AND INTRATHecal CHEMOTHERAPy WITH AGE- AND RISK-ADAPTED RADIATIoN thErapy FOR CHILDREN WITH NEwLY DIAGNOSED CNS ATypICAL TERTAID/RHABDOID Tumor (DfCI 02-294)

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INTRODUCTION: Atypical teratoid/rhabdoid tumor (ATRT) of the central nervous system (CNS) is a highly malignant neoplasm primarily affecting young children, with a historical median survival ranging between 6.5–10 months. Based on a successful pilot series of patients with newly diagnosed and recurrent disease (Zimmerman, J Neuro-oncol, 2003), a prospective multi-institutional trial was conducted using a modified IRS-III regimen, including intrathecal chemotherapy, for patients with newly diagnosed CNS AT/RT (DFCI 02-294). The early results of this prospective trial were previously published (Chi, JCO, 2009). We present the long-term survival outcomes. METHOD: Treatment was divided into five phases: pre-irradiation induction chemotherapy (Weeks 1-6); chemo-radiation (Weeks 7-12); consolidation (Weeks 13-18); maintenance (Weeks 19-44); and continuation therapy (Weeks 45-51). Intrathecal chemotherapy administration alternated between the intra-lumbar and intra-ommaya routes. Patients with M0 stage received focal conformal radiation therapy (RT) at the prescribed time to a total dose of 5400 cGy. For patients over the age of 3 years with M+ disease at diagnosis, 3600 cGy craniospinal irradiation (CSI) was prescribed, with boost to primary sites of disease to total dose of 5400 cGy. RESULTS: Of 25 children enrolled, 22 were evaluable. Median age at diagnosis was 2.5 years (0.2 - 19.5 yrs). Twelve primary tumors were located in the supratentorial compartment, 10 in the posterior fossa. GTR of the primary tumor were achieved in 11 patients. Sixteen patients had M0 disease, one M2 and five M3. All patients received intrathecal chemotherapy. Seventeen patients received RT. Of the 14 patients evaluable for chemotherapy response, the objective RR was 62%, and from RT, 38%. The 3-year EFS and OS are 36% and 46 +/- 13%, respectively. CONCLUSIONS: For this rapidly fatal disease, significant progress has been made in terms of improving and sustained survival. A future international study is planned to test the combination backbone regimen against high-dose chemotherapy/autologous stem cell transplant, incorporating the growing biologic data on rhabdoid tumors.

0134. RNA nanoparticle vaccines re-direct host-immunity against intracranial malignancies

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INTRODUCTION: Malignant brain tumors are the number one cause of cancer death in children necessitating the development of targeted therapeutics. RNA-nanoparticle (RNA-NPs) can meet this need through delivery of total tumor RNA (tRNA), targeting a broad repertoire of undefined and patient specific tumor antigens, to dendritic cells (DCs) inducing potent, non-toxic anti-tumor immunity. Since NPs have been used with limited toxicity in clinical-grade medicine, protect nucleic acids from degradation, and can be engineered to modulate immune responses, we have explored tRNA-loaded NPs as an attractive “off-the-shelf” vaccine platform to target primary DCs in order to stimulate host immunity to precipitate tumor efficacy. METHOD: The current study was undertaken to determine if vaccination with tRNA-NPs would recruit DCs to effectively cross-prime immune responses generating anti-tumor efficacy in pediatric brain tumors without the induction of intolerable autoimmunity. To achieve this end, we investigated RNA-NP localization, DC trafficking, and their engagement of innate and specific immune responses using model antigens in TCR transgenic mice. Subsequently, anti-tumor efficacy was evaluated in intracranial tumor bearing mice vaccinated with NPs complexed with tRNA-NPs extracted from murine medulloblastoma and glioma models. RESULTS: We screened commercially available and clinically translatable NP formulations and determined that the cationic liposome DOTAP was superior for delivery of RNA. Afterwards, we demonstrated the capacity to measure protein expression from RNA-NPs in vivo using a luciferase reporter assay and verified that RNA-NPs transfected DCs in vitro and in vivo. Furthermore, RNA-NPs were superior in the expansion of antigen specific T cells in vivo compared with positive control peptide vaccinations in complete Freund’s adjuvant. Finally, we established that RNA-NPs generate anti-tumor efficacy in established intracranial tumors that can be potentiated with immunomodulatory RNAs encoding for GM-CSF. CONCLUSIONS: RNA-NPs are a novel platform for inducing potent nontoxic anti-tumor immunity in tumor bearing mice and may be harnessed to provide a more effective and specific therapy critical in improving clinical outcomes for children affected by malignant brain tumors without adding further toxicity to existing treatments. This data will establish the preclinical feasibility, efficacy, and toxicity data to support the rationale for clinical development of tumor RNA-loaded NP vaccines for medulloblastomas, and has a wide range of clinical applicability for all malignancies that can be targeting using tRNA obtained from surgical resection of solid tumors.

0135. BET bromodomain inhibition of MYC-amplified medulloblastoma

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INTRODUCTION: Group 3 medulloblastomas are highly lethal tumors characterized by MYC amplification. They are notably resistant to standard chemotherapy and radiotherapy, yet the development of targeted therapies against these tumors has been hampered by a lack of compounds that directly target MYC. It was recently discovered that drugs targeting bromodomains, protein regions that recognize acetylated lysine residues such as those found on N-terminal histone tails, effectively shut down MYC-associated transcriptional activity. JQ1 is a compound that inhibits BRD4, a BET bromodomain containing protein expressed in Group 3 medulloblastomas. Here, we investigate BET bromodomain targeting for the treatment of Group 3 medulloblastoma. METHOD: We tested the efficacy of JQ1 in established and newly generated patient- and GEMM-derived Group 3 medulloblastoma cell lines and xenografts, in vitro and in vivo. Non-MYC-amplified cell lines and normal human SVZ-derived neural stem cells were tested as controls. We performed cell cycle profiling and analyzed markers for apoptosis, senescence and differentiation after treatment with JQ1 versus control. The effect of JQ1 on MYC expression and global MYC-associated transcriptional activity was assessed by qPCR and gene expression microarray profiling, respectively. In vivo efficacy of JQ1 was assessed in orthotopic xenografts established in immunocompromised mice. RESULTS: Treatment of MYC-amplified medulloblastoma cells with the active isomer of JQ1 (JQ1-S) resulted in decreased cell viability compared to treatment with the inactive isomer (JQ1-B). JQ1-S treatment downregulated MYC expression and MYC-associated transcriptional activity. JQ1-S treatment also resulted in a decrease in the percentage of cells transitioning through S phase compared to cells treated with JQ1-B. In contrast, no effect was observed with JQ1 on neural stem cells derived from the subventricular zone. We will present data from experiments which are currently underway examining the efficacy of JQ1 in vivo models. CONCLUSIONS: JQ1 represses MYC expression and MYC-associated transcriptional activity in Group 3 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-driven medulloblastomas. Furthermore, these data serve as rationale to rapidly move forward with early phase clinical trials examining the role of BET bromodomain inhibitors for children with these highly lethal tumors.

0136. Longitudinal Cortical Thickness Changes in Children Treated for Medulloblastoma

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INTRODUCTION: Children treated for medulloblastoma have neuroanatomical changes associated with neurocognitive deficits (IQ, attention, working memory, and processing speed). Based on a previous cross-sectional
study reporting atypical cortical thinning in patients 1-8 years after therapy, it was anticipated that higher radiation doses to cortex around the posterior fossa and more distal cortical regions still undergoing maturation would exhibit the greatest thinning. This study explored longitudinal differences in cortical thickness in patients before, during, and following therapy. METHOD: Ten patients with medulloblastoma (7.6-20.3 years; 5 male; 6 average and 4 high risk) treated on the SJMB03 protocol (http://clinicaltrials.gov/ct2/show/study/NCT00852502) were imaged at 12 and 24 months, and 10 age-matched controls (7.6-20.7 years; 7 male) were imaged three times 12 months apart. High-resolution 3D T1-weighted MR imaging was acquired on a 3T whole-body system and processed with the FreeSurfer software (http://surfer.nmr.mgh.harvard.edu) to assess cortical thickness. Statistical testing of cortical thickness differences between patients and controls was conducted at each time point using linear regression modeling with the QDEC package within FreeSurfer. All reported cortical differences were significant with p < 0.01. RESULTS: There was extensive cortical thinning in patients compared to controls at baseline, which may be attributed to disease, hydrocephalus, steroids, or surgery, but is present before either irradiation or chemotherapy. The extent of cortical regions with significant thinning decreased at 12 months and many regions were mostly resolved by 24 months. However, patients had some cortical areas which exhibited persistent thinning even 24 months after diagnosis. These regions included: bilateral superior frontal, right rostral middle frontal, left dorsal lateral frontal (pars orbitalis), bilateral superior and inferior parietal cortex, bilateral lateral occipital, left pericalcarine, and medial temporal. CONCLUSIONS: Children treated for medulloblastoma have significantly thinner cortex compared to their peers even before therapy begins. While cortical thinning resolves for most regions, there are select regions which are persistent through adulthood. These cortical regions with persistent thinning have been associated with neurocognitive functions such as attention and memory. Assessing connectivity (eg, diffusion tensor imaging) within known or suspected functional networks that involve the regions with cortical abnormalities may help identify a neuroanatomical model to characterize the impact of therapy and treatment in this vulnerable population.

INTRODUCTION: Medulloblastoma growth and recurrence are driven by a subpopulation of cells with the stem cell property of unlimited self-renewal. In Sonic hedgehog (Shh) subgroup tumours, these cells’ identity, and the mechanisms by which they propagate the tumour and resist therapy are poorly defined. We hypothesize that rare, therapy resistant Sox2+ stem-like cells propagate Shh-subgroup medulloblastoma. To test this hypothesis, we used the Patched1−/+ mouse model of medulloblastoma to evaluate stem (Sox2+), progenitor (DCX+) and neuronal (NeuN+) cell populations’ proliferative dynamics in primary tumours. METHOD: Tumours were enzymatically digested with trypsin, and imaged by MALDI TOF. Labeling of primary Patched1−/+ tumours was used to quantify cell populations’ proliferation and identify label-retaining cells. Apoptosis was assessed by IHC staining for active Caspase 3 and TUNEL. Intracranial injection of the antimitotic Cytarabine (Ara-C) into Patched1−/+ mice ablated dividing cells. In vivo limiting-dilution analysis (LDA) of cells from primary Patched1−/+; Sox2-EGFP tumours quantified the self-renewal potential of Sox2+ and Sox2− cells. Gene expression data from 64 primary medulloblastoma samples was obtained using the 4 × 44K feature Agilent Whole Human Genome Oligo Microarray. RESULTS: Chronic EdU pulse-chase experiments demonstrated the quiescent nature of Sox2+ cells versus the majority of tumour cells exhibiting rapid label acquisition and dilution. DCX+ progenitor-like cells are fast dividing, while NeuN+ cells are Ki-67−, yet labeled and lost EdU. Single EdU pulse-chase revealed NeuN+ cells are produced by differentiation of proliferating DCX+ cells. NeuN+ cells exhibit greater apoptosis than all tumour cells and Sox2+ cells. These cells were confirmed tumour-mutating cells by eliminating dividing cells enriched for Sox2+ cells, indicating therapy resistance. In human tumours, Sox2 is overexpressed within the Shh-subgroup where its expression negatively correlates with survival. CONCLUSIONS: For the first time, we have characterized self-renewal and proliferative heterogeneity in an in vivo brain tumour model. Our results suggest that Shh-subgroup medulloblastoma is propagated as an aberrant developmental process, where slowly dividing Sox2+ stem cells produce rapidly dividing progeny that differentiate into short-lived neuronal cells. Sox2−/Lo Ki67− are therapy resistance due to their quiescent nature. The negative correlation between Sox2 expression and survival in human tumours points to the clinical relevance of these findings. Our results suggest targeting slowly dividing stem cells will be required for lasting medulloblastoma cures.

**O138. IDH1 AND IDH2 MUTATIONS IN PEDIATRIC OLGODENDROGLIOMAS**

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**INTRODUCTION:** Pediatric oligodendrogliomas are rare tumors. IDH1 and IDH2 mutations are common in adult oligodendrogliomas, but a recent study in 14 pediatric oligodendrogliomas found none with mutations. METHOD: Methods: A retrospective review (1960-present) of pediatric oligodendrogliomas and low-grade gliomas at our institution. RESULTS: Results: We reviewed all pediatric oligodendrogliomas and low-grade gliomas at our institution. All samples were sequenced using ultra-deep targeted DNA sequencing (Sanford Burnham Preclinical Research). All 23 samples were sequenced (12 IDH1, 11 IDH2). IDH1 mutations were identified in 12 (52%) cases, whereas IDH2 mutations were identified in 11 (48%) cases. CONCLUSIONS: Our findings suggest that pediatric oligodendrogliomas have a different genetic profile compared to adult oligodendrogliomas and may represent a distinct disease entity.

**O139. DEVELOPMENT OF COMBINED PK/PD STUDIES OF RAF/MEK/mTOR INHIBITORS FOR THE TREATMENT OF PEDIATRIC LOW-GRADE ASTROCYTOMAS BY MALDI MASS SPECTROMETRY IMAGING**

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**INTRODUCTION:** Pediatric low-grade astrocytomas (LGA) account for the majority of pediatric central nervous system neoplasms. A wealth of studies has evidenced deregulations of the MAPK pathway in tumorigenesis of such tumors. This pathway is a conserved signaling cascade involving various protein kinases for signal transduction from the cell membrane to the nucleus to mediate biological functions such as cell growth, survival, and differentiation. MALDI mass spectrometry imaging has proven to be an effective method to reveal the two-dimensional spatial distribution of drugs, lipids and peptides in tissue and opens the way to potentially combine pharmacokinetic and pharmacodynamic analyses of RAF/MEK/mTOR inhibitors. RESULTS: Results: The MS analysis on the drug standards allowed us to optimize parameters for their analysis by MALDI MSI. Using heme as a natural marker of drug distribution was imaged from treated liver/blood sections using MALDI TOF/TOF instrument. 10 μm thickness of fresh tissue sections from pathway relevant orthotopic mouse models were treated with the RAF/MEK/mTOR inhibitors and their metabolites. Proteomic and lipidomic profilings of brain sections are in progress for a subset of the orthotopic mouse models treated with BBB penetrating drugs to assess tumor response to targeted treatment. CONCLUSIONS: The presented pharmacokinetic and pharmacolipidomics data combined with the study of drug distribution have the potential to provide a valuable advance for the identification of drug-responsive biomarkers in the development and implementation of novel and more effective therapeutic approaches. This study evidences the main input of MALDI MSI in drug repurposing and development for the treatment of brain cancers.

**O140. DISSECTING MOLECULAR AND PROGNOSTIC SUBTYPES OF GROUP 3 AND GROUP 4 MEDULLOBLASTOMAS**

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**INTRODUCTION:** Oligodendrogliomas and low-grade gliomas are a diverse group of brain tumors that are influenced by clinical factors that are not predicted by molecular subtypes. The current World Health Organization (WHO) classification consists of 2 main subtypes: IDH wild-type and IDH-mutant. However, IDH wild-type oligodendrogliomas are underrepresented in the literature. In this study, we aimed to characterize the genetic and clinical features of IDH wild-type and IDH-mutant oligodendrogliomas.

**METHODS:** This is a comprehensive retrospective review of 225 consecutive newly diagnosed low-grade tumors at our institution. The majority of patients had IDH wild-type tumors and the remainder were IDH-mutant tumors, with no statistical difference in the distribution of patient age at diagnosis, gender, race/ethnicity, or tumor pathology. The following variables were collected for each tumor: age at diagnosis, tumor grade, mutational status, survival, and follow-up data. Statistical analysis was performed using the Fisher’s exact test, Mann-Whitney U test, and 2-tailed Student’s t test. A p value of <0.05 was considered statistically significant.

**RESULTS:** Of 225 cases, 66% were IDH wild-type and 34% were IDH-mutant tumors. The median age at diagnosis was 33 years (range, 1-80 years) and 133 patients (60%) were male. The median follow-up was 28 months (range, 1-236 months). There was a significant difference in survival between IDH wild-type and IDH-mutant tumors, with a trend toward a longer overall survival in the IDH wild-type group (p = 0.05). There was no difference in survival between IDH wild-type and IDH-mutant tumors based on patient age at diagnosis, gender, or race/ethnicity. No statistical difference was observed in the distribution of tumor grade between IDH wild-type and IDH-mutant tumors.

**CONCLUSIONS:** IDH wild-type and IDH-mutant oligodendrogliomas are distinct subtypes with different clinical and genetic features. These findings may help guide the development of targeted therapies for these tumors.
INTRODUCTION: Integrated genomics approaches have revealed distinct genetics, transcriptomics, and prognosis of the four medulloblastoma subgroups. Clinically, Group 3 and Group 4 are characterized by frequent metastatic dissemination and an unfavorable prognosis. These tumors exhibit a reduced ability to elicit biologic intertumoral heterogeneity. Early evidence suggests that subtypes exist within Group 3 and Group 4, potentially accounting for this heterogeneity. Previous attempts to identify the underlying biology driving these subtypes, and to define prognostic subtypes have been underpowered by sample or small samples sizes included in earlier genomic studies. METHOD: To elucidate these subtypes, we performed a high-resolution genome-wide methylation profiling analysis using the Infinium HumanMethylation450 BeadChip in a discovery set of 361 frozen primary Group 3 or Group 4 medulloblastomas. We carried out comprehensive genome-wide copy number and gene expression analysis for matching samples. Unsupervised consensus clustering approaches were used to identify biological subtypes for each of the datasets. We integrated annotation across platforms using integrative clustering approaches, and delineated key driver genes and pathways using bioinformatic algorithms. Subtypes were correlated with clinico-pathological information. RESULTS: Unsupervised consensus clustering analyses of transcriptomic and methylation data revealed three distinct subtypes within both Group 3 and Group 4. Subtype-specific distributions of clinico-pathological, genomic, epigenetic and transcriptional characteristics were elucidated using an integrated approach. Poor prognosis was observed in a Group 3 cluster enriched in LCA histology, TGF-beta pathway deregulation, and MYC amplification. MYC status or M stage had no prognostic impact within this cluster. Group 3 infants defined an intermediate risk group. Intriguingly, a cluster comprising one third of Group 3 tumors delineated patients with a favorable prognosis, who would be considered high-risk based on current molecular stratification schemes. CONCLUSIONS: Integrated analyses of transcriptomic and genomic alterations and epigenetic distinct subtypes within Group 3 and Group 4. SNCAIP duplications and diploidy defined one Group 4 subtype, while the other clusters showed tri- or tetraploidy, and MYCN or CDK6 amplifications, respectively. Future research on medulloblastoma and the development of clinical trials should take into consideration these distinct subtypes of medulloblastoma to improve stratification and to define future avenues for rational, targeted therapy.

0141. FUNCTIONAL ANALYSIS OF THE H3.3-K27M MUTATION IN DIPG
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INTRODUCTION: Diffuse Intrinsic Pontine Gliomas (DIPG) is a rare but devastating pediatric brainstem tumor. It is usually diagnosed based on clinical symptoms and radiological detection of a tumor mass centrally located in the pons. Up to now efforts to treat this tumor have been unsuccessful. Our lab has been studying the genetics of DIPG with the hope of developing more effective treatment. Recent whole genome sequencing efforts by our lab and others have identified a highly recurrent K27M mutation in the histone variant gene H3F3A. Here we investigate the potential function of H3.3-K27M in tumorigenesis. METHOD: To investigate the role of H3.3-K27M in the initiation and/or maintenance of DIPG, we introduced the mutation into various cell lines including normal human astrocytes (NHAs) and neural stem cells (NSCs). To assess genome-wide changes in gene transcription we performed HT12 gene expression analysis using H3.3-K27M and WT- H3.3 over-expressing NHAs. Additionally, we tested the ability of these cells to grow in an anchorage independent manner and form tumors in the brainstem of immunocompromised mice. Immunohistochemistry of DIPG patient samples was performed to assess the global effect of H3.3-K27M on histone marks associated with activation and/or repression of gene expression. RESULTS: We observed a reverse translocation of H3.3-K27M to WT cell lines and confirmed H3.3-K27M as well as the sphere-forming potential of NSCs. Gene expression profiling of NHAs over-expressing H3.3-K27M or WT-H3.3 revealed widespread changes in gene expression affecting multiple pathways. Interestingly, these cells did not form tumors in an anchorage independent assay and have yet to form tumors in mice. Immunohistochemistry of human DIPG tissue suggests that H3.3-K27M positive tumors have altered levels of H3K27 histone marks. CONCLUSIONS: Our data suggests that while H3.3-K27M over-expressed is able to affect cell growth, tumors are unable to completely transform NHAs on its own. The effect of H3.3-K27M appears to be highly dependent on the cellular context and as such, future work will aim to elucidate how cooperation with other gene mutations, like those in p53, affect the tumorigenic potential of these cells.

0142. THE TYPE OF HISTONE H3 VARIANT K27M MUTATION DRIVES THE AGGRESSIVENESS OF DIFFUSE INTRINSIC PONTINE GLIOMA
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INTRODUCTION: DIPG is the most challenging brain tumor for biologists and oncologists. No significant improvement in the prognostication, diagnosis or treatment of this tumor has been made in the last 30 years. Recent high-throughput sequencing data have shown the frequent occurrence of mutations in the histone variants H3.3 (H3F3A) or H3.1 (HIST1H3B) but little is known of the biological changes accompanying these mutations. METHOD: The type of histone H3 variant mutations were characterized by Sanger Sequencing in 140 pediatric high-grade gliomas including 31 DIPG confirmed radiologically and systematically biopsied at diagnosis. Mutations were correlated with histological (oligodendrogial vs astrocytic), immunohistochemical (EGFR, P53, Olig2, MIB1), genomic (specific amplifications or losses), transcriptomic (mesenchymal or proneural) and survival data. Supervised clustering of gene expression according to the type of mutation was also performed. RESULTS: Mutations at K27M in histone H3 variants were found in 45/51 (88%) of DIPG. The K27M mutation was seen twice more frequently in H3F3A. While mutations in H3F3A are also present in thalamic or medulloblastomas, the mutations of HIST1H3B were only seen in DIPG. The type of histone H3 variant mutated was not linked with histology or GE profiling in DIPG. Amplification of PDGFR was more frequent in H3F3A (23% vs 4%, p = 0.054). Overall survival was significantly shorter in patients with H3F3A-mutated DIPG (median OS = 11 months vs 13 months, p = 0.03). CONCLUSIONS: Almost all true DIPG harbor a K27M mutation in one of the two histone variants. Our data do not show that the type of histone H3 variant K27M mutation is driving a specific gene expression profile or phenotype; however it may influence the behaviour of the tumor. Indeed, mutations in H3F3A characterize rapidly progressing DIPG. If confirmed in follow-up studies, this could be one of the first prognostic marker to be taken into account when judging the results of prospective trials.

0143. VALIDATION OF HEME AS A MARKER OF THE VASCULARITY FOR MALDI IMAGING OF THE BLOOD-BRAIN BARRIER IN BRAIN DEVELOPMENT
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INTRODUCTION: Development of drugs for brain tumor treatment is challenged by methodologies used to assess the ability of drugs to penetrate the blood-brain barrier (BBB). The blood-brain barrier is a natural protective boundary for the human brain from the entrance of exogenous toxins as well as potential drugs. Therefore, many drugs that target other cancers are not effective for brain tumors. Determining drug permeability is critical for brain cancer drug development. Using mass spectrometry imaging (MSI), we used heme as a new marker of brain vascularization and applied it to interpret the drug permeability to brain tumors. METHOD: Fluorescein and fluorescence isothiocyanate (FITC) were injected in mice through tail vein injection. For drug analysis, the mice were dosed with RAF265 and BKM120 through oral gavage. The mouse organs were flash frozen after dissection and the brain tissues were sectioned to 10 μm thickness. 2,5-Dihydroxybenzoic acid was prepared and deposited using the ImagePrep (BrukerDaltonics, Germany). Mouse brain sections were imaged by fluorescence microscope followed by MALDI TOF/TOF. Tissue sections were imaged with the Ultraflextreme MALDI TOF/TOF (BrukerDaltonics, Germany). Tissue sections were imaged at 20-150 μm spatial resolution. RESULTS: The fluorescence images of fluorescein and FITC were correlated with the heme and fluorophore images obtained from MALDI MSI, validating heme as an effective marker
to visualize the blood-brain barrier. RAF265, known to be effective in subcutaneous animal models, but not in orthotopic brain models, was observed to accumulate in the tumor region and ventricles. However, most RAF265 in the tumor was co-localized with the water-soluble tracer BKM120, which has high permeability, was observed to distribute more evenly throughout the brain without co-localizing with heme. CONCLUSIONS: Our experiments validated MALDI MSI to be an effective approach to image the blood-brain barrier using heme as the surrogate and investigate drug permeability to the tumor in a way that can significantly inform and guide drug re-purposing and development. The co-registration of RAF265 with heme signals in tumor region demonstrates that the majority of the drug dose is retained within the inflicted blood capillaries of the tumor, and not accessing the tumor tissue. However, BKM120 does not accumulate in the tumor region with high drug penetration and spreads out across the entire brain.

0144. INTEGRATIVE CLUSTERING REVEALS FOUR CLINICALLY AND MOLECULARLY DISTINCT SUBTYPES OF SHH MEDULLOBLASTOMA
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INTRODUCTION: Sonic hedgehog (SHH) medulloblastomas account for one-third of all patients and comprise the majority of infant and adult medulloblastomas. Although all tumors in the SHH subgroup show over-activity of the SHH pathway, this subgroup exhibits remarkable heterogeneity in its transcriptional signatures, somatic copy number alterations, and mutations. To date, few genomic studies have profiled sufficient cases to identify distinct molecular subtypes within this subgroup, making it difficult to define molecular targets for the development of novel treatment strategies. METHOD: To specifically address these issues, we performed comprehensive genome-wide gene expression analysis using the Affymetrix GeneChip Human Gene 1.0 ST Array and a high-resolution methylation profiling analysis using the Illumina Infinium HumanMethylation450 BeadChip in a discovery set of >250 primary SHH medulloblastomas. Unsupervised clustering was performed to delineate molecular subtypes for each of the datasets. Integrative clustering was used to incorporate profiling data from different platforms. Subtype-specific pathway analysis was carried using Ingenuity and Cytoscape. RESULTS: Unsupervised consensus clustering analyses of transcriptomic and methylation data revealed four distinct subtypes. SHH tumors reveal distinct methylation and expression signatures in infant and adult tumors further underlining age-specific differences in SHH tumor biology. Notably, both infant and adult SHH tumors can be further separated into two distinct clusters including different proportions of pediatric and adolescent patients. Subtype-specific distributions of clinicalopathological and molecular characteristics were elucidated using an integrated evaluation of the genome, epigenome, and transcriptome. Integrative clustering revealed a subset of SHH tumors characterized by 10q loss, specific gene expression and CpG methylation changes with a particularly poor prognosis. CONCLUSIONS: Collectively, our integrated analysis demonstrates that pediatric and adult SHH medulloblastomas are clinically, epigenetically, and transcriptionally, genetically, and prognostically distinct. A refined understanding of the subtypes within infant and adult SHH tumors may help to develop novel outcome prediction algorithms and define novel targets for molecular-based therapies.

0145. GLIAL TUMOR ORIGINS IN A BRAFV600E-DRIVEN MOUSE MODEL OF PEDIATRIC GLIOMA
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INTRODUCTION: Malignant astrocytoma is a leading cause of brain tumor related mortality in children. Recently we have shown that the activating mutations of BRAF, a member of the serin/threonine protein kinases, are present in ~20% of pediatric glioma gr. II-IV. In addition, concomitant homozygous deletion of CDKN2A, encoding tumor suppressor proline p14 and p16, was observed in 71% of such BRAFV600E-gliomas. METHOD: To generate a de novo mouse model bearing similar genetic mutations, we used hGFAP-cre that is expressed in astrocyte as well as oligodendrocyte precursor cells (OPC), to activate BRAFV600E in a cdkn2a null genetic background. RESULTS: We have previously shown that these mice over-produce astrocytes in spinal cord during fetal-early post-natal stages (Tien et al., 2012). In contrast, we report ongoing proliferation of OPC is persistent until around P21 when mice become neurologically symptomatic. We then treated these mice with a mutant BRAFV600E specific inhibitor PLX4720. By using 20mg/kg/day regime, the OPC population and the proliferation index revealed by ki-67 staining was significantly reduced. Currently, we are directly testing OPGs as a possible cell-of-origin through FACS purification of NG2+ cells. CONCLUSIONS: These findings will be used to determine competence of OPCs to give rise to BRAF-driven glioma and effects of BRAF small molecule inhibitors on this process.

0146. GENOMIC ANALYSIS OF DIFFUSE PEDIATRIC LOW-GRADE GLIOMA IDENTIFIES RECURRENT, ONCOGENIC MYBL1-TRUNCATING REARRANGEMENTS
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INTRODUCTION: Pediatric low-grade gliomas (PLGGs) are among the most common solid tumors in children. While unifying genetic events have been identified in some PLGG subtypes, including BRAF duplications in pilocytic astrocytomas BRAFV600E mutations in pleomorphic xanthoastrocytomas and gangliogliomas, the rarity and diversity of diffuse PLGGs has historically impeded determination of genetic driver events specific to each histopathological group. We have previously shown that diffuse low-grade gliomas are a particularly heterogeneous group of tumors revealing distinct methylation and expression signatures in infant and adult medulloblastomas, which suggests the potential for the development of novel treatment strategies. METHOD: To specifically address these issues, we performed comprehensive genome-wide gene expression analysis using the Affymetrix GeneChip Human Gene 1.0 ST Array and a high-resolution methylation profiling analysis using the Illumina Infinium HumanMethylation450 BeadChip in a discovery set of >250 primary SHH medulloblastomas. Unsupervised clustering was performed to delineate molecular subtypes for each of the datasets. Integrative clustering was used to incorporate profiling data from different platforms. Subtype-specific pathway analysis was carried using Ingenuity and Cytoscape. RESULTS: Unsupervised consensus clustering analyses of transcriptomic and methylation data revealed four distinct subtypes. SHH tumors reveal distinct methylation and expression signatures in infant and adult tumors further underlining age-specific differences in SHH tumor biology. Notably, both infant and adult SHH tumors can be further separated into two distinct clusters including different proportions of pediatric and adolescent patients. Subtype-specific distributions of clinicalpathological and molecular characteristics were elucidated using an integrated evaluation of the genome, epigenome, and transcriptome. Integrative clustering revealed a subset of SHH tumors characterized by 10q loss, specific gene expression and CpG methylation changes with a particularly poor prognosis. CONCLUSIONS: Collectively, our integrated analysis demonstrates that pediatric and adult SHH medulloblastomas are clinically, epigenetically, and transcriptionally, genetically, and prognostically distinct. A refined understanding of the subtypes within infant and adult SHH tumors may help to develop novel outcome prediction algorithms and define novel targets for molecular-based therapies.

0147. GENERATION OF ANTI-PDGFRα IMMUNOTOXINS FOR BRAINSTEM GLIOMA TREATMENT
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INTRODUCTION: Despite radiation and chemotherapy, the prognosis for children with diffuse brainstem gliomas is extremely poor. There is a need for effective therapeutic strategies to improve survival. Platelet-derived growth factor receptor alpha (PDGFRα) is identified as a potential therapeutic target in whole-genome profiling of pediatric brainstem glioma. Recombinant immunotoxins (RTIs) are composed of an Fv that binds to an antigen on a target cell, thus killing only the cancer cells. We have generated an RTI that targets PDGFRα for treating these highly malignant tumors. METHOD: We have constructed an RTI, PD1-P58KDEL, by fusing an anti-PDGFRα scFv, 2.449.1.3 V1, to a truncated
of treated animals was 5.4 days longer than the control group. Results show potent cytotoxicity against PDGFRα-transfected cells and cells from human pediatric GBM xenografts. The median survival time was 7 months (range: 3.5–22 years). CONCLUSIONS: The PD1-PE38KDEL protein was efficacious in targeting the brainstem tumor surface antigen PDGFRα. Further investigations of this RIT as a therapy for brainstem glioma will be pursued in a convection-enhanced delivery animal model. This RIT not only has significant therapeutic potential for brainstem glioma, but for other PDGF-expressing malignancies as well.

0148. USING MAGNETIC RESONANCE IMAGING TO DETERMINE GADOLINIUM CONTRAST AGENT CONCENTRATION
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INTRODUCTION: Convection-enhanced delivery (CED) has the ability to deliver drugs, including macromolecules, into the brain with high concentration by bypassing the blood-brain barrier, which makes it a promising drug delivery method in the treatment of brain tumors including diffuse intrinsic pontine glioma. However, there are currently no adequate methods to evaluate in vivo drug distribution and local concentration in CED. This is analogous to not knowing pharmacokinetics in systemic delivery or dose and fields in radiotherapy. The feasibility of using CED to deliver Gd-DTPA was assessed in a murine model of glioma (K-145 glioma) using T2-weighted MRI to determine the concentration distribution. METHOD: Gd-DTPA standards (12 concentrations) were used to optimize scanning sequences for longitudinal relaxation time (T1) and transverse relaxation time (T2) on a 7 Tesla scanner. T1 was determined by using the following relationship: \( S = \frac{g}{T_1} \), where \( S \) is the signal intensity, \( g \) is the scaling coefficient and \( T_1 \) is a function dependent on the imaging sequence. Standard curve was obtained by fitting R1 values to a linear model. Then, CED-glioma model was infused with Gd-DTPA and scanned and the optimized sequence to determine Gd-DTPA concentration. RESULTS: In Gd-DTPA standards, R1 values were mapped for each concentration and R2 values were deducted. Gd-DTPA concentrations and R2 values follow an excellent linear correlation. Using this linear relationship, Gd-DTPA concentrations in the hydrogels at different spatial locations and time points after infusion were determined. CONCLUSIONS: Gd-DTPA concentration can be determined using R1 values from MRI scans. Further studies will apply this method to in vivo determination of Gd-DTPA concentration, which will require compensating for the difference in longitudinal relaxivities of Gd-DTPA in hydrogen and brain tissues. The results may improve CED by allowing clinicians to monitor and sustain agent concentrations at target sites for the treatment of brain tumors.

0149. PATTERNS OF PROGRESSION IN PEDIATRIC PATIENTS WITH HIGH-GRADe GLIOMA OR DIFFUSE INTRINSIC PONTINE GLIOMA TREATED WITH BEVACIZUMAB-BASED THERAPY AT DIAGNOSIS
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INTRODUCTION: Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor, is standard of care for recurrent glioblastoma multiforme (GBM) and is undergoing clinical trials in adults and children with newly-diagnosed high-grade gliomas (HGG), including diffuse intrinsic pontine glioma (DIPG). Sixty percent to 75% of adult GBM patients receiving bevacizumab at diagnosis or recurrence have been reported to have had good or minimal progression. It is believed that bevacizumab is efficacious in targeting the brainstem tumor surface antigen PDGFRα. Further investigations of this RIT as a therapy for brainstem glioma will be pursued in a convection-enhanced delivery animal model. This RIT not only has significant therapeutic potential for brainstem glioma, but for other PDGF-expressing malignancies as well.

0150. DISTINCT IMMUNE CELL INFILTRATE PROFILES ACROSS PEDIATRIC BRAIN TUMOR TYPES
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INTRODUCTION: Emerging evidence exists for anti-tumor immune regulation in the central nervous system. Microarray-based studies by our laboratory confirm that the immune control of tumor progression extends to the “immunoprivileged” central nervous system, identifying prognostic immune gene signatures in primary tumor specimens. Using a more diverse multicolor flow cytometric approach we have systematically characterized the phenotype, frequency and function of infiltrating immune cells across the spectrum of pediatric brain tumor types and normal brain. METHODS: A panel of dissaggregated primary pediatric brain tumor samples (13 ependymoma (EPN), 4 high grade glioma (HGG), 6 pilocytic astrocytoma (PA) and 6 medulloblastoma (MED)) and 5 normal brain samples obtained from epilepsy patients were analyzed by flow cytometry. Immune cell types analyzed included both myeloid and lymphoid lineages and their respective markers of polarization (M1/M2 and/or TH1/TH2 functional phenotypes). RESULTS: Immune parameters that distinguished each of the tumor types were identified. EPN and PA demonstrated significantly higher infiltrating myeloid and lymphoid cells compared to HGG, MED or normal brain. Both EPN and PA demonstrated an M1 functional phenotype compared to normal brain and MED. However, EPN was unlike PA in that it showed a TH2 functional phenotype, similar to normal brain, HGG and MED each exhibited M2 functional phenotypes likely linked to the murine models that were used. CONCLUSIONS: These results of this comparative tumor analysis demonstrate that different pediatric brain tumor types exhibit distinct immune microenvironments. This suggests that individual immunotherapeutic approaches need to be designed for each tumor type. Furthermore, the immune characteristics identified in this study can be manipulated in order to optimize the efficacy of such immunotherapies.

0151. PD0332991, a cdk4/6 INHIBITOR, INDUCES TUMOR REGRESSION AND EXTENDS SURVIVAL IN ATYPICAL TERATOID RHABDOID TUMOR (ATRT) AND MEDULLOBLASTOMA MOUSE MODELS
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INTRODUCTION: The cyclin dependent kinases 4 and 6 (cdk4/6)-dependent G1 checkpoint controls cell cycle progression in differentiated cells. Cdk4/6 activity is elevated in ATRTs and medulloblastomas. The vast majority of ATRTs have biallelic inactivating mutations in SMARCB1, which causes cell cycle progression by downregulation of p16INK4a and upregulation of cyclin D and E2F. Likewise, cyclin D expression is increased in most
medulloblastomas and is a poor prognostic indicator. Since these pathway aberrations lead to increased activity of cdk4/6, we assessed efficacy of a cdk4/6 inhibitor in ATRT and medulloblastoma mouse models. METHOD: The efficacy of a specific inhibitor of cdk4/6, PD0332991, was investigated using xenotransplantation of patient-derived xenotransplant (PDX) mouse models of ATRT and medulloblastoma, generated using freshly resected tumor tissue from pediatric patients at Seattle Children’s Hospital or Children’s Oncology Group (COG) sites, PDX0332991 (150 mg/kg or 75 mg/kg) was administered daily by oral gavage. Efficacy was assessed by measuring the difference in tumor size (subcutaneous xenografts) or survival (orthotopic xenografts) in drug-treated as compared to vehicle-treated mice. Pharmacodynamic analyses involved investigation of the phosphorylation state of Rb protein and the extent of apoptosis. RESULTS: PD0332991 treatment caused significant reduction in subcutaneous ATRT and medulloblastoma tumors as compared to vehicle treatment. Rb phosphorylation at Ser678 was significantly reduced in drug-treated tumors. Likewise, Ki67, a marker of cell proliferation, was significantly reduced following drug treatment. In each of 3 survival studies using orthotopic medulloblastoma models, all vehicle-treated mice (n = 12-13) died, while all drug-treated mice (n = 11-13) survived for the study duration (10-28 days). In the ATRT orthotopic mouse model, 13/17 drug-treated mice were alive at the end of the study (45 days) as compared to 1/17 vehicle-treated mice. There was no apparent toxicity associated with drug treatment. CONCLUSIONS: Inhibition of cdk4/6 is a promising therapeutic strategy for both ATRT and medulloblastomas. Treatment with PD0332991 resulted in significant tumor regression in subcutaneous tumor models and a highly significant survival advantage in orthotopic models. Pharmacodynamic analyses indicate that PD0332991 is engaging with cdk4/6, with a significant reduction of Rb phosphorylation and an increase in apoptosis. Interestingly, inhibition of cdk4/6 would be expected to have a cytostatic effect, several lines of evidence point towards a cytoreductive effect in vivo. The results of this study strongly support use of PD0332991 in a clinical trial for pediatric ATRT and medulloblastoma patients.

0152. DRUGGING MYCN THROUGH AURORA KINASE A TO TARGET MEDULLOBLASTOMA
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INTRODUCTION: Amplification or mis-expression of MYC proteins contributes to a range of pediatric brain cancers including most subtypes of medulloblastoma. MYC proteins are tightly regulated at the level of protein stability. Stabilization of MYC protein in particular proceeds through a kinase-independent function of the mitotic kinase, Aurora Kinase A, with expression of Aurora Kinase A itself reported to correlate negatively with survival in medulloblastoma (Neben et al., Cancer Research 2004). METHOD: An array of MYCN-driven cancer cell lines were acquired through the UCSC Cell Culture Core. Lentiviral and bacterial constructs were generated to express MYCN and mutants were generated by PCR-based site-directed mutagenesis (Stratagene). Aurora A was purified from overexpressing bacteria, crystals were generated by hanging drop vapor diffusion. Standard chemical synthesis and crystallography were performed. Western blots and in vitro Aurora A kinase activity were performed by antibody specific approaches using antibodies to MYCN (Abcam), Aurora A, Histone H3, phospho-Histone H3 (all from Cell Signaling), and GAPDH (millipore). RESULTS: We synthesized and screened a novel class of inhibitors to identify agents that abrogated degradation of MYCN protein across various MYCN-expressing cell lines. These structures demonstrated that a bulky chemical moiety in CD323 (our lead compound) clashes sterically with structures in the amino terminus of Aurora Kinase A, resulting in a hinge-like movement of the amino-terminal lobe away from the carboxy terminal lobe of Aurora A. In comparing structures of free Aurora Kinase, Aurora Kinase bound to clinical inhibitors, and Aurora Kinase bound to CD323, the degree of hinge movement correlated directly with degradation of MYCN. CONCLUSIONS: These structural data, coupled with mammalian cell line and biochemical data, establish a potential to augment MYCN significantly above the effects of simple nonamolar level inhibition of Aurora Kinase A kinase activity. This class of inhibitors, which disrupts stabilizing interactions between Aurora A and MYCN, represents new candidate agents to target medulloblastoma and other MYC-driven cancers.

0154. A HUMAN NEURAL STEM CELL MODEL OF MYC-DRIVEN MEDULLOBLASTOMA REVEALS GLUTAMINE METABOLISM AS A POTENTIAL THERAPEUTIC TARGET
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INTRODUCTION: Medulloblastoma comprises four subgroups with different associated mutations and clinical prognoses. Group 3 tumors have increased MYC levels and the worst clinical prognosis. MYC overexpression in cancer lead to increased glutamine metabolism and glutamine addiction. We hypothesized that human neural stem cell derived from the developing cerebellum transfused with MYC and cooculata targeting oncogenes would create a genetically accurate model of group 3 medulloblastoma for pre-clinical testing. We further hypothesized that MYC-driven medulloblastoma would exhibit increased glutamine metabolism and be sensitive to glutaminase. METHODS: MYC cerebellar derived human neural stem cells with lentivirus coding for c-MYC, dominant negative R248W, p53, constitutively active AKT, and hTERT. We also created neuroisomers expressing subsets of these constructs. We measured proliferation in vitro with BrdU and MTS assays and generated xenografted mice with subcutaneous xenografts or tumors. Tumors were processed for histology and stained with immunohistochemistry for markers of neuronal and glial differentiation. After treatment with the glutamine anti-metabolite (2S)-Amino[(5S)-3-chloro-4,5-bis(2-oxo-ethyl)-5-methanesulphonyl (acvin), we determined apoptosis by flow cytometry (cell cycle and annexin staining) and cleaved caspase 3 immunofluorescence. We determined glutaminase expression by western blotting. RESULTS: R248W/p53/MYC/AKT/hTERT transformed cerebellar stem cells formed aggressive orthotopic xenograft tumors with large cell/anaplastic histology and spinal metastases. Control MYC-alone and R248W/p53/MYC/hTERT transduced cells formed tumors with increased latency and decreased penetrance. MYC expression positively correlated with increased expression of glutaminase. Acvin decreased cell proliferation in MYC-containing neuroisomers compared to those without MYC (p < 0.012). Acvin treatment of MYC expressing cells led to a 65% increase in apoptosis compared to MYC-negative cells. Forced low-MYC UW228 medulloblastoma cell line also led to increased glutaminase expression and increased sensitivity to acvin (p<0.03). CONCLUSIONS: Human cerebellar stem cells can be transformed by c-MYC and cooperating oncogenes, resulting in a model that recapitulates group 3 medulloblastoma histologically. These cells and classical high-MYC medulloblastoma cell lines are sensitive to glutamine anti-metabolites, while non-MYC driven medulloblastoma and SV40 immortalized human cerebellar stem cells are resistant. Our in vitro and in cell human neural stem cell model can suggest that glutamine metabolism may be a therapeutic target in MYC-driven medulloblastoma, and that glutamine anti-metabolites may be valuable clinical agents.
0156. BRAF STATUS IN PERSONALIZING TREATMENT APPROACHES FOR PEDIATRIC GLIOMAS
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INTRODUCTION: Pediatric low-grade gliomas (PLGGs) represent an excellent platform for personalized approaches to cancer treatment. Activation of mitogen protein kinase (MAPK) and phosphatidylinositol-3’ kinase (PI3K)/Akt signaling pathways of agents that target these key growth regulatory pathways, BRAF, a kinase within the MAPK pathway, is activated by missense mutation (V600E) in ~20% of grade 2-4 pediatric gliomas. In addition to BRAF/MEK/MAPK, ERK, the PI3K/AKT/mTOR signaling cascade is activated in ~half of PLGGs. In the current study, we asked whether knowledge of individual tumor BRAF genotype can guide selection of the best combination of signaling inhibitors for individual pediatric gliomas.

METHOD: We used glioma cell lines containing BRAF(V600E) (AM83) or wild type BRAF (SF188, SF8628), and normal human astrocytes immortalized with hTERT and E6/E7 expressing retrovirus. Signaling inhibitors used were everolimus (RAD001, mTOR inhibitor, 4µM), PLX4720 (BRAF(V600E) specific inhibitor, 5 microM), AZD6244 and GDC-0973 (MEK inhibitors, 1 microM), and 0.5% DMSO as control. Cell cycle distribution was determined 24 hours after treatment using flow cytometry to quantify BrdU and 7-AAD staining according to FITC BrdU Flow Kit (BD). Apoptosis was assayed 72 hours after treatment by flow cytometry quantification of Annexin V-FLICA+PL GG cells. RESULTS: BRAF(V600E) tumor cell lines were significantly more sensitive to apoptosis and cell cycle arrest in BRAF(V600E) cells, compared to single agents. Pediatric glioma lines SF188 and SF852 were also sensitive to everolimus, AZD6244 and GDC-0973, but not PLX4720. Combination of everolimus, AZD6244 or everolimus/GDC-0973 were more effective than single agent treatments. No single agent or combination affected apoptosis or cell cycle in NHA. CONCLUSIONS: The use of BRAF status to determine effective combination therapies is an important consideration for personalized treatment of pediatric low-grade gliomas. Combination of PLX4720 and everolimus or PLX4720 and MEK inhibitor are equally efficacious and superior to single agent treatment, but we consider the former combination may be better tolerated for long-term treatment of children with PLGGs. For BRAF WT PLGGs, combination of everolimus with MEK inhibitor is superior to single agent therapies and would represent our treatment of choice for PLGGs with this genotype in future clinical trials. We next look for optimal treatment of KIAA1549:BRAF-expressing PLGGs.

0157. NOVEL ROLE FOR ENHANCING IMMUNOTHERAPY AGAINST PEDIATRIC BRAIN TUMORS USING HEMATOPOIETIC STEM CELLS
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INTRODUCTION: Adoptive T-cell therapy is an effective treatment modality for solid tumors, leading to objective clinical responses, including >40% durable complete response rate of metastatic lesions within the CNS. We have pioneered a platform of adoptive cellular therapy (ACT) employing total tumor RNA pulsed dendritic cells to expand polyfunctional tumor-reactive T-lymphocytes in vitro. The anti-tumor efficacy of adoptively transferred T-cells is significantly enhanced under myeloblastic host conditioning and hematopoietic stem cell rescue, leading to significant survival benefit and cures. The role of HSCT in immunotherapy is unexplored, but we demonstrate that HSCT-T cell interactions in adoptive immunotherapy lead to enhanced anti-tumor efficacy. METHOD: Primary dendritic cells derived from murine bone marrow were pulsed with total RNA derived from a syngenic high-grade astrocytoma that we transduced with retroviral vector expressing luciferase (Luc+) and used to generate tumor-reactive T lymphocytes (TTRNA-T cells) ex vivo. TTRNA-T cells were adoptively transferred into intracranial tumor bearing mice following myeloblastic host conditioning and HSCT rescue. RESULTS: In a highly invasive murine astrocytoma model, ACT and HSCT co-transfer, resulted in significant prolongation of median survival and cures of established tumors. These studies revealed greater T-cell infiltration of intracranial tumors in mice receiving MA + HSCT and long term T-cell persistence within the tumor microenvironment.

Specific co-localization and persistence of tumor-reactive T-cells and HSCTs were observed within the tumor microenvironment for >60 days post-transfer. Mechanistic studies demonstrated that HSCTs significantly enhance intratumoral localization of T-cells via the MIP1e/CCR5 axis, showing a direct correlation between the presence of HSCT-derived chemokines and increased numbers of tumor reactive T-cells at the tumor site. CONCLUSIONS: Our novel data reveals that HSCT-T cell interactions with tumor microenvironment modulate immunotherapy by guiding the intratumoral localization of T-cells, thereby playing a role in facilitating the eradication of infiltrative malignant gliomas.

0158. MALIGNANT TRANSFORMATION OF A DESMOPLASTIC INFANTILE GANGLIOGLIOMA: COMPARATIVE GENETIC HYBRIDIZATION ANALYSIS OF THE PRIMARY GLIOMA AND TRANSFORMED MALIGNANT GLOBLASTOMA
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INTRODUCTION: DIG (Desmoplastic Infantile Ganglioglioma) is a rare neocortical neoplasm classified as WHO grade I tumor under or neuronal and mixed neuronal glial tumors (under 2007 World Health Organization brain tumor classification.). It is usually considered to have a good prognosis but 40% of the cases require additional medical, radiation and/or further surgical intervention and 15% of infants and children develop leptomeningeal spread or diffuse into malignant glial transformation. This case is described in literature but no studies have been conducted to detect the genetic alterations associated with the transformation. METHOD: A DIG tumor in a 2 mo old boy that showed aggressive behavior, requiring debulking initially at 2.5 mo age and subsequently at 10 mo age following tumor progression, was treated with chemotherapy, and subsequently by observation. At 5 yrs age the patient presented with recurrent seizures in association with malignant transformation into glioblastoma. Chromosome microarray analysis (CMA) using oligo array was performed on the biopsy specimen obtained at 2 mo age and on the subsequent transformed malignant glioblastoma. RESULTS: Following the clinically stable course of 7.5 years duration at glioblastoma transformation additional debulking and chemotherapy was employed. One year subsequently, the child remains stable with moderate neurological deficits. CMA using oligo array performed on the biopsy specimen obtained at 2 mo age did not show any significant abnormality, however there were significant genomic deletions and duplications associated with the glioblastoma from the same tumor subsequently. These included multiple genomic losses (4q, loss of chromosome Y, deletion of 6q) and a gain of 5q. Amplification of 12q14 involving the genes GL1 and CDK4 was identified. CONCLUSIONS: The complexity of cytogenetic abnormalities is suggestive of genomic instability and indicative of an aggressive tumor progression. Deletion of 4q, the loss of chromosome Y, and amplification of GL1 and CDK4 genes are associated with pediatric glioblastomas; (CDK4 is part of Rb/p16INK4a/cyclin-D/CDK4 pathway also associated with malignant transformation of low grade gliomas (ganglioglioma); and deletion of 5q are frequently found in pediatric malignant astrocytomas. Our case illustrates the rare recurrency of DIG with malignant transformation into glioblastoma and its associated genetic alterations.

0159. DEVELOPMENTALLY REGULATED ANTIGENS FOR IMMUNOLOGIC TARGETING OF MEDULLOBLASTOMA SUBTYPES
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INTRODUCTION: Medulloblastoma (MB) remains incurable in one third of patients despite aggressive multi-modality standard therapies. Emerging evidence from integrated genomic studies has suggested MB variants arise from deregulation of pathways affecting proliferation of progenitor cell populations within the developing cerebellum. Immunotherapy presents a promising alternative by specifically targeting cancer cells and to date, there have been no successful immunologic applications targeting MB. Using total embryonic RNA as a source of tumor rejection antigens is attractive because it can be delivered as a single vaccine, target both known and unknown fetal proteins, and can be refined to preferentially treat distinct MB subtypes. METHOD: We have created two transplantable, target both known and unknown fetal proteins, and can be refined to preferentially treat MB subtypes. CONCLUSIONS: Our novel data reveals that HSC-T cell interactions with tumor microenvironment within the tumor microenvironment modulate immunotherapy by guiding the intratumoral localization of T-cells, thereby playing a role in facilitating the eradication of infiltrative malignant gliomas.

Specific co-localization and persistence of tumor-reactive T-cells and HSCTs were observed within the tumor microenvironment for >60 days post-transfer. Mechanistic studies demonstrated that HSCTs significantly enhance intratumoral localization of T-cells via the MIP1e/CCR5 axis, showing a direct correlation between the presence of HSCT-derived chemokines and increased numbers of tumor reactive T-cells at the tumor site. CONCLUSIONS: Our novel data reveals that HSCT-T cell interactions with tumor microenvironment modulate immunotherapy by guiding the intratumoral localization of T-cells, thereby playing a role in facilitating the eradication of infiltrative malignant gliomas.
0160. BMI1 REGULATES GLI ACETYLATION THROUGH CULLIN3 IN MEDULLOBLASTOMA STEM CELLS

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INTRODUCTION: Constitutive activation of the Shh pathway in neural progenitor cells of the external granular layer of the cerebellum is a frequent driver of brain tumour initiating cells (BTICs) in medulloblastoma (MB). Recently, we have shown that activation of the Shh pathway leads to an increase in production of BMI1, a polycomb repressor which promotes‘stemness’in BTICs. Our current work demonstrates that up-regulation of BMI1 by the Shh pathway leads to repression of cullin3 transcription. Cullin3 has been shown to be downregulated in HDAC1, leading to increased acetylation of the Glis transcription factors which can further activate the Shh pathway. METHOD: We use MB BTICs from cell lines and primary tumours, grown in neural stem cell media. BMI1 and CUL3 expression is modulated using overexpression (OE) and lentiviral-mediated shRNA knockdown (KD) vectors. Subsequently, mRNA and protein levels of downstream genes are assessed by RT-PCR and Western blotting (WB). GlI acetylation is assessed by immunoprecipitation using anti-acetyl lysine antibody on WB. Chromatin immunoprecipitation (ChIP) using BMI1 antibody on CD15+ cells and subsequent PCR amplification of CUL3 promoter regions will elucidate BMI1 occupancy of CUL3 promoter. Functional significance of CUL3 knockdown in MB BTICs will be assessed in vivo, using a NODSCID xenograft model. RESULTS: To date, ChIP analysis has demonstrated that BMI1 leads to a reduction of CUL3 protein and a corresponding increase of HDAC1 in Med8a tumor spheres. CONCLUSIONS: Our data suggests the existence of a positive feedback loop whereby BMI1 can repress transcription of the CUL3 E3 ligase. This in turn leads to reduced ubiquitination of HDAC1 and increased GlI deacetylation. Deacetylated Glis further activate BMI1 transcription, driving the positive feedback system. We have previously demonstrated MB BTICs to be Shh-receptive cells that respond to ligand produced by bulk tumour. Our future investigations will be directed towards the identification of pathways that might lead to aggressive activation of the Shh pathway and produce a bona fide BTIC, providing a novel set of drug targets for future therapies.

0161. SURVIVAL ADVANTAGE WITH EVEROLIMUS (RAD001) COMBINED WITH A SELECTIVE BRAFV600E INHIBITOR IN A XENOGRAFT MODEL OF BRAFV600E-MUTANT PEDIATRIC GLIOMA

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INTRODUCTION: Pediatric gliomas commonly recur after resection, and, as a result, these tumors are frequently treated with adjuvant chemotherapy or radiation. We have previously shown that the PI3K/Akt/mTOR pathway is activated in pediatric brain tumors, and this activation confers tumor sensitiv-
ity to mTOR inhibitors. Similarly, we have previously demonstrated that activating BRAFV600E mutation, that occur in as much as 20% of pediatric-grade II-IV gliomas, are responsive to V600E-targeted chemotherapy. In the current study, we have investigated the efficacy of mTOR + BRAFV600E combination therapy, using in vitro and in vivo approaches. METHOD: BRAFV600E-targeted inhibitor PLX4720 and mTOR inhibitor everolimus (RAD001) were used. In vitro cell proliferation and apoptotic response of inhibitor treatments were examined using clonogenic assays and flow cytometry.

In vivo, mice with flank xenografts, established with a BRAFV600E mutant pediatric low-grade glioma (BT40), were randomized to receive vehicle only (control), monotherapy, or a combination of PLX4720 + everolimus. Animals were euthanized per protocol, and followed for survival and response to treatment by monitoring tumor volume, and by immunohistochemical analysis of tumor tissues for proliferation (Ki-67) and apoptotic response indicators. RESULTS: Clonogenic assay results showed synergistic activity with the combination of PLX4720 and BRAFV600E gliomas. Flow cytometry revealed increased GI arrest in BRAFV600E cells treated with combined inhibitors, in relation to treatment with monotherapy. Cells with wild-type (WT) BRAF were nonresponsive to PLX4720, and combination therapy did not improve upon the anti-tumor effects of everolimus monotherapy for BRAFVT cells. In the in vivo model of BRAFV600E glioma, combined treat-
ment with PLX4720 + everolimus led to a statistically significant survival advan-
tage in relation to either monotherapy. In vivo results indicated increased apoptosis and DNA damage in BRAFV600E mutant cells from combina-
tion therapy. CONCLUSIONS: To our knowledge, this is the first in vivo demonstration of combinatorial activity of a BRAFV600E (PLX4720) and mTOR inhibitor (everolimus), as applied to potential glioma treatment. Our results suggest that apoptosis, DNA damage and cell cycle arrest are enhanced through the simultaneous blockage of both the mTOR and MEK/ERK pathways in mediator of tumor cell killing.

0162. DEVELOPMENT AND ANALYSIS OF A GENETIC MODEL OF CHOROID PLEXUS TUMOR

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INTRODUCTION: Choroid plexus tumors are intraventricular papillary neoplasms arising from choroid plexus epithelium. They occur most often in childhood and comprise 10–20% of all brain tumors in infants. Choroid plexus papillomas is a benign tumor, whereas choroid plexus carcinoma is malignant and most commonly found in pediatric population. Despite good prognosis for choroid plexus papilloma after complete surgical removal, incompletely resected or inaccessible papillomas or choroid plexus carcinomas have been associated with poor outcomes. Previous studies implicated Notch signaling in choroid plexus tumorigenesis. In this study, we developed and characterized a genetic model of choroid tumors based on Notch pathway activation. METHOD: Math1-Cre transgenic strain was bred with Rosa26-NICD1 strain that express Notch1 intracellular domain (NICD1) in a Cre-dependent manner. Math1-Cre/Rosa26-NICD1 animals were further crossed to GFP reporter strain to label Math1-positive lineage from rhombic lip. Tumor cell proliferation, differentiation and survival were analyzed by immunohistochemical and immuno blotting analysis. Gene expression was examined by quantitative RT-PCR analysis and immunostaining. RESULTS: Math1-positive rhombic lip lineage was detected in a small fraction of choroid plexus epithelium of 4th ventricle. A hyperplasia in the choroid plexus epithelium derived from Math1-Cre/Rosa26-NICD1 animals was observed in Math1-Cre/Rosa26-NICD1 animals. Morphological analysis revealed papillary and intraventricular growth of epithelial cells that exhibited enhanced proliferation compared to control animals in early postnatal period. Tumor cells expressed Lmx1a, Otx2 and Aquaporin 1, markers for choroid plexus epithelial cells. In addition, tumor cells express acetylated tubulin, suggesting the presence of cilia structures. Gene expression analysis demonstrated that tumor cells express NICD1 and exhibit constitutively active Notch signaling. CONCLUSIONS: Math1-positive rhombic lip lineage contributes to choroid plexus epithelium and is sensitive to Notch1-driven tumor formation. We have developed a genetic model of choroid plexus tumor that exhibits characteristics similar to pediatric choroid plexus tumors. We will continue to examine mechanisms and signaling pathways that drive tumor cell proliferation and malignant transformation in this model. Study of choroid tumors in this novel model will bring crucial insights into the mechanisms driving tumor formation and new directions for therapeutic development.

0163. ESTABLISHING ZEBRAFISH MODELS OF CNS PRIMITIVE NEUROECTODERMAL TUMORS

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INTRODUCTION: Primitive Neuroectodermal Tumors (PNETs) of the CNS are rare, aggressive childhood neoplasms whose molecular and cellular origins are not known. Recent integrative genomic approaches on a large collection of hemispheric PNETs have revealed at least 3 distinct PNET subtypes: Primitive-neuroepithelial, Oncogeneal and Mesenchymal. These studies suggest that
Distinct cellular origins and oncogenic events drive the formation of different PNET subtypes. By modeling these specific changes in the neuroectoderm of the zebrafish, we have generated a novel vertebrate CNS tumor model that closely resembles the human oligodendroglial PNET. METHOD: To model CNS PNETs, we analyzed published gene expression databases to identify potential oncogenic events and markers for cell of origin. For example, oligodendroglial PNET subtype tumors have elevated expression of SOX10 and OLIG2, suggesting a glial precursor origin. In addition, NF2 overexpression and p53 pathway mutations are associated with PNET tumorigenesis. To target these changes in sox10-expressing progenitor cells, we generated transgenic lines that express fluorescently tagged human NRAS under the control of the sox10 promoter in a p53 mutant background. CNS tumor onset was monitored by mCherry fluorescent resonance and characterized at histopathological levels. RESULTS: Tg(Sox10:mCherry;NRAS): p53 animals develop CNS tumors by 8 weeks of age with 50% penetrance. Tumors grow aggressively throughout the CNS and surrounding tissues, with animals succumbing to disease by 38 weeks. Pathological analysis showed small round blue cell tumor histology with IHC consistent with human PNET tumors (synaptophysin, GFAP, Nestin). Differential gene expression analysis based on RNA-Seq from 8 different fish PNET tumors and controls shows activation of genes involved in midbrain-hindbrain, glial development. Cross-species comparisons of PNET gene expression signatures reveal highly conserved genetic programs drive oligodendroglial PNET formation. CONCLUSIONS: This is the first zebrafish model of a human brain tumor and the first model of the oligodendroglial PNET subtype. These models can be used to identify conserved molecular mechanisms involved in PNET formation and invasion. We are also generating inducible PNET models using CRE/LOX approaches that will allow us to produce thousands of these tumor animals at low cost for future in vivo drug screens.

0164. PKL1 INHIBITION LEADS TO INACTIVATION OF THE RSK/YB-1 PATHWAY AND MAY SERVE AS A SURROGATE FOR SALL4 MOLDS IN CLINICAL TRIALS

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INTRODUCTION: Polo-like kinase-1 (PLK1) is essential for the sustained growth of brain tumors. Our laboratory previously published that PLK1 inhibition with either siRNA or the small molecule BI-2536 suppresses the growth of glioblastoma multiforme (GBM) and medulloblastoma (MB) in vitro and in orthotopic mouse models. More recently, PLK1 was also found to be associated with relapse and poor survival in pediatric MB patients. The underlying molecular mechanisms of PLK1’s role in promoting tumor growth and drug resistance remained largely undefined. Moreover, a robust surrogate marker(s) for PLK1 inhibition is currently lacking in clinical trials.

METHOD: Gain of function and loss of function approaches were used to examine PLK1’s downstream signaling activities. Human recombinant PLK1 was transfected into HeK293 cells and signaling changes were assessed by immunoblotting. To examine downstream PLK1-regulated signaling, we used a dominant negative inhibition of p38 MAPK in GBM cells. SILAC into SF188 glioblastoma cancer cell tumors. We utilized antibody arrays to assess the impact of PLK1 inhibition on the proteome. The second generation PLK1 pharmacological inhibitor BI-6727 was also used to inhibit PLK1 in brain tumor cells lines to address changes in signaling and cell growth. RESULTS: In HEK293 cells, transfecition of hPLK1 induced the activation of RSK/ YB-1 pathway, which belongs to the MAPK signaling cascade. Using antibody arrays, the MAPK pathway was also implicated in PLK1 signaling. Inhibiting PLK1 with siRNA blocked p-RSK and p-YB-1. Likewise, BI-6727 inhibited this pathway in a dose-dependent manner. In primary pilocytic astrocytoma cells PLK1 was expressed at low levels. Therefore we are transfecting PLK1 into the cells to activate the RSK/ YB-1 pathway. Cell growth and response to therapy will be addressed. Conversely, PLK1 will be inhibited in primary GBM and the effect on the pathway will be reported. CONCLUSIONS: There are several PLK1 inhibitors in clinical trials for the treatment of adult cancers, however, they have not been evaluated in children with brain tumors. One of the challenges is discerning which of these agents or markers should be actively assessed in preclinical models.

0166. MODELING PEDIATRIC GLIOMA SUBGROUPS IN MICE AND HUMANS

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INTRODUCTION: Transcriptomic subgroups of childhood GBMs are associated with IDH1 (R132H) mutations, H3F1A (K27/H34) mutations, and PDGFRA amplifications. Most pediatric GBMs (except PDGFRA amplified tumors) displayed mutations in the p53 gene. The location and age at diagnosis suggest that GBM subgroups arise from different progenitor cells. We and others have previously shown that the proneural tumor oligodendroglioma can arise from oligodendrocyte progenitor cells (OPCs). We have validated data from other groups showing that astrocytomas can arise from neural stem cells (NSCs). Development of pediatric gliomas has traditionally been modeled using mouse models rather than human precursor cells. METHOD: We use the RCAS/tva-a system to drive expression of PDGFB-BB, R132H mutation, K27 mutation, and G44 mutation. We infect OPCs and NSCs through injecting infected retroviral injections into the developing rat brain and proliferation by postnatal ages in mice displaying p53 mutations. Isolation of precursors from different regions followed by infection of tv-a expressing cells allows us to study effects on differentiation, self-renewal, and proliferation. Gene expression and methylation of infected cells are characterized. Lentiviral infection of cultured human fetal OPCs and NSCs with PDGFB, R132H mutation, K27 mutation, and G44 mutation in combination with shRNA against p53 is used to induce transformation. RESULTS: Stereotactic injections demonstrated that OLIG2-expressing cells represent a target for transformation in both mouse and human cells. OLIG2-tva-a/p53 mutant mice infected with RCAS-PDGFB-BB developed high-grade gliomas after 2-4 months. Infection of OPCs in vitro using RCAS-PDGF-BB virus resulted in a rapid expansion along with a block in differentiation. Tumors generated by OLIG2-expressing differentiated glial cells and maintained as a glioma. As the most widely expressed cycling cell population, OPCs is a likely origin for different childhood gliomas. Our studies also suggest that a block in differentiation is an early event in gliomagenesis. Transformation of human precursor cells in vitro followed by xenografting into mice represent a model to study sequential events during gliomagenesis.

0167. PROLIFERATIVE PROGENITOR CELLS OF THE POSTNATAL PONS: IMPLICATIONS FOR PONTINE GLIOMAGENESIS

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INTRODUCTION: Diffuse Intrinsic Pontine Glioma (DIPG) is a fatal pediatric brain tumor of unknown developmental origin. The present study...
describes the postnatal growth of human pons and the proliferative progenitor cells of the postnatal human and mouse pons, providing a developmental context in which to identify candidate cells of origin for DIPG. METHOD: We followed the growth of human brainstem by MRI analysis of normal children. We then characterized proliferation and lineage analysis by immunohistochemistry in pediatric human post-mortem samples and in mouse brainstem, co-staining proliferative cells for various markers of astrocyte or oligodendrocyte lineage. RESULTS: We observed that proliferation levels in postnatal human pons are greatest in neonates and decline through infancy; cells of the basis divided more robustly than tegmentum. This correlates with MRI data that show the pons to grow fastest in the first months of life, and faster in basis than tegmentum, then slowing its growth in childhood (despite minimal proliferation after 6 months). Proliferation in postnatal mouse pons is also restricted to childhood, peaking shortly after birth, and is greater in basis than tegmentum. The mouse pons contains several anatomically and molecularly distinct proliferative progenitor populations that decline at different ages. CONCLUSIONS: The early postnatal pons contains a bimodal postnatal proliferative progenitor population, particularly in the ventral pons, which may account for extremely rapid regional growth during the first months of life. Ongoing work will identify the lineage association of proliferative cells in the pons and whether targeting these cells is sufficient for de novo gliomagenesis.

0168. UNIQUE PATTERNS OF PROGRESSION IN RECURRENT PRIMITIVE NEUROECTODERMAL TUMORS
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INTRODUCTION: Ependymoma (EPN) is the 3rd most common brain tumor in children. Despite standard therapy, most children with EPN relapse and ultimately die of their disease. Numerous molecular characteristics of newly diagnosed EPN have been elucidated, but the biology of relapsed EPN is largely unexplored. METHOD: We used transcriptome microarray (Affymetrix U133plus2) to analyze 13 matched pairs (26 total samples) of EPN previously reported posterior fossa sub-groups (Groups A and B) and reveals transcriptomic similarity (347 differentially expressed) among relapsed EPN tumors. We then performed hierarchical clustering (Affymetrix U133plus2) to analyze 13 matched pairs (26 total samples) of newly diagnosed and relapsed pediatric EPN. Normalization and unbiased hierarchical clustering were performed using Bioconductor R, and ontology analyses were performed using DAVID. RESULTS: Clustering confirmed previously reported posterior fossa sub-groups (Groups A and B) and reveals unique progression patterns. Tumors initially presenting as Group A (n = 8) change significantly at recurrence, evidenced by progression of three recurrences to Group B (10%) differentially expressed genes (p < 0.05; fold ≥1.5). Conversely in Groups B, recurrent tumors cluster side-by-side with their primary (n = 5), implying transcriptomic similarity (347 differentially expressed). Ontology (actin cytoskeleton) and specific gene (NELL2, LAMA2) analyses of differentially expressed genes support progression from Group A to Group B phenotype at recurrence, and enhancement of group B signatures at recurrence in those initially presenting as Group B. CONCLUSIONS: We are the first to report subgroup re-designation of recurrent pediatric EPN. We are also the first to identify subgroups of progression between sub-groups in Group A tumors exhibit more transcriptomic change and become more similar to Group B tumors at recurrence. This suggests that therapy at recurrence should be tailored to the Group B phenotype.

0169. REFINED RADIOLOGICAL RESPONSE CRITERIA FOR EVALUATING LEPTOMENINGEAL METASTASIS - LM RESPONSE SYSTEM
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INTRODUCTION: The Y-box-binding protein-1 (YB-1) is an oncogenic transcription and translation factor that is highly expressed in adult and pediatric glioblastoma (GBM) but not in normal brain tissues. It is functionally associated with a ribosomal S6 kinase (RSK) (90S ribosomal S6 kinase (90S (R)) when cells are treated with temozolomide (TMZ). We previously reported that expression of YB-1 at recurrence in tumors from GBM patients before and after TMZ treatment (n = 14 pairs) by immunohistochemistry, YB-1 localization was evaluated in drug refractory GBM cell lines (SF188 and BT74), using immunocytochemistry and in orthotopic xenografts by immunohistochemistry. To understand how YB-1 contributes to TMZ resistance, we used pRT-PCR arrays to profile gene expression changes in SF188 cells. POST-YB-1 and BMI-1 silencing. RSK was inhibited with siRNA or the small molecule inhibitor BI-D1870. Primary GBM isolates were cultured in monolayer and as neurospheres. RESULTS: YB-1 was expressed in all of the tumors before TMZ treatment and therapy did not reduce expression. TMZ-treated SF188 cells continue to proliferate with retaining YB-1 transcriptional activity. Silencing YB-1 or BMI-1 induced GADD45a and RAD17, which are genes responsible for recognizing DNA damage. This occurs through the polycomb repressor BMI-1. The expression of these genes are reactivated by inhibiting the YB-1 pathway suggesting that this may be a new avenue to sensitize GBM cell lines to TMZ. In summary, YB-1 functions impose TMZ resistance by suppressing the expression of genes that recognize DNA damage, which prevents the GBM cells from undergoing apoptosis.

0170. THE Y-BOX BINDING PROTEIN-1 CONVEYS TEMOZOLOMIDE RESISTANCE BY THE INHIBITING DNA DAMAGE RESPONSE PATHWAY THROUGH THE POLYCOMB REPRESSOR BMI-1
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INTRODUCTION: The Y-box-binding protein-1 (YB-1) is an oncogenic transcription and translation factor that is highly expressed in adult and pediatric glioblastoma (GBM) but not in normal brain tissues. It is functionally associated with a ribosomal S6 kinase (RSK) (90S ribosomal S6 kinase (90S (R)) when cells are treated with temozolomide (TMZ). We previously reported that expression of YB-1 at recurrence in tumors from GBM patients before and after TMZ treatment (n = 14 pairs) by immunohistochemistry, YB-1 localization was evaluated in drug refractory GBM cell lines (SF188 and BT74), using immunocytochemistry and in orthotopic xenografts by immunohistochemistry. To understand how YB-1 contributes to TMZ resistance, we used pRT-PCR arrays to profile gene expression changes in SF188 cells. POST-YB-1 and BMI-1 silencing. RSK was inhibited with siRNA or the small molecule inhibitor BI-D1870. Primary GBM isolates were cultured in monolayer and as neurospheres. RESULTS: YB-1 was expressed in all of the tumors before TMZ treatment and therapy did not reduce expression. TMZ-treated SF188 cells continue to proliferate with retaining YB-1 transcriptional activity. Silencing YB-1 or BMI-1 induced GADD45a and RAD17, which are genes responsible for recognizing DNA damage. This occurs through the polycomb repressor BMI-1. The expression of these genes are reactivated by inhibiting the YB-1 pathway suggesting that this may be a new avenue to sensitize GBM cell lines to TMZ. In summary, YB-1 functions impose TMZ resistance by suppressing the expression of genes that recognize DNA damage, which prevents the GBM cells from undergoing apoptosis.

0171. MYC DRIVEN HUMAN NEUROSphere MODELS OF PRIMITIVE NEUROECTODERMAL TUMORS ARE SENSITIVE TO INHIBITORS OF GLUTAMINE METABOLISM
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INTRODUCTION: The MYC family of proteins promotes proliferation and an aggressive phenotype in diverse cancers. Primitive Neuroectodermal Tumors (PNETs) are high grade tumors that arise throughout the neuroaxis. One subset of PNETs in MYC amplified and some PNETs are known to express LIN28A, a key regulator of MYC. MYC expression in tumors leads to increased reliance on glutamine metabolism (i.e. Warburg effect). We hypothesize that MYC-driven PNET tumors will up regulate glutamine metabolism. We secondarily hypothesize that glutaminease inhibitors will selectively target MYC-driven PNET. METHOD: We used the MYC -positive PFSK cell line and a human neural stem and progenitor cell model of MYC-transformed PNET to test our hypothesis. Neurospheres derived from developing human cerebral cortex were transduced using lentiviral coding for c-MYC, dominant negative p53, sibling, and control cells. Neurosarcoma, University of British Columbia, Vancouver, BC, Canada, before developing gliomas.
constitutively active AKT, and hTERT. Controls were non-MYC immortalized cortex neurons. MYC and glutaminase expression were determined by western blot. The glutaminase inhibitors DON (6-Diazoo-5-oxo-L-norleucine) and acivicin (2S)-Aminoisobutyric acid (3S)-choloro-4,3-5-dibydro-1,2-oxazole-5-yl)[ethanolic acid) were used to disrupt glutamine metabolism. We measured cell proliferation using MIT assay and BrdU. We evaluated apoptosis using flow cell cycle analysis and cleaved caspase 3 immunofluorescence. R248Wp53/MYC/AKT/hTERT neurosphere cells treated with DON showed a 48% reduction in BrdU incorporation compared to untreated cells (p < 0.01). DON and acivicin treatment of R248Wp53/MYC/AKT/hTERT neurosphere cells led to an increase in the apoptotic fraction of cells, as measured by flow cytometry (26% increase in apoptosis with DON treatment vs. 44% increase with Acivicin treatment). In comparison, non-MYC driven human neurospheres did not show an increase in apoptosis with DON or acivicin treatment. CONCLUSIONS: MYC expression in conjunction with other oncogenic hits can transform human neural stem and progenitor cells, creating a model of aggressive PNET. This MYC-driven PNET model is susceptible to glutamine metabolic inhibitors, while cortical neurospheres immortalized with oncogenes other than MYC are resistant to these inhibitors. We are currently further investigating the mechanism of growth inhibition using BrdU analysis, cell cycle analysis, and cleaved caspase 3. Murine orthotopic xenograft experiments investigating the efficacy of DON and acivicin treatment in MYC-driven PNET are currently underway in our laboratory. These experiments suggest that glutamine metabolism may be a therapeutic target in PNET.

0172. GENETIC ABOLITION OF THE BRAF ONCOGENE DRIVES BRAF-TRANSFORMED ASTRONOMY COMPLEXES IN A MURINE MODEL OF BRAF-INDUCED BRAINSTEM ASTROCYTOMA

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INTRODUCTION: The p53 tumor suppressor mediates cellular responses (growth arrest and/or apoptosis) in response to ionizing radiation or genotoxic damage. We have shown that MYC can transform neurosphere cells from primary human neural stem cells. MYC overexpression or acetylation is sufficient to transform primary human neurosphere cells. MYC overexpression driven neurosphere cells showed increased MYC and glutaminase compared to non-MYC driven neurosphere cells. R248Wp53/MYC/AKT/hTERT transduced human neurosphere cells formed aggressive PNET-like tumors in orthotopic xenograft models. R248Wp53/MYC/AKT/hTERT transduced human neurosphere cells treated with DON showed a 48% reduction in BrdU incorporation compared to untreated cells (p < 0.01). DON and acivicin treatment of R248Wp53/MYC/AKT/hTERT neurospheres led to an increase in the apoptotic fraction of cells, as measured by flow cytometry (26% increase in apoptosis with DON treatment vs. 44% increase with Acivicin treatment). In comparison, non-MYC driven human neurospheres did not show an increase in apoptosis with DON or acivicin treatment. CONCLUSIONS: MYC expression in conjunction with other oncogenic hits can transform human neural stem and progenitor cells, creating a model of aggressive PNET. This MYC-driven PNET model is susceptible to glutamine metabolic inhibitors, while cortical neurospheres immortalized with oncogenes other than MYC are resistant to these inhibitors. We are currently further investigating the mechanism of growth inhibition using BrdU analysis, cell cycle analysis, and cleaved caspase 3. Murine orthotopic xenograft experiments investigating the efficacy of DON and acivicin treatment in MYC-driven PNET are currently underway in our laboratory. These experiments suggest that glutamine metabolism may be a therapeutic target in PNET.

0173. LncRNA PROFILE OF GLIOBLASTOMA REVEALS THE POTENTIAL ROLE OF LncRNAs IN CONTRIBUTING TO GLIOBLASTOMA PATHOGENESIS

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INTRODUCTION: LncRNAs have recently emerged as a major class of regulatory molecules involved in a broad range of biological processes and complex diseases. Our aim is to identify important lncRNAs that might play an important role in contributing to glioblastoma (GBM) pathogenesis by conducting lncRNA and mRNA profiles comparison between GBM and normal brain tissue. METHOD: The differentially expressed lncRNA and mRNA profiles of the tissue between GBM patient and age-matched donor without GBM diseases were analyzed via microarray. We proposed a novel model for (2S)-Aminoisobutyric acid-GBM mRNA targeting relationships that combined the potential targets of the differentially expressed lncRNAs with the differentially expressed mRNAs abundance data. Bioinformatic analysis of the predicted target genes (gene ontology, pathway and network analysis) were done for further research. RESULTS: The lncRNA microarray reveals differentially expressed lncRNAs between the GBM and normal brain tissues. In the GBM group, 654 lncRNAs were upregulated and 654 were downregulated (fold change ≥ 4.0 or ≤0.25, P < 0.01). We found 104 matched lncRNA-mRNA pairs for 91 differentially expressed lncRNAs and 84 differentially expressed gene-similar gene-pathway expressions. These analyses showed a significant change in PPAR pathways in the GBM group compared with the normal brain group (P < 0.05). By further conducting lncRNA gene network analysis, we found that the ASLNC22381 and ASLNC208181 might play important roles via their target mRNA in the recurrence and malignant progression of GBM.

0174. H3.3 K27M ACCELERATES PDGF-INDUCED BRAINSTEM GLIOMAGENESIS IN VIVO

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INTRODUCTION: Diffuse Intrinsic Pontine Glioma (DIPG) is an incurable pediatric brain tumor with an extremely poor prognosis. Recently, K27M H3.1 and H3.3 mutations were discovered to occur in 78% of DIPG patients and are indicative of a poor prognosis. This mutation non-constitutively activates the specific, highly conserved lysine histone tail residue 27 to mimic an active regulatory state. Mutations at this residue in vertebrates accelerate development of PDGF-induced brainstem gliomagenesis. The goals of this study are to determine: 1) whether H3.3K27M is sufficient to induce brainstem gliomas, 2) if H3.3K27M accelerates PDGF-induced brainstem gliomagenesis, and 3) if H3.3K27M reduces global H3K27me3 levels in vivo. METHODS: Mice were infected with the brainstem glioma-inducing virus in vivo. We are currently investigating the mechanism by which H3.3K27M is cooperating with PDGF-induced brainstem gliomagenesis. Furthermore, H3.3K27M is sufficient to reduce global H3K27me3 levels in vivo. We are currently investigating the mechanism by which H3.3K27M is cooperating with PDGF-induced brainstem gliomagenesis.

0175. A PHASE I/II STUDY OF ADOPTIVE T-CELL THERAPY (ALT) AND DC VACCINATION (DCV) DURING RECOVERY FROM MYELOABLATIVE CHEMOTHERAPY AND HEMATOPOIETIC STEM CELL TRANSPLANTATION (HDC + ASCT) OR NON-MYELOABLATIVE CONDITIONING (NMA) IN PATIENTS (PTS) WITH RECURRENT CENTRAL PNETS

Sridharan Gururangan, Gerald Grant, Tim Driscoll, Gerald Archer, James Herndon, Henry Friedman, Joanne Kurtzberg, Darell Bigner, Sridharan Gururangan, Gerald Grant, Tim Driscoll, Gerald Archer

INTRODUCTION: Outcome following standard therapy for children with recurrent central nervous system tumors is poor. Recently, K27M H3.1 and H3.3 mutations were discovered to occur in 78% of DIPG patients and are indicative of a poor prognosis. This mutation non-constitutively activates the specific, highly conserved lysine histone tail residue 27 to mimic an active regulatory state. Mutations at this residue in vertebrates accelerate development of PDGF-induced brainstem gliomagenesis. The goals of this study are to determine: 1) whether H3.3K27M is sufficient to induce brainstem gliomas, 2) if H3.3K27M accelerates PDGF-induced brainstem gliomagenesis, and 3) if H3.3K27M reduces global H3K27me3 levels in vivo. METHODS: Mice were infected with the brainstem glioma-inducing virus in vivo. We are currently investigating the mechanism by which H3.3K27M is cooperating with PDGF-induced brainstem gliomagenesis.

REF-MATCH PROTOCOL

Sridharan Gururangan, Gerald Grant, Tim Driscoll, Gerald Archer, James Herndon, Henry Friedman, Joanne Kurtzberg, Darell Bigner, John Sampson, and Duane Mitchell, Duke University Medical Center, Durham, NC, USA

INTRODUCTION: Outcome following standard therapy for children with recurrent central nervous system tumors is poor. Recently, K27M H3.1 and H3.3 mutations were discovered to occur in 78% of DIPG patients and are indicative of a poor prognosis. This mutation non-constitutively activates the specific, highly conserved lysine histone tail residue 27 to mimic an active regulatory state. Mutations at this residue in vertebrates accelerate development of PDGF-induced brainstem gliomagenesis.
required to improve outcome. We have developed a novel platform for the expansion of polyclonal populations of tumor-specific T cells using amplified tumor RNA pulsed DCs that has shown considerable efficacy in preclinical models of invasive brain cancers. We are conducting a phase I/II study to assess the feasibility, safety, and estimate the efficacy of ALT + DCV following recovery from HDC + ASCR (group A) or NMA (group B) in patients with recurrent PNET (RE-MATCH Protocol, FDA IND BB-14058, Duke IRB #18020). Eligible patients are enrolled if they received 4 cycles of standard induction chemotherapy prior to either HDC + ASCR (carboplatin, thiotepa, and etoposide) (group A) or NMA using carboplatin, fludarabine (group B) are evaluated (3 x 10^6, 10^7, 10^8) cells/Kg with a single dose of intracranal DCV (107 cells). Safety evaluation for dose limiting toxicity (DLT) assessment is two weeks past the third biweekly vaccine with monthly vaccines thereafter as determined by CT or MRI. TSV: 19 subjects have been enrolled by IRB consent for collection of tumor tissue. Tumor RNA was successfully amplified from 13/13 recurrent tumor samples. Six samples were removed for histology other than PNET or no viable tumor on biopsy or resection. We have generated sufficient DCs for at least 3 vaccinations per T cell to the targeted dose of 12 in 13 enrolled subjects. Seven subjects have received immunotherapy; 4 subjects at 3 x 10^6 T cells/Kg (Group A-1, Group B-3), and 3 subjects in Group B at 3 x 10^7 T cells/Kg, There have been no DLTs observed. CONCLUSIONS: ALT + DCV therapy is feasible and preliminary evidence demonstrates the safety of this treatment approach. Accrual continues on the phase I component of the study along with clinical and immunologic evaluation.

0176. NG2 GROULAMEN IN PEDIATRIC DIFFUSE INTRINSIC PONTINE EPIGLOMIA AND ITS ROLE IN TUMORIGENICITY IN VIVO Meli Frangos1, Vinay Nadavalli1, Dharmil Kambhampati1, Oren Becher2, Towy MacDonald3, Ravi Bellamkonda4, Roger Packer5, and Javad Nazzarian5. 1Children’s National Medical Center, Washington, DC, USA; 2Duke University School of Medicine, Durham, NC, USA; 3Emory Children’s Center, Atlanta, GA, USA; 4Georgia Institute of Technology, Atlanta, GA, USA; 5Johns Hopkins School of Medicine, Baltimore, MD, USA; 6National Cancer Institute, Bethesda, MD, USA

INTRODUCTION: Pediatric brainstem glioma (BSG) is one of the most difficult cancers to treat accounting for 10-20% of all pediatric central nervous system tumors. BSGs may occur anywhere along the brainstem, recognized into two main groups: diffuse intrinsic pontine gliomas (DIPGs) and focal brainstem gliomas. DIPGs represent about 80% of BSGs with a peak onset of six to nine years of age. DIPGs invade throughout the pons and may spread to other brainstem areas. To further understand the pathophysiology of the disease, a genetically engineered (PDGFβ-expressing) BSG mouse and a xenograft model have been established. METH: Protein from BSG mouse tumor and normal specimens were processed and submitted for MS/MS proteomic analysis using LTQ-Orbitrap-XL. Isolated peptides identified using the Sequest algorithm in the Bioworks browser and uniprot data base were submitted to quantitative and subgroup analysis using ProteoIQ and Partek Genomics Suite. Proteins of interest were validated using human DIPG mouse and BSG specimens by western blot and immunohistochemical staining. RESULTS: 19 subjects have been enrolled on a screening protocol. RESULTS: We show high expression of NG2 in murine model of brainstem glioma as well as 80% of pediatric DIPG specimens tested. shRNA-mediated knockdown of NG2 reduces cellular migration in vitro. NG2 expression is defective (symmetric) in mitotic cells in vitro and in vivo which is consistent with observations in adult gliomas. Injection of NG2 expressing neurospheres into brains of P2 mice results in highly aggressive brainstem tumors (leading to death within 3-7 weeks) providing a solid model for testing preclinical evaluations. Furthermore, we show selective delivery of liposomal nanoparticles to brainstem of our robust BSG mouse model. CONCLUSIONS: We introduce a robust murine model of brainstem glioma that is developed using NG2 expressing cells. High expression of NG2 in a subset of DIPGs and its defective expression may provide novel approaches for treating DIPGs and BSGs.

0177. AN OPEN-LABEL, TWO-STAGE, PHASE II STUDY OF BEVACIZUMAB AND LATAPINIB IN CHILDREN WITH RECURRENT OR REFRACTORY EPENDYMOMA: A COLLABORATIVE PEDIATRIC EPIGLOMIA RESEARCH NETWORK STUDY (CERN) Mariko DeWire1, Maryam Fouladi1, Clinton Stewart2, Cynthia Wetmore3, Cynthia Hawkins3, Carmen Jacobs4, Ying Yuan4, Stewart Goldman5, Kari Rives6, Mariko DeWire7, Maryam Fouladi1, Clinton Stewart2, Cynthia Wetmore2, Mariko DeWire1, Maryam Fouladi1, Clinton Stewart2, Cynthia Wetmore2, Mariko DeWire1, Maryam Fouladi1, Clinton Stewart2, Cynthia Wetmore2, Mariko DeWire1, Maryam Fouladi1, Clinton Stewart2, Cynthia Wetmore2, Stewart Goldman5, Tobey MacDonald3, Ravi Bellamkonda4, Roger Packer1, and Javad Nazzarian5. 1Children’s National Medical Center, Washington, DC, USA; 2Duke University School of Medicine, Durham, NC, USA; 3Emory Children’s Center, Atlanta, GA, USA; 4Georgia Institute of Technology, Atlanta, GA, USA; 5Johns Hopkins School of Medicine, Baltimore, MD, USA; 6National Cancer Institute, Bethesda, MD, USA

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0178. COMPARATIVE MOLECULAR ANALYSIS OF PEDIATRIC DIFFUSE INTRINSIC PONTINE EPIGLOMIA REVEALS TUMOR SUBTYPES WITH DIFFERENTIAL ACTIVITY OF SONIC HEDGEHOG PATHWAY Amanda Saratasi1, Sreedhanya Nadavalli1, Kendall Snyder1, Madhuri Kambhampati2, Jordan Hall3, Eric Raabe4, Kathy Warren5, Roger Packer2, and Javad Nazzarian1. 1Children’s National Medical Center, Washington, DC, USA; 2Duke University School of Medicine, Durham, NC, USA; 3Emory Children’s Center, Atlanta, GA, USA; 4Georgia Institute of Technology, Atlanta, GA, USA; 5Johns Hopkins School of Medicine, Baltimore, MD, USA; 6National Cancer Institute, Bethesda, MD, USA

INTRODUCTION: Diffuse Intrinsic Pontine Glioma (DIPG) is a highly morbid form of pediatric brainstem glioma. Molecular characterization is limited due to lack of tissue. Recent investigations suggest possible molecular subtypes may account for the historical poor response to therapy. We previously generated protein profiles of CSF and formalin fixed DIPG tumor specimens to characterize patterns of protein expression. Here, we present the first comprehensive tissue proteome of fresh frozen DIPG tumor specimens (n = 16) and normal brain tissue (n = 10). We characterize differential protein expression in DIPG tumor specimens, and compare these to gene expression and DNA methylation profiles of the same tissue. METHOD: Normal brain and tumor tissue was collected intraoperatively or post-mortem. Extracted total tissue protein was quantified by spectrometry (MS/MS) via LTQ-Orbitrap-XL and iTRAQ based group comparison. Genes and gene expression data from the sequenced tumor genome of 16 DIPG patients were detected using whole-genome Human HT-4 v12 Gene Expression Bead ChIP arrays. Quantitative and statistical analysis was performed with GenomeStudio, ProteoIQ, and Partek Genomics Suite. Functional analysis was performed using Ingenuity Pathways Analysis. Gene and protein expression was validated via western blot and immunohistochemical staining of tumor and normal brain tissue. RESULTS: 1,918 differentially expressed genes were detected in DIPG tumors (p < 0.05, FC > 2 or < 0.2). Unsupervised clustering revealed two distinct subgroups with differential SHH activity (GLI1 z-score ≥ 2.800 or ≤ -2.000) and expression of GLI1, GLI2, GLI3, P16, CDH1 and SMO. Protein profiling revealed high expression of TLR11, TLR6, EEF3 (FC > 2), with differential SHH pathway (GLI1 z-score -0.626 vs. 2.254) and protein expression COL1A2, LMNA, MAP4, NES, NRCAM, STMN1, and TNC between subgroups (ANOVA, p < 0.05, FC > 2 or < -2). Concordant differences in DNA methylation were detected in related genes, including GLI1, FOXF1, SMO, SHH, and SUFU.
019. CORRELATION OF CSF AND SERUM BIOMARKERS OF OXIDATION IN PEDIATRIC BRAIN TUMOR PATIENTS

Joshua Thompson1, Andrea Griesinger1, Nicholas Foreman1, Sarah Rush2, Angela Higginbotham1,1 University of Colorado, Aurora, CO, USA;2 Akron Children’s Hospital, Akron, OH, USA

INTRODUCTION: There is interest in identifying biological markers (bio-markers) that can be measured for diagnostic, prognostic, and evaluating response to therapies. Increases in measures of oxidative stress in CSF have been shown to be an indicator of brain injury and these could be useful in our patient population, although measuring these markers in the CSF poses an obstacle as CSF is not routinely collected during therapy. In order to use markers of oxidative stress, less invasive methods of monitoring are needed. We hypothesized that markers of oxidative stress would correlate with CSF levels providing a non-invasive method of monitoring. METHOD: One measure of oxidative stress is determined by the concentration of F2 isoprostanes, a prostaglandin-like compound formed through spontaneous peroxidation of lipids present in high concentrations in phospholipids. We collected matched pairs of CSF and serum from patients at the time of primary tumor resection. F2 isoprostane levels were measured in each sample by a competitive enzyme-linked immunos assay (ELISA) (Cayman’s 8-Isoprostane EIA Kit). Correlation between the CSF and serum values was determined using a Pearson’s correlation. RESULTS: Reproducible and reliable values were able to be obtained from both the serum and CSF samples from patients with multiple tumor types. We also found a strong correlation of F2 isoprostane between CSF and serum with an R-value of 0.75. CONCLUSIONS: This study demonstrates a correlation between serum and CSF levels of F2 isoprostane, a marker that has been previously shown to correlate with levels of oxidative brain injury. This study will allow for further investigations into the practical applicability of oxidative markers in children with CNS tumors as our study has shown that serum levels correlate with those in CSF and provide a less invasive mechanism for study. Future studies are needed to determine a correlation between the levels of these oxidative markers in the serum and the long-term outcomes in children with CNS tumors.

0180. mTORC1 and mTORC2 pathway activity in pilocytic astrocytoma. METHOD: We used formalin-fixed tissue embedded human pilocytic astrocytoma tissues with immunohistochemistry in tissue microarrays. We also studied in vitro the effect of mTORC1 blockade in pediatric low grade glioma cell lines. RESULTS: We evaluated mTORC1 and mTORC2 pathway activity in pilocytic astrocytoma using an in vitro study. We evaluated mTORC1 and mTORC2 pathway activity in pilocytic astrocytoma.

0181. IDENTIFICATION OF AREG, SHH, MMP12 AND MMP9 AS NOVEL THERAPEUTIC TARGETS IN CRANIOPHARYNGIOMA

Jacob Gump1, Andrew Donson2, Diane Birks3, Michael Handler2, Nicholas Foreman3, and Todd Hankinson2;1 University of Colorado Anschutz Medical Campus, Aurora, CO, USA;2 Children’s Hospital Colorado, Aurora, CO, USA

INTRODUCTION: Neurological consequences of cranioopharyngioma (CPA) and its treatment condemn children with this disease to the lowest overall quality of life of any primary pediatric brain tumor population. This is due to irreversible neurological damage, including hypothalamic obesity, panhypopituitarism, and blindness. However, CPA is woefully understudied due to its low mortality (~90% 5-year survival), clinical complexity and recalcitrance to laboratory study. CPA is the most common non-glial pediatric brain tumor (~6%). Yet, there are no proven pharmacologic therapies for CPA, limiting treatment to surgery and radiation. Here, we identify rational targets for CPA therapy.

METHOD: This pilot study employed gene expression microarray analysis to study 8 samples of snap-frozen pediatric CPA to identify genes that were substantially overexpressed relative to normal human brain, normal pituitary and a panel of pediatric brain tumors. Overexpression was confirmed by western blotting for protein levels and by quantitative RT-PCR. We also analyzed each sample for activating mutations in B-catenin by sequencing the hot-spot region in exon 3. Clustering analyses were also conducted using microarray data from CPA and other CNS tumors to reveal gene expression patterns and signatures in the CPA samples. RESULTS: We identified the overexpression of 4 targetable gene products in this panel of CPA samples. Amphiregulin (AREG) mRNA was overexpressed by an average of 37-fold above normal brain tissue and other pediatric brain tumors. We also identified 162-fold overexpression of sonic hedgehog (SHH). Furthermore, CPA was shown to be 4-fold increased in MMP12 (MMP-12) and MMP9 at levels 1400 times and 900 times higher, respectively, than any other tumor tested. The overexpression of these genes was confirmed by QPCR and using western blot analysis, which demonstrated that these target genes were expressed at levels not observed in other pediatric CNS tumors. CONCLUSIONS: Each of these molecules represents a rational therapeutic target for CPA. Medications targeting these molecules are in or approaching clinical trials but have not yet been tested against CPA. These preliminary findings will be confirmed in larger pediatric CPA tumors, with the goal of providing robust biological data that can drive subsequent clinical trials. Treatments and outcomes for CPA have remained essentially unchanged for decades; targeted therapy has the potential to change this paradigm.

0182. INTEGRATIVE GENOMIC ANALYSES IDENTIFY RECURRENT STRUCTURAL ALTERATIONS IN ATYPICAL TERATOMATOUS RHABDOID TUMORS (ATRTs)

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INTRODUCTION: ATRTs (Atypical teratoid rhabdoid tumours) represent one of the most aggressive pediatric brain tumours. However, paradoxically, ATRTs have been reported to exhibit balanced genomes with only recurrent somatic alterations of the SMARCB1 locus on chr22. To better define molecular mechanisms underlying ATRT biology we comprehensively interrogated 63 ATRTs using an integrated genomics approach. METHOD: 63 ATRTs were investigated using a combination of OmniQuad ultra-high resolution SNP genotyping (n = 39) and whole-genome/exome sequence (n = 24) analyses. Copy number and structural alterations were mapped using orthogonal methods including circular binary segmentation (dChip, Partek Suttes), CREST, BreakDancer and Pindel analyses. Alterations were validated by targeted re-sequencing using the Sanger method and/or MiSeq and Ion Torrent platforms. RESULTS ATRTs exhibited distinctive chromosomal imbalances that are distinct from other pediatric CNS tumors with exception of loss of function mutations in SMARCB1 (15 SNVs in 63 tumours). Significantly, ATRTs exhibited a predominance of structural events (~3.13/tumour) including recurrent losses of LRP1B (chr2q22.12), CDH13 (chr16q23.3, BCR (chr21q22.13), CHEK2 (chr22q12.12), MLK1 and EP100 (chr22q13.12). We also detected inter-chromosomal translocations of EARS2/TRAM1 ([t;8;9][t;13;16p12]) and CDH13 with a non-coding locus ([t;16;11][t;23;p13]). Notably a majority of ATRTs (48/63) exhibited structural alterations of SMARCB1 including 2
novel intra-chromosomal events identified by WGS that were not detectable by MLPA or exon sequence analyses. CONCLUSIONS: Somatic structural alterations drive recurrent genetic events in ATRT. Our integrated high resolution WGS analysis demonstrates complex interactions among cortical and subcortical networks, including thalamus, prefrontal cortex, cingulate, and striatum. CONCLUSION: Improved neuroimaging demonstrated complex interaction between development and adverse brain function changes in children treated for medulloblastoma. Ventral visual processing was disrupted in patients, consistent with high radiodoses to cortex and commissural white matter connections around the posterior fossa. However, activation was affected broadly in patients, suggesting that disease and treatment lead to widespread brain changes. Connectivity mapping is an important complement to conventional structural and functional imaging, and promises to advance understanding of effects of disease and treatment on white and gray matter substrates of subsequent cognitive deficits, and the impact of interventions on brain function.

0185. PROMOTER METHYLATION-DEPENDENT SILENCING OF CELL CYCLE TUMOR SUPPRESSORS IN AGGRESSIVE PNET IS OVERCOME BY PERIFOSINE AND HDAC INHIBITORS

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INTRODUCTION: Primary neuroectodermal tumors (PNETs) are frequently found to have amplifications of Cyclin D and Cyclin-dependent kinase genes, which are major driving factors for the high mitotic rate and aggressive growth of these tumors. Previously our labs have shown that perifosine treatment of medulloblastoma and supratentorial PNET cultures and orthotopic xenografts induces dramatic p21-dependent growth arrest in PNET tumor models. To better understand the mechanism of induction of p21 following treatment, studies were performed to better characterize the process of transcriptional activation of p21 and the related cell cycle inhibitor p16. METHOD: Establishment of new cell and xenograft models from PNET tumors allowed characterization of the molecular mechanisms involved in p16 induction. Primary tumor tissue, short-term PNET cultures and their xenografts were examined for p21 and p16 expression by quantitative RT-PCR prior to and following treatment with perifosine, CUDC101, SAHA and valproic acid, compared to non-tumor CNS stem cell cultures and normal brain samples. Experiments were subjected to bisulfite sequencing and the results compared. Changes in expression levels of p21, p16 and cell lineage and differentiation markers were assayed by Taqman qRT-PCR assay, confocal imaging and western blotting. RESULTS: The mean latency and duration, respectively, were 19.5 and 4.5 days. In all cases of mutism, the tumor was in the midline. Medulloblastoma accounted for 3 (7.1%) of 90 patients with SJMB03 protocol therapy included comprehensive neuropsychological evaluations (baseline, +1 year, +2 year) included comprehensive neuropsychological testing and neuroimaging. Imaging included a battery of five MRI tasks to probe ventral visual function in reading, diffusion tensor imaging to characterize white matter, and high-resolution structural MRI. Task activation (SPM, http://www.fil.ion.ucl.ac.uk/spm/) and connectivity (GIFT, http://fmrib www.mr.northysoftware/gif/) were analyzed with clinical and behavioral covariates. RESULTS: Reading performance was diminished in patients (eg, reading fluency: patients = 93.3 [62 – 130], controls = 111.6 [82 – 175]) and patterns of brain activation during orthographic processing were altered in children who survive medulloblastoma. Activation was altered in neocortical networks for reading, and changes in activation were associated with performance on standardized tests of reading performance. Functional connectivity during orthographic processing revealed remarkable interactions among cortical and subcortical networks, including thalamus, prefrontal cortex, cingulate, and striatum. CONCLUSION: Improved neuroimaging demonstrated complex interaction between development and adverse brain function changes in children treated for medulloblastoma. Ventral visual processing was disrupted in patients, consistent with high radiodoses to cortex and commissural white matter connections around the posterior fossa. However, activation was affected broadly in patients, suggesting that disease and treatment lead to widespread brain changes. Connectivity mapping is an important complement to conventional structural and functional imaging, and promises to advance understanding of effects of disease and treatment on white and gray matter substrates of subsequent cognitive deficits, and the impact of interventions on brain function.
INTRODUCTION: Medulloblastoma is the most common malignant pediatric brain tumor, and it comprises four molecular subgroups: WNT, SHH, Group 3, and Group 4 - which have distinct biological, clinical, and genetic characteristics. Current treatment regimens stratify all medulloblas-
toma patients into risk groups based on clinical features including patient age, tumour histology, and metastatic stage. Recent reports of the stark genetic differ-
ences among the four subgroups of medulloblastoma suggest that subgroup-specific biomarkers may improve the risk-stratification of patients.

METHOD: Molecular biomarkers for risk-stratification were identified from a copy-number profiling screen by single nucleotide polymorphism arrays on a discovery set (n = 629), consisting of samples acquired retrospec-
tively from 64 centers across the globe. Tissue microarrays for select biomarkers were performed in an independent validation set (n = 453). Combined risk-stratification models were designed based on clinical and cyto-
genetic biomarkers identified by multivariate Cox proportional hazard analy-
yses on the discovery set, and the models were subsequently tested on the validation set. RESULTS: Subgroup status was strongly predictive of patient survival; it improved the predictive accuracy of a multivariate survival model on top of known clinical biomarkers. WNT patients had good survival irrespective of clinical biomarkers. With the exception of metastatic stage, most clinical biomarkers were differentially associated with survival across the remaining subgroups. Similarly, most known cyrogenetic biomarkers were only prognostic within specific subgroups (e.g. MYC and MYCN ampli-
fications). Our clinical-cytogenetic risk schema can identify low-risk (almost Wnt-like) Group 3 patients, and high-risk (almost Group 3-like) SHH patients using only tools available in a modern neuropathology laboratory. CONCLUSIONS: Molecular subgroups and genomic aberrations in medulloblas-
toma are powerful biomarkers of prognostic outcome. Combining subgroup and cytogenetic biomarkers with established clinical risk factors can substantially improve survival prediction, even in the context of heterogeneous clinical treatments. Critically, the prognostic significances of biomarkers are often restricted to specific subgroups. By examining >1000 patients, we have identified several novel cyrogenetic markers that identify very high risk, and very low risk groups of patients. The identified cyrogenetic biomarkers can behave as high-priority candidates for prospective multicentre trials.

INTRODUCTION: During cerebellar development, the Sonic hedgehog (Shh) signaling pathway drives the proliferation of granule cell precursors (GCPs). Aberrant activation of Shh signaling causes over-proliferation of GCPs leading to medulloblastoma, an aggressive pediatric brain tumor. Although the tissue-restricted Shh-binding protein Boc associates with the Shh receptor Patched1 to mediate Shh signaling, whether Boc plays a role in medulloblastoma tumorigenesis is unknown. METHOD: n/a RESULTS: Here, we show that BOC is upregulated in the SHH subgroup of human medulloblastoma and that Boc upregulation is associated with the early steps of medulloblastoma tumorigenesis in a mouse model of medulloblastoma. We further show that Boc upregulation in GCPs induces their proliferation. Conversely, Boc inactivation decreases the size of medulloblastoma tumors and reduces the progression of early medulloblastomas into advanced tumors. We find that Boc regulates tumor size by controlling the expression of genes important for cell proliferation. Remarkably, Boc also regulates Patch1 loss-of-heterozygosity, an important event in the progression from early to advanced medulloblastoma. CONCLUSIONS: Taken together, our results indicate that Boc modulates medulloblastoma progression and that targeting tissue-restricted components of the Shh signaling pathway, such as Boc, can prevent cancer development.

INTRODUCTION: Poor outcome in medulloblastoma is driven in part by MYC-amplified / Group 3 medulloblastomas. These tumors are notably resis-
tant to standard chemotheraphy and radiotherapy, even at maximally tolerated doses. Understanding which molecular pathways, when targeted, result in a biologically significant effect will help guide the development of novel thera-
pies for children with these lethal tumors. METHOD: We performed a luciferase-based cell viability screen on a patient-derived MYC-amplified me-
dulloblastoma cell line against a library of 1,982 biologically and structurally diverse compounds. We identified 115 "hits" from the primary screen and per-
formed secondary validation of these compounds against four additional MYC-amplified medulloblastoma cell lines and a counter screen in normal subventricular zone derived neural stem cells. Chemical genomic signatures of selected candidates were generated and gene set enrichment analysis was performed against databases containing canonical pathways and chemical genomic signatures of other well-annotated drugs. Further confirmation of in-
ferred biological activity was performed using standard bench assays. RESULTS: We identified several classes of compounds that effectively inhibit-
ated MYC-associated transcriptional activity and decreased viability in Group 3 medulloblastoma cells. These included proteasome inhibitors, aldehyde dehy-
drogenase inhibitors, HDAC inhibitors and several neuroactive ligand recep-
tor agonists. Despite the variation of mechanism of action across these compounds, the overlaps of their gene expression profiles identified enrichment of genes corresponding to cellular response to ROS. Increased ROS was confirmed by DNA damage staining and inhibition of their biological effects by co-treatment with NAC, a ROS scavenger. CONCLUSIONS: Using a chemical biology and chemical genomics approach, we have identified several classes of compounds that shut down MYC transcriptional activity and effectively inhibit MYC-amplified medulloblastoma growth and survival. Though these individual classes of compounds target different proteins/path-
ways, they all converge on cellular response to ROS. Our approach highlights various mechanisms, including proteasome inhibition and inhibition of alde-
hyde dehydrogenase activity, which may be exploited in ROS-based therapies, and such agents should be considered for further preclinical and clinical develop-
ment.

INTRODUCTION: AT/RTs are aggressive early childhood brain tumors that are characterized by the functional loss of SMARCB1 through biallelic deletion and/or mutation. A recent study using Whole Exome Sequencing of DNA (WES) identified an extremely low mutation rate in AT/RTs, and no genes altered recurrently other than SMARCB1 (Lee et al, 2012). These findings suggest that factors other than genomic instability are important drivers of AT/RT tumorigenesis. Here we report on the extensive genomic findings and to look for alterations in the AT/RT transcriptome. METHOD: DNA was extracted from 5 AT/RT patient samples and 5 matched germline samples and used for WES on the Illumina platform. Reads were aligned using the Burrows-Wheeler-Alignment algorithm, and variants were called using the Genome Analysis Tool Kit. Whole transcriptome sequencing of polyA+ RNAs (RNA-seq) was performed on the Illumina platform using 4 of the tumors and 2 of the germline samples used for WES. The RNA-seq reads were aligned using GSNAP, and differential expression of genes and isoforms was examined using Cufflinks tools, DESeq and DEXSeq. RNA-seq reads were also used to verify genomic alterations identified by WES. RESULTS: WES resulted in a mean coverage of the tar-
targeted genome of 51.6 MB, with over 80% of sites achieving " callable" coverage of 10X or greater. Mean mutation rate was .21 mutations per 1000 RT samples. Differential exon usage in AT/RT tran-
scripts was examined using Cufflinks tools, DESeq and DEXSeq. RNA-seq results were therefore used to verify genomic alterations identified by WES. Results were against databases containing canonical pathways and chemical genomic signatures and to look for alterations in the AT/RT transcriptome. METHOD: DNA was extracted from 5 AT/RT patient samples and 5 matched germline samples and used for WES on the Illumina platform. Reads were aligned using the Burrows-Wheeler-Alignment algorithm, and variants were called using the Genome Analysis Tool Kit. Whole transcriptome sequencing of polyA+ RNAs (RNA-seq) was performed on the Illumina platform using 4 of the tumors and 2 of the germline samples used for WES. The RNA-seq reads were aligned using GSNAP, and differential expression of genes and isoforms was examined using Cufflinks tools, DESeq and DEXSeq. RNA-seq reads were also used to verify genomic alterations identified by WES. RESULTS: WES resulted in a mean coverage of genome of 51.6 MB, with over 80% of sites achieving " callable" coverage of 10X or greater. Mean mutation rate was .21 mutations per 1000 RT samples. Differential exon usage in AT/RT samples. Differential exon usage in AT/RT compared to normals was seen in 1% of genes. CONCLUSIONS: The RNA sequencing results closely matched those reported previously by Lee et al (2012). In spite of the very low level of genomic alterations in these tumors, RNA-seq revealed substantial heterogeneity across the AT/RT transcriptome, both at the gene and isoform levels. The results of this study suggest that loss of SMARCB1 does not result in genomic instability, but does impact the transcriptome in ways that are often occurrence-specific. The reason(s) underlying this heterogeneity remain to be discovered.
0190. CHARACTERIZATION OF RADIATION-INDUCED BRAIN INJURY IN SURVIVORS OF PEDIATRIC BRAIN TUMORS USING MAGNETIC RESONANCE SPECTROSCOPY

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INTRODUCTION: Children receiving radiation therapy for malignant brain tumors are at risk for developing long-term sequelae including endocrinopathies, cognitive deficits, and secondary malignancies. These deficits take years to develop hampering the ability to conduct efficient clinical trials with toxicity endpoints and develop interventions to mitigate these effects. Magnetic resonance spectroscopy (MRS) is a non-invasive imaging technique that can characterize a variety of brain injuries. We explore the MRS characteristics of patients with radiation-induced brain injury. METHOD: Survivors of pediatric brain tumors who received cranial irradiation and underwent clinical MRS of either frontal or parietal white matter using a standard single voxel PRESS sequence during routine MR follow-up were included. Comparisons were made with anonymized control data. Means and standard deviations were calculated and 2-sided Student t-tests were performed for 15 measures. Bonferroni correction was used for multiple comparisons. RESULTS: 10 patients were identified with 14 white matter spectra. Compared to controls (p ≤ 0.001). CONCLUSIONS: Although the number of spectra was small in this very heterogeneous cross-sectional cohort, there appear to be significant differences in the metabolic profiles which may correlate with the pathogenesis of radiation-induced brain injury. Elevated choline/creatine ratios, for example, have been observed in neuroinflammatory conditions. Studies with more patients are currently ongoing.

0191. ABRIGATION OF T CELL TGF-B SIGNALING RESTRICTS MEDULLOBLASTOMA PROGRESSION

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INTRODUCTION: Transforming growth factor beta (TGF-β) is a pleiotropic cytokine regulator of the immune system, and is required for development of suppressive T regulatory cells (Tregs). Immunosuppression via the secretion of TGF-β is a potent mechanism for human cancers to evade the immune response. We sought to determine the impact of genetically ablating TGF-β T cell signaling on progression of medulloblastoma, a highly malignant pediatric brain tumor that is the most common childhood central nervous system cancer. METHOD: A mouse model expressing a dominant-negative form of the TGF-β receptor type II under T cell regulatory control (TGFbRII-DNR) was utilized to inhibit T cell TGF-β signaling. This transgenic expresses a type II TGF-β receptor that lacks the intracellular kinase domain and acts as a dead-end decoy receptor to bind TGF-β ligand at the cell membrane. These mice were bred with mice carrying a mutant smoothened transgene (SmoA1). These animals have constitutively active Sonic hedgehog signaling and thereby depleting Tregs that promote tumor growth. Because TGFbRII-DNR mice lack functional Tregs, these results raise the possibility of a translational approach to medulloblastoma immunity by blocking TGF-β signaling and thereby depleting Tregs that promote tumor growth.