(Group 3 MB) is the deadliest with only 30% long term survival. Irradiation for Group 3 Medulloblastoma is required for long term survival of children. Methods to enhance the effect of irradiation against Group 3 MB are an active area of investigation. Immunotherapy using the anti-CD47 treatment has shown promise in treating Group 3 MB. We recently demonstrated that irradiation significantly enhanced anti-CD47-mediated phagocytosis of high-grade glioma cells *in vitro*. Furthermore, mice engrafted with human high-grade glioma that received anti-CD47 combined with irradiation showed a significant increase in the survival rate and a significant decrease in tumor growth than those that received a single treatment. We have now extended these studies to demonstrate the enhancement of anti-CD47-dependent phagocytosis of human Group 3 MB with irradiation. We also analyzed normal human neural stem cells exposed to the same treatments to assess for the potential toxicity that uniquely exists with this treatment combination.

MBRS-32. TOPOISOMERASE II B INDUCES NEURONAL, BUT NOT GLIAL, DIFFERENTIATION IN MEDULLOBLASTOMA

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BACKGROUND: We previously reported that Gli3, which was a downstream molecule of Sonic Hedgehog signal, induced neuronal and/or glial differentiation in some types of medulloblastoma (desmoplastic/nodular medulloblastoma and medulloblastoma with extensive nodularity), and patients of medulloblastoma with neuronal differentiation showed favorable prognosis, but those with glial differentiation tended to show miserable prognosis (Miyahara H, Neuropathology, 2013). This time, we focused on Topoisomerase II β (Top2β), which was reported to induce neuronal differentiation and inhibit glial differentiation, and examined the expression of Top2β in medulloblastomas with neuronal and glial differentiations. METHODS: We assessed the expression of Top2β, NeuN, and GFAP using triple fluorescent immunostaining method in medulloblastoma samples with both neuronal and glial differentiations. Furthermore, the expression of Top2β, H3K4me2, and H3K27me3 were also assessed, because Top2βwas positively or negatively regulated by H3K4me2 and H3K27me3, respectively. RESULTS: Many large nuclei in the nodules, in which differentiated cells were seen, was visualized by Top2β. The Top2β signals were seen in NeuN+ cells but not GFAP+ cells. H3K4me2 signals were visualized in Top2β+ large nuclei, but H3K27me3 and NeuN+ large nuclei were distributed independently. CONCLUSIONS: These results indicate that Top2β may be a molecule associated with neuronal, but not glial, differentiation of medulloblastoma cells. Drugs targeting histone modification enzymes such as EZH2 inhibitors are possible therapeutic targets as a differentiation-inducing therapy for medulloblastoma.

MBRS-33. TEMPORARY RESTORATION OF P53 ACTIVITY DURING FRACTIONATED RADIOTHERAPY IN A GROUP3 MEDULLOBLASTOMA GEMM REPRESENTS A POWERFUL TOOL FOR RADIOBIOLOGY STUDIES

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TP53 pathway alterations are well-described events in medulloblastoma (MB) and are predictive of poor clinical outcome. Alterations are rare at diagnosis in Group3 (Gr3) and Group4, but enriched in Sonic Hedgehog and WNT subgroups. However, *TP53* mutations are observed in all subgroups at relapse. Radiation therapy, along with surgery and chemotherapy, represents the standard of care treatment for MB. Loss of p53 function correlates with increased resistance to radiation in several cancers conferring poor survival for patients. In this study, we exposed the MYCN-driven/Trp53^{kiki} (with tamoxifen-inducible p53 activation) Gr3 MB GEMM to a clinically relevant fractionated radiation therapy (RT) regime, to assess the role of p53 in Gr3 radio-resistance and relapse. Mice exhibiting tumour progression (bioluminescence (BLI) signal >10^9 photons/second) were randomized to treatment groups. A small animal radiation research platform was used to deliver CT-guided cranio-spinal irradiation (CSI) and a cranial boost (CB). Mice were followed for survival and tumour burden tracked using BLI. Bodyweight was monitored to evaluate treatment tolerability. Full dose radiation therapy (54Gy CB, 36Gy CSI, α/β=10) or dose modulation (12Gy CB, 8Gy CSI) was performed. The results showed comparable primary tumour regression in response to RT in p53 inactive and active backgrounds, followed by imminent relapse or prolonged remission respectively. No significant acute toxicity was observed. Temporary activation of p53 during RT improved tumour-free survival and decreased the incidence of relapse. In conclusion, we developed a new model which will help improve understanding of the radiobiology of high-risk MB and future preclinical trials.

MBRS-37. RECURRENT ACTIVATING MUTATIONS OF AKT GENES IN WNT-ACTIVATED MEDULLOBLASTOMAS

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Medulloblastoma (MB) can be classified into four distinct molecular subgroups (WNT group, SHH group, group 3, and group 4). Medulloblastoma of the WNT subgroup (WNT-MB) are commonly associated with favorable prognosis. Prospective molecular analysis based on a combination of CGH-array, targeted NGS and Nanostring-based subgrouping on 272 MB was conducted. Our custom targeted NGS panel of 75 genes includes genes recurrently affected in MB together with actionable genes with therapeutic purpose including some involved in the PIK3/AKT signaling pathway. Among the 272 MB analyzed, 26 cases (9.6%) belonged to the WNT subgroup based on *CTNNB1* mutations, monosomy of chromosome 6 and Nanostring-based molecular subgrouping. Our targeted NGS revealed three hotspot activating mutations in *AKT3* in WNT-MB and only one cases in another MB subgroup (in a group 4 MB; among the 33 cases of confirmed group 4 MB in our cohort). We subsequently performed Sanger sequencing of the hotspot Glu17 codon of *AKT1*, *AKT2*, and *AKT3* in 42 additional WNT-MB. This analysis revealed six additional activating mutations of AKT genes (four *AKT3* and two *AKT1* hotspots mutations) in WNT-MB. Altogether, we report 9/68 (13.2%) cases of WNT-MB with AKT genes mutations (two mutations in *AKT1* and seven mutations in *AKT3*). Our molecular analysis revealed AKT hotspot mutations that presumably activate the PIK3/AKT signaling pathway in WNT-MB. Even though WNT-MB is the subgroup of MB with the most favorable prognosis, this result emphasizes a possibility of targeted therapy that need to be further explored *in vitro* and *in vivo*.

MBRS-38. MOLECULAR CLASSIFICATION AND CLINICAL CHARACTERISTICS OF 236 MEDULLOBLASTOMAS IN JAPAN

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Recent intensive genomic and molecular biological analyses have made a consensus that medulloblastomas (MBs) are at least classified into four core subgroups, and the new 2016 WHO brain tumor *classification* has introduced the *classification* of MBs genetically defined in addition to classical histopathological diagnosis. To establish a nationwide network of a molecular diagnosis system for pediatric brain tumors, the JPMNG co-organized by the Japan Society for Neuro-Oncology and the Japanese Society for Pediatric Neurosurgery have started the clinical researches in

2012, and we have summarized results of molecular analysis of Japanese MBs. Total 236 primary MBs have been subclassified by gene expression profile using the NanoString nCounter system or DNA methylation array, and their single nucleotide mutations and copy number aberrations have been also examined. Mean follow up time was 68.9 months. Proportion of four core subgroups were WNT (16.9%), SHH (25.4%), Group 3 (17.4%) and Group 4 (40.3%), respectively. In cases of less than 3 years old, no WNT have been found and 63.2% cases were SHH. In cases between 3 to 17 years old, Group 4 is the most (47%), and these trends is almost consistent with published references. *TP53* mutations were identified in 23.3% of SHH, and they were significantly poor prognosis. Metastatic or *MYC* gain Group 3 MBs were poor prognosis, while Group 4 MBs with loss of chromosome 11 or whole chromosomal aberration were good prognosis. These findings reveal molecular properties of Japanese MBs and will contribute to develop new therapeutic strategies.

MBRS-39. MAP4K4 CONTROLS PRO-INVASIVE SIGNALING IN MEDULLOBLASTOMA CELLS

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The molecular mechanisms contributing to distant dissemination and local recurrence of medulloblastoma, the most common malignant brain tumor in childhood, are poorly understood and no targeted anti-invasion therapies exist till date. We explored regulators and effectors of MAP4K4, a pro-invasive kinase overexpressed in MB and associated with metastatic progression in different solid malignancies. MAP4K4 is upregulated both at mRNA and protein levels in primary pediatric brain tumors compared to normal cerebellum. MAP4K4 is required for growth factor- and irradiation-induced migration and invasion of medulloblastoma cells. It furthermore promotes turnover and activation of the receptor tyrosine kinase c-Met and of the ß1 integrin adhesion receptor [1]. To characterize these clinically relevant consequences and to identify druggable targets of MAP4K4 function, we profiled the interactome of MAP4K4 in starved and growth factor stimulated medulloblastoma cells. To systematically address MAP4K4 impact on receptor expression and turnover, we determined the MAP4K4-dependent surface proteome in medulloblastoma cells. We found that MAP4K4 is part of the striatin-interacting phosphatase and kinase (STRIPAK) complex and that STRIPAK component striatin 4 is controlling cell motility and invasiveness in medulloblastoma cells. Invasiveness of medulloblastoma cells is abrogated by a truncation mutant of MAP4K4 lacking the striatin 4 interaction domain. We furthermore found that MAP4K4 mediates growth factor-induced surface expression of solute carriers and immunomodulatory proteins involved in chemoresistance and immune evasion. Thus, our study identified MAP4K4 as a missing link between pro-tumorigenic growth factor signaling and tumor cell functions relevant for disease progression. It may help identifying druggable vulnerabilities in medulloblastoma cells to restrict tumor growth and dissemination. 1. Tripolitsioti, D. *et al*., *Oncotarget* **9**, 23220–23236 (2018).

MBRS-42. YB-1 - A NOVEL THERAPEUTIC TARGET IN HIGH-RISK MEDULLOBLASTOMA?

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Medulloblastoma relapse occurs in 30–40% of patients and is typically fatal. The emergence of therapy resistant sub-clones likely plays a major role in a large proportion of recurrent medulloblastoma. Y-box binding protein 1 (YB-1) is a multifunctional transcription/translation factor and known onco-protein. Overexpression has been described in numerous cancers, where elevated expression and nuclear accumulation correlates with disease progression, metastasis and drug resistance. Genomic analysis of a large medulloblastoma cohort revealed YB-1 up-regulation across all subgroups of medulloblastoma, where elevated expression correlated with poor survival. Immunohistochemical staining of patient tissue microarrays displayed significant YB-1 expression, with a high proportion (83%) of patients exhibiting nuclear accumulation. High YB-1 expression was also observed at both protein and RNA level across medulloblastoma cell lines, with expression highest in Group 3 and 4. Hence, we hypothesised that YB-1 plays a role in medulloblastoma chemoresistance and progression. Treatment of Group 3 (HDMB-03 and D283MED) and SHH (DAOY) cell lines with vincristine and cisplatin and analysis of cellular localisation by nuclear/cytoplasmic fractionation and immunofluorescence demonstrated that YB-1 undergoes nuclear translocation in response to these standard medulloblastoma chemotherapy agents. Chromatin immunoprecipitation (ChIP) analysis of untreated Group 3 cell lines (D283MED and HDMB-03) demonstrated considerable YB-1 interaction with an inverted CCAAT box in the *ATP-binding cassette subfamily B member 1* (*ABCB1)* promoter. RT-PCR analysis of ABCB1 following vincristine and cisplatin treatment revealed differences in transcript expression, indicative of different YB-1 promoter interactions dependent on chemotherapeutic treatment. Our results highlight YB-1 as a novel candidate chemoresistance driver in medulloblastoma.

MBRS-43. ELUCIDATING HOW NOVEL EXTRACELLULAR MATRIX SUBTYPES DIFFERENTIALLY IMPACT THE SURVIVAL OF MEDULLOBLASTOMA SUBGROUPS

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Medulloblastoma (MB) is the most common malignant paediatric brain tumour and frequently exhibits metastasis and chemoresistance. MBs are categorised into four molecular subgroups (WNT, Sonic hedgehog, Group 3 and Group 4), each associated with different demographics and clinical features. We have shown that the expression of specific extracellular matrix proteins in the brain tumour microenvironment differ between subgroups. A prime example is laminin (an ECM glycoprotein) the expression of which correlates with good overall survival in the SHH subgroup and poor overall survival in Group 4. Our aim is to determine the cause of this difference in survival. Candidate laminin-responsive-genes (LRGs) were identified using the Cavalli data set and RNA-Seq analysis of MB cells grown on 3D hydrogels with and without laminin. The role of laminin in the regulation of MMPs and the other LRG candidates was investigated by qRT-PCR, western blotting and zymography in 2D and long-term 3D-hydrogel assays. Thus far we have shown that in CHLA-01-R (metastatic Group 4 cell line) three of our LRGs are upregulated in response to laminin in 2D, as well as in preliminary 3D studies. Additionally, we have observed a unique MMP9 secretion profile of SHH cells grown in 3D compared to 2D, suggesting that our 3D assay allows us to observe relevant phenotypes absent in 2D culture. We are now in the process of identifying which of these LRG candidates are involved in metastasis and chemoresistance. This will enable the elucidation of novel therapeutic targets and crucially increase our understanding of MB-microenvironment interactions.

MBRS-44. TIME, PATTERN AND OUTCOME OF MEDULLOBLASTOMA RELAPSE ARE ASSOCIATED WITH TUMOUR BIOLOGY AT DIAGNOSIS AND UPFRONT THERAPY: A COHORT STUDY

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Disease relapse occurs in ~30% of children with medulloblastoma, and is fatal in the majority. We sought to establish whether clinico-molecular characteristics at diagnosis are associated with the nature of relapse, subsequent disease-course, and whether these associations could inform clinical management. We surveyed the clinical features of medulloblastoma relapse (time-to-relapse, pattern-of-relapse, time-to-death and overall outcome) in 247 centrally-reviewed patients who relapsed following standard-upfront-therapies. We related these to clinico-molecular features at diagnosis, prognostic factors, and first-line/relapse treatment. Patients who received upfront craniospinal irradiation (CSI-treated) displayed prolonged time-to-relapse compared to CSI naïve patients ($p<0.001$). Similarly, in CSI naïve patients, CSI at relapse, alongside re-resection and desmoplastic/nodular histology, were associated with long-term survival. In CSI-treated patients, the nature of relapse was subgroup-dependent. Local-nodular relapse patterns were enriched in relapsed-MB_{SHH} patients ($p<0.001$), but a notable proportion (65%) also acquired distant-diffuse disease ($p=0.010$). MB_{Group3} relapsed quickly (median 1.3 years), MB_{Group4} slowly (median 2.1 years). Distant-disease was prevalent in MB_{Group3} and MB_{Group4} relapses (90%) but, in contrast to relapsed-MB_{SHH}, nodular and diffuse patterns of distant-disease were observed. Furthermore, nodular disease was associated with a prolonged time-to-death post-relapse ($p=0.006$). Investigation of second-generation $MB_{Group3/4}$ subtypes refined our understanding of heterogeneous relapse characteristics. Subtype VIII had prolonged time-to-relapse; subtype II a rapid time-to-death. Subtypes II/III/VIII developed a significantly higher incidence of distant-disease at relapse, whereas subtypes V/VII did not. The nature of medulloblastoma relapse are biology