

(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  $\beta$  (Top2 $\beta$ ), which was reported to induce neuronal differentiation and inhibit glial differentiation, and examined the expression of Top2 $\beta$  in medulloblastomas with neuronal and glial differentiations. **METHODS:** We assessed the expression of Top2 $\beta$ , NeuN, and GFAP using triple fluorescent immunostaining method in medulloblastoma samples with both neuronal and glial differentiations. Furthermore, the expression of Top2 $\beta$ , H3K4me2, and H3K27me3 were also assessed, because Top2 $\beta$  was 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 Top2 $\beta$ . The Top2 $\beta$  signals were seen in NeuN+ cells but not GFAP+ cells. H3K4me2 signals were visualized in Top2 $\beta$ + large nuclei, but H3K27me3 and NeuN+ large nuclei were distributed independently. **CONCLUSIONS:** These results indicate that Top2 $\beta$  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<sup>kiki</sup> (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<sup>9</sup> 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,  $\alpha/\beta=10$ ) 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 case 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