Abstracts

multiple PCR-based NGS panel was negative for alteration of the SMO, PTCH1 and CTBNN1 genes. Further molecular characterization via methylation profiling demonstrated the sonic hedgehog (SHH) molecular subtype. Proof of principle in the clinic, however, ultrasound was performed and identified congenitally absent right kidney; audiometry evaluation was unremarkable. Discussion: In patients with Gorlin syndrome, cases of unilateral renal agenesis in association with germline SHH-pathway mutations have been reported in the literature. However, renal agenesis is rarely associated with unilateral Gorlin syndrome in the absence of a SHH mutation. Our patient’s renal agenesis may represent a rare bilateral manifestation of Gorlin syndrome and the development of renal agenesis in a patient with Gorlin syndrome has not been previously reported.

EMBR-20. ELONGATION CONTROL OF MRNA TRANSLATION DRIVES GROUP 3 MEDULLOBLASTOMA
Alberto Deladelu1, Gian Luca Negri2, Que Xi Wang1, Albert Huang1, Silvanian Siddiqui3, Chunyang Zhang1, Yue Zhou1, Sofya Langman1, Andrea Lisovskiy1, Volker Hovestadt2, Michael Taylor1, Gabriel Lepriver2, and Poul Sorensen1; 1BC Cancer Research Centre, Vancouver, BC, Canada; 2Massachusetts General Hospital, Boston, MA, USA; 3Arthur and Sonia Labatt Brain Tumor Research Centre, Toronto, ON, Canada, 4Hennich Heine University, Duesseldorf, Germany

Medulloblastoma (MB) is the most common pediatric intracranial tumor accounting for 15% of all childhood brain cancers. Our understanding of MB development and its genetic amplifications of the MYC oncogene are predictors of adverse outcome in MB, underscoring a dire need for novel and more effective therapeutic approaches. The let-7 family of small non-coding RNAs (miRNAs) is known to inhibit tumor progression and regulate metabolism by targeting and degrading several cellular mRNAs, including MYC. Indeed, let-7 miRNAs are frequently repressed in several cancer types, including in MYC-driven MB. We previously reported that the mRNA translation elongation regulator mammalian eukaryotic elongation factor 2 kinase (eEF2K) is a pivotal mediator of cancer cell adaptation to nutrient deprivation. In the current work, we identified a potential binding site for let-7 miRNAs on the eEF2K untranslated region (UTR). In addition, eEF2K mRNA and let-7 miRNA expressions negatively correlate in MB, suggesting a potential regulation of the former by the latter. Let-7 miRNAs transfection decreases eEF2K mRNA and protein levels (by ~40–50%). Down-regulation of luciferase activity by let-7 miRNAs is impaired upon mutation of the let-7 binding site on the eEF2K 3’UTR. Inhibition of eEF2K mRNA and protein induction by let-7 miRNAs increases survival in vitro under nutrient deprivation, altering their mRNA translation rates. Knockout of eEF2K increases survival of MYC-amplified MB xenografts when mice are kept under calorie restricted diets. We conclude that let-7 miRNAs degrade the eEF2K transcript and binding to its 3’UTR, indicating that let-7 repression in MYC-driven MB is partially responsible for increased eEF2K levels. Moreover, the let-7-eEF2K axis constitutes a critical mechanism for MYC-driven MB adaptation to acute metabolic stress, representing a promising therapeutic target. Future therapeutic strategies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs, as eEF2K activity appears critical under metabolic stress conditions.

EMBR-21. CLINICALLY TRACTABLE OUTCOME PREDICTION OF GROUP 3/4 MEDULLOBLASTOMA BASED ON PTDS2 IMMUNOHISTOCHEMISTRY: A MULTICOHORT STUDY
Alberto Deladelu1, Christopher Dunham2, Maria Rita Huang3,1,4, Kevin Brown1, Jonathan Chan5, Katerina Gwon6, Jason Mooff7, David J. Gourley8, Betty H. Ng9, 1BC Cancer Research Centre, Vancouver, BC, Canada; 2Children’s Hospital, Philadelphia, PA, USA; 3University of Bern, Bern, Switzerland; 4St. Lukes’ Clinical Research Center for Children, Moscow, Russia; 5Russian Federation; 6Neurological NN Burdenko Institute, Moscow, Russian Federation; 7German Cancer Research Center (DKFZ), Heidelberg, Germany; 8The Arthur and Sonia Labatt Brain Tumor Research Centre, Toronto, ON, Canada

Background: International consensus and the 2021 WHO classification recommend recognizing eight molecular subtypes among Group 3/4 medulloblastoma (representing ~60% of tumors). However, very few clinical centers worldwide possess the technical capabilities to determine DNA-methylation patterns or other molecular parameters of high-risk for Group 3/4 tumors. As a result, biomarker-driven risk stratification and therapy assignment constitutes a major challenge in medulloblastoma research. Here, we identify an immunohistochecmistry (IHC) signature that can be applied in multiple steps to the development of the tumor, based on the recognition of four different cell types, including pediatric MB. Although in vivo studies provide a promising proof-of-concept for the therapeutic targeting of BMI1 in Group 3 MB, mice that receive treatment eventually succumb to their disease. This study provides the foundation for clinical validation of small molecule inhibitors targeting BMI1 in vivo clonal tracking technology was leveraged to profile in vivo clonal dynamics of Group 3 MB in response to the establishment of chemoradiotherapy resistant clones and in combination with PTDS2. Comparison of clonal composition of the tumors extracted from the brains and spines post-treatment revealed the persistence of a small number of clones with the ability to escape therapy and drive subsequent therapeutic resistance. In order to better understand molecular susceptibilities of MB cells post BMI1 inhibition, we undertook an in vitro genome-wide CRISPR/Cas9 screening to identify context-specific MB regulatory pathways to be synergistically targeted along with BMI1. By comparing the results of the in vitro genome wide CRISPR/Cas9 screen to the essential genes in human neural stem cells (hNSCs), we identified several context specific regulators of mTOR, AKT and drug resistance pathways. Future therapeutic strategies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs, as eEF2K activity appears critical under metabolic stress conditions.

EMBR-22. RATIONAL DEVELOPMENT OF SYNERGISTIC THERAPIES ALONGSIDE BMI1 INHIBITION FOR GROUP 3 MEDULLOBLASTOMA
David Bakshishyuan1, Ashley A Adle1, Chitra Venugopal1, Kevin Brown1, Katherine Chan5, Maleha A Qazi5, Chirayu Chokshi1, William D Gowney1, David J. Gourley1, Betty H. Ng9, 1BC Cancer Research Centre, Vancouver, BC, Canada; 2McMaster University, Hamilton, ON, Canada; 3University of Toronto, Toronto, ON, Canada

Medulloblastoma (MB) is the most common pediatric brain tumor. Of its four distinct molecular subgroups, Group 3 MBs are associated with increased risk of recurrence, metastasis and overall poor patient outcome. In recent years, small molecule inhibitors targeting BMI1 have shown to be efficacious against several types of malignant tumors including pediatric MB. Although in vivo studies provide a promising proof-of-concept for the therapeutic targeting of BMI1 in Group 3 MB, mice that receive treatment eventually succumb to their disease. This study provides the foundation for clinical validation of small molecule inhibitors targeting BMI1 in vivo clonal tracking technology was leveraged to profile in vivo clonal dynamics of Group 3 MB in response to the establishment of chemoradiotherapy resistant clones and in combination with PTDS2. Comparison of clonal composition of the tumors extracted from the brains and spines post-treatment revealed the persistence of a small number of clones with the ability to escape therapy and drive subsequent therapeutic resistance. In order to better understand molecular susceptibilities of MB cells post BMI1 inhibition, we undertook an in vitro genome-wide CRISPR/Cas9 screening to identify context-specific MB regulatory pathways to be synergistically targeted along with BMI1. By comparing the results of the in vitro genome wide CRISPR/Cas9 screen to the essential genes in human neural stem cells (hNSCs), we identified several context specific regulators of mTOR, AKT and PLK1 pathways. The combined treatment alongside PTDS2 has demonstrated synergistic efficacy against MB cells with minimal toxicity in NSCs in vitro and is currently being evaluated in preclinical studies. This study provides the foundation for clinical validation of small molecule inhibitors synergistic with PTDS2 to improve the durability of remissions and extend survival of patients with treatment-refractory Group 3 MB.
Medulloblastoma (MB) is the most common pediatric central nervous system malignancy. Although the current standard of care leads to ~70% patient survival, the therapies are highly toxic, leading to life-long side effects, and recurrence due to therapeutic resistance is fatal. We sought to investigate mediators of radiation response in mouse models for the Sonic hedgehog (SHH) subgroup MB as well as human pediatric MB cell lines. We previously identified Y-box binding protein 1 (YB1) as a downstream effector of YAP-mediated MB radiation resistance. YB1 is a crucial, yet understudied, protein highly expressed across all 4 subgroups of MB. Through its DNA- and RNA-binding cold shock domain, YB1 mediates both transcriptional and post-transcriptional effects on five MB cell lines at nanomolar concentration range, independent of its p53 mutational status. Cells treated with YF11 inhibitor were arrested in G2/M phase. Apoptosis was observed on Annexin V flow cytometry 24h after treatment, followed by necrosis after 48h in p53-wildtype cells. In contrast, treated p53-mutant cells underwent apoptotic cell death which additionally resulted in cell death mechanisms upon YF11 inhibition was confirmed on immunoblotting by upregulated p53 expression and presence of cleaved-PARP and DNA-damage marker in p53-wildtype cells, indicative of apoptosis. While induced p53 expression after ionizing radiation was observed in both p53-wildtype and p53-mutant cells, treated p53-mutant cells underwent apoptosis, while treated p53-wildtype cells predominantly underwent apoptosis.

EMBR-24. YB1 IS CRITICAL FOR MEDULLOBLASTOMA TUMOR MAINTENANCE AND DNA REPAIR FOLLOWING THERAPEUTIC INTERVENTION
Leon McWearr, Anna Kenney, Victor Chen, and Tiffany Huang; Emory University, Atlanta, GA, USA

Medulloblastoma (MB) is the most common pediatric central nervous system malignancy. Although the current standard of care leads to ~70% patient survival, the therapies are highly toxic, leading to life-long side effects, and recurrence due to therapeutic resistance is fatal. We sought to investigate mediators of radiation response in mouse models for the Sonic hedgehog (SHH) subgroup MB as well as human pediatric MB cell lines. We previously identified Y-box binding protein 1 (YB1) as a downstream effector of YAP-mediated MB radiation resistance. YB1 is a crucial, yet understudied, protein highly expressed across all 4 subgroups of MB. Through its DNA- and RNA-binding cold shock domain, YB1 mediates both transcriptional and post-transcriptional effects on five MB cell lines at nanomolar concentration range, independent of its p53 mutational status. Cells treated with YF11 inhibitor were arrested in G2/M phase. Apoptosis was observed on Annexin V flow cytometry 24h after treatment, followed by necrosis after 48h in p53-wildtype cells. In contrast, treated p53-mutant cells underwent apoptotic cell death which additionally resulted in cell death mechanisms upon YF11 inhibition was confirmed on immunoblotting by upregulated p53 expression and presence of cleaved-PARP and DNA-damage marker in p53-wildtype cells, indicative of apoptosis. While induced p53 expression after ionizing radiation was observed in both p53-wildtype and p53-mutant cells, treated p53-mutant cells underwent apoptosis, while treated p53-wildtype cells predominantly underwent apoptosis.

EMBR-25. GENOME-WIDE GENETIC AND EPIGENETIC ASSESSMENT OF GROUP 4 MEDULLOBLASTOMA FOR IMPROVED, BIOMARKER DRIVEN, PROGNOSTIC VIA RISK-STRATIFICATION
Jared Goddard1, Ana Santa-Caste1, Emily Southworth1, Stephen Crouser1, Idona Martin-Guerrero2,3, Miguel Garcia-Ariz1,4, Aurora Navajas1, Franck Bourdeaut1, Christelle Dufour1, Tobias Goshczik3, Torsten Pietsch3, Dan Williamson1, Simon Bailey5, Ed Schwalbe5,6, Steven Clifford7, and Debbie Hicks1; Wolfson Childhood Cancer Research Centre, Newcastle University, Newcastle upon Tyne, UK; Biocruces Health Research Institute, Barakaldo, Spain; 2Department of Genetics, Physio Anthropology and Animal Physiology, University of the Basque Country, Bilbao, Spain; 3Department of Pediatric Hematology and Oncology, Hospital Vall d’Hebron, Barcelona, Spain; 4Humphrey Oei Institute of Cancer Research, National Cancer Center Singapore, Singapore; 5Pediatric Brain Tumor Research Office, SingHealth-Duke-NUS Academic Medical Center, Singapore; 6KK Hospital Singapore; 7Institute of Molecular and Cell Biology, A*STAR, Singapore; 8Cancer and Stem Cell Biology Program, Duke-NUS Medical School, Singapore

Introduction: KIF11, a mitotic kinesin, is a component responsible for assembly and maintenance of mitotic spindle during mitosis. Tumor cells can upregulate KIF11. Inhibition of KIF11 results monopolar spindle formation, resulting in monoastral mitosis in cells. This activates the spindle assembly checkpoint, cells are arrested and prevented from entering cell cycle, resulting in cell death via apoptosis or necrosis, cell division with aneuploidy or mitotic slippage without division into tetraploid G1 phase. Methods: We hypothesized that the effect of KIF11 inhibition on medulloblastoma (MB) is dependent of its p53 mutational status. Results: Our findings on Hoechst staining demonstrated a small molecule inhibitor of KIF11 which induced apoptosis in p53-wildtype MB cells at 48h (p<0.0001), was able to trigger mitotic catastrophe (p = 0.0010) in p53-mutant MB cells at 24h and subsequent necrosis (p=0.0039) at 48h. KIF11 inhibitor exerted anti-proliferative effects on five MB cell lines at nanomolar concentration range, independent of its p53 mutational status. Cells treated with KIF11 inhibitor were arrested in G2/M phase. Apoptosis was observed on Annexin V flow cytometry 24h after treatment, followed by necrosis after 48h in p53-wildtype cells. In contrast, treated p53-mutant cells underwent apoptotic cell death which additionally resulted in cell death mechanisms upon KIF11 inhibition was confirmed on immunoblotting by upregulated p53 expression and presence of cleaved-PARP and DNA-damage marker in p53-wildtype cells, indicative of apoptosis. While induced p53 expression after ionizing radiation was observed in both p53-wildtype and p53-mutant cells, treated p53-mutant cells underwent apoptosis, while treated p53-wildtype cells predominantly underwent apoptosis.