markers classified patients in three groups (WNT, SHH and non-WNT/non-SHH) in 98% of cases. PCR-based method confirmed results from IHC in 81.5%. Additionally, we were able to detect WNT activation in 2 patients, previously classified as SHH. For both cases, the presence SHH was confirmed by microdissection. 6 further confirmed WNT subgroups. Integration of these three techniques resulted in the following frequencies: WNT (13.0%), SHH (38.9%), group 3 (9.5%), group 4 (20.3%) and non-WNT/non-SHH (18.5%). From 40 patients of all subtypes, the snRNA-seq identified an overall survival on average 10% for low, intermediate and high-risk groups were 100%, 60% and 20%, respectively (p<0.05), based only in molecular criteria, which confirmed the prognostic importance of this method. CONCLUSIONS: At an estimated cost of $220 per patient, we are able to implement central molecular diagnosis for the incorporation into a prospective clinical trial protocol in Latin America.

MBRS-67. ROLE OF CYCLIN DEPENDENT KINASE-9 IN MYC-ENHANCED MEDULLOBLASTOMA
Krushna Madhavan1, Bethany Voe1, Ettiene Danis1, Iliano Balakrishnan2, Angela Pierce1, Dong Wang1, Ahmed Abdel-Hafiz2, Natalie Sergeova2, Nathan Dahl1, Sujatha Venkataramani1, and Rajeev Lu1
University of Colorado Denver Anschutz Medical Campus, Aurora, USA,1University of Colorado Denver Anschutz Medical Campus, Aurora, USA,2University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA,3The Children’s Hospital of Colorado, Aurora, CO, USA
Myc is highly expressed in group 3 medulloblastoma (Myc-MB) and in cell growth, proliferation and oncogenesis by directly promoting the activity of RNA polymerases (RNA Pol). Myc driven RNA Pol II activity is maintained by BCL-6 via BORC. Transcription Elongation Factor (TEF) catalytic core consists of cyclin dependent kinase-9 (CDK9) and Cyclin T, that phosphorylate and release RNA Pol II into active elongation. CDK9 is over expressed in group 3 MB suggesting that Myc may be vulnerable to inhibition by CDK9. The exact mechanism is not completely known in MB. Genetic depletion of CDK9 suppressed Myc-MB cell clonogenicity in vitro and tumor growth in vivo. CDK9 by two clinically relevant inhibitors, Avuociclib and AZD4573, suppressed clonogenicity and cell self-renewal of Myc-MB xenografts. CDK9 in Myc-driven downregulated Myc and RNA Pol II phosphorylation at Ser2 and Ser5, and, upregulated P21. Further, mice with orthotopic xenografts treated with CDK9 inhibitors survived significantly longer than control mice. RNA-Seq-based gene set enrichment analysis showed that CDK9 depletion decreased Myc-driven transcriptional programs and enhanced differentiation networks. Chip-Seq for Pol2 and Myc, demonstrated that the Myc-driven aberrant transcriptional input can be reversed via CDK9 depletion. These findings highlight the role of CDK9 in Myc-driven pathogenesis and that its inhibition is critical to the treatment of Myc-MB.

MBRS-68. SINGLE NUCLEUS RNA-SEQUENCING DECIPHERS INTRATUMORAL HETEROGENEITY IN MEDULLOBLASTOMA WITH EXTENSIVE NODULARITY (MBEN)
David N. Ghasemi1, Konstantin Okonechnikov1, Jan-Philipp Malm2,3, Kati Lappalainen1, Katharina Bauer1, Michelle S. Liberek1, Laura Giese2,3,4, Maja K. Maas1,5,1Marcel K. Richter1,6, David A. Jones3, Andreas von Deimling4, Stefan M. Pfister2,4, Andrey Korshunov6,7, and Kristian W. Paigen1,6
1Hopp-Children’s Cancer Center Heidelberg (KiTZ) and Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, 2Single-cell Open Lab, German Cancer Research Center (DKFZ), Heidelberg, Germany, 3Division of Chromatin Networks, German Cancer Research Center (DKFZ) and BioQuant, Heidelberg, Germany, 4Hopp-Children’s Cancer Center Heidelberg (KiTZ) and Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, 5Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands, 6Department of Neuropathology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany, 7Department of Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany, 8Department of Pediatric Oncology, Hematology, and Immunology, University Hospital Heidelberg, Heidelberg, Germany
Medulloblastoma (MB) with extensive nodularity (MBEN) represent a rare subtype of cerebellar tumors of infancy which comprise two histologically distinct components, nodular reticulin-free zones and inter-nodular reticulin-rich regions. We applied single nucleus RNA-sequencing (snRNA-seq) using the 10X Genomics and the SMARTseq V2 protocols, bulk RNA-sequencing, DNA-methylation profiling and DNA-panel sequencing to ten histologically confirmed MBEN specimens. All tumors were classified as SHH medulloblastoma. Genomic data of two MBEN cases showed a strongly proliferating, SHH-like subset of cells in MBEN, which might represent the driving cell population in these malignancies, while direct analyses of nodular and inter-nodular regions did not reveal any significant differences. These findings suggest that both components originate from the same cell of origin but represent different cellular development stages.

MBRS-69. METABOLITE PROFILING OF SHH MEDULLOBLASTOMA IDENTIFIES A SUBSET OF CHILDHOOD TUMOURS ENRICHED FOR HIGH-RISE RISK BIOMARKERS AND CLINICAL FEATURES
Christopher Bennett1,2, Sarah Kohe1, Florence Burte3, Heather Rose1,2,3, Debbie Hicks1, Ed Swallow1, Stephen Crouser1, Lisa Storer2,1Anbarasu Louraudusamy1, Martin Wilson1, Shivaram Arula4, Dipayan Mitra5, Robert Dineen1, Simon Bailey3,5, Daniel Williamson3, Richard Grundy3, Steven Clifford6, and Andrew Pett2,1
1University of Birmingham, Birmingham, United Kingdom, 2Birmingham Children’s Hospital, Birmingham, United Kingdom, 3Newcastle University Centre for Cancer, Newcastle upon Tyne, United Kingdom, 4Northumbria University, Newcastle upon Tyne, United Kingdom, 5University of Nottingham, Nottingham, United Kingdom, 6The Hope Children’s Factor b (poduct), Liverpool, United Kingdom, 7Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom, 8Great North Children’s Hospital, Newcastle upon Tyne, United Kingdom
SHH medulloblastoma patients have a variable prognosis. Infants (<3–5 years at diagnosis) are associated with a good prognosis, while disease-course in childhood is associated with specific prognostic biomarkers (MBEN and LU). We previously showed that MYC amplification, TERT promoter mutations, LCA histology (3/5) and a lower proportion of high-risk markers - LCA histology (3/5) and clinical and molecular features were compared between clusters. Two clusters were observed. A significantly higher concentration of lipid was observed in Cluster 1 (t-test, p<0.012). Cluster 1 consisted entirely of childhood-SHH while Cluster 2 included both childhood-SHH and infant-SHH subtypes. Cluster 1 was enriched for high-risk markers – LCA histology (3/5), MYCN amplification (2/7 vs. 0/5), TERT promoter mutations (0/7 v. 3/5) and metastatic disease - whilst having a lower proportion of TERT promoter mutations (0/7 v. 3/5) than Cluster 2. These pilot results suggest that it is possible to identify childhood-SHH patients linked to high-risk clinical and molecular biomarkers using metabolite profiles and (ii) these may be detected non-invasively in vivo using magnetic resonance spectroscopy.

MBRS-70. FUNCTIONAL DEPENDENCY BETWEEN REST AND DNMT1 IN MEDULLOBLASTOMA
Shinji Maegawa1, Tara Dobson1, Yue Lu2, Marcos Estoc1,2
1Ari Harmanci2, and Vidya Gopalakrishnan1,2,1Department of Pediatrics, University of Texas, MD Anderson Cancer Center, Houston, TX, USA, 2Department of Epigenetics and Molecular Carcinogenesis, University of Texas, MD Anderson Cancer Center, Houston, TX, USA, 3Department of Leukemia, University of Texas, MD Anderson Cancer Center, Houston, TX, USA, 4School of Biomedical Informatics, Center for Precision Health, University of Texas Health Science Center, Houston, TX, USA, 5Department of Molecular and Cellular Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX, USA
Medulloblastomas exhibit poor neuronal lineage specification. Expression of REST Silencing Transcription Factor (REST), a repressor of neurogenesis, is aberrantly elevated in human sonic hedgehog (SHH) medulloblastomas. Constitutive REST expression in mice (RESTTG) drives SHH pathway activation and neuroectodermal differentiation in the context of Ptc8b haplosufficiency (Ptc8b<), implicating it as a driver of tumorgenesis. Tumor formation in Ptc8b<–RESTTG mice showed significantly decreased latency and increased penetrance compared to that in Ptc8b<– mice. Since REST silences gene expression by chromatin remodeling, we sought to identify cooperating epigenetic changes that contributed to its oncogenic activity.
MBRS-71. ATAXIA TELANGIECTASIA AND RAD3-RELATED PROTEIN ATTENUATES DNA DAMAGE AND IS A THERAPEUTIC TARGET IN MYC-DRIVEN MEDULLOBLASTOMA
Ahmed Abdel-Hafiz1, Krishna Madhavan1, Ilango Balakrishnan1, Angela Pierce1, Dong Wang1, Ettene Danis1, Natalie Serkova1, Supatha Venkataraman1, and Rajeev Nellan1; Children’s Hospital and Medical Center, Omaha, NE, USA, 2University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA, 3University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA, 4The Children’s Hospital of Colorado, Aurora, CO, USA

Group 3 medulloblastosomas (Myc-MB), and particularly the 3γ subtype, have the worst prognosis and show a 5-year overall survival of less than 40%. Group 3 tumors are often accompanied by Myc amplification and have a higher rate of metastatic disease and relapse. Unfortunately, therapeutic strategies to target Myc have remained elusive. Furthermore, the relapse of the MB has been linked to DNA replication stress. Ataxia telangiectasia and Rad3-related protein (ATR) senses persistent DNA damage, which in turn leads to DNA replication stress, and activates damage checkpoints, thereby leading to increased cell survival. ATR is highly expressed in MB and is thought to contribute to disturbed DNA replication to protect genomic integrity. Yet, the exact underlying mechanisms involving ATR are still unclear. The activation of ATR (ATR) in ATRD738, suppressed clonogenicity and cell self-renewal in Myc-MB. ATRi in Myc-MB cell lines downregulated Chk1 and upregulated P21. ATR also induced cell cycle arrest and increased apoptosis in Myc-MB cell lines. Further, mice with orthotopic xenografts treated with ATR inhibitor survived significantly longer than control mice. High-throughput drug screening showed ATRi to be synergistic with chemotherapeutic agents including gemcitabine, cisplatin, and topotecan. The treatment of Myc-MB cells with ATR inhibitor in combination with gemcitabine and with radiation increased in expression of DNA damage markers. These findings emphasize the role of ATR in alleviating DNA replication stress and that its inhibition is critical to the treatment of Myc-MB.

MBRS-72. MIr-212 FUNCTIONS AS A TUMOR SUPPRESSOR GENE IN GROUP 3 MEDULLOBLASTOMA VIA TARGETING NUCLEAR FACTOR I/B (NFIB)
Naveen Kumar Parmal1, Ranjana Kanchan1, Peetani Attri1, Ramakanth Venkata1, Ishwor Thapa1, Mohd Nassar1, Surinder Batra1, and Sidharth Mahapatra1,2; 1University of Nebraska Medical Center, Omaha, NE, USA, 2Children’s Hospital and Medical Center, Omaha, NE, USA

Medulloblastosoma (MB), the most frequent malignant pediatric brain tumor is divided into four primary subgroups, i.e. wingless-type (WNT), sonic hedgehog (SHH), group 3, and group 4. Haplosufficiency of sonic hedgehog (SHH) in group 3, and c-myc amplification distinguishes high-risk group 3 tumors and are associated with rapid recurrence and early mortality. We sought to identify the role of mir-212, which resides on overlapping toxicities with other targeted agents like bevacizumab despite their potential combined therapeutic benefit. METHODS: A retrospective review of patients treated with MEK +/- BRAF inhibitors and bevacizumab from 2015–2019 was conducted. Data collected included demographics, tumor type, neurofibromatosis status, treatment duration, reason for concurrent treatment, and toxicities. RESULTS: Fifteen patients aged 3–24 years old (median age 14 years) were identified. Diagnoses included five high-grade gliomas, four low-grade gliomas, four benign nerve sheath tumors, and one ependymoma. Nearly half (46.7%) were positive for neurofibromatosis type 1. Three patients were treated with a BRAF + MEK inhibitor with twelve were treated with a MEK inhibitor combined with bevacizumab. Duration of treatment ranged from 16–420 days (median 119 days). Reasons for concomitant therapy included progressive disease with neurologic decline (46.7%), painful benign nerve sheath tumors (26.7%), and visual loss with optic pathway gliomas (26.7%). Toxicity while on concurrent therapy included one episode of grade 1 left venricular dysfunction, one grade 1 bleeding episode, and one grade 2 wound complication. There were no episodes of hypertension, thrombosis, GI perforations, or hemorrhages. CONCLUSIONS: Our preliminary suggests bevacizumab in combination with MEK and BRAF inhibitors can be used

PRECLINICAL MODELS/EXPERIMENTAL THERAPY/DRUG DISCOVERY
MODL-01. SAFETY IN CONCOMITANT USE OF MEK AND BRAF INHIBITORS WITH BEVACIZUMAB
Shelvy Winzinger, Ashley Sabas, Molly Hemenway, Anandani Sells1, and Jean Mulcahy-Ley; Univ of CO SOM, Children’s Hospital Colorado, Aurora, CO, USA

BACKGROUND: MEK and BRAF inhibitors are increasingly common treatments for pediatric nervous system tumors. While effective in blocking Ras/Raf/MEK/ERK pathway activation driving tumor progression, the side effect profile differs from traditional cytotoxic chemotherapies. Little data exists on overlapping toxicities with other targeted agents like bevacizumab despite their potential combined therapeutic benefit. METHOIDS: A retrospective review of patients treated with MEK +/- BRAF inhibitors and bevacizumab from 2015–2019 was conducted. Data collected included demographics, tumor type, neurofibromatosis status, treatment duration, reason for concurrent treatment, and toxicities. RESULTS: Fifteen patients aged 3–24 years old (median age 14 years) were identified. Diagnoses included five high-grade gliomas, four low-grade gliomas, four benign nerve sheath tumors, and one ependymoma. Nearly half (46.7%) were positive for neurofibromatosis type 1. Three patients were treated with a BRAF + MEK inhibitor with twelve were treated with a MEK inhibitor combined with bevacizumab. Duration of treatment ranged from 16–420 days (median 119 days). Reasons for concomitant therapy included progressive disease with neurologic decline (46.7%), painful benign nerve sheath tumors (26.7%), and visual loss with optic pathway gliomas (26.7%). Toxicity while on concurrent therapy included one episode of grade 1 left venricular dysfunction, one grade 1 bleeding episode, and one grade 2 wound complication. There were no episodes of hypertension, thrombosis, GI perforations, or hemorrhages. CONCLUSIONS: Our preliminary suggests bevacizumab in combination with MEK and BRAF inhibitors can be used