and therapy-dependent, providing immediate translational opportunities for improved disease management through biology-directed surveillance, post-relapse prognostication and risk-stratified selection of second-line treatment.

MBRS-45. TWIST1 AND ABCB1 ARE FUNCTIONAL DETERMINANTS OF METASTASIS IN MEDULLOBLASTOMA

Alice Cardall[1], Franziska Linke[1], Ian Kerr[2], and Beth Coyle[1]; [1]Children's Brain Tumour Research Centre, School of Medicine, University of Nottingham Biodiscovery Institute, Nottingham, United Kingdom, [2]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

Paediatric medulloblastomas (MB) are frequently metastatic, resulting in a poor prognosis for the patient. Of the four MB subgroups, group 3 patients present with the highest rates of metastasis and worst outcomes. The mechanisms behind the metastatic process are poorly understood, limiting our ability to develop novel therapeutic treatments. We hypothesised that the epithelial-mesenchymal transition (EMT) transcription factor TWIST1 and the multidrug efflux pump ABCB1 (ATP-binding cassette subfamily B member 1) synergistically drive MB metastasis. TWIST1 protein expression was analysed in patient tissue microarrays by immunohistochemistry. High TWIST1 expression was associated with metastatic patients (p=0.041). Physical and functional interactions between TWIST1 and ABCB1 were investigated using chromatin immunoprecipitation (ChIP) and a 3D migration and invasion model. ChIP analysis confirmed TWIST1 binding to the *ABCB1* promoter in SHH (ONS-76) and group 3 (D283MED and HD-MB03) metastatic cell lines. TWIST1 and ABCB1 were inhibited in HDMB03 cells with harmine and vardenafil respectively, resulting in attenuated cell migration in the 3D model. Western blot and qRT-PCR analysis of harmine treated cells confirmed a reduction in ABCB1 protein and gene expression. Overall our data reveals TWIST1 and ABCB1 to be key targets for MB metastatic disease. Using bioinformatics analysis and ChIP sequencing, additional TWIST1 downstream targets are now being identified and compared across the metastatic cell lines (ONS-76, D283MED and HD-MB03). This data will provide a deeper insight into the pathways associated with MB metastases, enabling personalised treatment approaches for patients with metastatic disease.

MBRS-46. CHARTING NEOPLASTIC AND IMMUNE CELL HETEROGENEITY IN HUMAN AND GEM MODELS OF MEDULLOBLASTOMA USING SCRNASEQ

Andrew Donson[1,2], Kent Riemondy[3], Sujatha Venkataraman[1,2], Ahmed Gilani[4], Bridget Sanford[2], Andrea Griesinger[1,2], Vladimir Amani[1,2], Todd Hankinson[5,1], Michael Handler[5,1], Jay Hesselberth[3], Timothy Gershon[6], Robert Wechsler-Reya[7], Nicholas Foreman[1,2], and Rajeev Vibhakar[1,2]; [1]The Morgan Adams Foundation Pediatric Brain Tumor Research Program, Children's Hospital Colorado, Aurora, CO, USA, [2]Department of Pediatrics-Hematology and Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [3]RNA Biosciences Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [4]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [5]Department of Neurosurgery, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [6]Department of Neurology, University of North Carolina, Chapel Hill, NC, USA, [7]Sanford Burnham Prebys Medical Discovery Inst., La Jolla, CA, USA

We explored cellular heterogeneity in medulloblastoma using single-cell RNA sequencing (scRNAseq), immunohistochemistry and deconvolution of bulk transcriptomic data. Over 45,000 cells from 31 patients from all main subgroups of medulloblastoma (2 WNT, 10 SHH, 9 GP3, 11 GP4 and 1 GP3/4) were clustered using Harmony alignment to identify conserved subpopulations. Each subgroup contained subpopulations exhibiting mitotic, undifferentiated and neuronal differentiated transcript profiles, corroborating other recent medulloblastoma scRNAseq studies. The magnitude of our present study builds on the findings of existing studies, providing further characterization of conserved neoplastic subpopulations, including identification of a photoreceptor-differentiated subpopulation that was predominantly, but not exclusively, found in GP3 medulloblastoma. Deconvolution of MAGIC transcriptomic cohort data showed that neoplastic subpopulations are associated with major and minor subgroup subdivisions, for example, photoreceptor subpopulation cells are more abundant in GP3-alpha. In both GP3 and GP4, higher proportions of undifferentiated subpopulations is associated with shorter survival and conversely, differentiated subpopulation is associated with longer survival. This scRNAseq dataset also afforded unique insights into the immune landscape of medulloblastoma, and revealed an M2-polarized myeloid subpopulation that was restricted to SHH medulloblastoma. Additionally, we performed scRNAseq on 16,000 cells from genetically engineered mouse (GEM) models of GP3 and SHH medulloblastoma. These models showed a level of fidelity with corresponding human subgroup-specific neoplastic and immune subpopulations. Collectively, our findings advance our understanding of the neoplastic and immune landscape of the main medulloblastoma subgroups in both humans and GEM models.

MBRS-47. RAPID MOLECULAR SUBGROUPING OF MEDULLOBLASTOMA BASED ON DNA METHYLATION BY NANOPORE SEQUENCING

Julien Masliah-Planchon[1], Elodie Girard[2], Philipp Euskirchen[3], Christine Bourneix[1], Delphine Lequin[1], Maud Blanluet[1], Jean-Baptiste Aillaud[1], Francois Doz[4], Franck Bourdeaut[5,6], and Olivier Delattre[5,1]; [1]Unité de Génétique Somatique, Institut Curie, Paris, France, [2]U900, Institut Curie, Paris, France, [3]Department of Neurology, Charité - Universitätsmedizin Berlin, Berlin, Germany, [4]SIREDO Center, France, France, [5]SIREDO Center, Paris, France, [6]RTOP Department, Paris, France

Medulloblastoma (MB) can be classified into four molecular subgroups (WNT group, SHH group, group 3, and group 4). The gold standard of assignment of molecular subgroup through DNA methylation profiling uses Illumina EPIC array. However, this tool has some limitation in terms of cost and timing, in order to get the results soon enough for clinical use. We present an alternative DNA methylation assay based on nanopore sequencing efficient for rapid, cheaper, and reliable subgrouping of clinical MB samples. Low-depth whole genome with long-read single-molecule nanopore sequencing was used to simultaneously assess copy number profile and MB subgrouping based on DNA methylation. The DNA methylation data generated by Nanopore sequencing were compared to a publicly available reference cohort comprising over 2,800 brain tumors including the four subgroups of MB (Capper et al. Nature; 2018) to generate a score that estimates a confidence with a tumor group assignment. Among the 24 MB analyzed with nanopore sequencing (six WNT, nine SHH, five group 3, and four group 4), all of them were classified in the appropriate subgroup established by expression-based Nanostring subgrouping. In addition to the subgrouping, we also examine the genomic profile. Furthermore, all previously identified clinically relevant genomic rearrangements (mostly *MYC* and *MYCN* amplifications) were also detected with our assay. In conclusion, we are confirming the full reliability of nanopore sequencing as a novel rapid and cheap assay for methylation-based MB subgrouping. We now plan to implement this technology to other embryonal tumors of the central nervous system.

MBRS-48. IDENTIFICATION OF NOVEL THERAPEUTIC APPROACHES FOR MYC-DRIVEN MEDULLOBLASTOMA

Kübra Taban[1,2], David Pauck[1,2], Mara Maue[1,2], Viktoria Marquardt[1,2], Hua Yu[3], Olivier Ayrault[3], Daniel Picard[1,2], Jasmin Bartl[1,2], Nan Qin[1,2], Arndt Borkhardt[2], Guido Reifenberger[4], and Marc Remke[1,2]; [1]Division of Pediatric Neuro-Oncogenomics, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), partner site University Hospital Düsseldorf, Düsseldorf, Germany, [2]Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [3]Institut Curie, PSL Research University, CNRS UMR, INSERM, 91405, Orsay, France, [4]Institute of Neuropathology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Medulloblastoma (MB) is the most common malignant brain tumor in children and is frequently metastatic at diagnosis. Treatment with surgery, radiation and multi-agent chemotherapy may leave survivors of these brain tumors with long-term deficits as a consequence. One of the four consensus molecular subgroups of MB is the MYC-driven group 3 MB, which is the most malignant type and has a poor prognosis under current therapy. Thus, it is important to discover more effective targeted therapeutic approaches. We conducted a high-throughput drug screening to identify novel compounds showing efficiency in group 3 MB using both clinically established inhibitors (n=196) and clinically-applicable compounds (n=464). More than 20 compounds demonstrated a significantly higher anti-tumoral effect in MYC^{high} (n=7) compared to MYC^{low} (n=4) MB cell models. Among these compounds, Navitoclax and Clofarabine showed the strongest effect in inducing cell cycle arrest and apoptosis in MYC^{high} MB models. Furthermore, we show that Navitoclax, an orally bioavailable and blood-brain barrier passing anti-cancer drug, inhibits specifically Bcl-xL proteins. In line, we found a significant correlation between *BCL-xL* and *MYC* mRNA levels in 763 primary MB patient samples (Data source: "R2 https://hgserver1.amc.nl"). In addition, Navitoclax and Clofarabine have been tested in cells obtained from MB patient-derived-xenografts, which confirmed their specific efficacy in MYC^{high} versus MYC^{low} MB. In summary, our approach has identified promising new drugs that significantly reduce cell viability in MYC^{high} compared to MYC^{low} MB cell models. Our findings point to novel therapeutic vulnerabilities for MB that need to be further validated *in vitro* and *in vivo*.