gene expression, variant signatures and estimate ecDNA copy number in the medulloblastoma tumor sample. We identified 12 distinct clusters in the human tumor, 5 of which were determined to be normal non-tumor [OSC1] cells, as identified by specific cell type markers, and 7 of which were determined to be tumor cells. Enrichment of ecDNA was restricted to only one of these tumor clusters. In addition, we also performed the same multiome single-cell analyses in an orthotopic xenograft mouse model derived from this SHH MB patient tumor. In the PDX, 17 clusters were identified, all of which were determined to be tumor cells and enriched for ecDNA. Our preliminary results indicate that tumor cells with ecDNA in the human tumor (particularly the ecDNA enriched cluster) almost exclusively account for [OSC2] the cells in the corresponding PDX, emphasizing the aggressiveness of ecDNA containing cells.

MEDB-67. SUBGROUP SPECIFIC ANALYSIS OF CELLULAR METABOLISM IN MEDULLOBLASTOMA

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INTRODUCTION: Molecular subgrouping of Medulloblastoma (MB) has expanded our understanding of its biology and the impact on clinical parameters. However, detailed analysis of inter- and intratumoral heterogeneity on a metabolic level is currently lacking. Within this study, we aimed at improving our understanding of metabolic heterogeneity between the MB subgroups, between samples within these subgroups and how these differences affect prognosis. METHODS: We analyzed metabolic characteristics of four MB cohorts covering 1,804 samples in total. In 911 samples (ICGC and MAGIC cohort), we explored metabolic programs on RNA level. In two cohorts (ICGC and G3/G4 samples from the HIT cohort; n=1,035) we examined genetic alterations on DNA level. Furthermore, single-cell RNA-sequencing data of six samples were used to explore intratumoral metabolic heterogeneity. Inter- and intratumoral heterogeneity were correlated to clinical data. RESULTS: Using publicly available gene signatures, we discovered significant differences in metabolic gene expression comparing established MB subgroups. Three metabolically distinct clusters of G3/G4 samples could be defined by unsupervised analyses in two independent cohorts. We were able to confirm our finding of intertumoral metabolic differences on single-cell RNA level. Additionally, our analysis revealed the possibility of sample-specific metabolic features. On DNA level, we identified regulatory genes with known role in MB development to be predominantly associated with lipid metabolic processes. After all, lipid metabolism and metabolism of nucleotides in MB have prognostic value and correlate with the outcome of patients. CONCLUSION: Our data highlight the importance of metabolic properties in MB. We show the distinct metabolic signatures are clinically relevant and, thus, might provide opportunities for novel target-directed therapeutic options in the future.

MEDB-68. ANALYSIS OF TELOMERES LENGTH AND ALTERNATIVE LENGTHENING OF TELOMERES (ALT) IN MOLECULAR SUBGROUPS OF INFANT MEDULLOBLASTOMA

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We investigated the association between the molecular profile and telomere length in a infant medulloblastoma (iMB) cohort, retrospectively studied. Activation of telomeres maintenance mechanisms was analyzed to determine whether the senescence escape triggered by telomere-elongation mechanisms could explain the aggressivity of some iMB belonging to the same molecular subgroup. Interestingly, several telomerase- and ALT-targeted therapies have recently been tested on pediatric cancers and might represent a promising strategy for the future treatment of aggressive telomerase- or ALT-positive iMB. We analyzed a cohort of 50 FFPE tissues from young MB patients (age ≤ 3); IHC, FISH, and an Illumina 850K methylation profile were used to identify molecular subgroups. Telomere length was measured using Telo-quantitative FISH, and image analysis was performed using TFL-Telo software. Three distinct telomere intensity categories (low (L), medium (M), and high (H)) were identified by comparing neoplastic- to endothelial-cell signals in each sample. ATRX loss and TERTp mutation/methylation were investigated using IHC and Sanger sequencing/methylation-specific PCR. SHH-MBs accounted for 59% of our cohort, while Group3/4-MBs accounted for 41%; no WNT-MBs were detected. ALT was found to be activated in 10% of iMBs and was not exclusive to any molecular subgroup, implying that it could be a potential mechanism associated with aggressive behaviour in a subset of iMBs. Promising results have been found in the telomere length distribution among the iMB molecular subgroups: SHH iMBs had a higher frequency of High (H) telomeres length (85%) than NON-SHH/NON-WNT iMBs (p=0.046), which were more frequently associated with Medium (M) telomeres length. CONCLUSIONS: ALT activation in infant MBs (10%) could be a novel target for risk-stratification and personalized therapy. It may be useful to examine ALT as a potential predictor of aggressive behaviour and as a promising novel therapeutic approach for a subset of these tumors in the diagnostic workup.

MEDB-69. CLINICAL AND MOLECULAR META-ANALYSIS OF THREE MAJOR MEDULLOBLASTOMA CLINICAL TRIALS (ACNS0331, SJMB03, ACNS0332) UNCOVERS NOVEL STRATEGIES TO IMPROVE RISK-STRATIFIED THERAPY

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BACKGROUND: Given the vast molecular heterogeneity present within medulloblastoma (MB) and considerable differences in therapy, we performed a meta-analysis of three large, recently published, prospective clinical trials (ACNS0331, SJMB03, ACNS0332) comprising 898 children with newly-diagnosed MB to shape future therapy. METHODS: Molecular subgroups, subtypes, and copy number variations were uniformly procured from DNA methylation profiles and mutations from next-generation sequencing. Patients were stratified into six clinically homogeneous groups for cross-trial comparisons: (1) ACNS0331_LDCSI - patients with non-metastatic (M0), non-residual (R0), non-anaplastic MB treated with low-dose (LD) craniospinal irradiation(CSI); (2) ACNS0331_SDCSI - patients with M0R0 non-anaplastic MB treated with standard-dose(SD) CSI; (3) SJMB03_SDCSI - patients with M0R0 non-anaplastic MB treated with SDCSI; (4) SJMB03_HDCSI - patients with metastatic (M+) MB treated with high-dose (HD) CSI; (5) ACNS0332_HDCSI - patients with M+ MB treated with HDCSI; (6) ACNS0332_HDCSI_Carbo - patients with M+ MB treated with HDCSI and carboplatin. RESULTS: 803 (WNT=125, SHH=122, G3=189, G4=367) of 898 patients formed the cohort. No significant difference was observed between the event-free survival (EFS) from ACNS0331_SDCSI and SJMB03_SDCSI or from SJMB03_HDCSI and ACNS0332_HDCSI when analyzed as a whole or by subgroup. ACNS0331_LDCSI outcome was inferior to the combined ACNS0331_SDCSI + SJMB03_SDCSI cohorts (p<0.001) and in G3 (p=0.030). ACNS0332_HDCSI_Carbo EFS was superior to ACNS0332_HDCSI + SJMB03_HDCSI only in G3/G4_subtype III (p=0.045). Additional molecular risk factor analysis identified M0R0 G3/G4_subtype VII and SHH without high-risk features as very low risk (>90% EFS) and M0R0 G3/G4_subtype III as high risk (<40% EFS).CONCLUSION: The comparable results observed across trials presents a welcome opportunity to reduce toxicity by eliminating excessive doses of chemotherapy (i.e. vincristine, cisplatin, and cyclophosphamide) from therapy. Furthermore, these results support molecularly driven risk classification as the means for a better, more-refined, treatment stratification.

MEDB-70. METABOLISM MEDIATED RADIATION RESISTANCE IN MYC-DRIVEN MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most prevalent malignant brain tumor in children and demonstrates a high level of heterogeneity. Treatment for MB includes chemotherapy and radiation often resulting in long-term morbidity. MYC-driven MB, are high-risk tumors with poor long-term survival and increased susceptibility to develop recurrent tumors. Recurrent MB is far more aggressive with limited treatment options leading to a 5-year survival rate of 12%. To understand what drives MYC-amplified MB relapse we performed single-cell RNA sequencing of irradiated MB xenograft tumors. We identified an overall enhancement of metabolic activity in radiation-resistant cells. We further observe enhanced wild-type IDH1 and IDH2 expression in two clusters, which coincide with hypoxia and Nestin expression, marking a stem-cell like niche. Stem-like cancer cells are notori-

ously resilient against radiation therapy. Furthermore, wtIDH1 and IDH2 represent a unique target in radiation-resistant MB which has not previously been identified. Wild type IDH1/IDH2 are more recently shown to promote tumor proliferation and mediate metabolic reprogramming through the production of oncometabolites and substrates that functionally alter chromatin structure and gene transcription. We hypothesized that MYC modulation of wtIDH1/IDH2 facilitates metabolic reprogramming and promotes radiation-resistant cell populations. We show the change in the structural integrity of chromatin altered in radiation-resistant MB by metabolic adaptation and the effect of disrupting IDH1/IDH2 activity. We further compare these results to the chromatin profile of patient primary and matched relapsed MB samples at the single-cell level. We demonstrate that targeting IDH1/2 with chemical inhibitors suppresses MB cell growth. Our results disclose insights into the development of radiation resistance and provide a potential therapeutic target for the treatment of relapsed MYC-MB.

MEDB-71. MOLECULAR CHARACTERISATION OF GROUP 4 MEDULLOBLASTOMA IMPROVES RISK-STRATIFICATION AND ITS BIOLOGICAL UNDERSTANDING

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Group 4 (MB_{Grp4}) accounts for ~40% of medulloblastoma and the majority of non-WNT/non-SHH cases, yet its underpinning biology is poorly understood, and survival outcomes are not sufficiently explained by established clinicopathological risk factors. We investigated the clinical and molecular correlates of MB_{Grp4}, including second-generation methylation non-WNT/non-SHH subtypes (I-VIII) and whole chromosome aberration (WCA) subtypes (defined by chromosome 7 gain, 8 loss, and 11 loss; WCA-favourable risk [WCA-FR] ≥2 features, WCA-high risk [WCA-HR] ≤1 feature). A clinically-annotated MB_{Grp4} discovery cohort (n=378) was assembled from UK CCLG institutions, collaborating centres and SIOP-UKCCSG-PNET3/HIT-SIOP-PNET4 clinical trials. Contemporary molecular profiling integrating methylation/WCA subtypes and next-generation sequencing was performed. Survival modelling was carried out with patients >3 years old who received craniospinal irradiation (n=336). Association analysis confirmed relationships between methylation and WCA subtypes. Subtypes VI and VII were enriched for WCA-FR ($p<0.0001$) and aneuploidy, whereas subtype VIII was defined solely by i17q ($p<0.0001$). Whilst we observed an overall low mutational burden, WCA-HR harboured recurrent mutations in genes involved in chromatin remodelling ($p=0.007$). No gene-specific events were associated with disease risk, however integration of both methylation subtype and WCA groups enabled improved risk-stratification survival models that outperformed current schemes. The optimal MB_{Grp4}-specific model stratified patients into: favourable-risk (local disease, subtype VII or subtype VI with WCA-FR; 39/194 [20%], 97% 5-year PFS), very-high-risk (metastatic disease with WCA-HR; 71/194 [37%], 50% 5-year PFS) and high-risk (remaining patients; 84/194 [43%], 67% 5-year PFS). Findings were validated in independent cohorts. Comprehensive clinico-molecular assessment of MB_{Grp4} provides important understanding of its clinical and biological heterogeneity. Our novel MB_{Grp4} stratification scheme removes standard risk disease and identifies a favourable risk group (20% of MB_{Grp4}) with potential for therapy de-escalation. Current therapeutic strategies are insufficient for the very-high risk group (encompassing 37% of MB_{Grp4}), for whom novel therapies are urgently required.

MEDB-72. MOLECULAR CHARACTERIZATION OF MEDULLOBLASTOMAS IN A SINGLE INSTITUTION

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INTRODUCTION: The four molecular groups (WNT, SHH, Group 3 and Group 4) in medulloblastoma have been well established for the past decade. New subgroups within the four principal molecular groups have recently been discovered and recognized by WHO classification of Central Nervous System Tumours (5th edition). Subgroups were reported to have distinct somatic copy-number aberrations and clinical outcomes. This further classification could be helpful to refine prognostication and potentially provide risk stratification for treatment planning. AIM: To interrogate archival medulloblastoma samples using Oncoscan Microarray Assay, correlate with clinical features and consider the assay for clinical use. METHODS: Thirty-one archival samples with histological diagnosis of medulloblastoma and molecular grouping results from NanoString were retrieved and evaluated with Oncoscan Microarray Assay. Twenty-six were subjected to DNA methylation profiling to compare the results. Eight cases also had molecular data from next-generation sequencing (NGS) done with the in-house Ampliseq Childhood Cancer Panel. Correlation was made with clinical characteristics and outcomes of these 31 patients. RESULTS: OncoScan microarray showed distinct differences in the copy number profiles of the 31 medulloblastoma samples. Seventeen samples could be further classified into one of 12 subgroups. However, further subgrouping was challenging without first determining the main molecular group especially amongst non-WNT/SHH tumours. DNA methylation results provided corroboration with the Oncoscan subgrouping results in 25 of 26 samples. NGS panel detected additional genetic alterations in 5 of 8 samples. CONCLUSIONS: Oncoscan Microarray Assay showed potential in providing additional molecular information for further subgrouping of medulloblastoma, but was insufficient for determining the main molecular groups. Moving forward, molecular characterization could instead be done through use of NGS panel and DNA methylation, which provides tumour epigenetic profiling on top of copy number variants. These could be used alongside the NanoString platform, which is performed routinely for all medulloblastomas at our centre.

MEDB-73. LIPID METABOLISM AS A THERAPEUTIC VULNERABILITY IN BET INHIBITOR-RESISTANT MEDULLOBLASTOMA

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MYC-driven medulloblastomas are a particularly devastating group of pediatric brain tumors that exhibit resistance and continued progression despite standard of care treatments. Our preclinical work identified BET-bromodomain inhibitors as a potentially promising new class of drugs for children with medulloblastoma and other MYC-driven cancers, providing rationale to evaluate these agents in clinical trials. However, treatment with BET inhibitor (BETi) alone is unlikely to be sufficient to cure, with most tumors evolving to acquire resistance to single-agent targeted therapies. We applied an integrative genomics approach to identify genes and pathways mediating BETi response in medulloblastoma. These studies revealed that MYC-driven medulloblastoma cells with acquired resistance to BETi reinstate transcription of essential genes suppressed by drug and exhibit changes in cell state and new vulnerabilities not present in drug-sensitive cells. We now have a growing body of evidence showing that BET inhibition downregulates the expression of key lipid metabolism genes and metabolism-related signaling pathways, and that medulloblastoma cells with adaptive resistance to drug differentially express and exhibit preferential dependency on specific lipid metabolic genes and transcriptional regulators. Our studies explore the possibility of exploiting these metabolic vulnerabilities to overcome BETi resistance and provide a more efficacious upfront therapy.

MEDB-74. SERIAL ASSESSMENT OF MEASURABLE RESIDUAL DISEASE IN MEDULLOBLASTOMA LIQUID BIOPSIES

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