multiplex 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. Prior to initiation of chemotherapy, renal ultrasound was performed and identified congenitally absent right kidney; audiology evaluation was unremarkable. Discussion: In patients with Gorlin syndrome, cases of unilateral renal agenesis in association with germline SHH-pathway mutations have been reported [1]. SHH signaling is implicated in multiple steps in the development of the urinary system [2]. Outside Gorlin syndrome, however, to the best of our knowledge unilateral renal agenesis coinciding with SHH-driven MB has not been reported. Our patient notably lacks any clinical stigmata of Gorlin syndrome (skeletal abnormalities, skin pits, macrocephaly) and does not exhibit the characteristic germline genetic abnormalities that define GS (PTCH1 mutation or 9q22.3 microdeletion). There are important treatment implications for patients with the constellation of abnormalities we describe here, particularly regarding the requisite frequent monitoring of renal function during multi-agent chemotherapy courses. Our patient tolerated chemoradiation well, and is currently on maintenance chemotherapy with favorable course to date.

EMBR-20. ELONGATION CONTROL OF MRNA TRANSLATION DRIVES GROUP 3 MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common pediatric intracranial tumor and leading cause of childhood related cancer deaths. Group 3 affiliation and 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 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 3' 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 significantly reduces survival of MYC-amplified MB cell lines 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 mRNA by 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 studies 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 TPD52 IMMUNOHISTOCHEMISTRY: A MULTICOHORT STUDY

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Background: International consensus and the 2021 WHO classification recognize 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 immunohistochemistry (IHC) marker as a clinically tractable method for improved medulloblastoma risk-stratification. Patients and Methods: We bioinformatically analyzed published medulloblastoma transcriptomes and proteomes identifying as a potential biomarker TPD52, whose IHC prognostic value was validated across three Group 3/4 medulloblastoma clinical cohorts (n = 387) treated with conventional therapies. Risk stratification and prediction capability were computed utilizing uni- and multivariate survival analysis. Newly developed risk classifiers including TPD52 IHC were compared to state-of-the-art risk stratification schemes in terms of prediction error, area under the time-dependent receiver operating characteristic (ROC) curves and C-statistic. Biomarker-driven prognostic stratification models identified were cross validated in different cohorts. Results: TPD52 IHC positivity represents a significant independent predictor of early relapse and death for Group 3/4 medulloblastoma (HRs between 3.67–26.7 [95% CIs between 1.00–706.23], $p = 0.05$, 0.017 and 0.0058). Cross-validated survival models incorporating TPD52 IHC with clinical features outperformed existing disease risk-stratification schemes, and reclassified ~50% of patients into more appropriate risk categories. Finally, TPD52 immunopositivity is a predictive indicator of poor response to chemotherapy (HR 12.66 [95% CI 3.53–45.40], $p < 0.0001$), suggesting important implication for therapeutic choices. Conclusion: The current study redefines the approach to risk-stratification in Group 3/4 medulloblastoma. Integration of TPD52 IHC in classification algorithms significantly improves outcome prediction and can be rapidly adopted for risk stratification on a global scale, independently of advanced but technically challenging molecular profiling techniques.

EMBR-22. RATIONAL DEVELOPMENT OF SYNERGISTIC THERAPIES ALONGSIDE BMI1 INHIBITION FOR GROUP 3 MEDULLOBLASTOMA

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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. These results suggest that additional mechanisms may underlie the maintenance of MB and underscores the main obstacle in treating a constantly evolving tumor. After initial preclinical validation of BMI1 inhibitor PTC-596, DNA barcoding clonal tracking technology was leveraged to profile *in vivo* clonal dynamics of Group 3 MB in response to the established chemoradiotherapy regimen alone and in combination with PTC-596. 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 tumor expansion. 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 PTC-596 has demonstrated synergistic efficacy against MB cells with minimal toxicity to hNSCs *in vitro* and is currently being evaluated in preclinical studies. This study provides the foundation for clinical validation of small-molecule inhibitors synergistic with PTC-596 to improve the durability of remissions and extend survival of patients with treatment-refractory Group 3 MB.

EMBR-23. KIF11 DEPENDENCY ON P53 MUTATIONAL STATUS IN MEDULLOBLASTOMA

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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 necrosis at 24h. Differences 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 inhibition of KIF11 and increased p53 expression were observed only after 48h, cleaved-PARP expression was detected as early as 24h in p53-wildtype, suggesting KIF11-independent, cleaved-PARP-mediated cell death at 24h. In contrast, treated p53-mutant cells showed decreased p53 expression and absence of cleaved-PARP and DNA-damage marker after 24h. Conclusions: Our results suggest that when mitotic arrest is induced, p53-mutant MB cells undergo mitotic catastrophe and necrosis while p53-wildtype MB cells predominantly undergo apoptosis.

EMBR-24. YB1 IS CRITICAL FOR MEDULLOBLASTOMA TUMOR MAINTENANCE AND DNA REPAIR FOLLOWING THERAPEUTIC INTERVENTION

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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 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 translational changes important for tumor maintenance and therapeutic response. We show that following ionizing radiation, YB1 mediates DNA repair through PARP and that PARP inhibition abrogates YB1-mediated DNA repair in cells overexpressing YB1. Additionally, through its inhibitory effects on p53, YB1 is capable of mediating anti-apoptotic effects in response to genotoxic insult. By targeting YB1 with short hairpin RNA, we show that cells are more amenable to ionizing radiation induced double strand breaks. Additionally, we utilize RNA binding protein immunoprecipitation sequencing to investigate post transcriptional regulation of RNAs bound by YB1. We show that YB1 binds numerous transcripts critical for the identity of early cerebellar progenitor cells, the putative cell of origin for SHH subgroup tumors, in addition to transcripts important for cell cycling and migration.

EMBR-25. GENOME-WIDE GENETIC AND EPIGENETIC ASSESSMENT OF GROUP 4 MEDULLOBLASTOMA FOR IMPROVED, BIOMARKER DRIVEN, PROGNOSTICATION AND RISK-STRATIFICATION

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Introduction: Medulloblastoma (MB) is the most common malignant brain tumour in children. The most frequent molecular subgroup, Group 4 (MB_{Grp4}) accounts for ~35/40% of cases, however it has the least understood underlying biology. Clinical outcomes are heterogeneous in MB_{Grp4} and are not accounted for by established clinico-pathological risk factors. There is now a requirement for a comprehensive study of MB_{Grp4}, considering established clinico-pathological features and novel molecular biomarkers to enhance risk-stratification and identify novel therapeutic targets. Methods: A clinically-annotated, retrospective MB_{Grp4} discovery cohort (n = 420) was generated from UK CCLG institutions, collaborating European centres and SIOP-UKCCSG-PNET3 and HIT-SIOP-PNET4 clinical trials. Contemporary, multi-omics profiling was performed. Focal and arm level copy number aberrations (CNAs) were determined from molecular inversion probe (MIP) or DNA methylation array which additionally provided next generation non-WNT/non-SHH (Grp3/Grp4) subtype classifications. Targeted next-generation DNA sequencing was performed to overlay the mutational landscape. Survival modelling was carried out with patients >3 years old who received craniospinal irradiation. Results: MB_{Grp4} subtypes were assigned to 88% of tumours with available data. Subtype VIII was strongly associated with i17q ($p<0.0001$). The favourable-risk cytogenetic signature (2 or 3 of; chromosome 7 gain, chromosome 8 loss and/or chromosome 11 loss) associated with both subtypes VI and VII ($p<0.0001$). *MYCN* amplifications were strongly associated with subtype V ($p<0.0001$) in addition to 16q loss ($p<0.0001$). The high-risk CNA group was enriched for mutations in genes involved in chromatin remodelling ($p<0.0001$). Risk factors were identified from multivariate survival modelling. Subtype and CNA groups contributed to improved risk-stratification models that outperformed current clinical schemes. Conclusion: Comprehensive genetic and epigenetic profiling in this large retrospective cohort has improved our understanding of the molecular and clinical heterogeneity within MB_{Grp4}. Incorporation of molecular biomarkers improved risk-stratification for MB_{Grp4}.

EMBR-27. NEOPLASTIC AND IMMUNE SINGLE CELL TRANSCRIPTOMICS DEFINE SUBGROUP-SPECIFIC INTRA-TUMORAL HETEROGENEITY OF CHILDHOOD MEDULLOBLASTOMA

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Medulloblastoma (MB) is a heterogeneous disease in which neoplastic cells and associated immune cells contribute to disease progression. To better understand cellular heterogeneity in MB we used single-cell RNA sequencing, immunohistochemistry and deconvolution of transcriptomic data to profile neoplastic and immune populations in childhood MB samples and MB genetically engineered mouse models (GEMM). Neoplastic cells clustered primarily according to individual sample of origin which is in part due to the effect of chromosomal copy number gains and losses. Harmony alignment of single cell transcriptomic data revealed novel MB subgroup/subtype-associated subpopulations that recapitulate neurodevelopmental processes and are associated with clinical outcomes. This includes photoreceptor-like cells and glutamatergic lineage unipolar brush cells in both GP3 and GP4 subgroups of MB, and a SHH subgroup nodule-associated neuronally-differentiated cell subpopulation. We definitively chart the spectrum of MB immune cell infiltrates, which reveals unexpected degree of myeloid cell diversity. Myeloid subpopulations include subgroup/subtype-associated developmentally-related neuron-pruning as well as antigen presenting myeloid cells. Human MB cellular diversity is recapitulated in subgroup-specific MB GEMM, supporting the fidelity of these models. These findings provide a clearer understanding of both the neoplastic and immune cell heterogeneity in MB and how these impact subgroup/subtype classification and clinical outcome.