MBRS-57. IDENTIFICATION OF *MYC*-DEPENDENT THERAPEUTIC VULNERABILITIES FOR TARGETING GROUP 3 MEDULLOBLASTOMA

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Group 3 medulloblastoma (MB_{Group3}) is a highly aggressive tumour characterised by *MYC* amplification and elevated expression (17% of MB_{Group3}). *MYC* amplification in MB_{Group3} confers a dismal prognosis using standard therapies, and there is an urgent unmet need for novel therapeutic approaches. The identification and targeting of *MYC*'s biological dependencies thus represents a promising strategy to treat *MYC*-MB_{Group3} tumours. Three independent isogenic *MYC*-regulable MB_{Group3} human cell-based models, in which elevated *MYC* expression can be directly down-regulated by doxycycline-inducible shRNAs, were developed and used initially to establish *MYC*-dependent growth of each model. Our novel models were then used to investigate *MYC*-dependent drug sensitivity, by characterising responses to a panel of candidate cancer therapeutics and small molecule inhibitors, including a high-throughput compound screen of >500 established/clinically-relevant small molecule inhibitors. This approach identified several specific, consistently observed, druggable *MYC*-dependencies (e.g. cell cycle regulators, DNA-damage response controllers, mitotic control machinery) with potential for the development of treatments against *MYC*-MB_{Group3} tumours. *PLK1*, *CHK1* and *AURK* were identified as prime candidate targets with consistent *MYC*-dependent response profiles. Subsequent validation of each candidate, by genetic and pharmacological target inhibition, confirmed their *MYC*-dependent effects, associated with downregulation of *MYC* and established target-dependent pharmacodynamic biomarkers/pathways. Results were consistent across all of our MB_{Group3} models. In summary, our novel models reveal druggable *MYC*-associated dependencies as a feature of MB_{Group3}. Our findings support the development of *PLK1*, *CHK1* and *AURK* inhibition as therapeutic approaches against *MYC*-dependent MB_{Group3}. Future work is now essential to validate our findings *in vivo*, to support the design of future clinical trials.

MBRS-59. SINGLE-CELL WHOLE-GENOME SEQUENCING DISSECTS INTRA-TUMOURAL GENOMIC HETEROGENEITY AND CLONAL EVOLUTION IN CHILDHOOD MEDULLOBLASTOMA

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Medulloblastomas harbor clinically-significant intra-tumoral heterogeneity for key biomarkers (e.g. *MYC/MYCN*, β-catenin). Recent studies have characterized transcriptional heterogeneity at the single-cell level, however the underlying genomic copy number and mutational architecture remains to be resolved. We therefore sought to establish the intra-tumoural genomic heterogeneity of medulloblastoma at single-cell resolution. Copy number patterns were dissected by whole-genome sequencing in 1024 single cells isolated from multiple distinct tumour regions within 16 snap-frozen medulloblastomas, representing the major molecular subgroups (WNT, SHH, Group3, Group4) and genotypes (i.e. *MYC* amplification, *TP53* mutation). Common copy number driver and subclonal events were identified, providing clear evidence of copy number evolution in medulloblastoma development. Moreover, subclonal whole-arm and focal copy number alterations covering important genomic loci (e.g. on chr10 of SHH patients) were detected in single tumour cells, yet undetectable at the bulk-tumor level. Spatial copy number heterogeneity was also common, with differences between clonal and subclonal events detected in distinct regions of individual tumours. Mutational analysis of the cells allowed dissection of spatial and clonal heterogeneity patterns for key medulloblastoma mutations (e.g. CTNNB1, TP53, SMARCA4, PTCH1) within our cohort. Integrated copy number and mutational analysis is underway to establish their inter-relationships and relative contributions to clonal evolution during tumourigenesis. In summary, single-cell analysis has enabled the resolution of common mutational and copy number drivers, alongside sub-clonal events and distinct patterns of clonal and spatial evolution, in medulloblastoma development. We anticipate these findings will provide a critical foundation for future improved biomarker selection, and the development of targeted therapies.

MBRS-60. THE ACTIONABLE GENOMIC LANDSCAPE OF RELAPSED MEDULLOBLASTOMA IS DEFINED BY MAINTENANCE AND ACQUISITION OF DRIVER EVENTS

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Medulloblastoma relapse (rMB) occurs in 30–40% of patients and is almost universally fatal. Understanding the genomic landscape of rMB, and its relationship to disease characteristics at diagnosis, will be essential to underpin the development of improved therapeutic strategies, delivered at both diagnosis and relapse. Utilising NGS and Illumina DNA methylation arrays, we interrogated the molecular landscape of >100 rMBs, alongside matched diagnostic samples (n>80), encompassing molecular subgroup, novel subtypes, copy number (CNV) and mutational variants. Molecular subgroup and novel subtypes were stable over disease-course. The majority of genomic aberrations were also maintained (total arm-level CNVs at relapse, 60% maintained/40% acquired; deleterious/driver mutations, 75% maintained/25% acquired). Importantly, however, the landscape of alterations differed markedly at relapse, through both selective maintenance and acquisition of specific gene and pathway aberrations. For instance, we observed significant enrichment of subgroup-specific events at relapse, including focal *CDK6/CDK14* amplifications (4/26 (15%) of MB_{Group4}) and *CDKN2A/CDKN2B* deletions (3/48 (6%) of MB_{SHH}). In contrast, mutations in DNA damage response pathways were commonly enriched across all molecular subgroups, most significantly in MB_{SHH} (~40% of rMB_{SHH}; *TP53*, 9/36 (25%); *ATM*, 5/36 (14%)). Driver events in rMB are characterised by both selective maintenance and acquisition across disease-course, and together combine to define its actionable genetic landscape. Evaluation of their clinical and biological significance will be essential to establish their potential (i) as biomarkers to direct disease management and (ii) as a basis for therapeutic strategies targeted against medulloblastoma relapse.

MBRS-61. MOLECULAR SUB-GROUPING OF PEDIATRIC MEDULLOBLASTOMA: CORRELATION WITH CLINICAL AND HISTOLOGICAL FEATURES, A SINGLE INSTITUTIONAL STUDY

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INTRODUCTION: Molecular subgroups of pediatric medulloblastomas are distinctive in infantile and non-infantile age-groups. METHODS: Real-time quantitative PCR based GEP of customized 12 protein-coding genes was performed on 206 FFPE childhood medulloblastoma samples. FISH for *MYC* amplification, monosomy 6 and sequencing for *CTNNB1* exon 3 mutation were done in relevant cases. H&E and reticulin-stained slides were used for histological subtyping. p53-protein immunoreactivity pattern was noted. RESULTS: Infantile (n=33) comprised 57.6% SHH-activated (desmoplastic: 73.7%; MBEN: 15.8% and classic: 10.5%), 21.2% group 3 (large cell/anaplastic [LCA]: 28.6% and none were desmoplastic) and 12% group 4. 40% of group 3 patients died of disease and 21% of the SHH-activated (all desmoplastic) had subsequent local recurrence. Non-infantile (n=173) comprised 19.4% WNT-activated, 12.9% SHH-activated (15% classic, 30% desmoplastic, 10% paucinodular), 19.4% group 3 (63.3% classic & 26.7% LCA), 48.4% group 4 (73.3% classic, 5.3% desmoplastic, 10.7% paucinodular & 1.4% LCA), and non-WNT/non-SHH (NWNS), NOS (n=14,9%) and unclassified (n=4,2.6%). None of WNT-activated were desmoplastic/LCA histology. Non-infantile WNT-activated and group 3 MBs showed 90% monosomy 6 & *CTNNB1* mutation, and 16.7% *MYC*-amplification respectively. 17.4% (13% spinal, 4.4% local) WNT-activated, 31% (12.5% local, 18.5% distant [spinal: 12.5%, intracranial:6%]) SHH-activated, 27% (18% both spinal and local, 9% spinal) group 3 and 31.5% (7.4% local, 5.5% intracranial, 11.2% spinal, 7.4% both spinal and local) group 4 showed metastases during follow up. CONCLUSIONS: SHH-activated and group 3 are the common infantile subgroups but group 4 is not non-existent in infantile age. No desmoplastic (including paucinodular) histological subtype is of WNT- activated and group 3.