

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

Gemma Llargués-Sistac<sup>1</sup>, Janet Lindsey<sup>1</sup>, Shanel Swartz<sup>1</sup>, Matthew Selby<sup>1</sup>, Alaiide Morcavallo<sup>2</sup>, Simon Bailey<sup>1</sup>, Louis Chesler<sup>2</sup>, Christopher J. Lord<sup>3</sup>, Daniel Williamson<sup>1</sup>, and Steven C. Clifford<sup>1</sup>; <sup>1</sup>Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Newcastle Upon Tyne, United Kingdom, <sup>2</sup>Paediatric and Solid Tumour Biology and Therapeutics Group, The Institute of Cancer Research London, London, United Kingdom, <sup>3</sup>The Breast Cancer Now Toby Robins Breast Cancer Research Centre and CRUK Gene Function Laboratory, The Institute of Cancer Research London, London, United Kingdom

Group 3 medulloblastoma (MB<sub>Group3</sub>) is a highly aggressive tumour characterised by MYC amplification and elevated expression (17% of MB<sub>Group3</sub>). MYC amplification in MB<sub>Group3</sub> 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<sub>Group3</sub> tumours. Three independent isogenic MYC-regulable MB<sub>Group3</sub> 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<sub>Group3</sub> 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<sub>Group3</sub> models. In summary, our novel models reveal druggable MYC-associated dependencies as a feature of MB<sub>Group3</sub>. Our findings support the development of *PLK1*, *CHK1* and *AURK* inhibition as therapeutic approaches against MYC-dependent MB<sub>Group3</sub>. 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

Marina Danilenko<sup>1</sup>, Masood Zaka<sup>2</sup>, Claire Keeling<sup>1</sup>, Stephen Crosier<sup>1</sup>, Rafiqul Hussain<sup>3</sup>, Edward Schwalbe<sup>4</sup>, Dan Williamson<sup>1</sup>, Jonathan Coxhead<sup>3</sup>, Vikki Rand<sup>2</sup>, Simon Bailey<sup>1</sup>, and Steven Clifford<sup>1</sup>; <sup>1</sup>Wolfson Childhood Cancer Research Centre, Translational & Clinical Research Institute, Newcastle University Centre for Cancer, Newcastle upon Tyne, United Kingdom, <sup>2</sup>National Horizons Centre, Darlington, United Kingdom, <sup>3</sup>Genomics Core Facility, Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom, <sup>4</sup>Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom

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-tumoral 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-tumour 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

Stacey Richardson<sup>1</sup>, Rebecca M Hill<sup>1</sup>, Christopher Kui<sup>1</sup>, Janet C Lindsey<sup>1</sup>, Yura Grabovska<sup>1</sup>, Edward C Schwalbe<sup>1,2</sup>, Debbie Hicks<sup>1</sup>, Daniel Williamson<sup>1</sup>, Stephen Crosier<sup>1</sup>, Abhjit Joshi<sup>3</sup>, Stephen B Wharton<sup>4</sup>, Jordan Hansford<sup>5</sup>, Barry Pizer<sup>6</sup>, Maria Vinci<sup>7</sup>, Angela Mastronuzzi<sup>7</sup>, Andrea Carai<sup>7</sup>, Anthony Michalski<sup>8</sup>, Thomas S Jacques<sup>9</sup>, Simon Bailey<sup>1</sup>, and Steven C Clifford<sup>1</sup>; <sup>1</sup>Newcastle University Centre for Cancer, Newcastle upon Tyne, United Kingdom, <sup>2</sup>Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom, <sup>3</sup>Department of Neuropathology, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom, <sup>4</sup>Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, United Kingdom, <sup>5</sup>The Royal Children's Hospital Melbourne, Melbourne, Australia, <sup>6</sup>Institute of Translational Research, University of Liverpool, Liverpool, United Kingdom, <sup>7</sup>Department of Pediatric Onco-Hematology and Transfusion Medicine, Ospedale Pediatrico Bambino Gesù, Rome, Italy, <sup>8</sup>Department of Haematology and Oncology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, <sup>9</sup>Neural Development Unit, UCL Institute of Child Health, London, United Kingdom

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<sub>Group4</sub>) and *CDKN2A/CDKN2B* deletions (3/48 (6%) of MB<sub>SHH</sub>). In contrast, mutations in DNA damage response pathways were commonly enriched across all molecular subgroups, most significantly in MB<sub>SHH</sub> (~40% of rMB<sub>SHH</sub>; *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

Gauri Deshpande, Mamta Gurav, Omsheer Shetty, Vinayak Kadam, Vishal Chaubey, Tejpal Gupta, Aliasgar Moidy, Girish Chinnaswamy, and Sridhar Epari; Tata Memorial Centre, Mumbai, Maharashtra, India

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.