Simon Robinson[1], Darren Hargrave[6], Maria Vinci[7], and Chris Jones[1]; [1]Institute of Cancer Research, London, United Kingdom, [2]Royal Marsden Hospital, London, United Kingdom, [3]Our Lady's Children's Hospital, Dublin, Ireland, [4]The University of Queensland, Brisbane, Australia, [5]Institut de Recerca Sant Joan de Deu, Barcelona, Spain, [6]Great Ormond Street Hospital, London, United Kingdom, [7]Bambino Gesù Children's Hospital, Rome, Italy

Paediatric high-grade glioma comprise multiple biological and clinical subgroups, the majority of which urgently require novel therapies. Patient-derived models represent useful tools for mechanistic and preclinical investigations based upon their retention of key genetic/epigenetic features and their amenability to high-throughput approaches. We have collected ~100 i*n vitro* models representing multiple subtypes (H3.3/H3.2/H3.1K27M, H3.3G34R/V, BRAF, MYCN_amp, NTRK_fusion, hypermutator, others) established under 2D (laminin) and/or 3D (neurosphere) conditions, credentialed by phenotypic (growth, invasion/migration) and molecular (methylation array, DNA sequencing, RNAseq) comparison to the original tumour sample. These were derived from patients at our local hospitals (n=29), as part of national co-clinical trials (n=19), from international collaborating centres (n=11), or shared directly by research groups worldwide (n=45). These have variously been subjected to pharmacological (approved/experimental drug libraries) and/or genetic screening (whole-genome CRISPR) to identify specific biological dependencies. Many have been established as orthotopic xenografts i*n vivo* (PDX), with detailed pathological and radiological correlations with the clinical disease, and with tumorigenic latencies ranging from 48–435 days. This resource has allowed us to identify genotype-specific synthetic lethalities and responses to targeted inhibitors, including olaparib (PARP) with *ATRX*, nutlin-3 (MDM2) with *PPM1D*, AZD1775 (WEE1) with *TP53*, and CYC065 (CDK9) with *MYCN*-amplification. Combinatorial screening highlighted synergies in *ACVR1*-mutant DIPG between novel ALK2 inhibitors and ONC201 (DRD2). Rapid screening allows for feedback of drug sensitivities to treating clinicians at relapse, whilst mechanistic underpinning of these interactions and use of the models to identify specific mediators of resistance will allow for rational future trial design.

MODL-21. INTEGRATIVE APPROACHES IN FUNCTIONAL GENOMICS TO IDENTIFY GENETIC DEPENDENCIES IN PEDIATRIC BRAIN CANCER

Claire Sun, Caroline Drinkwater, Dhanya Sooraj, Gabrielle Bradshaw, Claire Shi, Dasun Fernando, Sarah Parackal, Daniel Gough, Jason Cain, and Ron Firestein; Hudson Institute of Medical Research, Clayton, VIC, Australia

The precise decoding of human genomes facilitated by the advancements in next-generation sequencing has led to a better understanding of genetic underpinnings of pediatric brain cancers. Indeed, it is now evident that tumours of the same type harbour distinct driving mutations and molecular aberrations that can result in different prognosis and treatment outcomes. The profounder insight into the the identity, amount and types of molecular aberrations has paved the way for the advent of targeted therapies in precision medicine. Nevertheless, less than 10% of pediatric cancer patients harbour actionable mutations. Strictly limited therapeutic options that are firstly available for brain cancers and secondly acceptable for children's development further impede the breakthrough in the survival rate in pediatric brain cancers. This underscores a desperate need to delve beyond genomic sequencing to identify biomarker coupled therapies that not only featured with treatment efficacy in the central nervous system but also acceptable side effects for children. The Hudson-Monash Paediatric Precision Medicine (HMPPM) Program focuses on utilising genetic profiles of patients' tumour models to identify new therapeutic targets and repurpose existing ones using high-throughput functional genomics screens (2220 drugs and CRISPR screen of 300 oncogenic genes). Using a large compendium of over sixty patient derived paediatric brain cancer models, we provide proof-of-concept data that shows an integrative pipeline for functional genomics with multi-omics datasets to perform genotype-phenotype correlations and, therefore, identify genetic dependencies. Herein, using several examples in ATRT, DIPG and HGG, we show how functional interrogations can better define molecular subclassification of tumours and identify unique vulnerabilities.

MODL-22. DEVELOPING A REAL-TIME PERSONALIZED DRUG TESTING PLATFORM FOR PEDIATRIC CNS CANCERS

Sandra Laternser[1], Chiara Cianciolo Cosentino[2], Justyna M Przystal[1], Susanne Dettwiler[3], Elisabeth Jane Rushing[3], Nicolas U Gerber[4], Ana Guerreiro Stücklin[4], Rachna Prasad[2], Michael Grotzer[4], Niklaus Krayenbühl[4], Sabine Müller[2,4], and Javad Nazarian[2,4]; [1]University Children's Hospital Zurich, DRIz, Oncology Department, Zurich, ZH, Switzerland, [2]University Children's Hospital Zurich, DRIz, Oncology Department, Zurich, ZH, Switzerland, [3]University Hospital Zurich, Institute of Pathology and Molecular Pathology, Zurich, ZH, Switzerland, [4]University Children's Hospital Zurich, Zurich, ZH, Switzerland

INTRODUCTION: The relatively small size of biopsied CNS tumors has presented a historical challenge for real-time drug screens. Moreover, in vivo assessment of drug response does not often benefit patients with aggressive gliomas given the relatively long time (>8 months) of tumor engraftment in the classic mouse PDX models. Here, we aimed to develop an innovative real-time in vivo and in vitro drug screening platform capable of analyzing a minimal number (<1E6) of cells obtained at biopsy. METHODS: Existing primary cells were used to test 6 different culture platforms. The top platform was selected and used to expand tumor cells obtained of DMG biopsy. Tumor cells were validated using the minION sequencing platform. Single and combination drug (n=7) screens were performed. Effective drugs were further evaluated in zebrafish PDX and non-tumor bearing models to assess efficacy and toxicity, respectively. RESULTS: A total of 8 biopsies were obtained. Successful cell expansion was achieved in 6/8 (75%) and a limited drug screen in 3/6 (50%) of cases. Single and combination drug (n=7) assays identified responder and non-responders to candidate drugs. Systemic toxicity of effective drugs was tested in non-tumor bearing zebrafish. Tumor cells were engrafted in zebrafish providing the opportunity for an in vivo screen. The entire process was completed within 21 days on average. CONCLUSIONS: A novel platform was developed for rapid in vitro and in vivo drug screens of tumor cells obtained at biopsy. This platform will provide the opportunity to establish personalized therapy for heterogeneous cancers including DMGs.

MODL-23. DNA METHYLATION AND COPY NUMBER VARIATION PROFILE FOR CHARACTERIZATION OF PEDIATRIC BRAIN TUMOR PRIMARY CELL LINES

Lucia Pedace[1], Maria Vinci[1], Simone Pizzi[1], Giulia Pericoli[1], Giuseppina Catanzaro[2], Luana Abballe[2], Francesca Del Bufalo[1], Ignazio Caruana[1], Francesca Diomedi Camassei[1], Sabrina Rossi[1], Felice Giangaspero[2], Elisabetta Ferretti[2], Andrea Ciolfi[1], Marco Tartaglia[1], Franco Locatelli[1], Angela Mastronuzzi[1], and Evelina Miele[1]; [1]Bambino Gesù Children's Hospital, Rome, Italy, [2]University of Rome Sapienza, Rome, Italy

BACKGROUND: In vitro models of pediatric brain tumors (pBT) are instrumental for both understanding the oncogenic molecular mechanisms and identifying/testing new therapeutic strategies. DNA methylation (DM) is a stable epigenetic modification recently used to classify tumors. We aim to apply DM and Copy Number Variation (CNV) profiling to characterize pBT primary cell lines and tumors. METHODS: We included 36 pBT tissues from different histology (13 LGG, 9 DIPG, 9 HGG, 3 MB, and 2 Ependymomas), paired to their derived primary cultures. Cultures were established in two-dimensional (2D) or three-dimensional (3D) condition, as stem-cell or in serum-supplemented medium. For 9 cultures, both early (P2-P3) and long-term passages (>P4) were considered. Samples were analyzed for DM and CNV profiles using Illumina EPIC arrays and data compared with those of the brain tumor classifier. RESULTS: At early passages all cells retained the same DM and genetic patterns of original tumors, with no differences related to 2D/3D methods or presence of serum in media. Primary cell lines analyzed at > P4 and cultured in serum diverged from the primary tumor. CONCLUSIONS: DM profiles and CNV are useful tools to detect the recapitulation of pBT-derived primary cell-lines from the original tumor. Whatever subgroups tested, results suggest that in vitro models should be passaged as little as possible to retain the epigenetic and genetic alterations of the tumors and thus to be considered relevant for basic and translational biology. Ongoing experiments are aimed to determine how stable DM and CNV are in other conditions/tumor subgroups.

MODL-24. AN ORGANOTYPIC CHUNK CULTURE TECHNIQUE TO STUDY DISEASE MECHANISM AND DEVELOP TARGETED THERAPEUTICS FOR PEDIATRIC ADAMANTINOMATOUS CRANIOPHARYNGIOMA

Trinka Vijmasi[1], Eric Prince[1], Astrid Hengartner[1], Susan Staulcup[2], Andrea Griesinger[3], Andrew Donson[3], Ahmed Gilani[4], Nicholas Foreman[3], and Todd Hankinson[2]; [1]Department of Neurosurgery, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [2]Department of Neurosurgery, Children's Hospital Colorado, Aurora, CO, USA, [3]Deparment of Pediatrics, Children's Hospital Colorado, Aurora, CO, USA, [4]Department of Pathology, Children's Hospital Colorado, Aurora, CO, USA

BACKGROUND: Advances in the treatment of Adamantinomatous Craniopharyngioma (ACP) face challenges with translation to clinical study due to the absence of robust culture models of the disease. We developed a technique for culturing human ACP tissue in an organotypic chunk culture format that retains the tumor microenvironment for a duration sufficient to evaluate potential targeted therapeutics. METHODS: Intraoperatively