from GCPs, maintains quiescent stem-like cells during the disease and contributes to tumor outgrowth at recurrence. We found that FGF2-FGFR signaling causes increased growth and tissue invasion through the FGFR adaptor protein FRS2 in SHH and group-3 medulloblastoma [1]. Thus, targeting of FGFR-FRS2 signaling could abrogate brain tumor growth and spread by repressing tumor-promoting functions that are induced by microenvironmental FGF2. Using virtual screening combined with functional validation, we identified protein-protein interaction inhibitors (F2i) that bind FRS2 and abrogate FGFR signaling to the MAP-ERK pathway. Consistent with the requirement of FRS2 for pro-invasive signaling downstream of FGFR1 in medulloblastoma, F2i also efficiently block FGF2-induced migration and invasion in medulloblastoma-derived cells. Selected F2i display excellent binding kinetics with a similar Kd as the natural ligand domain of FGFR and cause steric alterations in the targeted protein domain. On-target activity was confirmed by thermal proteome profiling. Neither *in silico* screening nor empirical testing revealed significant off-target activity of the compounds. No toxicity of F2i was observed in cell-based models with confirmed functional activity on invasion and MAPK activation. Thus, we identified novel, low molecular weight pharmacological protein-protein interaction inhibitors with an excellent potential to specifically block FGFR functions relevant for brain tumor progression. 1. Santhana Kumar et al., *CellReports* **23**, 3798–3812.e8 (2018).

MODL-15. THE COMBINATION TREATMENT OF PARP INHIBITOR AND TMZ, OR DAG WILL BE PROMISING TREATMENT IN SF8628

Shigeo Ohba, and Yuichi Hirose; Fujita Health University, Toyoake, Japan

Diffuse midline glioma, H3 K27M-mutant (DMG) is a newly defined entity. The prognosis of DMG is poor. Because surgical resection is often incomplete for DMG, radiotherapy and chemotherapy are important. Temozolomide (TMZ) is an alkylating agent that adds a methyl group to DNA (O6-guanine, N7-guanine, and N3-adenine). TMZ-induced cytotoxicity is mainly derived from O6-methylguanine, which is repaired by O6-methylguanine DNA methyltransferase (MGMT). It has been reported that most of DMG lacked MGMT promoter hypermethylation, which is thought to contribute to less effectiveness of TMZ to DMG. The purpose of the study is to explore the way to inhibit the proliferation of DMG. A DMG cell line, SF8628, was used for the experiments. SF8628 had the expression of MGMT and was revealed to be resistant to TMZ. Because N7-methylguanine and N3-methyladenine are repaired via base excision repair, poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitor combined with TMZ was considered to be effective to suppress the proliferation of SF8628. As expected, PARP inhibitor enhanced TMZ-induced cytotoxicity in SF8628. Dianhydrogalactiol (DAG) is a bifunctional DNA-targeting agent forming N7-alkylguanine and inter-strand DNA crosslinks. DAG reduced the clonogenicity of SF8628. Moreover, inhibition of homologous recombination enhanced the DAG-induced cytotoxicity in SF8629. The combination treatment of PARP inhibitor and TMZ, or DAG were revealed to be promising treatments in SF8628.

MODL-16. ABEMACICLIB, A SELECTIVE CDK4/6 INHIBITOR, RESTRICTS GROWTH OF PEDIATRIC GLIAL-LINEAGE TUMORS IN VITRO AND IN VIVO

Muh-Lii Liang[1], Tsung-Han Hsieh[2], and Tai-Tong Wong[3]; [1]Department of Neurosurgery, Mackay Memorial Hospital, Taipei, Taiwan, [2]Joint Biobank, Office of Human Research, Taipei Medical University, Taipei, Taiwan, [3]Department of Neurosurgery, Taipei Medical University Hospital, Taipei, Taiwan

BACKGROUND: Glial-lineage tumors constitute a heterogeneous group of neoplasms, comprising gliomas, oligodendrogliomas, and ependymomas, which account for 40%–50% of all pediatric central nervous system tumors. Advances in modern neuro-oncological therapeutics are aimed at improving neoadjuvant chemotherapy and deferring radiotherapy because radiation exposure may cause long-term side effects on the developing brain in young children. Despite aggressive treatment, more than half the high-grade gliomas (pHGGs) and one-third of ependymomas exhibit recurrence within 2 years of initial treatment. METHODS: By using integrated bioinformatics and through experimental validation, we found that at least one gene among CCND1, CDK4, and CDK6 was overexpressed in pHGGs and ependymomas. RESULTS: The use of abemaciclib, a highly selective CDK4/6 inhibitor, effectively inhibited cell proliferation and reduced the expression of cell cycle–related and DNA repair–related gene expression, which was determined through RNA-seq analysis. The efficiency of abemaciclib was validated in vitro in pHGGs and ependymoma cells and in vivo by using subcutaneously implanted ependymoma cells from patient-derived xenograft (PDX) in mouse models. Abemaciclib demonstrated the suppression of RB phosphorylation, downstream target genes of E2F, G2M checkpoint, and DNA repair, resulting in tumor suppression. CONCLUSION: Abemaciclib showed encouraging results in preclinical pediatric glial-lineage tumors models and represented a potential therapeutic strategy for treating challenging tumors in children.

MODL-17. SHP2 INHIBITORS SHOW ACTIVITY AGAINST NF1-DEFICIENT GLIOMAS AND ENHANCE MAPK PATHWAY INHIBITION IN BRAF-V600E MUTANT GLIOMAS

Daniel Muldoon[1], Guisheng Zhao[1], Carly Batt[1], Mallika Singh[2], and Theodore Nicolaides[1]; [1]New York University Langone Health, New York, NY, USA, [2]Revolution Medicines, Inc., Redwood City, CA, USA

INTRODUCTION: Activation of the RAS-MAPK signaling cascade is common in pediatric gliomas. Based on the role of SHP2 in RAS pathway signaling, we hypothesized that NF1-deficient pediatric glioma models would respond to SHP2 inhibitor monotherapy whereas BRAF-V600E gliomas would not. However, we postulated that the latter would exhibit increased sensitivity to a BRAF inhibitor (BRAFi) in combination with SHP2i. Here we demonstrate that the SHP2 inhibitors SHP099 and RMC-4550 (SHP2i) show significant single-agent activity in vitro against NF1-deficient glioma cells and that the combination of RMC-4550 with BRAFi shows increased activity in BRAF-V600E glioma cells relative to the single-agents. METHODS: Using a panel of NF1 mutant/deficient and BRAF-V600E mutant glioma cell lines we examined effects on cell viability and protein expression levels of total and phosphorylated MEK, ERK, and AKT. RESULTS: LN229 and U87 NF1-deficient glioma cells are sensitive to SHP2i alone but not A375 cells (melanoma, BRAF-V600E). Additionally, we show that in multiple BRAF-V600E glioma cell lines BRAFi sensitivity increases when combined with a SHP2i. Immunoblots show decreased expression of pERK and pMEK in LN229 cells following SHP2i exposure, while A375 cells maintain MAPK pathway signaling. A sustained decrease in the expression of pERK after 24 hours was observed in BRAF-V600E glioma cells with BRAFi in combination with SHP2i, consistent with relief of feedback inhibition. In vivo studies using orthotopic xenograft models are underway. CONCLUSION: SHP2i shows preclinical activity in vitro against NF1-deficient pediatric glioma cell lines as a single-agent and against BRAF-V600E gliomas in combination with BRAFi.

MODL-19. DIPG HARBOUR ALTERATIONS TARGETABLE BY MEK INHIBITORS, WITH ACQUIRED RESISTANCE MECHANISMS OVERCOME BY COMBINATORIAL UP- OR DOWN-STREAM INHIBITION

Elisa Izquierdo[1], Diana Carvalho[1], Alan Mackay[1], Sara Temelso[1], Jessica KR Boult[1], Valeria Molinari[1], Mark Stubbs[1], Sarita Depani[2], Patricia O'Hare[2], Simon P Robinson[1], Michael Hubank[3], Darren Hargrave[2], and Chris Jones[1]; [1]The Institute of Cancer Research, London, United Kingdom, [2]Great Ormond Street Hospital, London, United Kingdom, [3]The Royal Marsden Hospital, London, United Kingdom

The survival of children with DIPG remains dismal, with new treatments desperately needed. In the era of precision medicine, targeted therapies represent an exciting treatment opportunity, yet resistance can rapidly emerge, playing an important role in treatment failure. In a prospective biopsy-stratified clinical trial (BIOMEDE), we combined detailed molecular profiling (methylation BeadArray, exome, RNAseq, phospho-proteomics) linked to drug screening in newly-established patient-derived models of DIPG *in vitro* and *in vivo*. We identified a high degree of *in vitro* sensitivity to the MEK inhibitor trametinib (GI50 16-50nM) in samples which harboured genetic alterations targeting the MAPK pathway, including the non-canonical *BRAF*_G469V mutation, and those affecting *PIK3R1*. Treatment of PDX models and the patient with trametinib at relapse, however, failed to elicit a significant response. We generated trametinib-resistant clones (62-188-fold, GI50 2.4–5.2µM) in the *BRAF*_G469V model through continuous drug exposure, and identified acquired mutations in *MEK1/2* (*MEK1*_K57N, *MEK1*_I141S and *MEK2*_I115N) with sustained pathway up-regulation. These cells showed the hallmarks of mesenchymal transition, with overexpression of key proteins involved in invasion/migration, such as collagen-family proteins, integrins, MMPs and AHNAK2, amongst others. Resistant clones were conversely sensitive to the upstream receptor tyrosine kinase inhibitor dasatinib (GI50 36-93nM), and combinations of trametinib with dasatinib and the downstream ERK inhibitor ulixertinib showed synergistic effects *in vitro*. These data highlight the MAPK pathway as a therapeutic target in DIPG, and show the importance of parallel resistance modelling and rational combinatorial treatments likely to be required for meaningful clinical translation.

MODL-20. A BIOBANK OF ~100 PATIENT-DERIVED MODELS REPRESENTING BIOLOGICAL HETEROGENEITY AND DISTINCT THERAPEUTIC DEPENDENCIES IN PAEDIATRIC HIGH GRADE GLIOMA AND DIPG

Diana Carvalho[1], Alan Mackay[1], Sara Temelso[1], Elisa Izquierdo[1], Elisabet Potente Fernandez[1], Rebecca Rogers[1], Jessica Boult[1], Janat Fazal Salom[1], Natalie Simon[1], Matthew Clarke[1], Valeria Molinari[1], Ketty Kessler[1], Anna Burford[1], Lynn Bjerke[1], Mariama Fofana[1], Michael Hubank[1,2], Jane Pears[3], Andrew Moore[4], Angel Montero Carcaboso[5], Lynley Marshall[2], Fernando Carceller[2],

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