from GCPs, maintains quiescent stem-like cells during the disease and contributes to tumor outgrowth at recurrence. We found that FG2-FGFR signaling causes increased growth and tissue invasion through the FGFR adaptor protein FGFR3. We developed a group of modulators targeting FGFR3. Thus, targeting FGFR3 by an FGFR1-selective modulator MRS2 further reduces FGFR3 signaling and could abrogate brain tumor growth and spread by repressing tumor-promoting functions that are induced by microenvironmental FG2. Using virtual screening combined with functional validation, we identified potent FGFR3-targeting 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 robustly block FGF2-induced migration and invasion in medulloblastoma-derived cells. Selected F2i further display excellent binding kinetics with a similar Kd as the natural ligand domain of FGFR and cause additive 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 assays. We validated 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., Cell Reports 23, 3798–3812.e8 (2018).

MODL.15. THE COMBINATION TREATMENT OF PARP INHIBITOR AND TMZ, OR DAG WILL BE PROMISING TREATMENT IN SF8628
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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 from O6-methylguanine DNA methyltransferase, which is normally expressed in 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 present study is to explore the way to inhibit the proliferation in 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 (BER) and nucleotide-excision repair (NER), combined TMZ with TMZ was considered to be effective to suppress the proliferation of SF8628. As expected, PARP inhibitor enhanced TMZ-induced cytotoxicity in SF8628. Dianhydrogalactitol (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 SF8628. 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
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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 of the high-grade gliomas (pHGGs) and one-third of ependymomas exhibit recurrence within 2 years of initial treatment. METHODS: By using integrated bioinformatic analysis 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 genes, and growth inhibition, 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 demonstrates 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 NFI-DEFICIENT GLIOMA AND ENHANCE MAPK PATHWAY INHIBITION IN BRAF-V600E MUTANT GLIOMA
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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 NFI-deficient pediatric glioma models would respond to SHP2 inhibitor monotherapy whereas BRAFVE600E glioma models would not. We found 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 NFI-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 NFI 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 NFI-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. Immunoblot shows decreased expression of pERK and pMEK in LN229 cells following SHP2i treatment 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 loops using orthotropic xenograft models and inhibitors are underway. CONCLUSION: SHP2i shows preclinical activity in vitro against NFI-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 Izquierdo1, Daytona Carvalho1, Alan Mackay2, Sara Temelsol2, Jessica KR Boulit, Valeria Molinari1, Mark Stubbs3, Sarita Depani3, Patricia O’Hare4, Simon P Batt4, Daniel Moore4, Darren Hargreave2, and Chris Jones1; 1The Institute of Cancer Research, London, United Kingdom, 2Great Ormond Street Hospital, London, United Kingdom, 3The 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 promise expanding an existing 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 (methyltion 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) and combinations of others. Resistant clones were conversely sensitive to the upstream receptor tyrosine kinase inhibitor dasatinib (GI50 36–93nM), and combinations of both. In the 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 2.4–5.2 nM) in the non-canonical BRAFV600E glioma cell lines we examined effects on cell viability and protein expression of pERK and pMEK in LN229 cells following SHP2i exposure, non-canonical MEK INHIBITION IN BRAF-V600E MUTANT GLIOMAS.

MODL.20. A BIOBANK OF ~100 PATIENT-DERIVED MODELS REPRESENTING BIOLOGICAL HETEROGENEITY AND DISTINCT THERAPEUTIC DEPENDENCIES IN PAEDIATRIC HIGH GRADE GLIOMA AND DIPG
Diana Czeczuga1, Alan Mackay2, Sara Temesol2, Elisa Izquierdo1, Elisabet Potente Fernandez1, Rebecca Rogers1, Jessica Boulit, Janat Fazal Salomi3, Natalie Simon1, Matthew Clarke1, Valeria Molinari1, Katy Kessler1, Anna Burford1, Lynn Bjerke1, Mariama Fofoana1, Michael Hubank1,2, Jane Pears1, Andrew Moore4, Angel Montero Carcaboso, Lynley Marshall, Fernando Carrascal1,2

The survival of children with DIPG remains dismal, with new treatments desperately needed. In the era of precision medicine, targeted therapies promise expanding an existing 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 (methyltion 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) and combinations of others. Resistant clones were conversely sensitive to the upstream receptor tyrosine kinase inhibitor dasatinib (GI50 36–93nM), and combinations of both. In the 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 2.4–5.2 nM) in the non-canonical BRAFV600E glioma cell lines we examined effects on cell viability and protein expression of pERK and pMEK in LN229 cells following SHP2i exposure, non-canonical MEK INHIBITION IN BRAF-V600E MUTANT GLIOMAS.
MOIDL-21. INTEGRATIVE APPROACHES IN FUNCTIONAL GENOMICS TO IDENTIFY GENETIC DEPENDENCIES IN PEDIATRIC BRAIN CANCER
Ciara van den Draper, Dhanaya Sooraj, Gabrielle Bradshaw, Claire Shi, Dusan 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 disparate clinical outcomes. The profound insight into 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 cancers are treatable with currently available monotherapeutic options, and many asymptomatically growing tumors are still treated palliatively. Ongoing experiments are aimed to determine how stable DM and CNV are useful tools to detect specific biological dependencies. Many have been established as characteristic of a particular tumor type or rare (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 lethality and responses to targeted inhibitors, including osteosarcoma (PARP) with ATRX, notch3 (G834R/V) with PPMD, and EZH2 (WT) with TET1 and CYC065 (CDK9) with MYCN-amplification. Comboscreening highlighted synergies in ACVR1-mutant DIPG between novel ALK2 inhibitors and ONC021 (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.

MOIDL-22. DEVELOPING A REAL-TIME PERSONALIZED DRUG TESTING PLATFORM FOR PEDIATRIC CNS CANCERS
Sandra Latsner1, Chiara Giancoli Cosentino2, Justyna M Przystal1, Susanne Dettwiler1, Elisabeth Jane Rushing1, Nicolas U Gerber1, Ana Guerrero Snick1, Rachna Prasad1, Michael Grotzer1, Niklaus Krähenbühl2, Sabine Müller3, and Javad Nazarzad1,2,4,1University Children’s Hospital Zurich, DRI, Oncology Department, Zurich, ZH, Switzerland, 2University Children’s Hospital Zurich, DRI, Oncology Department, Zurich, ZH, Switzerland, 4University Hospital Zurich, 4University of Zurich, Zurich, Switzerland, 5University of Queensland, Brisbane, Australia

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 established mouse PDX models. Here, we aimed to develop a 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 miRQON sequencing platform. Single and combination drug (n=7) screens were performed. Effective drugs were further evaluated in zebrablack 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 zebrablack. Tumor cells were engrafted in zebrablack 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 vivo and in vitro drug screens of tumor cells obtained at biopsy. This platform will provide the opportunity to establish personalized therapy for heterogeneous cancers including DMGs.

MOIDL-23. DNA METHYLATION AND COPY NUMBER VARIATION PROFILE FOR CHARACTERIZATION OF PEDIATRIC BRAIN TUMOR PRIMARY CELL LINES
Lucia Pedace1, Maria Vinci1, Simone Pizzai1, Guila Pericoli1, Giuseppe Abbatte2, Francesca D’Armio1,2, Ignazio Caruana1, Francesca Diomedi Camasselli1, Sabrina Ross1, Felice Giangaspero2, Elisabetta Ferretti2, Andrea Colt2, Marco Tartaglia3, Franco Locatelli3, Angela Mastronuzzi3, and Eulivia Miele4,1Bambino Gesù Children’s Hospital, Rome, Italy, 2University of Rome Sapienza, Rome, Italy

BACKGROUND: In vitro models of pediatric brain tumors (pBT) are instrumental for both understanding the oncogenic 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 Ependyomas), 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 were compared to assess 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 model or presence of serum in culture 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 the tested result, suggestions suggest 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.

MOIDL-24. AN ORGANOTYPIC CHUNK CULTURE TECHNIQUE TO STUDY DISEASE MECHANISM AND DEVELOP TARGETED THERAPEUTICS FOR PEDIATRIC ADAMANTINOMATOUS CRANIOPHARYNGIOMA
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BACKGROUND: Advances in the treatment of adamantinomatous craniopharyngioma (ACP) face challenges with translation to clinical reality 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