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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

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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

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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

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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

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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

collected tumor tissue from pediatric ACP was cut into volumes of approximately 3 mm^3 and rested over a semi-permeable insert placed in the wells of a 6-well plate. Specimens were cultured in (1) Control media, media containing (2) Tocilizumab, (3) Trametinib, and (4) combination of Tocilizumab and Trametinib, for 24 and 96 hours. Specimens were harvested for paraffin embedding, protein and gene expression assays. Supernatants were collected to assay secreted components. Paraffin embedded specimens were sectioned and stained for H&E, Pan-CK, Beta-Catenin, cleaved Caspase-3, Ki-67, and Phospho-ERK. RESULTS: H&E staining revealed characteristic histologic features of ACP with epithelial cells with palisading nuclei, wet keratin and ghost cells. Tumor sections were markedly positive for epithelial cell markers, Pan-CK and Beta-Catenin. Ki-67 and cleaved Caspase-3 were restricted to a small fraction of cells, indicating low index of proliferation and apoptosis under the culture conditions. The response to drug treatments shall be determined using gene expression assays and evaluation of the secreted components. CONCLUSION: The organotypic chunk culture technique appears to maintain the viability and integrity of ACP tumors for several days and may serve as an appropriate model for pre-clinical studies to develop targeted therapeutics for pediatric ACP.

MODL-25. REPLICATION REPAIR DEFICIENT MOUSE MODELS PROVIDE INSIGHT ON HYPERMUTANT BRAIN TUMOURS, MECHANISMS OF IMMUNE EVASION, AND COMBINATORIAL IMMUNOTHERAPY

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Replication repair deficiency (RRD) is the leading cause of hypermutant brain tumours in children. RRD is caused by defects in one of four mismatch repair (MMR) genes and mutations in *POLE* or *POLD1*. Such tumours are resistant to common therapeutic agents and animal models are needed to study RRD in vivo and test novel therapies like immune checkpoint inhibitors (ICIs). To model RRD brain tumours specifically, we engineered a *Pole* mutant mouse model harbouring the S459F mutation (*PoleS459F*). We combined *PoleS459F* mice with conditional *Msh2* knockout (*Msh2LoxP*) and *Nestin-cre* mice. All *Nestin-cre+Msh2LoxP/LoxPPoleS459F/+* mice rapidly succumbed to posterior fossa brain tumours between 8.6 and 12.4 weeks. Importantly, tumours exhibited hallmark "ultrahypermutation" (~350 mutations/Mb) and the corresponding signatures characteristic of human combined MMR and *POLE*-proofreading glioblastoma. Interestingly, *Nestin-cre+Msh2LoxP/LoxPPoleS459F/S459F* mice failed to establish normal cerebella, suggesting such mutational loads may not support normal brain development. Furthermore, *OLIG2-cre+Msh2LoxP/LoxPPoleS459F/+* mice failed to develop tumors. Tumors transplanted into syngeneic vs immunocompromised animals egrafted well orthotopically in the mouse hindbrain but significantly less efficiently when engrafted subcutaneously. Furthermore, immunocompromised and subcutaneous tumors revealed striking differences in mutational burden and clonal architecture, suggestive of nonautonomous immunoediting. Finally, anti-PD1 was sufficient to treat subcutaneously engrafted tumors in immunocompetent animals. This first mouse model of immunocompetent, hypermutant brain tumors can be used to uncover unique characteristics of RRD tumour evolution and allow for immune based therapeutic preclinical testing. Experiments to assess combinational ICIs and other therapeutic interventions in orthotopically transplanted tumors will also be presented.

MODL-26. CHILDREN'S BRAIN TUMOR NETWORK: ACCELERATING RESEARCH THROUGH COLLABORATION AND OPEN-SCIENCE

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The Children's Brain Tumor Network (formerly known as Children's Brain Tumor Consortium- CBTTC) is a global organization pioneering a model of open-science medical research to improve treatment and discover cures. Started in 2011, our objective was to utilize a regulatory, agreement, and governance architecture to remove existing research barriers that slowed down the pace of research and collaboration. Our network now includes 17 institutions working together to empower research. As of December 2019, over 3,600 subjects have been enrolled resulting in collection of over 45,000 specimens. Clinical data collection is longitudinal and includes medical history, diagnosis, treatment, pathology slides and reports, radiology imaging and reports, and outcome data. The tissue is collected flash-frozen, in freezing media, and fresh for the generation of pre-clinical models including cell lines. Blood is collected from the subject, with blood or saliva collected from the parents for germline comparison. Additionally, the Children's Brain Tumor Network- Pediatric Brain Tumor Atlas has generated 952 WGS and RNAseq, 221 proteomics, with annotated clinical data. All of this data, both generated raw and processed data, has been made available broadly to the scientific community via cloud-based platforms, including the Gabriella Miller Kids First Data Resource Portal, Cavatica, and PedCbioportal. As of January 2020, we have 45 approved biospecimen requests and 80 genomic/molecular data requests. In summary, the Children's Brain Tumor Network's goal is to accelerate the pace of discovery by providing resources and expanding the network of scientists working towards a cure.

MODL-27. MEK INHIBITION WITH TRAMETINIB SLOWS PROGRESSION OF MEDULLOBLASTOMA AND ATYPICAL TERATOID RHABDOID TUMOR IN ORTHOTOPIC XENOGRAFT MURINE MODEL

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BACKGROUND: Combination of surgery, chemotherapy, autologous transplantation, irradiation constitutes treatment of CNS embryonal-cell tumors (Medulloblastoma-MBL, atypical teratoid rhabdoid tumor-AT/RT). Targeted agents to improve survival and decrease side effects are necessary. We hypothesize that inhibiting MAPK pathway in MBL and AT/RT may be beneficial. METHODS: IHC(pERK) was performed on clinical tumors. *Trametinib*(MEK inhibitor) was tested on MBL(UW228, D283, DAOY); AT/RT(CHLA06, BT12) cell-lines. Luminescent cell-viability assay was done(72 hrs) and with crystal violet assay(10 days). Orthotopic, xenografts of MBL and AT/RT were made in *NOD-Scid gamma* mice. Mice were given *Trametinib* daily by gavage for 6 weeks(0.6mg/kg b.w). Western blot was performed on protein from cell lines and tumor xenografts incubated with *Trametinib*. H&E staining was done on murine tumors. RESULTS: AT/RT(48%) and MBL(57%); Anaplastic(50%), Desmoplastic(40%), Classic(38%); Group 4(66%), Group 3(20%), SHH(55%), WNT(0%) showed presence of pERK(clinical samples). In-vitro, *Trametinib* completely abrogated the phosphorylation of ERK at 125nM in AT/RT and 50nM in MBL. The IC50 after 10 days exposure was 10nM for AT/RT and 35nM for MBL. *Trametinib* treated mice showed delay in tumor growth and significant survival advantage in both AT/RT (p=0.00336) and MBL (p=0.0069). Murine tumors showed decreased proliferation (H&E). CONCLUSION: *Trametinib* decreased cell proliferation, increased survival in our murine model in both MBL and AT/RT. Pre-clinical results indicate benefits in subgroups of AT/RT and MBL with active MAPK pathway.

MODL-28. IMMUNE PRIMING WITH INTERFERON-Γ COMBINED WITH EPIGENETIC MODULATION IN PEDIATRIC BRAIN TUMORS

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