MODL-09. FEASIBILITY OF ACUTE SLICE CULTURE-SINGLE CELL SEQUENCING DRUG SCREENING AS A TOOL TO SELECT THERAPY FOR CHILDREN WITH RELAPSED BRAIN TUMORS

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Children with relapsed brain tumors are less responsive to treatment. These children often receive therapies without having any robust predictive method of potential benefit. Acute slice culturing(ASC) is a methodology permitting freshly operated tumor to undergo a culturing process preserving the tumor's micro-environment. With the current study, we investigated the feasibility of obtaining therapeutically meaningful data in a timely manner (3–5 days), performing direct drug testing and single cell sequencing using ASC. Previously, we have combined *ex vivo* slices of intact, patient-derived Glioblastoma tissue with single-cell RNA-seq for small-scale drug screening and assessment of patient and cell type-specific drug responses. We generated slices from preclinical mouse glioma models and surgical specimens from adult Glioblastoma patients, as well as from children with relapsed Ependymomas, Medulloblastomas, and Gliomas. We demonstrated that these acute slices preserved both the tumor heterogeneity and tumor microenvironment observed in single-cell RNA-seq of cells directly isolated from tumor tissue. Testing drug responses, we then treated tissue slices from the Glioblastoma mouse models and different patients with multiple drugs and combinations. This technique allowed us to identify drug-induced transcriptional responses in specific subpopulations of tumor cells, patient-specific drug sensitivities, and drug effects conserved in both mouse and human tumors. Preliminary data suggests that we can apply this procedure within 5–7 days and provide real-time drug screening/single cell sequencing ASC results to Recurrent/ Progressive pediatric Low-Grade Gliomas, High Grade Gliomas, Ependymomas and Medulloblastomas.

MODL-11. COMPARISON OF HUMAN & MURINE PA/PXA CHARACTERISTICS

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Pediatric low-grade gliomas (pLGGs) are the most common brain tumors in children. Despite recent advances in the molecular characterization of this heterogeneous set of tumors, the separation of specific tumor types is still not fully established. Pilocytic astrocytoma (PA; WHO grade I) and pleomorphic xanthoastrocytoma (PXA; WHO grade II) are two pLGG types that can be difficult to distinguish based on histology alone. Even though their clinical course is different, they are often grouped as 'pLGG' in clinical trials (and therefore treated similarly). Based on a cohort of 89 human pediatric tumor samples, we show that PAs and PXAs have clearly distinct methylation and transcriptome profiles. The difference in gene expression is mainly caused by cell cycle- and development-associated genes, suggesting a key difference in the regulatory circuits involved in tumor growth. In addition to BRAF V600E, we found *NTRK* fusions and a previously unknown *EGFR:BRAF* fusion as mutually exclusive driving events in PXAs. Both tumor types show marked signs of immune cell infiltration, but with significant qualitative differences, which might represent therapeutic vulnerabilities. To pave the way for further research on PA and PXA, we developed corresponding mouse models using the virus-based RCAS system, which allows introduction of an oncogenic driver into immunocompetent mice for molecular and preclinical research. The murine tumors do not only histologically resemble their human counterparts but also show a similar growth behavior. Expression analysis revealed that the murine PXAs have a stronger gene signature of proliferation and immune cell infiltration compared to PAs.

MODL-12. DEVELOPMENT OF A NOVEL IMMUNOCOMPETENT MOUSE MODEL FOR DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse intrinsic pontine glioma (DIPG) is a devastating brain tumor affecting young children. Immunotherapies hold promise however the lack of immunocompetent models recreating a faithful tumor microenvironment (TME) remains a challenge for development of targeted immunotherapeutics. We propose to generate an immunocompetent DIPG mouse model through induced overexpression of interleukin 13 receptor alpha 2 (IL13Rα2), a tumor-associated antigen overexpressed by glioma cells. A model with an intact TME permits comprehensive preclinical assessment of IL13Rα2-targeted immunotherapeutics. Our novel model uses the retroviral avian leucosis and sarcoma virus (RCAS) for *in vivo* gene delivery leading to IL13Rα2 expression in proliferating progenitor cells. Transfected cells expressing IL13Rα2 and PDGFB, a ligand for platelet derived growth factor receptor, alongside induced p53 loss via the Cre-Lox system are injected in the fourth ventricle in postnatal pups. We validated the expression of PDGFB and IL13Rα2 transgenes *in vitro* and *in vivo* and will characterize the TME through evaluation of the peripheral and tumor immunologic compartments using immunohistochemistry and flow cytometry. We confirmed expression of transgenes via flow cytometry and western blotting. Comparison of survival dynamics in mice inoculated with PDGFB alone with PDGFB+IL13Rα2 demonstrated that co-expression of IL13Rα2 did not significantly affect mice survival compared to the PDGFB model. At time of application, we initiated experiments to characterize the TME. Preliminary data demonstrate establishment of tumors within and adjacent to the brainstem and expression of target transgenes. Preclinical findings in a model recapitulating the TME may provide better insight into outcomes upon translation to clinical application.

MODL-13. GENETICALLY ENGINEERED PIG MODEL OF RHABDOID TUMOR PREDISPOSITION SYNDROME-1

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Atypical teratoid/rhabdoid tumor (AT/RT) is the most common malignant CNS tumor of children below 6 months of age. The majority of AT/RT demonstrate genomic alterations in the *SMARCB1* gene. There are two major hurdles in the development of safe and effective treatments for AT/RT: first, the mouse models do not fully recapitulate the disease seen in patients and their predictivity of clinical efficacy is still unproven. Second, due to a small patient population, the ability to recruit enough patients for clinical trials is challenging. Genetic studies have demonstrated that germline deletion of *SMARCB1* exons 4 and 5 predisposes to AT/RT at an early age. Comparison of human, swine, and mouse *SMARCB1* genes show similarities in gene and protein structure, with 100% amino acid identity between swine and human SMARCB1 isoforms. Thus, we hypothesized that germline deletion of exons 4 and 5 will predispose heterozygote swine to AT/RT development. *SMARCB1*$^{+/-}$ founder pigs are obtained using a CRISPR/Cas9 mediated gene-editing of conventional crossbred swine embryos, followed by embryo transfer into female swine surrogates. They are evaluated for clinical criteria used to diagnose AT/RT and by MRI at 6, 12, and 24 months of age, followed by histopathology and molecular analysis of the tumors as they are detected. Generating a large animal model of AT/RT would represent a breakthrough in the field from a genomic, pathophysiologic, preclinical and therapeutic perspective.

MODL-14. SMALL MOLECULE TARGETING OF ONCOGENIC FGF2-FGFR SIGNALING IN BRAIN TUMORS

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FGF2, the ligand of FGF receptors (FGFRs), is expressed in the developing and adult brain. FGF2-FGFR1 signaling causes the induction and maintenance of cancer stem cells through ERK-dependent up-regulation of ZEB1 and Olig2 in glioblastoma. In SHH medulloblastoma, Olig2 triggers tumor initiation

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

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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 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

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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 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

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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 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

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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

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