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