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

Balesh Gampeta1, Sanaa Zennou2, Mengjiao Zhao3, James Garvin4, Chankeet Sethi5, Eileen Stark1, Peter Sims1, Peter Canoll1, and Stergios Zacharoulis1; 1New York Presbyterian/Columbia, New York, NY, USA, 2Memorial Sloan Kettering, New York, NY, USA, 3Columbia University Medical Center, New York, NY, USA

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 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 tumors. In ASC drug screening application, we observed similar cell cycle arrest in 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 brain 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

Alexander C. Sommerkamp1,2, Penubo Sun1,3, Annika K. Weifers4,5, Britta Ismer1,2, Kathrin Schramm1, Andrea Wittmann1,2, Jan Gronych1, Andrey Korschunov3, Andreas von Deimling4, Natalie Jäger1,5, Steven M. Poser1,2, David T. W. Jäger1, John Hopp Children’s Cancer Center Heidelberg (KiTZ), Heidelberg, Germany, 2Pediatric Glioma Research Group, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany, 3Division of Pediatric Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany, 4Department of Neuropathology, University Hospital Heidelberg, Heidelberg, Germany, 5Clinical Cooperation Unit Neuropathology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany, 6Department of Neuropathology, University Hospital Heidelberg, Heidelberg, Germany, 7Clinical Cooperation Unit Neuropathology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany, 8Division of Molecular Genetics, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

Pediatric low-grade gliomas (pLGGs) are the most common brain tumor in children. Despite recent advances in the understanding 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’ types that can be difficult to distinguish based on histology alone. Despite their clinical course, the molecular characterization of these tumors is largely unknown. In this study, we compared 10 PA and 10 PXA cases from different institutions and divided them into molecular subtypes using single-cell sequencing. We identified two distinct molecular subtypes within both PA and PXA tumors. The PA subtype was characterized by the presence of BRAF V600E mutation, whereas the PXA subtype was characterized by mutations in IDH1 and/or IDH2. In conclusion, this study provides a comprehensive molecular characterization of PA and PXA tumors, which will aid in the development of targeted therapies for these tumors.

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

Magge Sebali1, Markella Zannikou1, Katarzyna Pirch1, Liliana Ilur1, Oren Becher1, Irina Balyanskaia1, Ann and Robert H. Lurie Children’s Hospital, Chicago, IL, USA, 2Northwestern University Department of Neurological Surgery, Chicago, IL, USA

Diffuse pontine gliomas (DIPG) are 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 immunotherapies. We propose to generate an immunocompetent DIPG mouse model through induced expression 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 retroviral vector system, expressing PDGF and IL13Rα2 transgenes in vitro and in vivo will charac- terize 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 preclinical 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

Brian NJ1,2, C. Dustin Rubinstein3, Jennifer J. Meudt3, Jaclyn A. Biegel3, Alexander R. Judkins1, Brent P. Lehman1, Jamie L. Reichert1, Jerome Vitte4, Dhanansayan Shanmuganayagam5, and Marco Giovanni6; 1Department of Head and Neck Surgery, David Geffen School of Medicine at UCLA and Jonsson Comprehensive Cancer Center (JCCC), University of California Los Angeles, Los Angeles, CA, USA, 2Department of Pediatrics, Division of Pediatric Hematology/Oncology, David Geffen School of Medicine at UCLA, USA, 3Biology Department, University of Wisconsin-Madison, Madison, WI, USA, 4Biomedical & Genomic Research Group, University of Wisconsin-Madison, Madison, WI, USA, 5Department of Pathology and Laboratory Medicine, Children’s Hospital of Los Angeles, and Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

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 tumors have aggressive genomic alterations, the molecular classification of which is still not fully established. Studies have revealed that the genomic alterations in AT/RT are characterized by the loss of SMARCB1 expression, which is a tumor suppressor gene located on chromosome 22. SMARCB1 loss is typically associated with the deletion of 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 crossed 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 ONGENIC FGF2- FGR SIGNALING IN BRAIN TUMORS

Karthiga Sanithana Kumar1, Cyrrl Brunner2, Matthias Schuster3, Oliver Zerbel1, Michael Grotzer3, and Martin Baumeister3; 1University Children’s Hospital Zurich, Zurich, Switzerland, 2ETH Zurich, Zurich, Switzerland, 3University of Zurich, Zurich, Switzerland

FGF2, the ligand of FGFR 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 FG2-FGFR signaling causes increased growth and tissue invasion through the FGFR adaptor protein FRS2. In SHP2 +/− and G3 medulloblastoma F, F2i efficiently block FGFR-induced migration and invasion in medulloblastoma-derived cells. Selected F2i's display excellent binding kinetics with a similar Kd as the natural ligand domain of FGFR and cause steatotic 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 and 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. Santha Kumar et al., Cell Reports 32, 3798–3812.e9 (2018).

MODL.15. THE COMBINATION TREATMENT OF PARP INHIBITOR AND TMZ, OR DAG WILL BE PROMISING TREATMENT IN SF8628 Shigco Ohta, and Yuschi 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 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 this 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 (BER), the dephosphorylated DNA polymerase (DNA-Pol) combined 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 Meh-Lui Lian1; Tsung-Han Hsieh2, and Tai-Tong Wong3; 1Department of Neurosurgery, Mackay Memorial Hospital, Taipei, Taiwan, 2Joint Biobank, Office of Human Research, Taipei Medical University, Taipei, Taiwan, 3Department 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. Approaches in modern neuro-oncological therapies are aimed at improving neoadjuvant chemotherapy and delivering 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 integrative 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 expression of cell cycle-related and DNA repair proteins, 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 nude mice model. Abemaciclib demonstrated significant 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 Mudalige1, Guangsheng Zhou2, Callidate Mathil D’singh3, and Theodore Nicolasides4; 1New York University Langone Health, New York, NY, USA; 2Revolution 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 mutant gliomas would not. We showed 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 lines and that the combination of RMC-4550 with BRAFi shows increased activity in BRAF-V600E glioma cell lines 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 lines are sensitive to SHP2i alone but not A375 cells (melanoma, BRAF-V600E). Additionally, we showed 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 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 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 Izquierdo1,1 Diana Carvalho2, Alan Mackay3, Sara Temelso2, Jessica KR Boul1,1 Valeria Molinari2,1 Mark Stubbs2,3, Sarita Deparnay1,2, Patricio1,2, Michael Hammon2,3, Darren Hargrave2, 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 represent an exciting treatment opportunity, yet resistance can rapidly emerge, playing an important role in treatment failure. In a prospective phase 1/2 clinical trial (BIOMEDE), we combined deterministically 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 PIK3CA. Treatment of DIPX 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_K27N, 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 modeling 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 Carvalho1, Alan Mackay2, Sara Temelso3, Elisa Izquierdo4, Elisabet Potente Fernandez, Rebecca Rogers, Jessica Boul1, Janat Fazal Salomi, Natalie Simon1, Matthew Clarke1, Valeria Molinari1, Katy Kessler, Anna Burford, Lynn Bjerke, Mariama Fofana1, Michael Hubank1,3, Jane Pears, Andrew Moore4, Angel Montero Carcaboso1, Lynley Marshall, Fernando Carceller1