of Pittsburgh, Pittsburgh, PA, USA, [3]Baylor College of Medicine, Houston, TX, USA

Pediatric glioblastoma (pGBM) are incurable brain tumors with overall poor prognosis and response to treatments due to molecular and epigenetic heterogeneity. In particular, the MYCN subtype of pGBM are a highly aggressive form of GBM with a dismal median survival of only 14 months. Furthermore, this subtype is enriched with loss of the tumor suppressor genes TP53 and PTEN, leading to aberrantly active PI3K-AKT signaling pathway and DNA-checkpoint abnormalities. Here, we report the generation of a novel syngeneic mouse model that recapitulates the features of the MYCN subtype of pGBM. We isolated Sox2-Cre neural stem cells from C57BL/6 mice and transduced inverted retroviral-cassettes of the murine *Mycn* oncogene simultaneously with shRNA targeting tumor suppressor genes *p53* and *Pten*. Retroviral-cassettes are flanked by tandem *LoxP* sites arranged so that Cre recombinase expression inverts the cassettes in frame allowing for MYCN protein expression and loss of the P53/PTEN proteins. Transgene activation is accompanied with selectable cell surface markers and fluorescent tags enabling for fluorescent activated cell sorting (FACS) of the desired cell populations. Neural stem cells with MYCN protein expression and concurrent silencing of P53 and PTEN protein (NPP cells) result in significantly increased proliferation and activation of PI3K-AKT pathway as compared to control neural stem cells and have. Injection of NPP cells into the forebrain of immune competent C57BL/6 mice result in the formation of invasive high-grade gliomas with a lethal phenotype at ~50 days post injection. Using several next generation brain penetrant small molecule inhibitors of the PI3K-AKT pathway, we show inhibition of tumorigenesis *in vitro*. Moreover, we have identified several novel mechanisms of PI3KAKT treatment resistance and are currently identifying therapies that may overcome this resistance through RNA seq analysis. In summary, well defined genetic drivers of GBM can lead to informed mouse model generation to test promising therapies.

### TMOD-19. INDIVIDUAL SPECIFIC HUMAN GUT MICROBE COMMUNITIES INFLUENCE RESPONSE TO IMMUNOTHERAPY IN A HUMANIZED MICROBIOME MOUSE MODEL OF GLIOMA

Kory Dees, Hyunmin Koo, J Fraser Humphreys, Joseph Hakim, David Crossman, Michael Crowley, Louis Nabors, Etty Benveniste, Casey Morrow, and Braden McFarland; University of Alabama at Birmingham, Birmingham, AL, USA

Although immunotherapy works well in glioblastoma (GBM) pre-clinical mouse models, the therapy has not demonstrated efficacy in GBM patients. Since recent studies have linked the gut microbial composition to the success with immunotherapy for other cancers, we utilized a novel humanized microbiome (HuM) model in order to study the response to immunotherapy in a pre-clinical mouse model of GBM. We used five healthy human donors for fecal transplantation of gnotobiotic mice since it is now recognized that microbe strain level differences render individual humans with a unique microbial community composition. After the transplanted microbiomes stabilized, the mice were bred to generate 5 independent humanized mouse lines (humanized microbiome HuM1-HuM5). Analysis of shotgun metagenomic sequencing data from fecal samples revealed a unique microbiome composition with significant differences in diversity and microbial composition among HuM1-HuM5 lines. We next analyzed the growth of intracranial glioma cells in the HuM lines. All HuM mouse lines were susceptible to GBM transplantation, and exhibited similar median survival ranging from 19-26 days. Interestingly, we found that HuM lines responded differently to the immune checkpoint inhibitor anti-PD-1. Specifically, we demonstrate that HuM1, HuM4, and HuM5 mice are non-responders to anti-PD-1 resulting in the death of the mice from the intracranial tumors, while HuM2 and HuM3 mice are responsive to anti-PD-1 and displayed significantly increased survival compared to isotype controls. Bray-Curtis cluster analysis of the 5 HuM gut microbial communities revealed that HuM2 and HuM3 were closely related. Detailed taxonomic comparison analysis at the top 5 across all HuM mouse lines revealed that *Bacteroides cellulosilyticus* was commonly found between HuM2 and HuM3 with high abundances. The results of our study establish the utility of humanized microbiome mice as avatars to delineate features of the host interaction with gut microbe communities needed for effective immunotherapy against GBM.

### TMOD-20. A SWINE MODEL OF GLIOBLASTOMA INDUCED BY SOMATIC GENE MODIFICATION

Barbara Tschida, Dylan Duerre, Mandy Taisto, and Adrienne Watson; Recombinetics, Eagan, MN, USA

Glioblastoma (GBM) is the most common and malignant primary brain tumor. Novel therapeutic development for GBM is needed since the standard of care universally fails to cure patients and the five-year survival rate remains below 10%. GBM therapeutic development is hampered by the lack of relevant large animal models for preclinical studies. To mitigate this problem, we are developing a model of GBM in outbred, immune-proficient swine which have comparable brain size and anatomy to humans. We developed methods for introducing genome engineering tools to minipig brain cells *in vivo* by direct injection of gene delivery reagents to the lateral ventricle. Using this technique, we have delivered a combination of expression vectors for oncogenes and targeted nucleases to disrupt tumor suppressor genes commonly altered in human GBM to alter six major human GBM-associated signaling pathways in a cohort of minipigs (Ras, Pi3k, p53, Rb/E2F, Pdgf, and the alternative lengthening of telomeres (ALT) pathways). These minipigs are being monitored for tumorigenesis using a secreted reporter, detectable through a simple luminescence-based blood test. Resulting tumors will be examined molecularly to detect the pathway-associated alterations in tumor tissue and determine the resemblance to human GBM. We hypothesize that this somatic cell gene-modification platform we have developed in the minipig will facilitate the efficient production of brain tumors that histologically and genetically resemble human GBM. It will allow the production of tumors that are genetically heterogeneous, of specified molecular subclasses, containing therapeutic targets of interest, and in the context of genetic backgrounds of interest. This minipig model of GBM will be applied towards preclinical therapeutic studies, imaging studies using human clinical grade equipment, and surgical technique development, to improve clinical trial success rates and patient outcomes. Funding for this study is provided by the National Institutes of Health through SBIR grant # 1R43CA235837-01A1.

### TMOD-22. AKALUC BIOLUMINESCENCE OFFERS SUPERIOR SENSITIVITY TO TRACK IN VIVO GBM EXPANSION

Dominique Bozec, Anirudh Sattiraju, Alexandros Bouras, Joe Gerald Jesu Raj, Daniel Rivera, Yong Huang, Chrystian Junqueira Alves, Rut Tejero, Hongyan Zou, Constantinos Hadjipanayis, and Roland H. Friedel; Icahn School of Medicine at Mount Sinai, New York, NY, USA

Longitudinal tracking of tumor growth using non-invasive bioluminescence imaging (BLI) is a key approach for in vivo cancer studies, but the current method of firefly luciferase (Fluc) BLI has quantitative limitations, as it is only suited for detection of tumors of considerable sizes at advanced stage, typically in the order of $>10^5$ cells. Recently, Akaluciferase (Akaluc) has been developed as an alternative BLI system that offers higher signal strength and better light penetration of tissue due to its red-shifted emission. Here, we established Akaluc BLI as a new sensitive method for in vivo tracking of glioblastoma (GBM) expansion in intracranial transplant models. In multiple GBM cell lines, including the frequently used U87MG and GL261, as well as patient-derived glioma stem cells (GSC), we demonstrate that Akaluc-expressing GBM cells produced more than 50-times brighter BLI signals in vitro and up to 100-fold higher signal intensities in vivo over Fluc-expressing counterparts. The higher sensitivity of Akaluc BLI permits early in vivo detection of intracranial GBM transplants starting as early as 4 hours after implantation and with as little as 5,000 transplanted GSC. We also reveal a prolonged engraftment period in intracranial GSC transplants before wide dissemination into host brain parenchyma. Akaluc BLI is also advantageous for longitudinal monitoring of therapeutic effects of chemoradiation for GBM and detection of early phase of tumor relapse. Thus, Akaluc BLI offers an important addition to the tool box for cancer research. SIGNIFICANCE: The high sensitivity of Akaluc bioluminescence is a significant improvement for the non-invasive tracking of tumors in preclinical cancer studies, including detection of small incipient tumors and micro-metastasis.

### TMOD-23. A NOVEL BRAF V600E LOW-GRADE GLIOMA MOUSE MODEL HIGHLIGHTS EXOMIC AND TUMOR IMMUNE ALTERATIONS AND DIFFERING THERAPEUTIC RESPONSES IN LOW- AND HIGH-GRADE GLIOMAS

Anne Marie Barrette[1], Lasse Meyer[2], Yoko Hirata[1], Stefan Grossauer[3], Edbert Lu[4], Whitney Tamaki[4], Claudia Petritsch[5], and Wei Wang[4]; [1]Stanford University, Stanford, CA, USA, [2]DKFZ, Heidelberg, Germany, [3]Hospital Frankfurt Hoescht, Frankfurt, Germany, [4]UCSF, San Francisco, CA, USA, [5]Department of Neurosurgery, Stanford University, Palo Alto, CA, USA

Pediatric low-grade glioma (pLGG), the most common brain cancer in children, is difficult to treat especially at recurrence. The BRAF V600E mutation is the second most common mutation in pLGG, and in a high-risk group for progression is associated with deletion of the tumor suppressor CDKN2A. A better understanding of the factors contributing to progression, in particular the role of the immune infiltrate is needed, but studies have been hindered by the lack of low-grade glioma mouse models. We utilized transgenic mice with a *c*re-*a*ctivatable (CA) allele of BRAF V600E to generate endogenous models for low-grade gliomas. We found that BRAF V600E expression cooperates with hemizygous CDKN2A deletion to induce low-grade gliomas, with tumors forming at a greater latency than by

Downloaded from https://academic.oup.com/neuro-oncology/article/22/Supplement_2/ii232/5961314 by guest on 24 April 2024