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The development of effective treatments for malignant brain tumors has been hampered by the lack of robust preclinical models that faithfully capture the high inter- and intra-tumor heterogeneity of the human disease. Conventional cell lines lose the most common genetic abnormalities of glioblastoma (GBM), while primary cultured cells do not account for the influences of the microenvironment and the blood brain barrier on tumor biology and drug efficacy. These systems are also under strong selection pressure divergent from that *in vivo*, leading to reduced heterogeneity between cultured tumor cells and an overall shift away from *in vivo* characteristics. Here we describe a biobank of direct-from-patient derived orthotopic xenografts (GliomaPDOX) that preserve the diverse genetic and transcriptional landscapes found in human GBMs. A paired comparison between matched patients, GliomaPDOX, and short-term primary cell cultures revealed transcriptional changes associated with altered nutrient availability and non-tumor cell interactions, emphasizing the impact of the tumor microenvironment on *in vivo* gene expression. Further, GliomaPDOX models preserved signatures of differentiated brain cell types recapitulating the intratumor heterogeneity of non-stem and stem-like cells found in patient tumors. These results are in contrast to those found in gliomapshere culture systems, where signatures of differentiated brain cells are abolished. Collectively these data show that GliomaPDOX is a model system that preserves defining molecular and anatomical characteristics of GBM and is well-suited for translational research investigations.

TMOD-04. TARGETING A GLIOMA SPECIFIC lncRNA IN A HUMAN BRAIN ORGANOID TUMOR MODEL

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Long non-coding RNAs (lncRNAs) are transcripts >200 nucleotides long with essentially no protein coding potential. While certain lncRNAs play key roles in cancer and therefore represent an important class of therapeutic targets, very few lncRNAs have been studied in glioma. Here, we show that knockdown of a primate-specific lncRNA – referred to as *GTT1* – selectively inhibits the growth of both adult and pediatric glioma but does not adversely affect the viability of normal human glia and neurons. In a recent genome-scale CRISPR interference (CRISPRi)-based screen, we identified *GTT1* as one of 65 lncRNA genes that modify the growth of U87 glioblastoma cells. To prioritize lncRNAs for further study, we next performed a CRISPRi-based screen to identify lncRNA targets that also sensitize tumor cells to radiation. Both CRISPRi-mediated and antisense oligonucleotide (ASO)-mediated knockdown of *GTT1* inhibited the propagation of U87 cells in culture, and this effect synergized with radiation treatment. Furthermore, *GTT1* knockdown inhibited the growth of patient-derived glioma cells including adult glioblastoma and pediatric diffuse intrinsic pontine glioma (DIPG) in culture. Although *GTT1* is expressed in normal human brain, knockdown of this lncRNA in normal human astrocytes, cortical neurons and fetal forebrain tissues did not reduce cell viability. As *GTT1* is primate-specific, knockdown of this lncRNA in mouse xenograft models would not fully assess potential adverse effects. We therefore developed a human brain organoid model comprised of mature astrocytes and functional neurons. Patient-derived glioma cells engrafted into these organoids, and tumors grew in an infiltrative manner. *GTT1* knockdown in this brain organoid model strongly impaired tumor growth but did not reduce organoid viability. These studies identify *GTT1* as a glioma-specific therapeutic target and illustrate how this human brain organoid tumor model can be used to rapidly evaluate the tumor-specificity of novel therapeutics.

TMOD-05. GLIOMA-261 LUCIFERASE-EXPRESSING CELL LINE STIMULATES AN IMMUNOGENIC RESPONSE SIGNATURE IN AN IMMUNOCOMPETENT MURINE MODEL

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Immunocompetent murine models of glioma, such as GL261, are essential to assess immune therapy efficacy. The GL261 cell line expressing firefly luciferase (GL261RFluc), has been used to enable non-invasive tumor monitoring in preclinical studies. However, it is unclear if the GL261 and GL261RFluc cells are equivalent, particularly when applied to tumor immunology. C57BL/6 mice (n=20 per group, repeated once) underwent stereotaxic, intracranial implantation with GL261 or GL261RFLuc cells at 5x10^4cells/5uL and were assessed for survival. GL261 and GL261RFluc cell lines were assessed by CFSE proliferation assay. TGF-Beta2 ELISA and proteome profiler immunoassay were performed on equivalent cell culture lysates for cytokine analysis. Mice were implanted with GL261 or GL261RFluc cell lines, sacrificed at day 10, and brains were either disassociated, FACS stained, and sorted for immune cell surface antigens (n=4 each group) or formalin-fixed and paraffin embedded for immunohistochemistry (IHC, n=6 each group). Median survival for GL261 implanted mice was 20.9 ± 1.3 days with all animals progressing to a moribund state, while median survival was not reached for animals implanted with GL261RFluc cells (P=0.001). Quantitative IHC analyses of brains implanted with GL261RFluc cells showed significant increases in CD4 and CD8 cells but decrease in FoxP3 positive cells. Proliferation assays were equivalent. TGF-Beta2 ELISA showed significantly elevated levels in the GL261RFLuc cells. Proteomic profiler results demonstrated differential cytokine expression greater than 2-fold for over 25% of cytokines evaluated. The detected increases in chemoattractants CCL2 and CCL5 were particularly pronounced in GL261RFLuc cells. FACS analysis showed a trend of increased PD-L1 positive cells in brains implanted with GL261 cells, but this did not reach significance. GL261RFluc cells create an inflammatory immune microenvironment when implanted intracranially in C57BL/6 mice compared to GL261 cells. These findings suggest that investigators should avoid GL261RFluc cells when evaluating immune therapeutics in a C57BL/6 background.

TMOD-06. HIGH INCIDENCE OF TUMORS AFTER TREATMENT WITH A DNA-ALKYLATING AGENT IN MOUSE STRAINS COMMONLY USED IN PRE-CLINICAL STUDIES

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Glioblastoma (GBM) is commonly treated with the DNA-alkylating agent temozolomide (TMZ). Silencing O6-methylguanine-DNA methyltransferase (MGMT) by promoter hypermethylation predicts response to TMZ. Oral TMZ administration also affects tissues with low MGMT expression, such as bone marrow, resulting in high incidence of leukopenia and secondary leukemia in humans. Here we assessed the anti-tumor effect of TMZ in a panel of orthotopic GBM patient-derived xenografts (GBM-PDXs), and looked for evidence of secondary malignancy in the GBM-PDX lines with extended survival post-TMZ treatment, and also examined TMZ-treated naïve mice from two immunocompromised strains. First, a panel of 12 orthotopic GBM-PDX in athymic nude mice (NCRNU-F) were treated with 2-cycles of TMZ (40mg/kg/day for 5 days in a 21-day cycle) or with vehicle control gavage (n=10/treatment group). Treatment response was measured by log-rank test comparison of Kaplan-Meier survival curves. The untreated controls and TMZ-resistant PDXs succumbed to brain tumor burden immediately after treatment completion. TMZ-responsive GBM-PDX lines and GBM-naïve nude mice developed respiratory dysfunction 3–5 months after TMZ treatment. Histopathological analysis of lung tissue at autopsy revealed multiple foci of papillary adenocarcinoma (negative for human markers) in both lungs, at an incidence of 95% at 280 days in TMZ-treated nude mice, with no evidence of tumors in other organs. GBM-naïve untreated nude mice in the control group did not develop tumors after 1 year. TMZ treatment of severe combined immunodeficiency (SCID) mice resulted in 77% tumor incidence in the thymus and/or lungs in a GBM-PDX line, 3–4 months post-treatment, with a similar incidence for GBM-naïve treated mice, and no tumors observed in control mice after 9 months. These results present additional evidence of the tumorigenic potential of TMZ, particularly in the background of immunodeficiency. The development of lung/thymus tumors in TMZ-responsive GBM-PDX lines is an undesirable confounding factor not previously reported.

TMOD-07. LOCALIZATION OF ERLOTONIB RELATIVE TO MRI-BASED TUMOR EXTENT IN PDX GLIOBLASTOMA MODEL: TOWARDS A MATHEMATICAL MODEL FOR THE INTERFACE BETWEEN MRI AND DRUG DISTRIBUTION

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Clinical neuro-oncology relies on the hyperintensity of gadolinium (Gd) contrast agent on magnetic resonance imaging (MRI) in tumor regions to confirm that the blood-brain barrier (BBB) is locally compromised. While the extent of Gd hyperintensity may indicate that systemically administered drug is being distributed to the tumor regions, little is known about how a drug is distributed and how it may relate to the Gd hyperintensity. Add-

itionally, glioblastomas (GBMs) are diffusely invading neoplasms with a significant fraction of the overall tumor cells spread peripheral to the Gd abnormality, which raises uncertainty as to how or if the rest of the diffuse tumor is affected by drug. Given the gap in understanding drug delivery to the brain, we propose a quantitative approach to model drug delivery in GBM based on MRI and matrix-assisted laser desorption/ionization mass spectroscopy imaging (MALDI). T2-weighted (T2) and T1-weighted with Gd contrast (T1Gd) MRI images were acquired for an animal with the GBM12 orthotopic GBM patient derived xenograft (PDX) line dosed with 100mg/kg erlotinib. A T1Gd region of interest (ROI) captured the Gd-associated hyperintensity. MALDI was performed and aligned with MRI images. A Drug ROI was created to represent the increased intensity of erlotinib on MALDI images. Since the Drug ROI encompassed the T1Gd ROI, we subtracted the two ROIs to create a 'Drug No T1Gd' ROI, which represented the drug region beyond the edge of the T1Gd ROI. A 'Brain ROI' was created to represent the region of the brain outside of the drug's spread. The MALDI intensities for the three ROIs were all significantly different ($p<0.05$), with the T1Gd ROI having the highest mean, followed by the Drug No T1Gd and the Brain ROIs. By developing a quantitative understanding of drug distribution, we can make more robust predictions regarding treatment efficacy in the clinical setting.

TMOD-08. GROWTH IMPAIRMENT UNDER CONDITIONS FAVORING MITOCHONDRIAL OXIDATIVE METABOLISM IN A YEAST MODEL OF CANCER-ASSOCIATED ISOCITRATE DEHYDROGENASE MUTATION

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The use of the budding yeast *Saccharomyces cerevisiae* as a model system to study cancer allows for faster, more efficient elucidation of various molecular mechanisms, including mutation rate by fluctuation analysis, cell cycle analysis by flow cytometry, metabolism via growth rate analysis, and functional genomics via genomic array screening. The vast majority of low grade gliomas (LGGs) carry somatic mutations in isocitrate dehydrogenase 1 *(IDH1*) and 2 (*IDH2*) genes. *IDH1* and *IDH2* catalyze the oxidative decarboxylation of isocitrate to a-ketoglutarate (a-KG) in an $NADP^+$ dependent manner. A point mutation (R132H in IDH1 and R172H in IDH2) confers the neomorphic ability for the enzyme reduce a-KG to D-2-hydroxyglutarate (D2-HG). In *S. cerevisae*, the $NADP^+$ dependent isocitrate dehydrogenases are encoded by three different genes, *IDP1, IDP2* and *IDP3*. We have successfully generated a yeast model that carries the analogous mutation in the yeast IDP1 gene ($IDP1^{R148H}$). The allele was inserted at the HO locus, which does not alter the endogenous *IDP1* gene. In this way, the resulting strain carries both a wild-type and mutant allele of *IDP*, more closely mimicking the metabolic state of glioma cells. We have validated this insertion by PCR, sequencing, and tetrad analysis. The production of the mutant $IDP1^{R148H}$ protein was detected by Western blot. The $IDP1^{R148H}$ strain shows normal growth on glucose and galactose-containing solid media, but reduced growth on glycerol-containing solid media compared to parental or $IDP1^{WT}$ strains. Impaired growth of yeast when glycerol is the sole carbon source suggests a defect in mitochondrial oxidative metabolism. This observation is consistent with a previous yeast $IDP1^{R148H}$ model which showed extensive mitochondrial DNA loss and respiration defects. Taken together, we have developed a model of IDH-mutant LGGs in *S. cerevisiae* that can be further utilized to study molecular mechanisms underlying tumorigenesis of LGGs.

TMOD-09. TARGETING THE PI3K-mTOR PATHWAY AND ELUCIDATING MECHANISMS OF RESISTANCE IN A NOVEL AND RELEVANT ANIMAL MODEL OF GLIOBLASTOMA

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Pediatric glioblastoma (pGBM) and adult glioblastoma (aGBM), henceforth collectively referenced as GBM are incurable brain tumors with variable prognosis and response to treatments due to the intermolecular heterogeneity. In particular, the GBM MYCN subtype is a highly aggressive genetic group in both pediatrics and adults where patients have 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 PI3K-AKT pathway activation and DNA-checkpoint abnormalities. Here, we report the generation of a novel syngeneic GBM mouse model of the MYCN subtype. We isolated and transduced C57BL/6 Sox2-CRE neural stem cells (NSCs) with inverted retroviral-cassettes of the murine Mycn oncogene and shRNA targeting tumor suppressor genes p53 and Pten. The 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 and PTEN proteins. Transgene activation is accompanied with selectable cell surface markers and fluorescent tags enabling for fluorescent activated cell sorting (FACS) of desired cell populations. MYCN protein expression with concomitant silencing of P53 and PTEN protein leads to increased proliferation and formation of invasive high-grade gliomas when implanted into the frontal cortex of immune competent C57BL/6 mice and NOD-SCID mice. Using several next generation brain penetrant small molecule inhibitors of the PI3K-AKT pathway, we show tumor regression *in vivo*. Moreover, we have identified several novel mechanisms of PI3K-AKT treatment resistance and are currently identifying therapies that may overcome this resistance. In summary, well defined genetic drivers of GBM can lead to informed mouse model generation to test promising therapies.

TMOD-10. METABOLIC AND BLOOD-BRAIN BARRIER MARKERS FOR FLUORESCENCE-GUIDED SURGERY: SYSTEMATIC HIGH-RESOLUTION MICROSCOPY INVESTIGATION IN HUMAN RELEVANT EXPERIMENTAL GLIOMAS

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BACKGROUND. Fluorescence guidance with 5-aminolevulinic acid(5-ALA) increases resection extent and may benefit surgical outcomes in high-grade gliomas. We investigated if a combination of various fluorescence guidance techniques can provide further advantage. We systematically assessed and compared the fluorescent patterns of 5-ALA-induced protoporphyrin IX (PpIX), fluorescein sodium(FNa) and indocyanine green(ICG) to identify GL261 gliomas in mice and RFP-U251 gliomas in rats. METHODS. 5-ALA(5mg), FNa(5 mg/kg) and ICG(20mg/kg) were administered perioperatively. Fluorescence patterns were recorded with operative microscope, laser scanning confocal microscope, and confocal laser endomicroscope. Fluorescence was assessed quantitatively as a surface area of fluorescent positive tissue and qualitatively(false/true, positive/negative) as compared to HE-stained histology. RESULTS. FNa highlighted a larger surface area($18.0mm^2$) than white light($16.9mm^2$,$p=0.016$) or PpIX ($16.0mm^2$,$p=0.035$). Both 5-ALA and FNa showed inhomogeneous staining patterns: multiple areas of equal staining, when PpIX was present and FNa was not, and vice versa. ICG was visible in 8/31 tumor samples, all immediately after injection. ICG did not reveal clear tumor margins, but stained non-tumor tissue and nearby vasculature. FNa signal was stronger (tumor to background ratio (TBR)1.93 ± 0.56) compared to 5-ALA(1.52 ± 0.31;p CONCLUSION. ICG highlighted tumor only within the first few minutes and stained mostly hypervascularized areas. Confocal and surgical imaging revealed inhomogeneous tumor border staining with PpIX/FNa. Simultaneous administration of 5-ALA and FNA may provide additional benefit. Neither ICG, 5-ALA or FNa worked perfectly, emphasizing the need for more specific markers for fluorescence-guided brain tumor resection.

TMOD-11. IMAGING BASED INVASION METRIC PREDICTIVE OF RESPONSE TO ABT414 IN ORTHOTOPIC EGFRviii AMPLIFIED PATIENT DERIVED XENOGRAFTS

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BACKGROUND: Failed trials involving targeted therapies face a daunting task of understanding whether the root cause was inadequate targeting, resistance, or insufficient delivery to the tumor. Previous work with a biomathematical model has shown the prognostic value of an imaging based invasion metric, D/ρ (mm^2/yr), which is linearly correlated with the extent of tumor burden beyond the imaging abnormality. As this extent likely impacts the definition of sufficient drug delivery, we investigated whether this metric is able to predict response to ABT-414, an antibody drug conjugate targeting EGFR. METHODS: *Preclinical experiments:* After initial screening *in vitro* and in flank, the efficacy of ABT-414 was evaluated for three patient derived cell lines (PDXs), GBM6, GBM12, and GBM39, implanted orthotopically. All three showed strong response to ABT-414 in the previous experiments. In the orthotopic setting, GBM39 was very sensitive to therapy (> 155 days benefit), GBM12 was moderately sensitive (15–30 days benefit), and GBM6