

165 (g165). Two sgRNAs, g82 and g165, created an 83bp deletion in the genomic sequence that can lead to the production of a non-functional PD-L1 protein. A homology-directed repair template (HDR) containing an in-frame stop codon was designed to enhance PD-L1 knockout specificity and efficiency. Both g82 and g165 were cloned into the CRISPR/Cas9 plasmid, and was co-transfected with the added HDR template. T7E1, qRT-PCR and western blot analysis determined that the dual sgRNA CRISPR/Cas9 system knocked out both endogenous (80%) and exogenous (64%) PD-L1 in U87 cells and PD-L1 overexpression U87 cells, respectively. Deletion of PD-L1 reduced U87 migration and proliferation, while PD-L1 overexpression promoted tumor growth and tumor-associated macrophage polarization. Together, deletion of both membrane and cytoplasmic PD-L1 altered the PD-L1-associated immunosuppressive environment and prevented tumor progression and migration. Thus, a dual sgRNA CRISPR/Cas9 gene-editing system is a promising avenue for anti-GBM immunotherapy.

#### NGMA-3. USE OF MULTI-OMICS DATA TO INITIALIZE AND VALIDATE A CAUSAL MODEL OF GLIOBLASTOMA STEM CELL SIGNALING

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Glioblastomas and glioblastoma stem cells are heterogeneous with respect to mutations, gene expression, and response to drugs. To make predictive responses of individual GBM stem cell lines to drugs, we have constructed a causal model of glioblastoma stem cell signaling. The core model was built starting from pathways identified from TCGA mutation data with the addition of the Jak/STAT, Hedgehog, and Notch pathways. Elements and relations between them were validated and extended using the PCNet interaction database and the INDRA database which includes machine read extractions from the biomedical literature. The result is a high confidence executable model consisting of 209 element and 370 rules of interaction between the elements. Stochastic simulations of the model provide dynamic (quantile) changes in time and responses to perturbations. The output provides activity of individual nodes as well as a cellular output state of cell cycle progression, apoptosis, or differentiation. To simulate the responses of individual cell lines to kinase inhibitors, the model was initialized using DNA sequencing data, RNA-seq, and reverse phase protein array (RPPA) data from each cell line. Comparing the results of the simulations to the drug responses of 11 different kinase targets, the model was 88% accurate in predicting effects on growth and survival. The model was further tested by comparing the effects of Mek inhibition of each of the cell lines in model to the results observed in the RPPA data which overlap by 127 elements. In this case, there was 62% concordance between the model and data when binned into quintiles. Discrepancies between the model predictions and the data are being investigated to determine whether the model logic or extent needs to be revised to improve the model. This modeling approach is a step toward developing algorithms for personalized therapeutics for GBM based on multi-omics data.

#### NGMA-4. CREATION OF A MADR BRAIN TUMOR SINGLE-CELL ATLAS FOR EXAMINATION OF INTER-/INTRATUMOR HETEROGENEITY AND THE RESULTS OF GENETIC PERTURBATIONS IN A DIVERSE ARRAY OF BRAIN TUMOR SUBTYPES

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We have recently established mosaic analysis by dual recombinase-mediated cassette exchange (MADR), which permits stable labeling of mutant cells expressing transgenic elements from precisely defined chromosomal loci. MADR provides a toolkit of elements for combinatorial labeling, inducible/reversible transgene manipulation, VCre recombinase expression, and genetic manipulation of human cells. Further, we have demonstrated the versatility of MADR by creating glioma models with mixed, reporter-identified zygosity or with “personalized” driver mutations from pediatric glioma. For example, introducing H3f3a (aka H3.3) mutation variants with MADR regulates the spatiotemporal profile of glioma, and single-cell RNA and ATAC sequencing analysis demonstrates a recapitulation of developmental hierarchy seen in K27M mutant human glioma. Moreover, we have generated novel models of H3.3 WT glioma, H3.3 G34R glioma, and supratentorial ependymoma using patient-derived oncofusion transgenes. These models display a high degree of phenotypic fidelity and we now compare these models on a single-cell level with our previous models, mouse single-cell RNA glioma datasets from other studies, and human tumor cell transcriptomes. Our multi-omics approach includes integration of ChIP-seq/Cut&Tag datasets, single-cell ATAC, and single-cell Cut&Tag datasets.

Moreover, we have engineered a novel methodology for inducible gain-and loss-of function perturbation studies *in vivo*. Using ETS transcription factors as a proof-of-principle, we overlay these genetic perturbations on the glioma atlas to examine the gene networks altered by precise molecular manipulations.

We hope that these combined approaches will enable researchers to discover disease mechanisms with increased resolution and test therapeutics in credentialed pre-clinical disease models.

#### NGMA-5. AN *IN VIVO* FUNCTIONAL GENOMICS SCREEN TO IDENTIFY NOVEL DRIVERS OF LUNG-TO-BRAIN METASTASIS

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**INTRODUCTION:** Brain metastases, the most common tumors of the central nervous system, occur in approximately 20% of primary adult cancers. In particular, 40% of patients with non-small cell lung cancer develop brain metastasis. As systemic therapies for the treatment of non-small cell lung cancer become increasingly effective at controlling primary disease, patients are ironically succumbing to their brain metastases. This highlights a large unmet need to develop novel targeted therapies for the treatment of lung-to-brain metastases (LBM). We hypothesize that an *in vivo* functional genomic screen can identify novel genes that drive LBM. **METHODS:** To do this, we developed a patient-derived xenograft (PDX) mouse model of LBM using patient lung cancer cell lines. This PDX model of LBM enables the use of fluorescent and bioluminescent *in vivo* imaging to track the progression of lung tumor and brain metastases. **RESULTS:** We have performed an *in vivo* genome-wide CRISPR activation screening to identify novel drivers of LBM. We will derive candidate genes through mouse brain and lung tissue sequencing after mice reach endpoint. **EXPECTED AREA OF FINDINGS:** This platform will lead to potential therapeutic targets to prevent the formation of LBM and prolong the survival of patients with non-small cell lung cancer. **CONCLUSION:** To the best of our knowledge, this is the first genome-wide *in vivo* CRISPR activation screen searching for drivers of LBM using a PDX animal model. This study can provide a framework to gain a deeper understanding of the regulators of BM formation which will hopefully lead to targeted drug discovery.

#### NGMA-6. QUANTITATIVE MGMT PROMOTER METHYLATION INDEX INDICATES NON-LINEAR, PROGNOSTIC EFFECT IN GLIOBLASTOMA

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**BACKGROUND:** Epigenetic inhibition of the O6-methylguanine-DNA-methyltransferase (MGMT) gene has emerged as a clinically relevant prognostic marker in glioblastoma (GBM). Methylation of the MGMT promoter has been shown to increase chemotherapy efficacy. While traditionally reported as a binary marker, recent methodological advancements have led to quantitative methods of measuring methylation, allowing for clearer insights into methylation's functional relationship with survival. **METHODS:** A CLIA assay and bisulfite sequencing was utilized to develop a quantitative, 17-point MGMT promoter methylation index derived from the number of methylated CpG sites. Retrospective review of 242 newly diagnosed GBM patients was performed in order to discern how risk for mortality transforms as promoter methylation increases. Non-linearities were captured by fitting splines to Cox proportional hazard models, plotting smoothed residuals, and creating survival plots. Covariates included age, KPS, IDH1 mutation, and extent of resection. **RESULTS:** Median follow-up time and progression free survival were 15.9 and 9 months, respectively. 176 subjects experienced death. A one-unit increase in CpG methylation resulted in a 4% reduction in hazard (95% CI 0.93–0.99, P<0.005). Moreover, GBM patients with low levels of methylation (1–6 CpG islands) fared markedly worse (HR=1.62, 95% CI 1.03–2.54, P<0.036) than individuals who were unmethylated (reference group). Subjects with medium levels of methylation (7–12 sites) had the greatest reduction in hazard (HR=0.48, 95% CI 0.29–0.80, P<0.004), followed by individuals in the highest methylation tertile (HR=0.62, 95% CI 0.40–0.97, P<0.035). **CONCLUSION:** The extent of MGMT methylation shares a non-linear relationship with survival, suggesting conformation of the marker's protective effect. This finding challenges the current understanding of MGMT and underlines the clinical importance of determining its prognostic utility. Potential limitations include censoring, sample size, and extraneous mutations. Future research is warranted to examine whether the location of CpG site methylation contributes to a reduction in mortality hazard.