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**Personalization of T cell production for cellular immunotherapy**

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**OBJECTIVES/GOALS:** Utilize polymer-based fiber scaffolds and machine learning methods applied to patient biomarker data to enhance and personalize T cell expansion and production for T cell therapy in chronic lymphocytic leukemia. **METHODS/STUDY POPULATION:** Scaffolds are 1) generated from a co-polymer blend of PDMS and PCL with controlled fiber diameters and pore size, 2) coated with activating antibodies to CD3 and CD28, and 3) used to stimulate T cells from both healthy donors and CLL patients. CLL patients have pre-annotated mutation burdens and clinical biomarkers. T cell populations will be analyzed for expansion markers and phenotypes before, during, and after expansion. Cell functionality will be measured by cytokine secretion, cell cycle analysis, and fold expansion, with respect to platform parameters, and analyzed with inputs of disease markers and exhaustion profile of isolated T cells using regression and random forest classifiers. **RESULTS/ANTICIPATED RESULTS:** We previously showed that engineering the mechanical rigidity of activating substrates can enhance and rescue T cell expansion from exhausted populations. Now we aim to study a broader range of compositions and geometry of scaffolds with respect to capacity to expand CLL T cells. Preliminary data with fiber diameters ranging from 300 nm to 6 um confirm the effect of geometry in modulating expansion. A biorepository of T cells from 80 CLL patients have been isolated concurrently. Anticipated results include correlating exhaustion profile of T cells with clinical biomarkers and identifying markers associated with expansion on panel of platform parameters. **DISCUSSION/SIGNIFICANCE OF IMPACT:** T cell therapy has shown particular promise in treating blood cancers, yet significant percentage of T cells isolated from patients undergoing treatments are unresponsive to activation. A powerful tool is to predict if and how patient T cells can be robustly expanded on a personalized approach.

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**Quantifying pH buffering capacity and kinetics of tumor and healthy tissue to understand and exploit differences in biology**

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**OBJECTIVES/GOALS:** The purpose of this work is to investigate natural buffering capacity of liver tissue and tumors, to understand and exploit differences for therapy. Using this work, we will determine the concentrations of reagents (acids or bases) used in ablation treatment to optimize treatment by increasing tumor toxicity and minimizing healthy tissue toxicity. **METHODS/STUDY POPULATION:** For this preliminary study, two methods will be used: benchtop pH experiments ex vivo and non-invasive imaging using acidoCEST MRI in vivo. For ex vivo, two types of tissues will be tested: non-cancerous liver and tumor tissue from HepG2 inoculated mice (n = 10). After mice are euthanized, pH will be measured in tissue homogenates at baseline and then the homogenates will be placed in either acidic (acetic acid) or basic (sodium hydroxide) solutions with varied concentrations (0.5–10M) and time recorded until pH returns to baseline. **RESULTS:** Imaging, Mia PaCA-2 flank model mice (n = 10) will be imaged with acidoCEST MRI to quantify pH at baseline. Mice will then be injected intratumorally with (up to 100 µL of) acid or base at increasing concentrations and imaged to quantify pH changes in the tumor. **RESULTS/ANTICIPATED RESULTS:** For this study, buffering capacity is defined as the concentration threshold for which tissue can buffer pH back to within normal range. Non-cancerous tissue is likely to buffer a wider range of concentrations compared to tumor tissue. From the benchtop experiment, comparison of time-to-buffer will be made for each concentration of acid/base for the two tissue types. AcidoCEST MRI will provide in vivo buffering capacity and potentially demonstrate tumor heterogeneity of buffering capacity. For both experiments, a pH vs. concentration curve for the two tissue types will allow for comparison of ex vivo to in vivo experiments, which will differentiate contributions of local tissue buffering capacity from the full body’s natural bicarbonate buffer system that depends on respiration and blood flow. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The pH of the body must be maintained within a narrow range. With cancer, impairment in regulation of tumor metabolism causes acidosis, lowering extracellular pH in tumors. It remains unclear if pH plays a role in local recurrence or tumor toxicity. This work will determine if acidoCEST MRI can measure deliberate alteration of pH and how this change affects biology.
were significantly altered. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results reveal novel genetic targets that underlie plasticity of fear-memory circuitry via their contribution of NMDAR-mediated fear consolidation and can inform future strategies for targeting fear-related disorders like PTSD. CONFLICT OF INTEREST DESCRIPTION: Anantha Shekhar and Yvonne Lai are co-founders of Anagin, Inc., which is developing some of the related molecules for the treatment of PTSD.

Sirtuin 3 activation as a potential renoprotective therapy in a mouse model of Alport syndrome

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OBJECTIVES/GOALS: Sirtuin 3 (Sirt3), a mitochondrial NAD⁺-dependent deacetylase, is decreased in diverse models of kidney disease, and Sirt3 activation prevents disease progression in many of those models. We are investigating if pharmacological activation of Sirt3 ameliorates kidney disease in a mouse model of Alport syndrome. METHODS/STUDY POPULATION: Alport syndrome is a hereditary orphan disease arising from a defect in the collagen IV syndrome. METHODS/STUDY POPULATION: Alport syndrome is a hereditary orphan disease arising from a defect in the collagen IV syndrome. METHODS/STUDY POPULATION: Alport syndrome is a hereditary orphan disease arising from a defect in the collagen IV syndrome.

RESULTS/ANTICIPATED RESULTS: Col4a3 transgenic mice on both the 129X1/SvJ and C57BL/6J backgrounds. RESULTS/ANTICIPATED RESULTS: Col4a3 transgenic mice on both the 129X1/SvJ and C57BL/6J backgrounds. RESULTS/ANTICIPATED RESULTS: Col4a3 transgenic mice on both the 129X1/SvJ and C57BL/6J backgrounds.

Sirt3 expression is decreased in the renal cortices of Col4a3-/- mice at the mRNA (P < 0.0001, male; P < 0.0001, female) and protein levels (P < 0.0001, male; P < 0.0001, female) compared to Col4a3+/+ controls. All experiments had 5–9 mice per group. Results of the prevention study with nicotinamide riboside, a NAD⁺ precursor, are in process of designing a panel of TCR variants with the goal of identifying candidates that improve interaction of TCR with MHC class I. Preliminary studies using our unpublished structure of TIL1383I we identified non-mutated residues that contribute to the binding orientation of TIL1383I to the tyrosinase peptide presented by HLA-A2. Structural analysis of TIL1383I revealed key residues, particularly beta-chain residues E97, G101, L102, and K105, that are responsible for engaging the tyrosinase peptide bound to HLA-A2. The crystal structure of TIL1383I in complex with tyrosinase-HLA-A2 also highlighted its uncharacteristic binding geometry and we therefore hypothesize that this binding orientation is associated with the observed CD8 co-receptor independence of TIL1383I. Indeed, functional analysis with TIL1383I transduced CD8-positive and CD8-negative T cells, transduced T cells expressing a truncated CD8 lacking the intracellular LCK signaling domain, and tyrosinase peptide variants presented by HLA-A2 mutants outline this co-receptor independence. Combined with our interrogation of tyrosinase peptide cross-reactivity via a peptide positional scanning library approach, structure-guided design resulted in the identification of TIL1383I variants with improved binding affinities to the tyrosinase.