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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 bio-markers. T cell populations will be analyzed for exhaustion markers and phenotypes before, during, and after expansion. Cell function-ality 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 res-cue 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 bio-markers 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 natu-ral 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 treat-ment to optimize treatment by increasing tumor toxicity and minimiz-ing healthy tissue toxicity. METHODS/STUDY POPULATION: For this preliminary study, two methods will be used: benchtop pH experi-ments 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. For in vivo 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 concen-trations 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, lower-ing 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.

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Role of PSD95 and nNOS interaction in gene regulation following fear conditioning and implications for molecular mechanisms underlying PTSD

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OBJECTIVES/GOALS: Normal fear learning produces avoidance behavior that promotes survival, but excessive and persistent fear after trauma can lead to development of phobias and post-traumatic stress disorder (PTSD). Our goal is to understand the mechanism and identify novel genetic targets underlying fear responses. METHODS/STUDY POPULATION: Involvement of the amygdala in fear acquisition is well established and requires activation of N-methyl-D-aspartic acid recep-tors (NMDARs). At a cellular level, NMDAR activation leads to pro-duction of nitric oxide (NO) by a process mediated by interaction between postsynaptic density protein 95 (PSD95) and neuronal nitric oxide synthase (nNOS). To elucidate mechanisms underlying the role of the PSD95-nNOS-NO pathway in conditioned fear, here we use rodent behavioral paradigms, pharmacological treatment with a small molecular PSD95-nNOS inhibitor, co-immunoprecipitation, Western blotting, and RNA-sequencing. RESULTS/ANTICIPATED RESULTS: We show that fear conditioning enhances the PSD95-nNOS interaction and that the small-molecule ZL006 inhibits this interaction. Treatment with ZL006 also attenuates rodent cued-fear consolidation and pre-vents fear-mediated shifts in glutamatergic receptor and current den-sities in the basolateral amygdala (BLA). With RNA-sequencing, expression of 516 genes was altered in the BLA following fear expres-sion; of these genes, 83 were restored by systemic ZL006 treatment. Network data and gene ontology enrichment analysis with Ingenuity Pathway Analysis and DAVID software found that cell-cell interaction, cognition-related pathways, and insulin-like growth factor binding