

detectable viremia. IL-15SA Deep-Priming increased CTL expansion and persistence in peripheral blood which correlated with improved CD4⁺T-cell preservation. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Here we demonstrate the first *in vivo* analysis of IL-15SA Deep-Priming of HIV-Specific CTLs. These data suggest that Deep-Priming of patient T-cells can enhance *in vivo* function and persistence, leading to improved viral suppression; a significant advancement in the field of HIV cure research. **CONFLICT OF INTEREST DESCRIPTION:** Austin Boesch, Thomas Andresen, and Douglas Jones are employees of Torque. Darrell Irvine is a co-founder of Torque and Chairman of Torque's Scientific Advisory Board.

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Defining the role of non-canonical PIK3CA mutations in head and neck squamous cell carcinoma

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OBJECTIVES/GOALS: To characterize the oncogenic potential of HNSCC cell lines harboring 17 non-canonical *PIK3CA* mutations. **METHODS/STUDY POPULATION:** Non-canonical *PIK3CA* mutant constructs generated via site-directed mutagenesis are subcloned into doxycycline-inducible vector pLVX-Puro. Serum-dependent HNSCC cell line (PCI-52-SD1) is then stably transfected with vectors and undergo doxycycline-induction. Cell survival is determined by depriving cells of fetal bovine serum for 72 hours and quantifying remaining cells with 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Cell proliferation and migration is evaluated with colony formation assays and transwell assays respectively. **RESULTS/ANTICIPATED RESULTS:** To date, the survival behavior of eight non-canonical mutants was assessed. Three mutants – Q75E, V71I, and E970K – exhibited 18.7-26.7% greater survival rate relative to cells transfected with wild-type. Five mutants – R519G, Y606C, W328S, C905S, and M1040I – demonstrated survival rates that differed only by -4.3% to +6.6% relative to wild-type. We hypothesize the three activating mutants that exhibited increased survival will also demonstrate increased cell proliferation and migratory behavior whereas the three neutral mutants will not differ from control. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Ongoing HNSCC PI3K inhibitor trials could be more effective if all *PIK3CA* hyperactivation mutations are known. Identifying non-canonical mutation effects could result in greater efficacy if drugs are restricted only to those with activating mutations. **CONFLICT OF INTEREST DESCRIPTION:** JRG and DEJ are co-inventors of cyclic STAT3 decoy and have financial interests in STAT3 Therapeutics, Inc. STAT3 Therapeutics, Inc. holds an interest in a cyclic STAT3 decoy oligonucleotide. The remaining authors declare no conflicts.

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Development of a Mouse Model to study interactions between parental history of alcohol use and early life adversity on behavioral and neurobiological development of offspring

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OBJECTIVES/GOALS: Individuals with a family history of alcoholism (FH+) are more likely to develop an alcohol use disorder than

those with no such history. Early life adversity has a high coincidence with FH+ making pathogenic studies difficult in clinical studies. Here, we developed a mouse model to study pathogenic mechanisms underlying these risk factors. **METHODS/STUDY POPULATION:** Male and female C57BL6/J mice were exposed to increasing concentrations of ethanol (3-21%) or water for 15 days prior to breeding. Ethanol was not present during gestation. Offspring were either removed from the home cage and isolated for 3 hours or left undisturbed from postnatal days 1-21. Beginning on PND 56 offspring mice were assessed for clinically relevant behavioral disruptions in social behavior, cognitive working memory, locomotor activity, anxiety-like phenotypes, ethanol preference and binge drinking behavior. In a separate experiment, brains of Cx3cr2^{+/GFP}xCcr2^{+/RFP} mice from ELA or control conditions were collected every 7 days after birth for assessment of neuroinflammation and central immune cell morphology and density. **RESULTS/ANTICIPATED RESULTS:** Mice with a family history of ethanol exposure and ELA are predicted to exhibit behavioral changes (impaired working memory, reduced social behavior, increased anxiety-like behaviors, increased ethanol consumption) to a greater extent than mice with a family history of ethanol exposure or ELA alone. We expect markers of neuroinflammation (cytokine expression, immune cell activation) to predict the behavioral changes in these mice. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Alcohol consumption and stressful life events are known environmental precipitants to neuroinflammation, which in turns may predispose individuals to anti-social and risky behavior. A mouse model of these early postnatal conditions will allow basic scientists to unravel the biological underpinnings of the behaviors driven by these factors.

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Dissecting the role of microenvironment heterogeneity on metastatic tumor cell phenotype at an engineered metastatic niche

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OBJECTIVES/GOALS: Breast cancer metastases are stochastic and difficult to detect. Therapy is often ineffective due to phenotypic changes of tumor cells at these sites. We engineered a synthetic metastatic niche to study the role of phenotypic transitions in the microenvironment on tumor cell phenotype. **METHODS/STUDY POPULATION:** The engineered metastatic niche is composed of a porous polycaprolactone scaffold implanted subcutaneously in Balb/c mice. The mice received an orthotopic inoculation of 4T1 cells (murine triple negative breast cancer) in the fourth right mammary fat pad and the disease was allowed to progress for 7-21 days (pre-metastatic to overt metastatic disease). The scaffolds and lungs (native metastatic site) were explanted and analyzed by single cell RNA-seq via Drop-seq. Cell phenotypes were identified and tracked over time with the Seurat and Monocle3 pipelines. Assessment of the impact of these cell populations on tumor cell phenotype was conducted through Transwell co-cultures. **RESULTS/ANTICIPATED RESULTS:** Healthy scaffolds are primarily composed of macrophages, dendritic cells, and fibroblasts – consistent with a foreign body response. Despite differences in the lung and scaffold prior to tumor inoculation, both tissues were marked by >5-fold increase in neutrophils/MDSCs. Additionally, 79% of genes at the scaffold that significantly changed over time were also identified in the lung, indicating key similarities in niche maturation. However, many immune cells at the scaffold had distinct phenotypes, with pro-