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.

4535

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.

4026

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-

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inflammatory/cytotoxic characteristics. These changes clearly impacted tumor cell phenotype, as cells from the scaffold increased tumor cell migration and apoptosis *in vitro*. DISCUSSION/SIGNIFICANCE OF IMPACT: Early phenotypic changes at the engineered metastatic niche can identify signs of metastasis prior to colonization of tumor cells. Furthermore, dynamics of immune and stromal cells change throughout niche maturation, influencing tumor cell phenotype and may suggest targeted therapies. CONFLICT OF INTEREST DESCRIPTION: Lonnie Shea, Jacqueline Jeruss, and Grace Bushnell are named inventors on patents or patent applications.

4579

Distinct clinical and immunological responses to αPD-1, kαPD-L1 and αPD-L2 immunotherapy in B16 melanoma in aged versus young hosts includes T-cell stem cell effects and PD-L2 expression differences

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OBJECTIVES/GOALS: Aging is the biggest risk factor for cancer, yet little is known about cancer immunotherapy effects. Here we investigate melanoma response to αPD-1, αPD-L1 and αPD-L2 in young vs. aged hosts. We look at different immune outcomes as possible mechanism. METHODS/STUDY POPULATION: We tested αPD-1 (100 μg/mouse), αPD-L1 (100 μg/mouse) or αPD-L2 (200 μg/mouse) in aged (18-24 months) and young (3-8 months) mice challenged orthotopically with B16. Tumors and draining lymph nodes (TDLN) were analyzed by flow. Bone marrow-derived DC were generated with GM-CSF. RESULTS/ANTICIPATED RESULTS: We reported that αPD-1 treats young and aged with B16 and αPD-L1 only treats young. aPD-L2 treated B16 in aged but, remarkably, not young, the first anti-cancer single agent immunotherapy exhibiting this property. Efficacy in young (aPD-1, aPD-L1) and aged (aPD-1, aPD-L2) correlated with increased T cell stem cells (TCSC) and total tumor-infiltrating lymphocytes (TIL), but TCSC differed by age and treatment (*e.g.*, distinct CCR2, CXCR5, CXCR3, PD-1 and TIM- expression). Aged expressed significantly more T-cell PD-1 and up to 40-fold more PD-L2 versus young in myeloid and NK cells, and TCSC. Bone marrow-derived DC experiments suggest aged DC are destined for high PD-L2 versus young. DISCUSSION/SIGNIFICANCE OF IMPACT: Treatment differences in aged vs. young could depend on immune checkpoint or TCSC differences. We are now identifying mechanisms for increased PD-L2 and contributions to aPD-L2 efficacy in aged, and testing TCSC effects. Our work can improve cancer immunotherapy in aged hosts and further provide important insights even in young hosts.

4071

Dynamic Control of Tumor Vessels Augments Antitumor Responses

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OBJECTIVES/GOALS: Our overall objective is to develop a directly observable and reproducible method of enhanced blood flow through tumor vessels (i.e. dynamic control) at the time of systemic treatment delivery. Our central hypothesis is that the dynamic control of tumor vessels will improve (1) systemic drug delivery and (2) effector cell trafficking to target tumor. METHODS/STUDY POPULATION: B16 melanoma cells were inoculated into C57BL/6 (B6) mice (male and female) in both regional (hind leg) and systemic (flank) models. Dynamic control consisted of an IV saline bolus (500 ul) and phenylephrine (10 ug). Tumor vessel response was observed in real-time through window chambers using intravital microscopy (IVM). Dynamic control was combined with melphalan (20 mg/ml) either regionally (isolated limb perfusion) or systemically. Outcomes included tumor growth, survival, IHC, and toxicity. Dynamic control will be combined with adoptive transfer of effector T cells. B6 mice will be inoculated with B16/OVA (flank with window chamber) and treated with fluorescently labeled (calcein), OVA-specific CD8+ T cells from OT-1 transgenic mice. IVM, IHC, and flow cytometry will be used to measure T cell trafficking. RESULTS/ANTICIPATED RESULTS: Dynamic control (1) restored blood flow in non-functional tumor vessels and (2) increased and then transiently reversed blood flow in functional vessels. Vessel diameters did not change, suggesting that shunting of systemic blood to the tumor vasculature accounted for the observed changes. Dynamic control augmented tumor responses in our regional therapy model of melanoma. Increases in DNA adduct formation (melphalan mechanism of action) detected by IHC, decreased tumor growth, and increased survival were observed with dynamic control. There was no increased limb toxicity. Similarly, dynamic control augmented responses in our systemic therapy model (decreased tumor growth and improved survival). We anticipate that dynamic control will improve trafficking of effector T cells in the next set of experiments. DISCUSSION/SIGNIFICANCE OF IMPACT: Heterogeneous responses to systemic therapies represent a major gap in current cancer treatment. An essential requirement for any effective therapy is its ability to reach tumor via the tumor-associated vasculature. We have therefore developed an approach to enhance drug delivery (dynamic control), which we also plan to test in clinical trials.

4578

Early life stress promotes chronicity of experimental colitis

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OBJECTIVES/GOALS: The overall goal of this study was to determine the effect of early life stress (ELS) on the intestinal CD4+ T cell immune compartment, at homeostasis and after induction of experimental Inflammatory Bowel Disease (IBD). METHODS/STUDY POPULATION: We used a mouse model of ELS, maternal separation with early weaning (MSEW). We used IL-10 reporter mice to enable analysis of IL-10-producing cells. Mice were examined on postnatal day 28 to determine the impact of ELS on gut regulatory T cells. Plasma levels of corticosterone (rodent stress response hormone) was determined by ELISA. Colitis was induced in MSEW and normal rear (NR) mice via intraperitoneal injection of α-IL-10R every 5 days until day 15. Mice were euthanized on days 20 and 30. Colonic tissue sections were stained for histological analysis. Remaining tissue was further processed for flow cytometric analysis of CD4+ T cells and innate lymphoid cells. RESULTS/ANTICIPATED RESULTS: Plasma corticosterone was elevated in MSEW mice compared to their NR counterparts at 4 weeks of age. We observed that the MSEW stress protocol does not affect the baseline colonic CD4+ T cell or innate lymphoid cell populations. There was a reduction in the intestinal

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