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.

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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

Myrna G Garcia[1], Alvaro Padron, Yilun Deng, Harshita Gupta, Aravind Kancharla, Ryan Reyes, and Tyler Curiel
[1]University of Texas Health Science Center San Antonio

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.

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Dynamic Control of Tumor Vessels Augments Antitumor Responses

Emmanuel M Gabriel, Assistant Professor[1], Deborah Bahr[2], Sanjay Bagaria[2], Debrabata Muhkopadhyay[2], and Keith Knutson[2]
[1]Mayo Clinic; [2]Mayo Clinic Florida

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.

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Early life stress promotes chronicity of experimental colitis

Rachel Quinn Muir[1], Barbara J. Klocke[1], Kasi C. McPherson[1], Jeremy B. Foote[1], Jennifer S. Pollock[1], and Craig L. Maynard[1]
[1]University of Alabama at Birmingham

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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