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512 TERMINALLY EXHAUSTED CD8+ T CELLS POTENTIATE THE TOLERGENIC TUMOR MICROENVIRONMENT AS FUNCTIONAL SUPPRESSORS
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Background Blockade of co-inhibitory ‘checkpoint’ molecules, PD-1 and CTLA-4, has induced impressive clinical responses in advanced tumors; yet only in a subset of patients.1-3 Limited success with checkpoint blockade therapy suggests other cell extrinsic or intrinsic mechanisms may be dampening an effective immune response. Cytotoxic CD8+ T cells (CTL) encountering chronic antigen and metabolic restriction can differentiate to a terminally exhausted state (Texh), marked by hyporesponsiveness and metabolic, epigenetic, and transcriptional dysfunction.4-8 While enrichment of this population in tumor is a negative prognostic factor,9-10 it remains unclear whether Texh are non-functional or instead possess tolerogenic or suppressive properties. Transcriptional profiling of tumor-infiltrating PD1-Tint (progenitor exhausted) CTL versus PD-1hiTIM3+ (terminally exhausted; Texh), reveals that exhausted cells express a pattern of genes associated with immune suppression. We hypothesize that Texh potentiate the suppressive microenvironment of solid tumor by autoregulation and inhibition of local immune responses.

Methods T cell populations were isolated from murine melanoma-B16-F10 or a lab-generated melanoma clone of the spontaneous BREF/PTEN model–by expression of inhibitory receptors and assayed in tandem in microsuppression assays. Murine melanoma clones with inhibited oxidative metabolism were generated by CRISPR-Cas9 deletion and validated for ablated mitochondrial respiration by extracellular flux analysis. Enforced expression of CD39 in effector T cells was attained by murine retroviral vector delivery.

Results When sorted directly from tumor, PD-1hiTIM3+ Texh, but not progenitor exhausted PD-1int CTL, induce marked suppression of T cell effector responses, comparable to Foxp3+ Treg from the same environment. Expression of the ectonucleotidase, CD39, is uniquely expressed in Texh and increases as T cells differentiate towards exhaustion. Genetic deletion of CD39 in Texh eliminates the regulatory phenotype of tumor-infiltrating Texh and enforced CD39 expression on effector T cells can inhibit T cell receptor signaling and downstream function. CD39 expression correlates with exposure to hypoxia and Texh sorted from tumors engineered to be less hypoxic displayed a significant loss of suppressive capacity. Our data suggest that tumor hypoxia enforces Hif1α-dependent expression of CD39 which depletes extracellular ATP contributes to generation of immunosuppressive adenosine, and has been previously associated with terminal exhaustion.11-13

Conclusions Our data support a model that as CTL progress to terminal exhaustion, hypoxic exposure enforces the upregulation of CD39, providing Texh a mechanism to suppress proinflammatory processes. These findings suggest Texh are not solely dysfunctional but rather are deleterious to antitumor immunity and may need to be drastically reprogrammed or deleted in order to alleviate immunosuppressive functions.

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0512

513 CD26 ENZYMATIC ACTIVITY MODULATES EFFICIENT MIGRATION OF ADOPTIVELY TRANSFERRED T CELLS TO SOLID TUMORS
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Background The inadequate ability of adoptively transferred T cells to eradicate solid tumors limits their use in treatments for patients afflicted with those cancers. Efforts to improve ACT for solid tumors aim to identify strategies that poised T cells for optimal response. We have previously identified a specific subset of CD4 T cells which express high levels of the ubiquitous ectoenzyme dipeptidyl peptidase-4 (DPP-4), also known as CD26, that produce a tremendous antitumor response in solid tumor models. We therefore sought to investigate the importance of CD26 on T cells destined for ACT.

Methods We adoptively transferred tumor specific CD26+ T cells into melanoma tumor-bearing CD26-/- mice, and continued the CD26 enzymatic activity of the donor cells in vivo with sitagliptin, an established competitive inhibitor of CD26.

Results Tumors in sitagliptin-treated mice eventually reached study endpoint, while tumors untreated mice were regressed for 130+ days. Tumor infiltration of donor cells and host CD8 and CD4 cells was diminished with sitagliptin treatment. A 32-plex cytokine array of blood plasma revealed a diminished profile of cytokines and chemokines, indicating that the inflammatory response of the T cells was dampened with sitagliptin treatment. Further experiments characterized the ability of CD26+ T cells to respond to tumor trafficking signals with