Background While cancer immunotherapy has mainly focused on exploiting CD8 T cells given their role in the direct elimination of tumor cells, increasing evidence highlights the crucial roles played by CD4 T cells in anti-tumor immunity. However, very few high frequency, the lack of robust algorithms to predict peptide binding to MHC class II molecules and the high polymorphism of MHC class II molecules render the study and use of circulating tumor antigen-specific CD4 T cells challenging. In this regard, the HLA-DRB3*02:02 gene encoding an HLA allele that is expressed by half of the Caucasian population, offers a way to identify CD4 T cell-defined tumor antigens to antimitic drugs. Cancer Res 2014;74(20):5878–5890.

Methods Here, we aim to identify, isolate and functionally characterize ‘quasi-universal’ human tumor antigen-specific HLA-DRB3*02:02-restricted CD4 T cells in cancer patients. Using an algorithm we recently developed in house,1 tumor-associated antigenic peptides binding to this allele are identified. We have generated a large collection of HLA-DRB3*02:02-restricted CD4 T cell clones of different tumor antigens specificities. We will perform in vitro co-cultures of CD4 T cell clones with tumor cells to measure cytokine secretion, their tumor cell killing and their phenotypic profile (PD-1, TIM3, TIGIT, 4-1BB, CD40L, LAG3, VISTA, OX40). We will sequence and clone the TCR of the most promising candidates for adoptive cell transfer therapy. Lastly, we will directly evaluate the presence of these cells ex-vivo and longitudinally monitor them in patients.

Results N/A

Conclusions Together, these results should contribute valuable targets for coordinated CD4 and CD8 T cell-based immunotherapy of cancer.

REFERENCE


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0510
TERMINALLY EXHAUSTED CD8+ T CELLS POTENTIATE THE TOLEROGENTIC TUMOR MICROENVIRONMENT AS FUNCTIONAL SUPPRESSORS

Paolo Vignali *, Kristin DePeaux, McLane Watson, Ashley Merk, Nicole Scharping, Greg Delgoffe. 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 (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 simply non-functional or instead possess tolerogenic or suppressive properties. Transcriptional profiling of tumor-infiltrating PD-1int (progenitor exhausted) CTL versus PD-1hiTIM-3+ (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 depletion 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 Hif1a-dependent expression of CD39 which depletes extracellular ATR 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 exposes 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 anti-tumor immunity and may need to be drastically reprogrammed or deleted in order to alleviate immunosuppressive functions.

REFERENCES
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[Abstract Image]