Title: T Cell Receptor Avidity Dictates CD8+ T cell function in Pancreatic Cancer

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Immunotherapy with agonistic αCD40 + PD-1/PD-L1 inhibition holds promise for treatment of the highly lethal malignancy pancreatic ductal adenocarcinoma (PDAC). Our goal is to identify the properties of MHC class-restricted tumor-specific T cell receptors (TCRs) that impact T cell differentiation and immunotherapy outcomes in PDAC. We cloned 4 TCRs from the most clonally expanded T cells in orthotopic PDAC-bearing control mice, or mice treated with αCD40 and/or αPD-L1. Here, we demonstrate that all 4 topmost TCRs are specific to the identical tumor neoepitope, but exhibit vast differences in tetramer binding and responsiveness to peptide antigen. In particular, TCR clones expanded by αPD-L1 largely fail to bind tetramer but are the predominant clonotype in tumors and exhibit robust functional responses. Next, we targeted the 4 cloned tumor-specific TCRs to the endogenous TCR alpha constant (Trac) locus in murine zygotes using a CRISPR-Readi approach, generating 4 independent mouse strains with physiological regulation of defined tumor-specific TCRs. We delineated T cell maturation, phenotype, and function in these T cell receptor exchange (TRex) mice, which now serve as a source of donor naïve or effector T cells to identify TCR biophysical and mechanical properties that confer robust antitumor immunity in PDAC alone and in combination with immunotherapy. Together, the novel TRex mice we generated will elucidate optimal TCR traits for durable immunologically targeting of PDAC.