659 T CELL RECEPTOR EXCHANGE BY ZYGOTE ENGINEERING RESULTS IN PHYSIOLOGICAL T CELL RESPONSES FOR THERAPEUTIC USE IN PANCREATIC DUCTAL ADENOCARCINOMA

Meagan Rollins*, Jackson Raynor, Ebony Miller, Ellen Spartz, Walker Lahr, Adam Burrack, Yun You, Branden Morarity, Beau Webber, Ingunn Stromnes. *University of Minnesota, Minneapolis, MN, United States*

Background Pancreatic ductal adenocarcinoma (PDA) is a lethal malignancy characterized by a highly suppressive tumor microenvironment. Despite this, engineered T cell therapy has promise for effectively targeting PDA. To identify the underlying mechanisms of antigen-specific engineered T cell immunosuppression in PDA, we create novel TCR knock-in mouse models for a robust and standardized source of naïve mesothelin (Msln)-specific T cells.

Methods Specifically, we integrate two murine mesothelin-specific TCRs into the physiologic Trac locus in primary murine T cells and zygotes using CRISPR/Cas9 and rAAV expressing the TCR DNA. Simultaneously using CRISPR/Cas9, Msln was disrupted to circumvent T cell tolerance.

Results This strategy resulted in the rapid generation of homozygous TCR Trac knock-in mice and with homozygous null mutations in Msln. In these TCR-exchanged (TRex) mice, most T cells expressed the 1045 (high affinity) or 7431 (low affinity) as determined by tetramer staining. TRex T cells exhibit a naïve phenotype and rapidly differentiate into effector T cells upon antigenic stimulation. While the high affinity 1045 TCR elicits function in CD4 T cells, the lower affinity 7431 T cells exhibit a higher functional avidity and less TCR downregulation when antigen is limiting. Historical TCR transgenic T cells, in which the TCR is randomly integrated into the genome, exhibit increased PD1, CD25, and CD69, decreased functionality, and a bias to CD25-Foxp3+ Treg as compared to T cells from TRex mice. Further, TCR Trac integration in primary T cells retain superior function following repetitive antigenic stimulations retrovirally transduced T cells. Adoptive transfer of 1045 TRex T cells significantly prolongs survival of mice bearing autochthonous PDA. When combined with a vaccine, 1045 TRex T cells cause involution of the fibroinflammatory tumor stroma.

Conclusions In sum, we rapidly generate mice that physiologically express the desired TCR, circumventing the shortcomings of standard T cell engineering strategies and TCR transgenic models.

Ethics Approval University of Minnesota Institutional Animal Care and Use Committee approved all animal studies to Dr. Ingunn Stromnes (2005-38115A.) Generation of TCR knockin (KI) animals was performed in the Mouse Genetic Laboratory at the University of Minnesota.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.659