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Engineering of regulatory T cells for immune tolerance

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The adaptive immune system has evolved to specifically recognize and destroy a virtually infinite variety of pathogens (non-self), while remaining unresponsive towards tissues (self). A subset of helper T lymphocytes dedicated to suppressing immune responses, regulatory T cells (Tregs) are essential to maintain self tolerance and immune homeostasis.

Manipulating human Tregs offers the opportunity to modulate the immune system with antigen specificity in organ transplant rejection and autoimmunity. Yet, antigen-specific Tregs are vanishingly rare. Moreover, the best antigen target is often unknown. Synthetic biology can be used to impart a desired specificity to human Tregs, greatly expanding what targets can be pursued using Treg-based therapies. Chimeric antigen receptors (CARs) are synthetic immune receptors comprising an extracellular antibody-based antigen-binding domain and an intracellular signaling domain. By combining T cell signal 1 and signal 2 in its endomain, a CAR allows for potent T cell activation directly downstream of antigen recognition.

Infusion of regulatory T cells (Tregs) engineered with a chimeric antigen receptor (CAR) targeting donor-derived human leukocyte antigen (HLA) is a promising strategy to promote transplant tolerance. To explore this strategy, we generated a fully humanized anti-HLA-A2 CAR by grafting the complementarity-determining regions (CDRs) of a human monoclonal anti-HLA-A2 antibody into the framework regions of the Herceptin 4D5 single-chain variable fragment and fusing it with a CD28-CD3zeta signaling domain. We then generated anti-HLA-A2 human CAR Tregs by targeting the endogenous T-cell receptor (TCR) *via* CRISPR/Cas9 and introducing the HLA-A2 CAR gene in the TCR alpha constant locus using homology-directed repair. These HLA-A2 CAR human Tregs maintained both Treg phenotype (FOXP3 and HELIOS expression) and suppressive function *in vitro*. Moreover, they selectively accumulated in HLA-A2-expressing human islets transplanted into immunodeficient NSG mice. Strikingly, HLA-A2-CAR Tregs delayed xenogeneic graft-*versus*-host disease only in the presence of HLA-A2, expressed either by co-transferred peripheral blood mononuclear cells)PBMCs) or by the recipient NSG mice. Altogether, genome-engineered mono-antigen-specific HLA-A2 CAR Tregs localize to HLA-A2-expressing grafts and exhibit antigen-dependent *in vivo* suppression, independent of TCR expression. To further develop precision Treg cell therapies for transplant tolerance, ongoing work interrogates CAR Treg-mediated

suppression of islet graft rejection in humanized mice. In addition, whole transcriptomic analysis at the population and single-cell levels is being conducted to dissect the intricacies of CAR signaling in human Tregs and conventional T cells, aiming to maximize CAR Treg therapies.