

Towards A Cure For Type 1 Diabetes (T1D): Human Chimeric Antigen Receptor (CAR) T Cell Ablation Of Diabetogenic Autoreactive T Cells

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Purpose: Diabetes substantially increases the risk of impaired wound healing, infection, and flap necrosis, creating major challenges in reconstructive surgery. These complications are especially pronounced in type 1 diabetes, where autoreactive T cells mediate disease by recognizing and destroying pancreatic β cells. Among these pathogenic T cell clones, the 1E6 T cell receptor is a well-characterized human autoreactive TCR found in most patients with T1D, making it an attractive target for selective immune elimination.

To address this, we developed a modular peptide–MHC chimeric antigen ligand (CAL) platform to redirect CAR T cells against specific T cell clones, here, 1E6 TCR-expressing T cells. In vitro and in vivo, CAL-guided CAR T cells selectively eliminated autoreactive targets with antigen-restricted activity and no detectable off-target toxicity. This strategy provides a proof-of-concept for precise depletion of pathogenic T-cell clones and may offer a broadly adaptable approach for autoimmune disease, alloreactivity in transplantation, and other T-cell–mediated disorders.

Method: We evaluated the ability of CAL-directed CAR T cells to eliminate 1E6 TCR-expressing human T cells in vitro and in vivo. For in vitro studies, 1E6 transduced JRT3 cells or primary human T cells were co-cultured with anti-biotin CAR T cells and matched CALs at multiple effector-to-target ratios, with mismatched CALs and vehicle controls included for comparison. Readouts included CAL binding and internalization, T-cell activation markers (CD69 and CD137), degranulation (CD107a), and flow cytometry-based cytotoxicity. For in vivo studies, NSG-A2 mice were engrafted with 1E6 luciferase-labeled target cells and then treated with CAR T cells and matched CALs. Tumor burden was monitored by bioluminescence imaging.

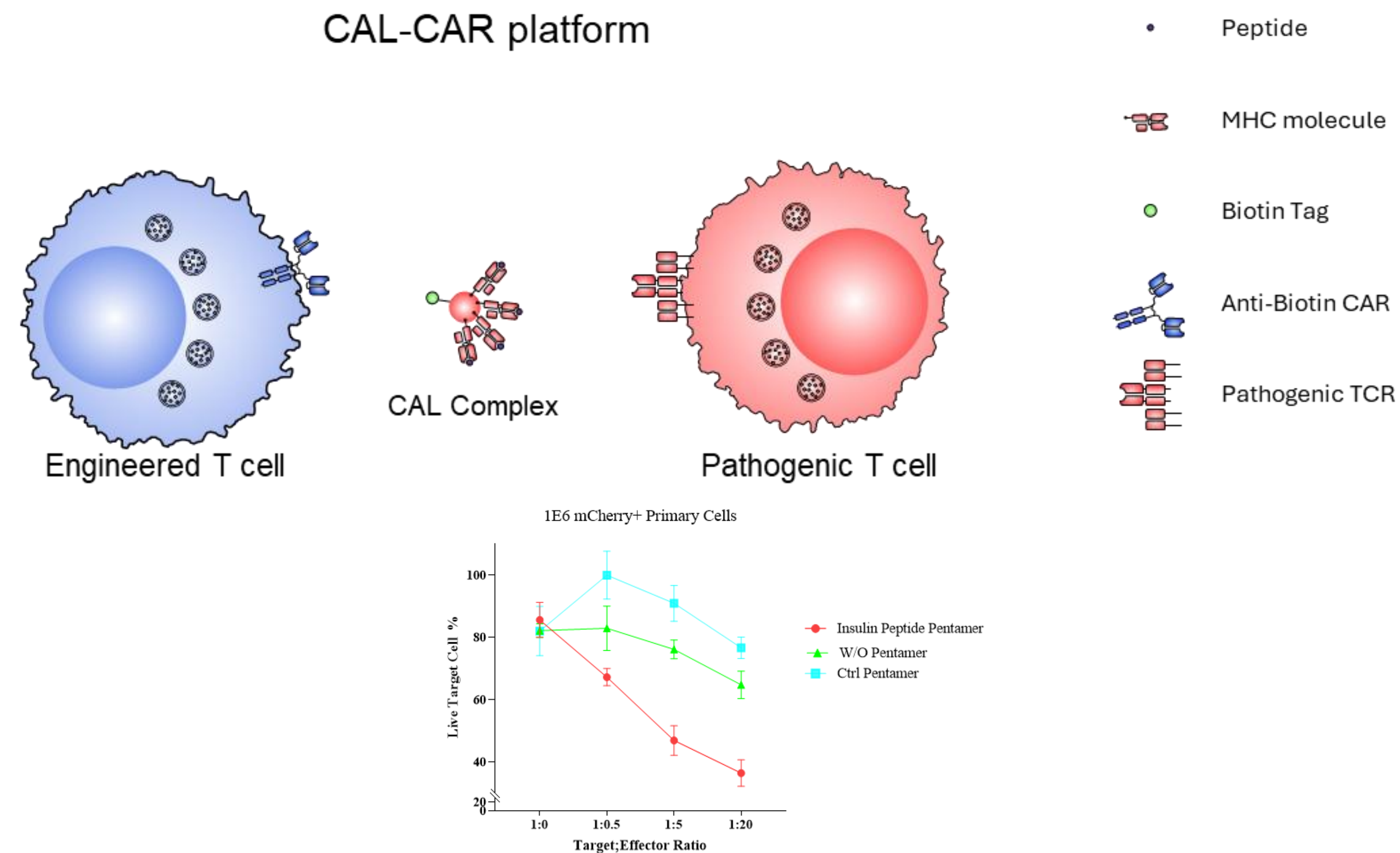


Fig. B) CAL-CAR T cells mediate efficient and antigen-specific elimination of 1E6 TCR-transduced primary target cells across multiple effector-to-target ratios. In the presence of insulin peptide pentamer, CAL-CAR T cells induced robust target cell killing, whereas cells remained largely unaffected when cultured with control pentamer bearing an irrelevant peptide or in the absence of pentamer, demonstrating strict antigen dependence of the response.

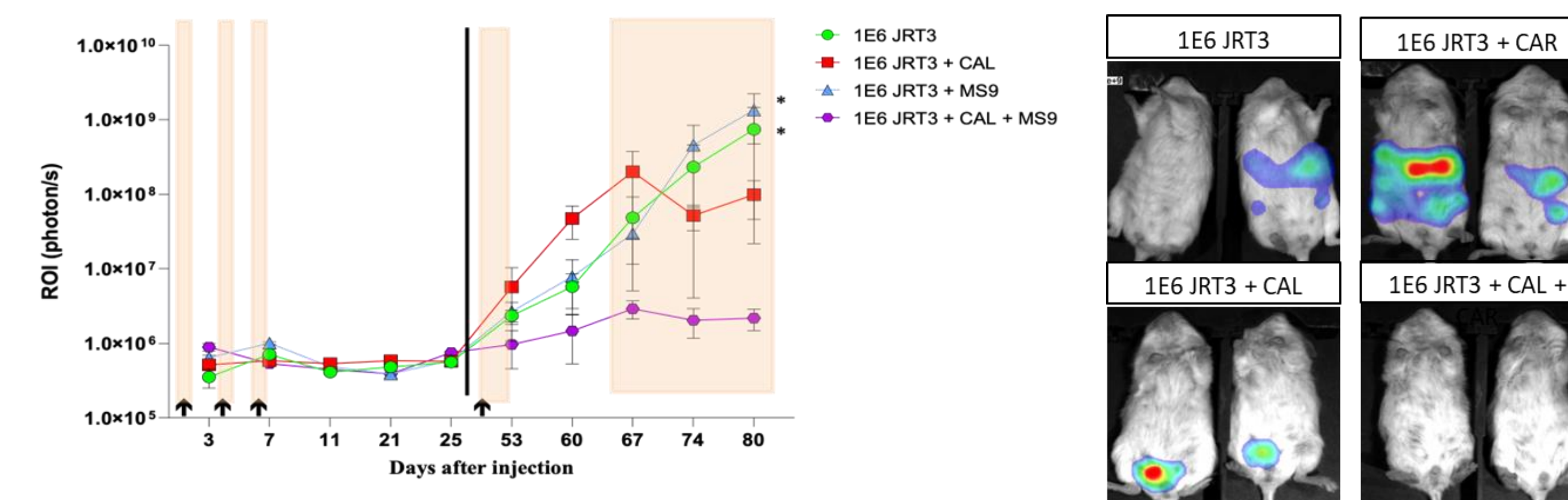


Fig. C) In vivo, the CAL-CAR system efficiently eliminated 1E6 TCR-transduced JRT3 cells when all CAL-CAR components were present, resulting in a marked reduction in bioluminescent signal over the 60-day observation period. In contrast, in the absence of CAL or CAR T cells, 1E6 JRT3 cells disseminated throughout the mouse body, with progressively increasing bioluminescence. The left panel shows longitudinal bioluminescence imaging over time, and the right panel compares bioluminescent intensity among groups at day 60.

Results: In vitro, CAL-guided CAR T cells showed robust, antigen-restricted activity against 1E6-expressing targets across a range of effector-to-target ratios. Matched CALs triggered strong activation, degranulation, and cytotoxicity, while mismatched CALs remained inert, confirming that killing depended on specific pMHC recognition rather than nonspecific CAR activation (Fig. B).

In vivo, CAL-directed CAR T-cell treatment eliminated luciferase-positive 1E6 targets in NSG-A2 mice, with near-complete loss of bioluminescent signal and improved survival compared with controls. CAR T cells persisted at low levels in the spleen, and no off-target toxicity was observed, supporting the platform's specificity and translational potential (Fig. C).

Discussion & Conclusion: These findings demonstrate that CAL-guided CAR T cells can selectively eliminate human autoreactive T-cell clones with strong antigen specificity in both in vitro and in vivo models. By separating target recognition from CAR specificity, this modular platform offers a flexible strategy for addressing pathogenic T cells without broad immune suppression, an important advantage in diseases where preserving host defense is essential. In reconstructive surgery, this approach is especially relevant because reducing autoimmune-driven inflammation and immune dysregulation may improve wound healing, reduce infection risk, and support flap survival in patients with diabetes.

More broadly, this study establishes a proof-of-concept for using pMHC-based CALs to retarget CAR T cells against disease-defining T-cell populations. Because the pMHC component can be swapped to match different TCR specificities, the platform is inherently adaptable to other autoimmune diseases, alloreactive T cells in transplantation, and potentially pathogenic T-cell responses in infectious settings. Together, these data support CAL-directed CAR T-cell therapy as a precision immunotherapy framework for selectively removing harmful T cells while preserving the remainder of the immune repertoire.