

CAR T Therapy for Tolerance Induction in Vascularized Composite Allotransplantation: Antigen-Specific Protection of Ova Skin Grafts via Cal-guided OT-I CAR T-cells

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Introduction

Vascularized composite allotransplantation (VCA) offers major reconstructive potential, but its long-term success is limited by the need for lifelong systemic immunosuppression. While immunosuppressive drugs reduce rejection, they also increase the risks of infection, malignancy, metabolic complications, and impaired wound healing, which are especially problematic in reconstructive patients. These limitations have driven interest in antigen-specific tolerance strategies that selectively target the immune cells responsible for rejection while preserving overall immune function. Chimeric Antigen Ligands (CALs) provide a modular platform for this purpose. CALs combine a high-avidity peptide–MHC complex with a molecular tag that recruits anti-tag CAR T cells, allowing antigen-specific targeting of pathogenic T cells. In this study, we used a class I CAL bearing H-2K^b/SIINFEKL to target OT-I CD8⁺ T cells in a murine OVA skin graft model and tested whether CAL-guided CAR T cells could protect grafts and promote tolerance induction. We aimed to determine whether CAL-guided CAR T cells could selectively eliminate OT-I alloreactive T cells, prolong antigen-bearing graft survival, and reduce graft infiltration. We also evaluated whether CAL alone could contribute to graft protection, supporting the possibility of a long-acting biologic approach to tolerance induction. More broadly, we sought to establish a modular and adaptable platform for antigen-specific immune editing in VCA.

Methods

B6 RagKO recipient mice were conditioned with 2 x 5.5 Gy irradiation and received tail skin grafts from Act-mOVA or wild-type C57BL/6J donors. Mice were adoptively transferred with 1.5 x 10⁶ MACS-purified OT-I CD8⁺ splenocytes intravenously and treated with CAL plus universal anti-FITC CAR T cells, CAL alone, and irrelevant CAL, CAR T cells without matched CAL, and wild-type grafts. Endpoints included graft survival, engraftment index, and mid-study analysis of graft infiltrates and cytokine profiles.

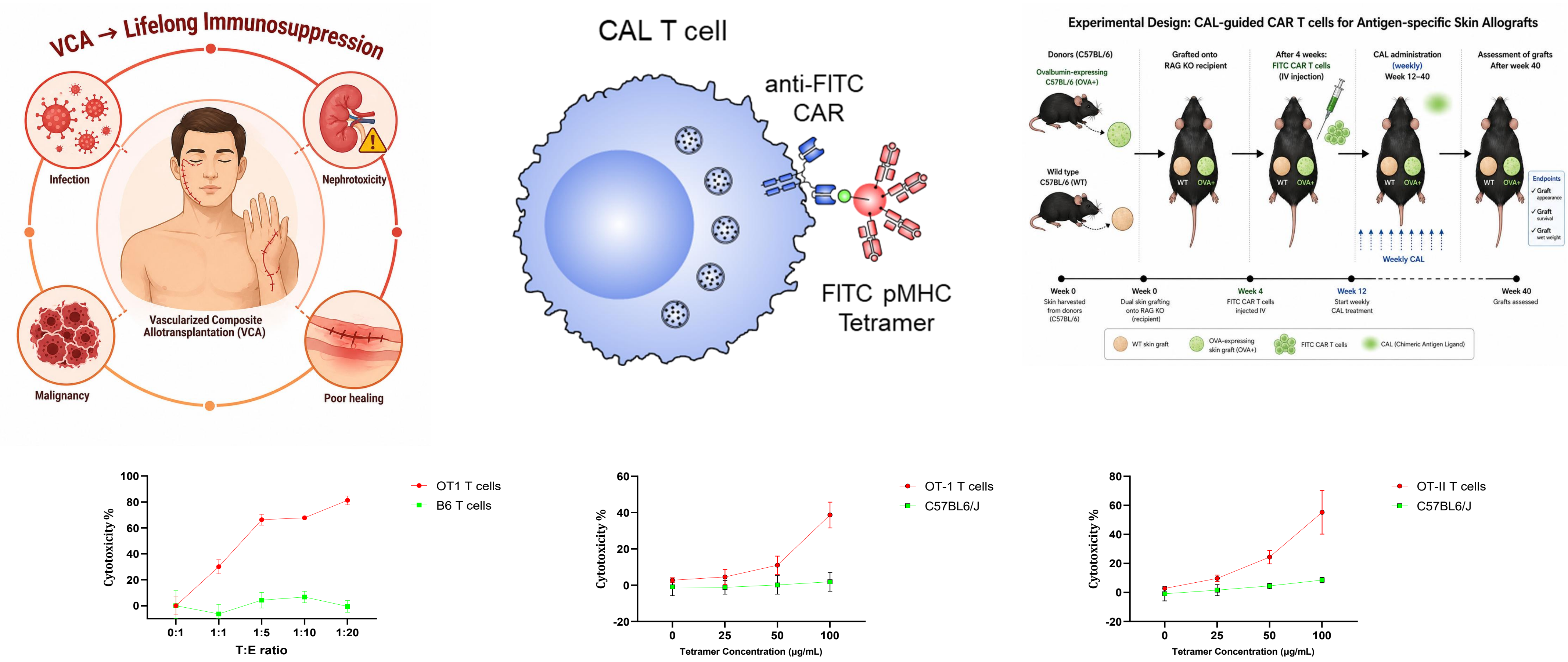


Figure D. CAL-CAR T cells specifically target and eliminate OT-I or OT-II T cells in vitro in an effector-to-target ratio–dependent manner. Increasing E:T ratios enhanced cytotoxic activity against cognate targets, whereas noncognate controls remained largely unaffected, demonstrating antigen–specific killing by the CAL–CAR system.

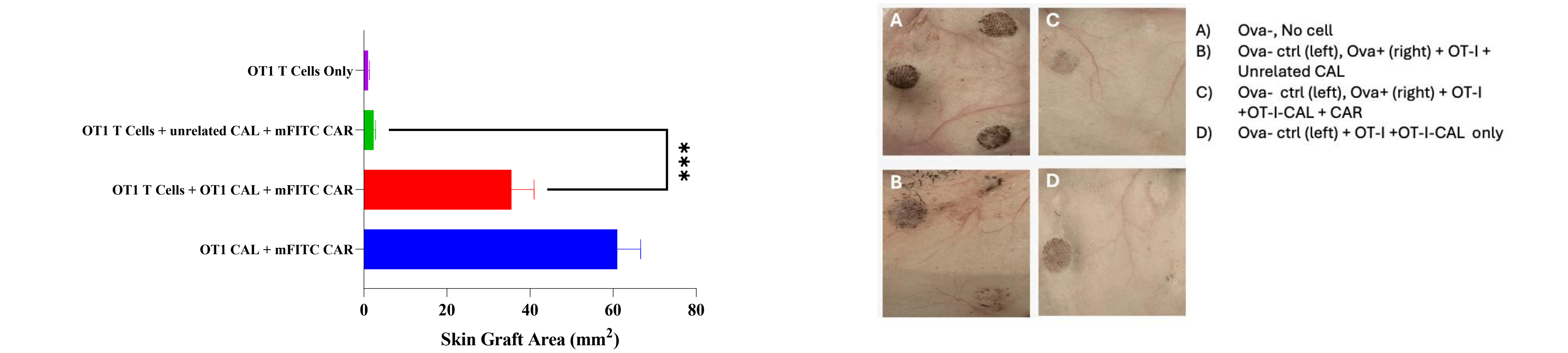


Figure E. The CAR-CAL system effectively eliminated ovalbumin-expressing skin grafts while leaving wild-type skin grafts intact, demonstrating antigen-specific protection of non-target tissue.

Results: CAL plus CAR T cell therapy prolonged the survival of OVA-expressing grafts in an antigen-restricted manner, while wild-type grafts were unaffected. Treated grafts showed reduced OT-I infiltration, increased CAR localization at the graft interface, and a cytokine profile consistent with dampened effector activity, indicating selective suppression of the alloimmune response. In vitro, CAL-guided CAR T cells exhibited robust, antigen-specific cytotoxicity against OT-I targets, whereas irrelevant CAL was inert. Together, these findings demonstrate that the CAL-CAR system can selectively target pathogenic T cells while preserving non-target tissues, supporting its potential as a precision tolerance-inducing strategy.

Conclusion

This study shows that tagged, high-avidity class I CALs can protect antigen-bearing skin grafts by directing anti-tag CAR T cells to eliminate OT-I clones. The prolonged graft survival and reduced graft infiltration support the feasibility of antigen-specific immune editing as a strategy for VCA. More broadly, CAL-guided CAR T therapy represents a flexible platform for tolerance induction that can be adapted to other transplant and autoimmune settings by changing the pMHC cargo. The additional activity of CAL alone suggests that a long-acting CAL biologic may also have therapeutic potential.