

Oxford Transplant Foundation Pump-priming Grant Report

Title

Engineering human regulatory T cells with a universal adaptor for programmable antigen targeting

Introduction

Chimeric antigen receptor (CAR) cell therapies are constrained by their single antigen specificity. To address this limitation, SNAP CARs, which can covalently bind to different benzylguanine-conjugated antibodies and thereby redirect cell specificity against different targets, have been developed. This strategy may be useful for programming regulatory T cells (Tregs) to target different human leukocyte antigen (HLA) molecules for use in transplantation with varying HLA mismatches. We aimed to generate SNAP-CAR Tregs capable of targeting different HLA molecules, to broaden the applicability of antigen-specific Treg therapy in transplantation.

Methods

Tregs were isolated from peripheral blood mononuclear cells (PBMCs) obtained from healthy donor leukocyte cones, expanded, then transduced using lentiviral vectors to express SNAP-CARs. Transduced cells were sorted for SNAP-CAR Tregs and further expanded (Figure 1). Anti-HLA-A2 and anti-HLA-B7 antibodies were conjugated to benzylguanine via an amine coupling reaction. Dose titration was performed to optimise preloading of SNAP-CAR Tregs with benzylguanine-conjugated antibodies.

Phenotypic markers were compared between SNAP-CAR Tregs and autologous, polyclonally expanded, unedited Tregs (apTregs) expanded in parallel. Treg-specific demethylated region (TSDR) demethylation was assessed using bisulphite PCR followed by next-generation sequencing. Stimulation assays were performed by co-culturing SNAP-CAR Tregs (with or without preloading with benzylguanine-conjugated antibodies) or apTregs, with either target antigen-expressing K562 cells or wild-type K562 cells. In alloantigen suppression assays, HLA-A2 negative CD3-enriched responder PBMCs were cultured with allogeneic HLA-A2 positive CD3-depleted stimulator PBMCs and Tregs at varying ratios.

Results

SNAP-CAR Tregs and apTregs were phenotypically similar with high demethylation at the TSDR (Figure 2A and B). SNAP-CAR Tregs preloaded with benzylguanine-conjugated antibodies (anti-HLA-A2 or anti-HLA-B7) showed increased CD25 and CD69 expression and elevated IL-10 secretion when co-cultured with target antigen-expressing K562 cells. This activation was antigen-specific, as it was not observed with unloaded SNAP-CAR Tregs, apTregs, or when preloaded cells were stimulated with wild-type K562 cells (Figure 2C and D, n=4-5 donors). SNAP-CAR Tregs preloaded with anti-HLA-A2 antibodies demonstrated a trend towards enhanced suppressive function compared with apTregs (Figure 2E, n=3 donors, p=0.06).

Conclusion

SNAP-CAR Tregs can be specifically activated after preloading with the appropriate antibodies and show evidence of enhanced suppressive function compared to apTregs in an HLA-A2 alloantigen suppression assay. This technology may be harnessed to alter Treg specificity on demand, enabling the targeting of varying HLA mismatches in transplantation.

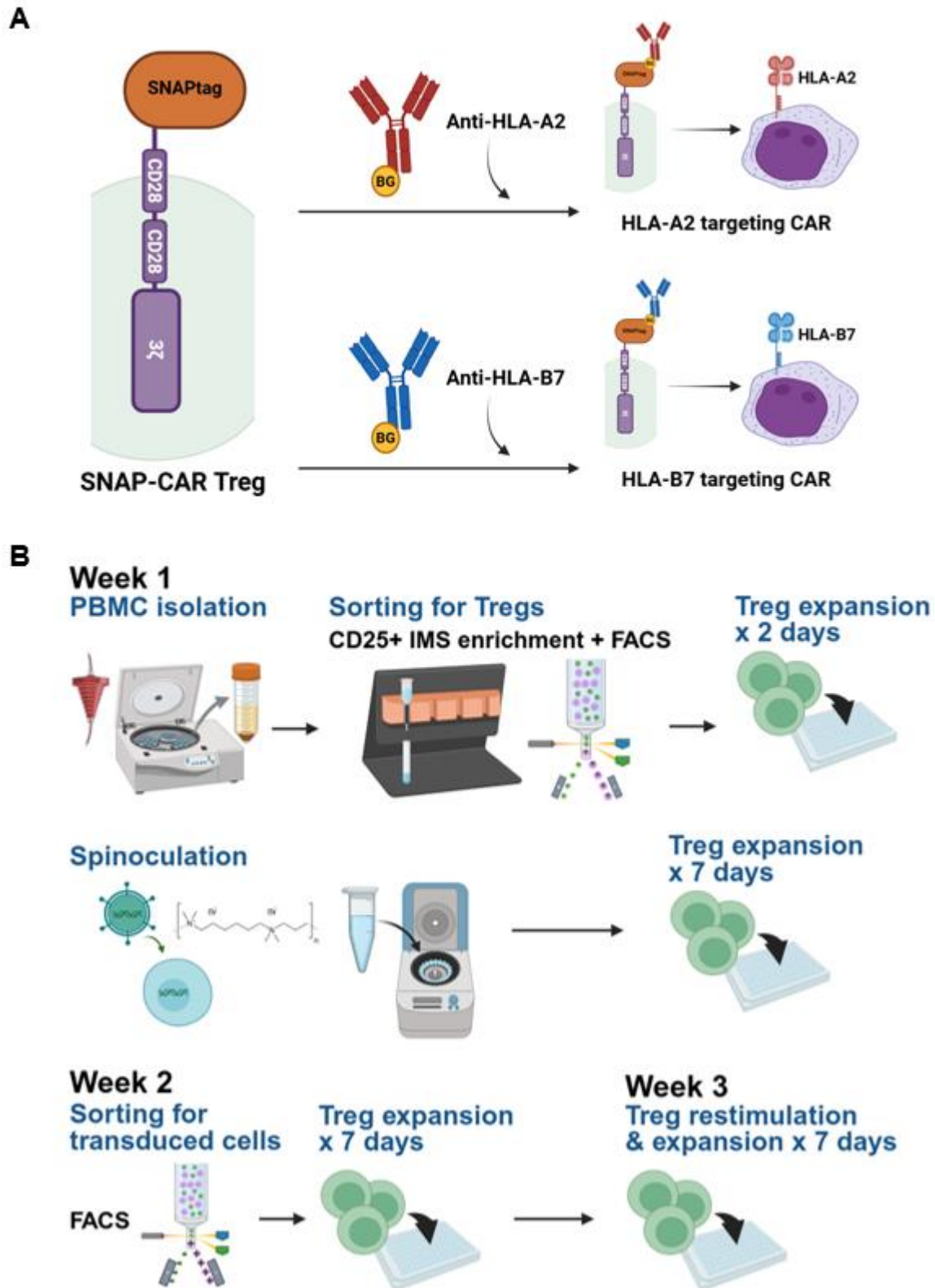


Figure 1. Generation of SNAP-CAR human regulatory T cells (Tregs). (A) Schematic of SNAP-CAR Treg targeting of different human leukocyte antigens using interchangeable benzylguanine-conjugated antibodies. (B) Workflow for Treg isolation, expansion, lentiviral transduction and enrichment of transduced cells.

Output

Publications

1. **Ho QY**, Hashimoto H, Hester J, Issa F. Vortex-actuated pre-enrichment accelerates human regulatory T cell sorting and improves early fitness. *Molecular Therapy Advances*. 2026 Mar 12;34(1):201667. doi: 10.1016/j.omta.2026.201667.

Conference abstracts

1. **QY Ho**, H Hashimoto, J Hester, F Issa. Abstract ID number 43: Human regulatory T cells with a modular receptor for programmable antigen targeting. [Abstract and Poster Presentation] In International Society for Cell & Gene Therapy (ISCT) Asia 2026; Singapore
2. **QY Ho**, H Hashimoto, J Hester, F Issa. Abstract ID number 1172: Vortex-actuated cell sorting boosts expansion of CRISPR-edited human regulatory T cells. *Cytotherapy*. 2026 May; 28(5)S102606:234 doi: 10.1016/j.jcyt.2026.102606 [Abstract, Poster, and Elevator Pitch Presentation] In ISCT Annual Scientific Meeting 2026; Republic of Ireland.
 - Invited for encore presentation (Abstract, Poster and Elevator Pitch Presentation) at ISCT Asia 2026; Singapore
3. **QY Ho**, J Hester, F Issa. Engineering human regulatory T cells with a universal adaptor for programmable antigen targeting via CRISPR-Cas12a transfection [Abstract and Oral Presentation] In Oxford Academic Kidney Network Symposium 2026; United Kingdom.
4. **QY Ho**, H Hashimoto, J Hester, F Issa. PP_374: Vortex-actuated cell sorting boosts human regulatory T cell throughput and growth. *Transpl Int*. 2025;38(Suppl 1):363. doi: 10.3389/ti.2025.13627 [Abstract and Poster Presentation] In ESOT Congress 2025, United Kingdom.

Next steps

1. A manuscript detailing our in vitro results is being prepared for submission to a peer-reviewed journal.
2. An HLA-A2 mouse skin transplantation model has been established using HLA-A2 transgenic NSG mice procured with this grant. Plans to refine the model further and to evaluate the SNAP-CAR Tregs in vivo are under discussion.



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