Antigen-Specific T Cells for Assessing Immune Modulating Therapeutics

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Introduction

Antigen-specific T cells play a key role in the adaptive immune system, as they can identify and eliminate cells presenting their specific antigen. Their targeted nature makes them valuable for testing immune-modulating therapies designed to either boost or suppress immune activity in diseases like cancer and autoimmunity. A major obstacle in evaluating antigen-specific T cell responses is having readily available cells to study the impact of immune modulators in real time. To address this, we have developed well-characterised transgenic and endogenous antigen-specific 'Thaw & Go' T cell banks that facilitate the evaluation of new immune modulators.

Generation of TCR-T Cells

Donors were selected based on HLA genotype compatibility with the antigen of interest, ensuring TCR-T cells will recognise their specific antigen when presented by the specific HLA genotype.

CD4+ or CD8+ T cells were isolated from cryopreserved PBMC (supplied by BioIVT, LLC) and transduced with a lentiviral vector encoding the TCR construct. An upstream tag enabled selection of successfully transduced cells. The cells were expanded using a proprietary protocol, and then characterised and cryopreserved.

Characterisation of the cells included transgenic TCR expression, and T cell exhaustion and activation markers.

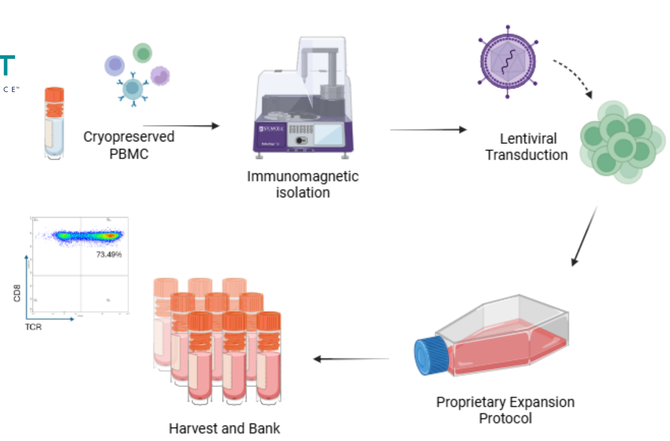
> **15-fold Expansion**

>50% antigen specific TCR expression

Yield> 2.3x10^8 cells per bank

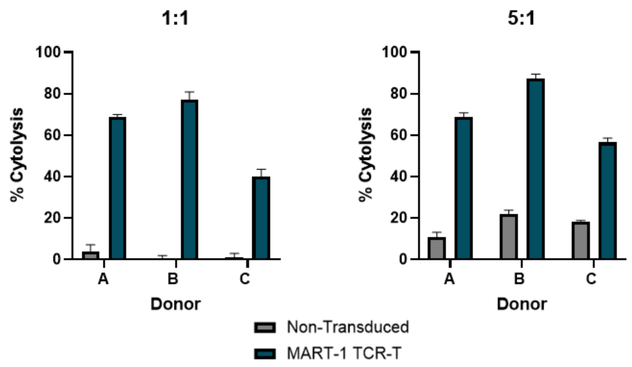
10 million cells per vial

35-85 vials per bank

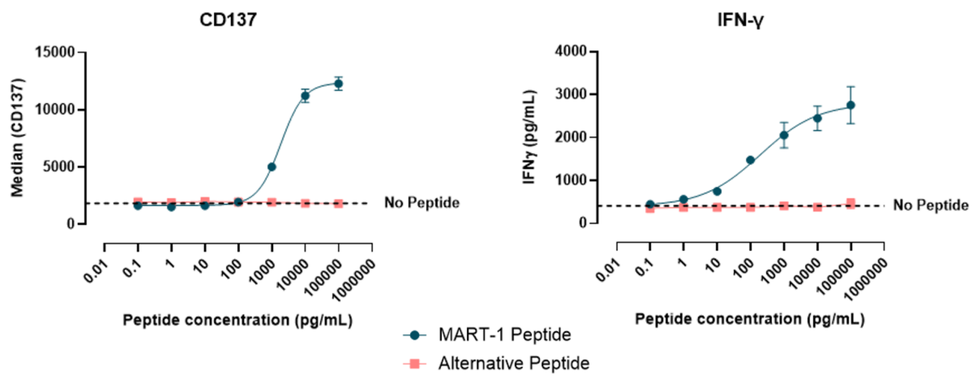


Functional Assessment of TCR-T Cells

MeWo cells are fibroblast-like cells isolated from a patient with malignant melanoma and have been reported to express the melanoma-associated antigen, MART-1 (Melan A). These cells were employed as target cells and co-cultured with CD8+ T cells engineered to express a transgenic MART-1-specific TCR at two E:T ratios (1:1 and 5:1), enabling antigen specific recognition and functional assessment of the TCR-T cells.

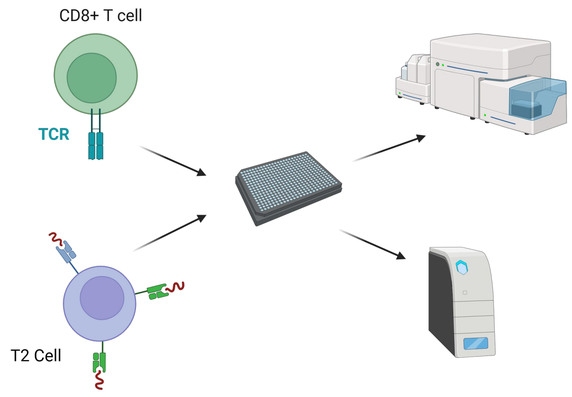


T2 cells were pulsed with either a MART-1 peptide, or an alternative non-specific peptide to evaluate antigen cross-reactivity. These peptide-loaded T2 cells were co-cultured with CD8+ T cells expressing a transgenic TCR specific for MART-1. T cell activation was assessed by measuring CD137 expression on CD8+ TCR+ cells, as well as assessing IFN-γ production.

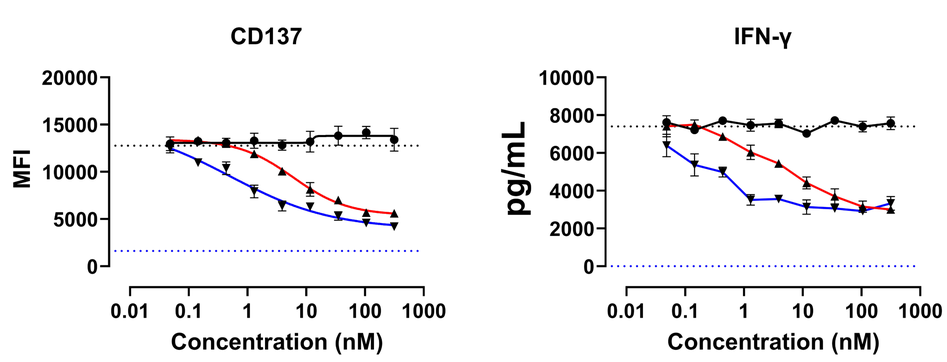


TCR-T Cells for Assessment of Immune Modulators

TCR-T cells were employed to evaluate the effects of immune modulators. T2 cells, which express HLA-A2 and can present specific antigens to cytolytic CD8+ T cells, were employed as the target cells. The T2 cells were pulsed with the antigen of interest and co-cultured with TCR-T cells in the presence of a concentration series of T cell inhibitors, enabling functional assessment of immune modulation.



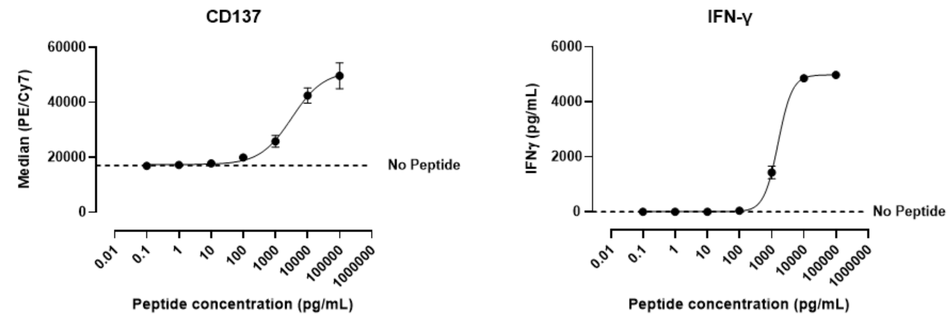
Large cell numbers per vial allow high-throughput screening runs, testing multiple samples in 384-well assays



Endogenous Antigen-Specific T Cells

An alternative strategy is to generate antigen-specific T cells by expanding CD8+ T cells that endogenously express a TCR specific to the target antigen. These cells offer enhanced biological relevance by maintaining native TCR expression, without the need to introduce a second TCR.

Naïve CD8+ T cells were isolated from cryopreserved PBMC and expanded following a proprietary protocol. Functional activity was assessed using HLA-A1+ K562 cells pulsed with CMV peptide as antigen-presenting targets. Co-culture with CMV-specific endogenous antigen-specific T cells enabled evaluation of antigen-specific response.



Summary

We have created 'Thaw & Go' banks of TCR-T cells (from PBMC supplied by **BioIVT, LLC**), ideal for high-throughput evaluation of immune-modulating therapies. These well-characterised cells exhibit specificity to their target antigen and can be assessed through a range of functional assays. Moreover, we have established an effective approach to expand endogenous antigen-specific T cells, providing a biologically relevant alternative. Finally, we are currently producing further cell banks, including Tregs and γδ T cells, to offer valuable resources for a wider range of applications and to support faster candidate selection and advance drug discovery efforts.

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