TCR Discovery and Detection of Antigen-Presentation on Cells using the Dextramer® Technology

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Introduction

To successfully develop and apply T cell-based immunotherapies, the specificity and sensitivity of the selected TCR must first be validated before proceeding to clinical development. Here, the detection and quantification of antigen-presenting cells (APC) is important for **1)** stratification and selection of patients with demonstrated expression of the target antigen, **2)** confirming tissue-specific expression of the target antigen, and **3)** monitoring target expression during treatment. To support such efforts, we have developed high-avidity TCR Dextramer® reagents to allow the detection of peptide presentation by APCs. This study presents a complete workflow for TCR discovery, followed by the generation and use of TCR Dextramer® as an analytical tool for evaluating target expression on the cell surface of APCs.

Conclusions

- Peptide (p)MHC-specific TCR sequences can be identified using dCODE Dextramer® reagents in a simple workflow.
- TCR functionality can be verified by making soluble TCR monomers and evaluate pMHC recognition in an artificial cell system.
- TCR Dextramer® reagents can be used to detect peptide presentation at the surface of antigen-presenting cells, here shown for peptide pulsed T2 cells and PBMC samples.
- Peptide presentation is detectable ≥2 nM peptide-pulsing concentration on T2 cells, ≥20 nM on PBMC samples.
- TCR Dextramer® is ideally suited to develop novel techniques for the detection of antigen presenting cells.

Identify Candidate TCR Sequences using dCODE Dextramer® Technology

MHC dCODE Dextramer® reagents are barcoded with DNA oligonucleotides, enabling highly precise single-cell multi-omics analysis of antigen-specific CD8+ or CD4+ T cells, providing simultaneous information on TCR recognition, gene expression, surface protein expression, and TCR sequence via V(D)J sequencing.

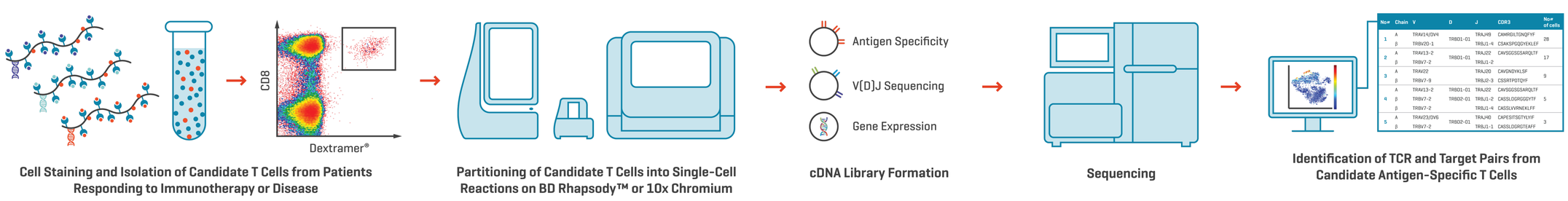
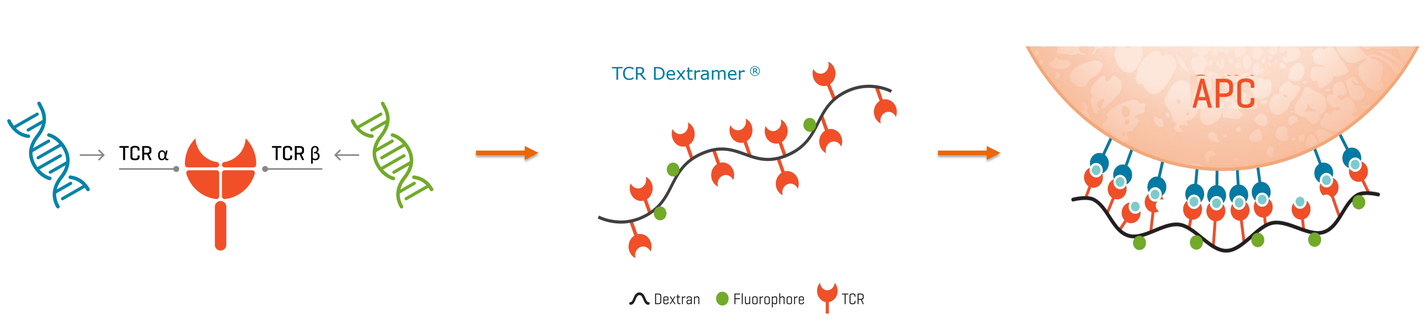


Fig. 1. Using dCODE Dextramer® Technology to identify candidate TCR sequences (with Gene Expression and Surface Markers).

Optimized Manufacturing of Soluble TCR Monomers and TCR Dextramer®



Soluble TCR Monomers are produced in *E. coli*, refolded, biotinylated, and purified with an optimized platform. Rigorously QC'ed Soluble TCR Monomers are attached to a fluorescent Dextramer® backbone. Antigen-presenting cells (APC) can be stained with TCR Dextramer® reagents like conventional pMHC Dextramer® reagents on T cells.

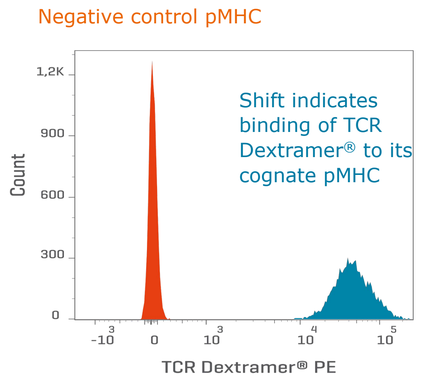
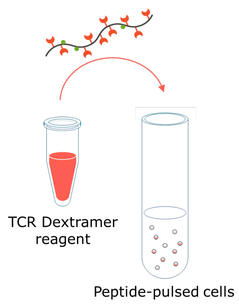


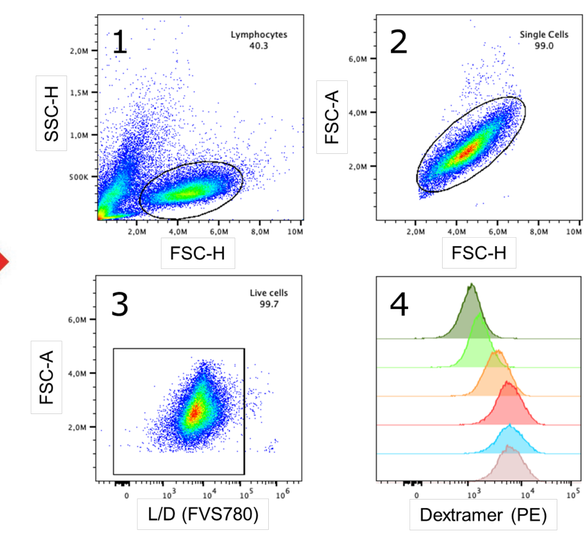
Fig. 2. Quality control of TCR Dextramer®. TCR functionality and specificity for the target pMHC is confirmed in an artificial cell system by flow cytometry.

Staining of peptide-pulsed cells using TCR Dextramer®

TCRs used in this study

TCR	TCR Specificity	K_d
A	HLA-A*02:01/ SLLMWITQV	48 pM
B	HLA-A*02:01/ SLLMWITQV	32 µM





Peptide-pulsed cells are stained with TCR Dextramer® reagents based on high and low affinity TCRs. The mean fluorescence intensity of the relevant cell population (lymphocytes/monocytes) is measured using flow cytometry.

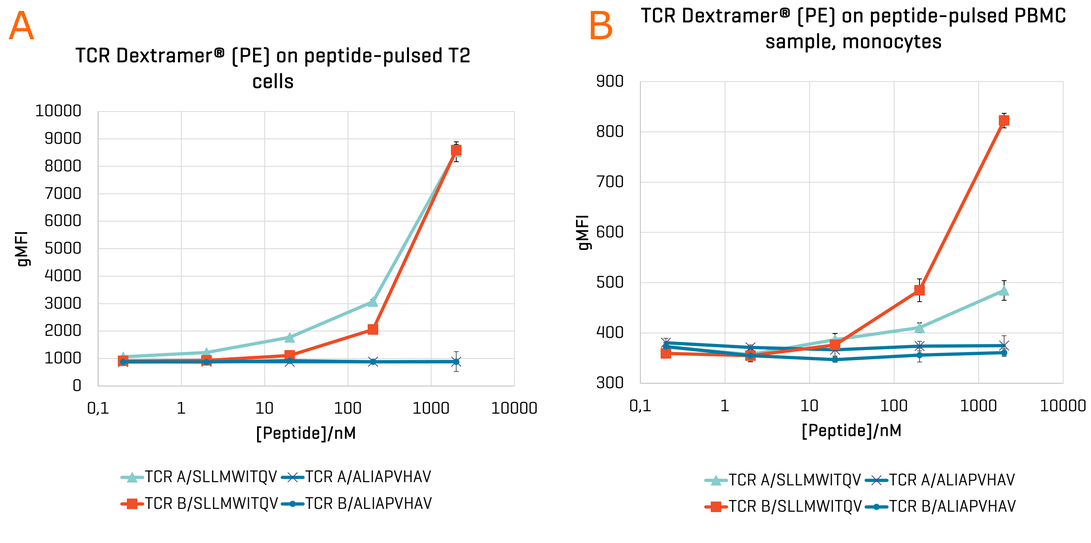


Fig. 3. TCR affinity impacts TCR Dextramer® binding.
A) Peptide titration on T2 cells, **B)** Peptide titration on PBMC sample; data shown for monocyte population. Target peptide: SLLMWITQV, Kd (HLA-A2) = 5.6 nM; control peptide: ALIAPVHAV, Kd (HLA-A2) = 7.0 nM.

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