Antigen-Specific Solutions for T-Cell Therapy Development and Manufacturing

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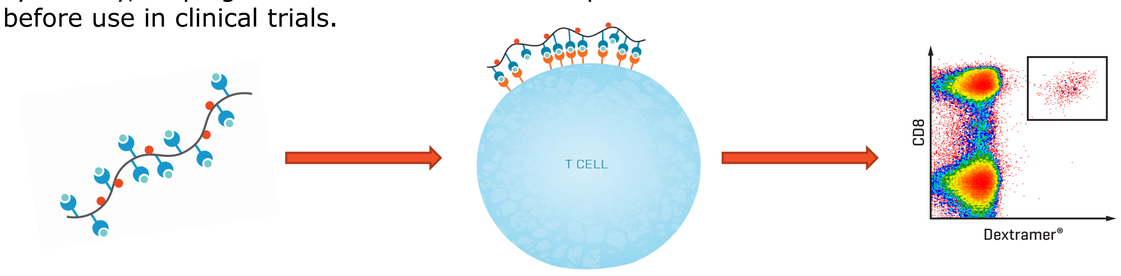
Introduction

The antigen-specific interaction between the T Cell Receptor (TCR) and cognate peptide MHC (pMHC) complex is at the heart of the development of successful T cell-based therapies. Understanding and characterizing the TCR:pMHC interaction is key to being able to demonstrate efficacy and investigate potential side effects of CAR and TCR cell therapies. The high avidity of the Dextramer® technology enables the reliable detection and quantification of engineered T cells (even at low frequency), allows the investigation of TCR cross-reactivity, and opens the possibility to develop TCR Dextramer® reagents for the detection of Antigen-Presenting Cells (APCs).

Quantification of Engineered T Cells with Dextramer® Technology

Figure 1. Quality Control and Lot Release Testing of T Cell-based Therapies. Establishing assays to evaluate TCR transduction and T cell number is essential for accurate dosing and minimizing the risk of side effects. Clinical-Grade (GMP) Dextramer® reagents enable both the identity and purity of T cells to be analyzed in a single step, using flow cytometry, helping to ensure that the infusion product meets defined lot release criteria before use in clinical trials.

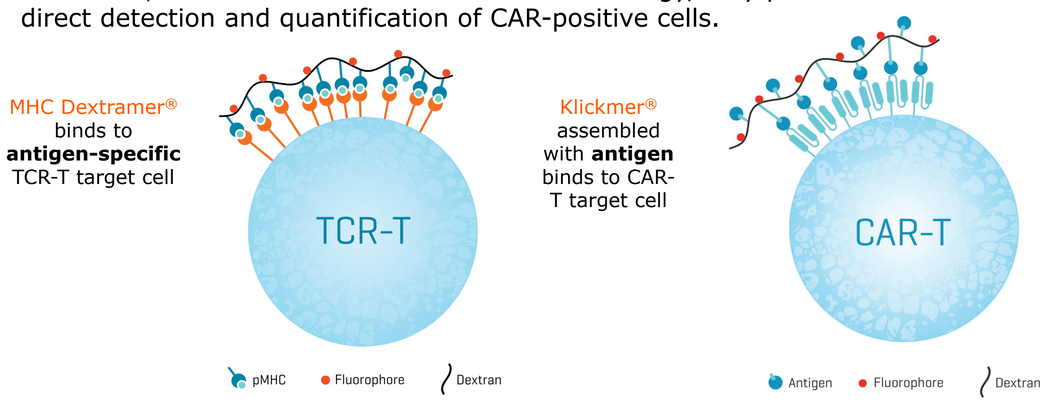


MHC Dextramer® reagent(s)

MHC Dextramer® binds to TCR on antigen-specific T cells

Assess % of target-specific Dextramer®-positive T cells by flow cytometry

Figure 2. Direct detection and quantification of TCR-T and CAR-T cells. MHC Dextramer® reagents enable the quantification of antigen-specific TCR-T cells. Similarly, Klickmer®, also based on the Dextramer® technology, may provide a sensitive solution for direct detection and quantification of CAR-positive cells.



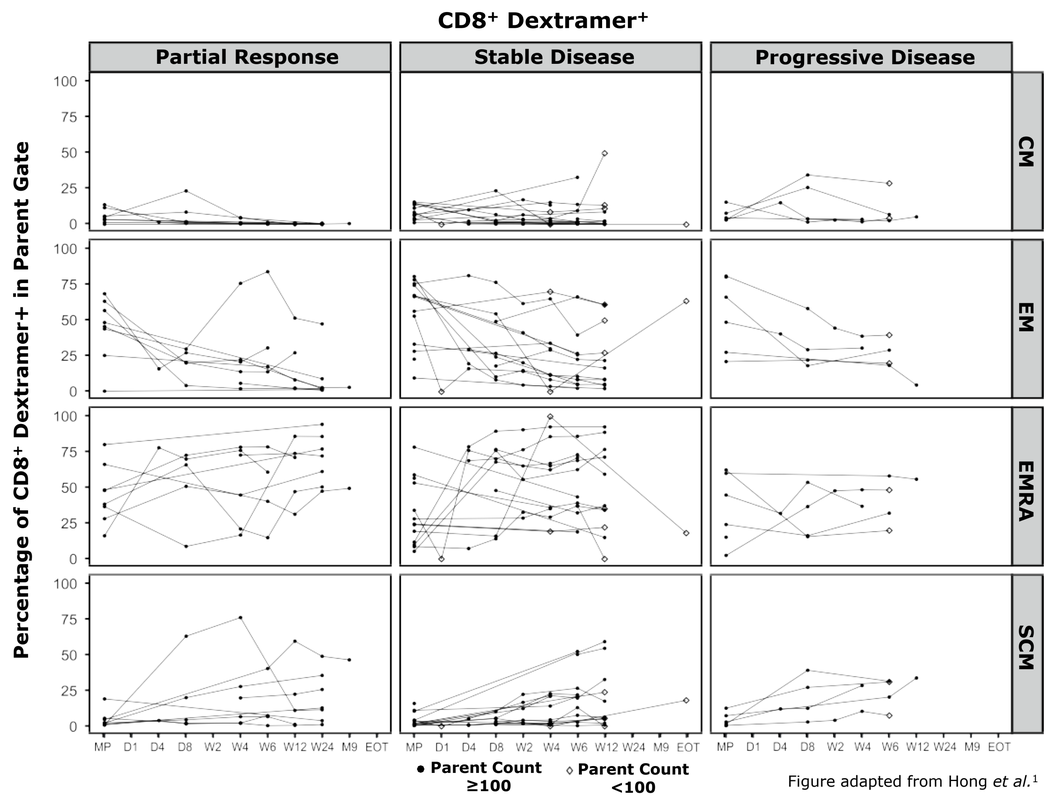


Figure adapted from Hong *et al.*[1]

Figure 3. Tracking infused T cells in patients. MHC Dextramer® (HLA-A2) was used to assess transduction of Adaptimmune's Autologous MAGE-A4-directed TCR-T cell therapy (afami-cel) product and investigate the kinetics of afami-cel persistence in post-infusion PBMC samples in a Phase I clinical trial[1].

CM = central memory
EM = effector memory
EMRA = effector memory RA+
SCM Stem cell memory

Screening for TCR Off-Target Cross-Reactivity

Screening candidate TCRs against a library of potential off-target antigens is vital to select TCRs that show the highest degree of specificity for their intended target and mitigate the risk of off-target toxicities in clinical trials. MHC Dextramer® Reagents can provide highly sensitive and accurate characterization of TCR performance in binding target and off-target epitopes, even for low-affinity interactions. The immunogenicity assessment can be scaled up in a 'TCR fingerprinting' approach[2].

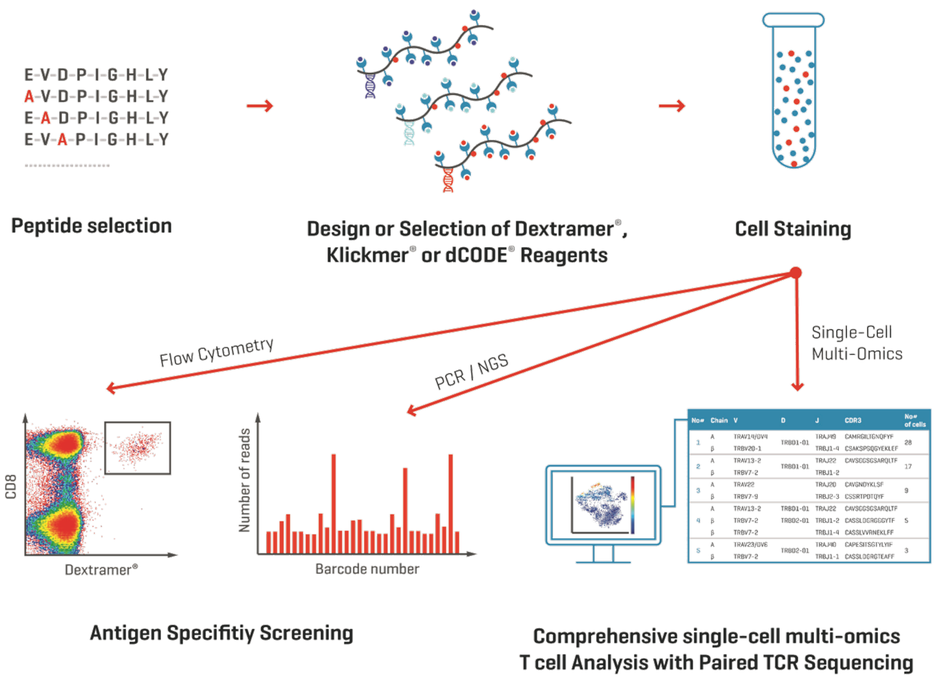


Figure 4. Cross-Reactivity Screening using barcoded dCODE Dextramer® technology to analyze a library of up to 1000 potential off-target antigens simultaneously. The library may include a panel of 'usual suspects' to avoid self-reactivity, a panel of epitopes from highly expressed proteins, in addition to sequence permutations of the target epitope.

Detection of Target Expression by TCR Dextramer®

The detection and quantification of APCs is important to:

- Stratify and select patients with demonstrated expression of the target antigen
- Confirm if the target antigen is present predominantly in the target tissue (and absent in normal tissues), thus avoiding potential toxicity
- Monitor the presence of the target antigen during treatment and possible tumor escape.

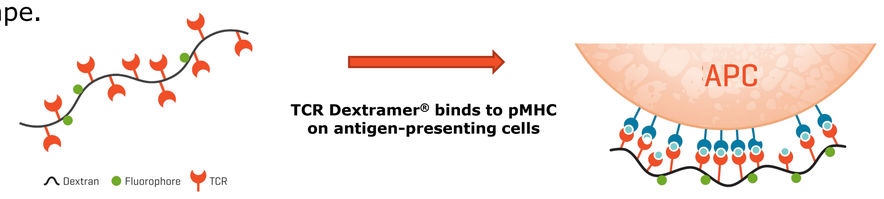


Figure 5. APC Detection using TCR Dextramer®.

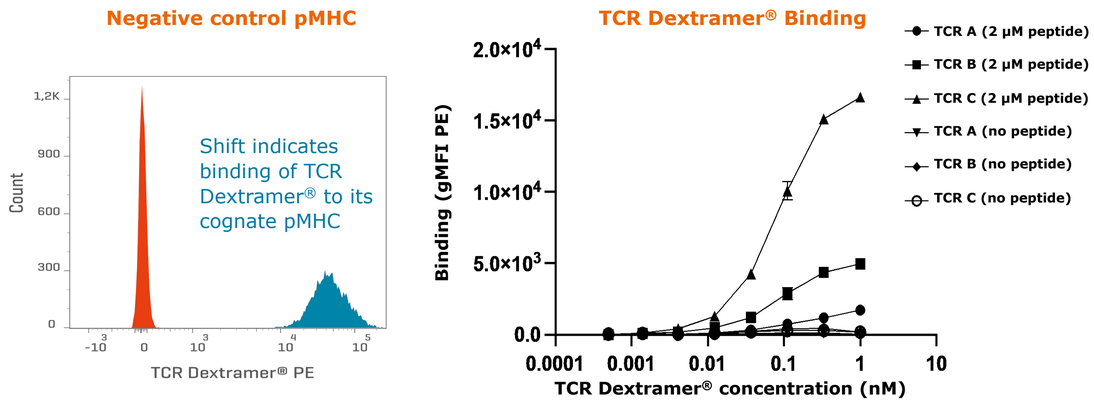


Figure 6. TCR Dextramer® Quality Control. TCR functionality and specificity for the target pMHC is confirmed in an *in vitro* cell surrogate system by flow cytometry.

Figure 7. TCR affinity impacts TCR Dextramer® binding. TCR Dextramer® with TCRs of different affinities towards their NY-ESO1 target[3] (A = wt, B = medium, C = highest affinity) stain peptide-pulsed T2 cells. High affinity TCR (C) gives highest staining signal.

Conclusions

- Dextramer® technology can help to demonstrate that the infusion product meets lot release criteria and enable infused T cells to be tracked in patients *ex vivo*
- Cross-reactivity screening to identify potential off-target antigens can be performed using barcoded dCODE Dextramer® reagents
- TCR Dextramer® is ideally suited to develop novel techniques for the detection and quantification of antigen presenting cells

References

1.Hong *et al.* Autologous T cell therapy for MAGE-A4+ solid cancers in HLA-A*02+ patients: a phase 1 trial. *Nat Med* **29**, 104–114 (2023). https://doi.org/10.1038/s41591-022-02128-z
2.Bentzen *et al.* T cell receptor fingerprinting enables in-depth characterization of the interactions governing recognition of peptide-MHC complexes. *Nat Biotechnol.* (2018). doi: 10.1038/nbt.4303.
3.Dunn, SM (2006) Protein Sci; 15(4):710-721.