From Target to Therapy – A Validated Workflow for Selecting the Right TCR

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Introduction

The development of T cell receptor (TCR)-based therapies – whether in a cellular or cell-engager format – depends on the identification of TCRs with high specificity for the target MHC:peptide complex (MHCp). However, T cells carrying TCRs of the relevant specificity are often rare, which complicates their efficient identification.

In this study, we present a TCR discovery workflow (Fig. 1) for the proliferation, identification and functionality validation of rare, MART-1-specific T cells starting from healthy donor material with undetectable levels of relevant T cells.

Workflow

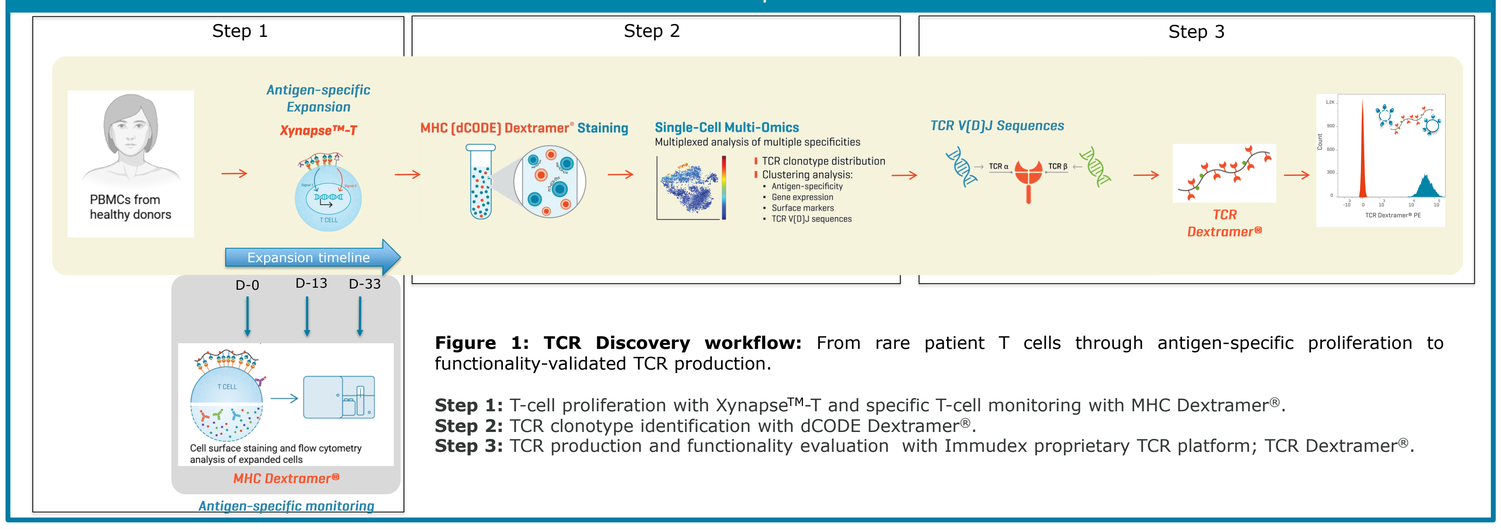


Figure 1: TCR Discovery workflow: From rare patient T cells through antigen-specific proliferation to functionality-validated TCR production.

Step 1: T-cell proliferation with Xynapse™-T and specific T-cell monitoring with MHC Dextramer®.
Step 2: TCR clonotype identification with dCODE Dextramer®.
Step 3: TCR production and functionality evaluation with Immudex proprietary TCR platform; TCR Dextramer®.

Step 1 – Rare T-Cell proliferation and monitoring

PBMC T-cell populations from two different donors (Donor #219 and Donor #268) were enriched by stimulating with the HLA-A*0201/MART-1 Xynapse™-T reagents (Fig. 2) in culture. MART-1-specific T cell (MART-1 T) proliferation was monitored by staining with fluorescently labelled HLA-A*0201 /MART-1 Dextramer® reagents and subsequent analysis by Flow cytometry (Fig. 3).

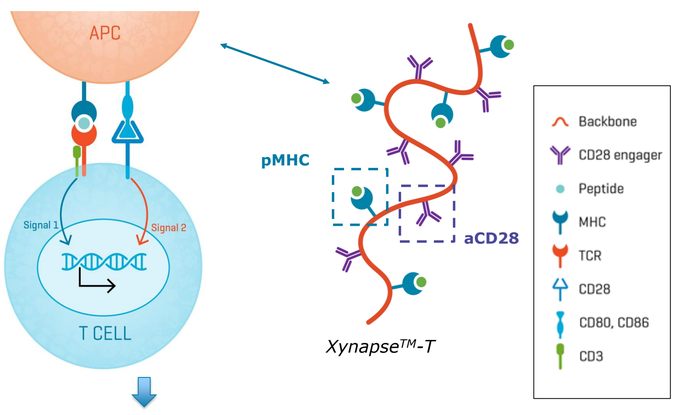


Figure 2: MART-1 specific Xynapse-T reagent for rare T-cell proliferation
Artificial antigen-presenting cell scaffolds (Xynapse™-T) were engineered by conjugating HLA-A*02:01/MART-1 complexes and CD28 co-engagers to a synthetic scaffold.

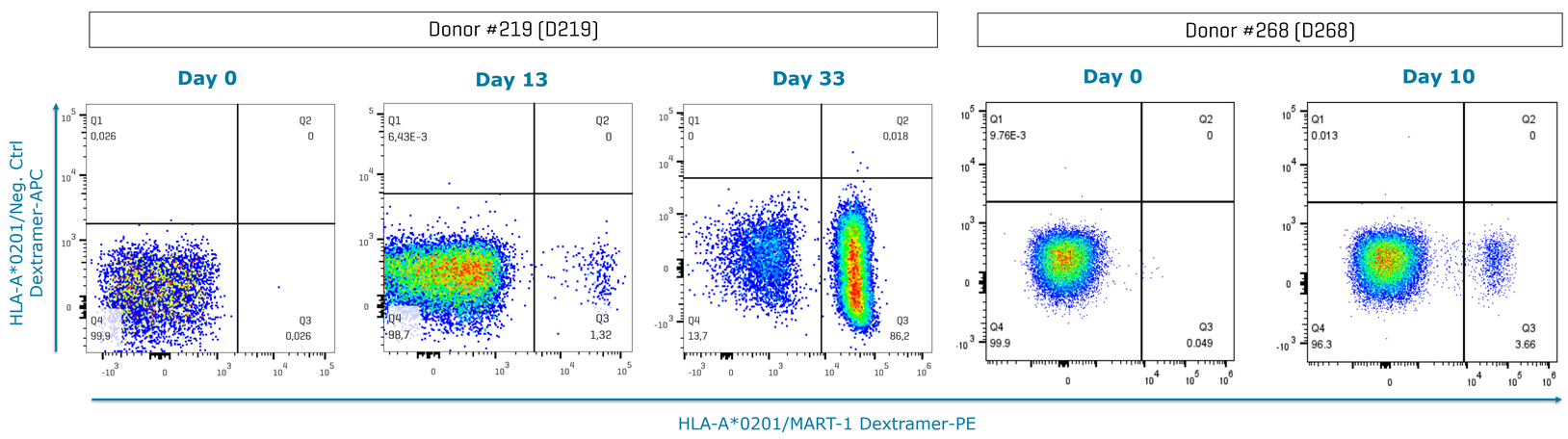


Figure 3: Rare MART-1 specific T-cell expansion monitoring:
PBMCs from two healthy donors, Donor #219 (D219) and Donor #268 (D268), were primed with HLA-A*0201/MART-1–specific Xynapse™-T.
D219 sample underwent Xynapse-T–mediated priming over a 30-day period with priming performed on day 0 and day 14. Flow cytometric analysis demonstrated that approximately 85% of cells at day 33 were MART-1–specific CD8⁺ T cells. D268 sample was primed with MART-1–specific Xynapse™-T on day 0 only and cultured for 11 days. Flow cytometric analysis revealed that approximately 3,5% of cells were MART-1–specific. Following completion of culture, cells were cryopreserved and subsequently thawed for single-cell analysis.

Step 2 - TCR clonotype identification

MART-1 TCR clonotypes were identified by staining cells from the enriched T cell cultures from D219 with DNA-barcoded HLA-A*02:01/ MART-1 dCODE Dextramer reagents and analyzing them on a BD Rhapsody™ single-cell multiomics platform.

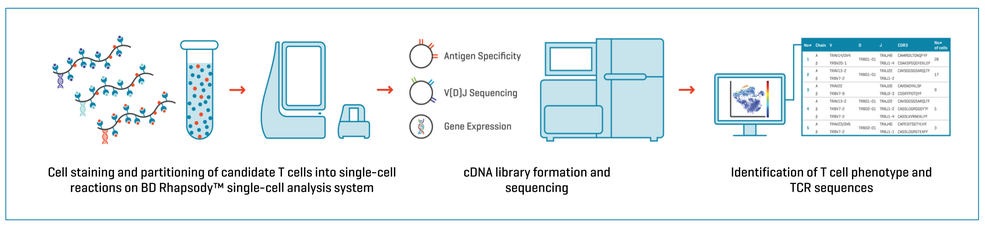


Figure 4: Workflow for identifying T cell phenotypes and TCR sequences.
Cells were stained with an AbSeq Immune Discovery panel (32 surface markers) and two dCODE Dextramer reagents: HLA-A*02:01/ELAGIGILTV (MART-1) and HLAA*02:01/ALIAPVHAV (neg.ctrl.). Cells were not sorted prior to single-cell analysis due to the high frequency of target cells in the sample after Xynapse-T priming and expansion. Analysis was performed on the BD Rhapsody single-cell system, generating libraries for targeted mRNA (immune profiling panel) and TCR sequencing along with the Immune Discovery panel and dCODE MHC Dextramer reagents.

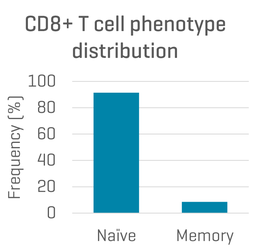


Figure 5: T cell phenotypes.
Most cells (91%) exhibit a naïve-like phenotype after stimulation and expansion.

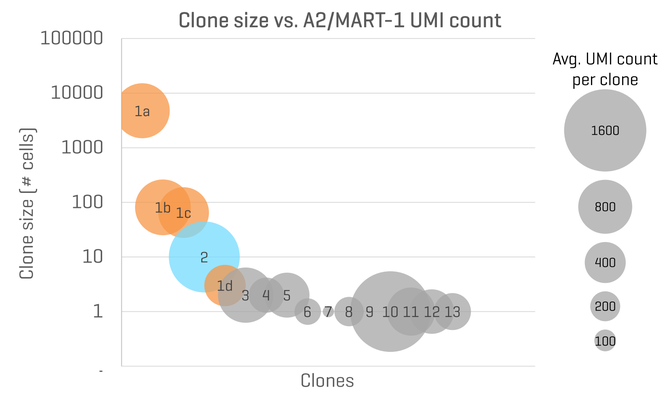


Figure 6: TCR clonotypes by size and A2/MART-1 staining level.
Sixteen distinct, HLA-A*02:01/MART-1-positive T cell clones were identified with the main clone family (1a-d) comprising 99.5% (1a: 96.5%) of all positive cells. The second, distinct clone (2) comprises 0.2% of all positive cells.

Clone ID	TRAV	TRAJ	TRBV	TRBJ	CDR3 [A;B]
1a	12-2*01	45*01	6-5*01	1-3*01	AVNPNSGGGADGLT; ASRSGQLVSGNTIY
1b			10-1*01		
1c	12-2*03		6-5*01		
1d			10-1*01		
2	12-2*01	6*01	6-5*01	1-5*01	ASSSGGSYIPT; ASSSGQGQPQH

Table 1: Main TCR clonotypes.
Clone family 1 (orange) consists of two clone pairs: Clones 1a and 1c express identical TCRs at the protein level. Similarly, clones 1b and 1d express identical TCRs. The 1a/c and 1b/d TCRs are nearly identical differing only in the TRBV segment, and due to the identical CDR3 sequences, all clones are considered part of the same functional family. Clone 2 differs from clone family 1 in both TRAJ and TRBJ as well as both CDR3 loops. Clonotypes 1a and 2 were chosen for further functional validation.

Step 3 - TCR production and validation

TCR molecules corresponding to MART-1 TCR clonotypes were refolded from material produced recombinantly in *E. coli*, and the corresponding TCR Dextramer reagents were evaluated for specific binding to HLA-A*02:01/MART-1-coated microbeads. (Fig 7).
TCR clone 1a could recognize the MART-1 coated beads but not the control proving its functionality and specificity. TCR clone 2 could not recognize the MART-1 coated beads or the control ones and is thus not functional. (Fig 8).

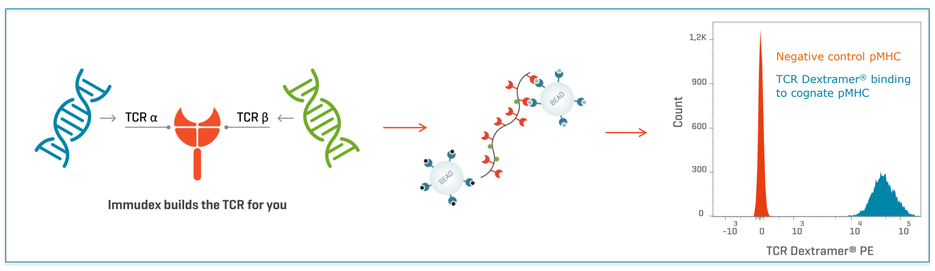


Figure 7: TCR production and functionality validation process

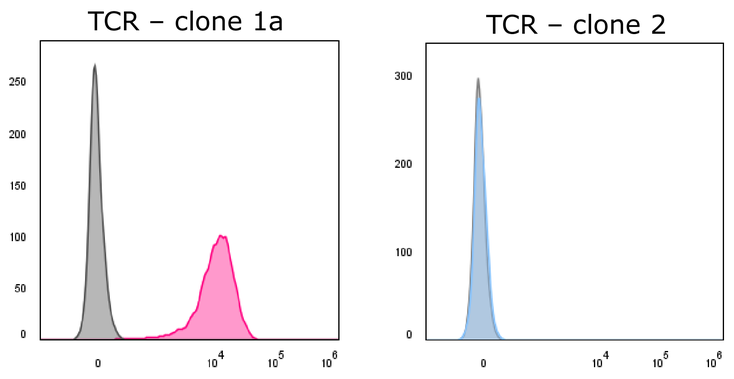


Figure 8: TCR functionality evaluation on HLA-A*02:01/MART-1-coated microbeads
TCR Dextramer reagents carrying TCR clone 1a or 2 were incubated together with HLA-A*0201/MART-1 coated beads or control beads. TCR Dextramer – clone 1a could recognize the HLA-A*0201/MART-1 coated beads (pink) but not the control beads (grey). TCR Dextramer – clone 2 could could not recognize HLA-A*0201/MART-1 coated beads (blue) nor the control beads (grey - overlapping).

Conclusions and perspectives

- We have built a pipeline enabling antigen-specific expansion and evaluation of rare T-cells in PBMC samples, followed by TCR clonotype identification, production, and functionality evaluation.
  - Xynapse™-T allows specific proliferation of rare T-cells.
  - dCODE Dextramer® allows TCR clonotype identification.
  - TCR Dextramer® platform allows production of TCR and functionality evaluation.

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