Multiomic characterization of T cell populations at the single-cell level utilizing sensitive dCODE™ Dextramer® Reagents and BD® AbSeq Ab-Oligos on the BD Rhapsody™ Single-Cell Analysis System

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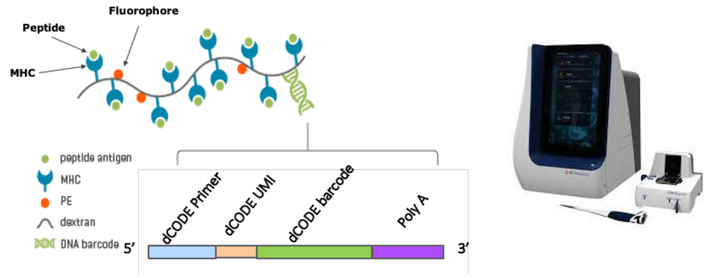
Abstract

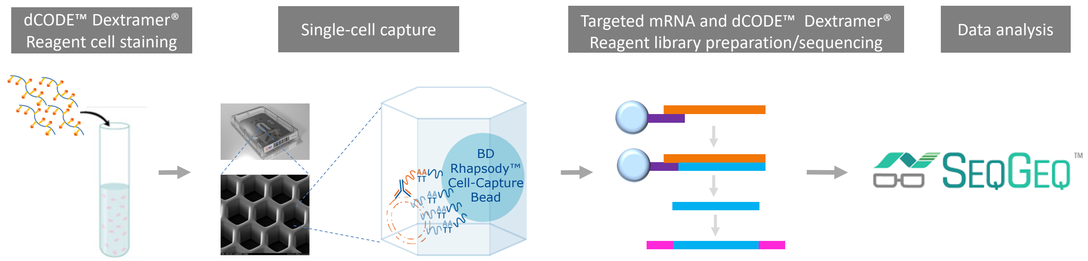
Adoptively transferred antigen-specific T cells have shown great efficacy in treatment of some virus-associated diseases and malignancies. A major driver of the development of adoptive T cell therapy has been our ability to successfully characterize the functional status and antigen specificity of T cells. However, this has been limited by inefficient detection of antigen-specific T cells possibly due to their low frequency and low binding affinities to known MHC-peptide complexes. Here, we aim to combine two powerful technologies, advanced dCODE™ Dextramer® Reagents from Immudex and single-cell multiomics analysis using the BD Rhapsody™ Single-Cell Analysis System, to detect and characterize disease-specific CD8⁺ T cells within thousands of PBMCs. Currently, we are able to identify over 350 mRNAs alongside a panel of over 20 BD® AbSeq Cell Surface Protein Markers that can be associated with T cell activation states. These data can be used to define T cell phenotypes alongside antigen specificity of enriched CD8⁺ Dextramer⁺ cells from a PBMC population. This study outlines our ability for high-resolution T cell profiling that has broader implications and utility in immuno-oncology, infectious diseases and autoimmunity.

Approach

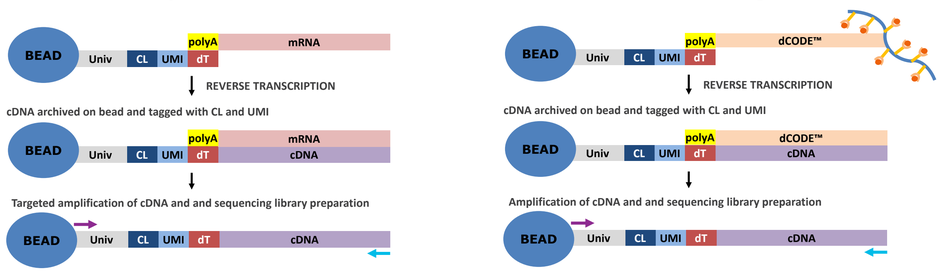
1A **Immudex dCODE™ Dextramer® Reagents design compatible with the BD Rhapsody System**



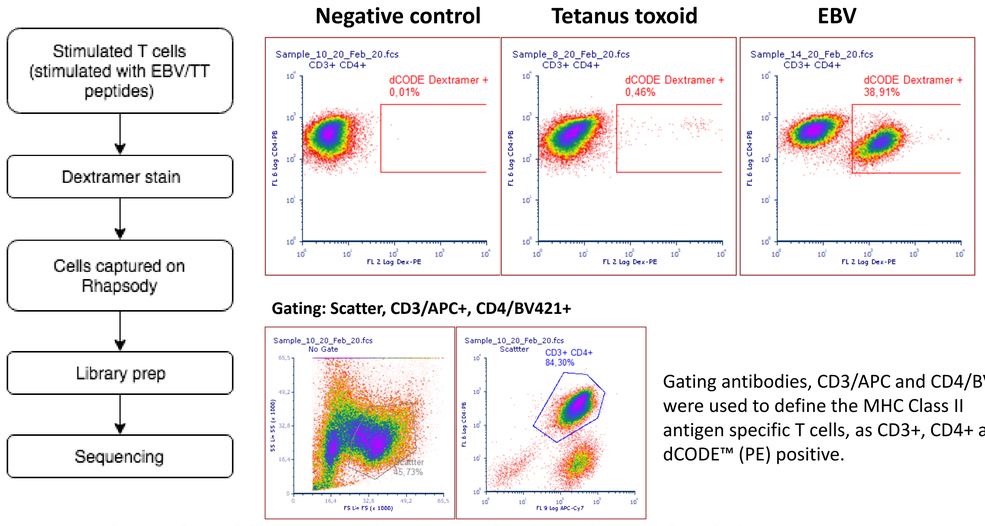
1B **BD Rhapsody System targeted workflow with dCODE™ Dextramer® Reagents**



1C **Beads with hybridized mRNA and dCODE™ retrieved from cartridge**



1D **Experimental Design: Human PBMCs stained with 3 Dextramers®**



Gating antibodies, CD3/APC and CD4/BV421 were used to define the MHC Class II antigen specific T cells, as CD3+, CD4+ and dCODE™ (PE) positive.

Figure 1. Experimental workflow and molecular mechanism of Immudex dCODE™ Dextramer® Reagents and BD Rhapsody System single-cell sequencing. (A) dCODE™ Dextramer® Reagents are compatible with the BD Rhapsody System. They contain a dCODE™-specific barcode that is associated with a specific antigen epitope that can be amplified and sequence simultaneously along with targeted mRNA. **(B)** and **(C)** Cells are first stained with a panel of dCODE™ Dextramer® Reagents followed by single-cell capture on the BD Rhapsody System that uses microwells to isolate individual cells prior to cell lysis. Upon lysis polyadenylated sequences from mRNA and dCODE™ Dextramers® (as shown in C) are captured on the beads. cDNA and library preparations for sequencing are completed followed by data analysis using SeqGeq™ Software **(D).** In this study, hPBMCs were stimulated with two peptide antigens, EBV and Tetanus toxoid (TT). Following stimulation the cells were simultaneously stained with dCODE™ EBV- and TT-specific Dextramers® as well as a negative control Dextramer® before capture on the BD Rhapsody System. Dextramer®-specific populations were examined using the BD Rhapsody System as well as by FACS, where the cells were labeled individually with the same dCODE™ Dextramer® Reagents. dCODE™ Dextramer® specificities were DRB1*0101, with the following antigen peptides: EBV / TSLYNLRRGTALA, TT / KIYSYFPSVISKV, Negative Control Clip / PVSKMRMATPLLMQA.

Results

Dextramer® Reagents can be detected on specific cell populations distinct from negative control

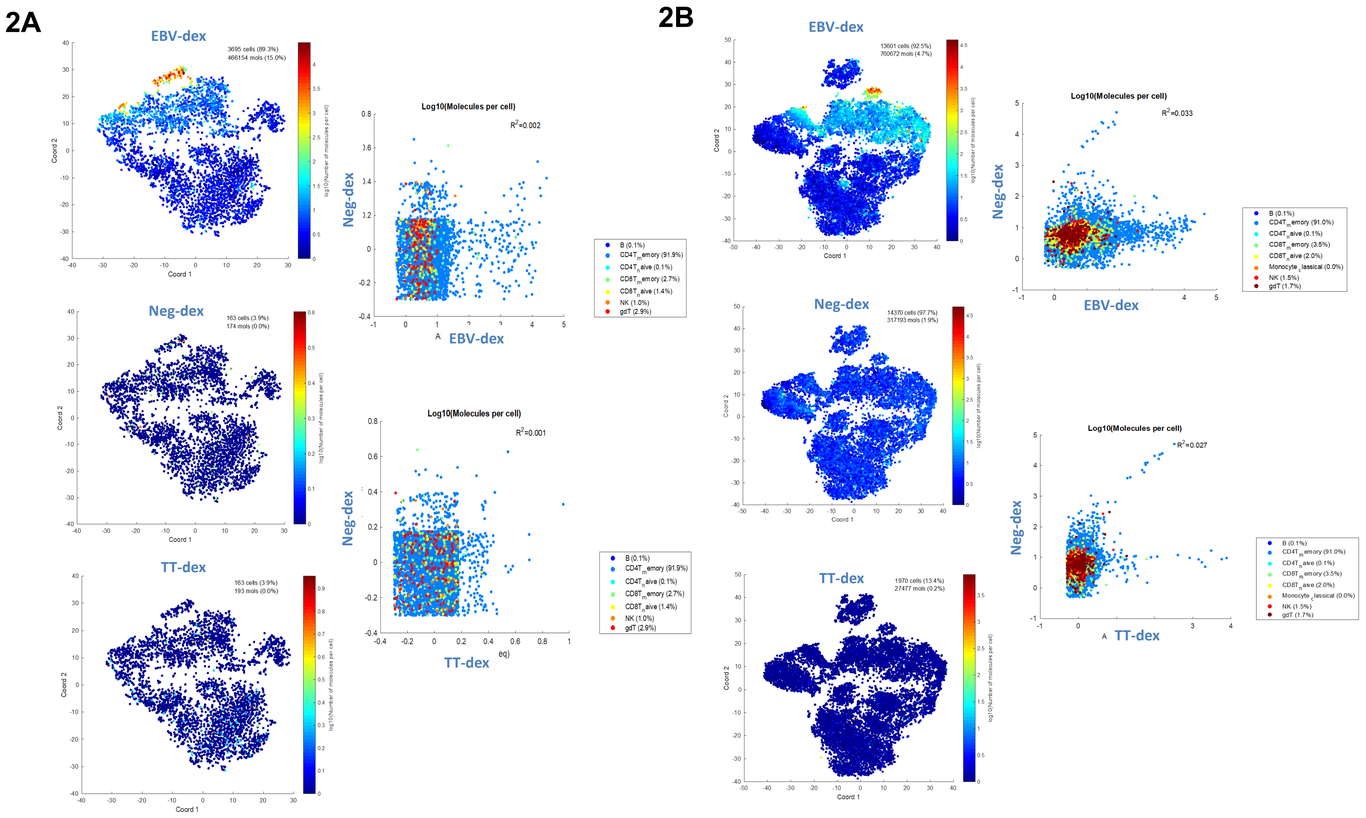
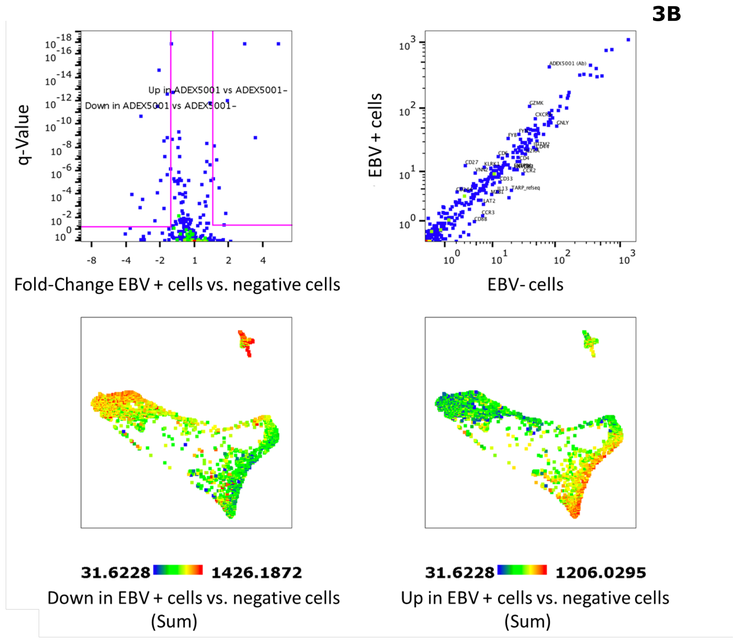


Figure 2. Antigen-specific T cells in hPBMC samples were identified using dCODE™ Dextramer® Reagents. Detection of EBV and TT dCODE™ Dextramer® shown in tSNE plots from two independent experiments using 6,000 cells in **(A)** and 30,000 cells in **(B)**. Neg-dex represent nonspecific binding from a negative control that can be distinguished from both EBV and TT dCODE™ Dextramers®.

Differential gene expression analysis shows a distinct transcriptional profile in dCODE™ Dextramer® positive T cells

3A



3B

Up Regulated

Feature	FC	q-Val	Mean Test	Mean Ctrl
ADEX5001 (Ab)	5.46	1.00E-17	435.06	78.88
CD27	3.43	1.24E-09	13.24	3.16
GZMK	2.76	1.00E-17	109.78	39.08
FYB	1.93	7.81E-13	34.83	17.57
VNN2	1.87	3.72E-03	9.96	4.86
CD244	1.83	3.82E-02	4.59	2.05
KLRK1	1.64	2.26E-05	12.24	7.09
FYN	1.56	1.00E-07	41.43	26.24
CXCR4	1.49	6.19E-09	72.09	48.18
CD6	1.46	4.27E-06	18.44	12.28

Down Regulated

Feature	FC	q-Val	Mean Test	Mean Ctrl
TARP_refseq	0.28	8.56E-05	4.91	19.98
CCR3	0.33	1.20E-03	1.48	6.49
CCR2	0.34	1.64E-11	9.66	30.56
CD8B	0.35	2.25E-04	0.99	4.71
IL13	0.45	1.67E-02	4.70	11.79
IFITM3	0.47	2.31E-12	11.12	24.76
LAT2	0.47	6.03E-05	2.67	6.75
CD44	0.49	1.78E-15	23.67	49.48
MZB1	0.50	5.44E-06	4.12	9.28
GNLY	0.53	1.29E-03	54.51	103.01
HAVCR2	0.54	2.36E-09	11.21	21.76
ENTPD1	0.55	4.72E-08	11.62	21.98
CD33	0.57	1.84E-06	6.97	12.96
CD4	0.57	1.25E-09	15.18	27.29
IFITM2	0.58	2.09E-13	25.20	44.44
IL2RA	0.62	5.75E-07	20.00	33.08

Figure 3. Gene expression analysis in EBV+ antigen specific T cells. Differential gene expression between Dextramer® EBV+ population vs. EBV-Negative can be identified. The sum of molecules from the upregulated and downregulated genes can be found in opposite coordinates in the TriMap space respectively showing that these cells have distinct transcriptional profiles.

Next Steps

- dCODE™ Dextramer® Reagents along with BD AbSeq Antibodies will be used to detect multiple antigen specific T cell populations in donor samples using the BD Rhapsody System. BD Targeted mRNA and AbSeq Antibody Panels will enable deep characterization of disease specific T cells in these donor samples.
- dCODE™ Dextramer® Reagents will also be used together with the BD Rhapsody CDR3 VDJ Single-Cell Protocol to provide the sequences of the variable regions of the detected disease-specific T cell receptors.

Conclusions

* dCODE™ Dextramer® Reagents are compatible with the BD Rhapsody System and can be used alongside targeted mRNA panels to identify antigen-specific cells.
* All dCODE™ Dextramer® Reagents used to stain cells were detected in sequencing experiments and further verified by FACS.
* We were able to identify distinct cell populations that have expression of positive but not negative Dextramers® even with low frequencies of antigen-specific T cells.