dCODE™ Dextramer® a new multimer technology for T-cell epitope profiling and single cell deep phenotyping using DNA, transcriptomic and genetic sequence analysis, on single cells, compatible with 10x Genomics Chromium workflow.

Kivin Jacobsen[1] , Liselotte Brix[1], Stéphane C. Boutet[2], Dagmar Walter[2], Josephine Y. Lee[2], Sarah E. B. Taylor[2], Katherine A. Pfeiffer[2]
[1]Immudex, Copenhagen, Denmark, [2]10x Genomics, CA, US

#116

Introduction:

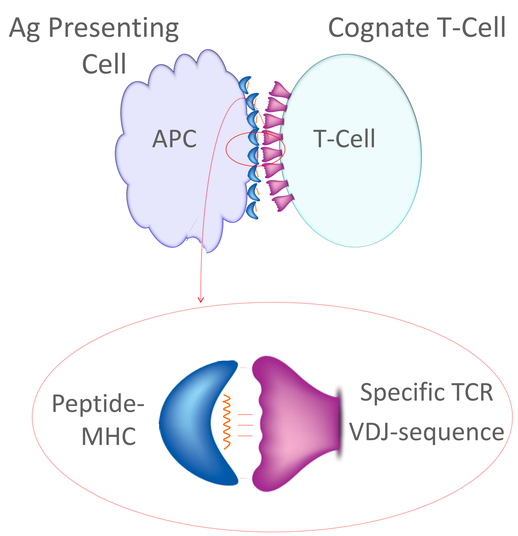
Identification of disease-specific T-cell epitopes is key to the development of many novel vaccines and immunotherapies. Profiling disease-specific T cells, emerging during a cellular immune response e.g. in tumor development or destruction, is an important aspect of personalized immunotherapy.

We have developed a technology, dCODE™Dextramer® (10x Compatible) for detection of antigen specific T-cells through DNA barcode and next generation sequencing.

Here we show data from staining with a library of 10 MHC dCODE™Dextramer® specificities, and two negative controls.

Due to the coupled surface staining of specific TCR, and the transcriptional analysis of the T-cells, paired peptide-MHC specificity and TCR α and ß chains are identified.

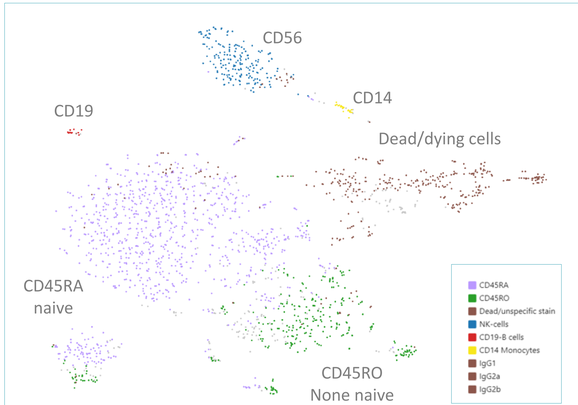
Paired TCR and VDJ TCR sequencing



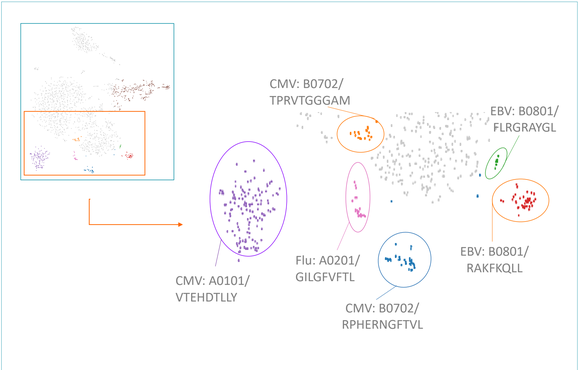
t-SNE representation of data

Data were clustered by gene expression, and by surface markers.
As the cells were sorted mostly T-cell markers are present. However a few cells of non T-cell linage are identified within the sorted sample. Population of "dead cells" (brown) correlated with high binding of all reagents, inc. the negative controls (antibody and dCODE Dextramer). Distinct detection of all Ag specific T-cells between CD45RA+(naïve) and CD45RO+(non-naïve) cells are evident, as expected for chronic or reoccurring virus infections.

Antibody t-SNE



Antigen specific T-Cell clusters



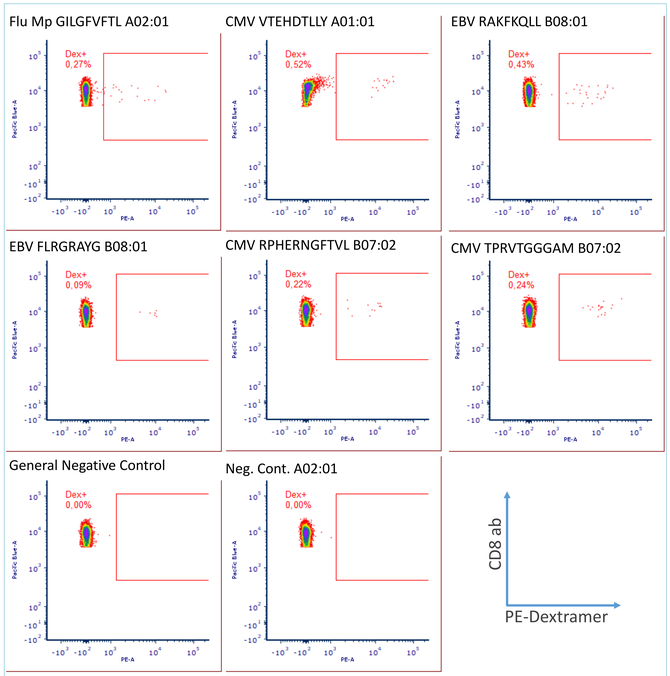
dCODE™Dextramer®panel, and staining

A HPBMC sample from a healthy donor (HLA-A01:01, HLA-A02:01, HLA-B07:02, HLA-B08:01) were stained according to established protocol, using a panel of 10 virus specific, and two negative control MHC dCODE™Dextramer® reagents, in combination with TotalSeq™C antibodies. (CD3, CD4, CD8a, CD14, CD56, CD19, CD25, CD45RA, CD45RO, Isotype-IgG1, Isotype-IgG2, Isotype-IgG2b from Biolegend).
The stained cells were sorted for MHC-dCODE positive cells (PE label) and loaded onto a 10x chromium controller using the Chromium single cell V(D)J Reagent kit with feature barcoding technology 10x genomics user guide (CG000186).
Analysis of the resulting next generation sequencing (Illumina) data, were performed using Cell Ranger, performing the, dimensional reduction, clustering, and t-SNE calculation, Loupe Cell Browser, and Loupe V(D)J Browser were used for visual analysis (all software by 10x Genomics).

dCODE™Dextramer®panel

Cat.no.	HLA	Peptide	Antigen
WB2132-PfBC	A0201	NLVPMVATV	pp65/CMV
WB2161-PfBC	A0201	GILGFVFTL	Flu MP/Influenza
WH2166-PfBC	B0702	RPPIFIRRL	EBV
WA2131-PfBC	A0101	VTEHDTLLY	CMV
WB2130-PfBC	A0201	GLCTLVAML	EBV
WB2144-PfBC	A0201	CLGGLLTMV	EBV
WI2148-PfBC	B0801	RAKFKQLL	EBV
WI2147-PfBC	B0801	FLRGRAYGL	EBV
WH2135-PfBC	B0702	RPHERNGFTVL	CMV
WH2136-PfBC	B0702	TPRVTGGGAM	CMV
NI3133-PfBC	B0801	AAKGRGAAL	gen. neg. control
WB2666-PfBC	A0201	ALIAPVHAV	A0201 neg .cont

Single MHC-Dextramer flow staining

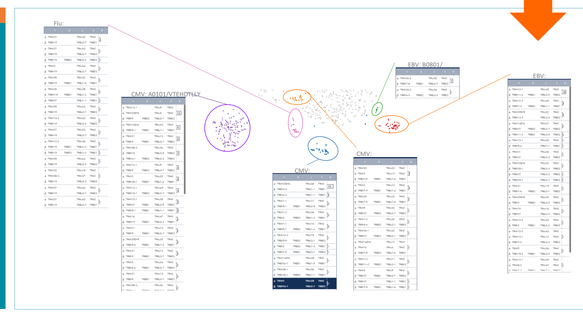


TCR clonotypes identified

Allele	A0201	A0101	B0801	B0801	B0702	B0702
Peptide	GILGFVFTL	VTEHDTLLY	RAKFKQLL	FLRGRAYGL	RPHERNGFTVL	TPRVTGGGAM
Antigen	Flu MP	CMV	EBV	EBV	CMV	CMV
TCR 1	4	12	9	7	15	5
TCR 2		9	3		3	2
TCR 3		8	3		2	2
TCR 4		6	2		2	
TCR 5		6	2		2	
TCR 6		6				
TCR 7		5				
TCR 8		4				
TCR 9		4				
TCR 10		3				
TCR 11		3				
TCR 12		3				
TCR 13		2				
TCR 14		2				
TCR 15		2				
TCR 16		2				
Single	15	23	15	1	6	8

TCR1-16 represent unique TCR clonotypes identified for each pMHC specificity. Numbers represent number of identical clonotype for each specificity. "Single" is the number of clonotypes only represented once for each specificity.
Only pMHC Dextramers that were found positive within the sample are recorded.

Paired TCR α ß chains identification



Summary:

Identification of pMHC specific TCR sequences reveals both know (reported in vdjdb.cdr3.net.) and new unknown TCR sequences for each MHC-Dextramer identified population of T-cells. This indicats high diversity of TCR's recognizing the same pMHC specificity. Identifying discrete cellular phenotypes that underlie immune receptor specificity and antigen-binding capabilities is critical for understanding of the adaptive immune response and its relation to disease.

Kivin Jacobsen, Immudex, Denmark , kj@immudex.com / CYTO 2019 poster #116