IMMUDEX®
PRECISION IMMUNE MONITORING

Dextramer® technology supporting the development of more potent and effective cell-based therapies

Valérie Bodemeyer, PhD, Technical Application Manager, Immudex
Mohammad Salem, PhD, Senior Pharma Business & Solution Manager, Immudex

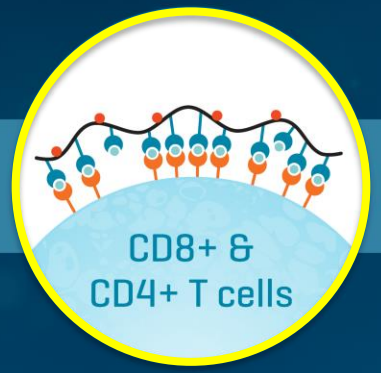
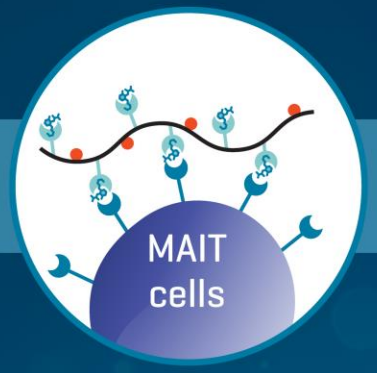
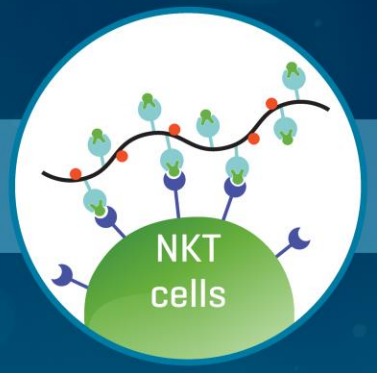
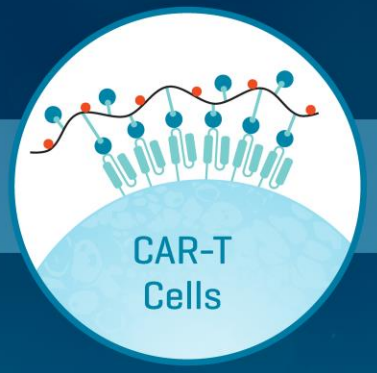
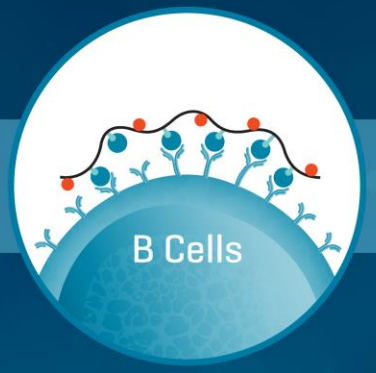
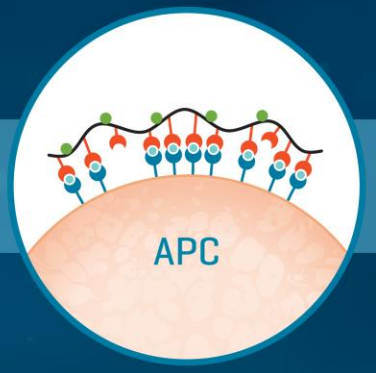
About us

- Reagents and Kits for antigen-specific cellular immune monitoring
- Services improving immune monitoring
  - Custom Solutions and Services
  - ***Coming soon*** *- Peptide-Binding Evaluation*
- Tools for exploring cellular immunity within:
  - Immuno-Oncology
  - Infectious Disease
  - Transplantation
  - Cellular Therapy
  - Autoimmunity
- From Basic research to Diagnostics
  - IVD Kit approved in EU + US
  - Clinical Grade (GMP) reagents

- Based in Copenhagen, Denmark
- North American operations based in Virginia, US

Enabling Characterization of Antigen-Specific Immune Cells

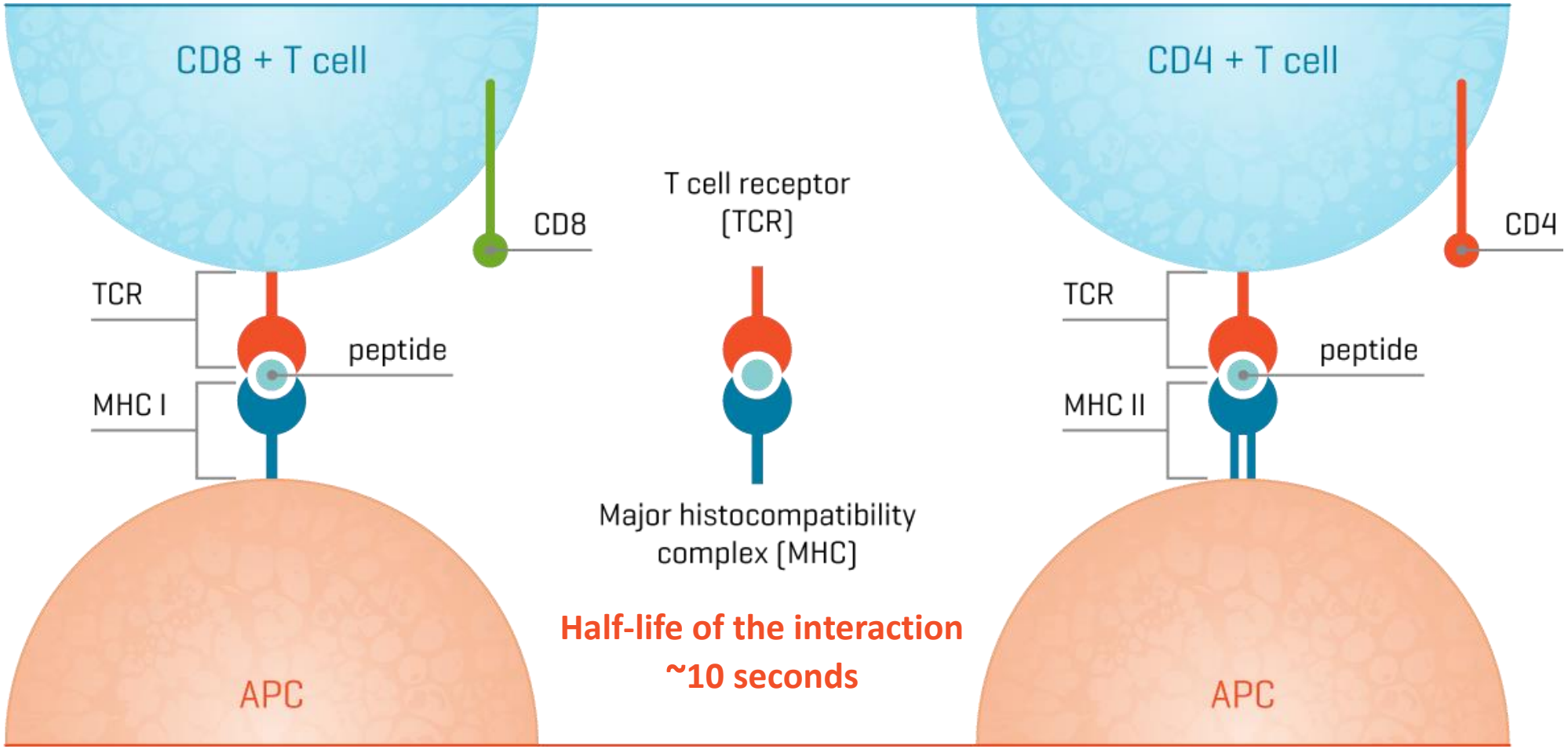
IMMUDEX®

Analyze antigen-specific T cells	Probe the unconventional T cell response		Create high-avidity multimers with any biotinylated molecule		Characterize your TCR and track antigen-presenting cells
					
MHC I & MHC II Dextramer®	MR1 Dextramer®	CD1d Dextramer®	Customized Dextramer®	Klickmer®	TCR Dextramer®
GMP available					

Principles of MHC-Peptide and TCR Interaction

The peptide presented on **MHC-I** molecule will stimulate a **CD8+ T** cell response

The peptide presented on **MHC-II** molecule will stimulate a **CD4+ T** cell response



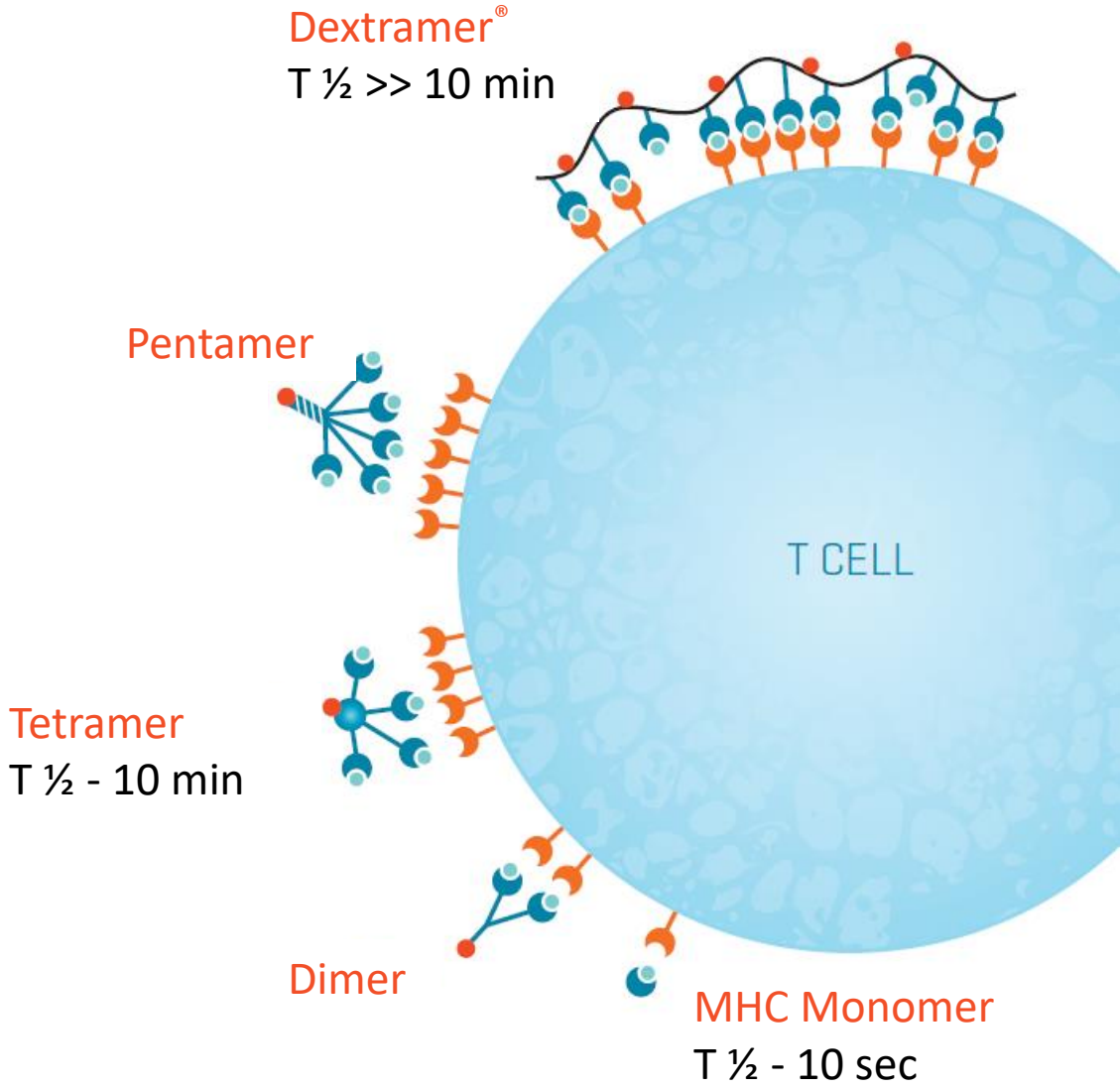
Half-life of the interaction ~10 seconds

For research use only. Not for use in diagnostic or therapeutic procedures

Advancement of MHC Multimer-Based Technologies

Advances in the MHC multimer field improve the detection of antigen-specific T cells:

- Higher-order multimers provide increased avidity in detection of antigen-specific T cells
- Higher-order dextran-based multimers enable detection of low-affinity T cells
- Increased number of fluorophores provides higher resolution when detecting T cells by flow cytometry
- DNA-barcoded multimers and NGS sequencing enhance multiplexing capabilities
- Single-cell multi-omics enables simultaneous acquisition of gene expression, phenotyping and antigen-specificity information

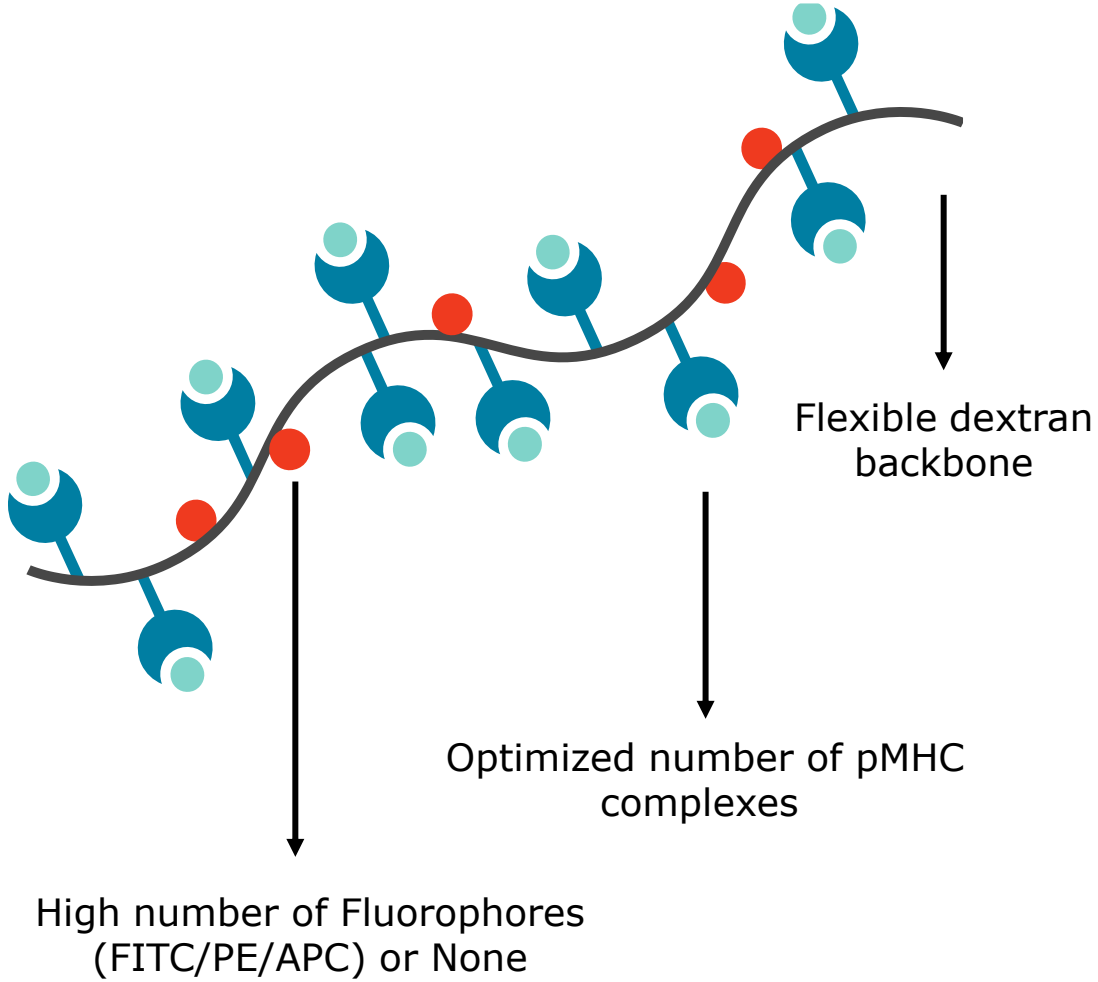


For research use only. Not for use in diagnostic or therapeutic procedures

MHC Dextramer® Technology – Reliable Detection of Antigen-Specific T-Cells by Flow Cytometry

Features of MHC Dextramer®

- Flexible dextran backbone provides stabilization of MHC Monomers
- Optimized number of MHC Monomers on dextran backbone enhances avidity
- High number of fluorophores provides enhanced resolution and minimal background staining
- High quality and consistency ensured by Immudex's quality control process



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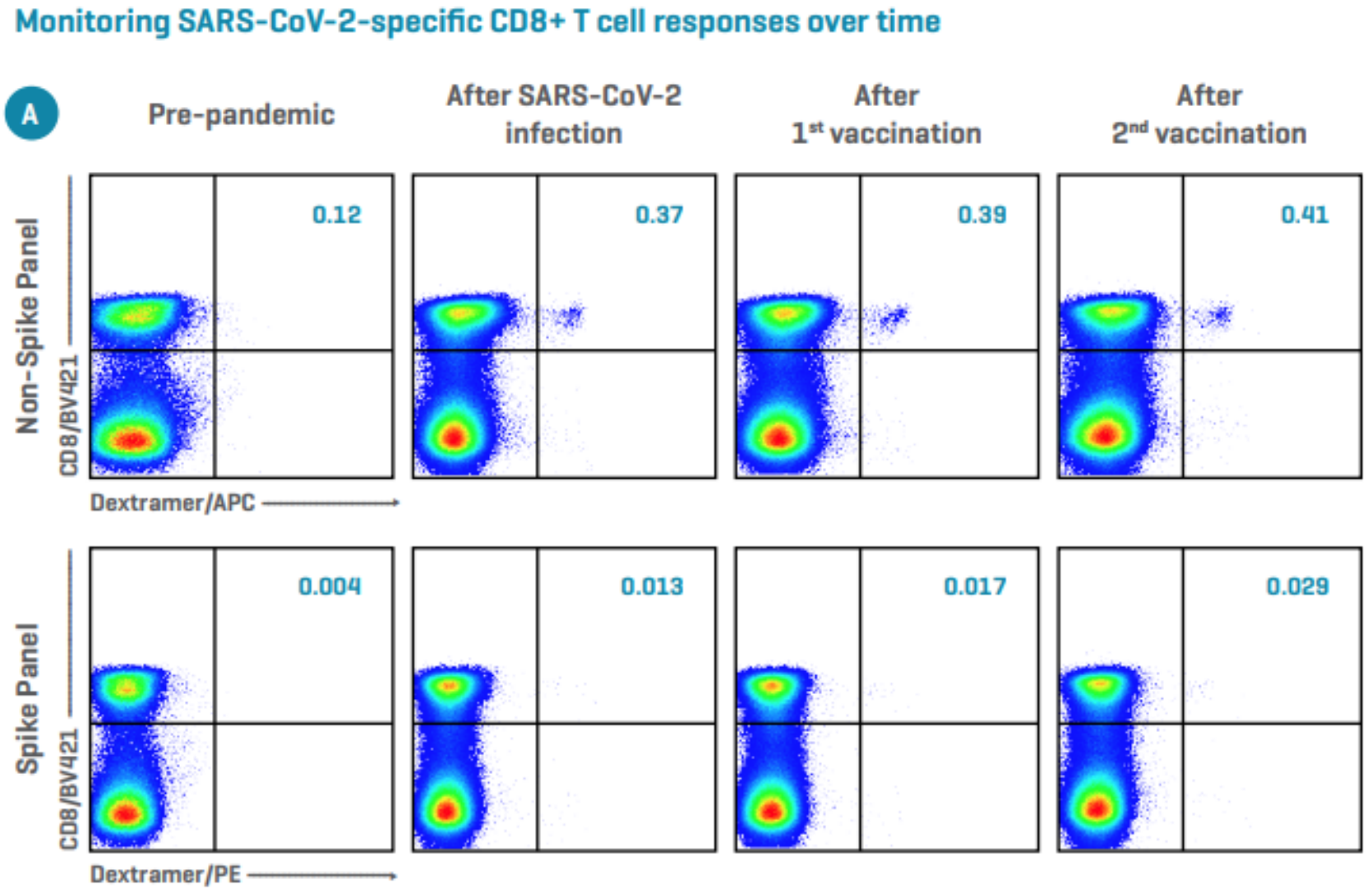
Example 1 – Longitudinal study of SARS-CoV-2 specific CD8+ T cell response



See full study here

Enabling Characterization of Antigen-Specific Immune Cells

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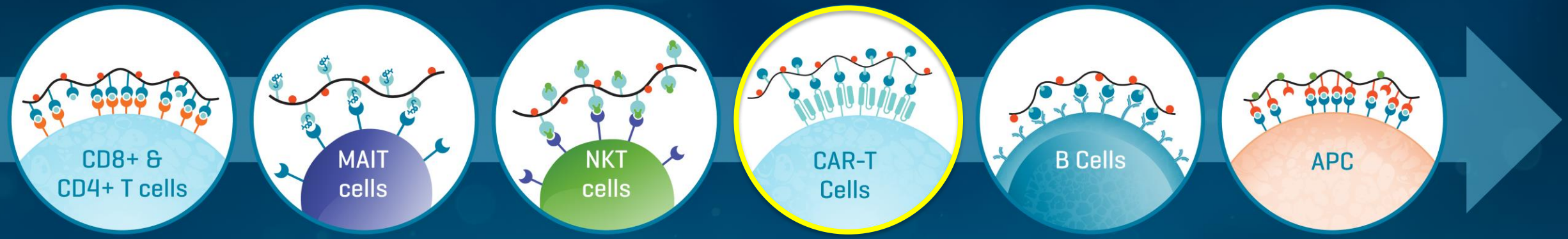
Probe the unconventional T cell response

Create high-avidity multimers with any biotinylated molecule

Characterize your TCR and track antigen-presenting cells



MHC I & MHC II Dextramer®

GMP available

MR1 Dextramer®

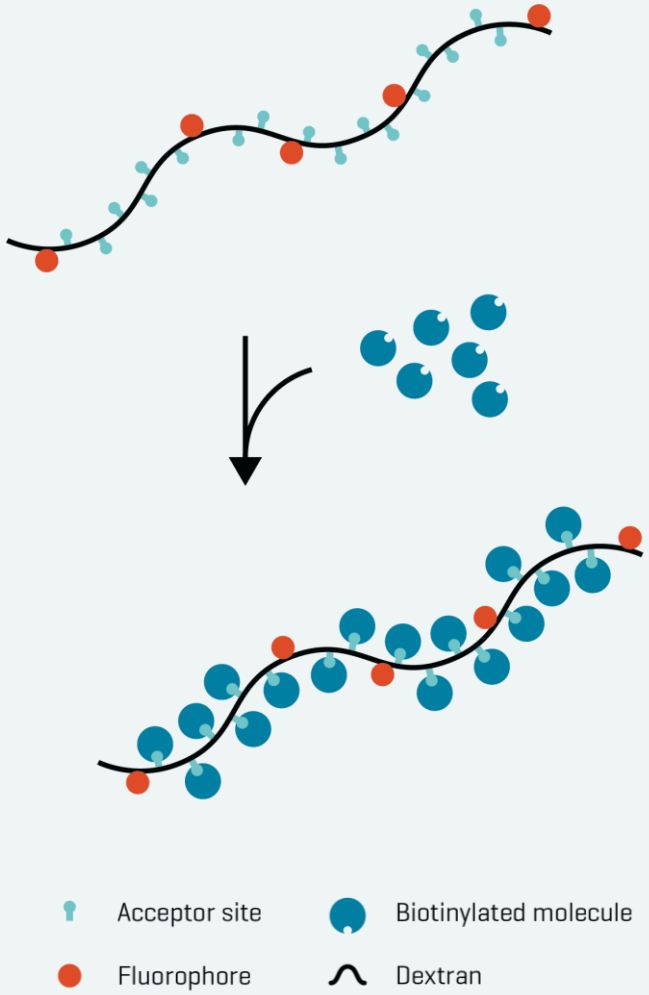
CD1d Dextramer®

Customized Dextramer®

Klickmer®

TCR Dextramer®

Klickmer® Technology



Customize your multimer

A variety of **biotinylated molecules** can be attached to the Klickmer™ enabling highly sensitive detection of antigen-specific immune cells.

Examples of molecules that can be bound, include:

- B-cell antigens
- TCRs
- Peptides
- Interleukins
- Immune stimulatory molecules
- Metal chelates/Lanthanides
- .. And much more!

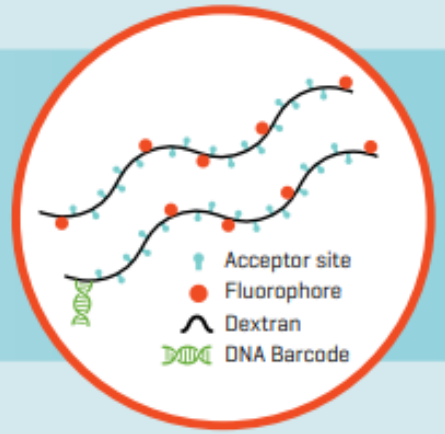
Immudex is the sole manufacturer and provider of dCODE Dextramer® reagents, and support related to these products is through Immudex. For research use only. Not for use in diagnostic or therapeutic procedures

Customize your reagent based on the prefered target

Choose your target antigen

CD19, BCMA, CD22...

Select reagent type



Flow cytometry:
BV421, FITC, PE, APC

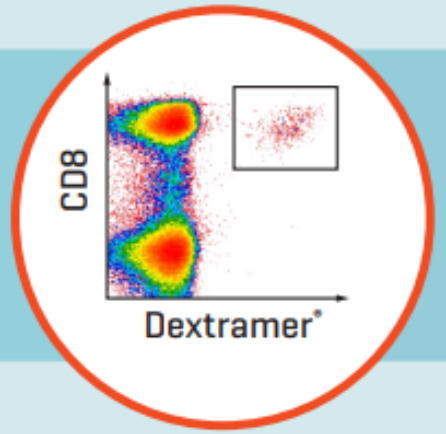
Single-cell multi-omics:
DNA barcode + PE

We make the reagent for you

Customized reagent design

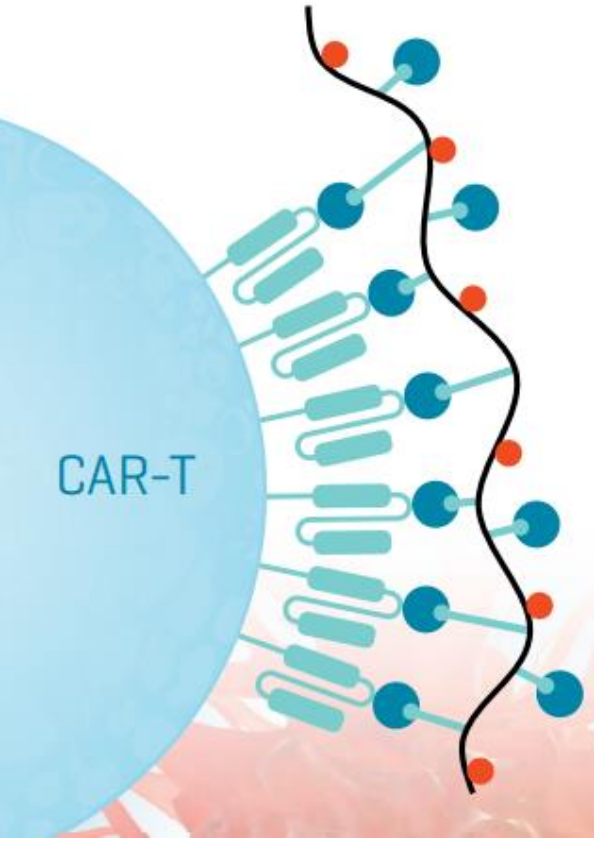
GMP possible

Quantify CAR-T cells



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

Applications in CAR-T Cell Therapy



- Direct CAR-T cell detection
- Enhanced sensitivity for low-affinity CAR-Ts, due to the high avidity of Dextramer® technology and multiple fluorophores
- Assessment of transduction levels
- Determine the % of CAR-T-positive cells
- Demonstrate that the infusion product meets defined lot release criteria
- Monitor kinetics and persistence of infused CAR-T cells in patient blood samples
- Deeper characterization of target-specific CAR-T cells with gene and surface marker expression by single-cell multi-omics

Example 2 – Detecting HLA-A2-specific CAR-Tregs

Study description

Goal: to examine the impact of IL-10 co-expression in engineered HLA-A2-specific CAR-Tregs.

Different CAR-Tregs were generated by lentiviral transduction of 4 different constructs: CAR specific to HLA-A2 alone (A2-CAR), IL-10 alone (IL10-poly), both constructs (IL10-A2-CAR), and neither construct (Poly). The transduction efficiencies were assessed by NIS-TRFP expression by FACS. Transduced cells were further expanded and assessed for phenotype and suppressive function.

To quantify the CAR expression, Tregs were stained with MHC I Dextramer® reagent specific for HLA-A2 /CINGVCWTV using flow cytometry, whereas IL-10 expression level was assessed by ELISA.

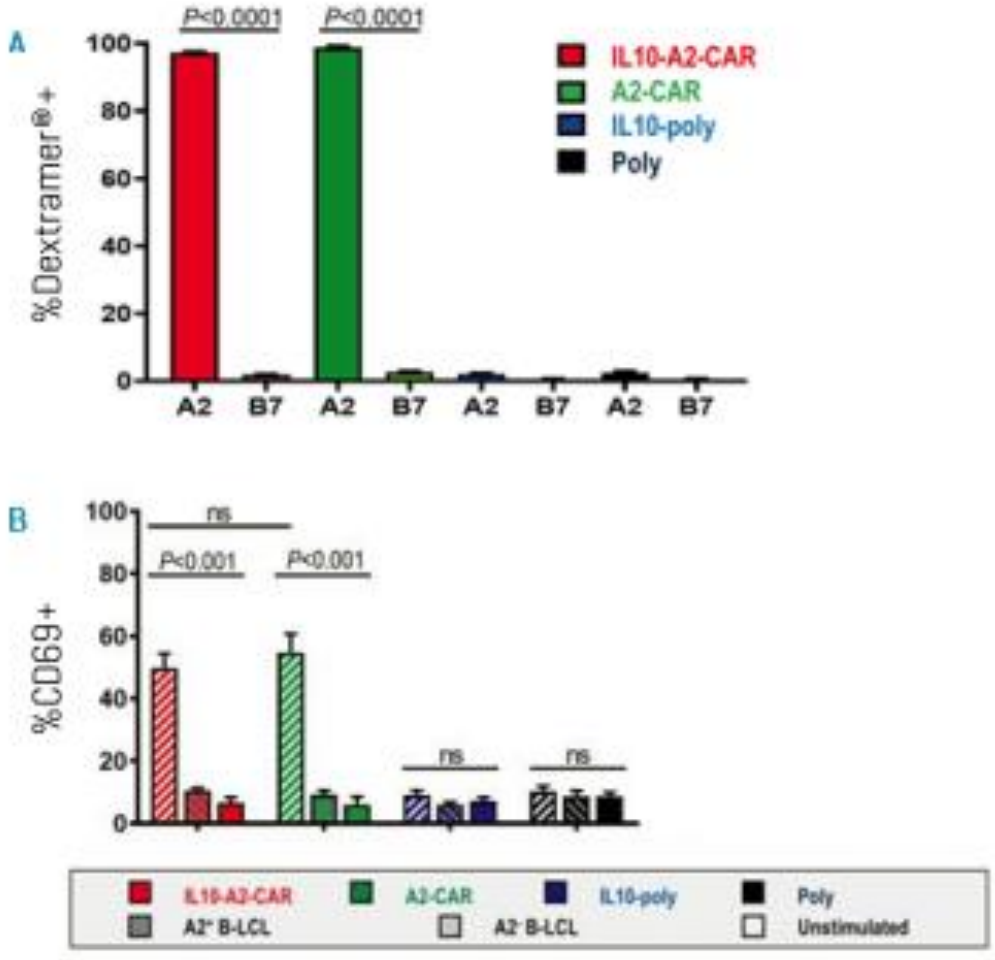


Fig.1.

A. HLA-A2/CINGVCWTV and HLAB7/APRGVRMAV (control) MHC I Dextramer® reagent were used to quantify CAR surface expression by flow cytometry.
B. Antigen-specific Treg activation was assessed by CD69 upregulation. Transduced Tregs were cultured with indicated B-LCLs for 18h followed by staining with anti-CD69 antibody and analysis by flow cytometry.

Mohseni et al. 2021 - European Journal of Immunology

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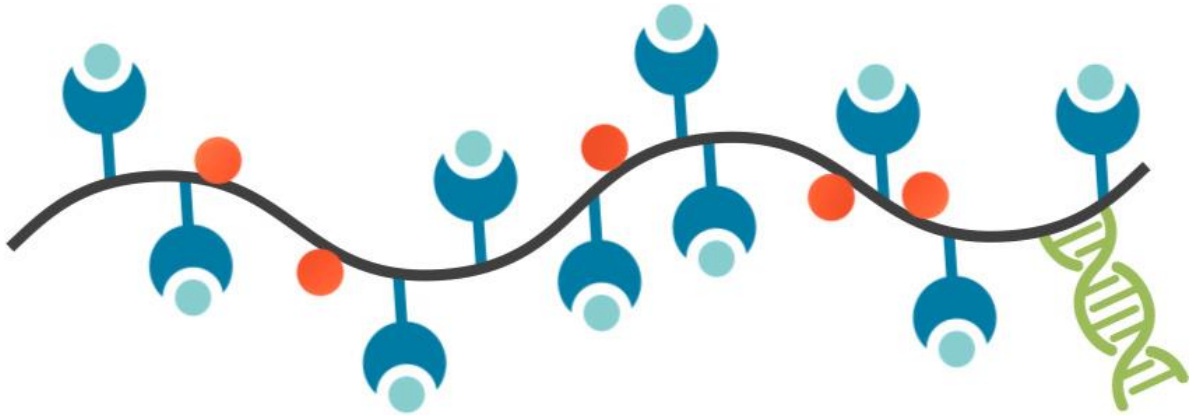
dCODE Dextramer® Technology – Unravelling the complexity of immune response through single-cell multiomics
IMMUDEX®

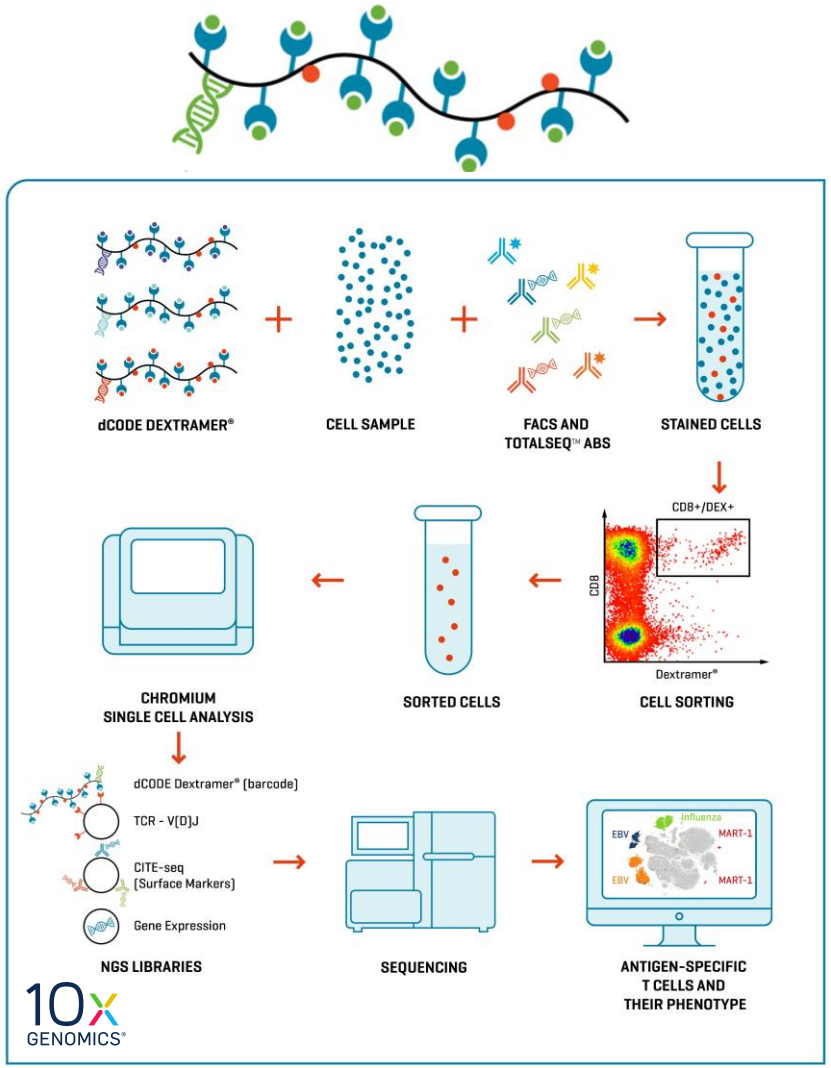
dCODE Dextramer® Technology
From Flow Cytometry to Single Cell Multiomics

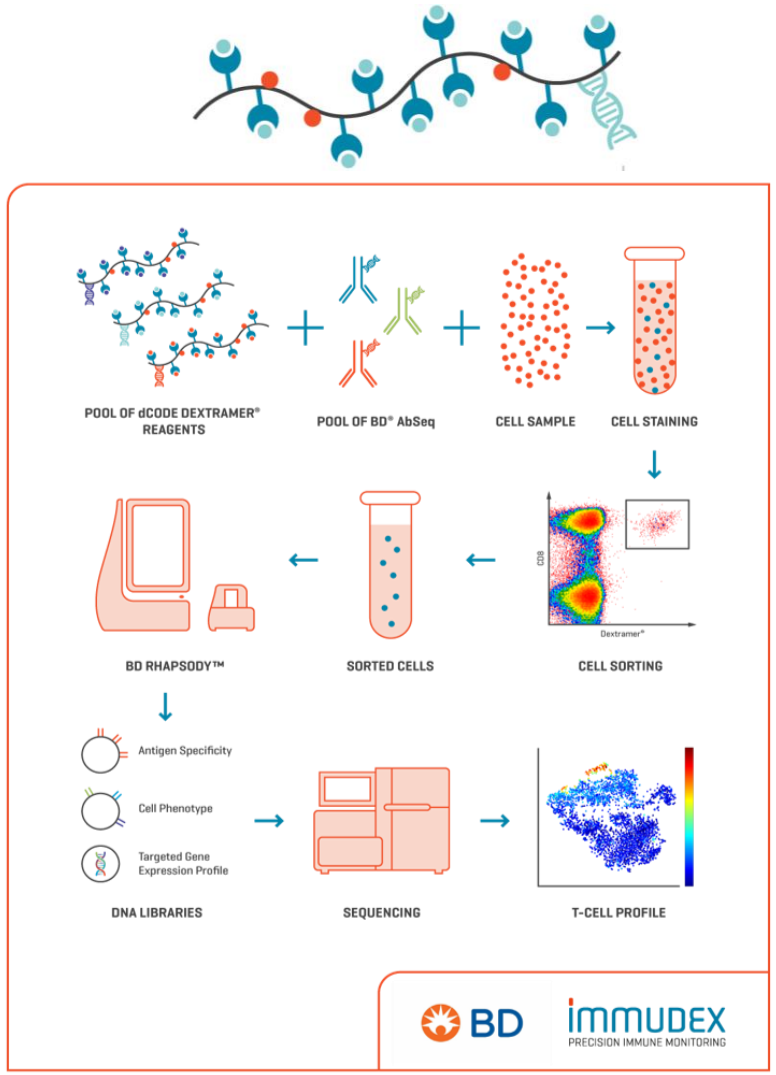


DNA barcode

- Unique DNA barcodes for each dCODE Dextramer®
- Tens to hundreds different dCODE Dextramer® used simultaneously
- PE fluorochrome for enrichment of low-frequency cells
- Can be combined with single-cell technologies (BD and 10x Genomics)

dCODE Dextramer®
10x and BD Workflows





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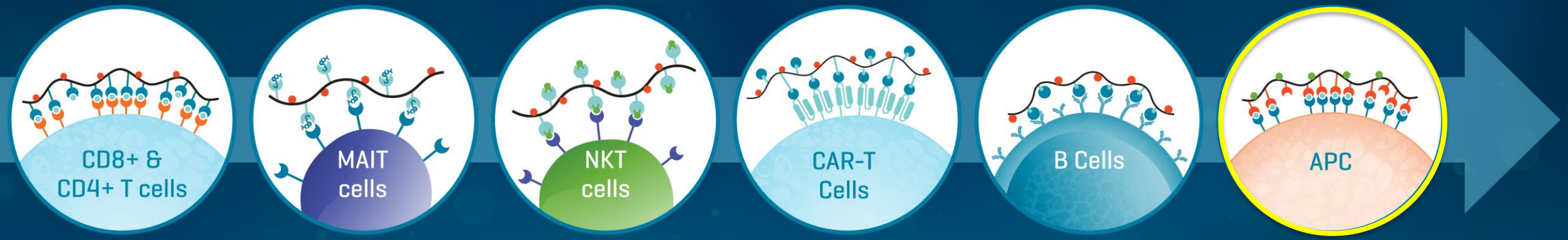

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GMP available

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CD1d Dextramer®

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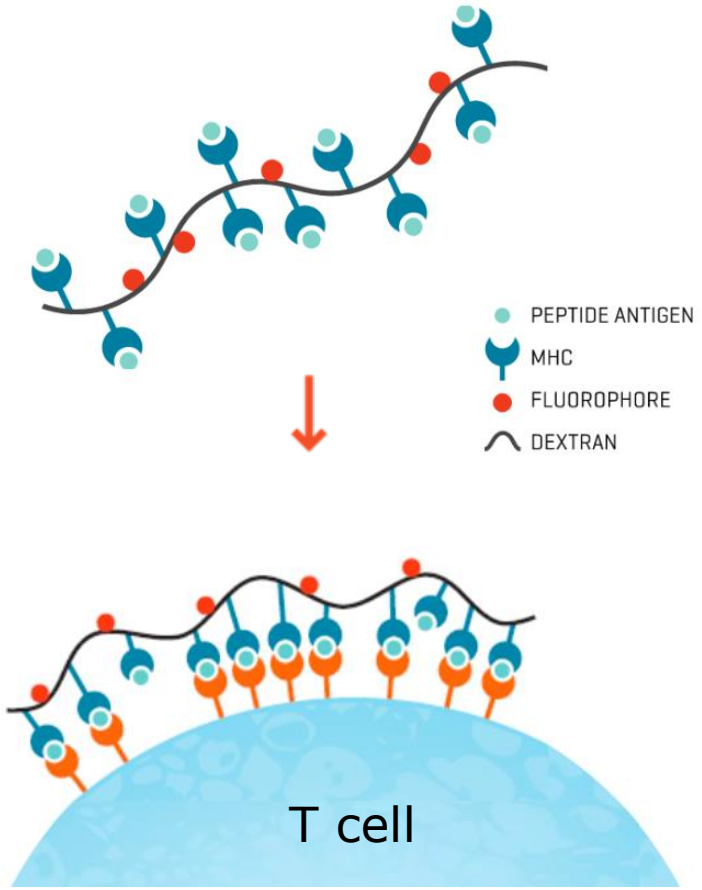
Klickmer®

TCR Dextramer®

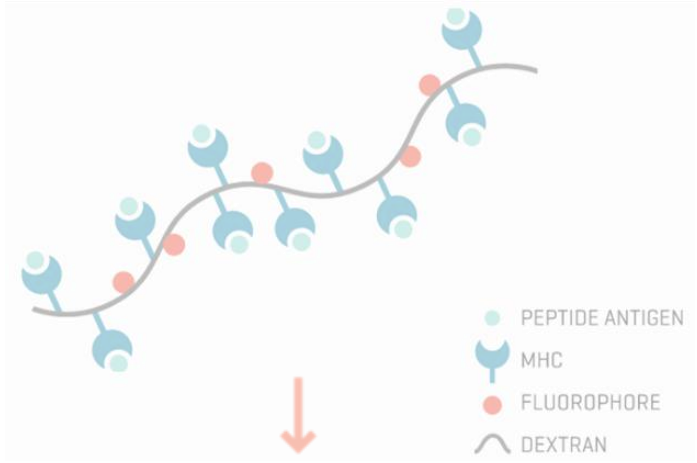
Detecting Antigen-Specific T Cells

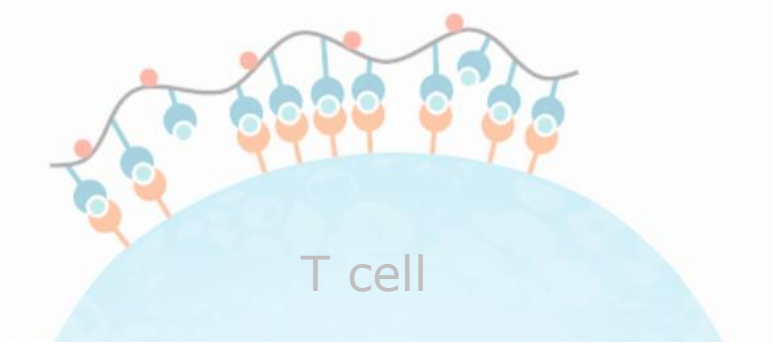
MHC Dextramer®



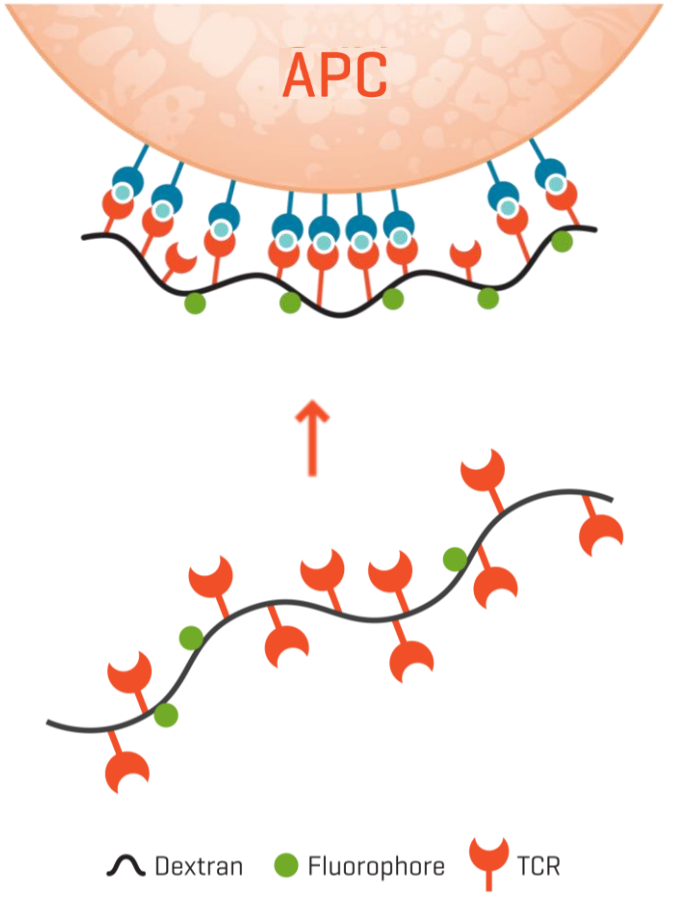
TCR Dextramer®

MHC Dextramer®





TCR Dextramer®

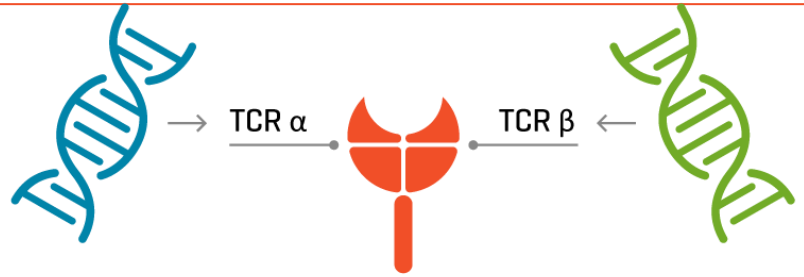


The Making of TCRs

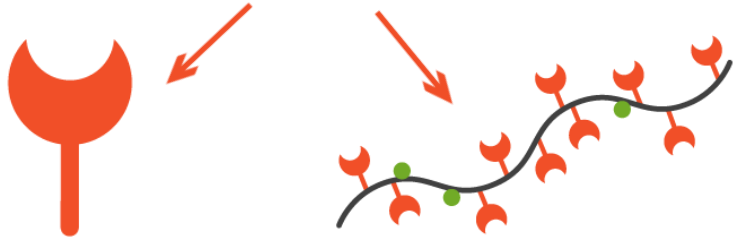
Published TCR sequences

or

Find TCR sequences with dCODE DEXTRAMER®

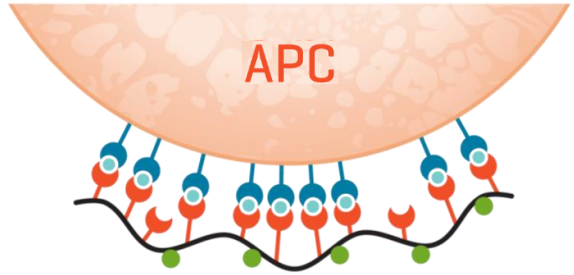


Immudex builds the TCR for you

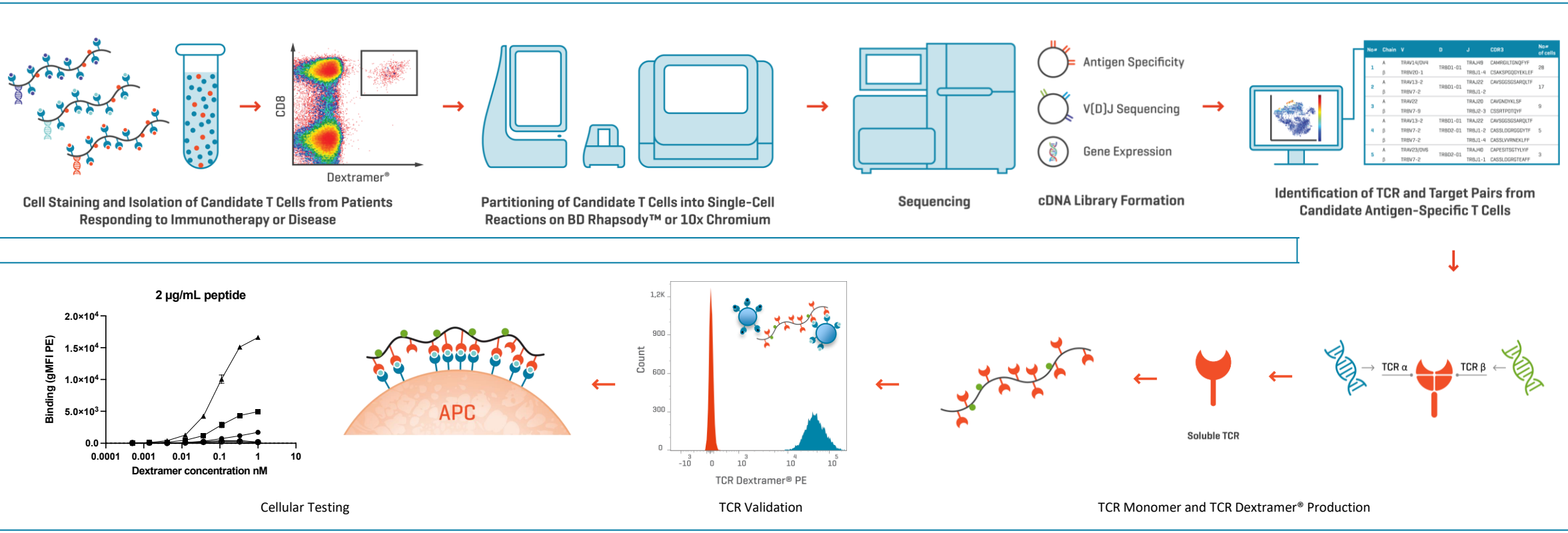


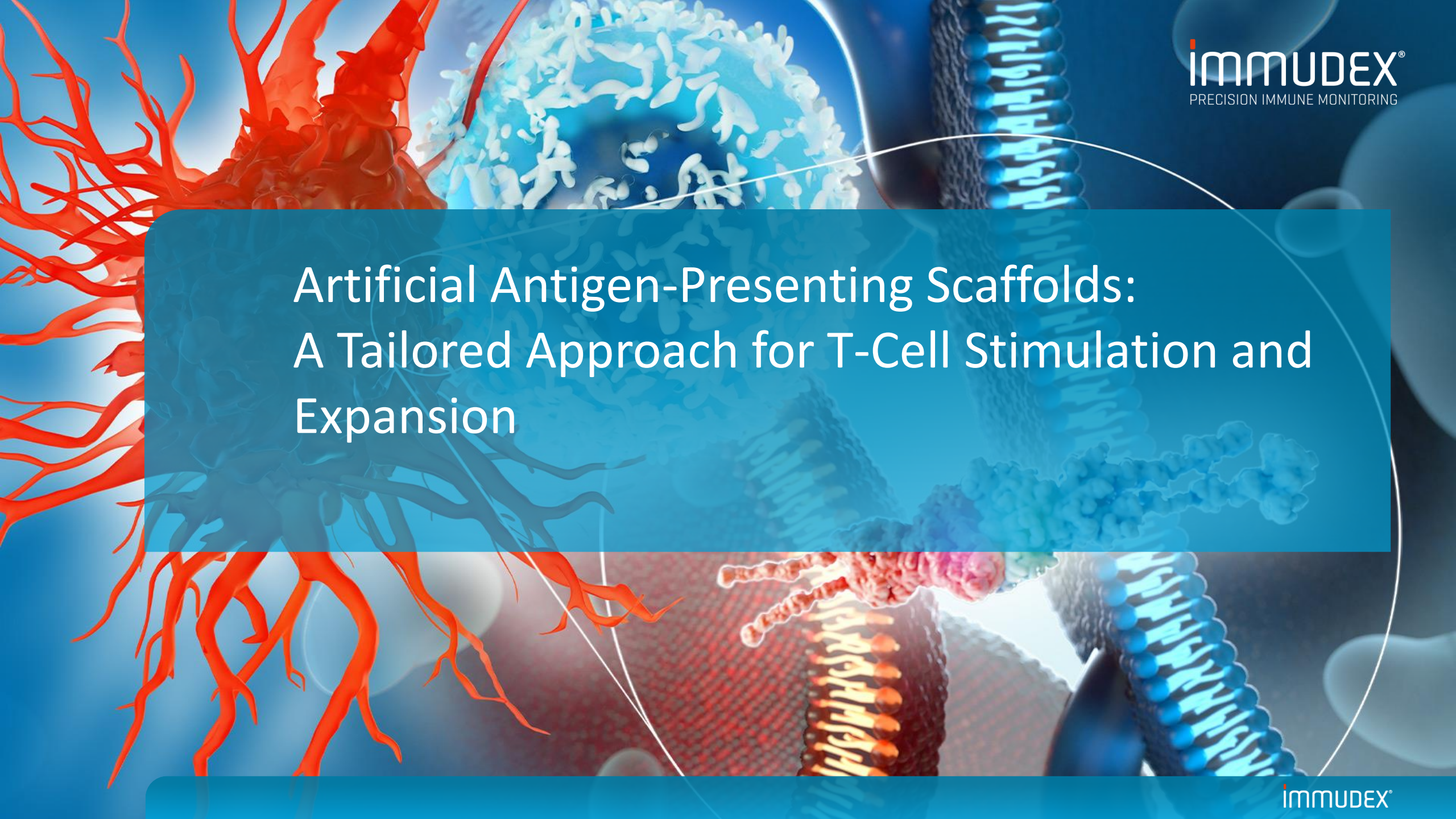
Soluble TCR

TCR-DEXTRAMER®



TCR DiVa – TCR Discovery & Validation
A complete workflow





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Artificial Antigen-Presenting Scaffolds: A Tailored Approach for T-Cell Stimulation and Expansion

PAX – New technology for evaluation of - Potency-Activation-eXpansion

AIM:

Develop robust reagent for stimulation and activation of specific T cells providing a technology:

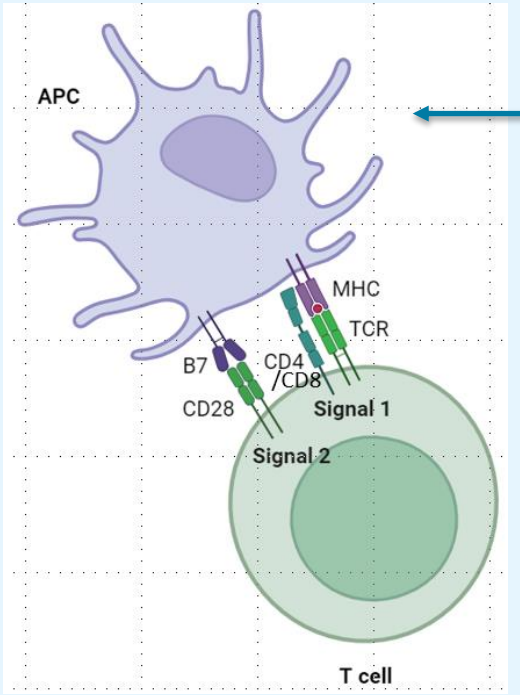
- Cell-free
- Simple
- Customized
- Standardized

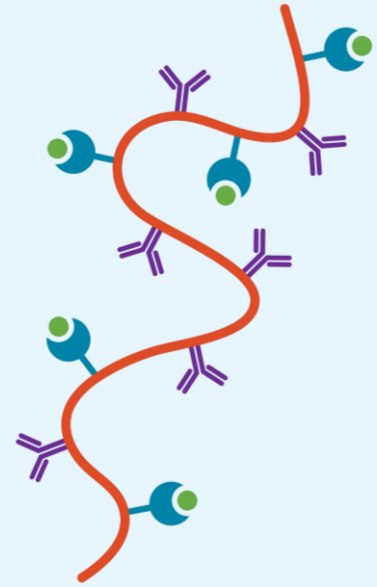
To be used to:

- Evaluate antigen-specific activation of T cells
- Expand antigen-specific T cells
- Explore effect of engineered co-stimulatory signals

Development Outline

- Construct artifical antigen-presenting scaffold (PAX reagent) able to stimulate antigen-specific T cells, providing:
  - Signal 1 – TCR-MHCp interaction
  - Signal 2 – CD28 engagement





Antigen-specific T cell activating scaffold

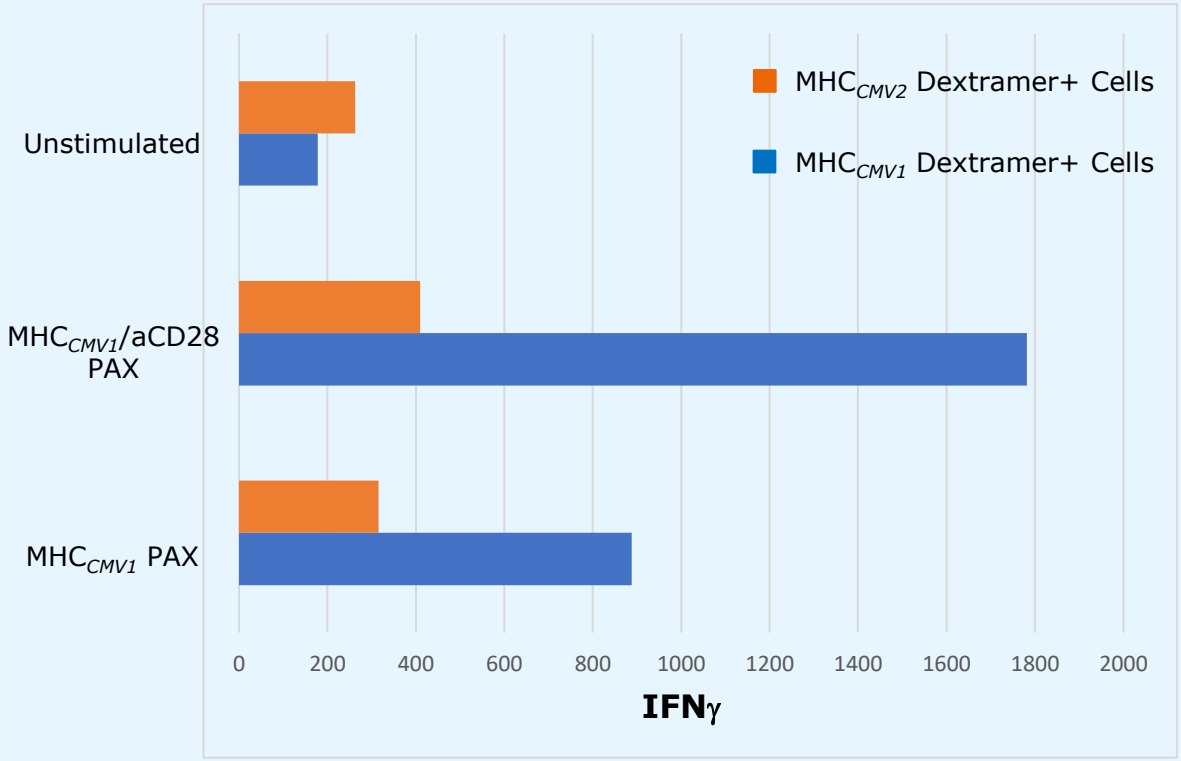
- Design assays evaluating
  - Potency and Activation
    - Activation marker on cell surface
    - Cytokine secretion
  - Expansion- Proliferation

MHCp/aCD28 PAX reagents can activate virus-specific T cells - inducing cytokine secretion

Evaluate induction of cytokine secretion

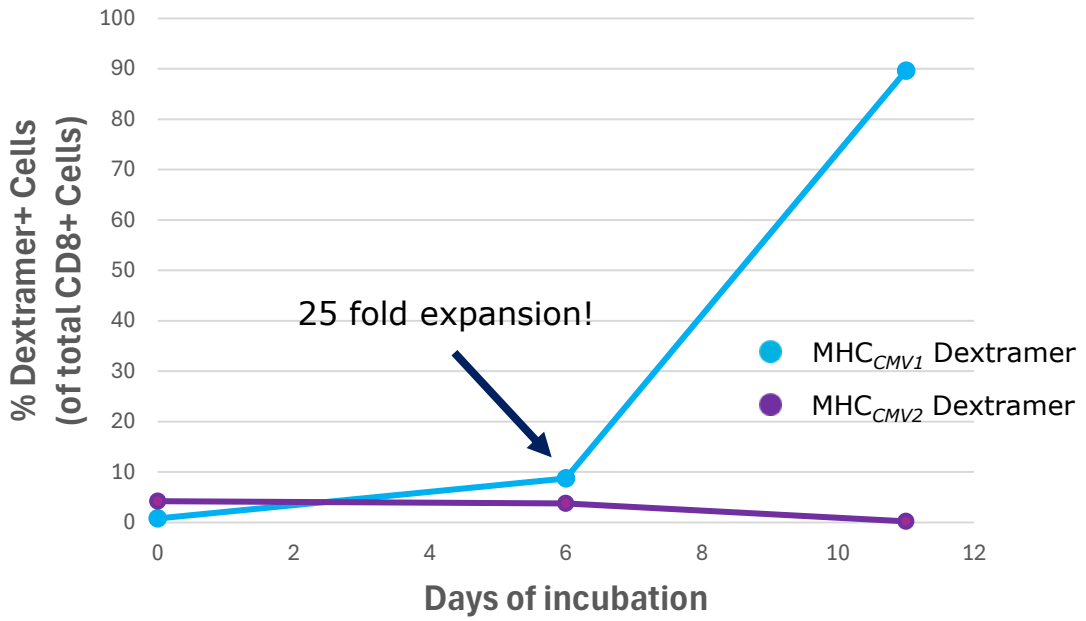
- CMV+ PBMC sample comprising T cell subsets specific for:
  - HLA-B*0702/TPRVTGGGAM (MHC_{CMV1})
  - HLA-B*0702/RPHERNGFTVL (MHC_{CMV2})
- Stimulate PBMC with:
  - MHC_{CMV1}/aCD28 PAX
  - MHC_{CMV1} PAX
  - No stimulation
- Assay - evaluate IFNγ production (Flow cytometry, ICS)
- **Conclusion**:
  - PAX reagent displaying MHCp + aCD28 can stimulate antigen-specific T cells to produce IFNγ
  - Highly antigen-specific
  - CD28 engagement improve activation

Stimulated CMV-specific T cells produce IFNγ

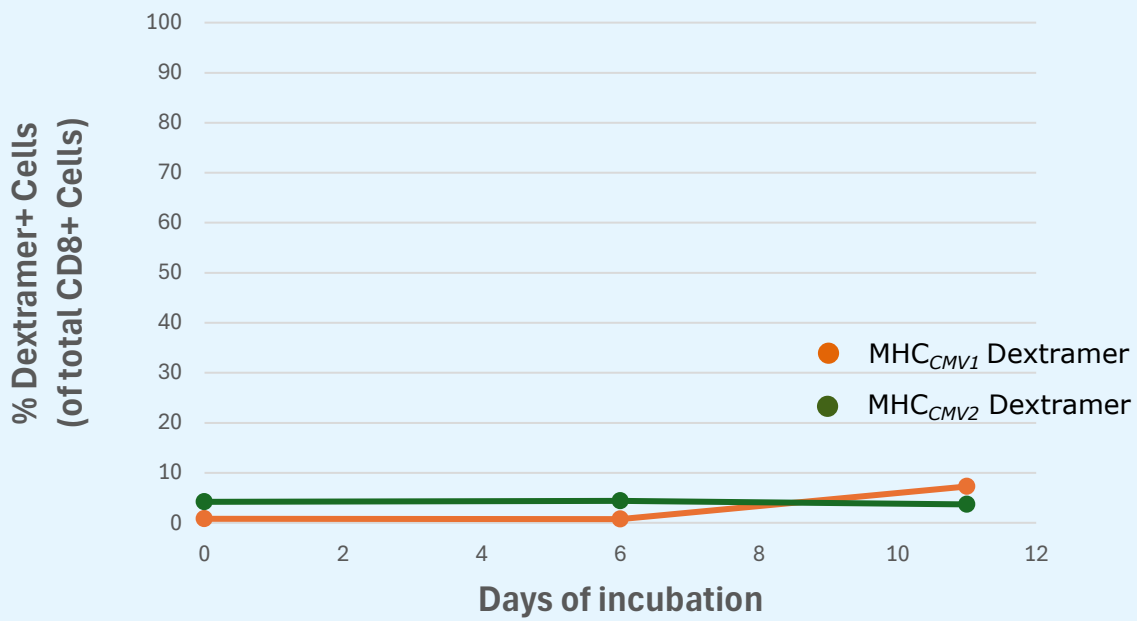


Antigen-specific expansion of T cells with PAX reagent

Stimulation with MHC_{CMV1} /aCD28 PAX reagent



Stimulation with MHC_{CMV1} PAX reagent (no aCD28)



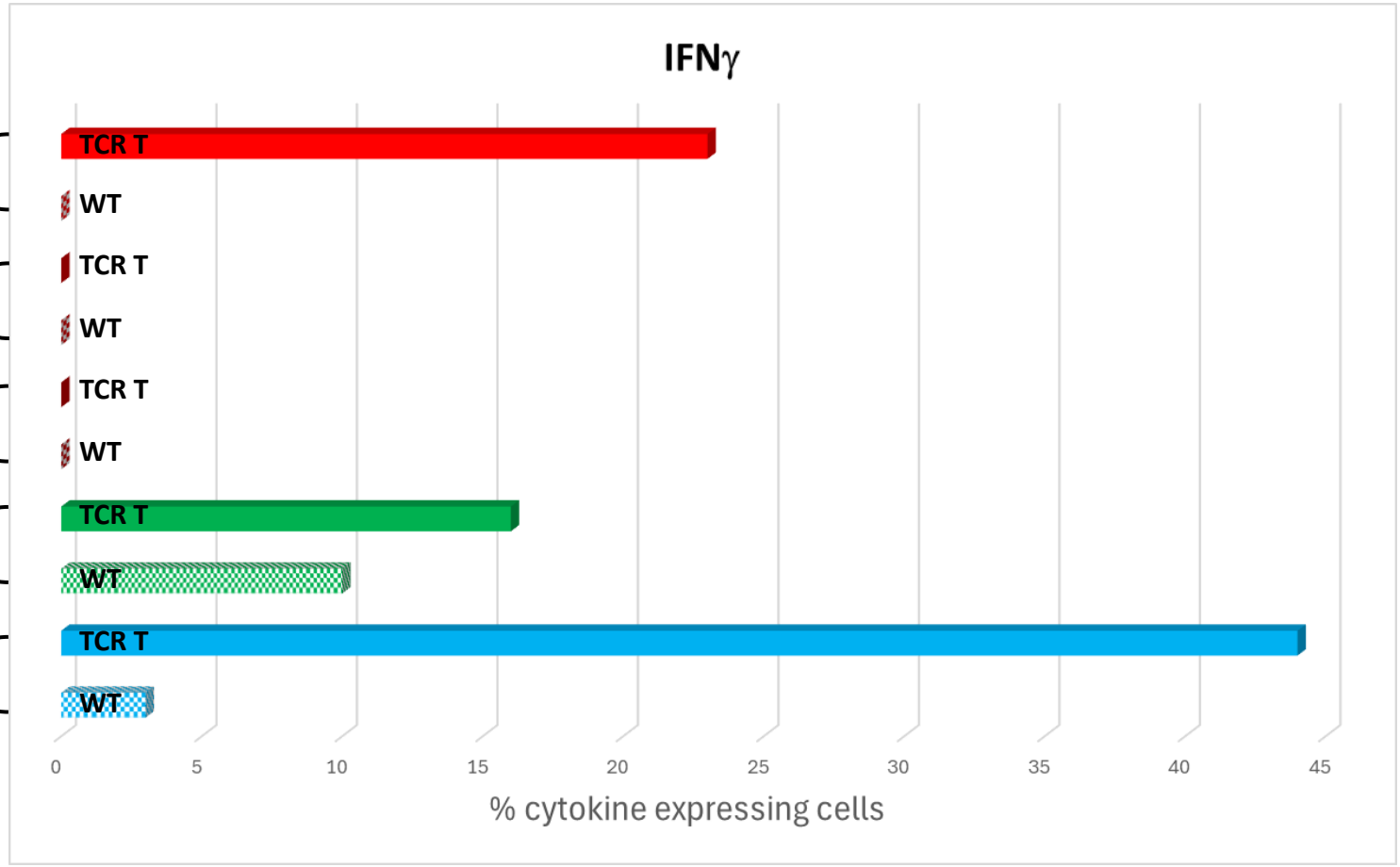
Conclusion:

- PAX Stimulation results in <u>proliferation</u> of virus-specific cells
- Proliferation is highly antigen-specific and CD28 dependent
- Similar results obtained w. naïve MART-1 specific cells

	Frequency of HLA-A*0201/MART-1 specific CD8+ T cells out of all CD8+ T cells		
Days of culture	D205	D215	D219
0	0,029	0	0,026
13	0,26	2,15	1
33	5,68	8,74	87,4

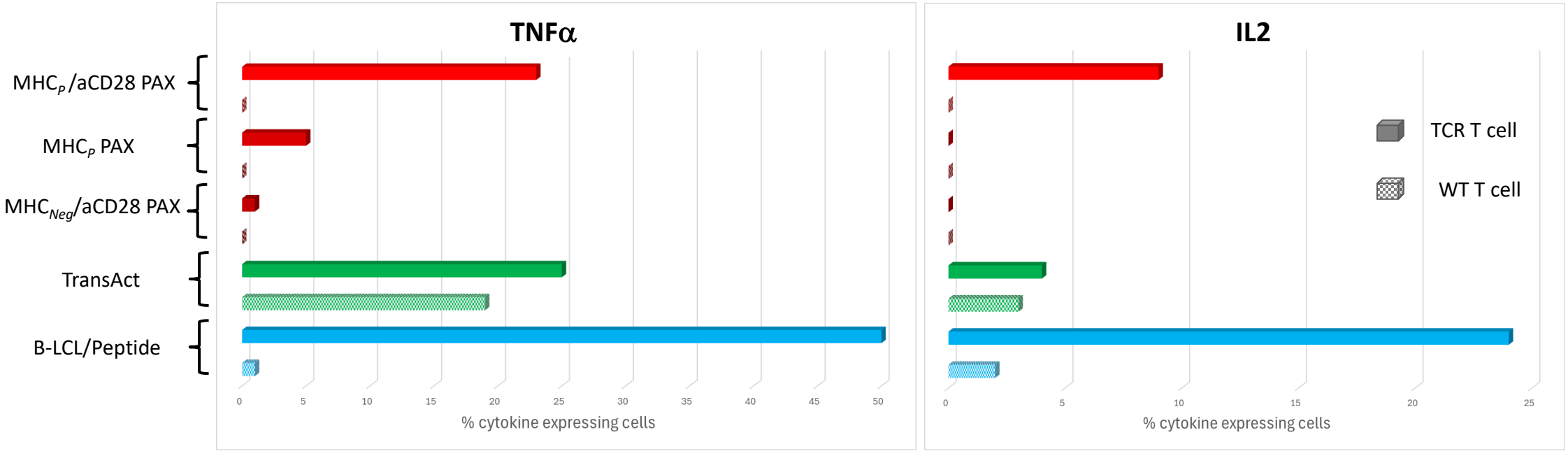
Antigen specific activation of TCR engineered T-cell with PAX reagent

- PAX reagents used for stimulation of TCR-T-cell product specific for MHC displaying cancer epitope
- Evaluate activation: expression of surface activation markers + cytokine production (Flow cytometry)



Data kindly provided by Collaborator

Antigen-specific activation of TCR-T cell line with PAX reagent



- PAX reagents can stimulate engineered T cells to
  - produce cytokines
  - upregulate activation surface markers (CD69, CD137)
- Activation is Highly MHCp-specific and CD28 co-stimulation dependent

Data kindly provided by Collaborator

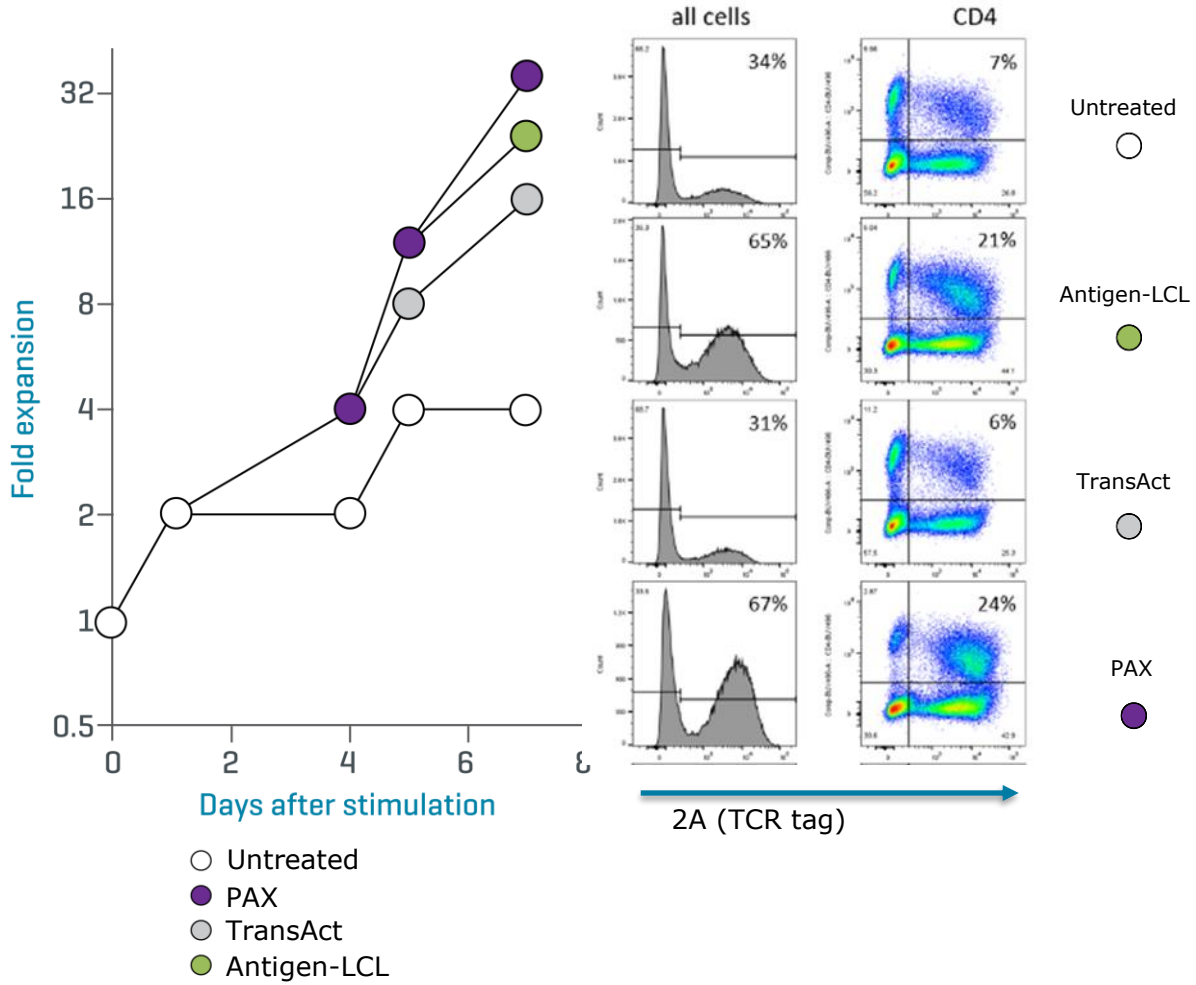
Antigen-specific expansion of TCRt engineered Cells with PAX reagent

PAX reagents can be used to expand and enrich for transfected/transduced T cells

Expansion is MHCp-specific, and the resulting cell product is highly enriched for engineered cells

Enrichment of engineered TCR expressing cells is comparable to what was achieved with peptide-pulsed antigen presenting cells

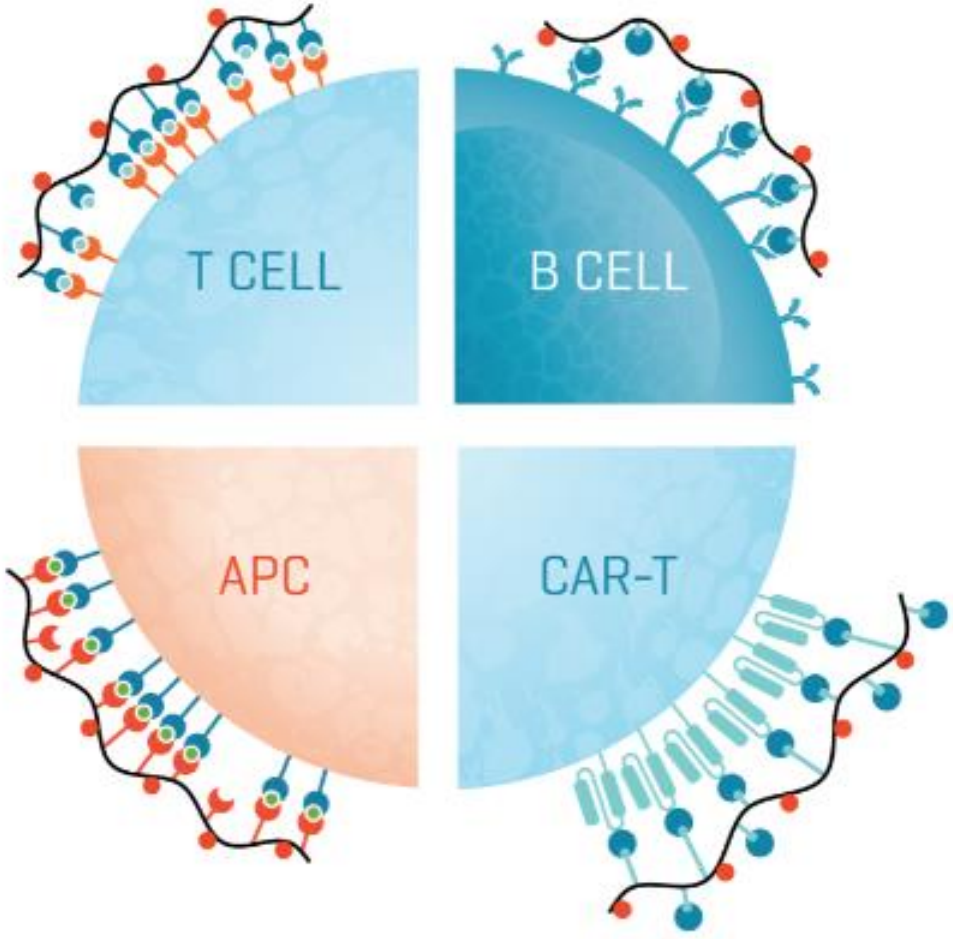
TCR engineered CD4+ Th cells are also efficiently expanded by PAX



Data kindly provided by Collaborator

TAKE HOME MESSAGE

- **Everything is customizable!** Alleles, peptides, barcodes…
- **Technical Support throughout your journey** from Reagent Design to Data Interpretation
- **Gain access to all our expertise** pMHC & TCR engineering, Assay Development, Quality Control and more



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Thank you for your attention and do reach out!

Valérie Bodemeyer, Technical Application Manager customer@immudex.com
Mohammad Salem, PhD, Senior Pharma Business & Solution Manager mas@immudex.com

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