dCODE Dextramer® (HiT) Reagents Allow Multiplex Screening of Large Epitope Panels

Immudex, internal data 2020

Background

dCODE Dextramer® (HiT) carries a unique DNA barcode, specific for the MHC-peptide complex displayed on the Dextramer® (**Fig. 1**). The MHC-peptide specificity can be identified by PCR and sequencing of the attached DNA barcode.

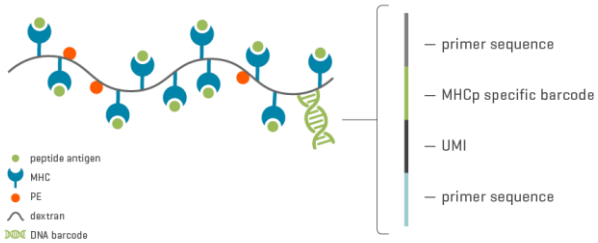

Fig. 1 dCODE Dextramer® (HiT)

Study Description

Goal: Detect antigen-specific T-cell populations in a human PBMC sample using a multiplexed panel of dCODE Dextramer® (HiT) reagents.

1. Healthy donor PBMC sample (haplotype A*02:01, A*29:02, B*35:01, B*57:01) stained with a pool of 56 MHC I dCODE Dextramer® (HiT) reagents displaying different viral and cancer epitopes. Each MHC-peptide specificity was made in duplicates, each with a different DNA barcode label.
2. Following staining, dCODE Dextramer®-positive and -negative cells were individually sorted by flow cytometry.
3. DNA barcodes bound to sorted cells were amplified by qPCR and sequenced. Specific enrichment of dCODE Dextramer®-positive cell populations was determined comparing with dCODE Dextramer-negative cells.
4. Positive antigen-specific populations were confirmed by flow cytometry.

Results

dCODE Dextramer® (HiT) reagents with MHC alleles matching the donor's haplotype detected four antigen-specific T-cell populations with a signal higher than the threshold (**Fig.2**). Results were confirmed by flow cytometry, which enabled identifying the same antigen-specific T-cell populations (**Fig.3**).

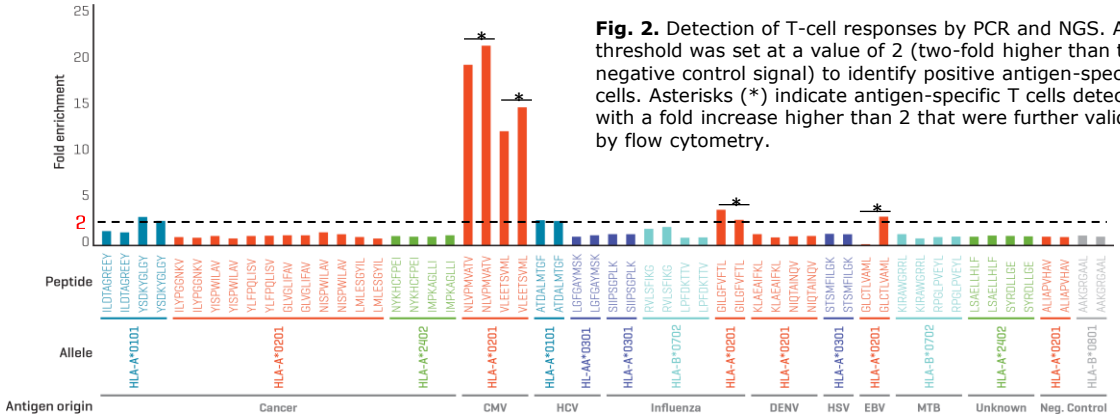

Fig. 2. Detection of T-cell responses by PCR and NGS. A threshold was set at a value of 2 (two-fold higher than the negative control signal) to identify positive antigen-specific T cells. Asterisks (*) indicate antigen-specific T cells detected with a fold increase higher than 2 that were further validated by flow cytometry.

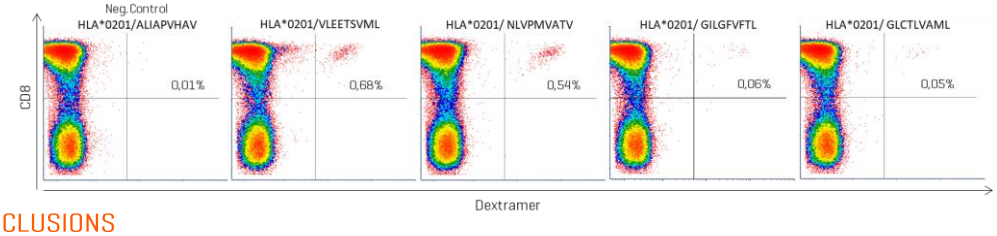

Fig. 3 Flow cytometry validation of the antigen-specific T cells detected by PCR and NGS.

Conclusions

- dCODE Dextramer® (HiT) technology enables the generation of highly multiplexed antigen-specificity data in a single experiment, allowing high-throughput epitope discovery and efficient neoantigen screening.
- Following identification, positive hits can be validated by flow cytometry and further analyzed using single-cell platforms for multi-omics analysis.