In vitro HLA peptide-binding assay and HLA tetramer combinatorial coding technique improve selection of candidate CD8+ T cell epitopes for immunotherapy

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Introduction

Narrowing down the list of potential (neo)epitopes and reducing the time of the identification process are currently major unresolved challenges.

Aim: To develop technology / assays which can speedup (neo)antigen identification and can be used for characterization / immunomonitoring of (neo)antigen-specific CD8+ T cell responses in limited patient material.

Results

1 HLA peptide-binding assay for finding potential CTL epitopes / target identification

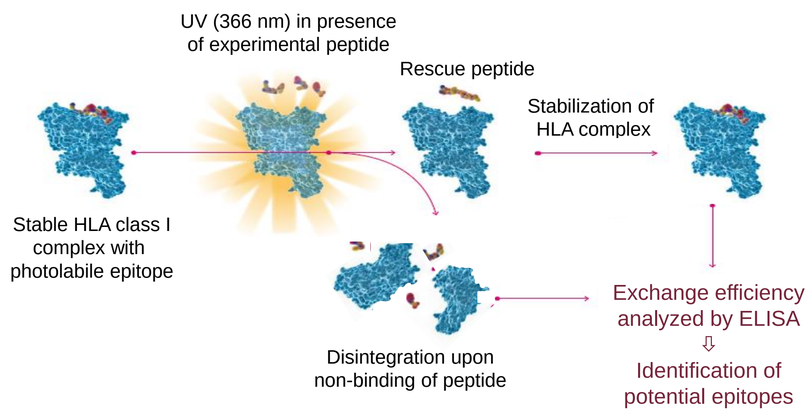

Figure 1. Principle of *in vitro* HLA peptide-binding assay using UV-induced peptide exchange*.

2 *In silico* vs *in vitro*: Ranking for best binders differs significantly

1. NetMHCpan cannot discriminate well between strong and weak binders
2. ~17% of *in silico*-predicted non-binders do bind
3. ~17% of *in silico*-predicted binders do not bind

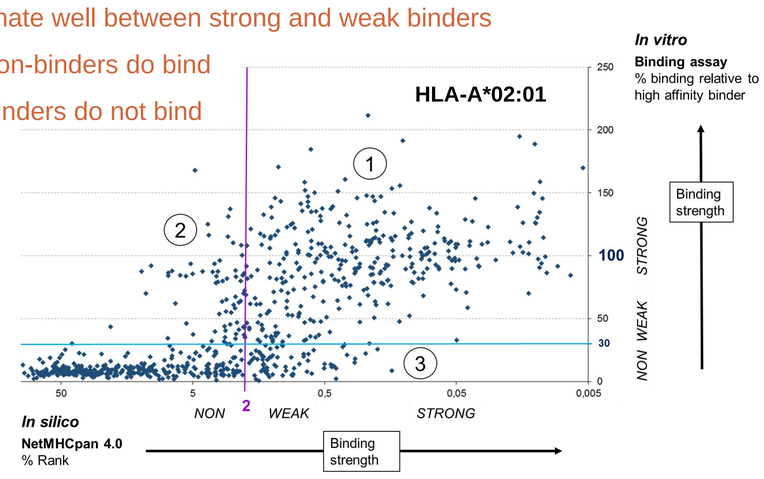

Figure 2. *In silico* analysis vs *in vitro* HLA peptide-binding. Peptides (*n*=795) were analyzed *in silico* for predicted binding and tested for real binding to HLA-A*02:01 using the *in vitro* HLA peptide-binding assay. Similar results were found for other HLA alleles (data not shown).

3 Ranking based on *in vitro* binding improved selection of candidate epitopes significantly (i.e. clustering of immunogenic peptides in the upper range of best binders)

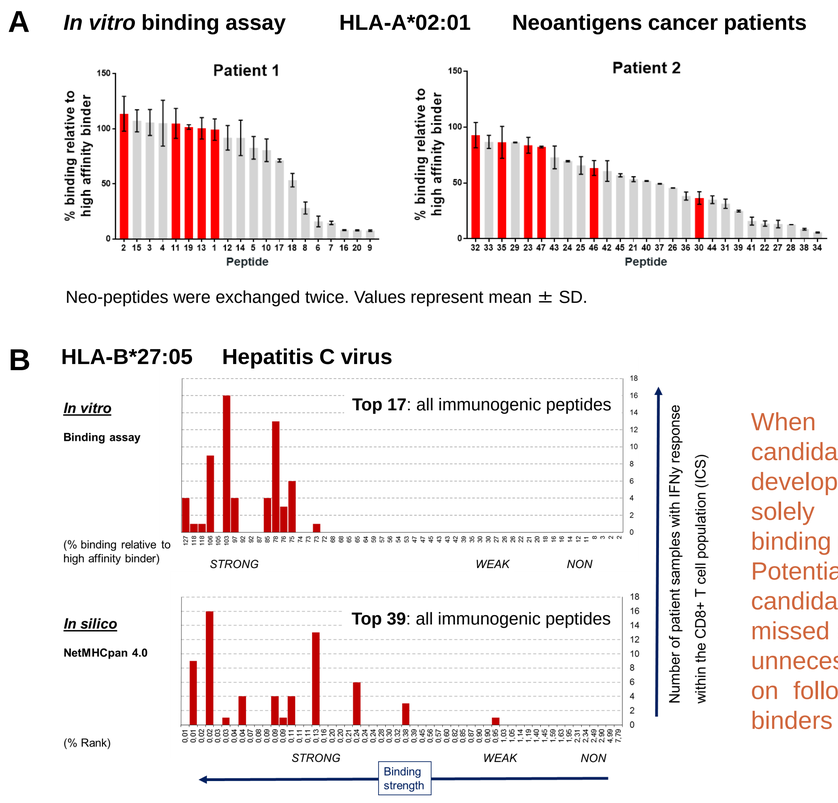

Figure 3. *In silico* binding prediction, *in vitro* binding and immunogenicity. (A) Neo-peptides (*n*=47) from 2 cancer patients predicted to bind to HLA-A*02:01 were tested for immunogenicity (Erlend Strønen et al. Science 2016; 352:1337-41), and binding using the *in vitro* HLA peptide-binding assay and subsequently ranked. Red bars represent immunogenic neoantigens, grey bars represent predicted neoantigens for which no *in vitro* T cell response could be detected. (B) HLA-B*27:05-restricted hepatitis C virus (HCV)- associated predicted binders (*n*=54) were tested for real binding and *in vitro* tested for immunogenicity using PBMCs from HCV patients (unpublished results from Jörg Timm, Institute of Virology, Essen, Germany). Rankings for best binding were compared based on *in silico* binding prediction and *in vitro* binding.

When selection of candidate epitopes for development is based solely on *in silico* binding prediction: Potential good candidates may be missed and resources unnecessarily spent on following up non-binders

4 HLA tetramer combinatorial coding (HTCC) technique* for identification of immunogenic peptides / immunomonitoring

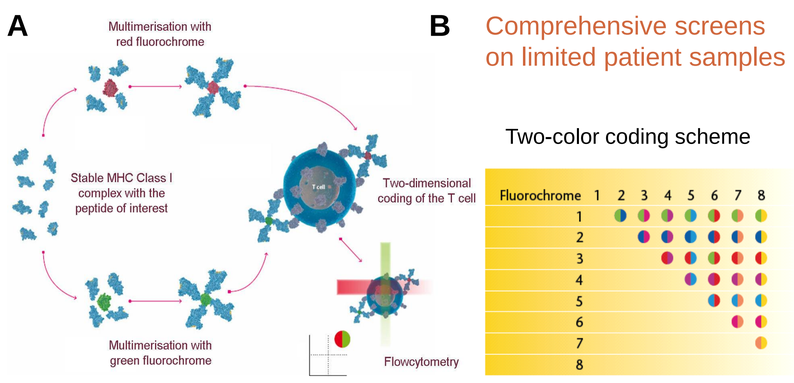

Figure 4. HTCC. (A) For each peptide-HLA combination 2 tetramers are created, each conjugated with a different fluorochrome (each peptide-HLA combination has a unique '2-color code'). Combinatorial codes are assembled on the T cell surface (tetramer staining) and visualized by flow cytometry. (B) Utilizing e.g. 8 different fluorochromes, a 2-color coding scheme results in 28 unique dual color combinations / cell stainings.

5 Simultaneous enumeration of up to 41 antigen-specific CD8+ T cell responses by HTCC including e.g. CD45RA and CD27

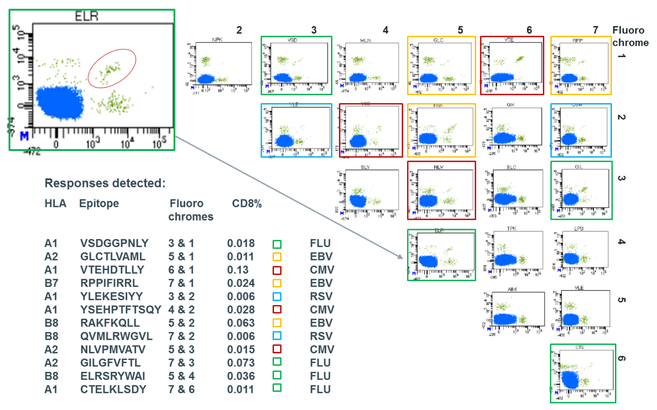

Figure 5. Example of parallel detection of T cell responses against 21 CD8 epitopes restricted to 4 different HLA alleles in a single PBMC sample of a healthy donor.

6 HTCC combined with phenotypic and intracellular markers allowing study of phenotypical and functional differences within and/or between antigen-specific T cell responses.

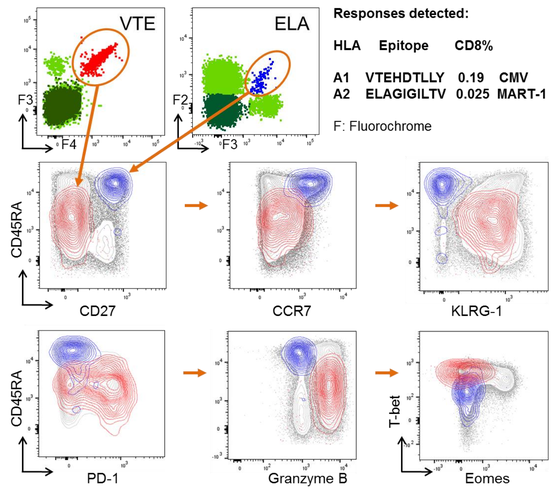

Figure 6. Example of results obtained combining HTCC with phenotypic (CD45RA, CD27, CCR7, KLRG-1, and PD-1) and intracellular (Granzyme B, T-bet, and Eomes) markers using a PBMC sample of a healthy donor.

7 HTCC combined with phenotypic and intracellular staining after peptide stimulation allowing study of phenotypical and functional differences within and/or between antigen-specific T cell responses.

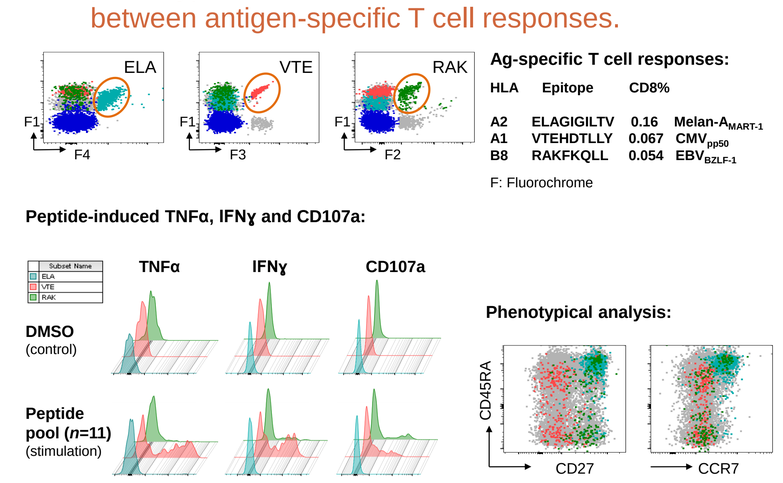

Figure 7. Example of results obtained combining HTCC with phenotypic (CD45RA, CD27, and CCR7) and intracellular (TNFα, IFNγ, and CD107a) markers using a peptide pool (*n*=11)-stimulated PBMC sample of a healthy donor.

Conclusions

1) Ranking based on *in vitro* HLA peptide-binding improved the selection of candidate CD8+ T cell epitopes significantly (best correlation with immunogenicity);
2) HTCC combined with phenotypic and/or intracellular markers contributes to in-depth characterization of a multitude of antigen-specific T cell responses in a single cell sample important for immunotherapeutic development and monitoring of immunotherapeutic interventions.

* : *Patent protected WO 2010/060439 and WO 2006/080837*

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