In vitro tools for epitope selection

TMB conference London 2019

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Sanquin

(Neo)-epitope selection
guided by in-vitro peptide-MHC binding assay

Characterizing antigen-specific T cells
in limited samples

What is **Sanquin**

Sanquin is the **blood supply organization**, with a **research institute** and products and services for **plasma medicines, diagnostics** and **reagents**.

Sanquin

Center of Excellence for Blood and Immunology

Blood Bank

Research

Diagnostic Services

Reagents

Plasma Products

Non-for-profit foundation, organized for public tasks and holding with Ltds
2791 employees, HQ in Amsterdam, The Netherlands

In vitro guided

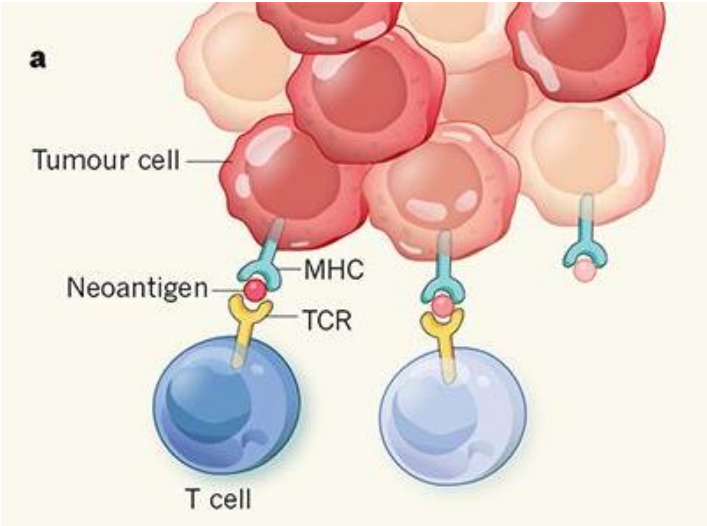
Relevant Epitope

Selection

Common aspects neo-epitope **identification**

Presence of **altered peptides** in tumor cells:

- **DNA/RNA Seq**
  - Tumor *versus* Healthy
  - Splicing variants
  - Viral
- RNA **expression** levels
- Post-transcriptional **modification**



Peptide **presentation** on cell

***In silico* predicted / measured**
- Peptide processing
- **Peptide-MHC** binding/stability

Mass-spectrometry
peptide elution & identification

Other **Immuno-biology** aspects for immunogenicity
Stimulation, Tolerance, Exhaustion

- **WT-neo** peptide differences
- **pMHC-TCR** binding
- Immunologic **history**
- Micro-**environment**

Common neo-epitope **selection process**

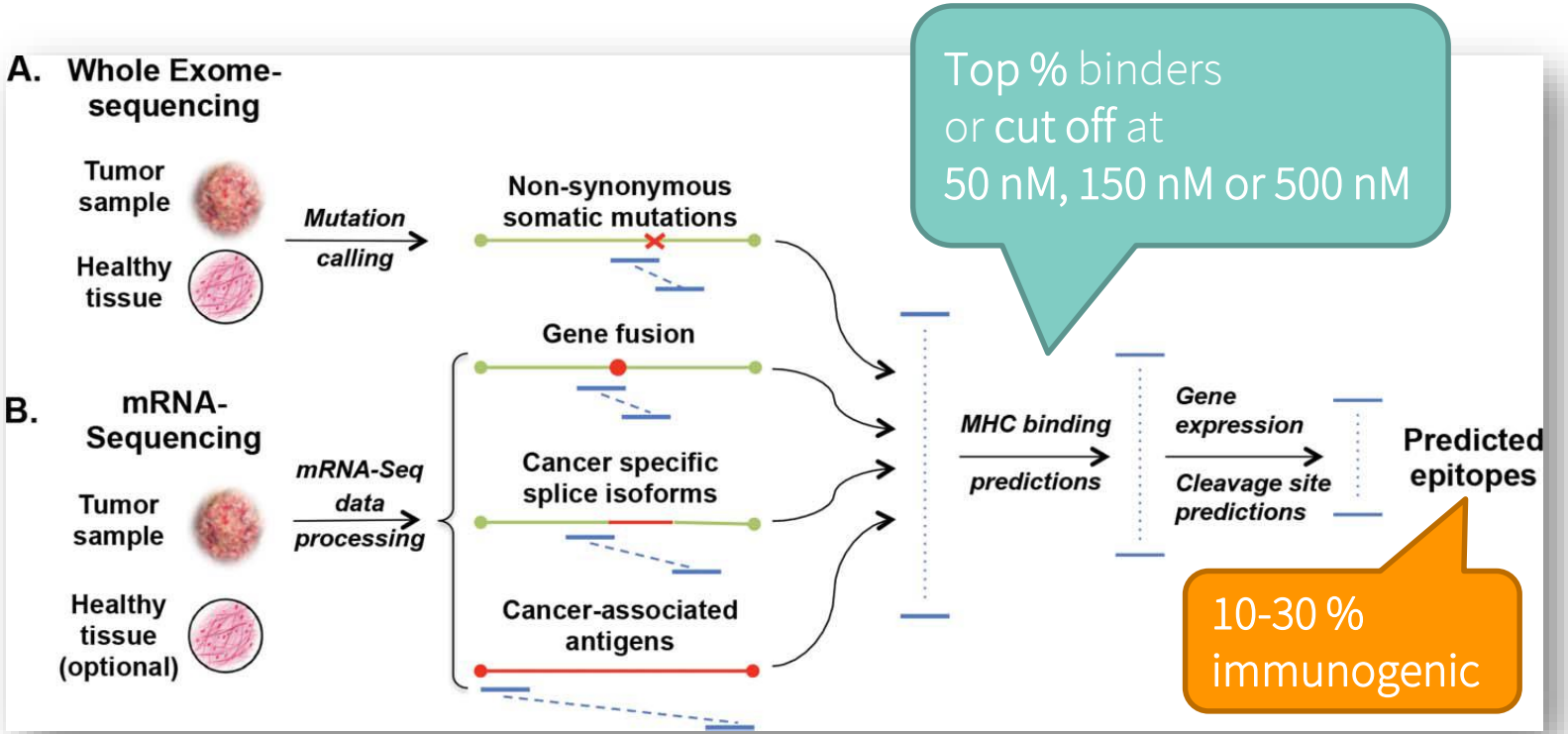
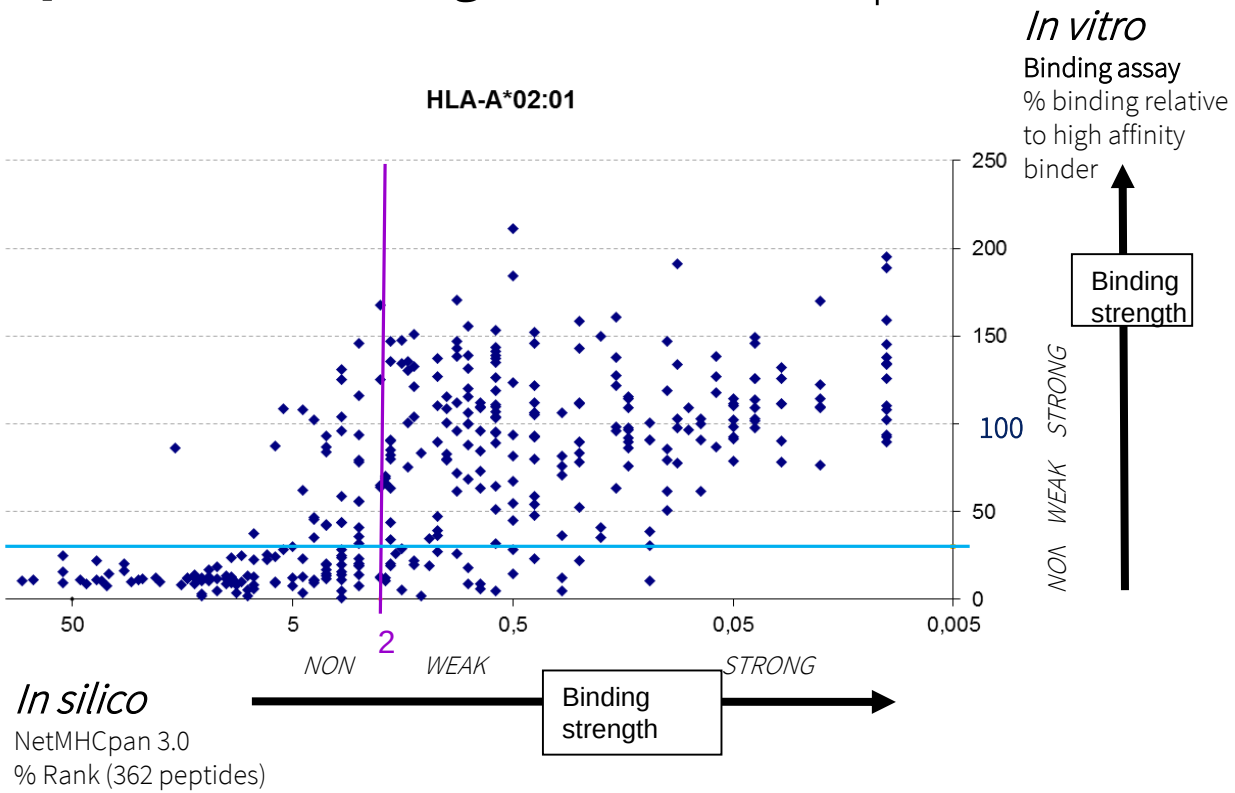


Image: Gfeller et al, 2016, OncoImmunology

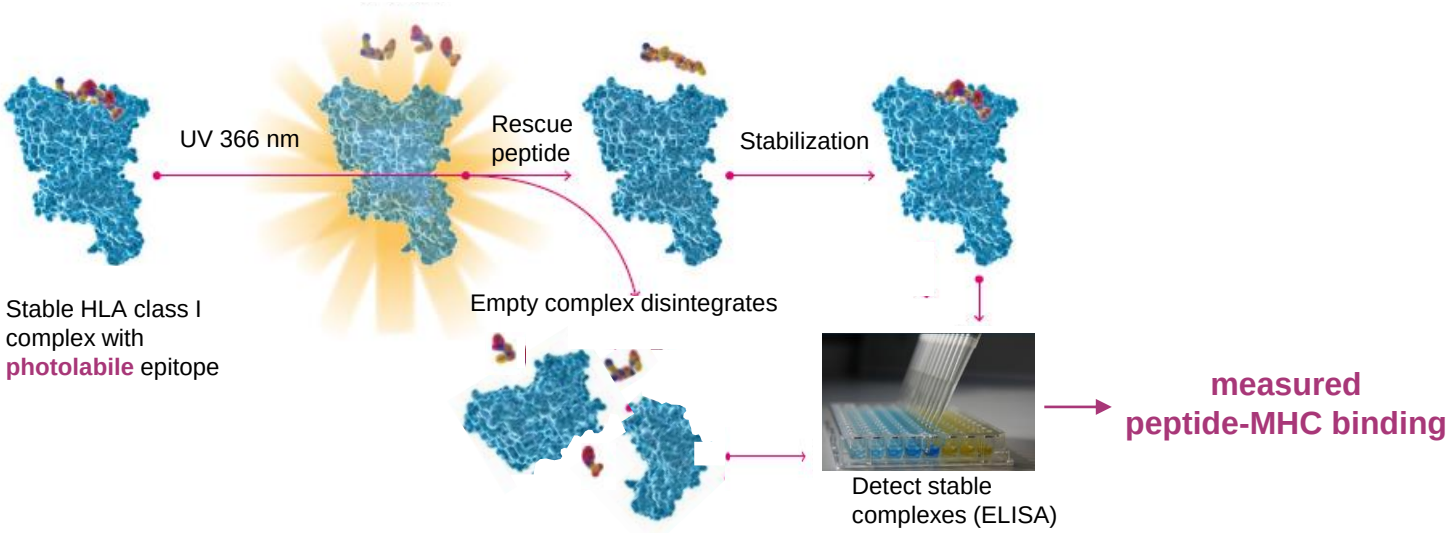
In vitro **measured peptide MHC binding versus** *in silico* prediction

In silico **prediction cannot discriminate well** between strong and weak binders

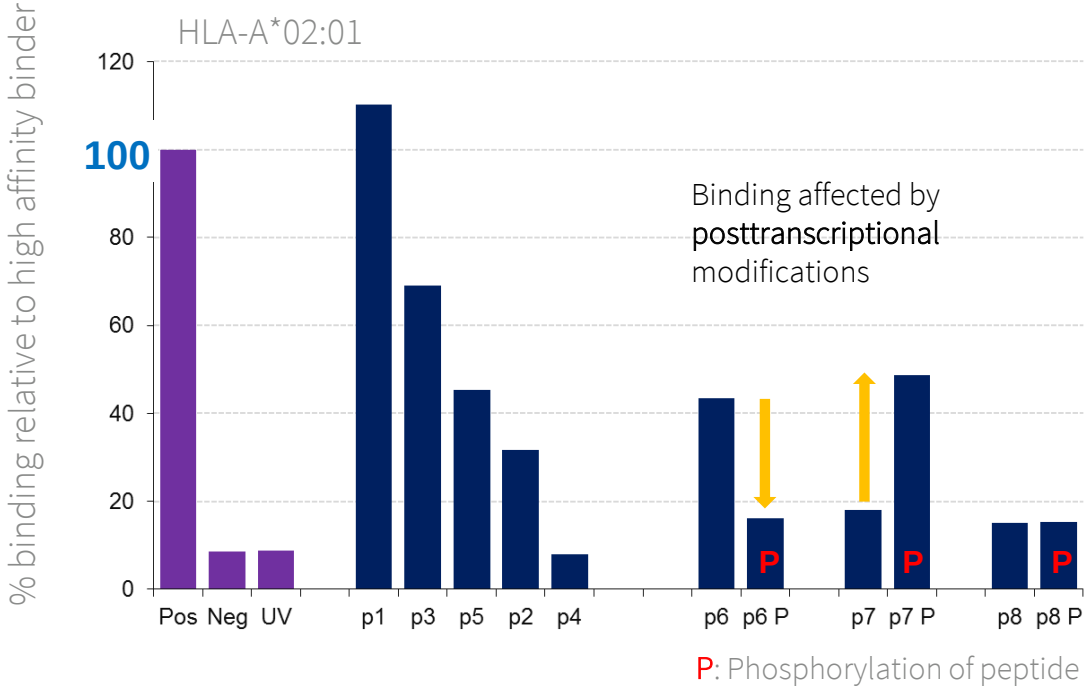


In vitro **assay** for high-throughput peptide-MHC binding

Peptide is essential for stability of HLA class I monomer



Measured *in vitro* binding of (modified) peptides to HLA class I



- **High-throughput**
- **Fast** (< 1 day)
- **Modification** compatible

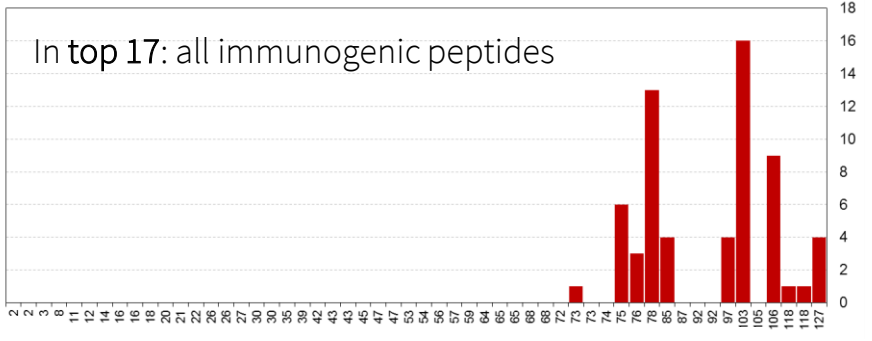
Ranking by *in vitro* binding improves selection of immunogenic epitopes

HLA-B*27:05
54 peptides
HCV

In vitro

Binding assay

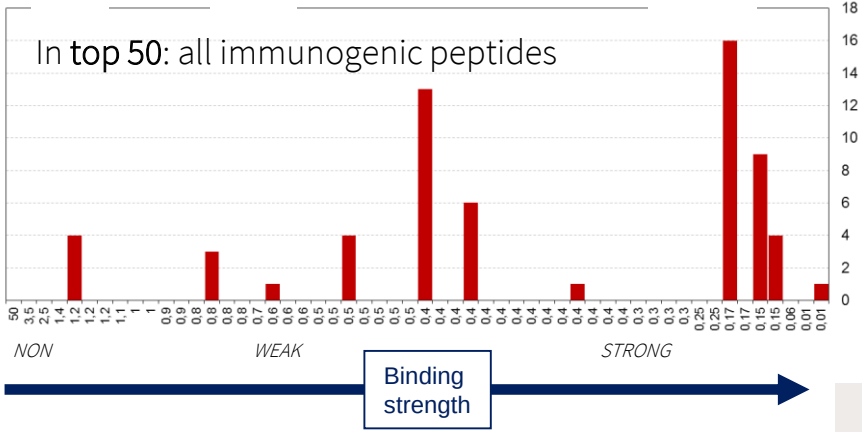
(% binding relative to high affinity binder)



In silico

NetMHCpan 3.0

(% Rank)



Number of patient samples with IFNγ response within the CD8+ T cell population (ICS)

Collaboration: Jörg Timm, Institute of Virology, Essen

Ranking by *in vitro* binding / stability improves selection of immunogenic neo-epitopes

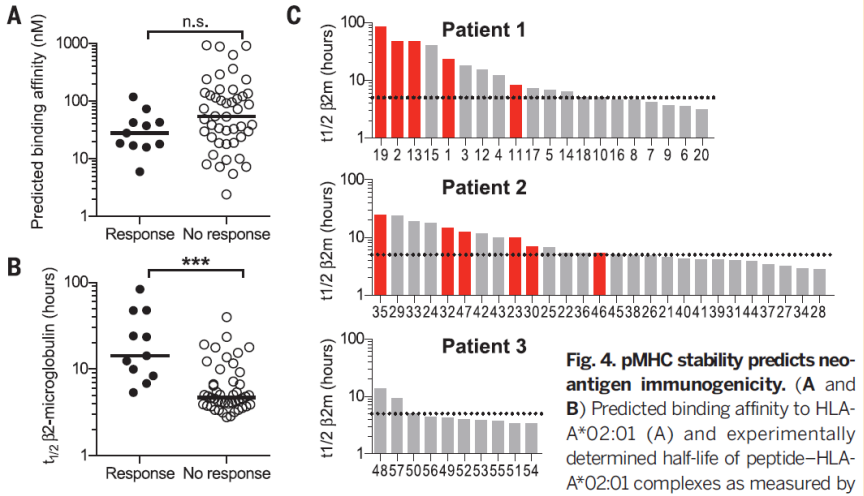
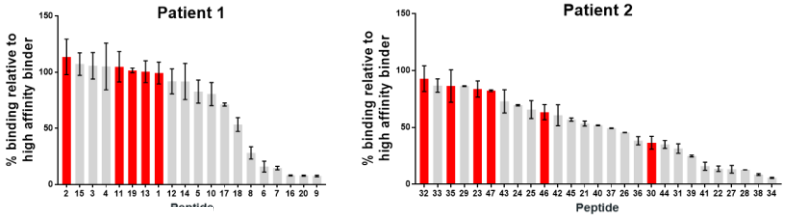


Fig. 4. pMHC stability predicts neoantigen immunogenicity. (**A** and **B**) Predicted binding affinity to HLA-A*02:01 (A) and experimentally determined half-life of peptide–HLA-A*02:01 complexes as measured by dissociation of β_2-microglobulin (B) for the 57 predicted neoantigens from patients 1, 2, and 3 that do or do not induce a T cell response. Peptide sequences and predicted affinities are listed in tables S1 to S3. (**C**) Red bars represent predicted neoantigens that were shown to be immunogenic; gray bars represent predicted neoantigens for which no T cell response could be detected. Dotted line represents suggested cutoff value of $t_{1/2}$ = 5 hours. Values in (B) and (C) represent means of triplicates. ***P < 0.0001 (Mann-Whitney U test), n.s., not significant.

Data from Stronen et al, Science, 2016

Sanquins *in vitro* binding assay



Remark: # of immunogenic peptides included **IF** only top 10 peptides would have been selected:

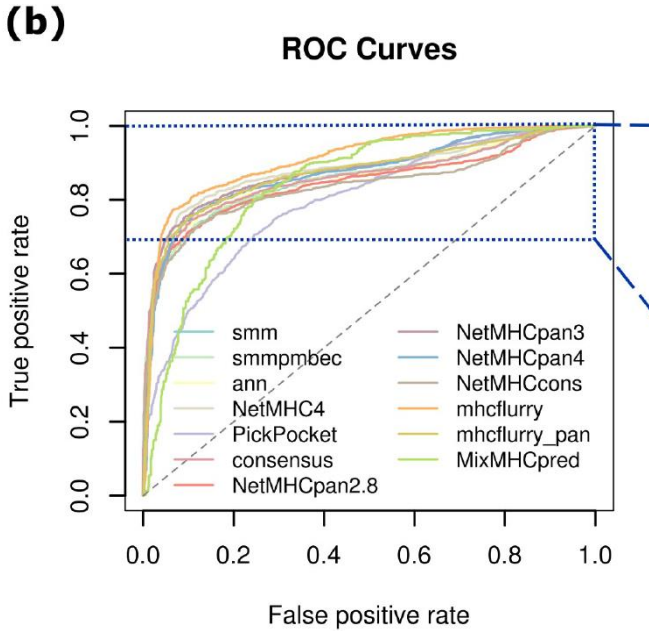
	Patient 1	Patient 2
Based on in silico:	3/5	2/6
Based on measured:	5/5	5/6

Systemetic study reliability of in silico MHC binding **prediction**

PLOS COMPUTATIONAL BIOLOGY

Zhao W, Sher X (2018)

Benchmarking MHC-presenting epitope prediction methods

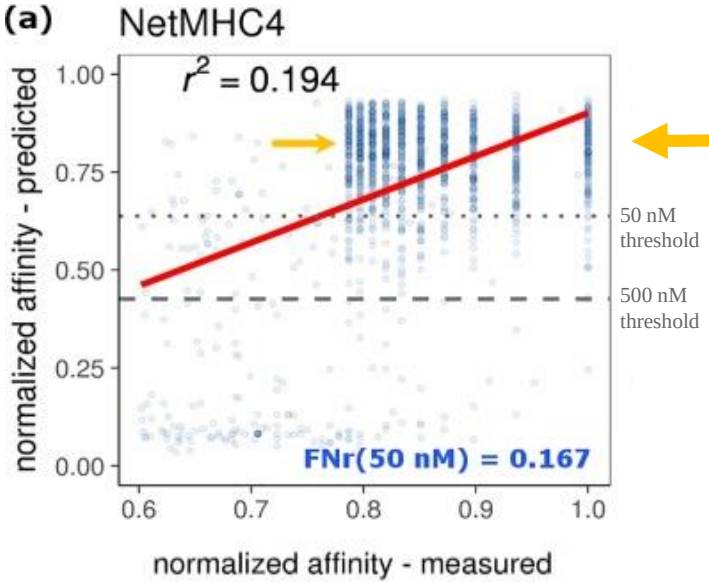


Binary classification performance
>65.000 peptides/ 32 alleles (IEDB database)

Binder versus Non-Binder
(cut-off IC50 = 500 nM)

Binary (Yes/No) prediction rather good
AUC >0.85 (although allele dependent)

Systemetic study reliability of in silico MHC binding **prediction**

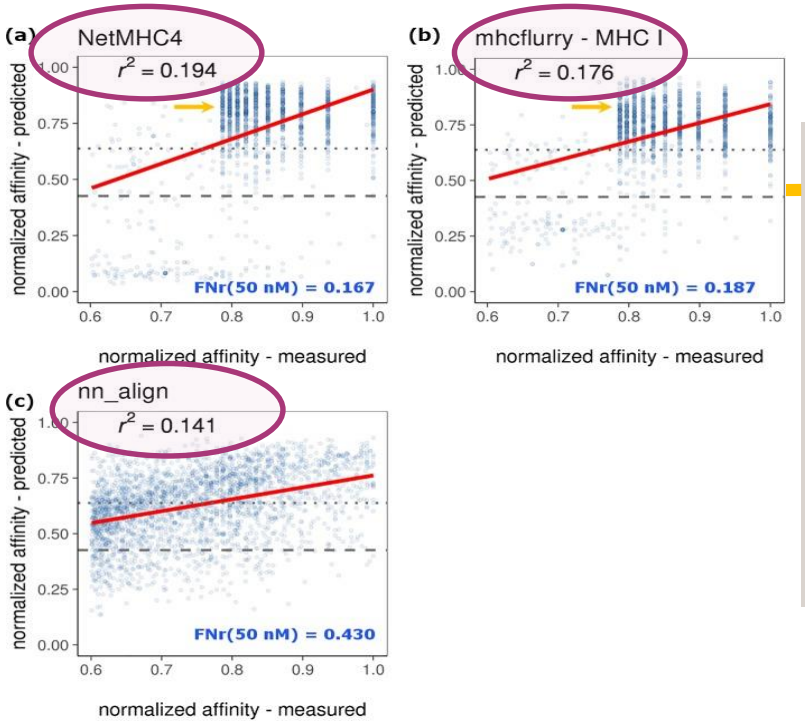


Diverged predictions for similarly well binding peptides
>65.000 peptides/ 32 alleles (IEDB database)

Priority ranking on prediction not reliable
AUC >0.85 (although allele dependent)

Zhao W, Sher X (2018)

Systemetic study reliability of in silico MHC binding **prediction**



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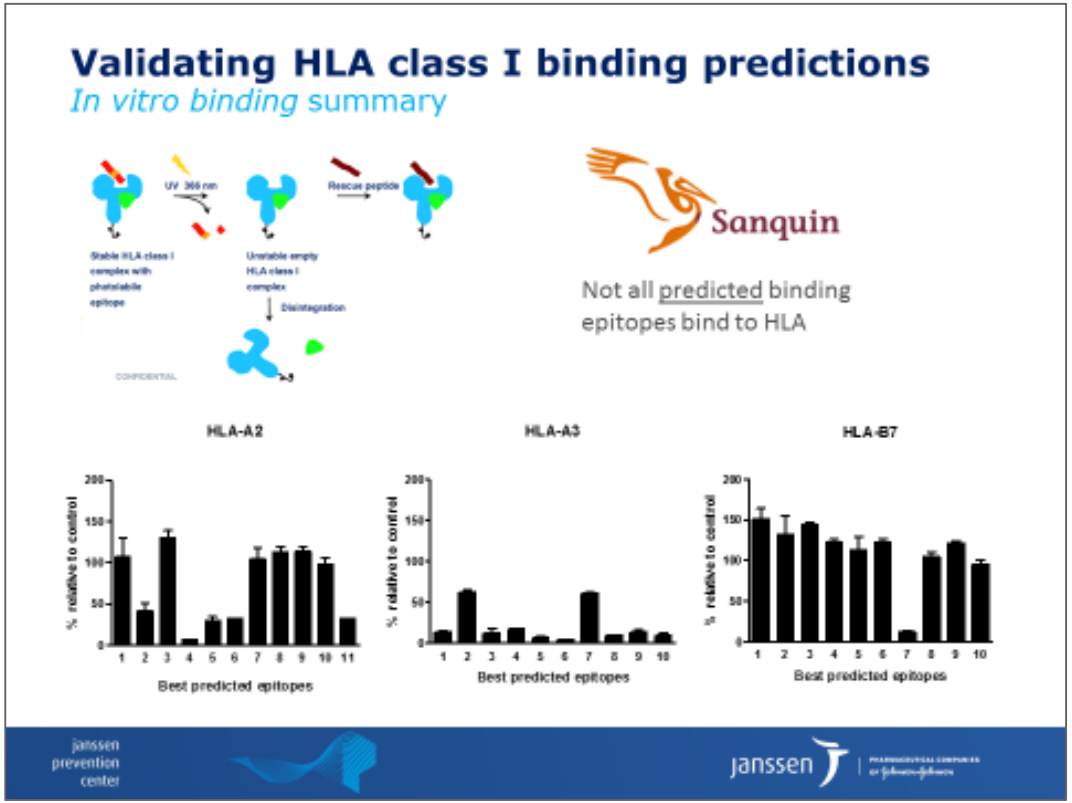
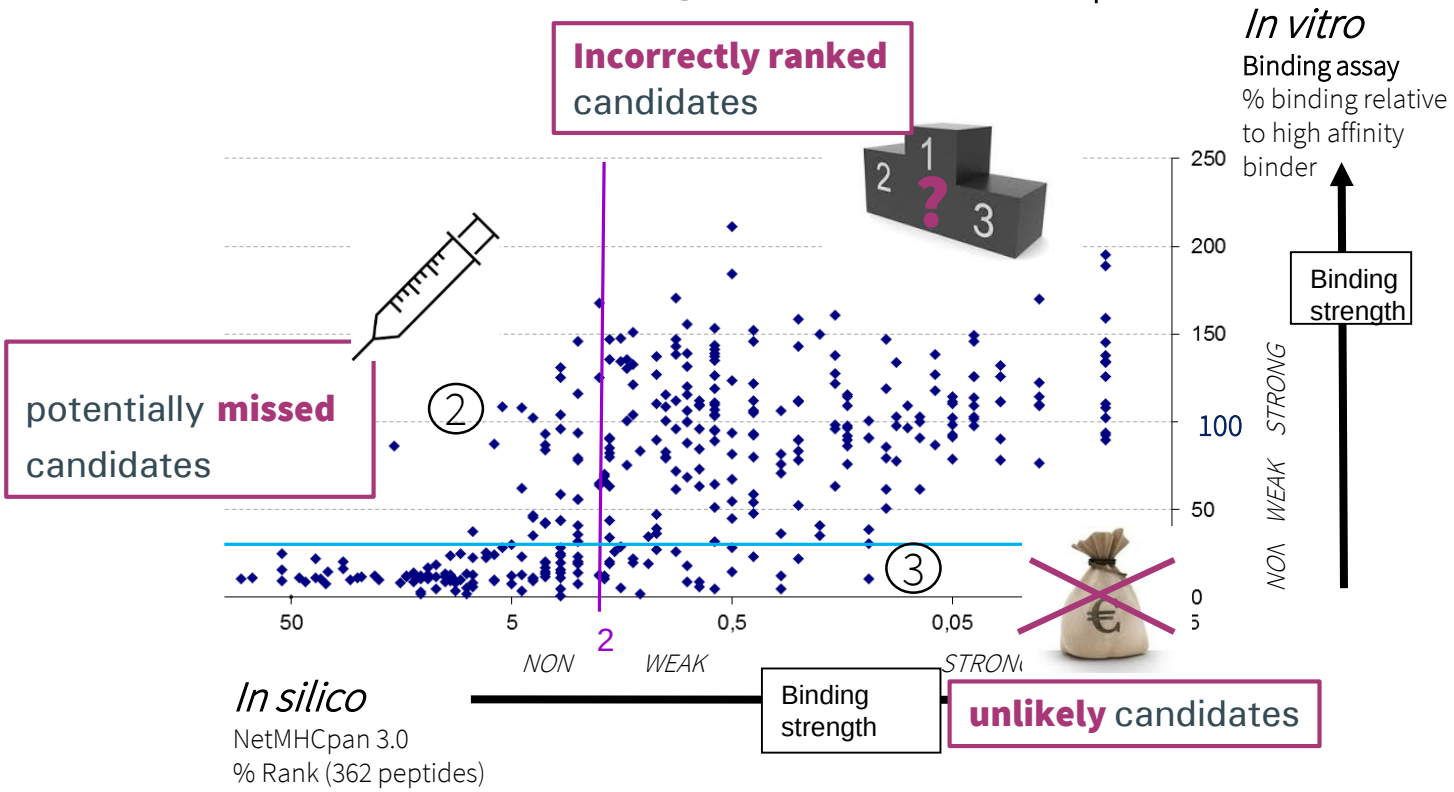
Zhao W, Sher X (2018)

- **In silico** prediction good for **Binding – Not binding** for most HLA alleles
  (~10-20% False positives)
- **In silico not reliable for ranking**
- Public and commercial predictors
- ***In vitro* binding assay improves selection**

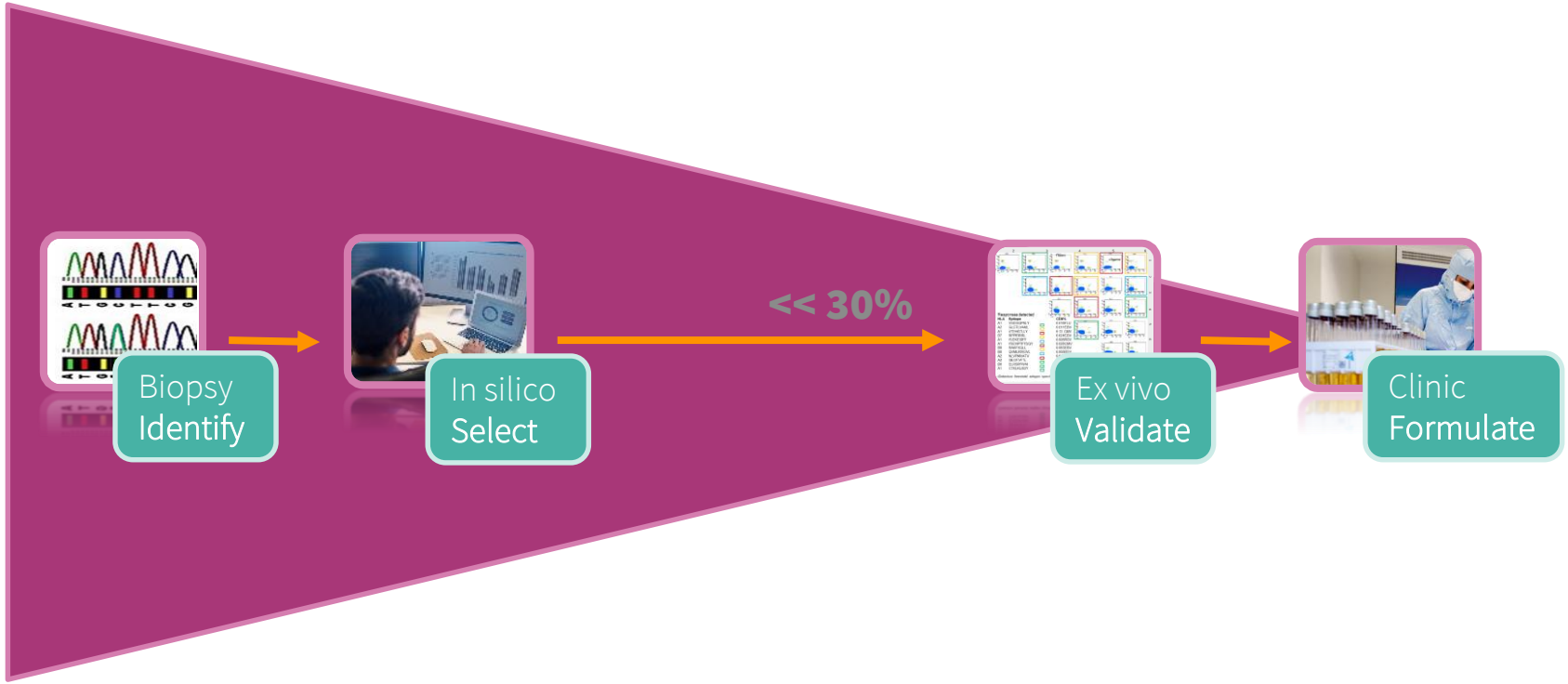


Peptides selected and ranked by commercial prediction software
Presented by Tiemessen, Janssen Prevention Center, 2016

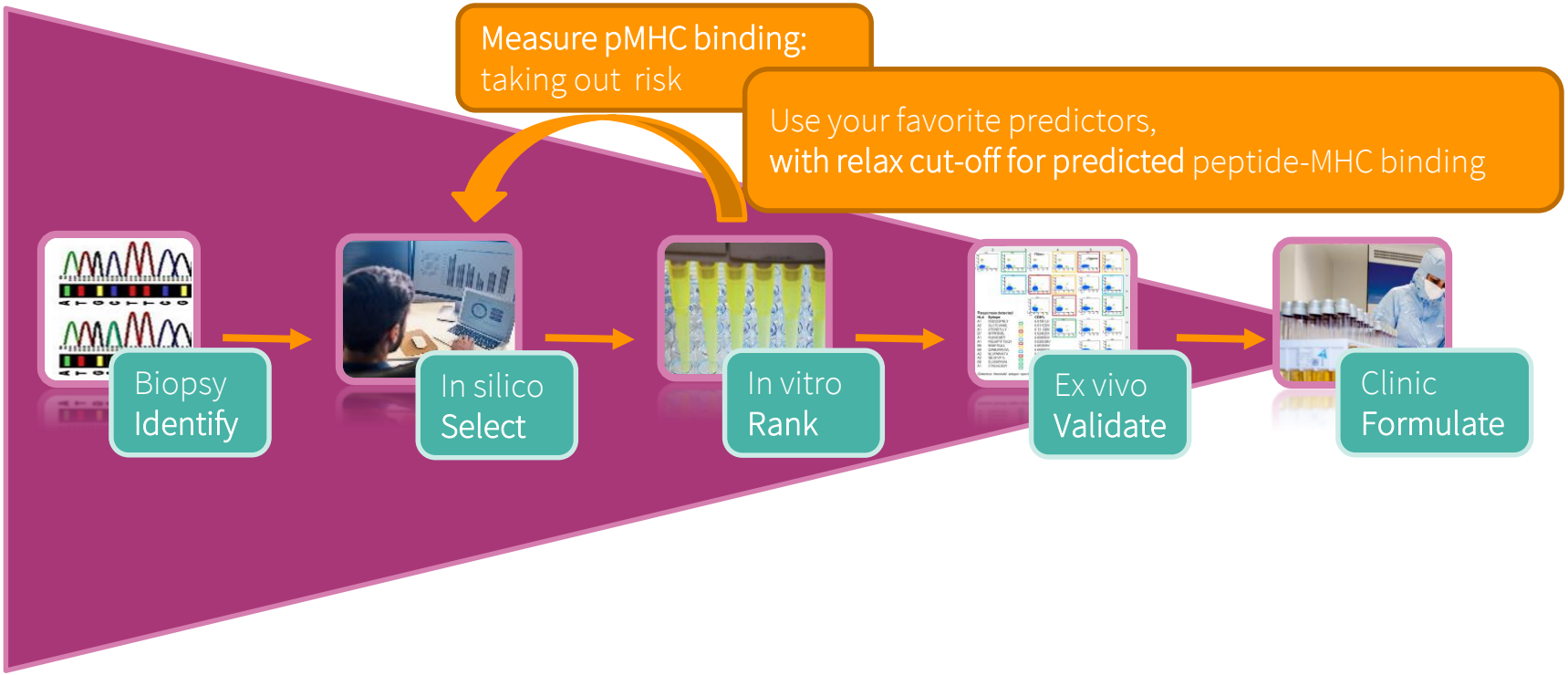
In vitro **measured peptide MHC binding versus** *in silico* prediction



Common neo-epitope **selection process**



***In vitro* guided** neo-epitope **selection process**

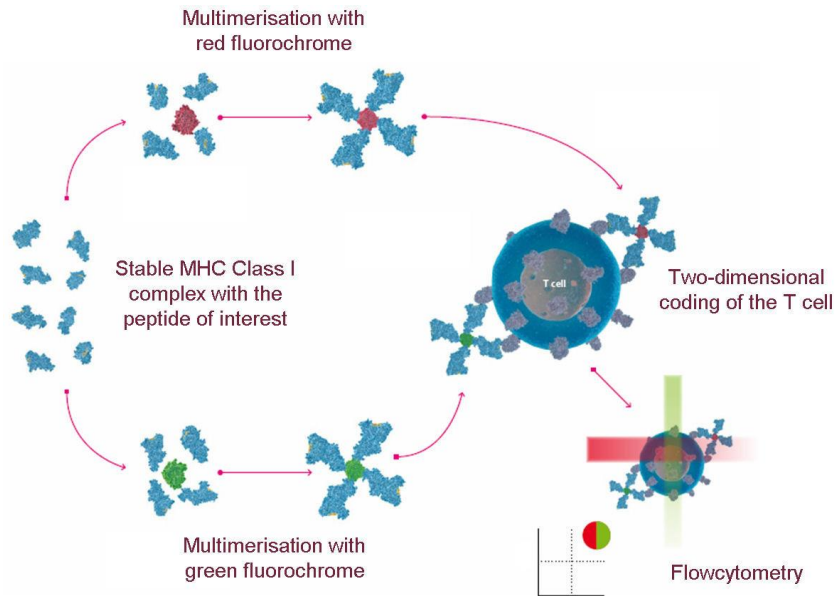


Ex vivo

Validation of Epitopes

by characterizing antigen-specific T cells

Detection of **multiple (neo)epitope specific** CD8 T cells **simultaneously**



Comprehensive screens
on limited patient samples

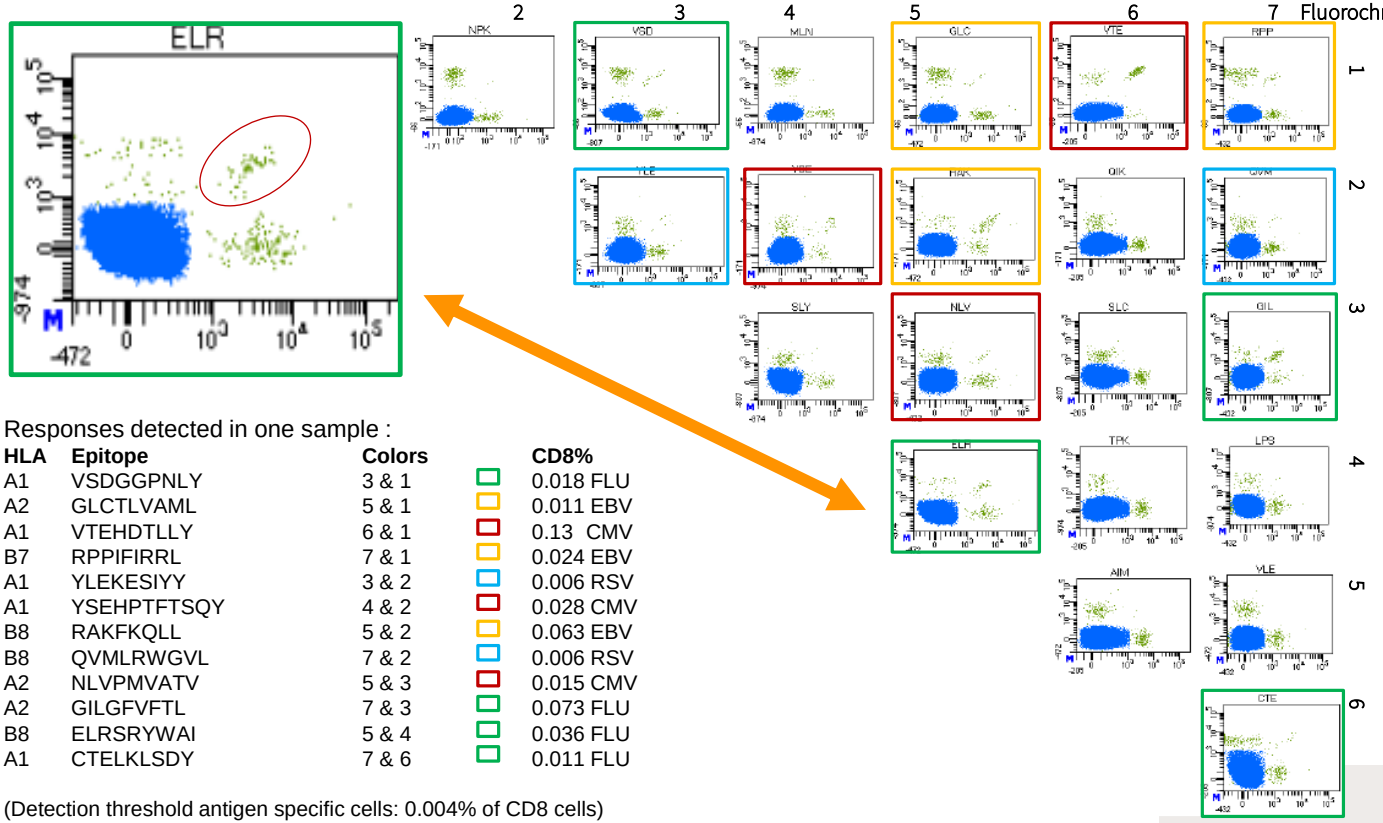
Two-color coding scheme

Fluorochrome	1	2	3	4	5	6	7	8
1								
2								
3								
4								
5								
6								
7								
8								

Confirmation of relevance of epitopes and monitoring treatment by HLA tetramer combinatorial coding (HTCC)

Patented in Europe, US and other regions

Detection of **multiple (neo)epitope specific** CD8 T cells **simultaneously**



Responses detected in one sample :

HLA	Epitope	Colors		CD8%
A1	VSDGGPNLY	3 & 1		0.018 FLU
A2	GLCTLVAML	5 & 1		0.011 EBV
A1	VTEHDTLLY	6 & 1		0.13 CMV
B7	RPPIFIRRL	7 & 1		0.024 EBV
A1	YLEKESIYY	3 & 2		0.006 RSV
A1	YSEHPTFTSQY	4 & 2		0.028 CMV
B8	RAKFKQLL	5 & 2		0.063 EBV
B8	QVMLRWGVL	7 & 2		0.006 RSV
A2	NLVPMVATV	5 & 3		0.015 CMV
A2	GILGFVFTL	7 & 3		0.073 FLU
B8	ELRSRYWAI	5 & 4		0.036 FLU
A1	CTELKLSDY	7 & 6		0.011 FLU

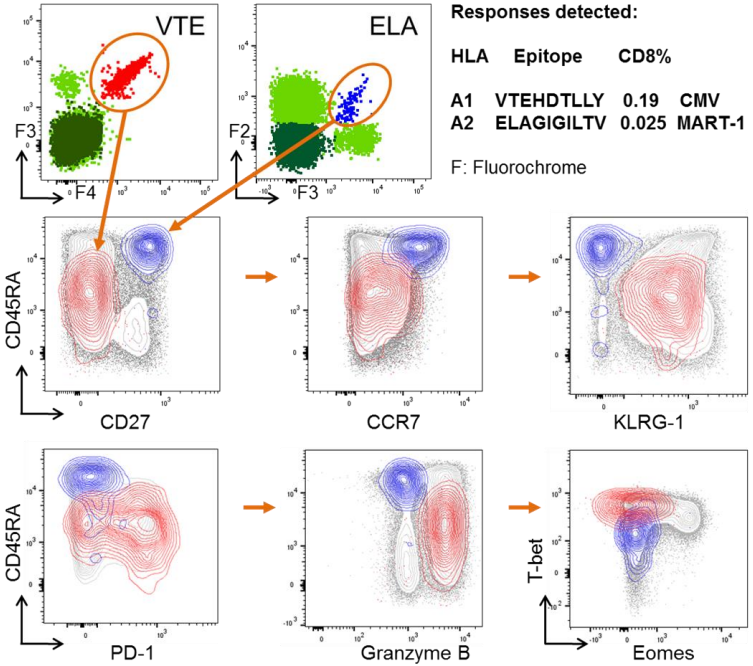
(Detection threshold antigen specific cells: 0.004% of CD8 cells)

Asses **type of response** for each antigen **in single patient** sample

Direct **ex-vivo** HTCC* &
- **Surface** markers
- **Intracellular** staining

Discriminating phenotypic and functional subpopulations

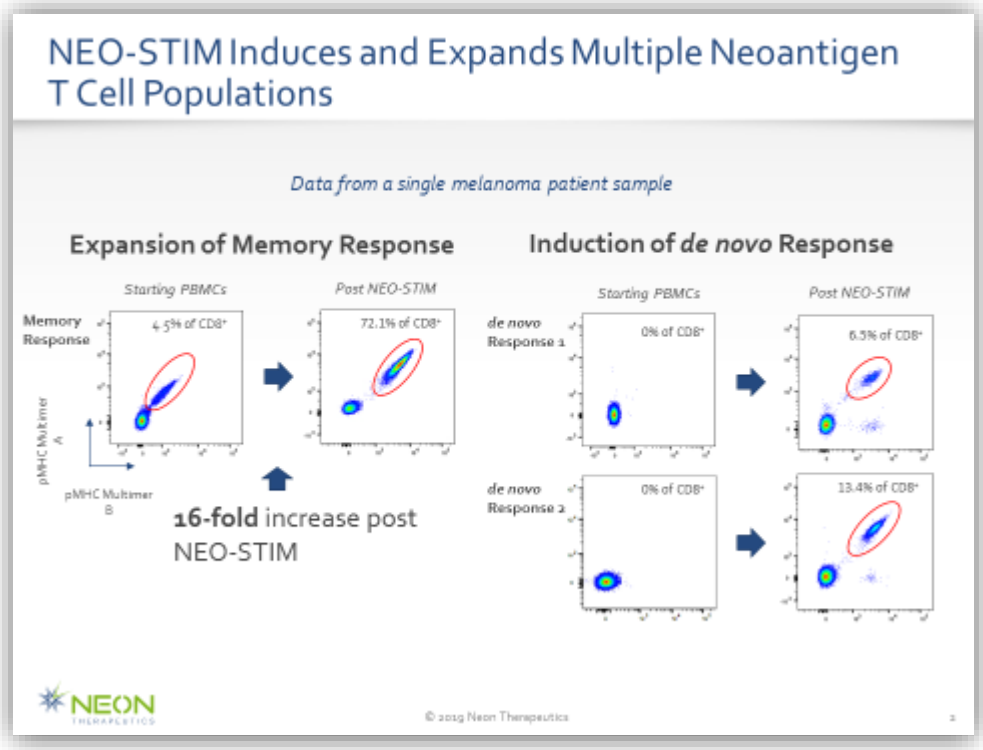
4*10^6 PBMC for subtyping response to **20-40 antigens** specificities **in one go**



HTCC: HLA tetramer combinatorial coding: Get more information out of your limited patient sample

Examples of use – assessing **pre-existing responses** and **treatment effect**

- Biomarker
- **Pre-existing response**
- Proof of mechanism
- **Effect of treatment**
- Immunomonitoring
- In depth characterization



Examples of use – assessing **pre-existing responses** and **treatment effect**

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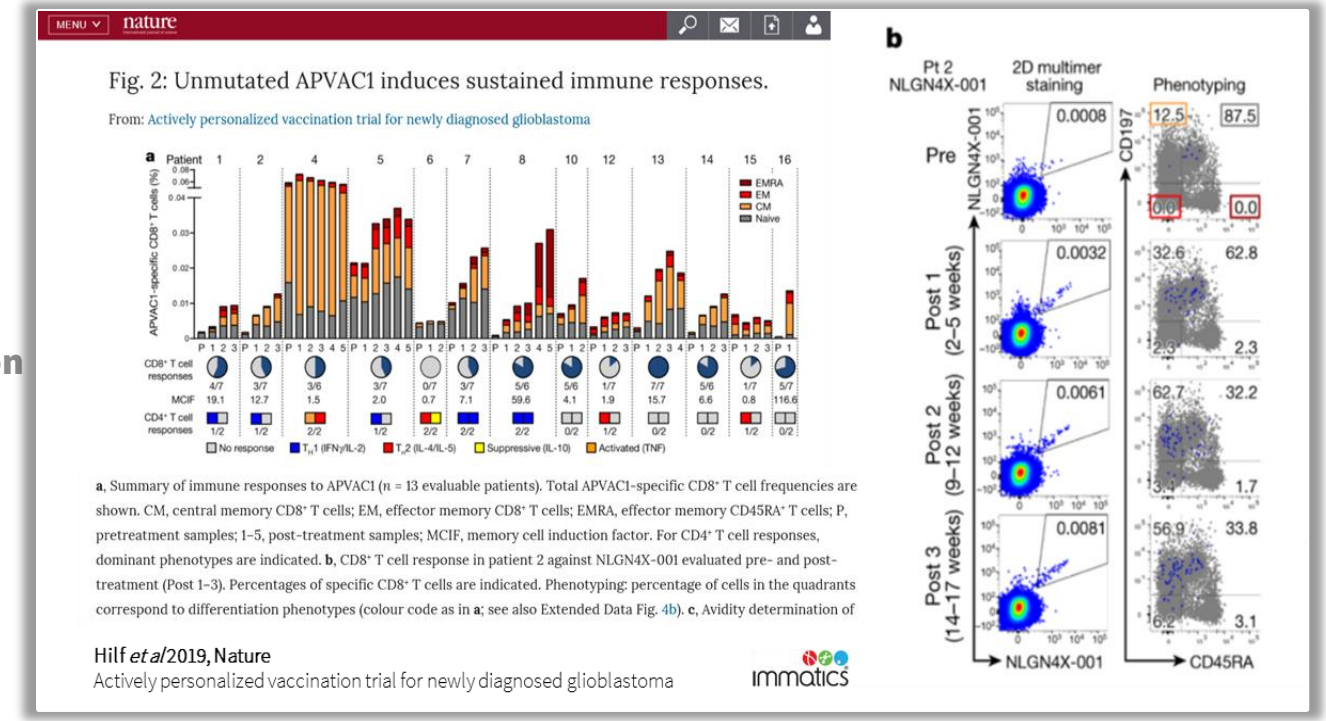


MENU nature

Fig. 2: Unmutated APVAC1 induces sustained immune responses.

From: Actively personalized vaccination trial for newly diagnosed glioblastoma

a, Summary of immune responses to APVAC1 (n = 13 evaluable patients). Total APVAC1-specific CD8$^+$ T cell frequencies are shown. CM, central memory CD8$^+$ T cells; EM, effector memory CD8$^+$ T cells; EMRA, effector memory CD45RA$^+$ T cells; P, pretreatment samples; 1–5, post-treatment samples; MCIF, memory cell induction factor. For CD4$^+$ T cell responses, dominant phenotypes are indicated. **b**, CD8$^+$ T cell response in patient 2 against NLGN4X-001 evaluated pre- and post-treatment (Post 1–3). Percentages of specific CD8$^+$ T cells are indicated. Phenotyping: percentage of cells in the quadrants correspond to differentiation phenotypes (colour code as in **a**; see also Extended Data Fig. 4b). **c**, Avidity determination of

Hilf *et al*/2019, Nature
Actively personalized vaccination trial for newly diagnosed glioblastoma

immatics

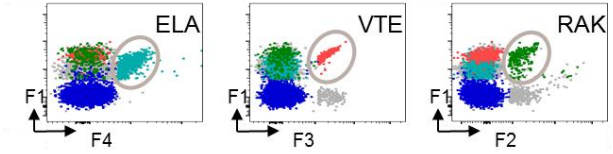
Study of **phenotypical and functional** differences **within and in-between** antigen-specific T cell responses

Combining **peptide stimulations** (11) with **cytokine** and **multimer-staining**

HTCC &

- **Surface** markers
- Intracellular **cytokines**

Detecting in depth features per antigen-specific T cell in small sample

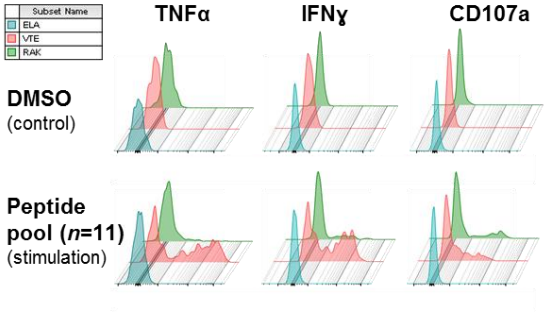


Ag-specific T cell responses:

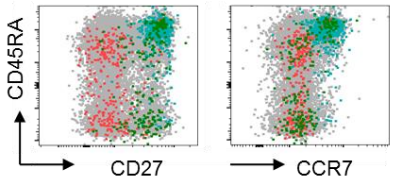
HLA	Epitope	CD8%	
A2	ELAGIGILTV	0.16	Melan-A_{MART-1}
A1	VTEHDTLLY	0.067	CMV_{pp50}
B8	RAKFKQLL	0.054	EBV_{BZLF-1}

F: Fluorochrome

Peptide-induced TNFα, IFNɣ and CD107a:

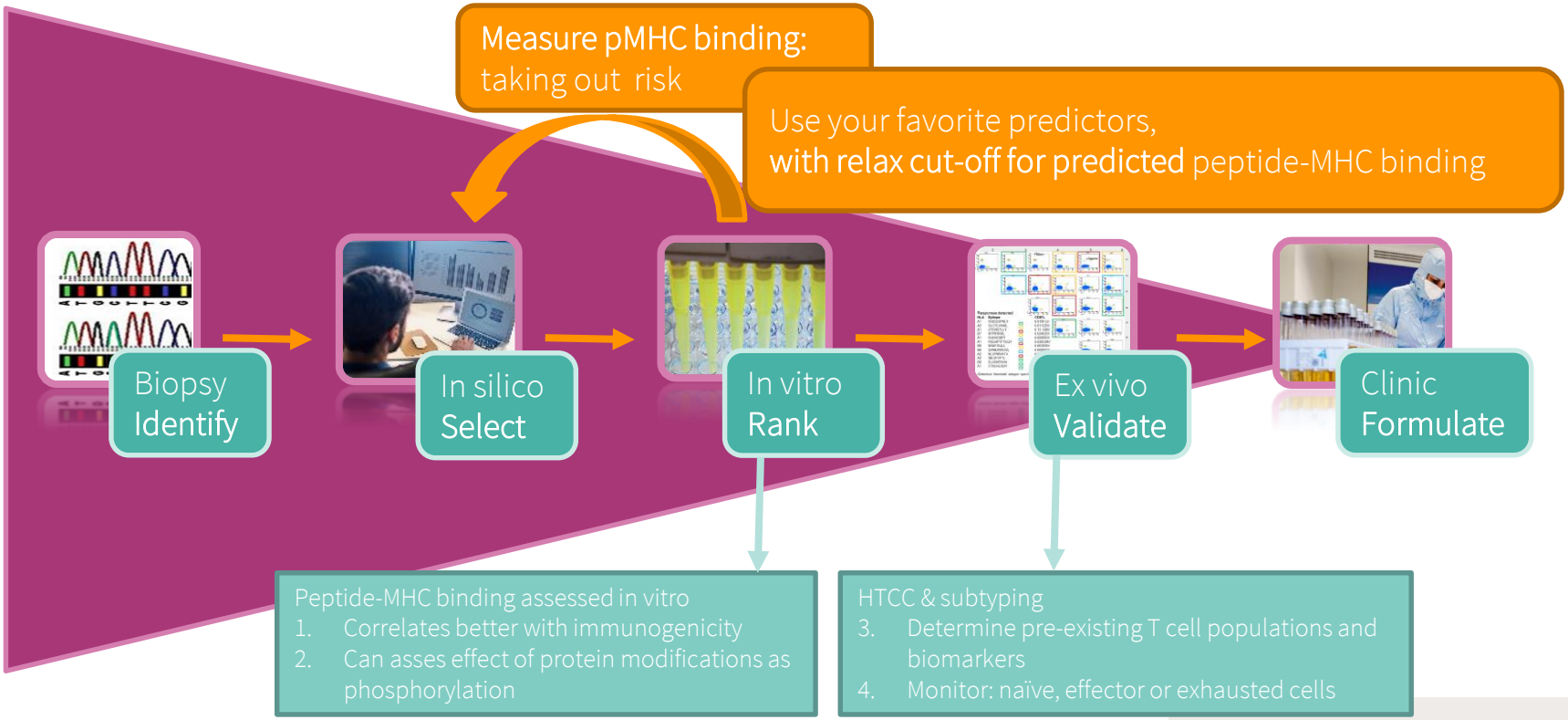


Phenotypical analysis:



3 of 11 peptide responses shown

***In vitro* guided** neo-epitope **selection process**

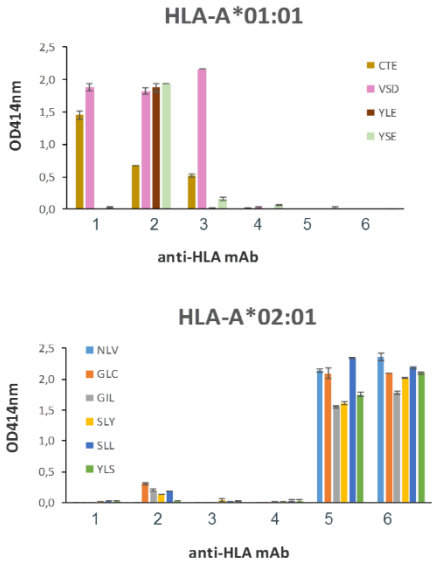


Asses **specificity and cross-reactivity** of “TCR”-mimes

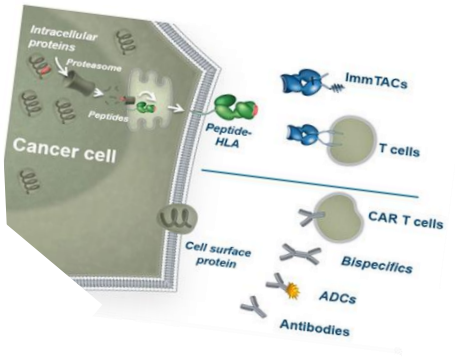
In vitro sTCR / recAb pHLA binding assay

assess binding of your TCR-mime to multitude of peptide-MHC complexes

- Determine specificity
- Assess cross-reactivity to non target



Differential binding characteristics anti-peptide/HLA mAbs



MHC team Sanquin Reagents BV
Wim van Esch
Juk Yee Mok
Giso Brasser
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Questions?

UV-peptide MHC exchange and HTCC are **patented** in Europe, US and other countries
For licenses: contact Astrid Visser - A.Visser@Sanquin.nl
Service testing provided by Sanquin (immunomonitoring@Sanquin.nl)
UV-exchangeable tetramers are available via Biolegend, USA

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