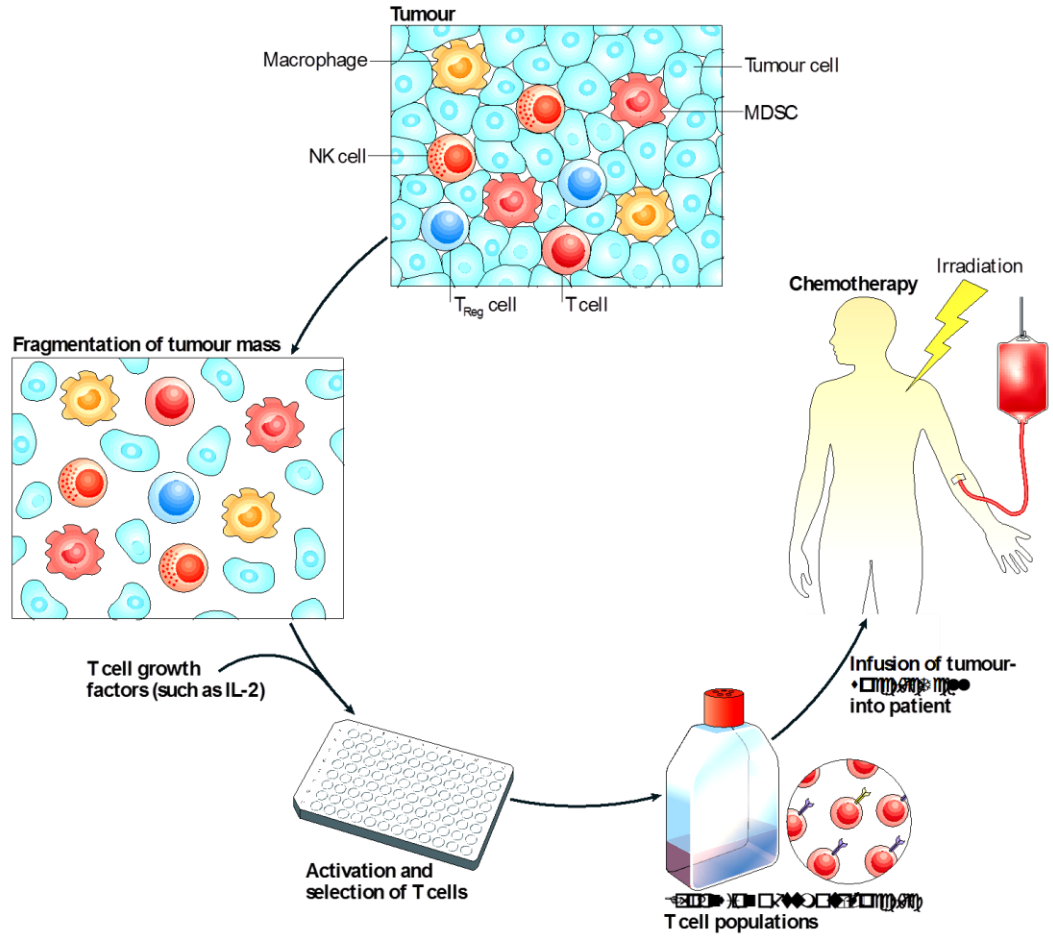
Developing T cell receptors with improved affinity

Nathalie Rufer, MD, PhD, PD

ESMO Symposium on Immuno-Oncology, November 21, 2014

Passive immunization approaches: adoptive T cell transfer

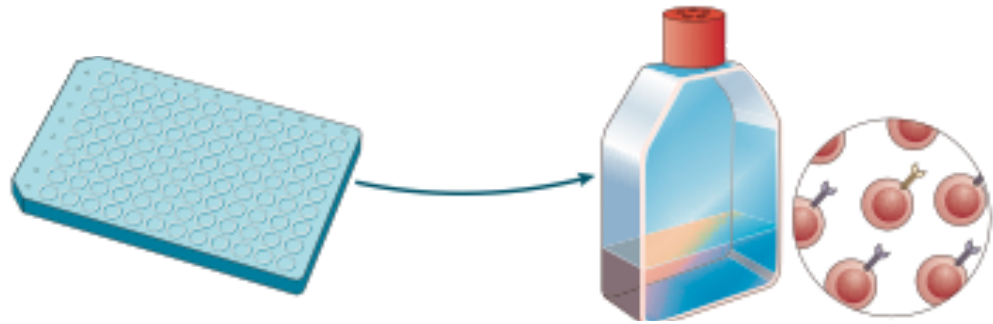
Immunotherapy aims at providing adequate numbers (frequencies) and to enhance the function of anti-tumor cytotoxic T cells while overcoming immune suppression and tolerance at the tumor site in cancer patients.



Restifo NP et al, Nat Rev Immunol, 12:269-281, 2012

Optimization of TCR:pMHC affinity against cancer cells

Most anti-(self) tumor-specific T cell responses are mediated by low avidity CD8 T cells due to mechanisms of central and peripheral tolerance.



Identification and selection of efficient anti-tumor T cells

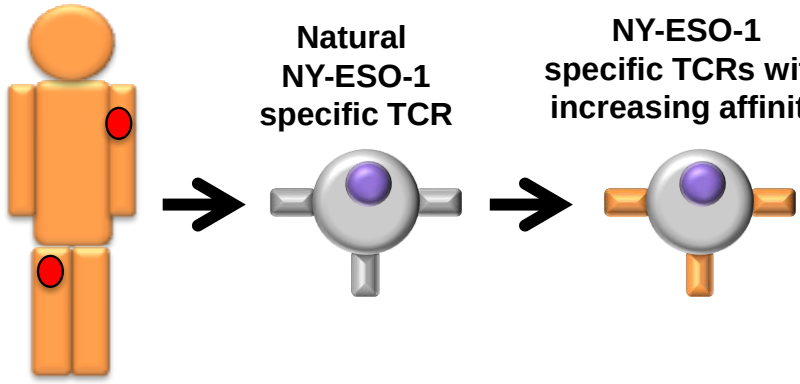
- T cell functionality
- Memory properties
- TCR affinity/avidity

Engineering T cells with increased TCR affinity against tumor antigens

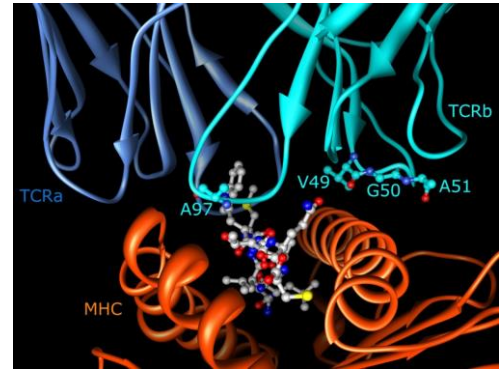
- **Phage-display approaches**
- **Rational in silico design approaches**
- **On-target and off-target side effects**

Restifo NP et al, Nat Rev Immunol, 12:269-281, 2012

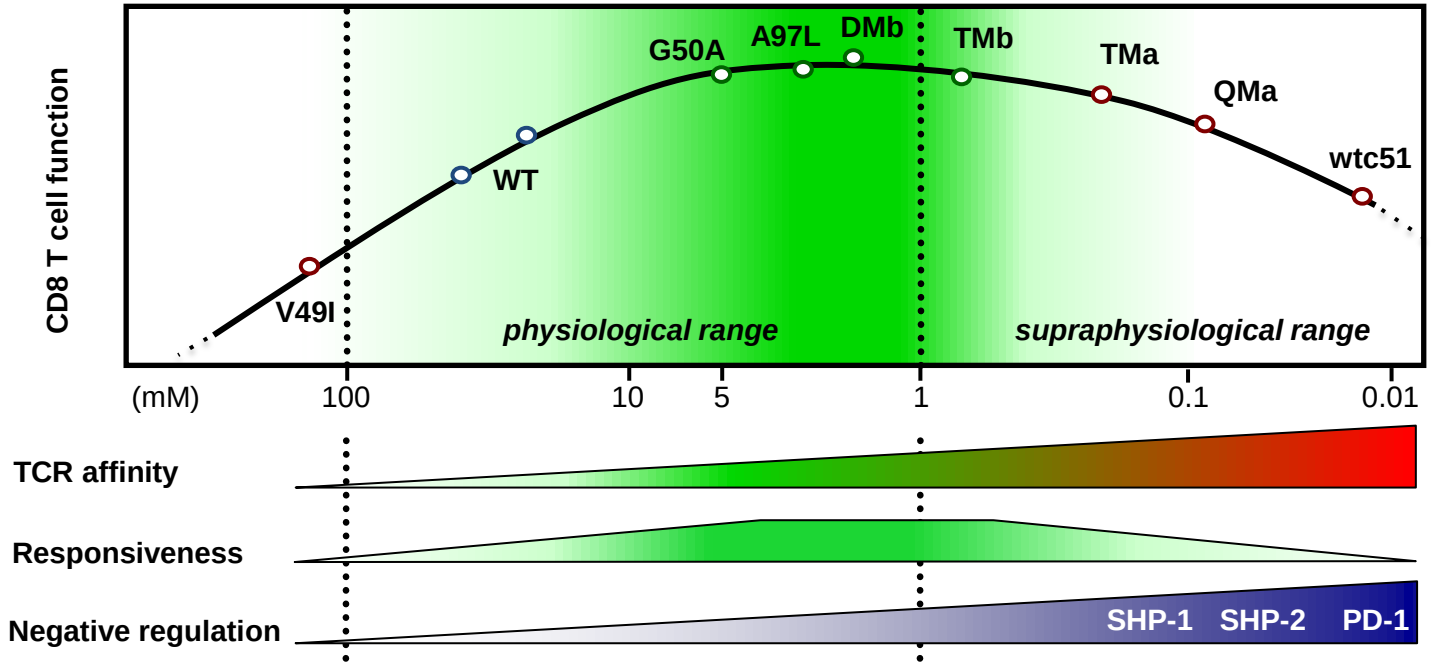
Aim 1: can we optimize TCR affinity and T cell function?



LAU 155 *Derré et al., PNAS, 2008*

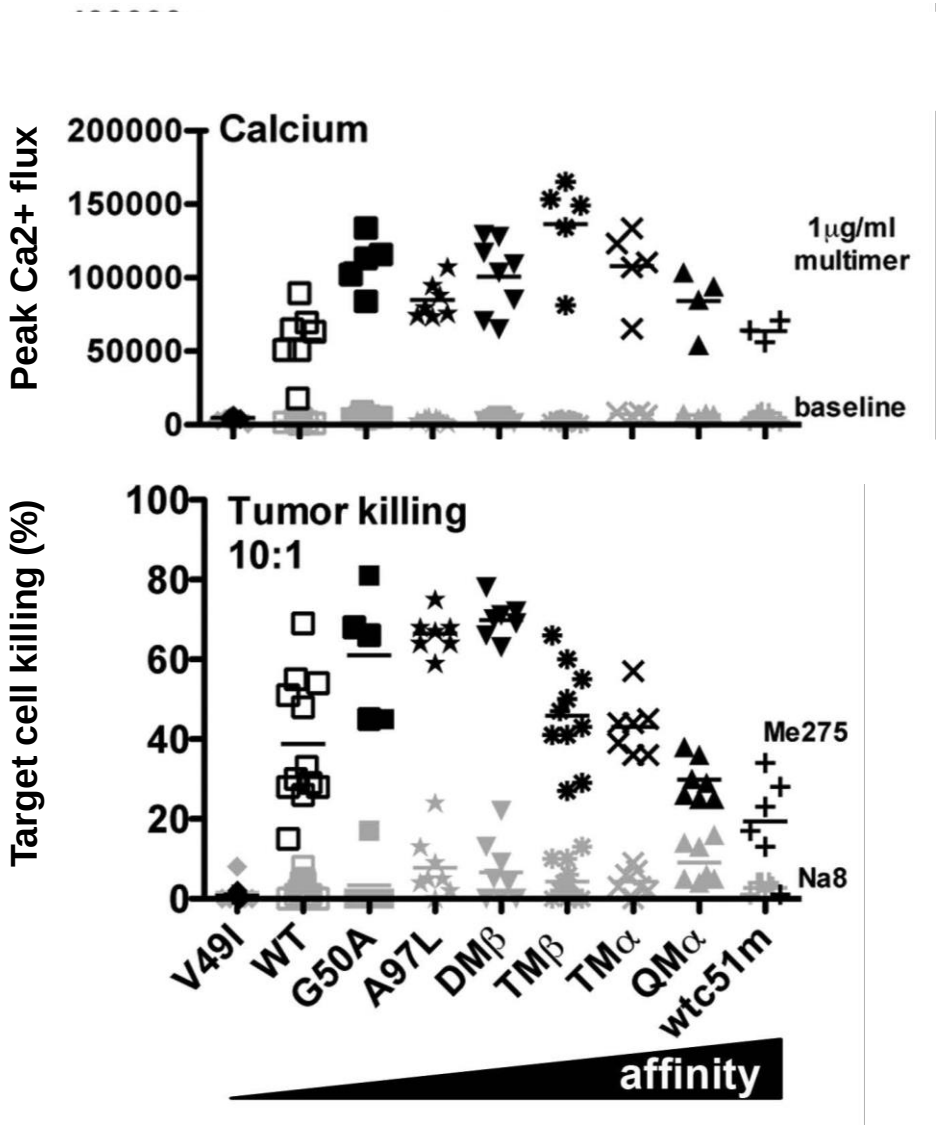


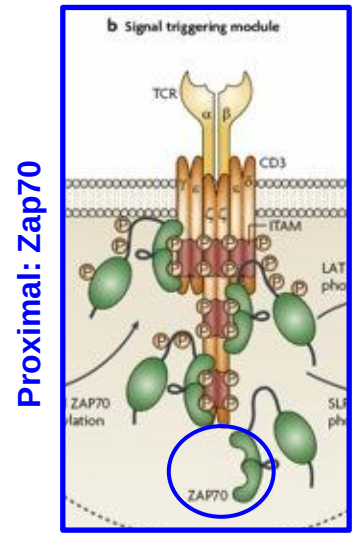
In silico structure-based approach
(Olivier Michielin's group)

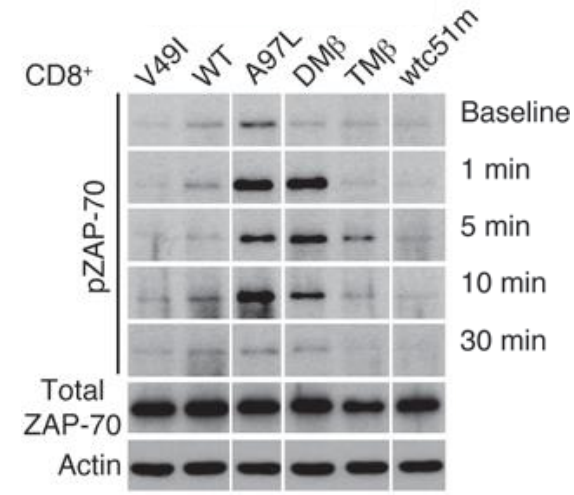


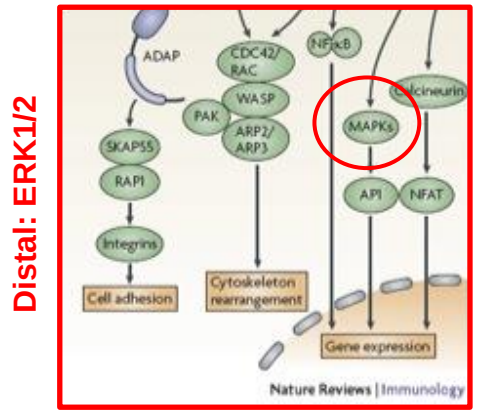
Schmid et al., J Immunol, 2010; Irving et al., J Biol Chem, 2012; Hebeisen et al., J Clin Invest, 2013

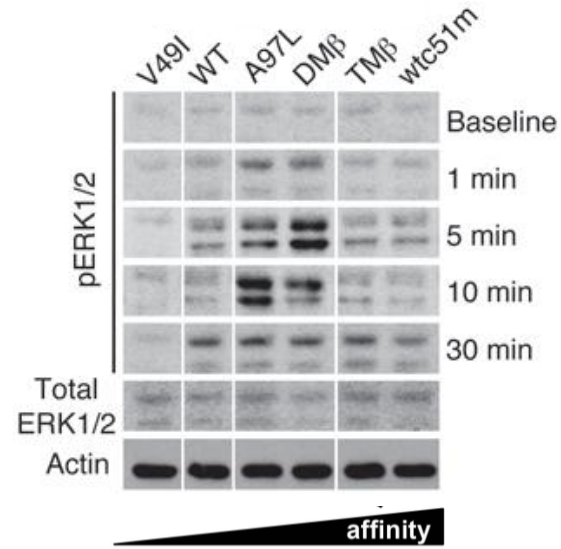
An affinity window for optimal T cell signaling and function





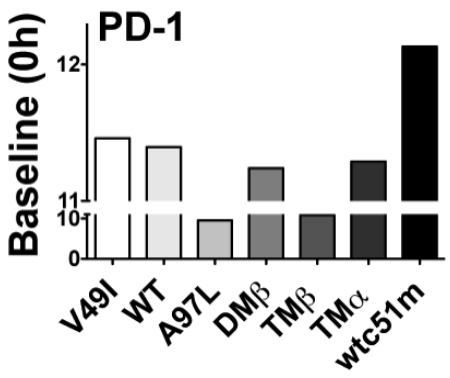


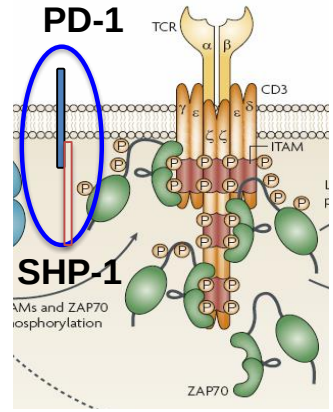


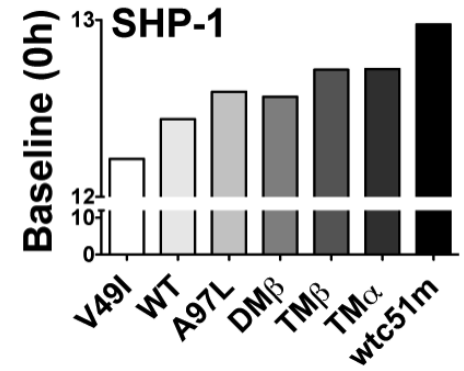


Hebeisen, Baitsch, Presotto, et al. J Clin Invest, 2013

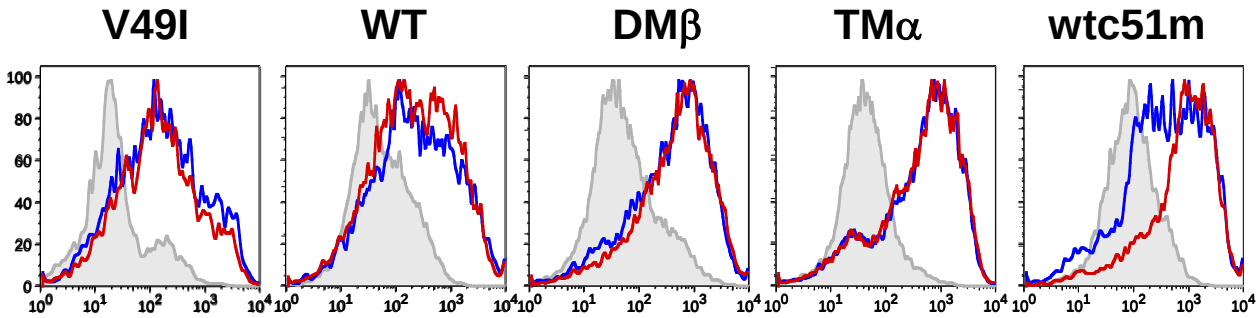
PD-1 inhibitory receptor and SHP-1/SHP-2 phosphatases





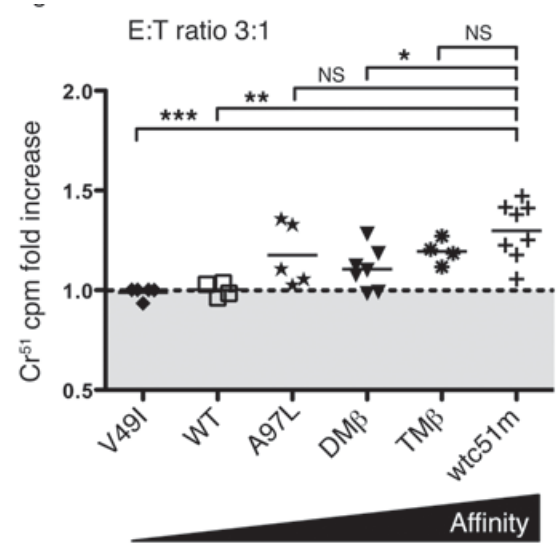


CD107 degranulation assays



anti-PD-L1 abs for 3-4 days of culture

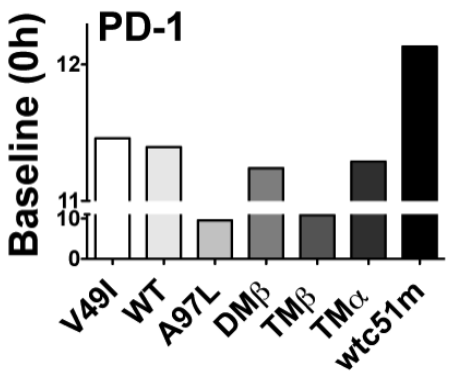
Killing assays

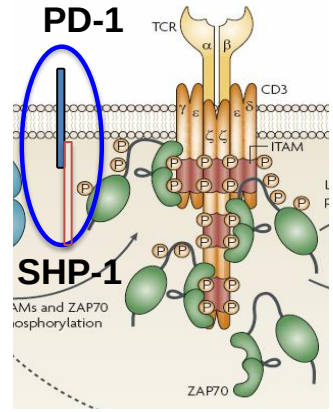


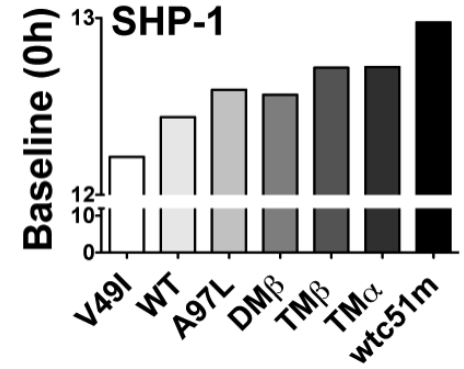
SSG (sodium stibogluconate)
50 ug/ml for 3-4 days of culture

Hebeisen, Baitsch, Presotto, et al. J Clin Invest, 2013

PD-1 inhibitory receptor and SHP-1/SHP-2 phosphatases







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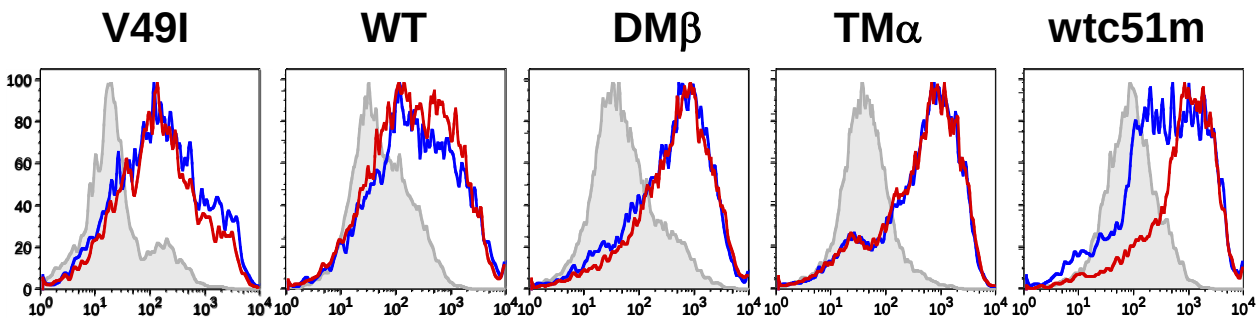
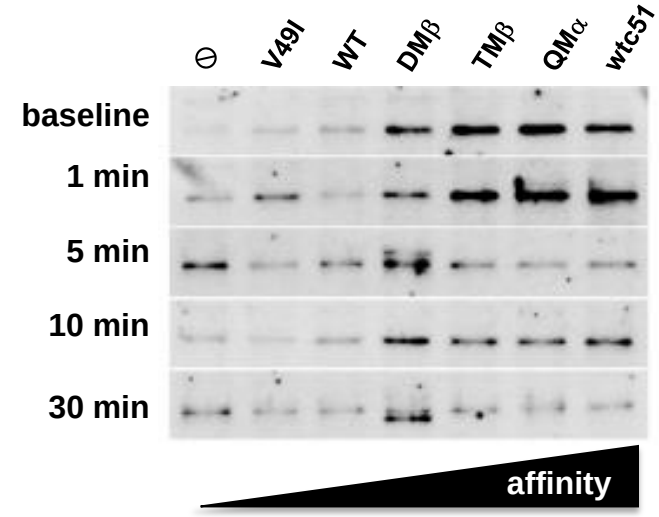


anti-PD-L1 abs for 3-4 days of culture

pSHP-2 (Y580)

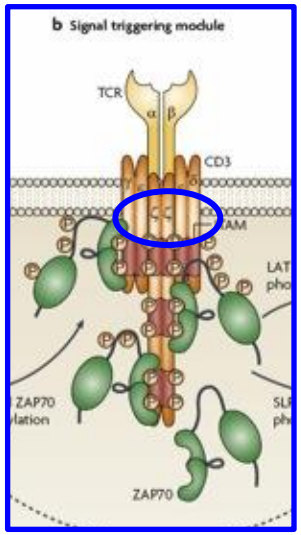


Hebeisen, Baitsch, Presotto, et al. J Clin Invest, 2013

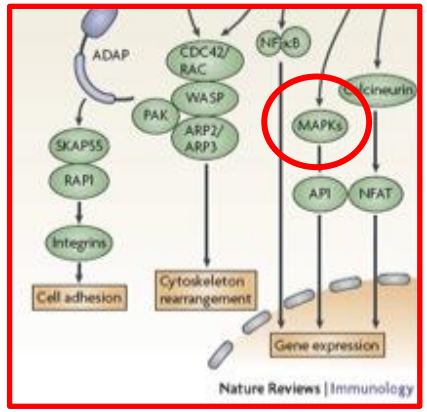
Presotto, Hebeisen et al. unpublished data

Mechanisms regulating T cells with enhanced TCR affinities

Proximal TCR-mediated signaling complex

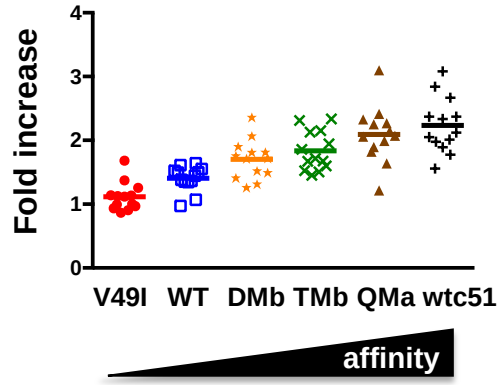


Distal signaling

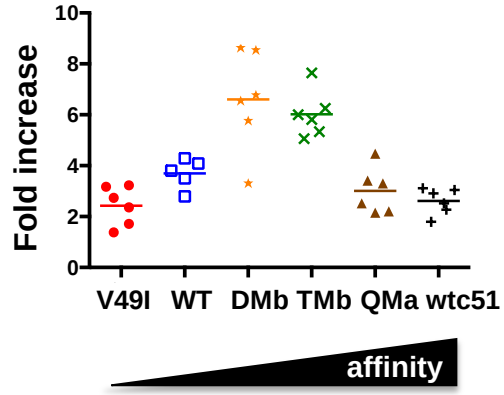


early post stimulation

pCD3z (Y142)

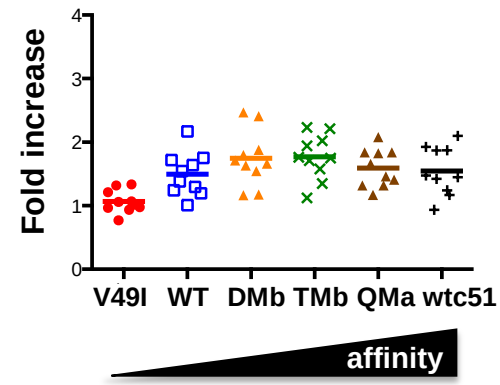


pERK1/2 (pY202/pY204)



late post stimulation

pCD3z (Y142)



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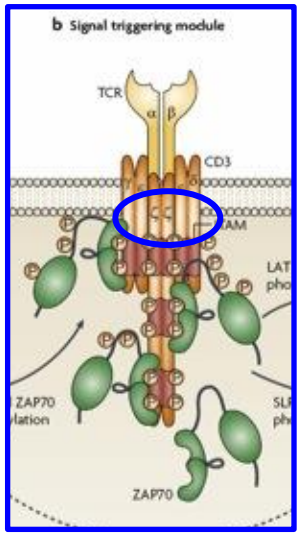
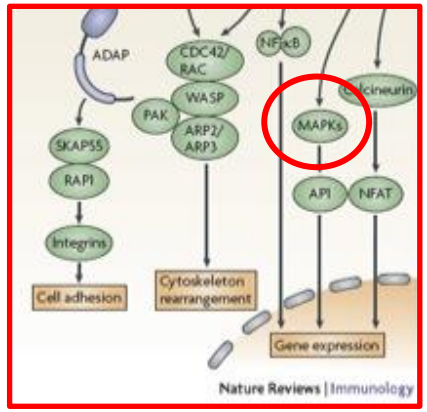
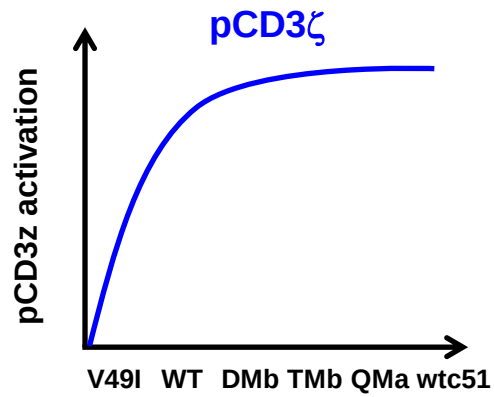


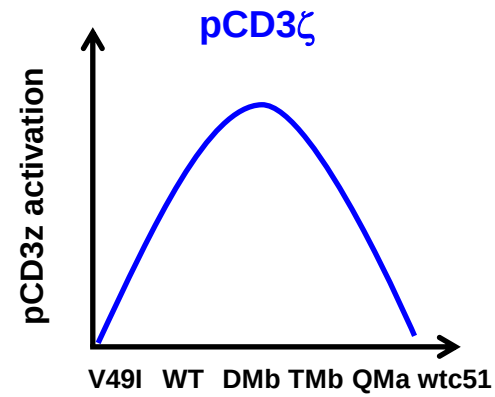
Distal signaling



early post stimulation

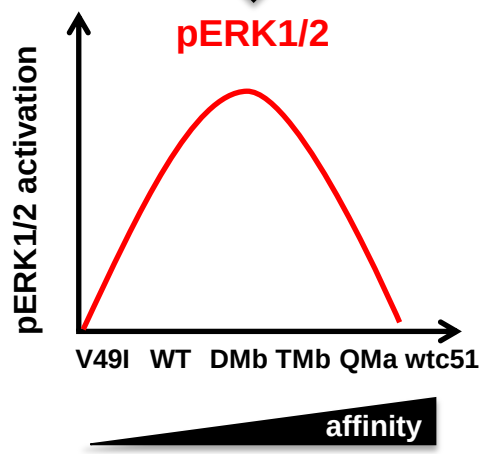


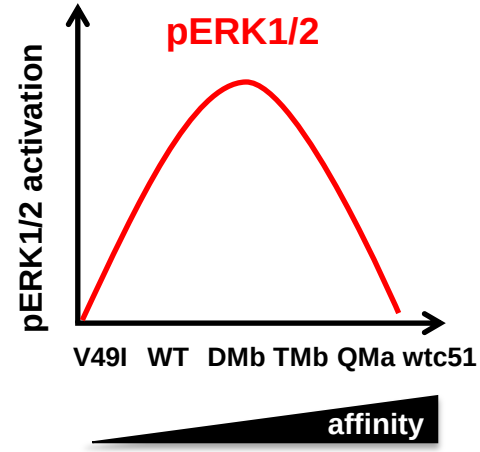
late post stimulation



1st level of regulation

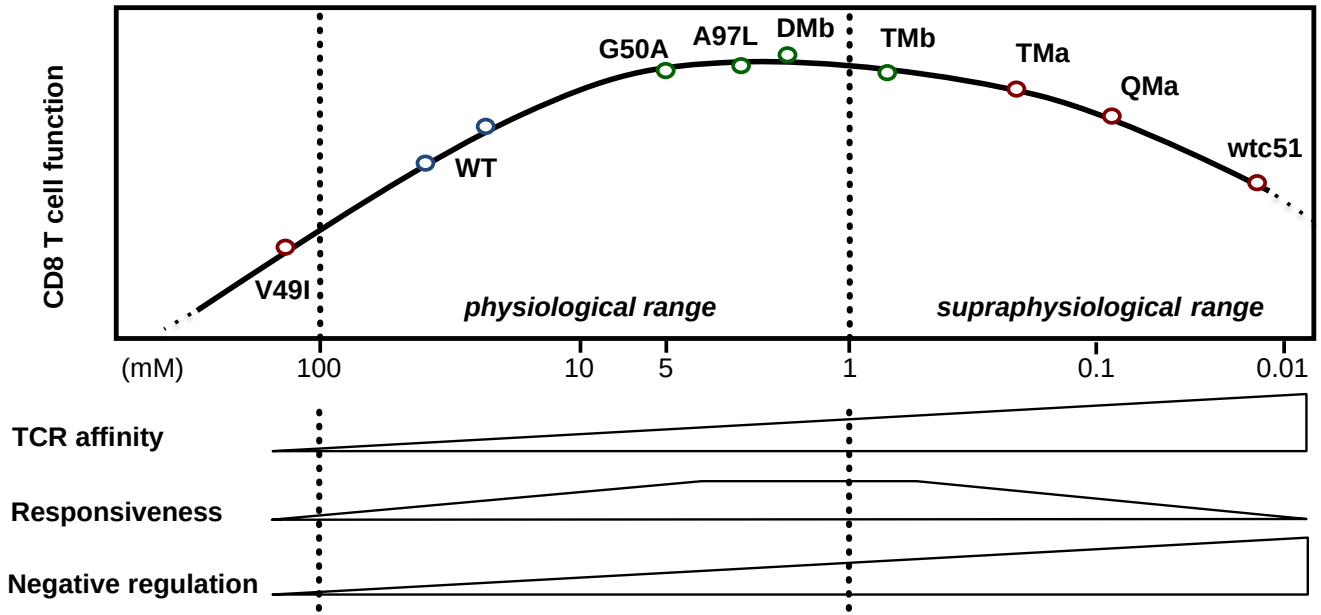
2nd level of regulation





Presotto, Hebeisen et al. unpublished data

Can we optimize TCR affinity and T cell function?

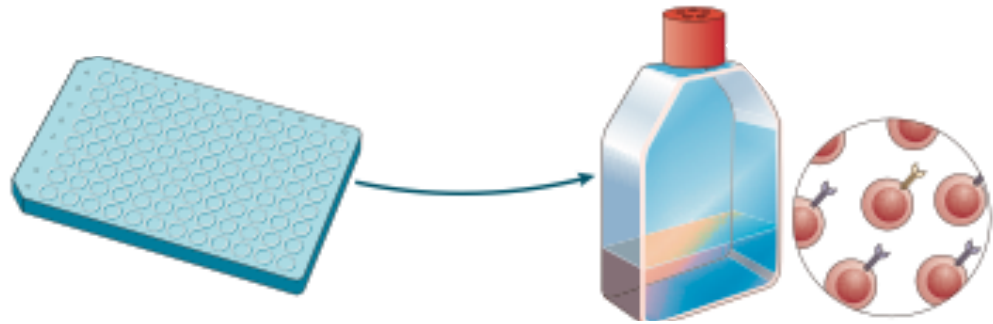


Optimal anti-tumor T cell responses occur within a well-defined TCR affinity window and are tightly controlled through several TCR affinity-mediated regulatory mechanisms

Highlight the importance of assessing TCR-pMHC affinity/avidity in relation to its functional efficacy for optimizing adoptive cell transfer

Optimization of TCR:pMHC affinity against cancer cells

Most anti-(self) tumor-specific T cell responses are mediated by low avidity CD8 T cells due to mechanisms of central and peripheral tolerance.



Identification and selection of efficient anti-tumor T cells

- **T cell functionality**
- **Memory properties**
- **TCR affinity/avidity**

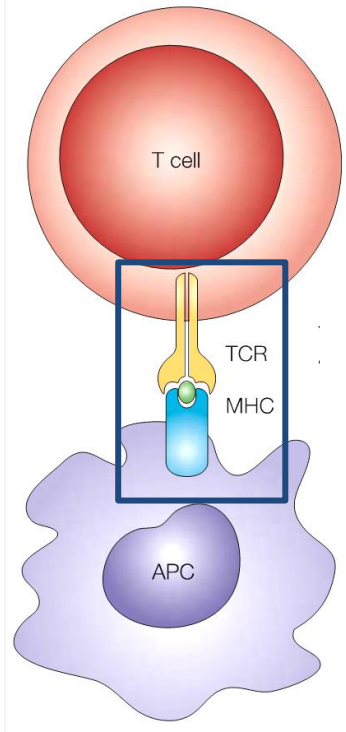
Engineering T cells with increased TCR affinity against tumor antigens

- **Phage-display approaches**
- **Rational in silico design approaches**
- **On-target and off-target side effects**

Restifo NP et al, Nat Rev Immunol, 12:269-281, 2012

TCR avidity is a major correlate of protection from disease

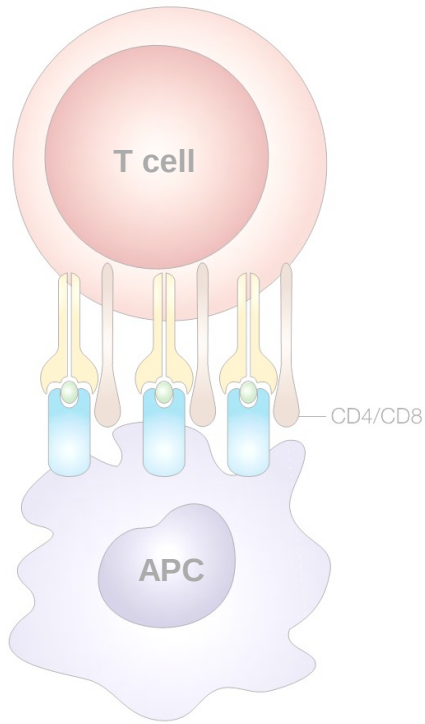
TCR affinity



Monomeric interaction
Strength of binding of
one pMHC and one TCR

Measured by SPR (Biacore)
using purified TCR molecules

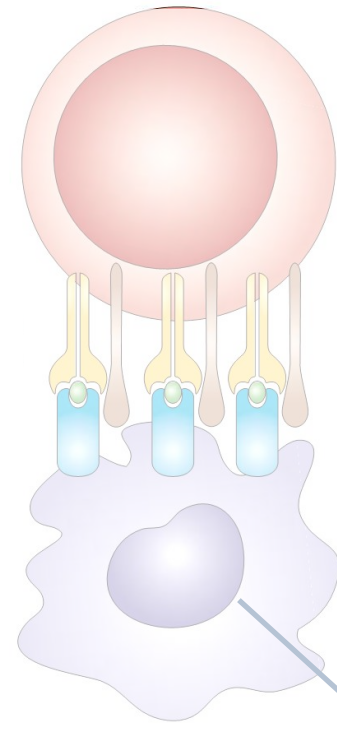
TCR avidity



Multimeric interaction
Strength of binding between
multiple TCRs and their pMHCs

Measured by multimers
or tetramers

Functional avidity



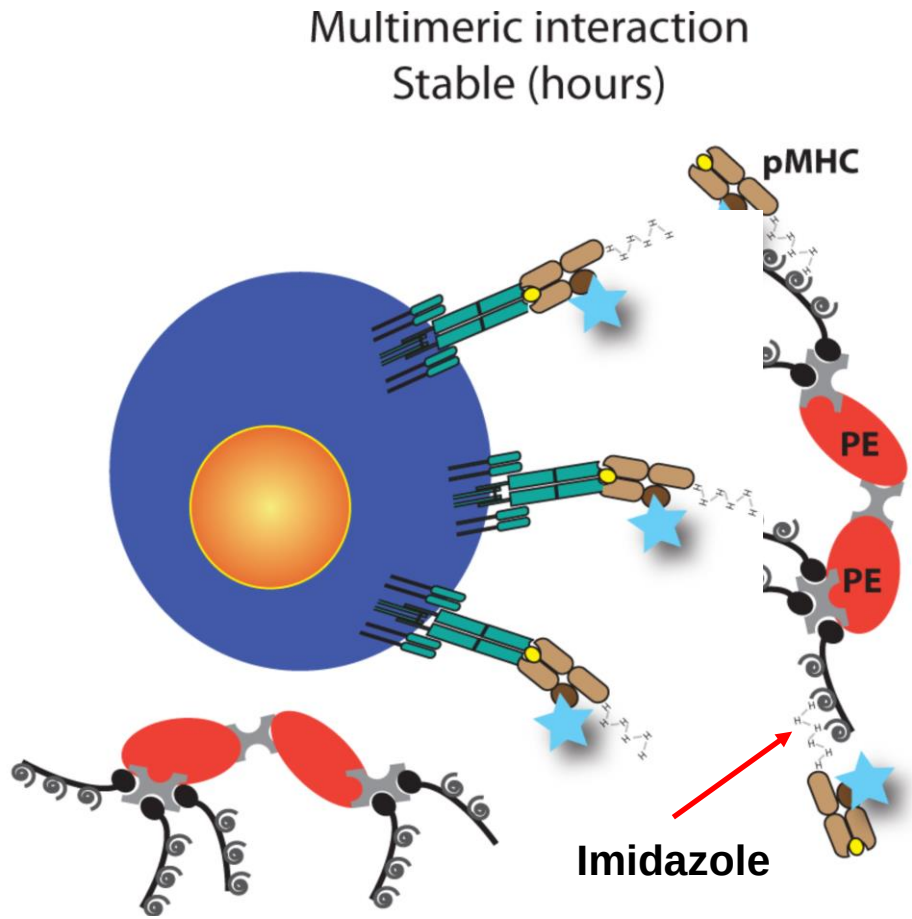
Antigen sensitivity
Relative kinetics of signaling
translating into biological function

Cytolytic function, proliferation
Cytokine production

Adapted from Nikolich-Zugich et al. Nature Rev Immunol, 2004

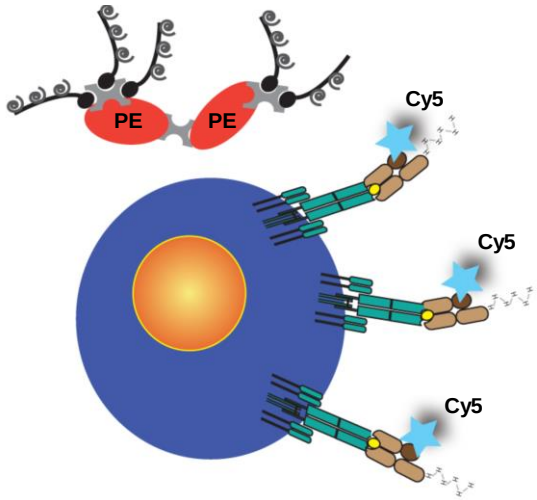
Aim 2: Can we identify and select for efficient anti-tumor T cells?

NTAmers
TCMetrix

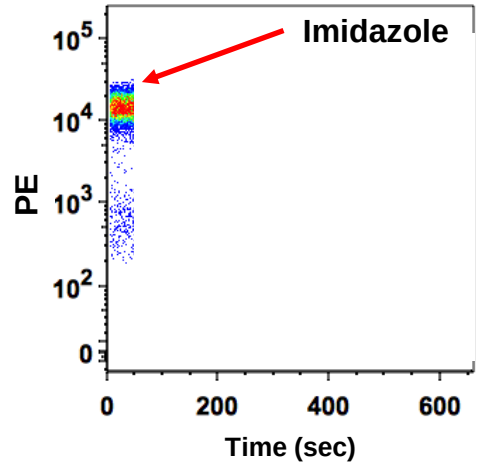


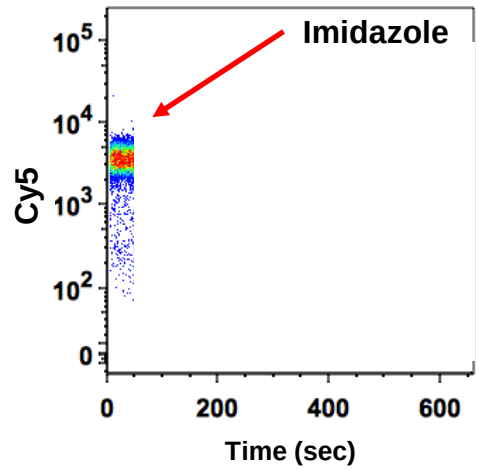
NTAmers for monomeric dissociation rate measurements

NTAmers
TCMetrix

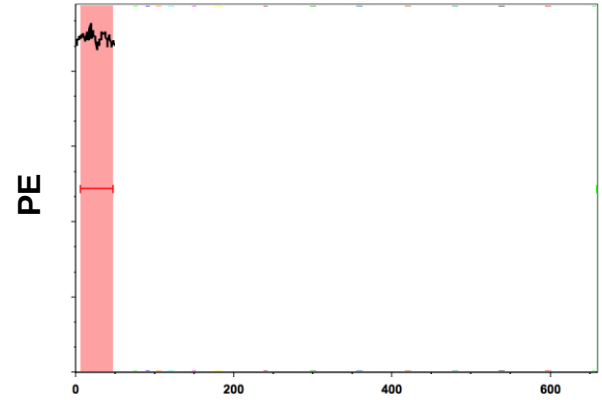


FACS

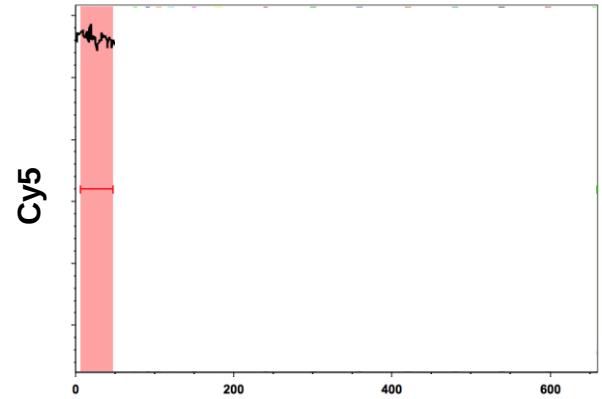




FlowJo Kinetics

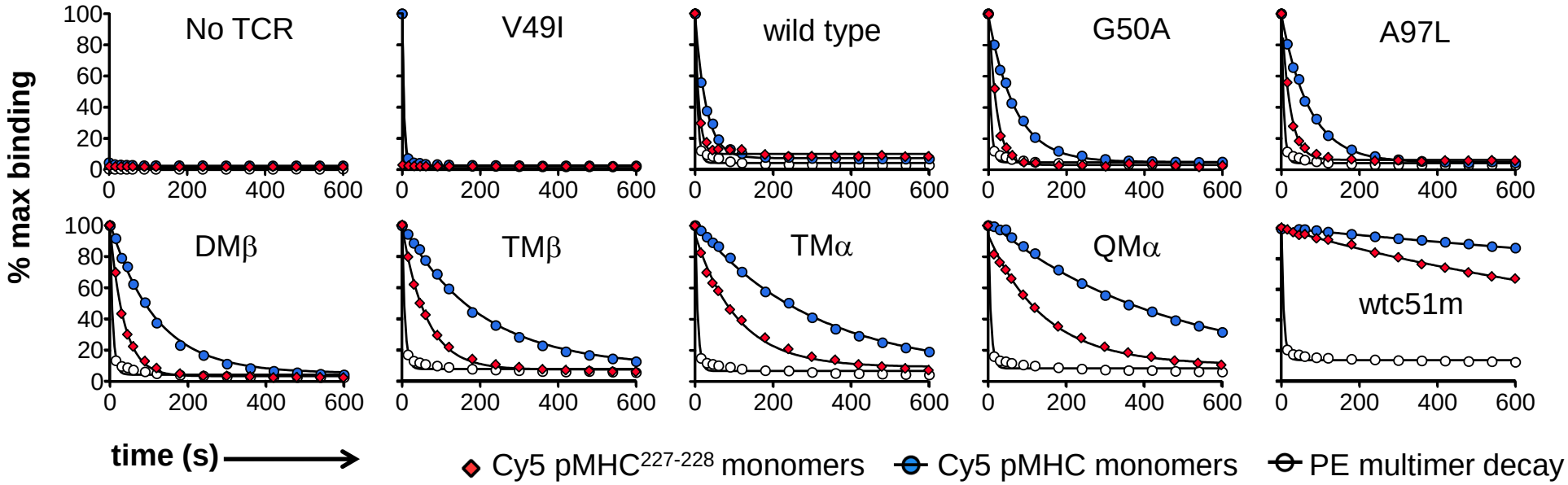


Rapid multimer decay (2-3 s)

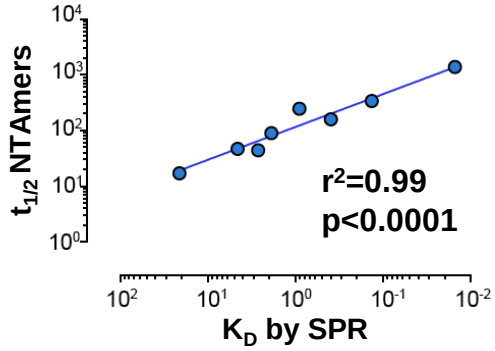


pMHC dissociation from TCR
→ k_{off} measurements

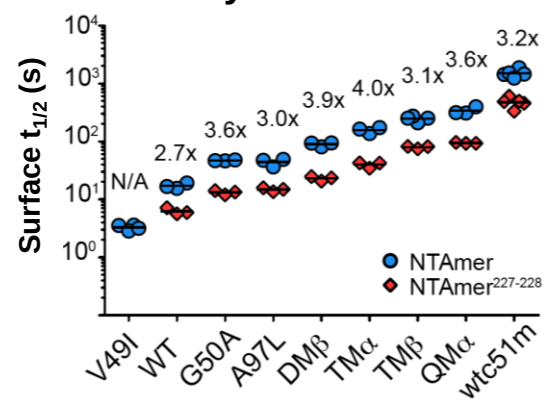
Correlations between NTAmers and SPR measured affinities



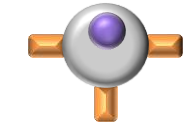
Strong correlation with SPR



CD8 prolongs TCR-pMHC half-life by a factor 3-4x

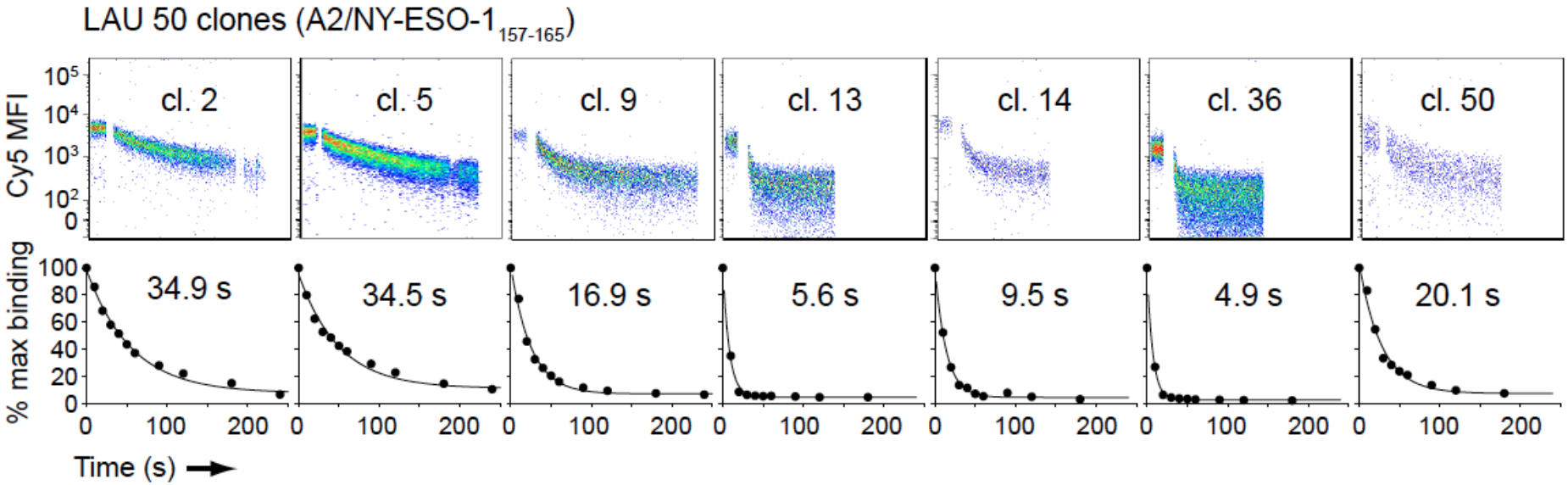


Panel of TCRs with increasing affinity

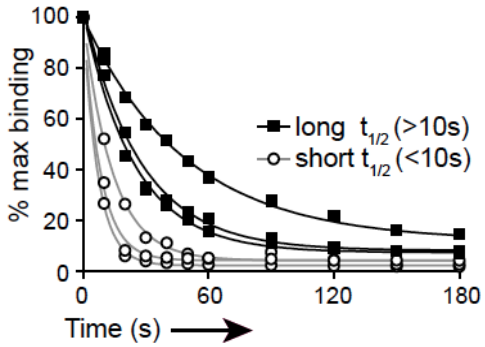


Hebeisen, Schmidt, Luescher et al., manuscript in submission

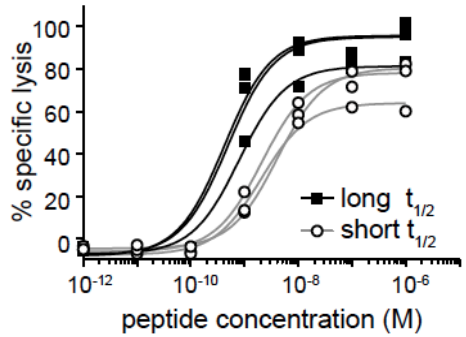
Assessing TCR:pMHC kinetics within the physiological range



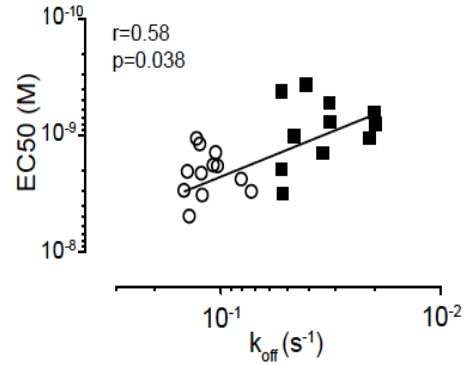
Monomeric $t_{1/2}$ analysis



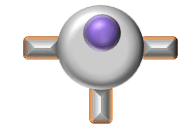
Killing assay



A2/NY-ESO-$1_{157-165}$

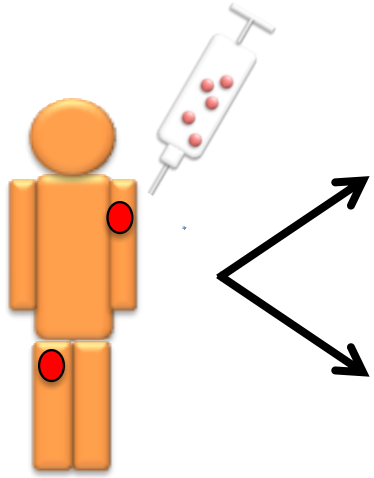


Panel of naturally occurring TCRs

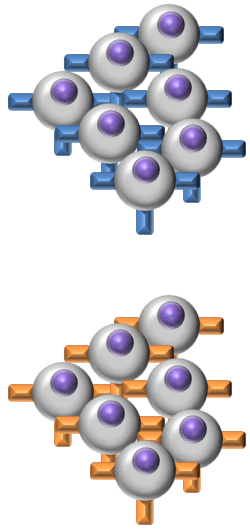


Hebeisen, Schmidt, Luescher et al., manuscript in submission

A clinically relevant model : impact of peptide vaccination



Vaccinated melanoma patients



Analog/ELA Melan-A_{26-35} peptide

5-10x stronger binding to HLA-A*0201

Native/EAA Melan-A_{26-35} peptide

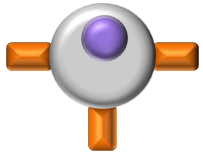
Adjuvant	Mechanism	T cell response
IFA CpG/TLR9	Ag + infl/depot + DCs	High frequencies

Vaccination	T cell frequency	TCR avidity	Poly functionality	Tumor recognition
Low affinity native EAAGIGILTV	+	?	+++	+++
High affinity analog ELAGIGILTV	++		+	+

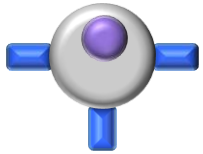
Speiser et al. PNAS, 2008; Speiser, Wieckowski et al., PNAS, 2011; Gupta et al., J Immunoth, 2012

NTAmers predict tumor-specific T cell responsiveness

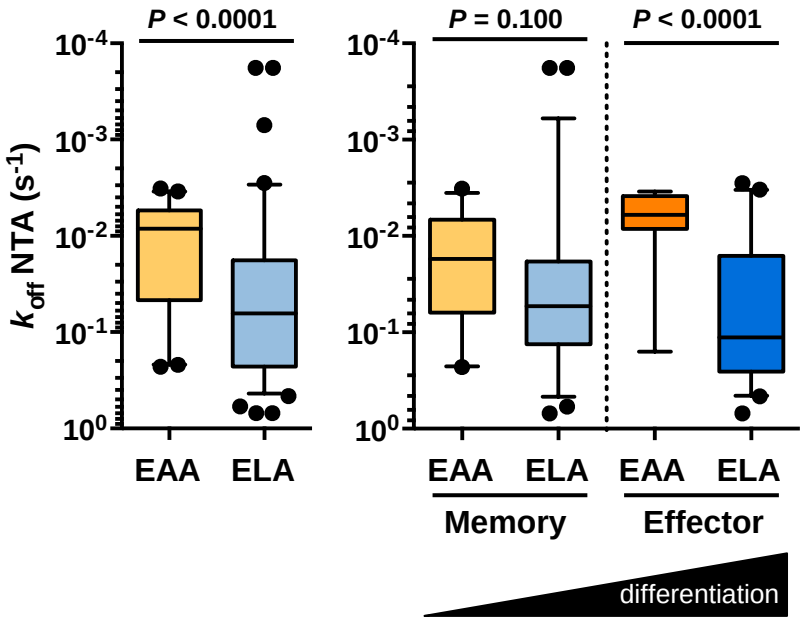
Large panel of vaccine-induced TCRs

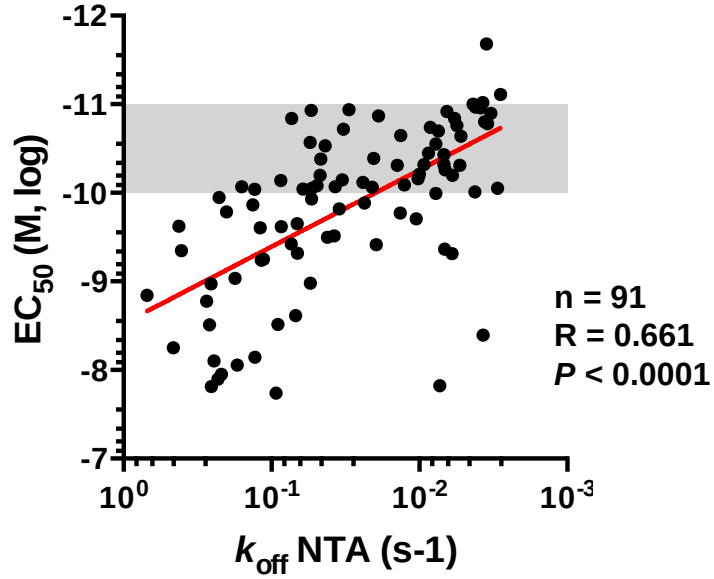


EAA
native peptide



ELA
analog peptide





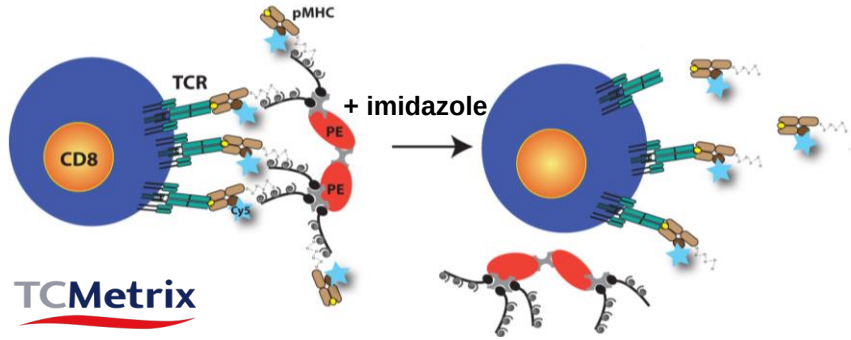
1. Native Melan-A peptide vaccine selects of a high avidity TCR repertoire

2. TCR avidity correlates with the functional competence of vaccine-induced tumor-specific T cells

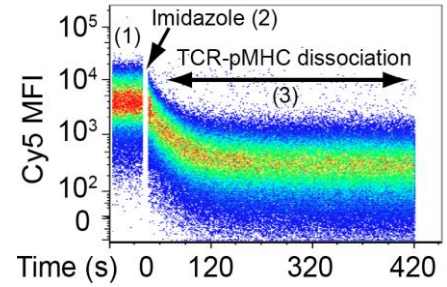
Gannon, Wieckowski et al., manuscript in preparation

Can we identify and select for efficient anti-tumor T cells?

Reversible NTAmers



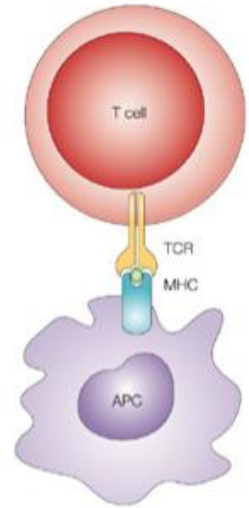
k_{off} analysis



NTAmer technology enables efficient and direct quantification of surface-based monomeric TCR:pMHC dissociation rates in a high-throughput manner

NTAmers accurately predicted T function, allowing the direct isolation and selection of rare functionally most-relevant CD8 T cells for adoptive cell transfer therapy

Collaborative network



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