

# T Cell Receptor Affinity and Avidity Defines Antitumor Response and Autoimmunity in T Cell Immunotherapy

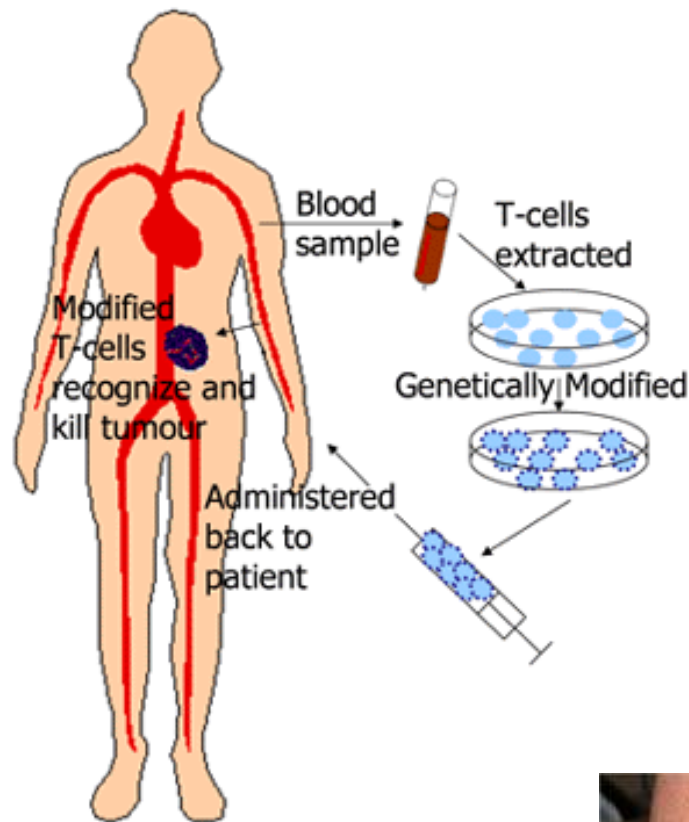
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# Challenges in immunizing against cancer

- Most tumor antigens are, although over-expressed on the tumor, non-mutated self (host) proteins.
- Our immune system is educated to tolerate self proteins.
- T-cells that express high affinity TCRs specific for self/tumor antigens are deleted in the thymus by negative deletion.

Morgan, RA. Cancer J. (2010)  
June, CH. J. Clin. Invest. (2007)  
Yee, C. Cancer J. (2010)

# Adoptive cell transfer (ACT) of antigen specific T-cells



- ▶ ACT with TILs achieves 49-72% objective response rate.
- ▶ Generation of tumor-specific T cells in this mode of immunotherapy is often limiting.
- ▶ ACT with TCR-engineered cells is promising but less efficient (25%).
- ▶ Not all cases result in complete and durable responses.



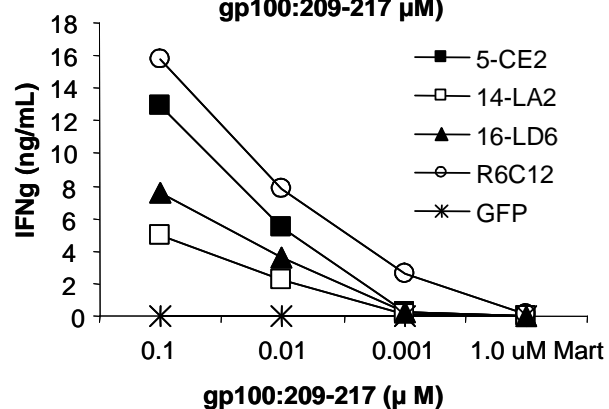
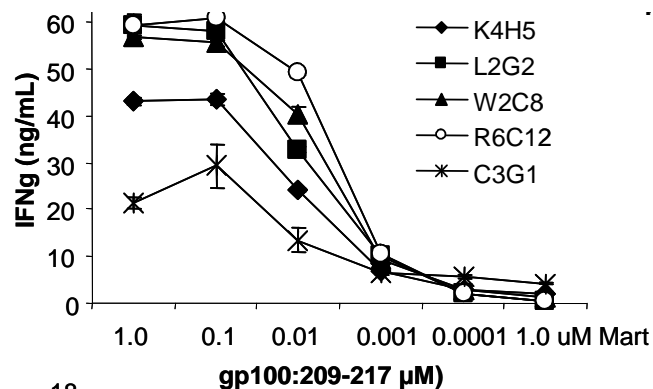
June, CH. *J. Clin. Invest.* (2007)  
Dudley et al., *J Clin Oncol*, 2008  
Yee, C. *Cancer J.* (2010)  
Morgan, RA. *Cancer J.* (2010)

## Can higher affinity TCRs render ACT more effective?

- Substantial evidence indicate a correlation between T cell functional activity and TCR affinity.
- Correlation remains controversial as higher affinity TCRs can lead to:
  - Stronger (Varela-Rohena, 2008).
  - Plateaued (Schmid et al. 2010, Tian et al., 2007)
  - Attenuated (Corse et al., 2010; Irving et al. 2012; McMahan, 2006)
- ACT using CD8+ T-cells is often associated with autoimmunity in mouse and humans (Palmer et al., 2008; Johnson et al., 2008; Yeh et al., 2009).

# A panel of A2/gp100-specific TCRs isolated from melanoma patients

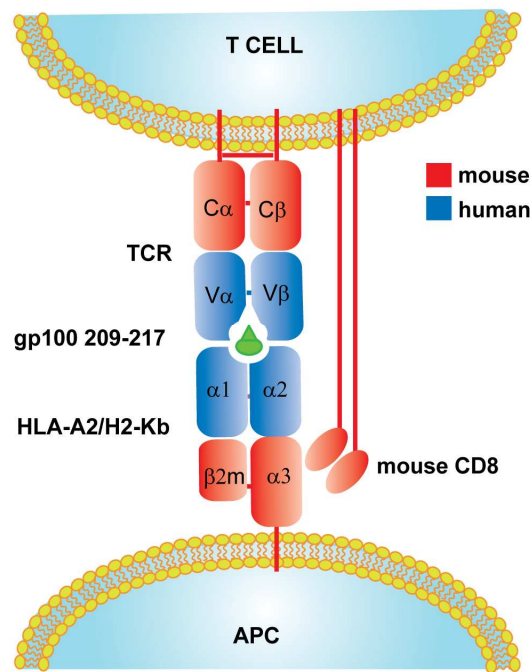
- Melanoma patients vaccinated with gp100 (2M) peptide + IL-2
- Isolated A2-gp100-specific TCRs by A2-gp100 tetramer sorting
- Cloned and sequenced TCR by RACE



TCR Name	TCR gene (IMGT®)		Source
	$\alpha$	$\beta$	
<b>19LF6</b>	19	19	PBL
<b>16LD6</b>	3	19	PBL
<b>R6C12</b>	41	12-3	PBMC
<b>K4H5</b>	17	27	PBMC
<b>5CE2</b>	12-1	27	PBMC
<b>L2G2</b>	12-2	7-9	PBL
<b>W2C8</b>	2	6-2	PBMC

# Generation of human/mouse chimeric TCRs

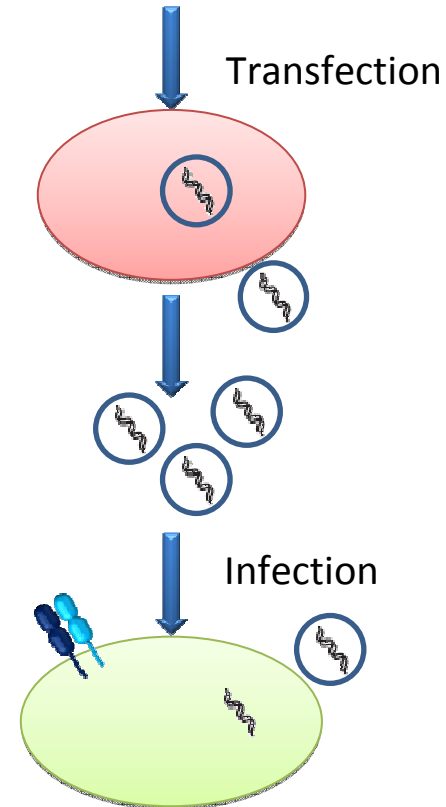
## Retroviral Constructs



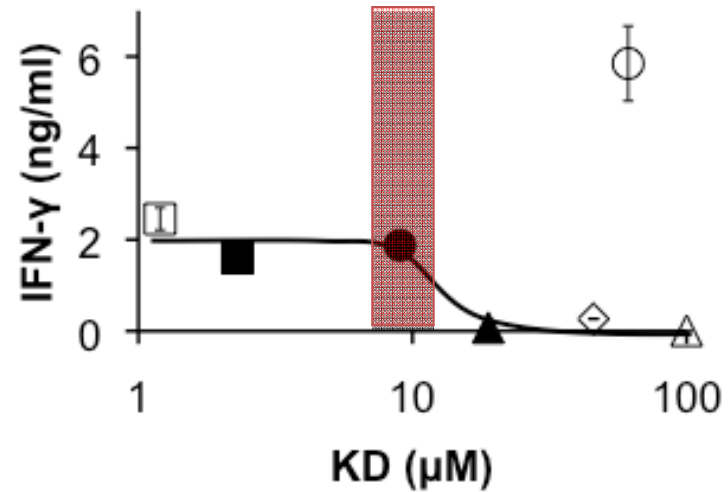
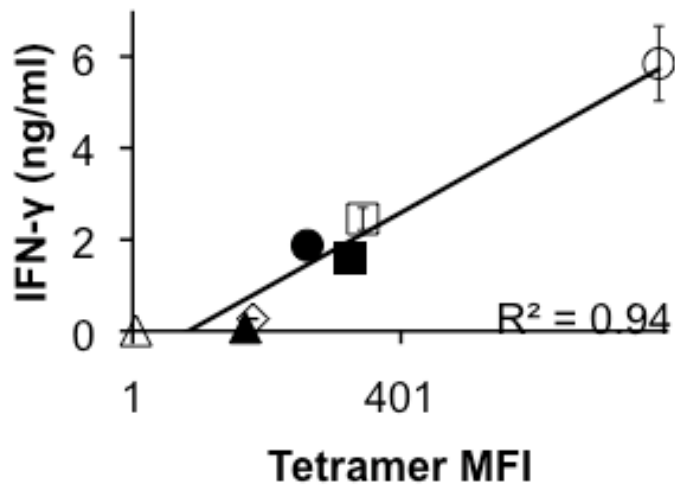
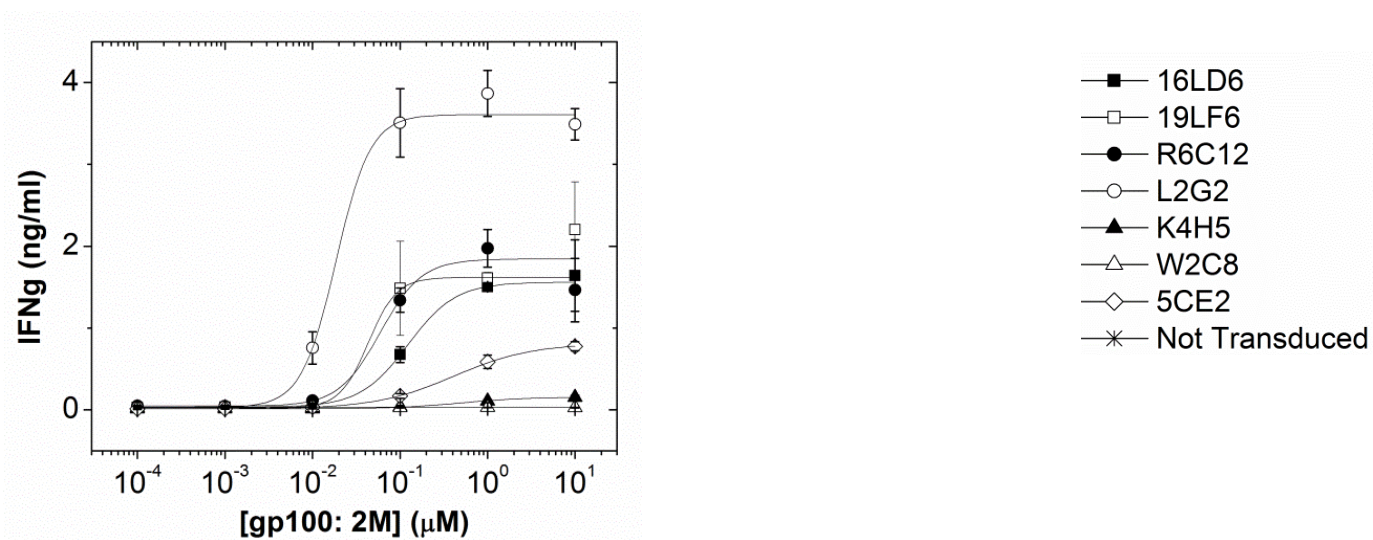
Phoenix Retrovirus  
Producer Cells

Retroviral Particles

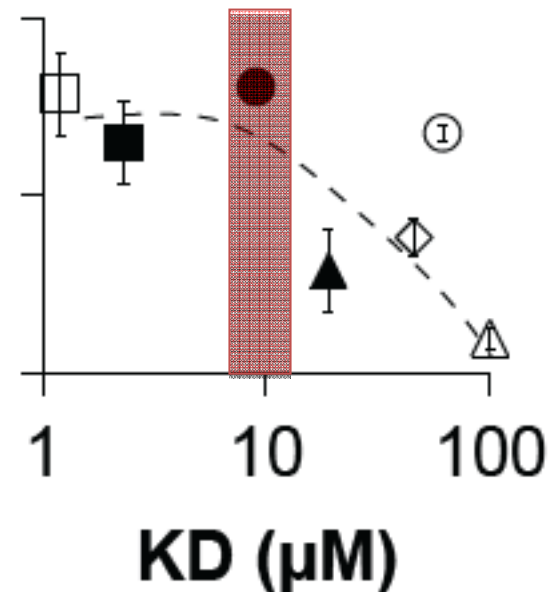
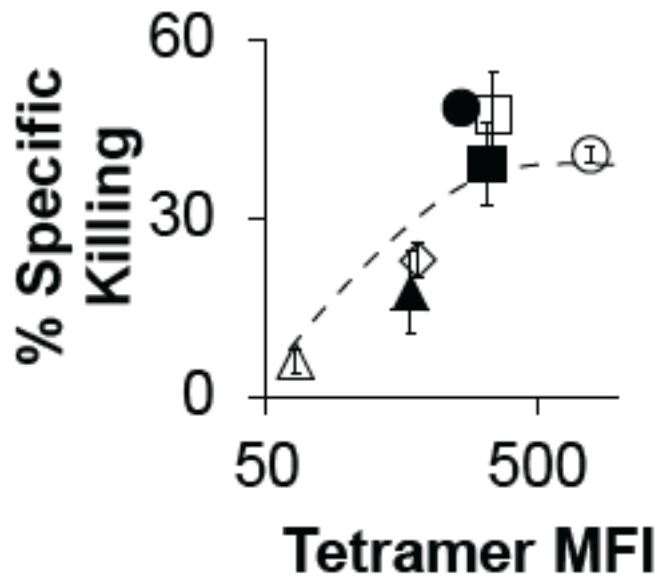
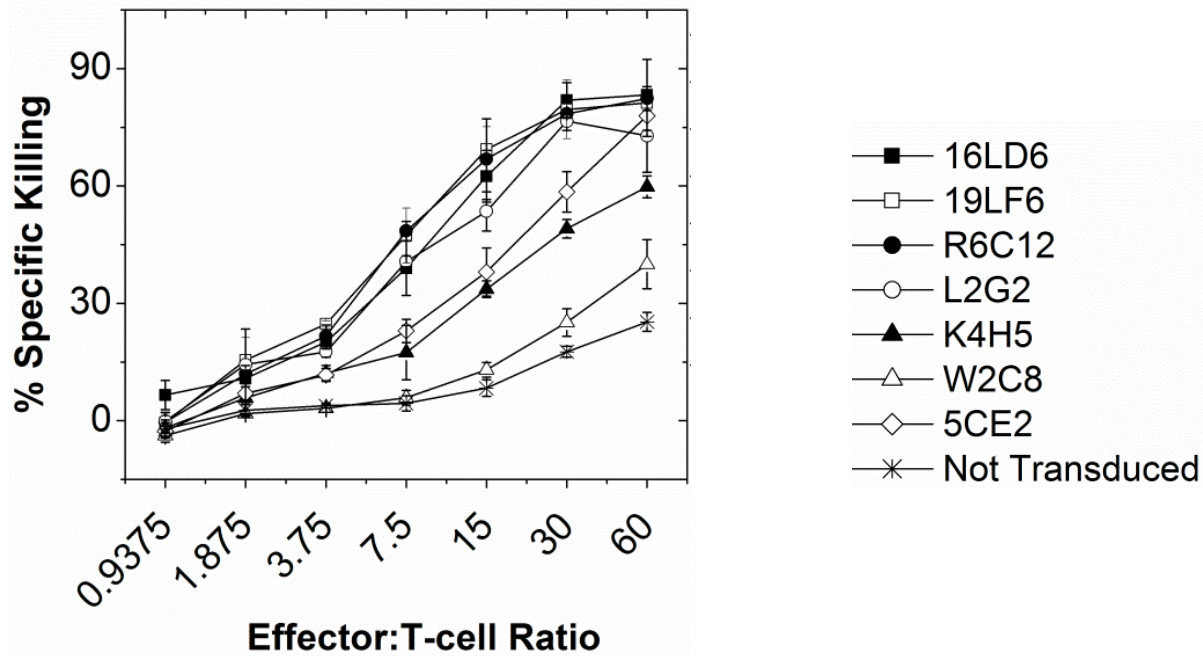
A2-K<sup>b</sup> naïve mouse  
CD8<sup>+</sup> T-cells



# Distal T-cell signaling events are correlated with tetramer binding affinities

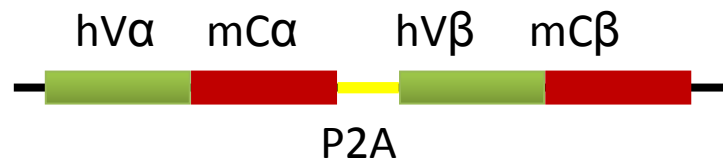
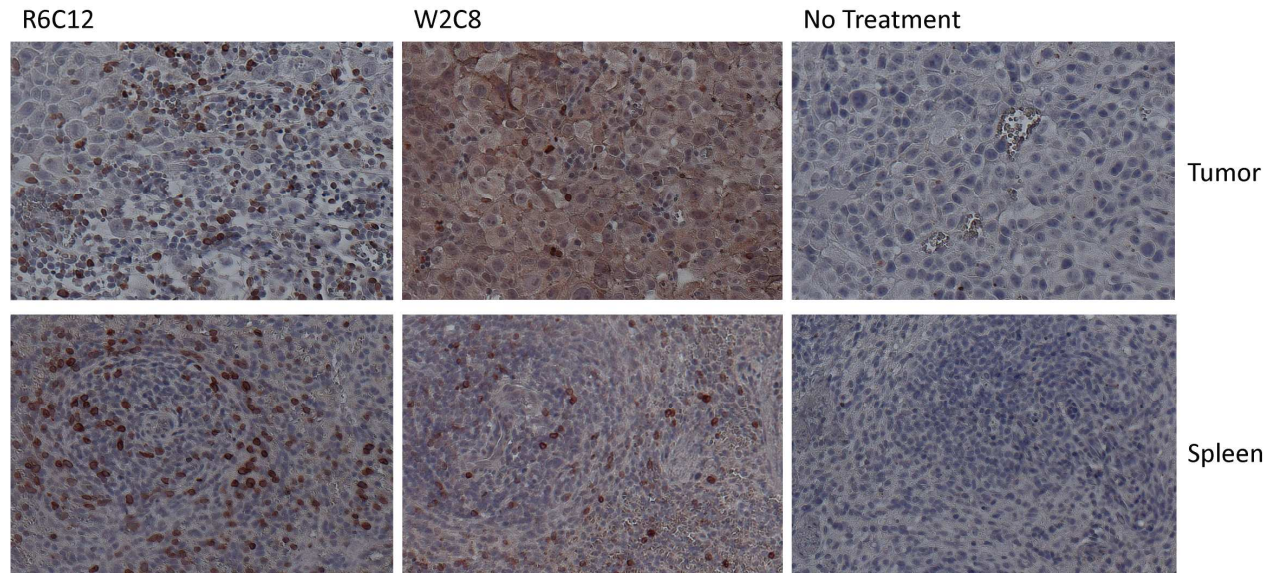


# Tetramer binding threshold for cytotoxicity

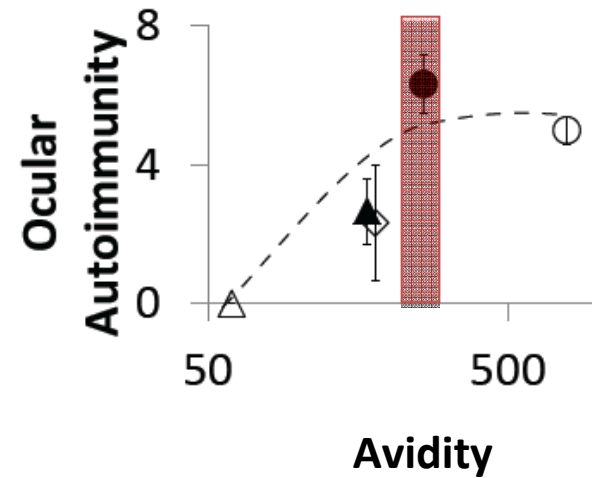
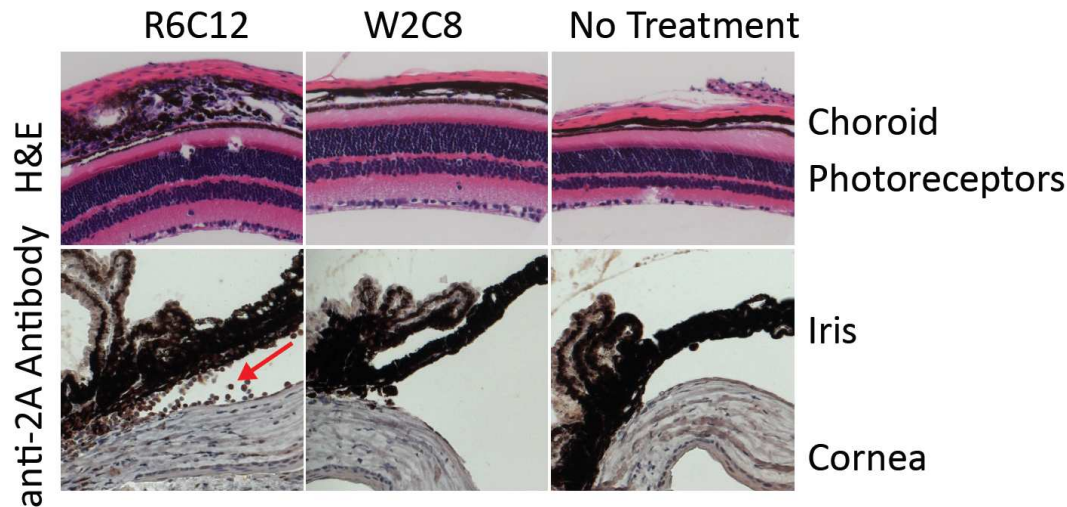
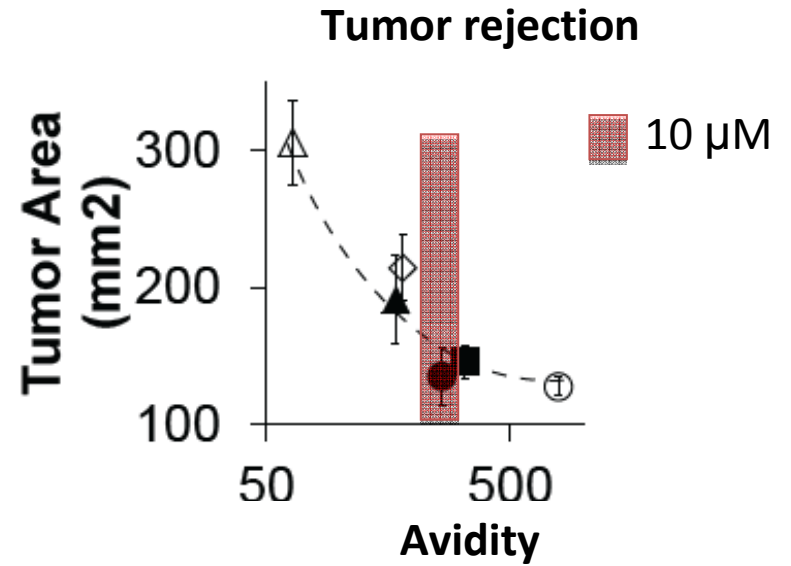
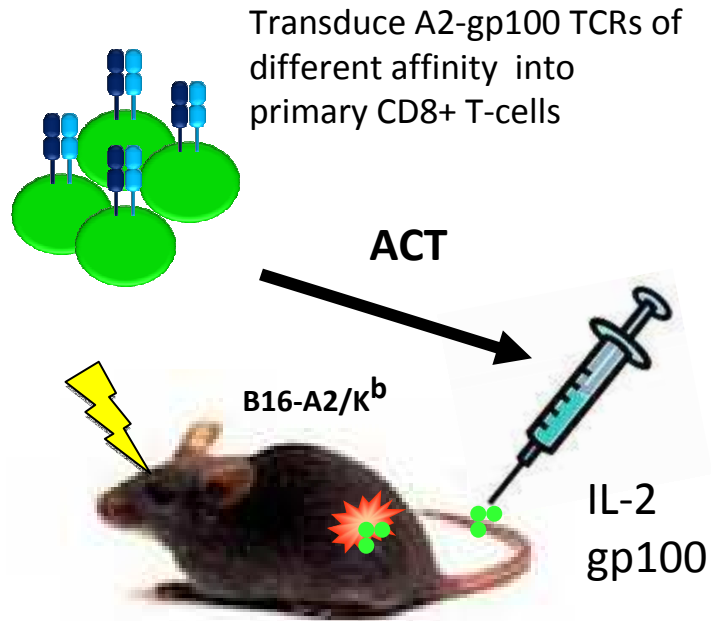




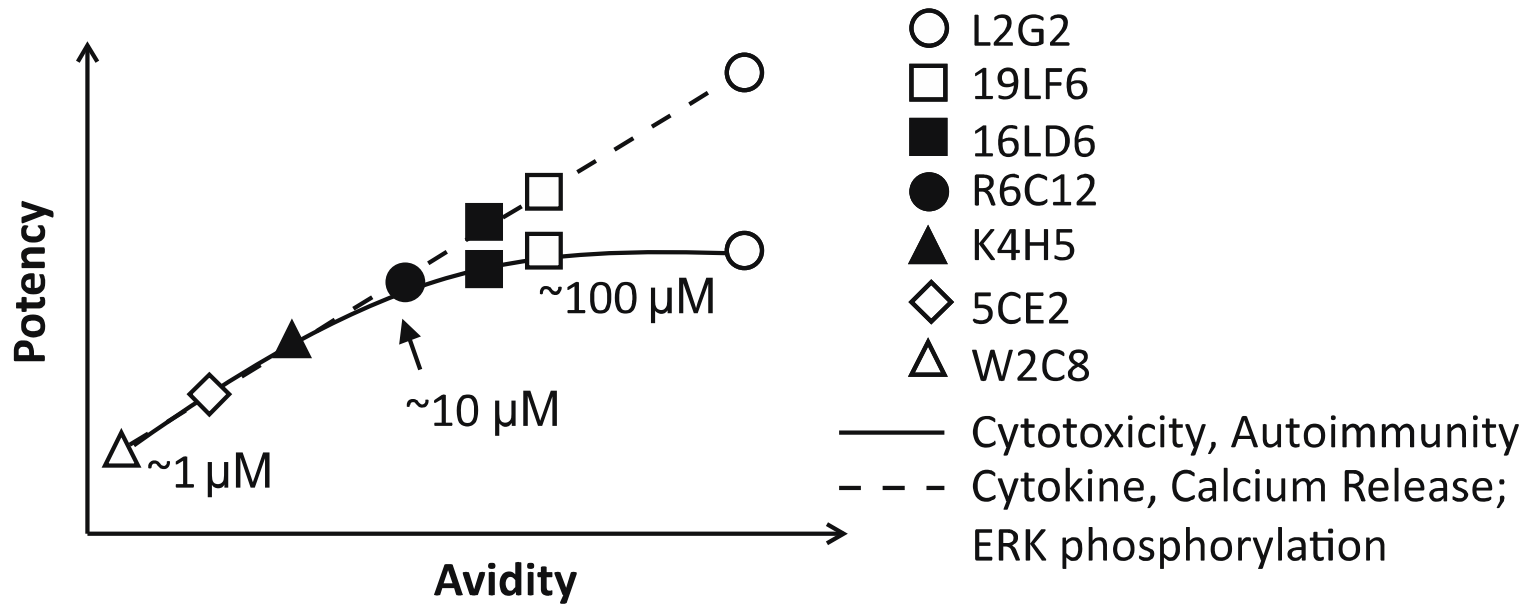
# Lack of tumor regression not due to lack of accumulation of T-cells in spleen and tumor



# Can higher affinity TCRs render ACT more effective?



# A TCR affinity threshold defines T cell functional activities induced by self-ligands



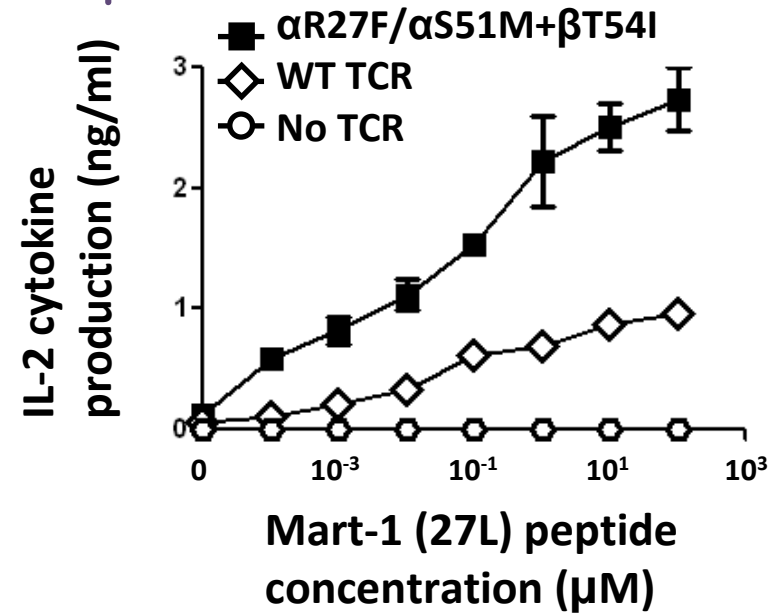
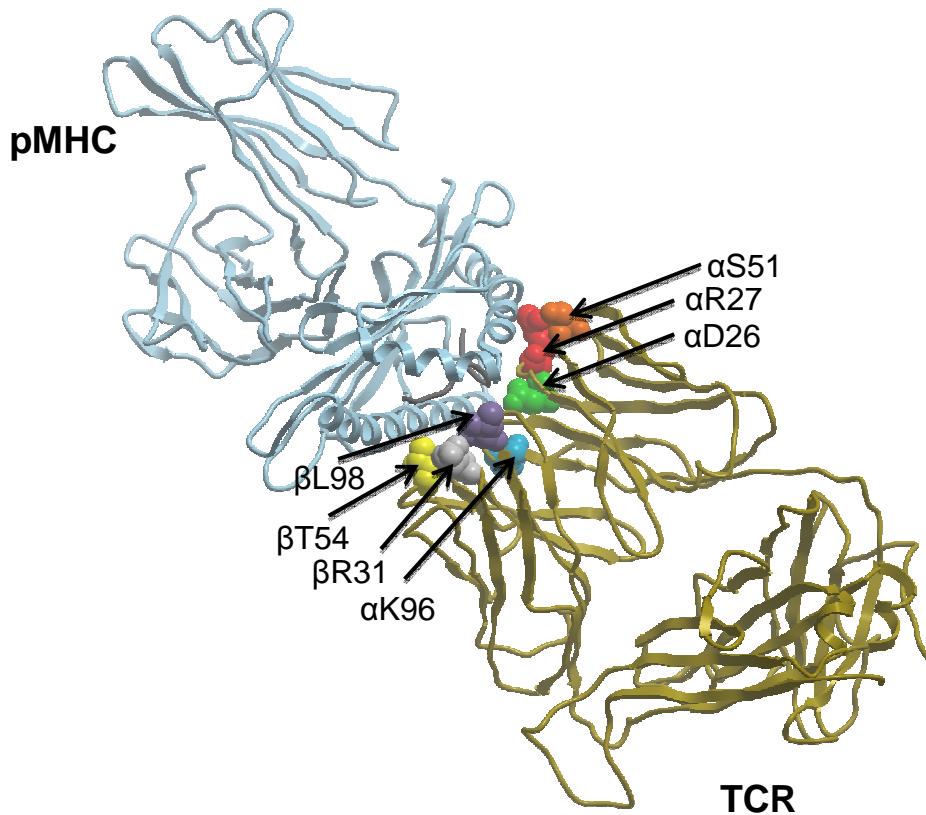
## Affinity threshold has clinical implications

TCR	KD ( $\mu\text{M}$ )	Objective cancer regressions	Cellular infiltrate into the eye
DMF4	29	13% (n=17)	0%
DMF5	5.6	30% (n=30)	55%
gp154	0.38	19% (n=16)	25%

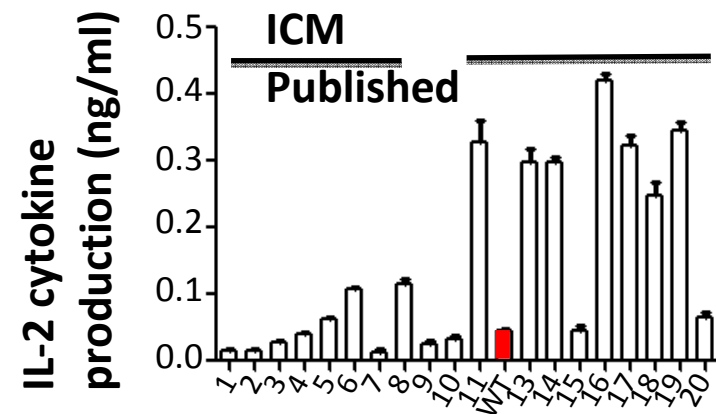
Autoimmune response is directly correlated with *in vivo* tumor rejection and plateau at the same affinity threshold (KD =10  $\mu\text{M}$ ).

Krogsgaard et al., *submitted*

# Specific increase in potency via structure-based design of a T cell receptor

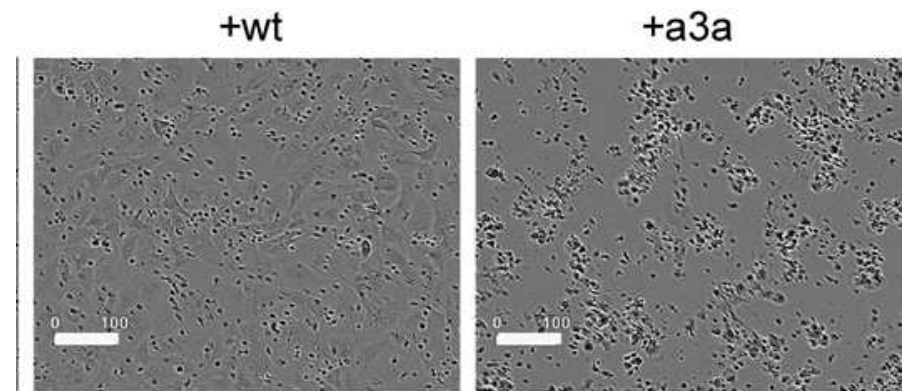
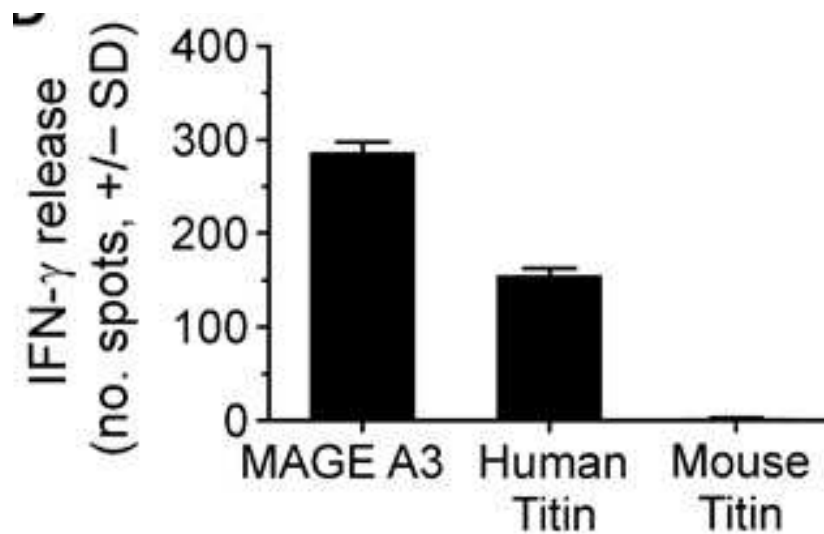


T2-A2/K<sup>b</sup> (self-peptide)



## Other safety concerns for engineered TCRs

MAGE-A3 engineered TCR (a3a) reacts with Titin to mediate cardiac arrest in patients



iCell cardiomyocytes

wt TCR: 500  $\mu$ M

a3a TCR: 2.3  $\mu$ M

Cameron et al., Sci Transl Med, 2013

# Conclusions

- T-cells with relative “high” affinity to self exist in the periphery.
- Above a certain T-cell affinity threshold increased activation is observed *in vitro* but plateau *in vivo*.
- Autoimmune response is directly correlated with *in vivo* tumor rejection and plateau at the same affinity threshold (KD =10  $\mu$ M).
- Strategies focusing on TCRs in the intermediate range (KD  $\sim$ 10  $\mu$ M) or targeting shared antigens could dampen the potential for autoimmunity during ACT.

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