

HelmholtzZentrum münchen

German Research Center for Environmental Health

High-affinity allo-restricted TCR for adoptive T cell therapy: selection and characterization

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SITC, November 2011

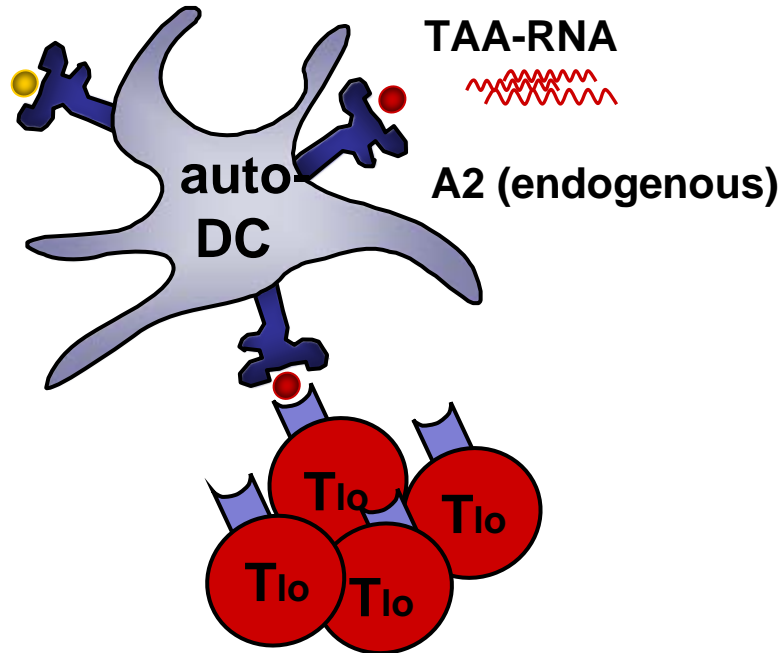
Presenter Disclosure Information

The following relationships exist related to this presentation:

My center holds IP on methods to generate high avidity T cells and selected TCRs derived using these methods

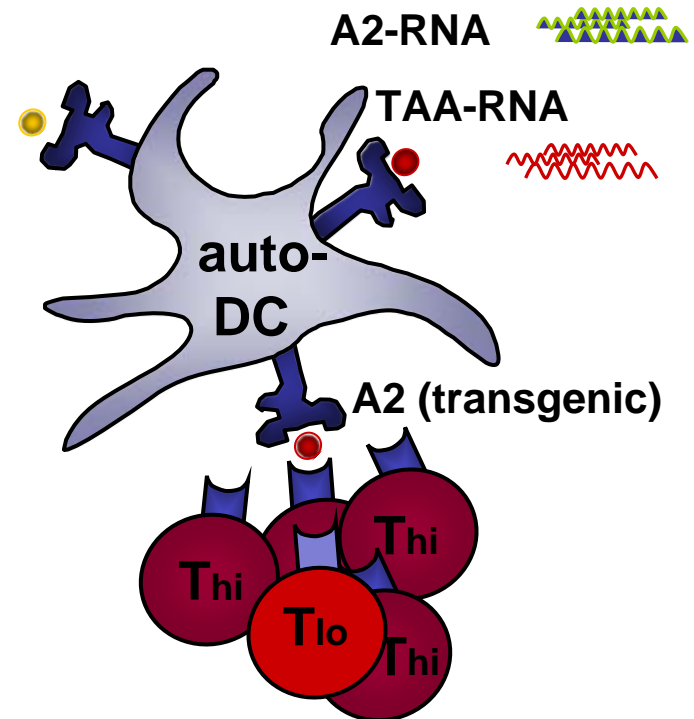
DC priming of high avidity CD8⁺ T cells

HLA-A2^{pos} donor



HLA-A2 is an *endogenous* gene
High affinity TCRs are deleted to
protect against autoimmunity

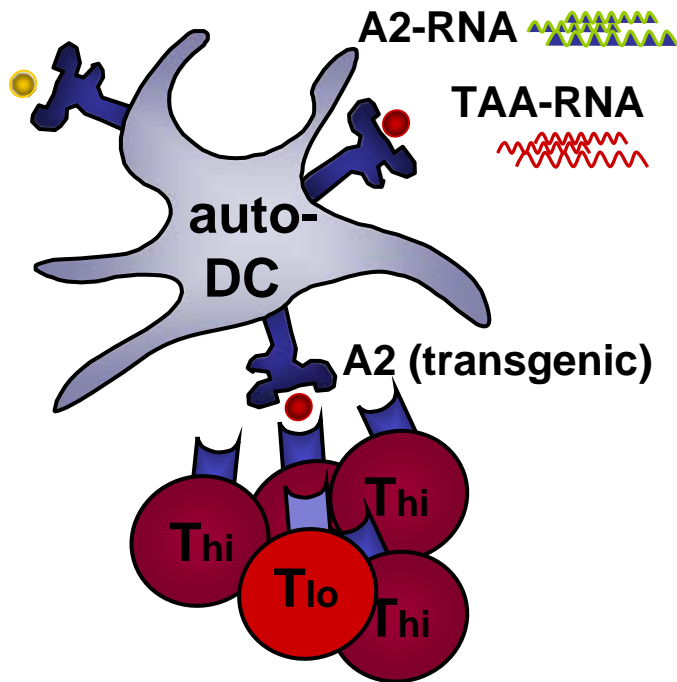
HLA-A2^{neg} donor



HLA-A2 is a *transgene*
Donor still has high affinity TCRs

Advantages of DC priming strategy

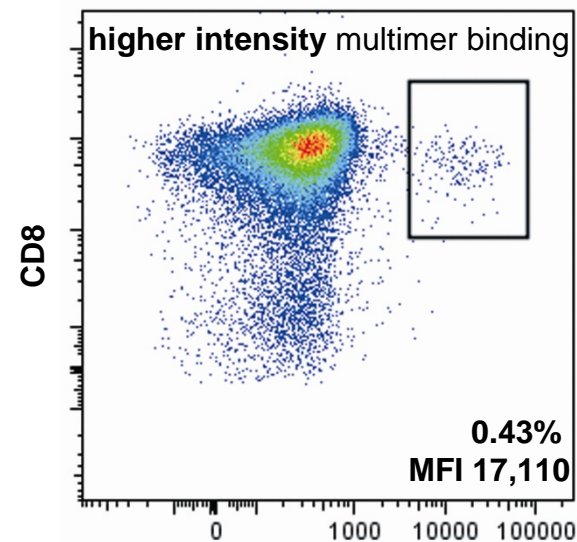
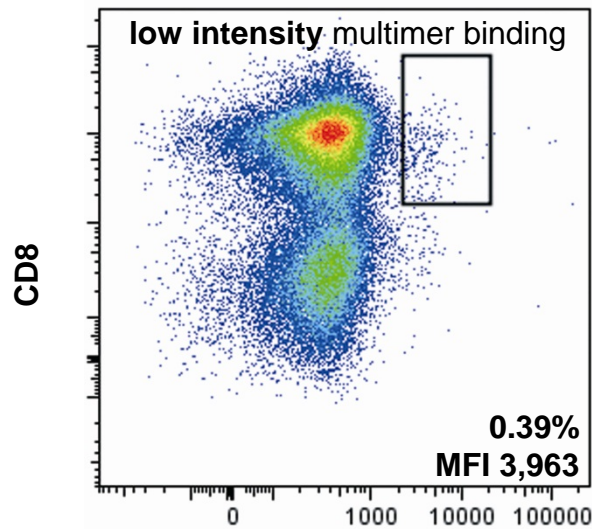
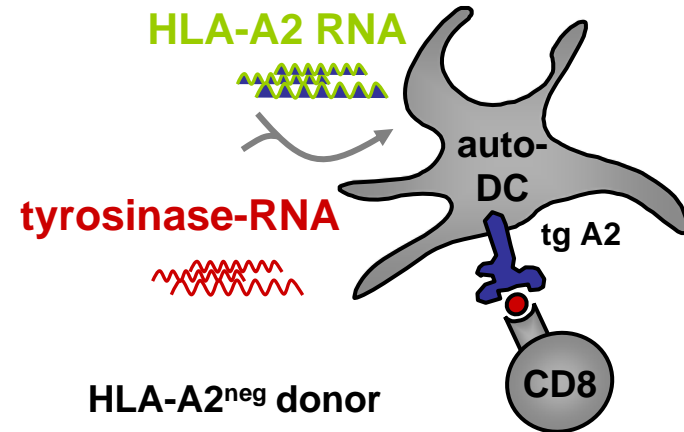
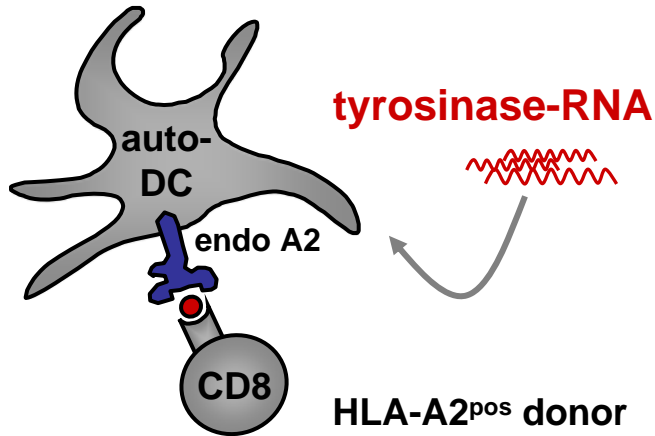
HLA-A2^{neg} donor



- optimal priming capacity of DC
- use any donor negative for selected MHC allele
- use any allogeneic class I or class II allele
- use any antigen available as cDNA

HLA-A2⁺ allo-restricted tyrosinase-specific CTL

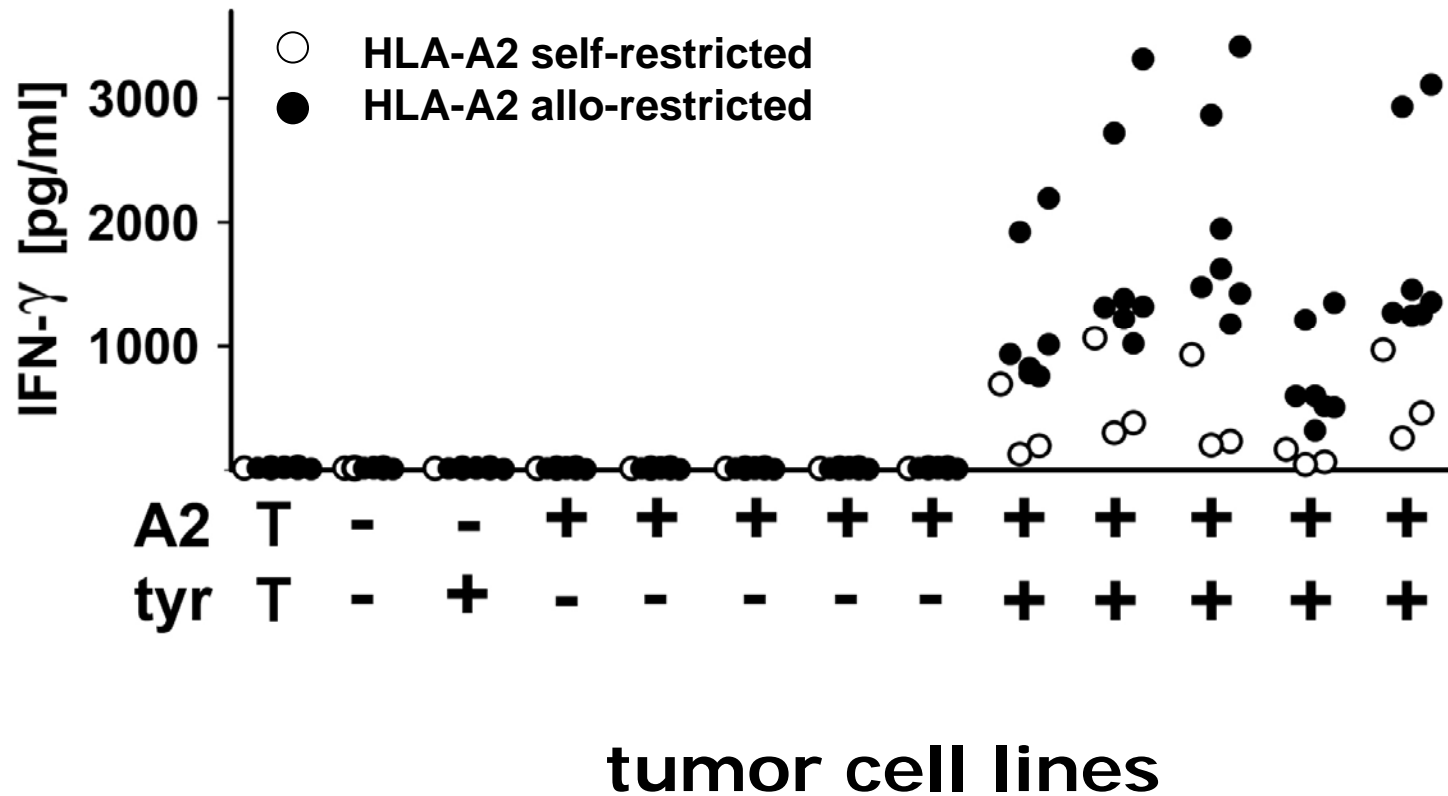
Higher intensity multimer binding by allo-primed CTL



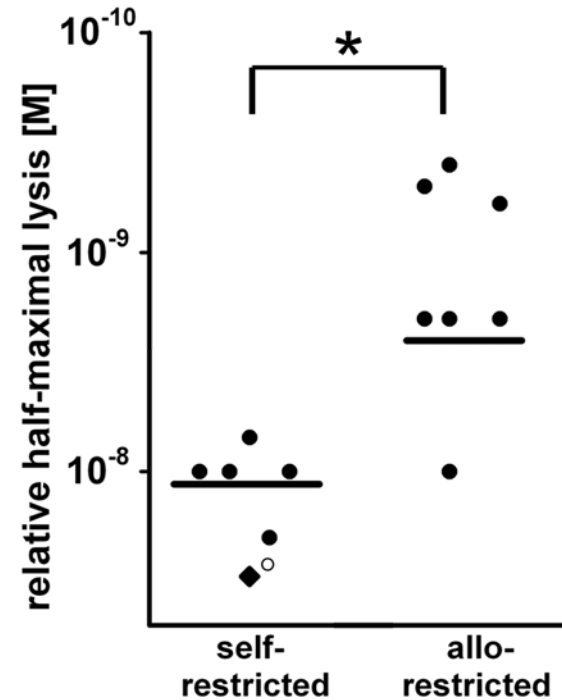
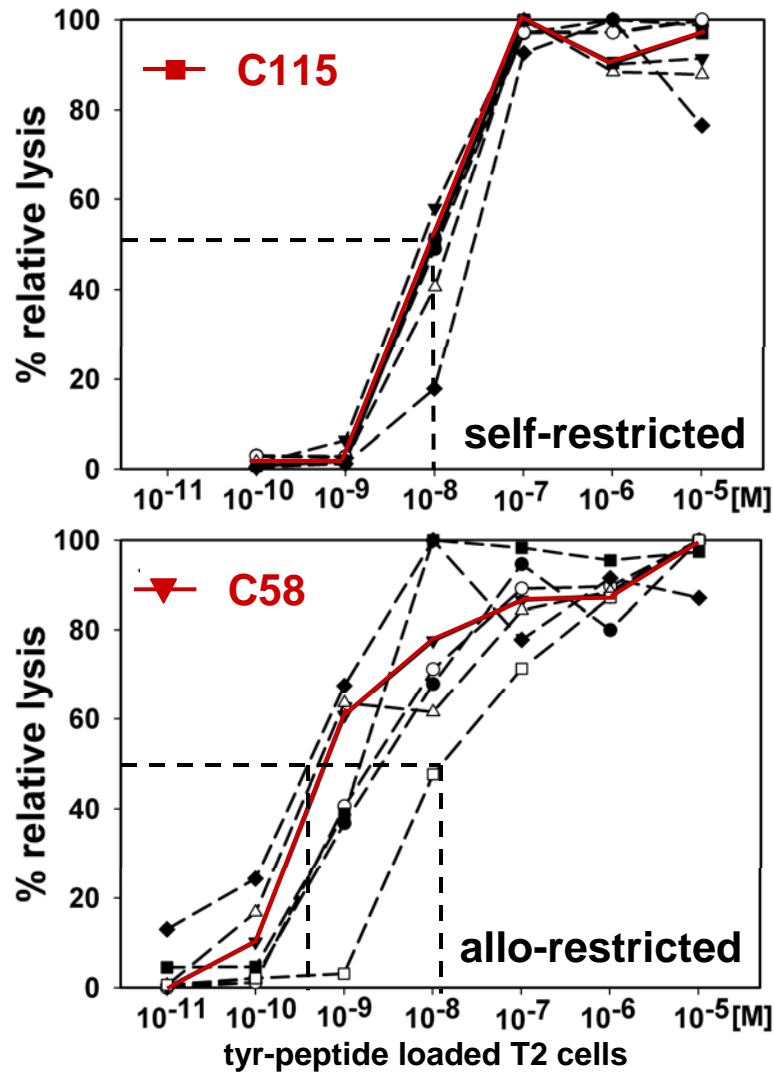
Distribution of self- and allo-restricted CTL clones

reactivity	self-restricted (HLA-A2 ⁺ donors)		allo-restricted (HLA-A2 ⁻ donors)	
no reactivity	21	55%	8	16%
allo-A2	0	0%	27	53%
A2-tyrosinase	17	45%	16	31%
total number	38	100%	51	100%

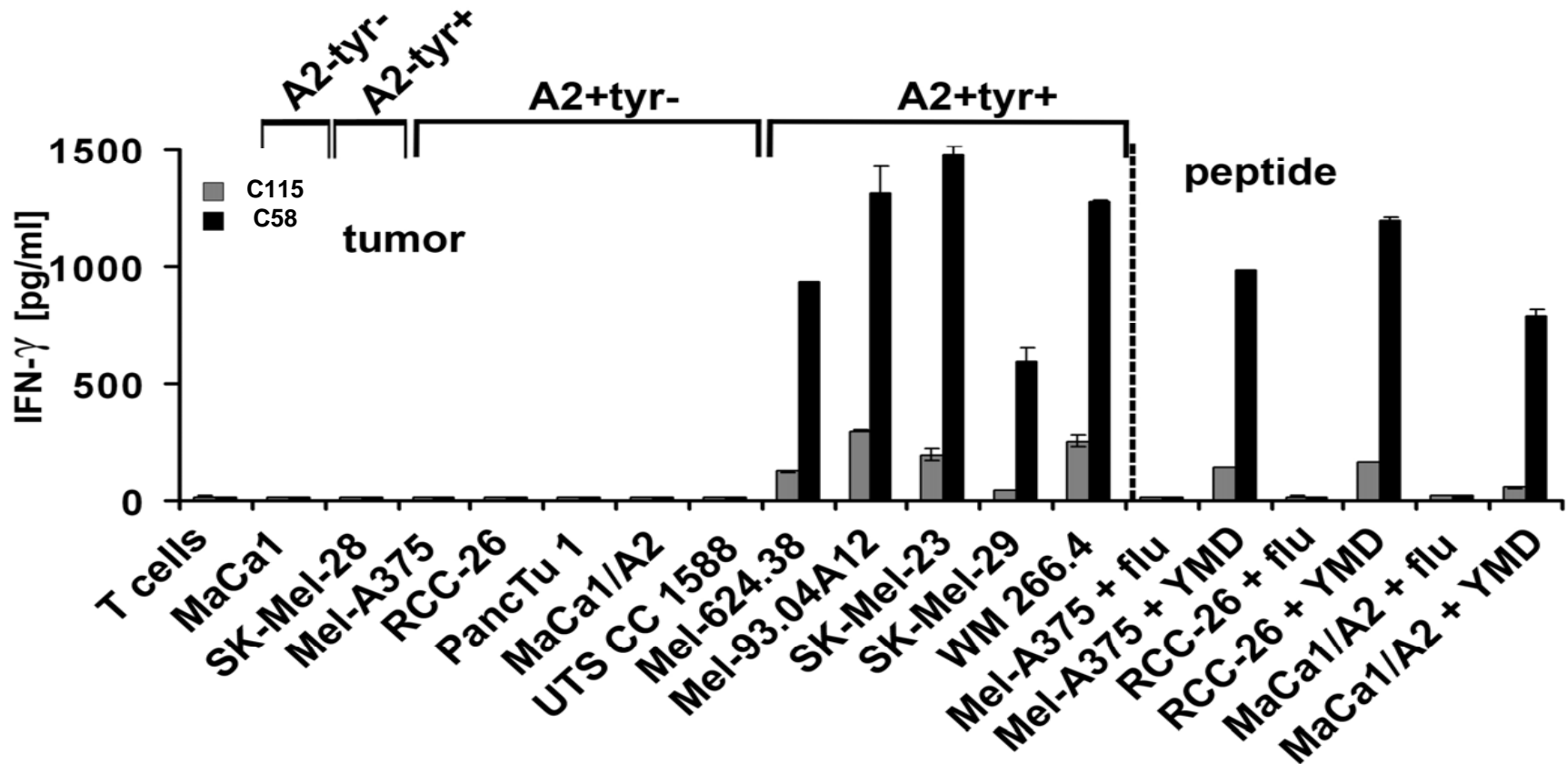
Specific recognition of melanoma lines



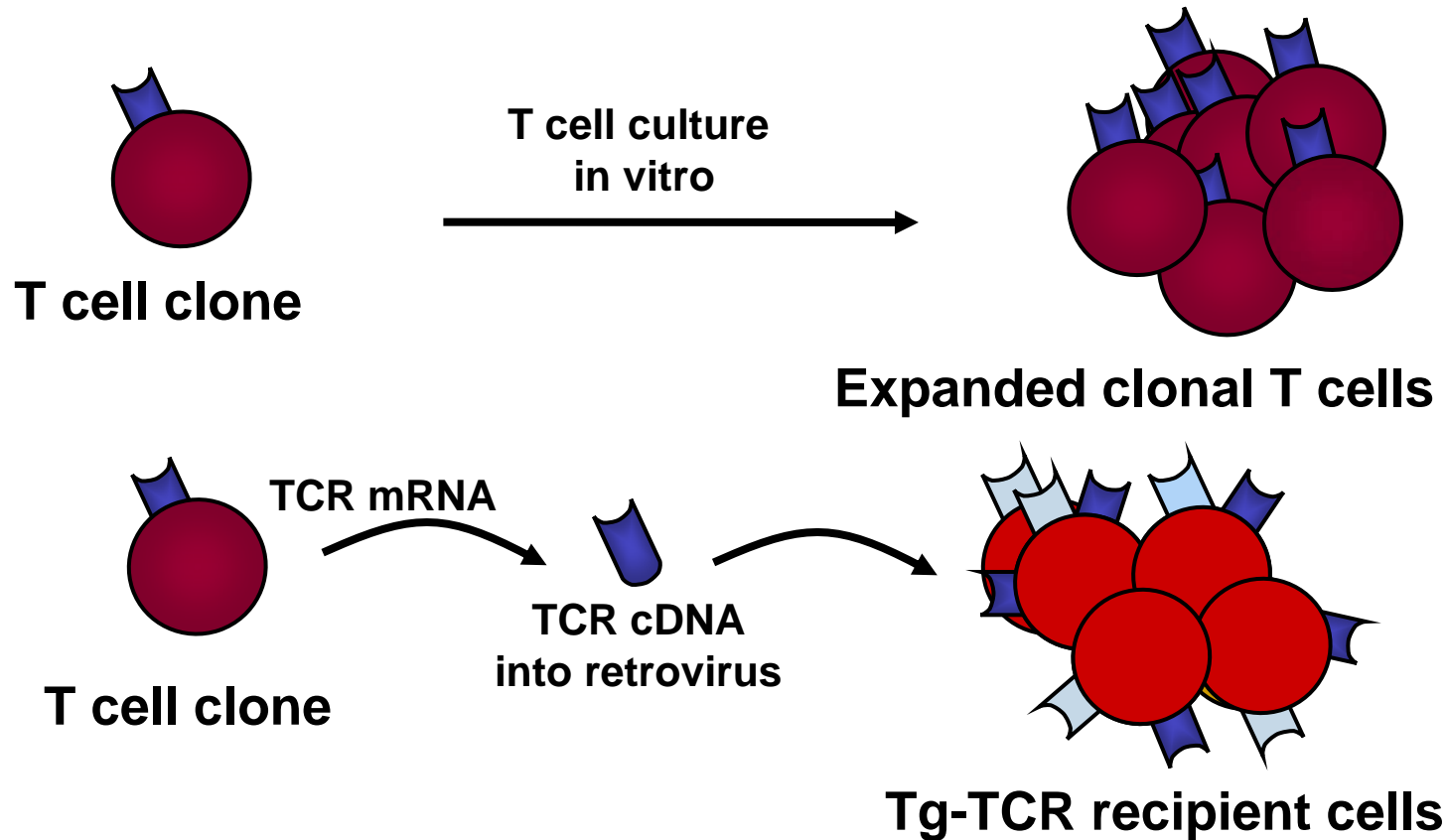
Allo-restricted clones show greater peptide sensitivity



Self- and allo-restricted CTL have identical specificities but different functional capacities

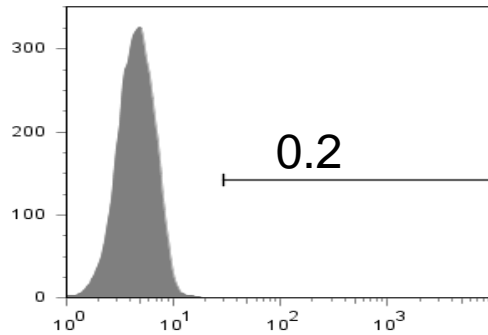


Transgenic TCR expression in PBL

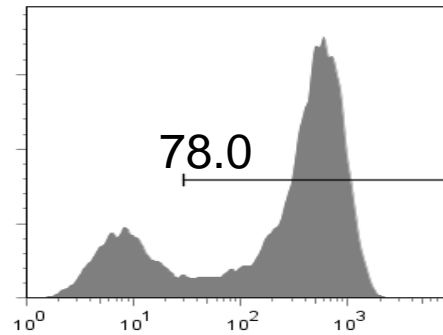


Transgenic TCR expression in recipient cells

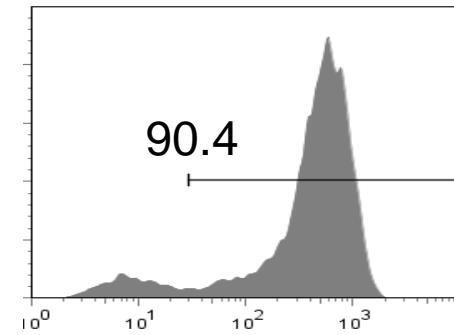
Jurkat 76 unmod.



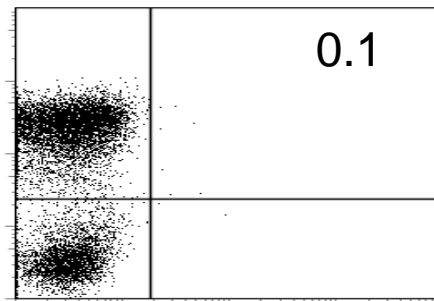
Jurkat 76 tgC115



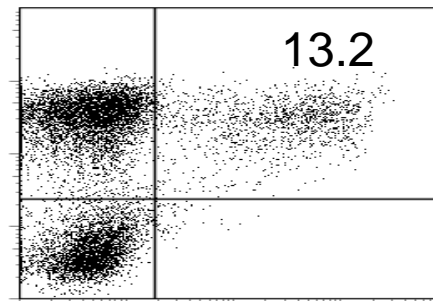
Jurkat 76 tgC58



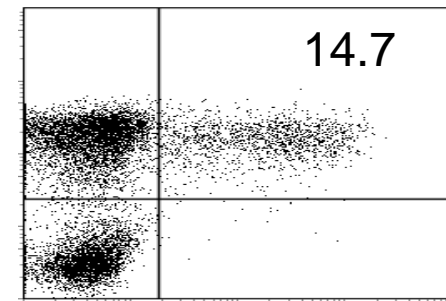
PBL unmod.



PBL TCR-C115



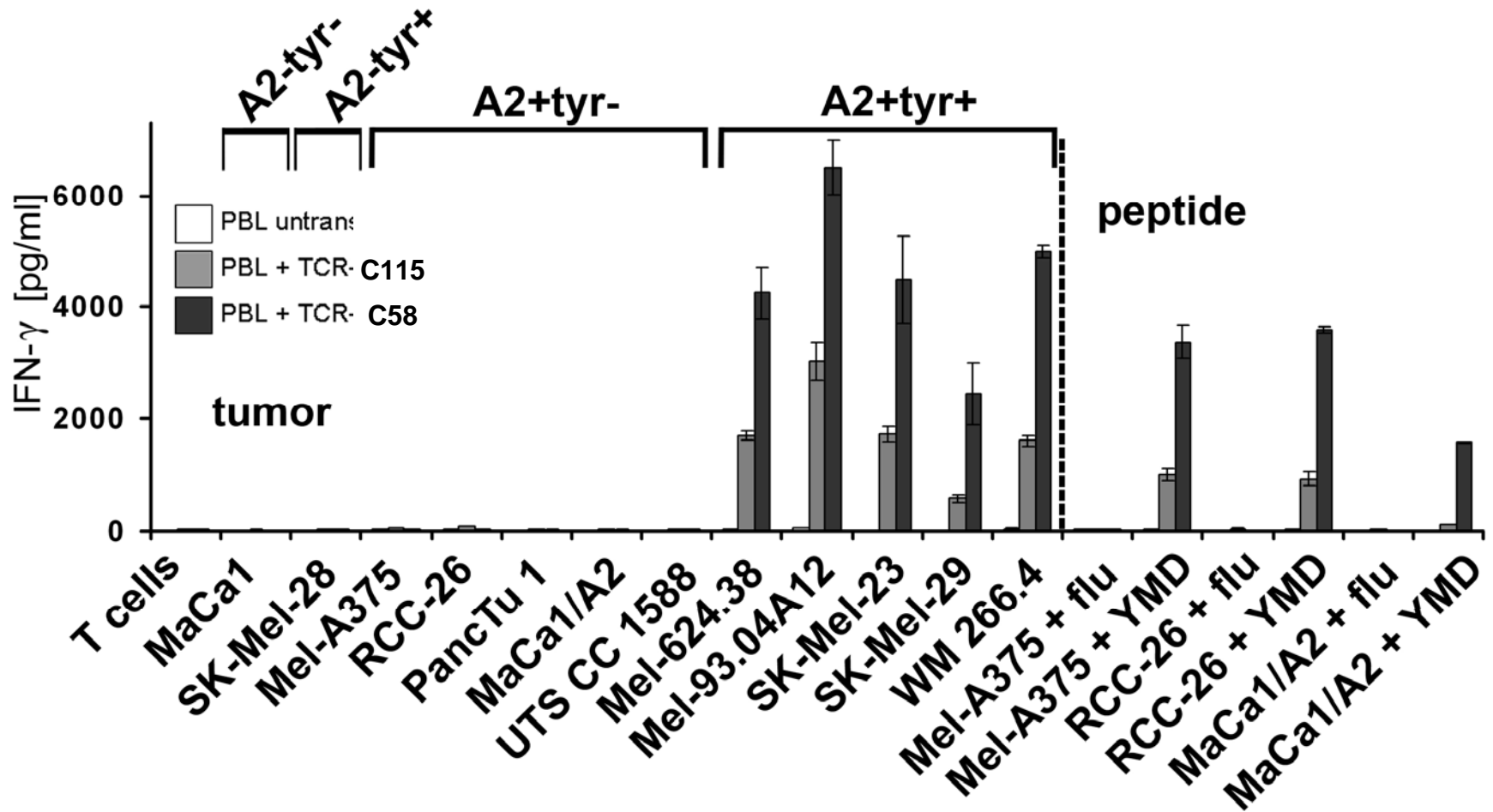
PBL TCR-C58



self-restricted

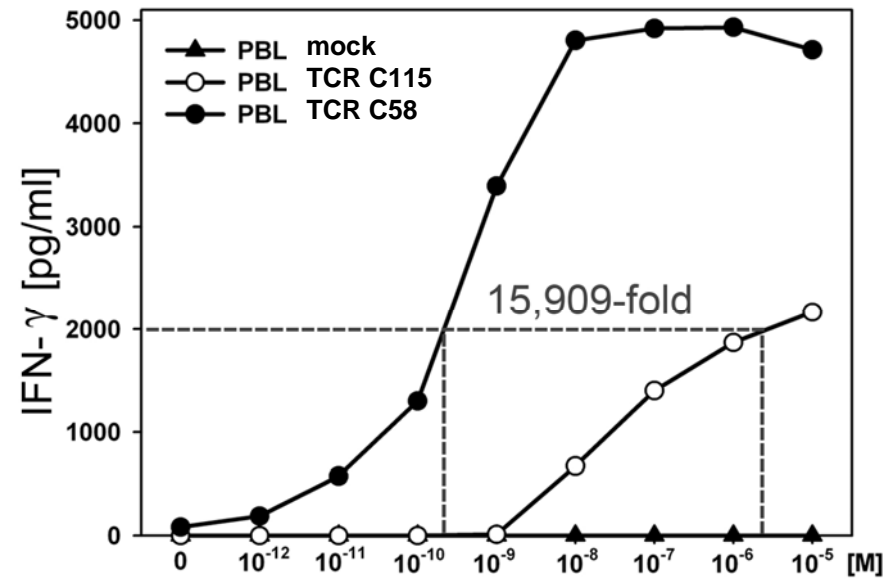
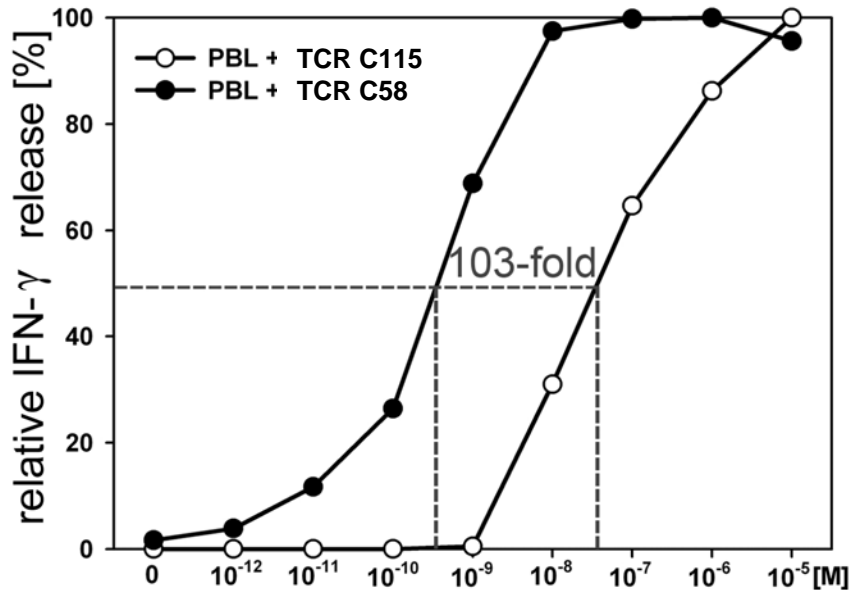
allo-restricted

Tumor-specific recognition by TCR-transgenic PBL



Allo-TCR shows superior peptide sensitivity

functional avidity



tyrosinase₃₆₉₋₃₇₇ pulsed T2 cells

Lessons learned from tyrosinase-specific T cells

- Allo-restricted CTL have superior peptide sensitivity
- Multimer binding does not necessarily correlate with peptide sensitivity and superior cell function
- PBL transduced with the allo-restricted TCR show superior functions
- DC priming can provide high affinity TCR for adoptive T cell therapy

Repertoire of allo-restricted TCR in development

Tyrosinase

Melan A

Survivin

HMMR

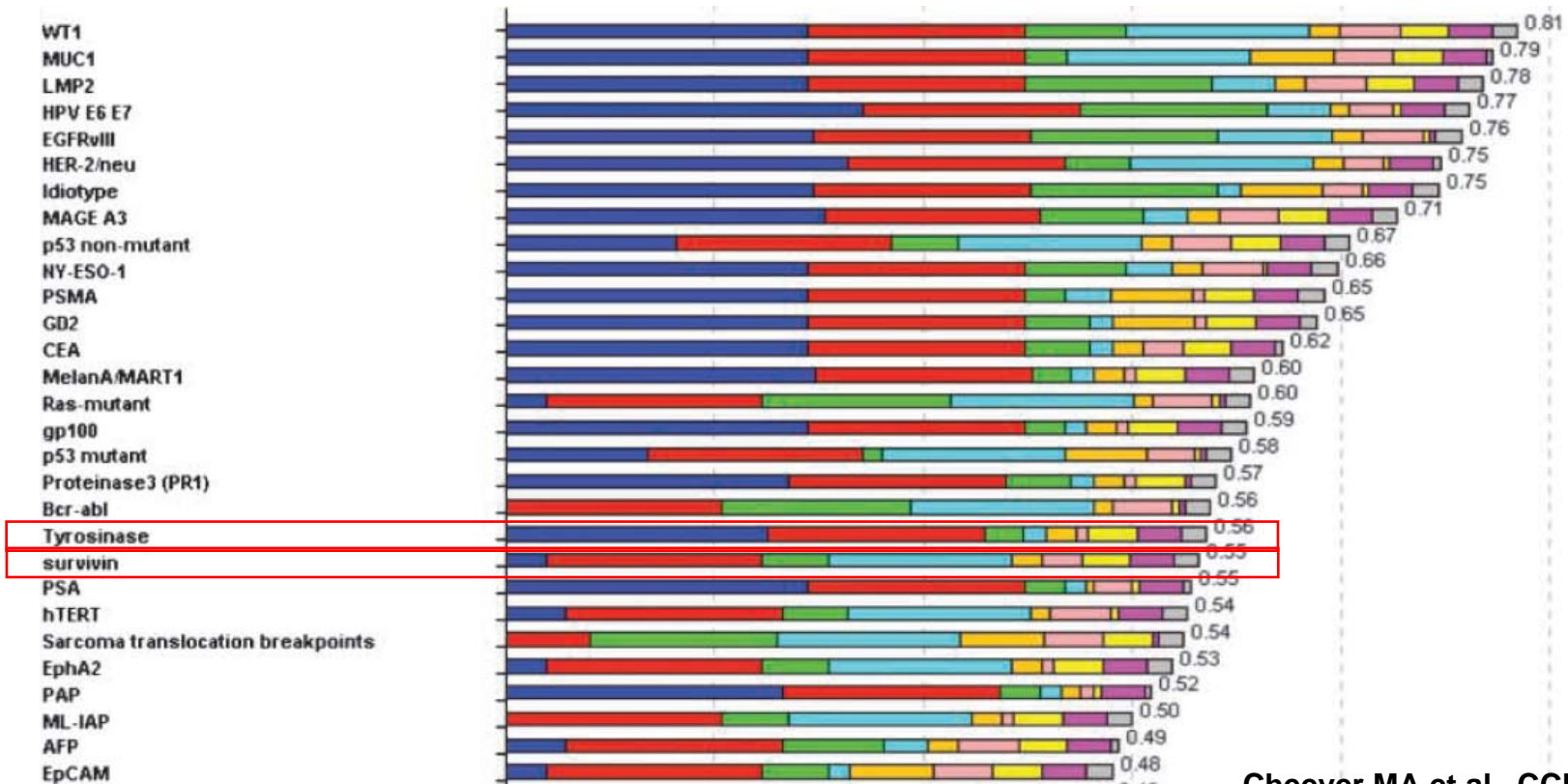
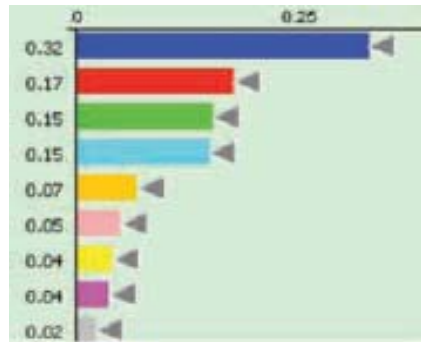
WT-1

NY-ESO-1 (non-HLA-A2 restricted)

Cancer-germline: 10 TAAs in progress

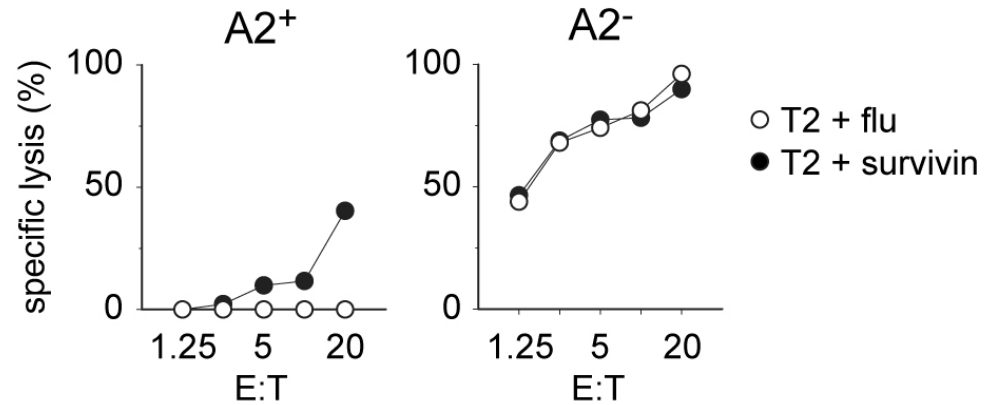
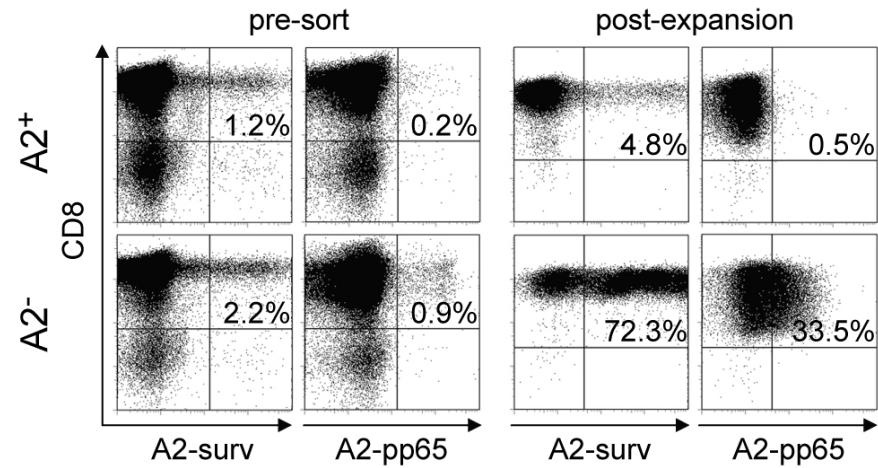
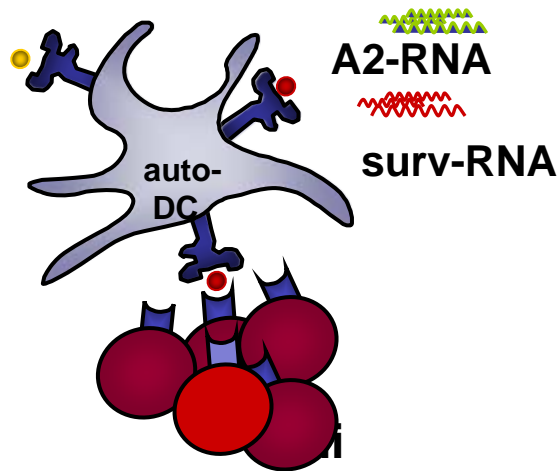
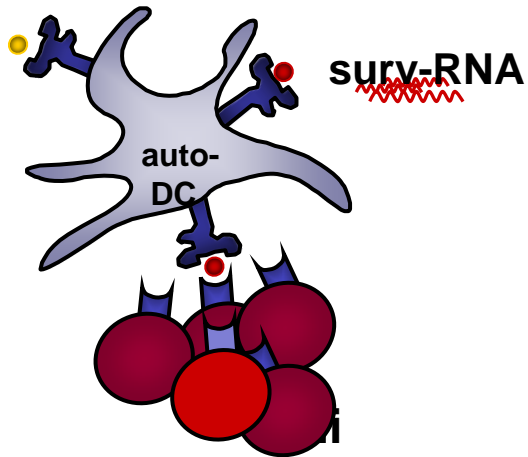
HLA-A2⁺ allo-restricted survivin-specific T cells

Ranking of tumor-associated antigens for vaccine development

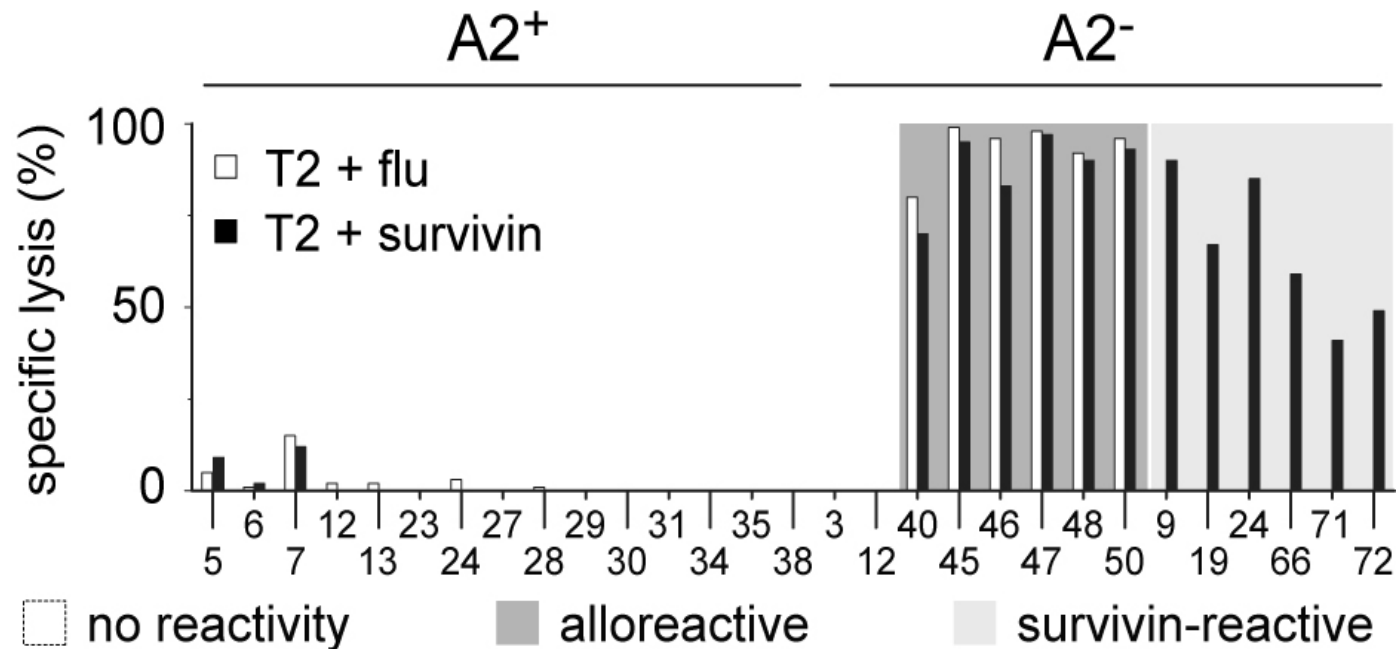


Cheever MA et al., CCR 2009

HLA-A2 self-restricted and allo-restricted survivin-specific T cell lines



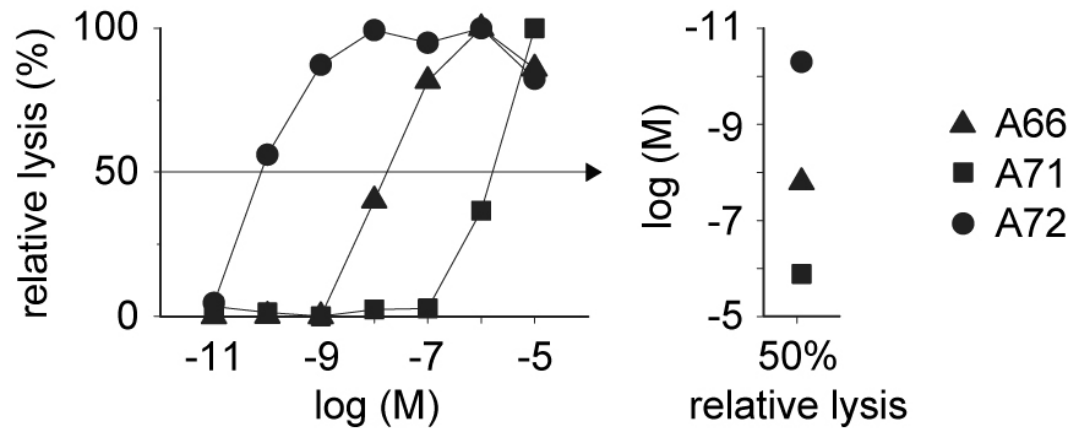
Screening of survivin-specific self-restricted and allo-restricted T cell clones



No detection of self-restricted survivin-specific CTL

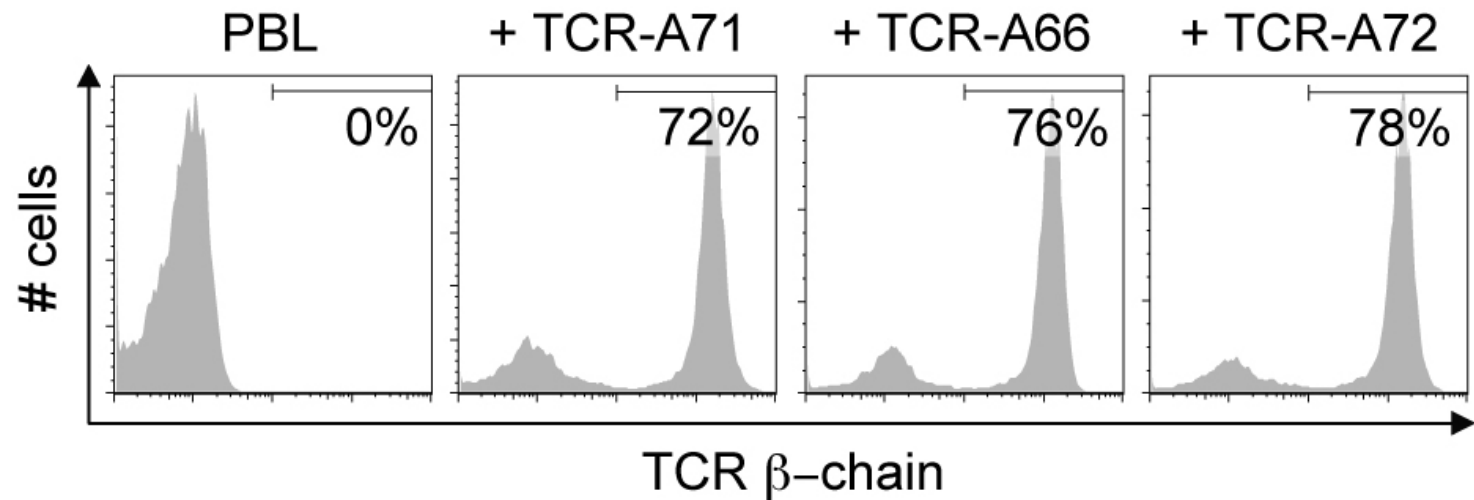
	A2 ⁺ (self-restricted)		A2 ⁻ (allo-restricted)	
no reactivity	46	100%	9	12%
alloreactive	0	0%	44	60%
survivin-reactive	0	0%	22	28%
total number	46	100%	74	100%

CTL display broad range of peptide sensitivities

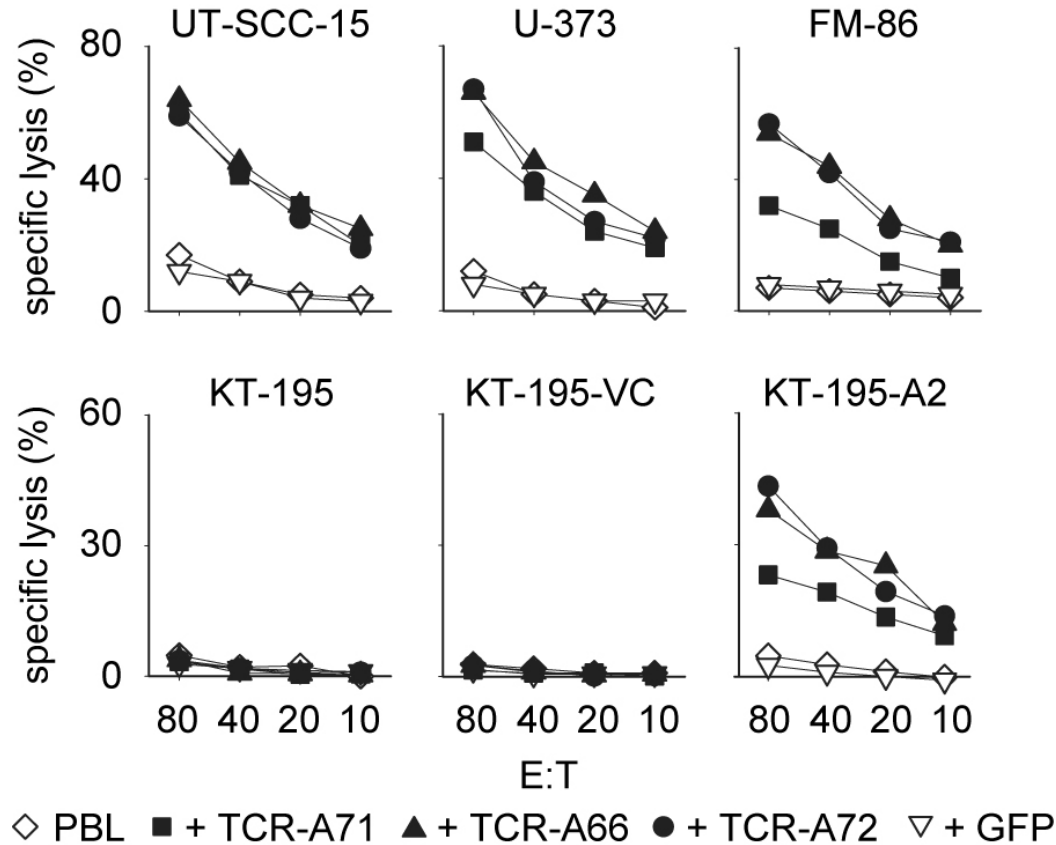


peptide sensitivity of
survivin₉₆₋₁₀₄-pulsed T2
cells

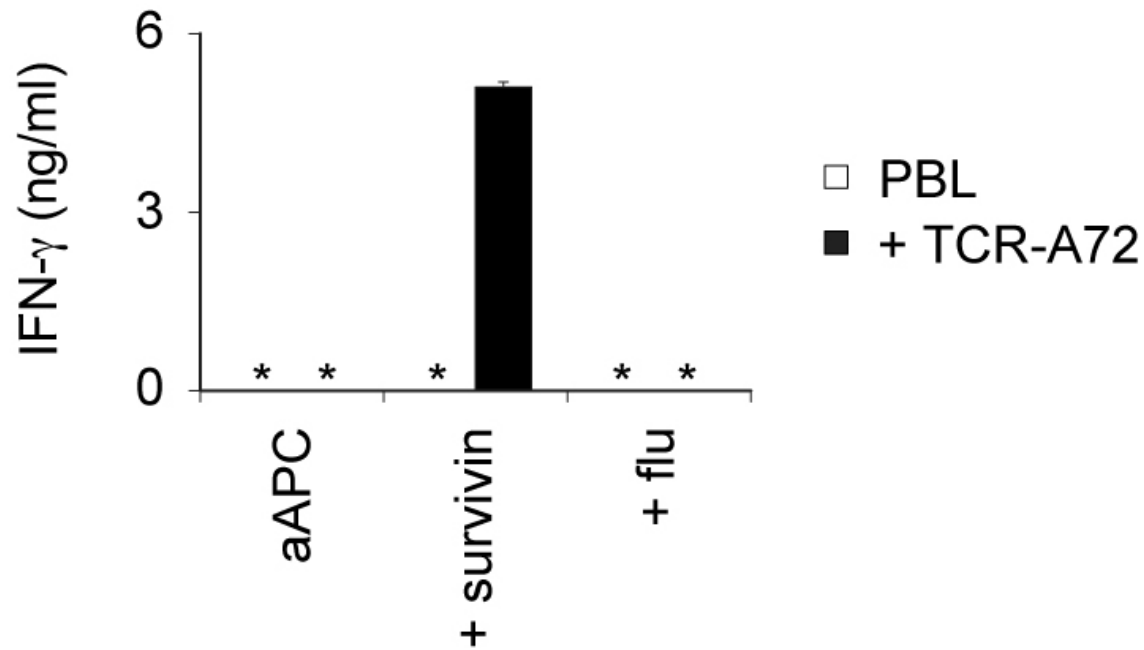
Transgenic TCR are well expressed on PBL



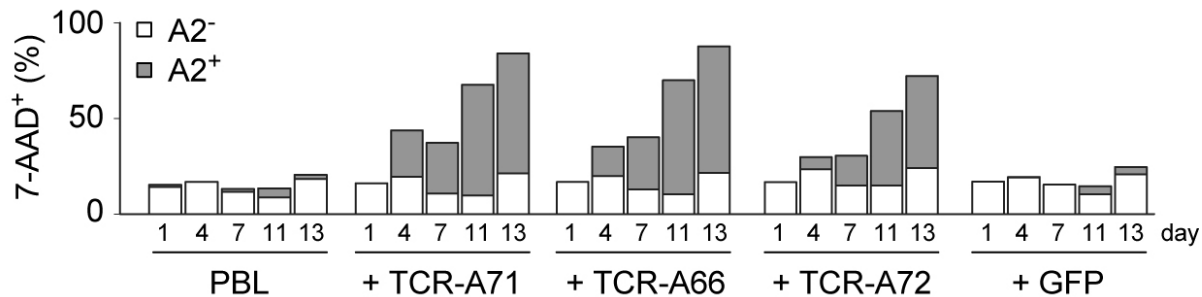
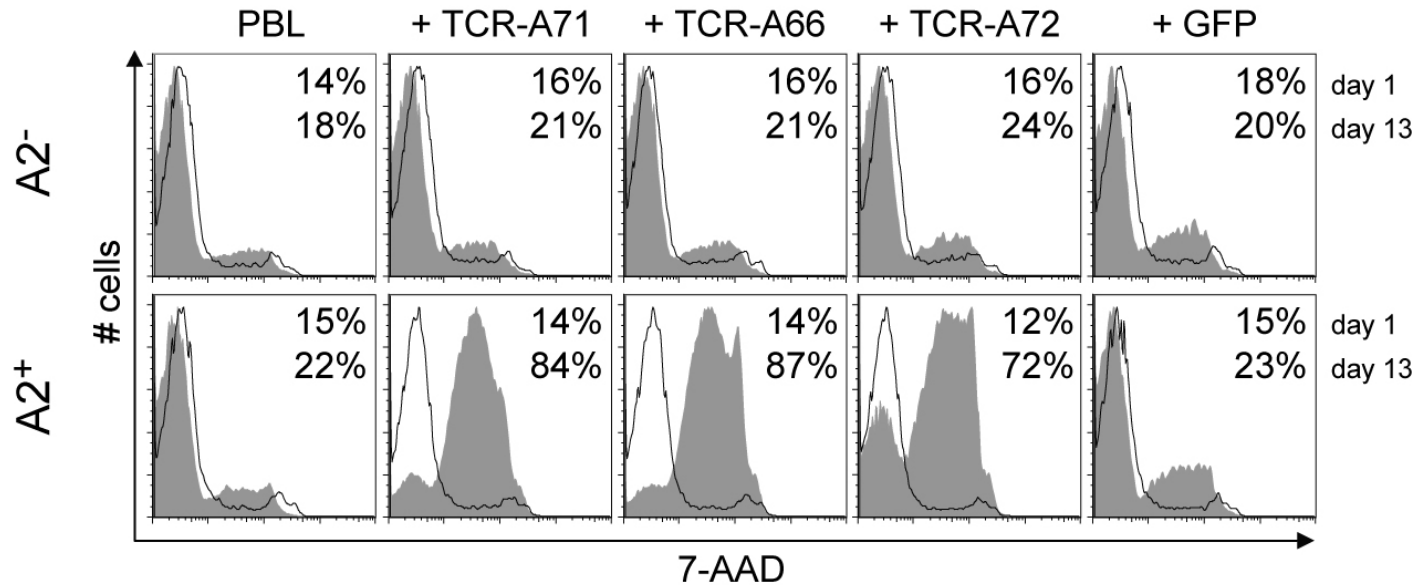
TCR-transgenic PBL kill survivin-positive tumor cells



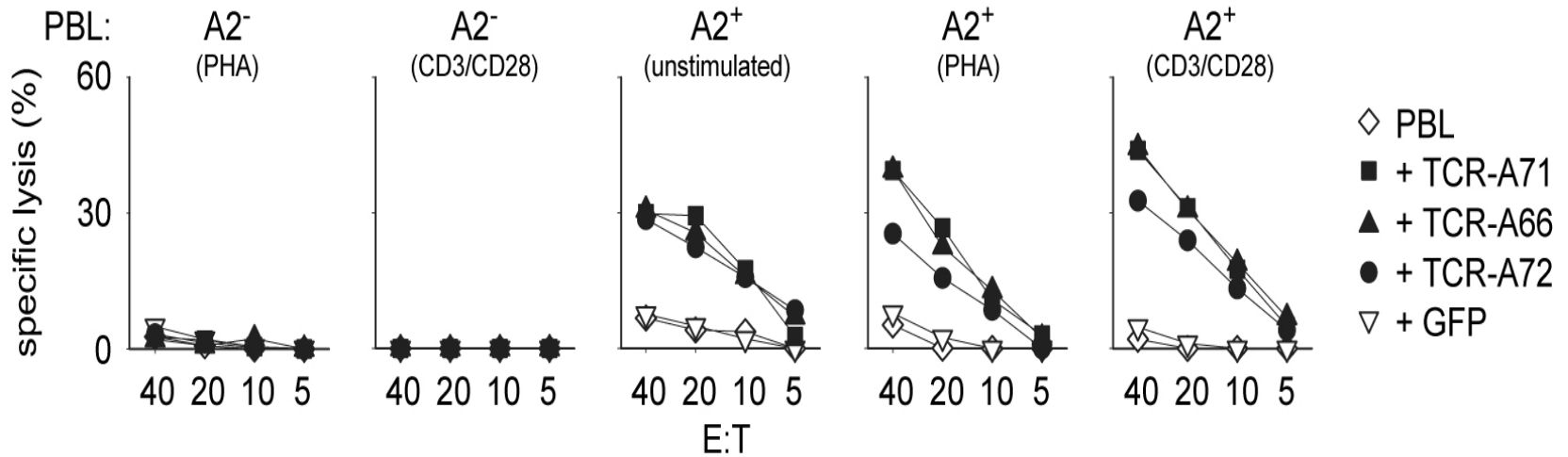
Tg-TCR recognition is survivin-peptide dependent



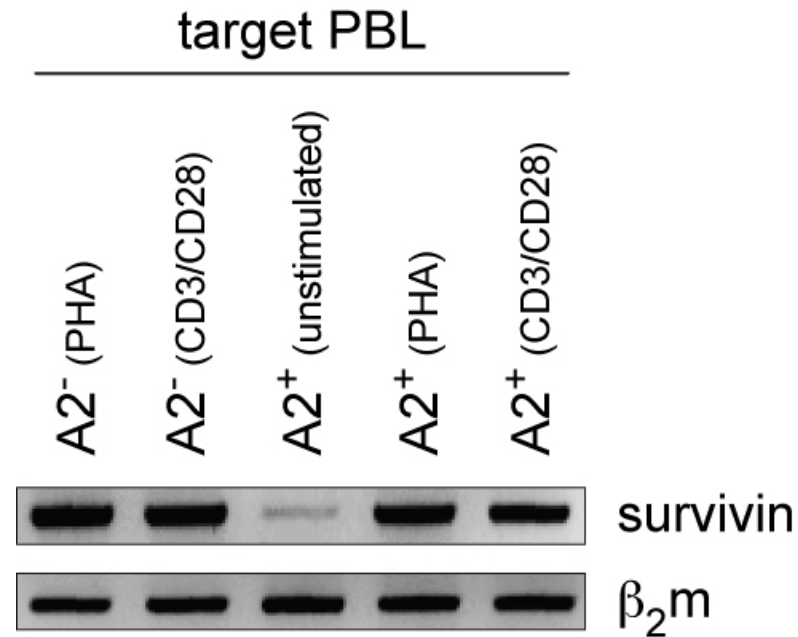
HLA-A2⁺ PBL with tg-TCR undergo high apoptosis



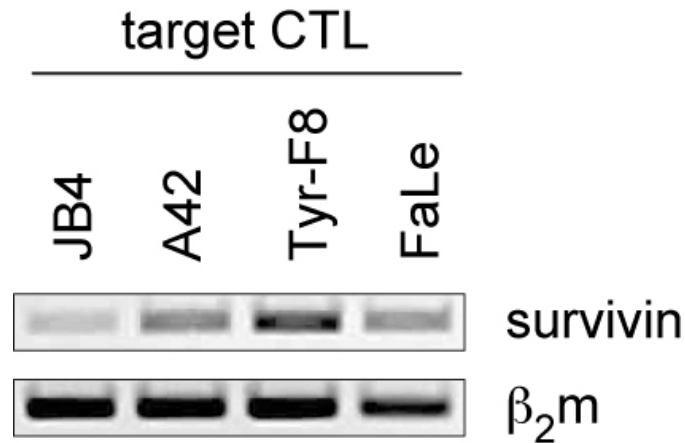
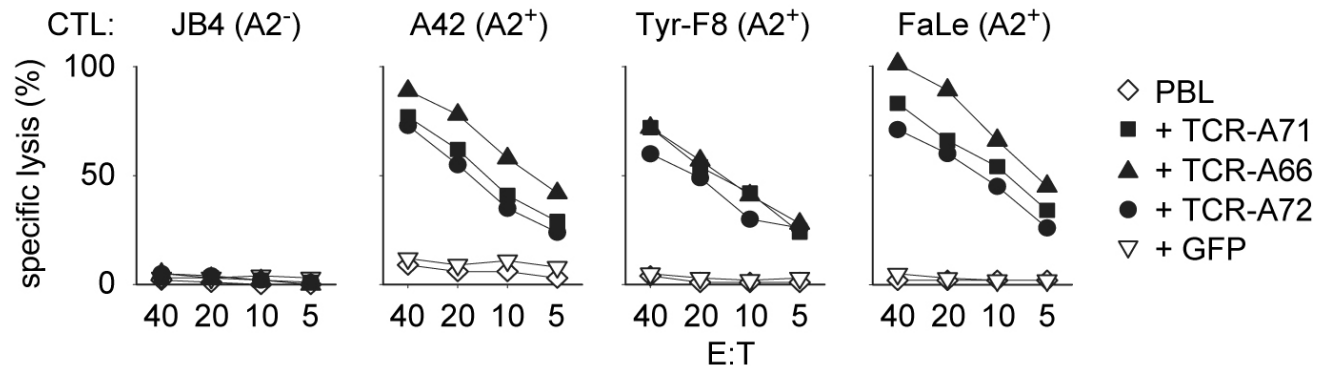
Survivin-specific tg-TCR kill HLA-A2⁺ PBL



Activated PBL express high levels of survivin transcripts



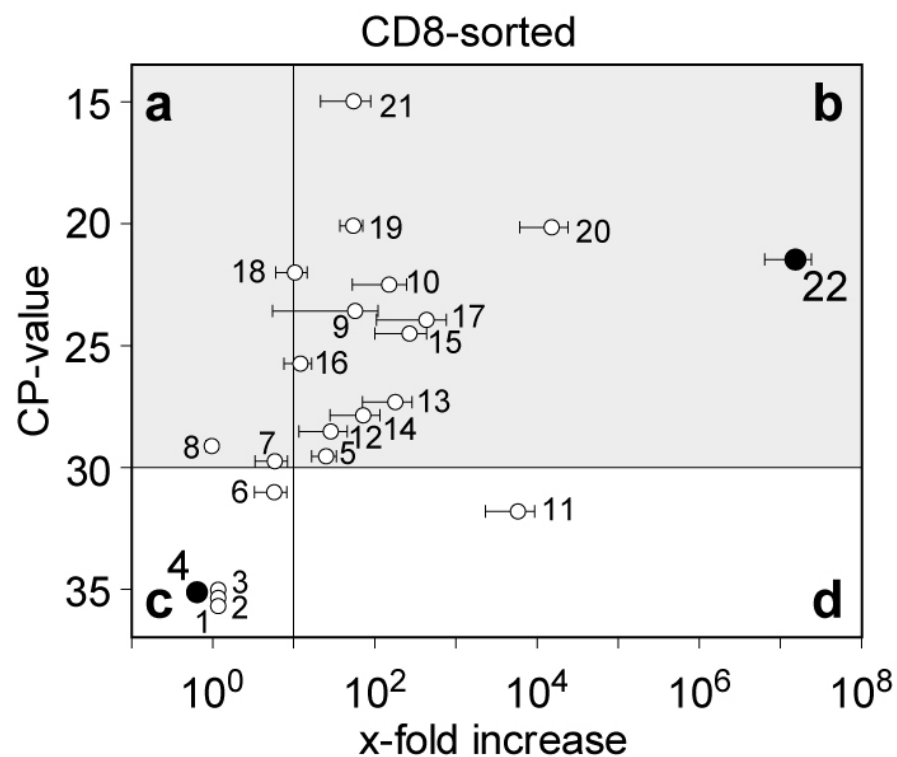
HLA-A2⁺ CTL are killed by TCR-transgenic PBL



Lessons learned from survivin-specific TCR

- Self-restricted survivin-specific T cells were not found
- Allo-restricted survivin-specific T cells were frequent and some had very high peptide sensitivities
- PBL transduced with the allo-restricted TCR showed excellent and specific effector cell functions
- MHC-restricted fratricide eliminates survivin as a tg-TCR specificity and raises questions regarding its use as a vaccine antigen

Wider implications for MHC-restricted fratricide?



- | | | | |
|----|----------------|----|----------|
| 1 | EGFR | 12 | EPCAM |
| 2 | MART-1/melan A | 13 | CA9 |
| 3 | NY-ESO-1 | 14 | hTERT |
| 4 | Tyrosinase | 15 | RGS5 |
| 5 | WT-1 | 16 | MUC-1 |
| 6 | EphA2 | 17 | VEGF |
| 7 | PSA | 18 | p53 |
| 8 | FLT3 | 19 | c-myc |
| 9 | CASP-1 | 20 | HMMR |
| 10 | CYP1B1 | 21 | NPM1 |
| 11 | PRAME | 22 | Survivin |

Acknowledgments

Institute of Molecular Immunology Helmholtz-Zentrum München

Susanne Wilde
Bernhard Frankenberger
Slavoljub Milosevic
Stefanie Spranger
Maja Buerdek

Leiden University Medical Center Department of Hematology

Mirjam Heemskerk

University of Tübingen

Stefan Stevanovic
Hans-Georg Rammensee

Institute of Immunology Max-Delbrueck-Centre for Molecular Medicine, Berlin

Daniel Sommermeyer
Matthias Leisegang
Wolfgang Uckert
Thomas Blankenstein

Institute of Medical Microbiology, Immunology and Hygiene, Technical University Munich

Dirk H. Busch
Matthias Schiemann
Florian Anderl

Supported by grants of the HGF Alliance for Immunotherapy of Cancer and the German Research Foundation (SFB-TR36)