

Translation of TCRs to the clinic –

MAGE-A1 TCR gene therapy
of Multiple Myeloma
as an example

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TCR Consortium



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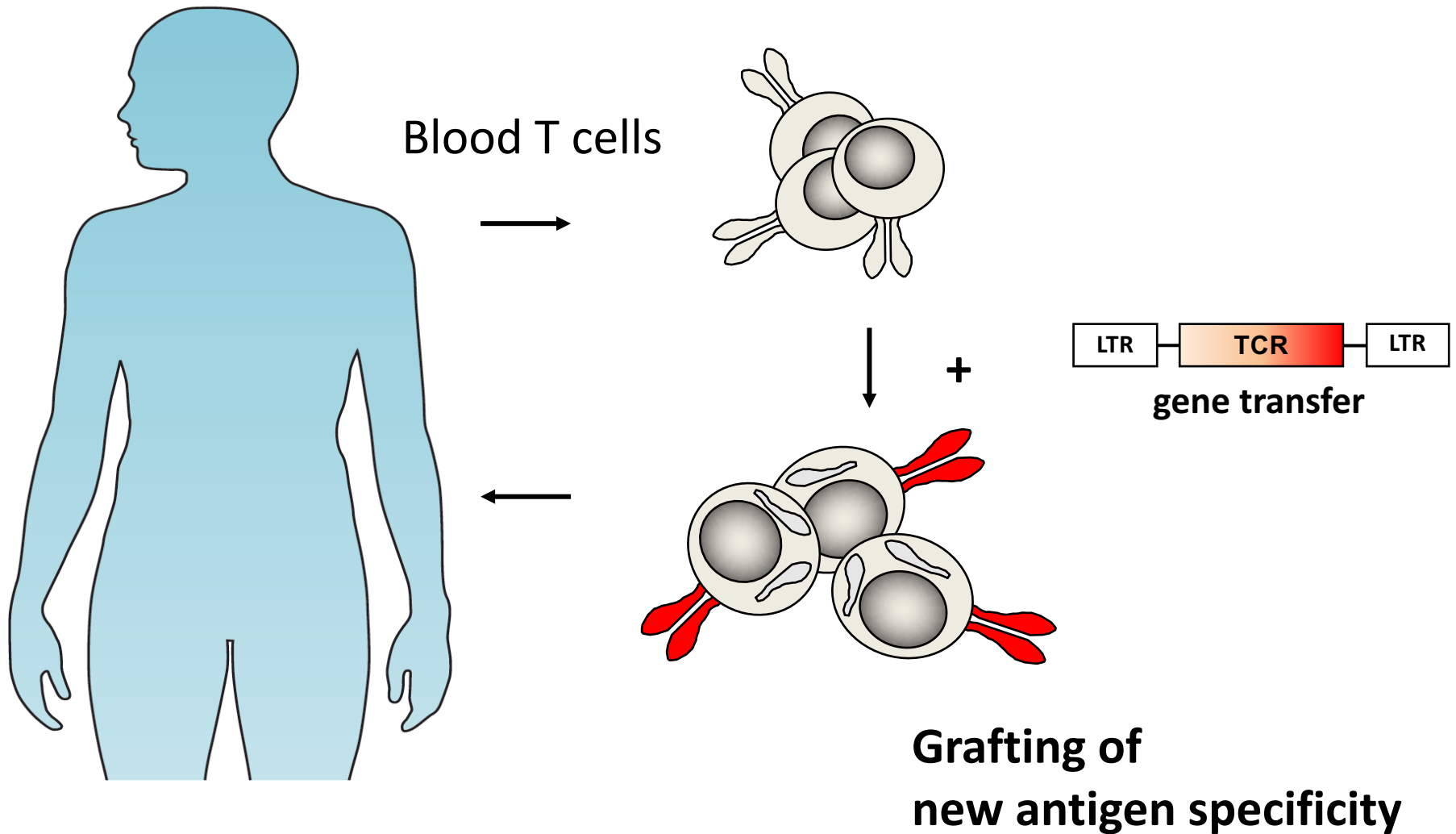
MAGE-A1 TCR gene therapy of Multiple Myeloma

BMBF program „Personalized Medicine“

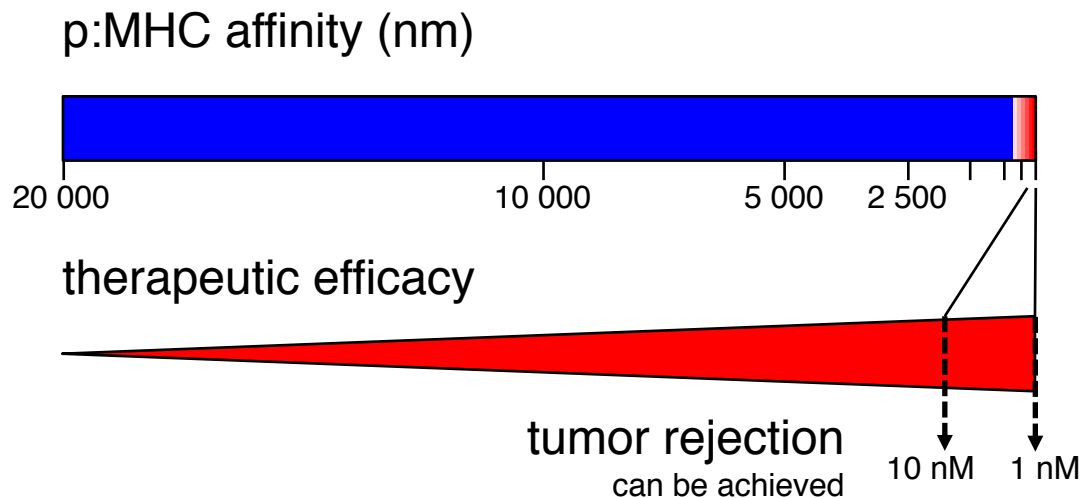
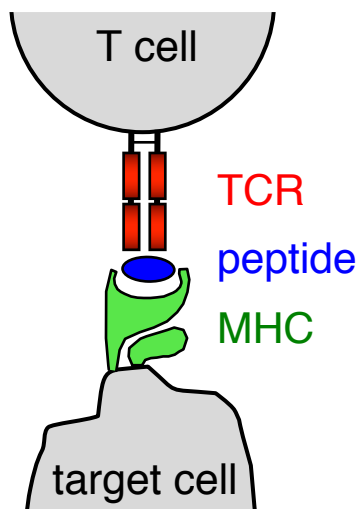


Federal Ministry
of Education
and Research

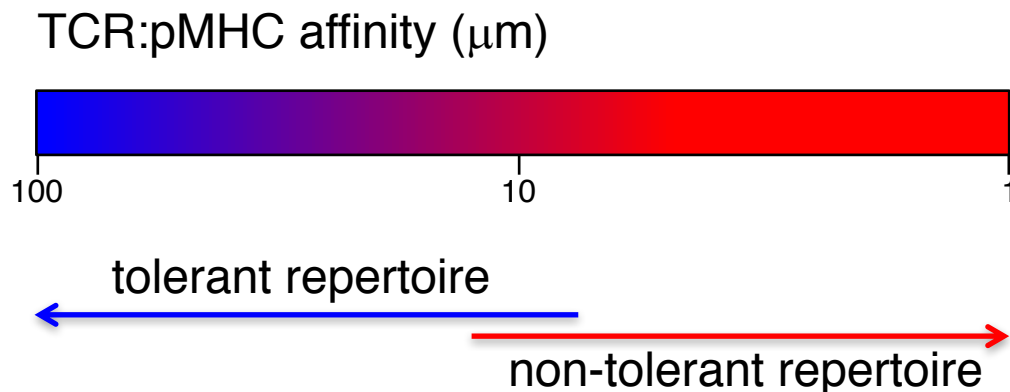
Redirected T cell therapy



Interaction between T cells and target cells is a three body problem: TCR - peptide - MHC



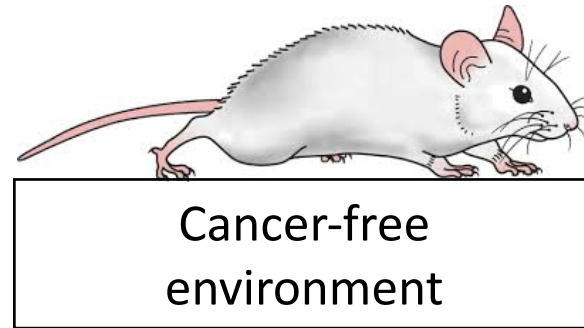
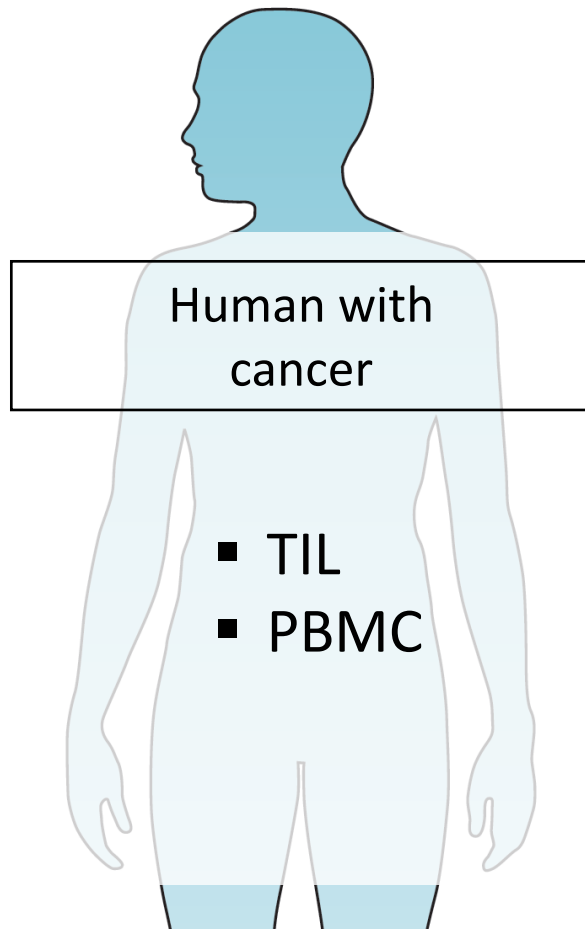
Cancer Cell 23: 516-526 (2013)



POINTS TO CONSIDER FOR ADOPTIVE T CELL THERAPY

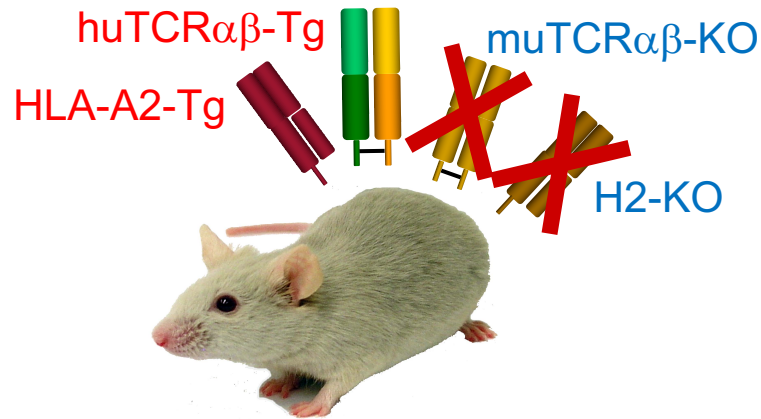
- best possible risk-benefit ratio for target antigen:
 - TSA (e.g. neoantigens) > CT > differentiation Ag
- most efficient rejection will occur when cancer cells are recognized as foreign:
 - TCRs with optimal-affinity

How to obtain therapeutic TCRs?



- humanized mouse

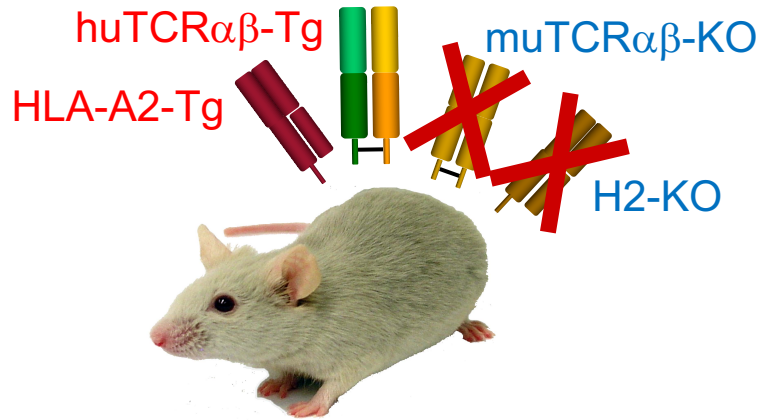
Transgenic mice with a diverse human T cell antigen receptor repertoire



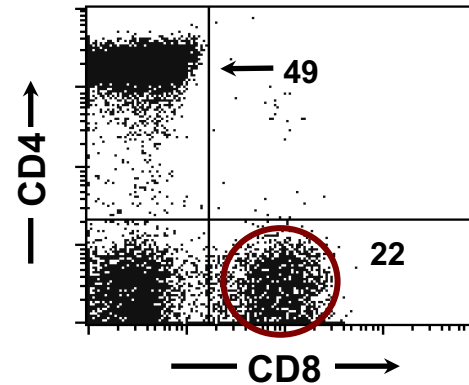
huTCR locus-Tg mice

- Mice are not tolerant for most human tumor antigens
- Human TCRs from non-tolerant repertoire in cancer free mice

Peripheral T cells in huTCR-locus Tg mice



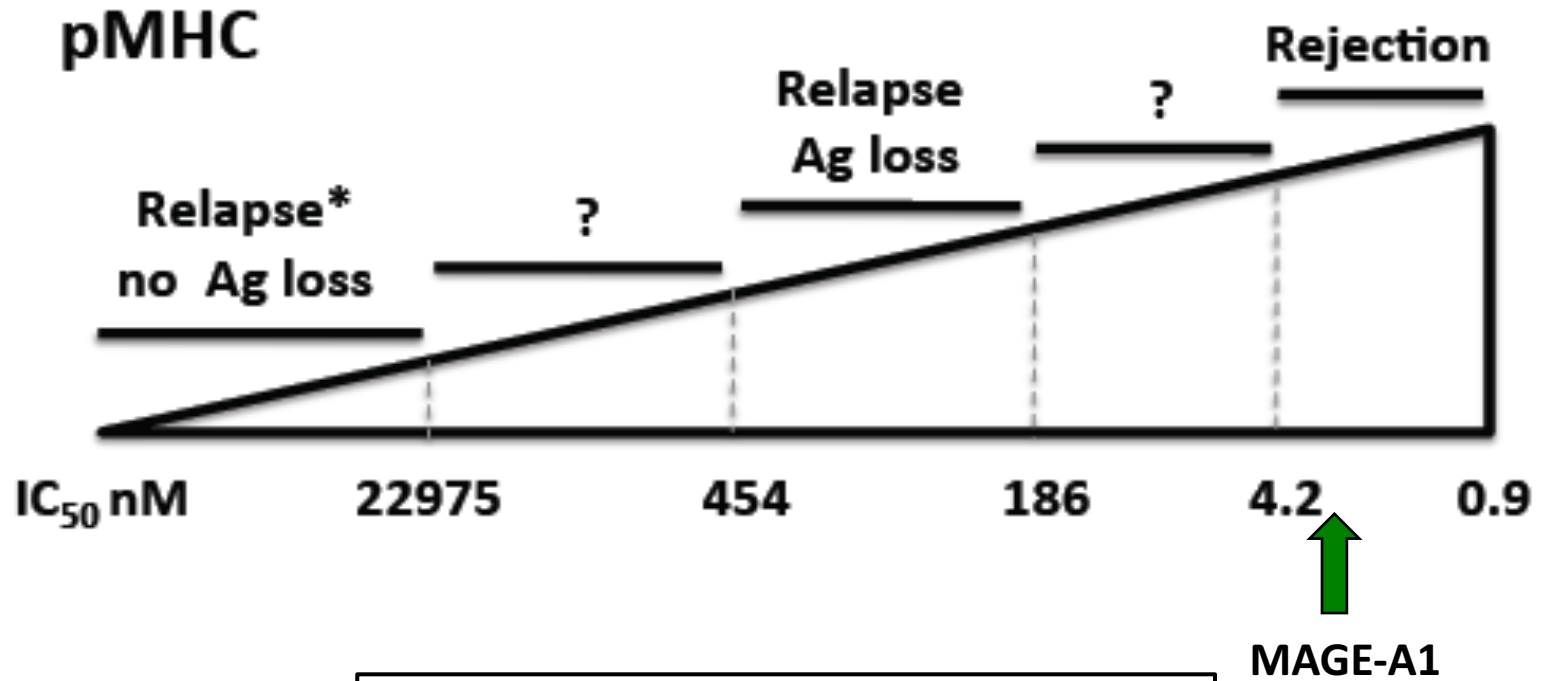
huTCR locus-Tg mice



MHC I: human
TCR: **human**

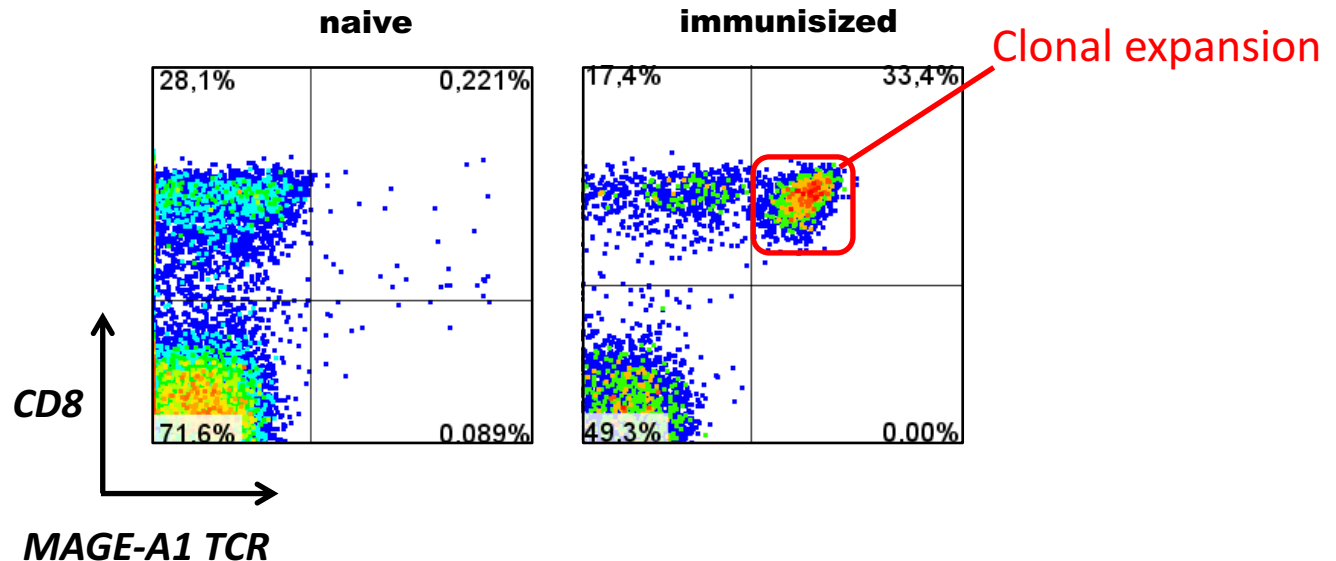
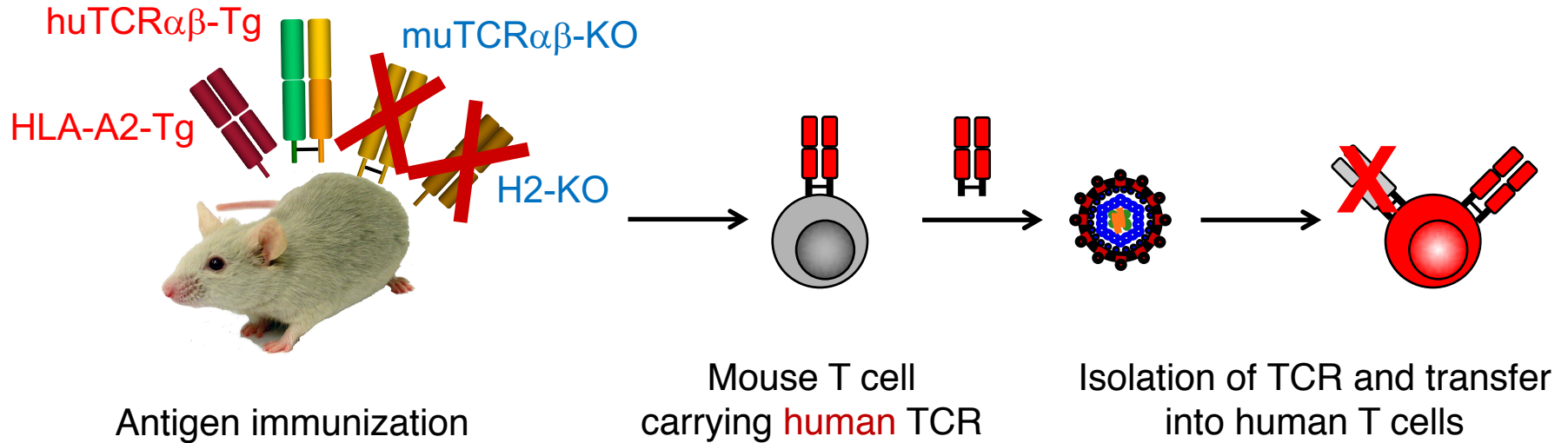
- CTL to various human TAA (>10)
- CTL to cancer viruses (>10)
- CTL to various human TSA (>30)

pMHC affinity is important



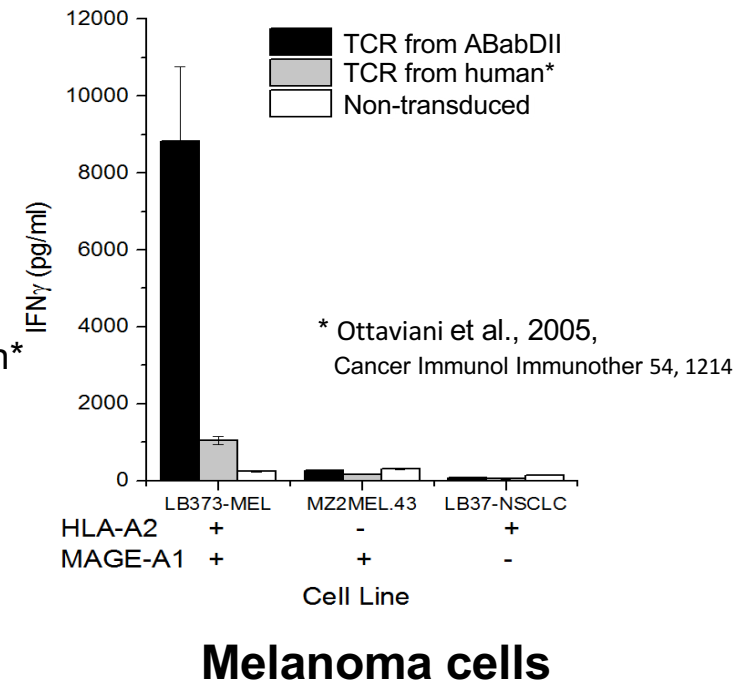
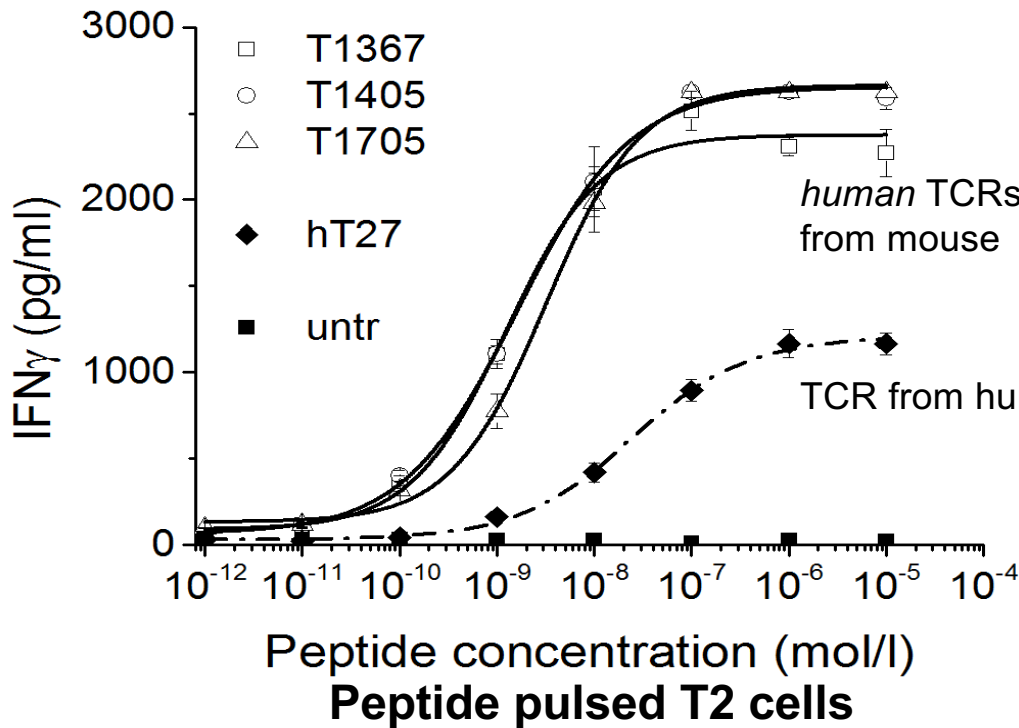
	270		300
Human	-ALAETSYV	KVLEYVIKV	SARVRFFFPSLREA-
Mouse	-AFAETSKMKVLQFFASINKTHPRAYPEKYAE-		
	*	*****	***
			*

Humanized mouse model for TCR generation



CANCER-ASSOCIATED ANTIGENS AS TARGET

Mouse-derived MAGE-A1 TCR has higher functional affinity



Timeline MAGE A1 TCR gene therapy of Multiple Myeloma

1. SA



Oct. 2013

2. SA



Oct. 2015

CTA (planned)



Q1 2017

R&D

Pre-clinical

Clinical



Data
summaries
Study
concept



IMPD
submission
Study synopsis
Manufacturing
licence

SAFETY OF T1367 T CELL THERAPY

Potential safety concerns

- MAGE-A1 expression in **healthy tissues**
- Allo-reactivity
 - T1367 was generated in mice expressing a **single HLA class I** molecule (in part applies also for an allogeneic human environment)
- cross-reactivity
 - **Promiscuous** TCR recognition has been reported
 - T1367 was positively and negatively selected based on a **mouse peptide repertoire**

MAGE-A1

- Limited expression in normal tissues (testis, placenta, embryonic tissue, **mTECs**)

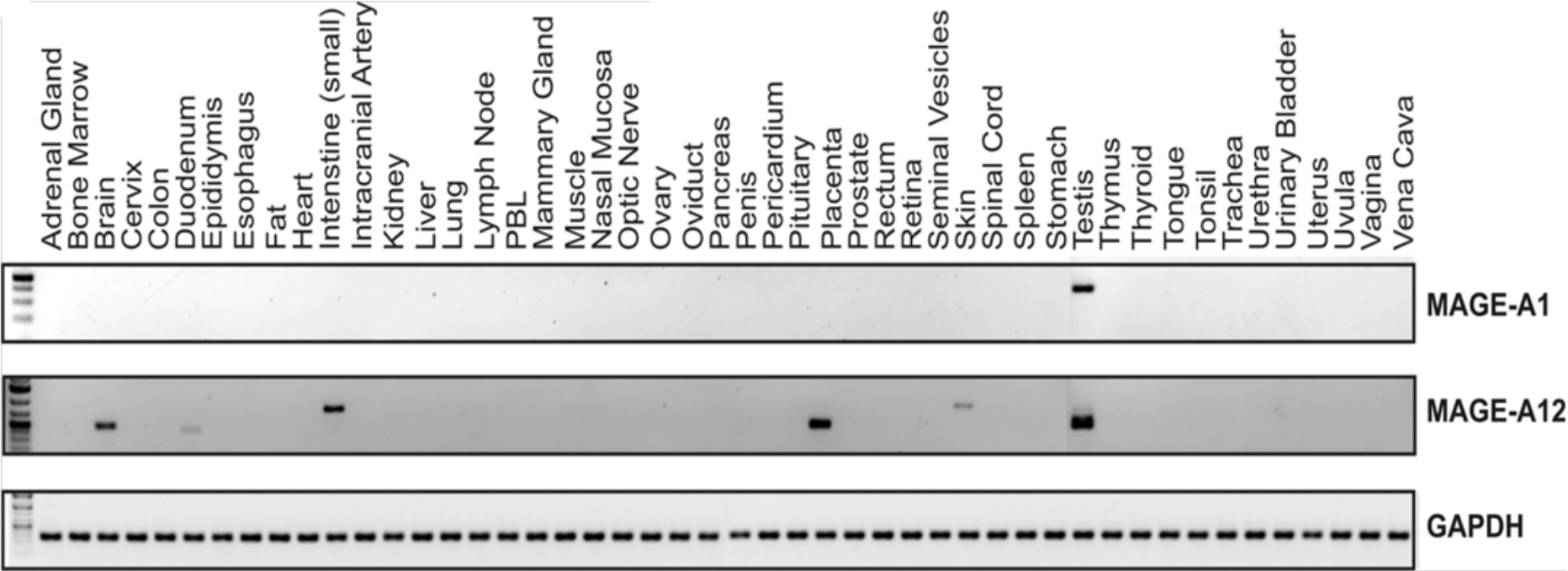


Expression in tumors:

- **Multiple myeloma**
- Lung cancer (NSCLC)
- Melanoma
- Breast cancer
- Colon cancer
- Hepatocellular carcinoma
- Cholangiocellular carcinoma

CANCER-ASSOCIATED ANTIGENS AS TARGET

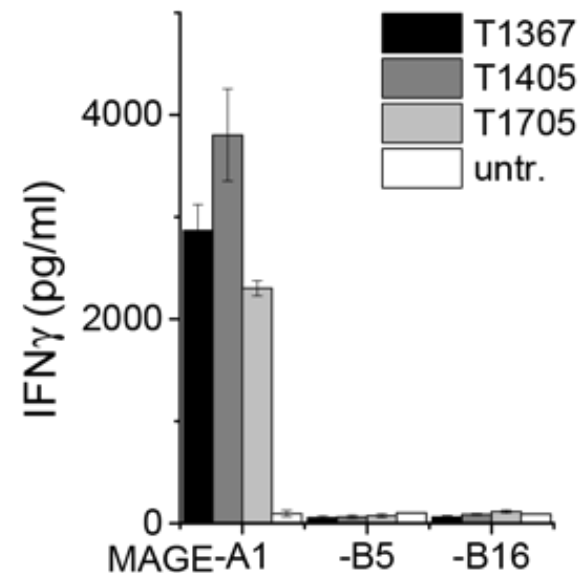
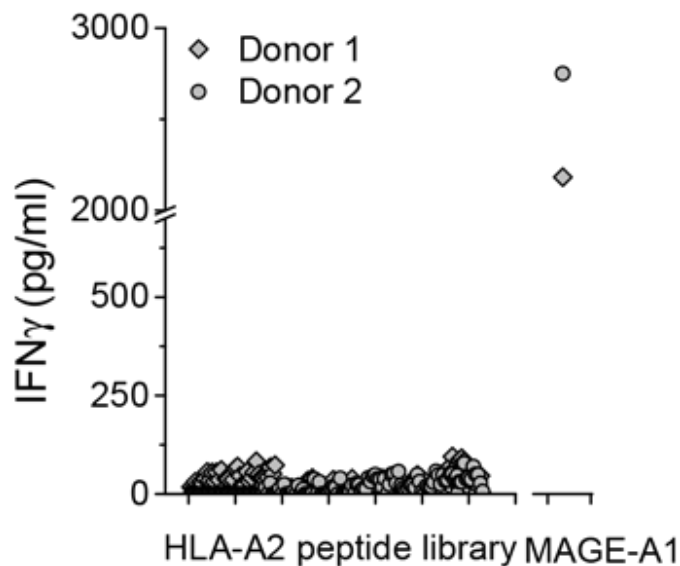
Restricted MAGE-A1 expression



CANCER-ASSOCIATED ANTIGENS AS TARGET

No evidence for off-target recognition

More than 100 HLA-A2
peptide ligands



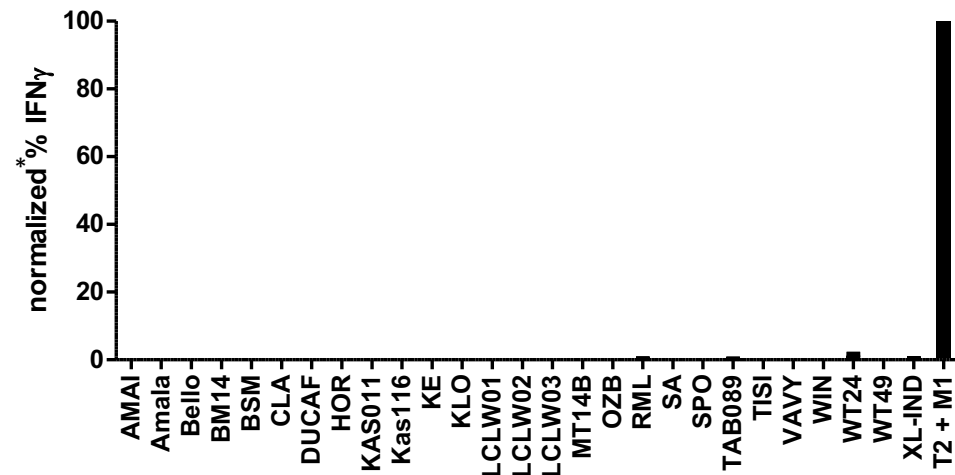
Peptide	Sequence	IC ₅₀ (nM)
MAGE-A1	KVLEYVIKV	4.69
MAGE-B5	KVLEYLAKV	4.11
MAGE-B16	KVLEFVAKV	4.97

CANCER-ASSOCIATED ANTIGENS AS TARGET

No evidence for allo-recognition

LCL panel

	HLA-A*		HLA-B*		HLA-C*	
AMAI	68:02		53:01		04:01	
AMALA	02:17:01		15:01:01		03:03:01	
Bello	02:02	11:01	41:01	52:01	12:02	17:01
BM14	03:01		07:02		07:02	
BSM	02:0101		15:010101		03:0401	
CLA	02:06:01	24:02	08:01	35	7	
DUCAF	30:02		18:01		05:01	
HOR	33:0301		44:0301		14:03	
KAS011	01:0101		37:01		06:02	
KAS116	24:020101		51:01		12:03	
KE	02:01	29:02	44:03	44:05	02:02	16:01
KLO	02:08	01:01:01:01	08:01:01	50:01:01	07:01:01:01	06:02:01:02
LCLW01	03:01	24:02	15:01	35:01	03:03	04:01
LCLW02	02:01	26:01	38:01	44:02	05:01	12:03
LCLW03	02:01	23:01	15:01	58:01	03:04	07:01
MT14B	31:01		40:01		03:04	
OZB	02:09	03:01:01	38:01	35:02	04:01:01	12:03:01
RML	02:04		51:0101		15:02	
SA	24:020101		07:0201		07:02	
SPO	02:01		44:02		05:01	
TAB089	02:07		46:01		01:02	
TISI	24:020101		35:08		04:01	
VAVY	01:01		08:01		07:01	
WIN	01:01		57:0101		06:02	
WT24	02:0101		27:0502		02:0202	
WT49	02:05:01		58:01:01		07:18	
XLI-ND	02:10	30:01	13:02	40:06:01:01	06:02	08:01

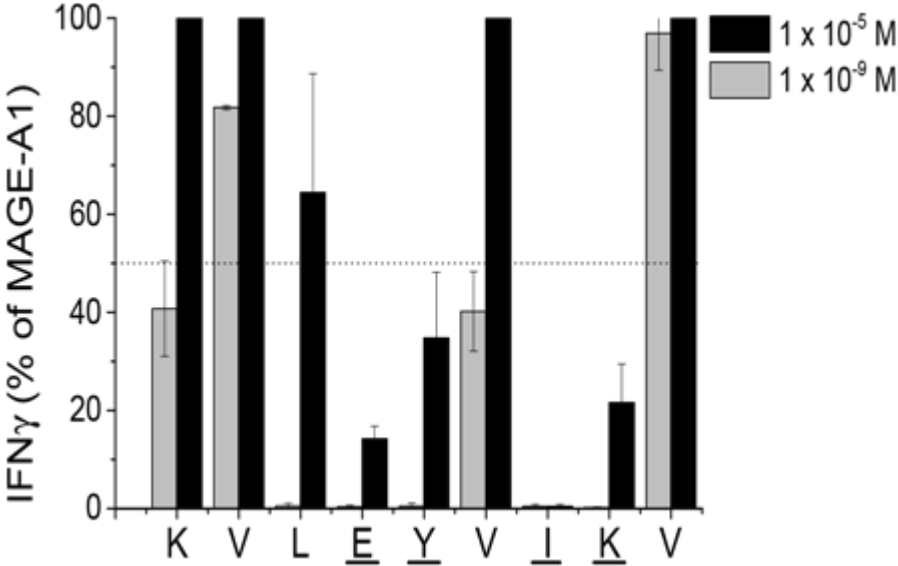


*Normalized to IFN γ production of MAGE-A1 loaded T2 cells

LCLs representing more than **75% for HLA-A***, **60% for HLA-B*** and **78% for HLA-C*** of the alleles within the German population

CANCER-ASSOCIATED ANTIGENS AS TARGET

MAGE-A1 recognition motif-related human self-peptides

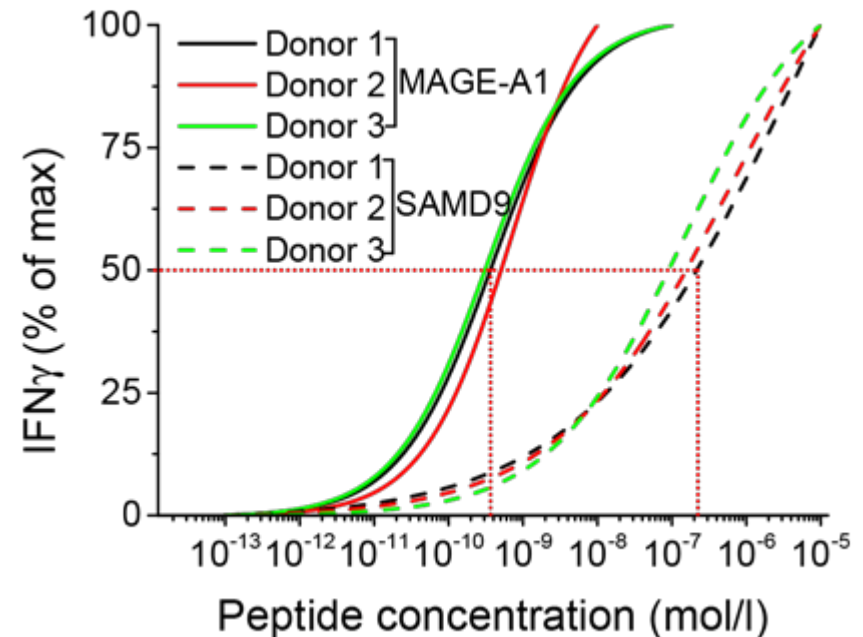
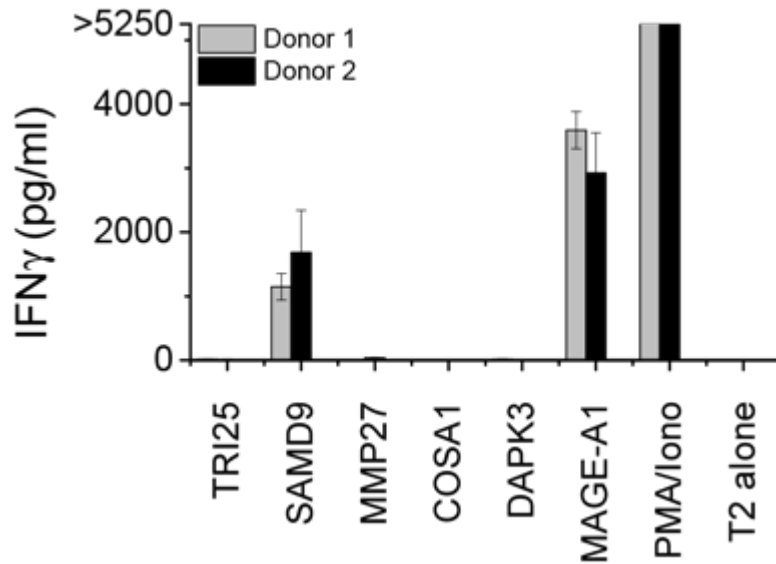


Alanine scan

Sequence	Gene Symbol	IC ₅₀ (nM)
X X X E Y X I K X	T1367 motif	
K V L E Y V I K V	MAGEA1	4.11
E L L E Y Y I K V	TRI25	23.34
G L L E Y L I K S	SAMD9	73.23
K Q F E Y D I K T	MMP27	834.74
R S L E Y D I K L	COSA1	4258.75
R L K E Y T I K S	DAPK3	5212.32

CANCER-ASSOCIATED ANTIGENS AS TARGET

SAMD9 is recognized at high peptide concentration

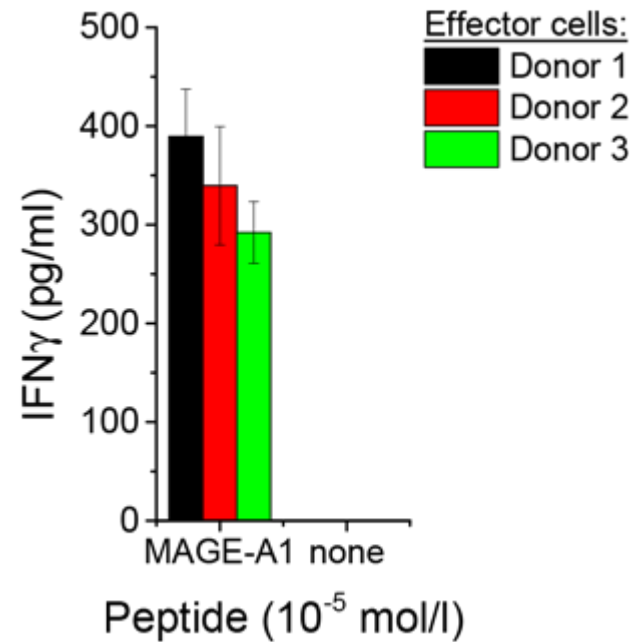
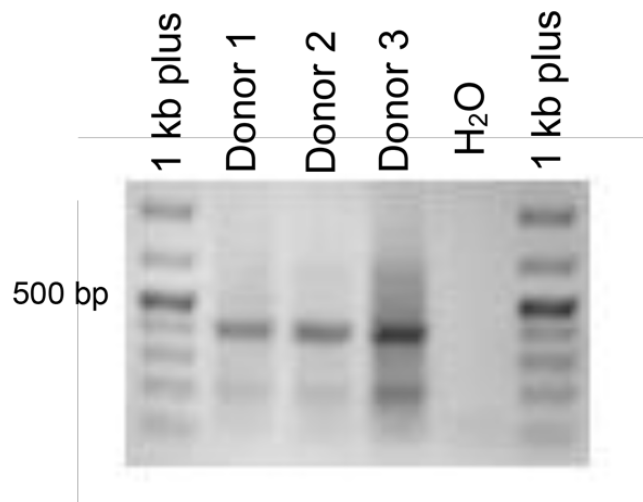


SAMD9: Sterile Alpha Motif Domain Containing Protein 9

CANCER-ASSOCIATED ANTIGENS AS TARGET

SAMD9 is not naturally presented

T cells

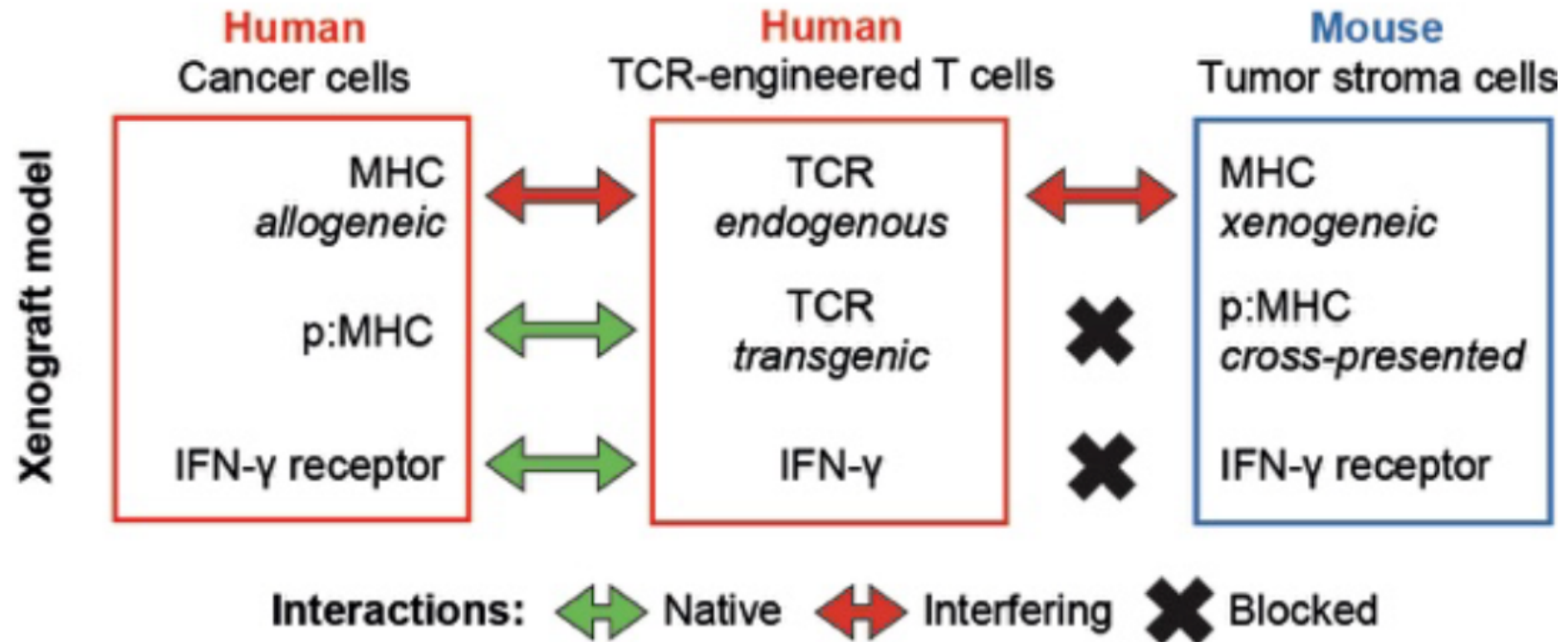


SAMD9: Sterile Alpha Motif Domain Containing Protein 9

Limitations in xenograft cancer models

Biodistribution and pharmacokinetics of T1367-transduced T cells can be addressed only in the autologous host, the MM patient

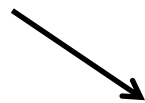
- human T cells in mice poorly expand or survive (species-specific factors)
- human T cells in NSG mice acquire functional activity, but may elicit lethal graft-versus-host disease (GvHD).



A pre-clinical model for analysis of TCR efficacy

1

Syngeneic mouse tumor cells expressing human antigen and HLA-A2

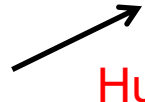
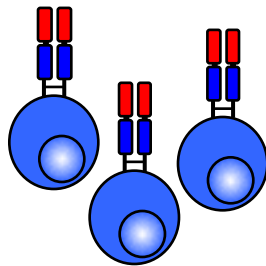


HLA-A2⁺

3

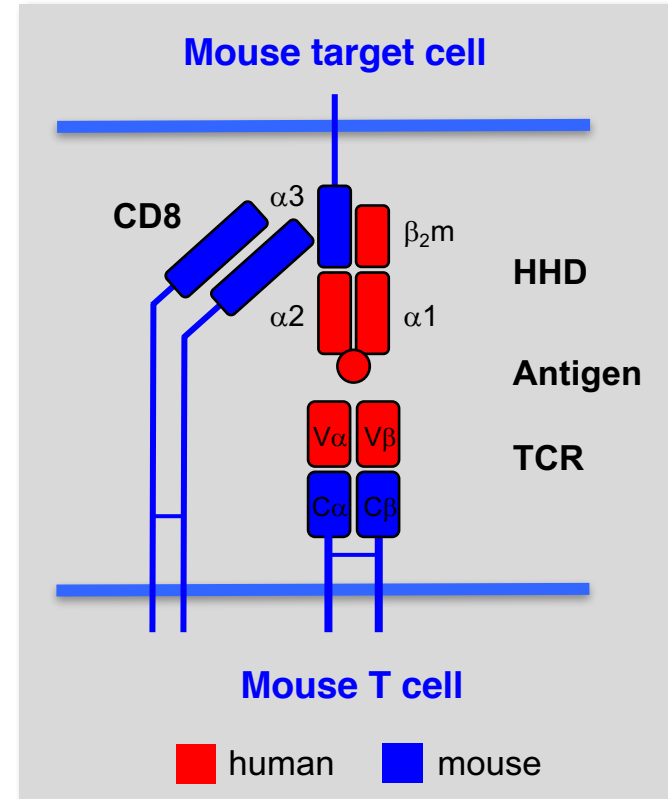


2



Human MHC-transgenic mouse (HHD)

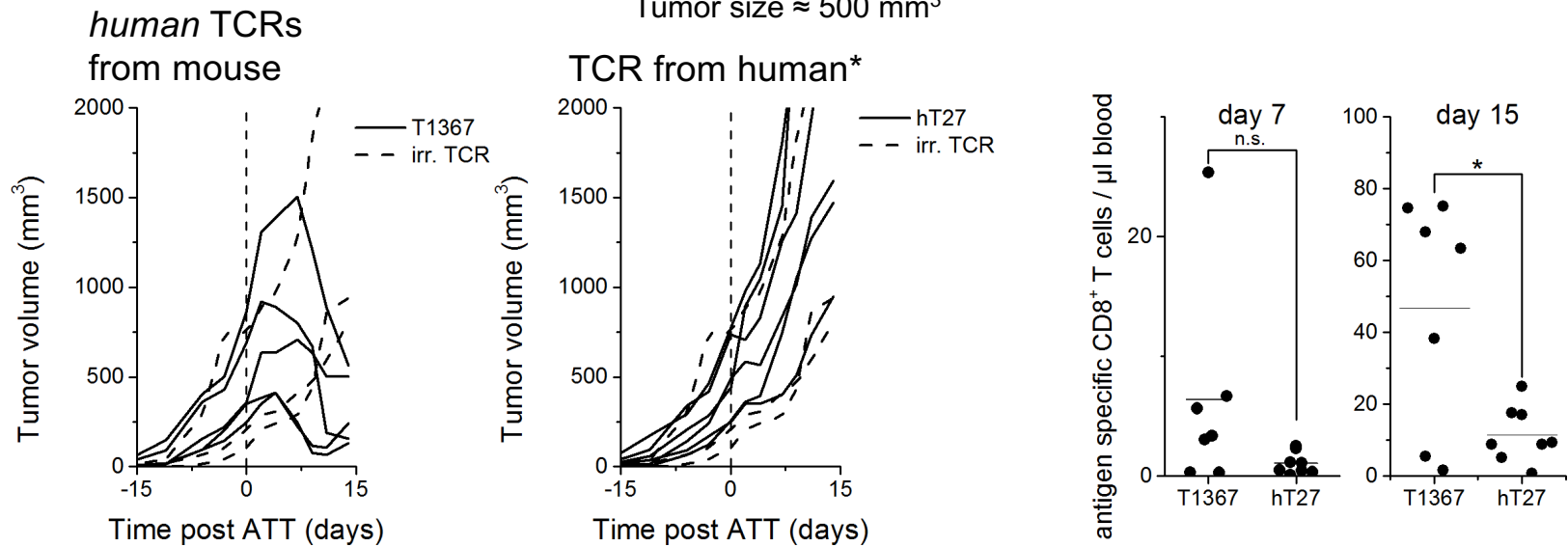
HLA-A2⁺ mouse T cells expressing human TCR



Does TCR gene therapy lead to rejection or relapse of tumor?

CANCER-ASSOCIATED ANTIGENS AS TARGET

In vivo function of TCR modified T cells

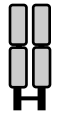


→ The TCR repertoire against MAGE-A1₂₇₈ is likely skewed towards low affinity in humans

MAGE-A1 TCR gene therapy: Study Information

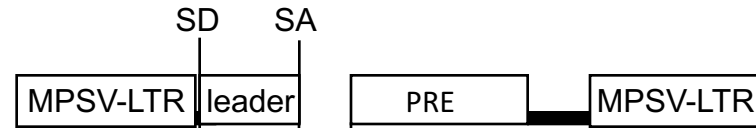
- Chimeric mouse/human TCR
- Target: Cancer-germline antigen
- Vector: Gamma retroviral MP71
- Indication: Relapsed/refractory Multiple Myeloma

TCR α - and β -chain gene expression cassettes



WT-1 TCR

MP71



Single chain

$\alpha + \beta$

TCR V α

TCR V β

IRES

$\alpha\beta$

TCR V α

IRES

TCR V β

$\beta\alpha$

TCR V β

IRES

TCR V α

P2A

$\alpha\beta$

TCR V α

P2A

TCR V β

$\beta\alpha$

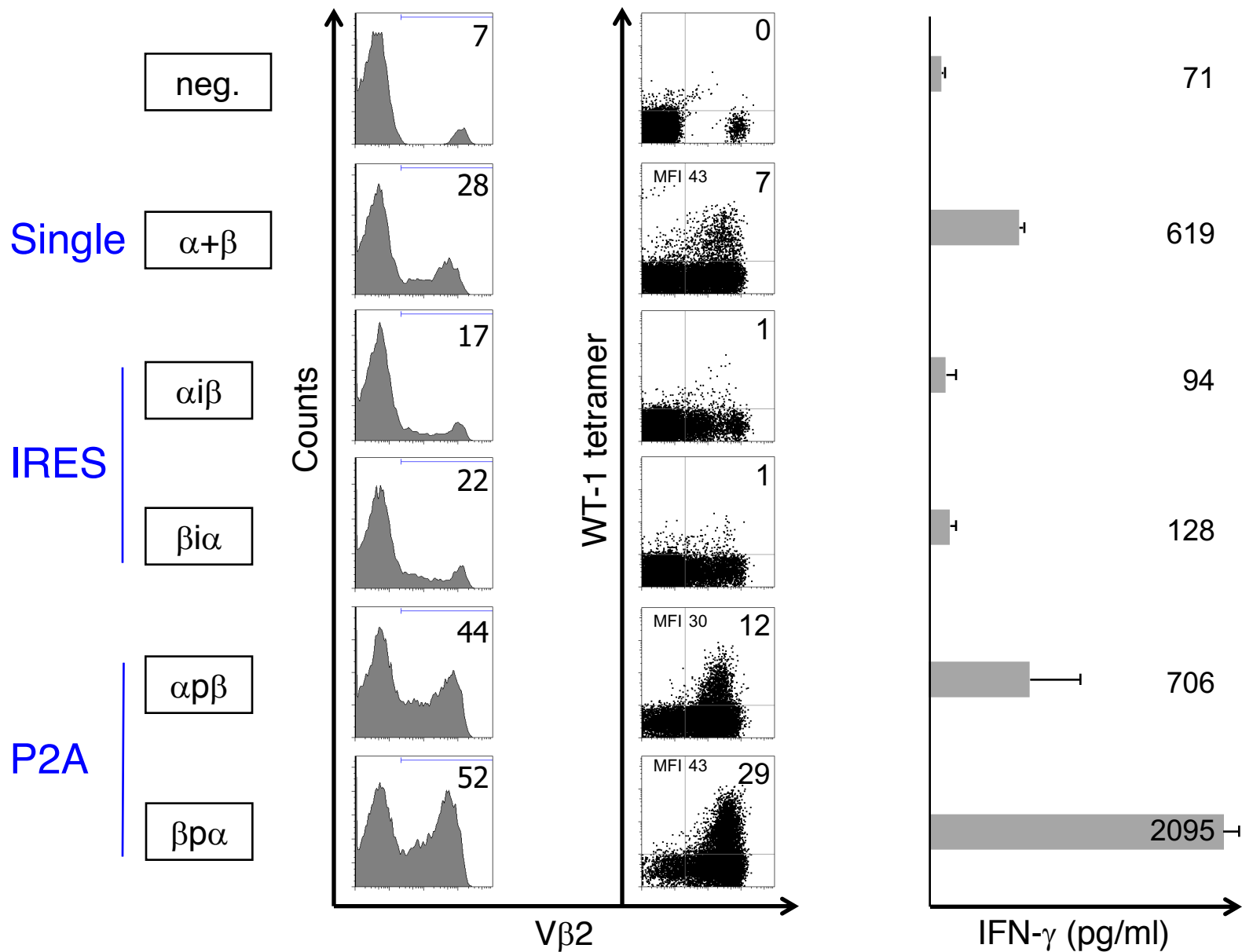
TCR V β

P2A

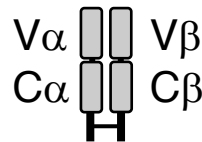
TCR V α



Transgene cassette determines TCR expression level



Optimization of TCR genes improves T cell functionality



Human TCR (wild type)



Murinization (mu)

Preferential pairing of tg TCR chains

(Cohen, 2006)



Disulfide bond (cys)

Preferential pairing of tg TCR chains

(Kuball, 2007)



Codon optimization (co)

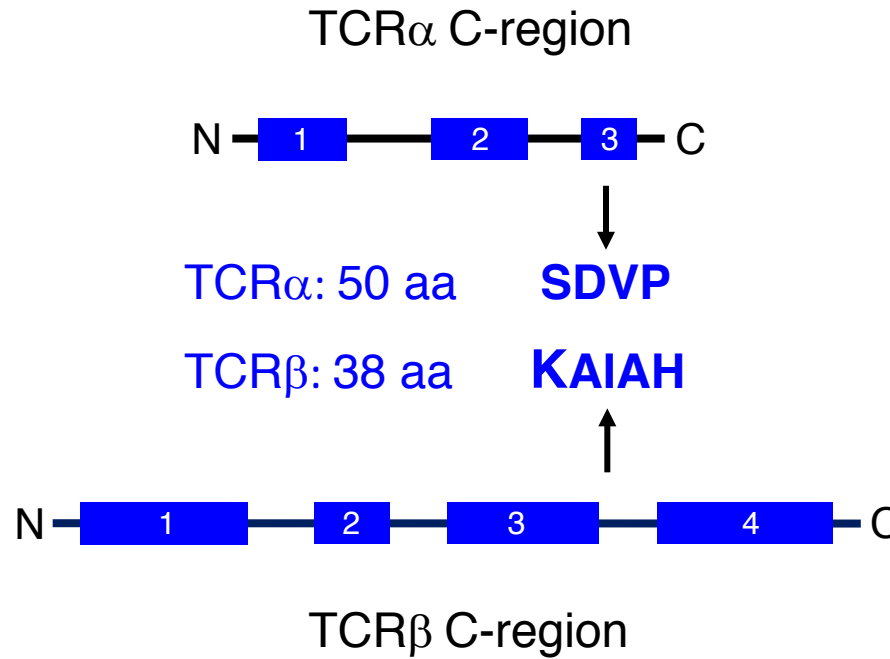
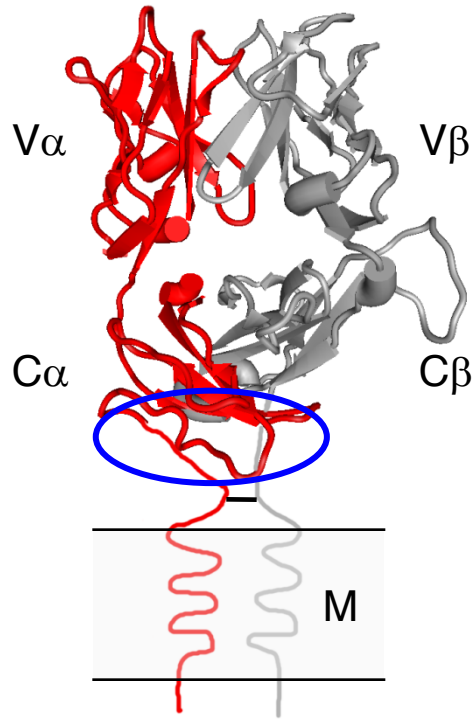
Enhanced tg TCR expression level

(Scholten, 2006)

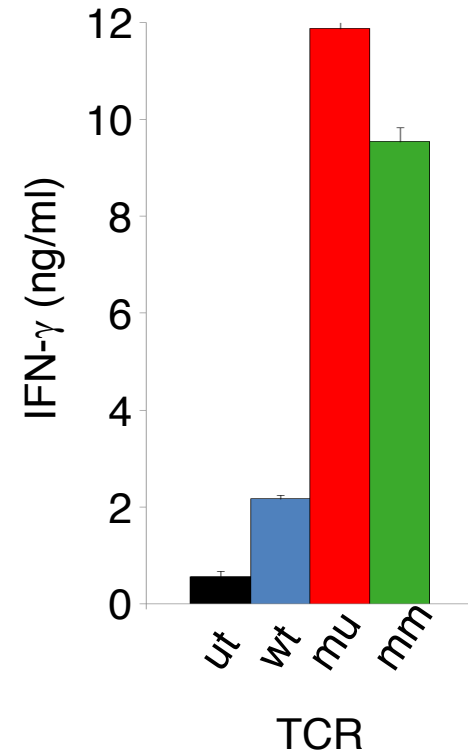
Others

- *increase the avidity of therapeutic TCR*
- *replace wild type TCR*

9 amino acids of the mouse TCR C-regions enhance human TCR expression

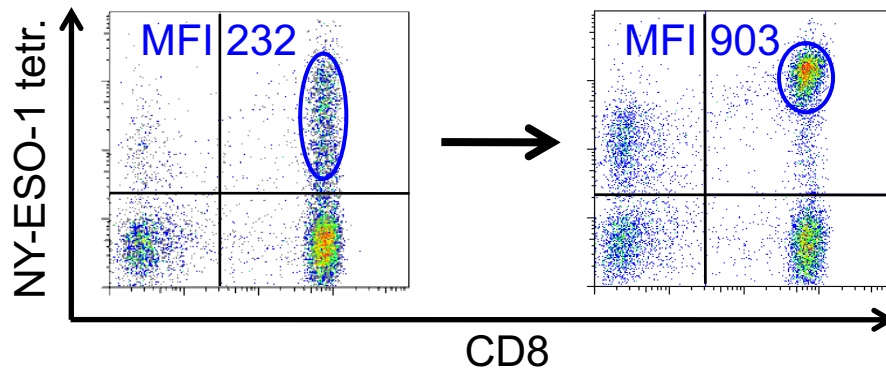
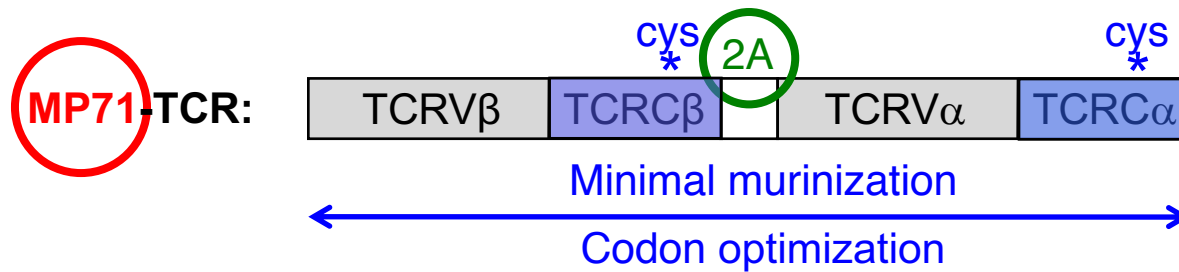


TCR53 tg T cells / RCC26



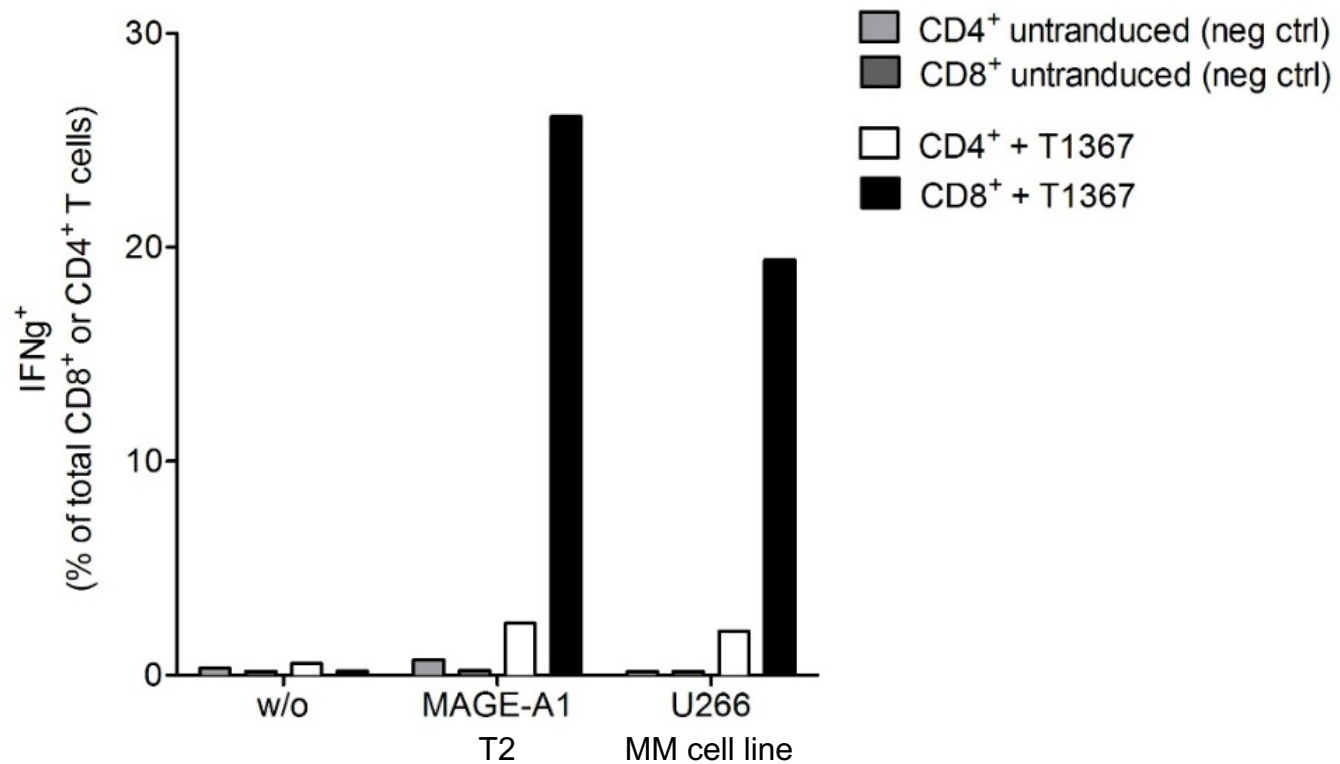
High-performance γ -retrovirus vector for TCR gene therapy

- Modification of retrovirus vector
- Optimization of TCR gene cassette
- Engineering of TCR genes

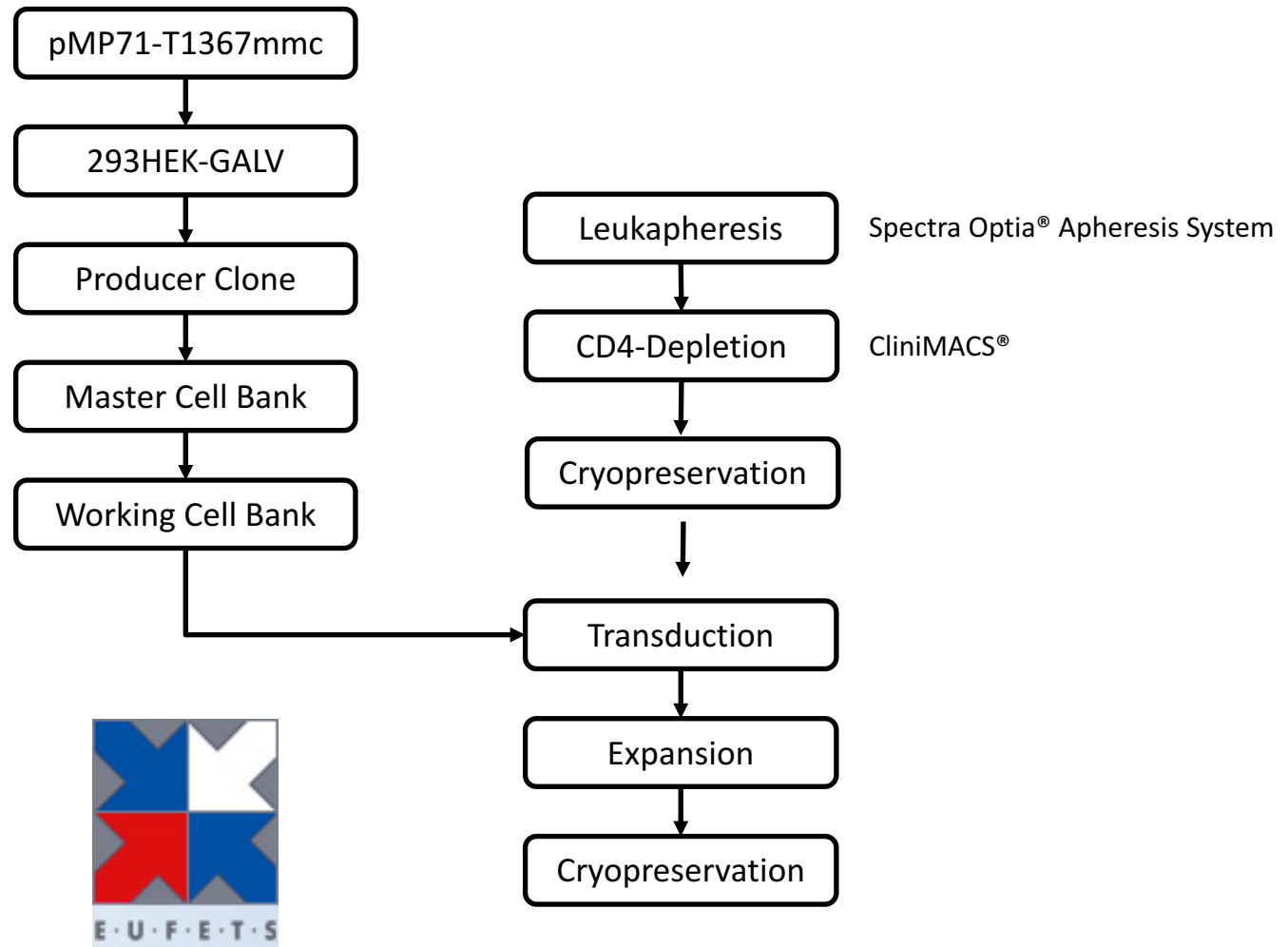


- TCR gene-modified T cells:**
- High expression level of tg TCR
 - Homogeneous population
 - High functional activity

MAGE-A1 TCR *T1367* is primarily active in CD8⁺ T cells



Manufacturing process



GMP Transduction Protocol

[Spectra Optia® Apheresis System](#)

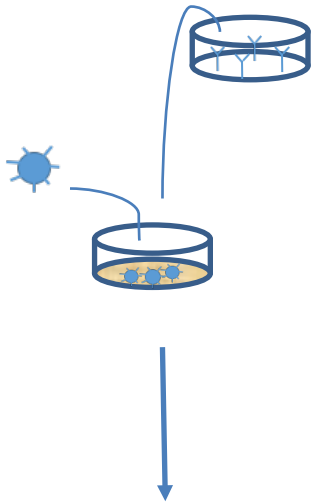
CD4⁺ cell-depletion with CliniMACS®, Miltenyi

PBMCs

Starting material

CD4⁻ cells

CD4 depl. leucapherisate

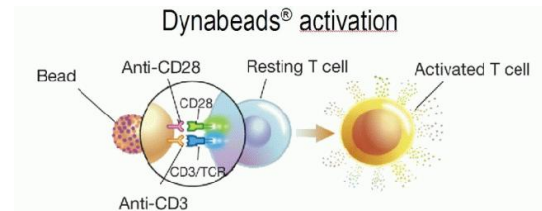


Adherence

depletion of monocytes

Activation

bead bound antibodies
+ Selection



Transduction

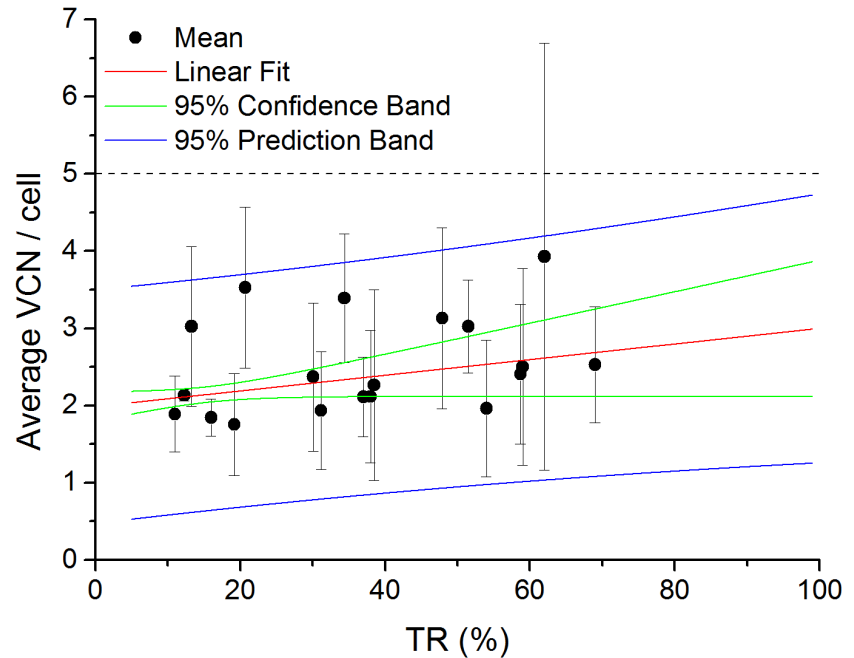
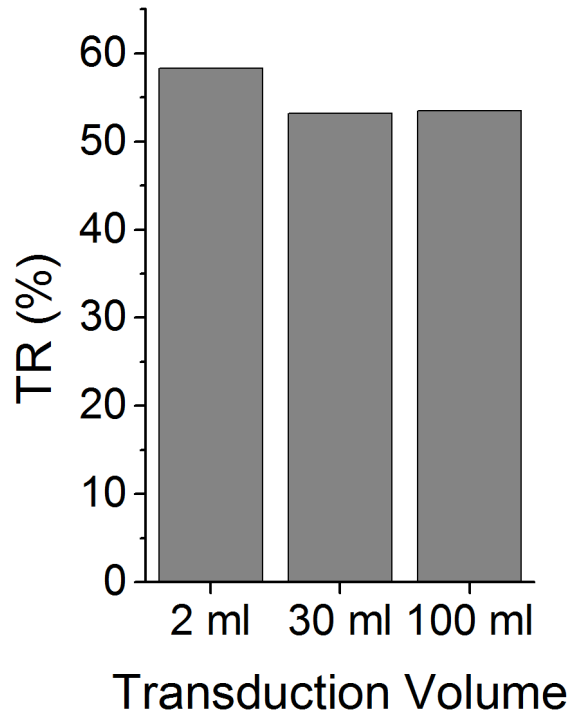
1x 90 min, 800g, 32°C
100 ml pure Virus

Cultivation

Up to 12 days in Wave Reactor

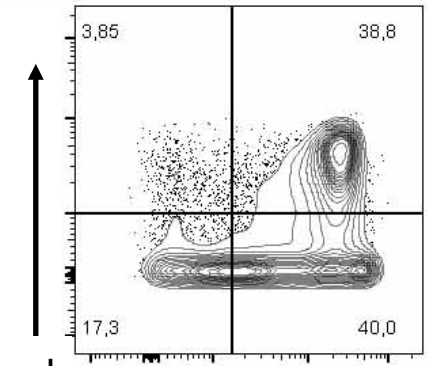
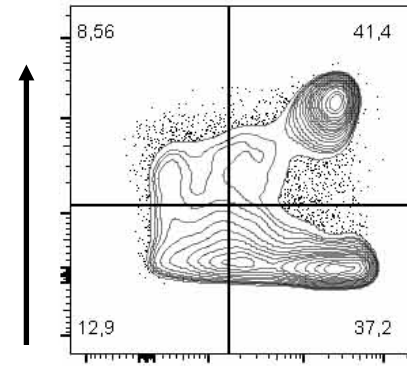
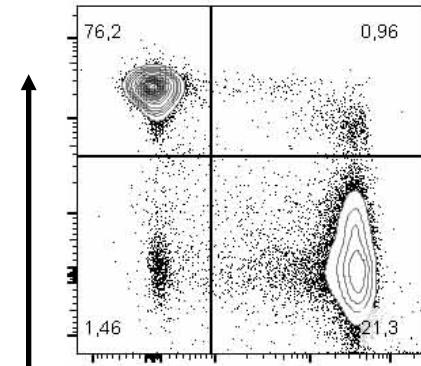


Mean pMP71 vector copy number per cell



MAGE-A1 TCR *T1367* transduced T cells have stem cell-like memory phenotype

Buffy coat
(control)

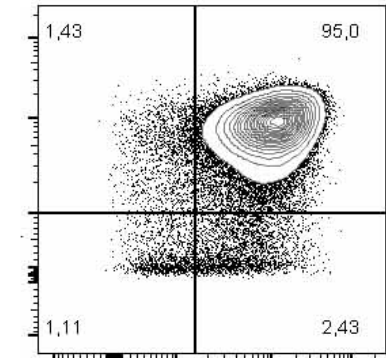
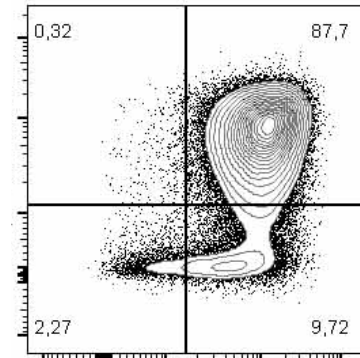
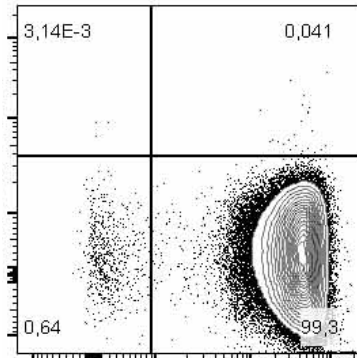


CD4

CCR7

CD62L

Leukapheresis
product,
RV transduced



CD8

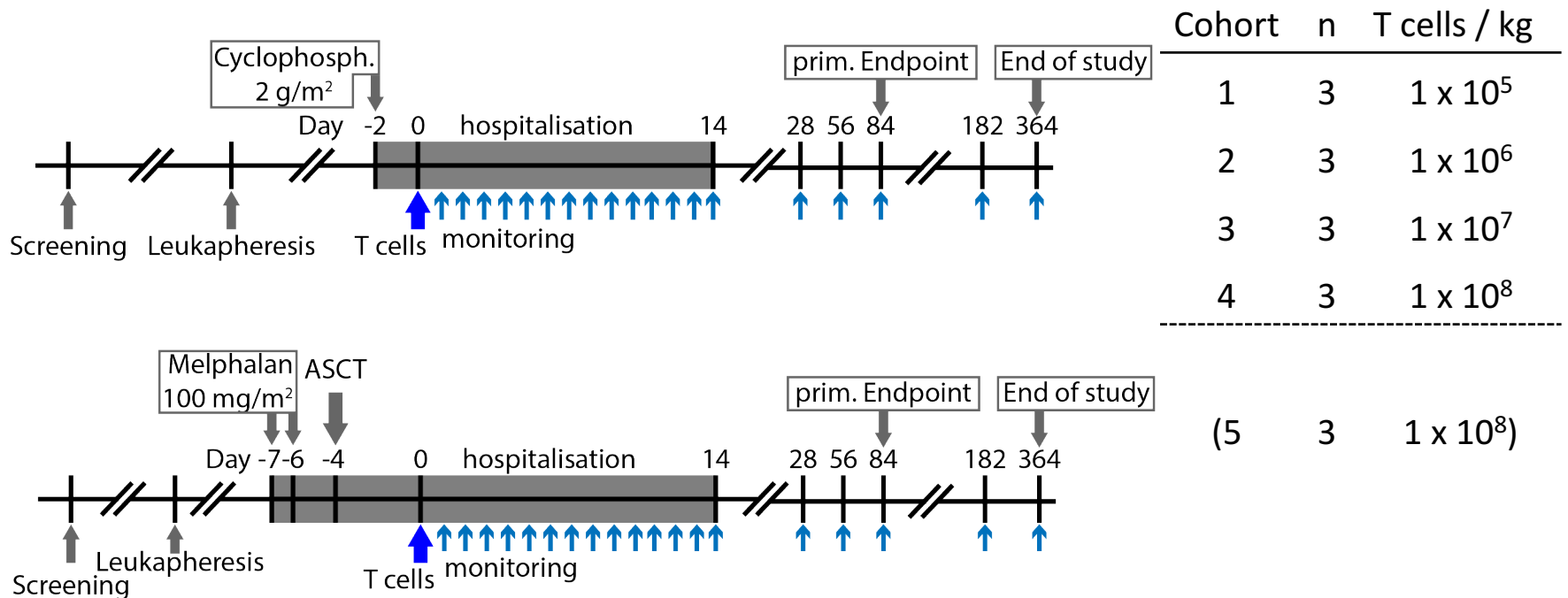
CD45RA

CD45RA

CD3+ CD8+ CD45RA+ CD45RO- CD62L+ CCR7+

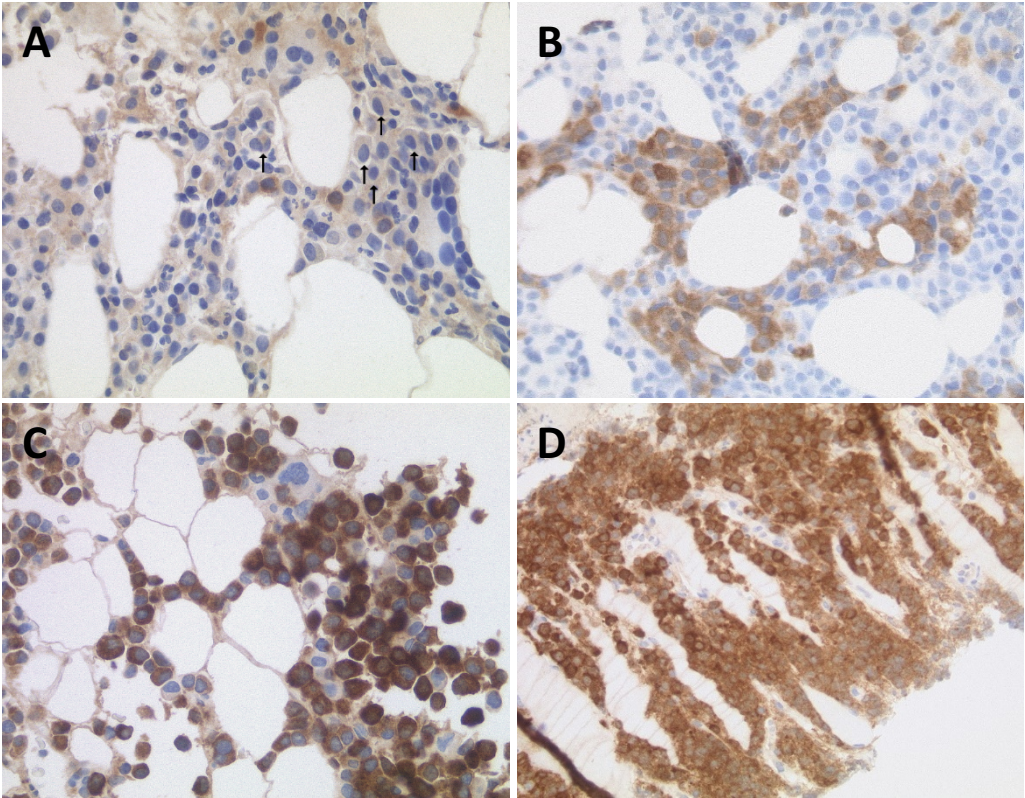
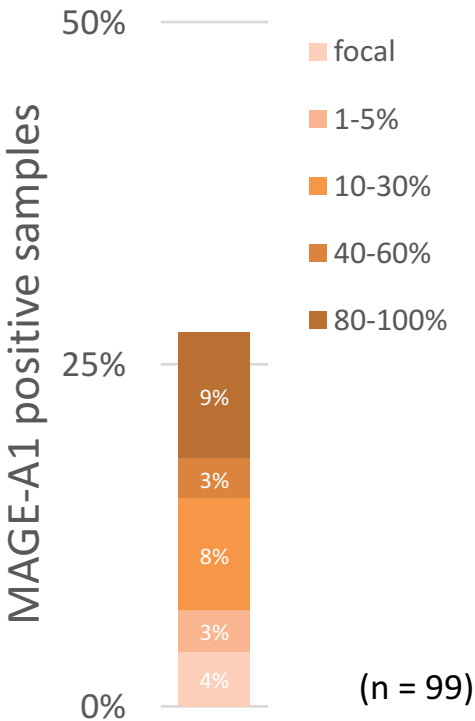
T CELL THERAPY OF MULTIPLE MYELOMA

Phase I trial of MAGE-A1⁺ Multiple Myeloma



CANCER-ASSOCIATED ANTIGENS AS TARGET

Identifying *MAGE-A1*⁺ myeloma



Patient selection

- Heterogenous expression pattern of MAGE-A1 within a tumor
- Correlation between extramedullary disease and homogenous MAGE-A1 expression
- Many patients with MAGE-A1 expressing myeloma are primary refractory to either a bortezomib or lenalidomide based regimen

Standardization of the manufacturing process: “Master Processes”

- technology platforms (GMP facility, medical device status)
- generic vectors documented in a dossier
(solution for bottlenecks for academia as a small customer)
- establishment of INDs for established products that can be used as a blueprint to develop processes and products that are derived from the existing process

Kathrin Borgwald
Sabrina Horn
Kristin Retzlaff
Katerina Thiede
Dana Hoser
George Papafotiou
Jaqueline Waldeck

Thomas Blankenstein
Matthias Obenaus
Elisa Kieback
Vivian Scheuplein
Wolfgang Uckert
Matthias Leisegang

Antonio Pezzutto
Lutz Uharek
Ioannis Anagnostopoulos

Principles and Applications of Adoptive T Cell Therapy



BMBF program „Personalized Medicine“



German Cancer Aid Priority Program ‘Translational Oncology’

*Exploring mutant immunogenic epitopes
for T cell therapy of cancer*



German Cancer Consortium (DKTK)



Berlin School of Integrative Oncology



THANK YOU!