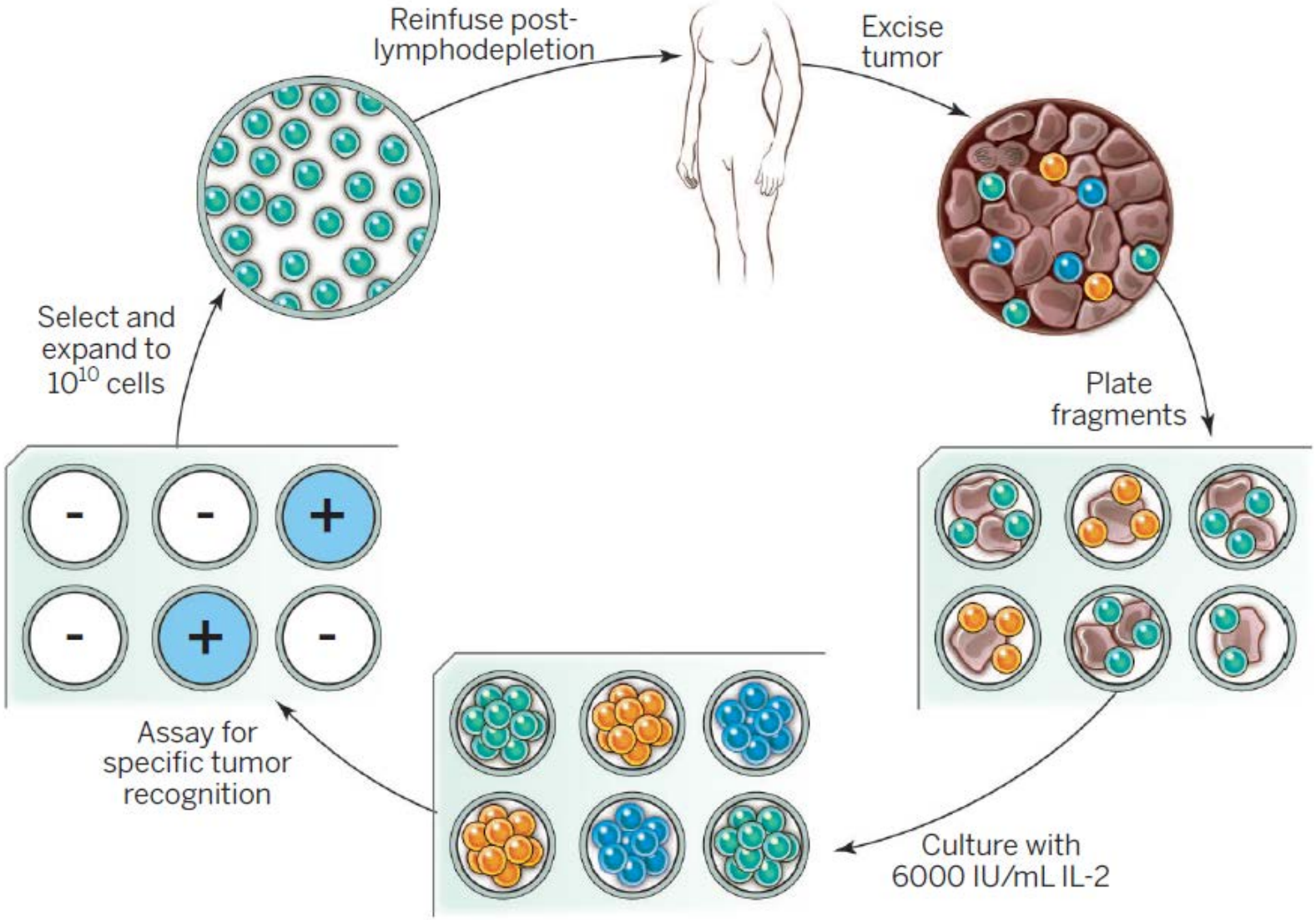
Adoptive Immunotherapy

Presented by Duo Li

12.05.2015

General schema for using the adoptive cell transfer of naturally occurring autologous TILs



Lymphodepletion prior to T cell transfer is followed by immune reconstitution

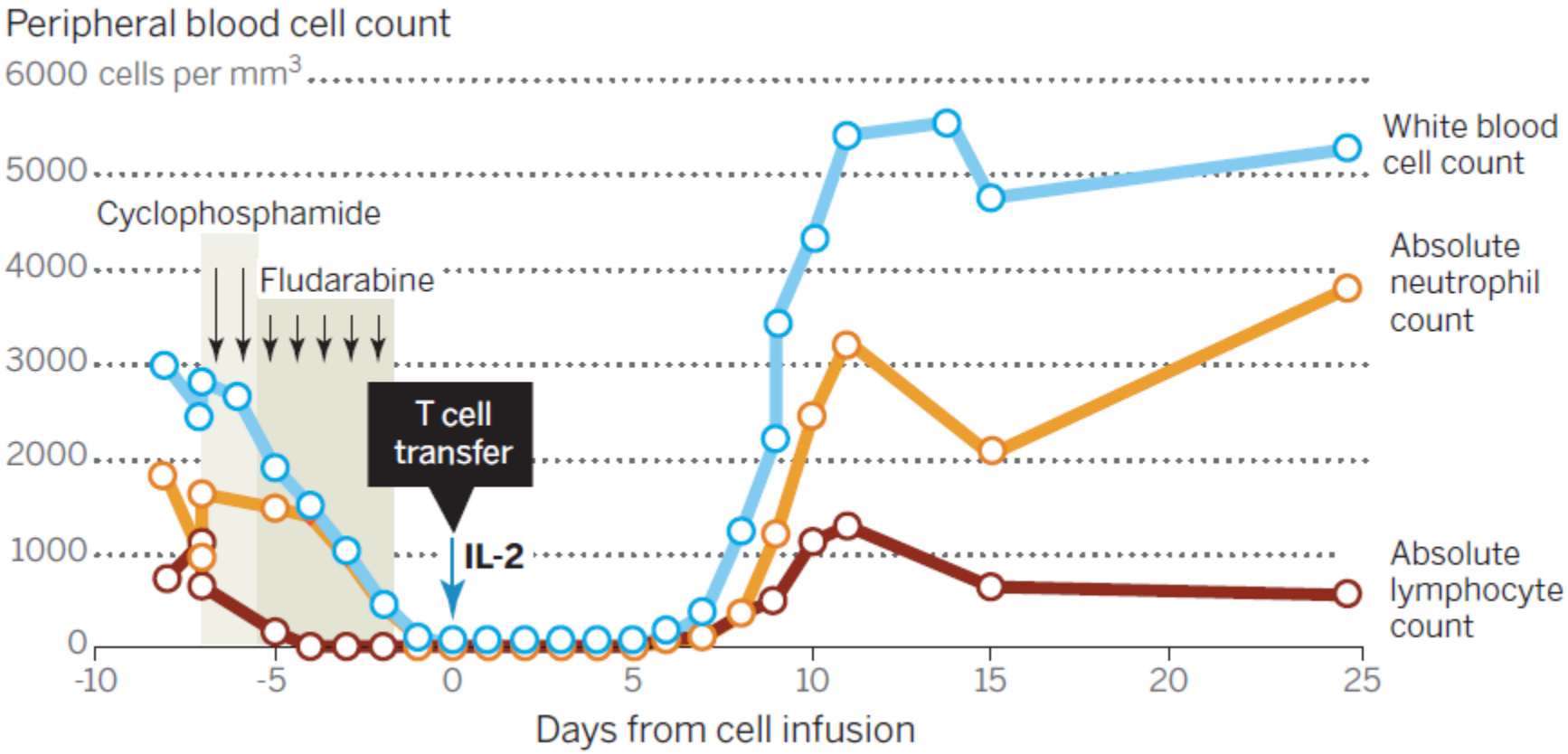
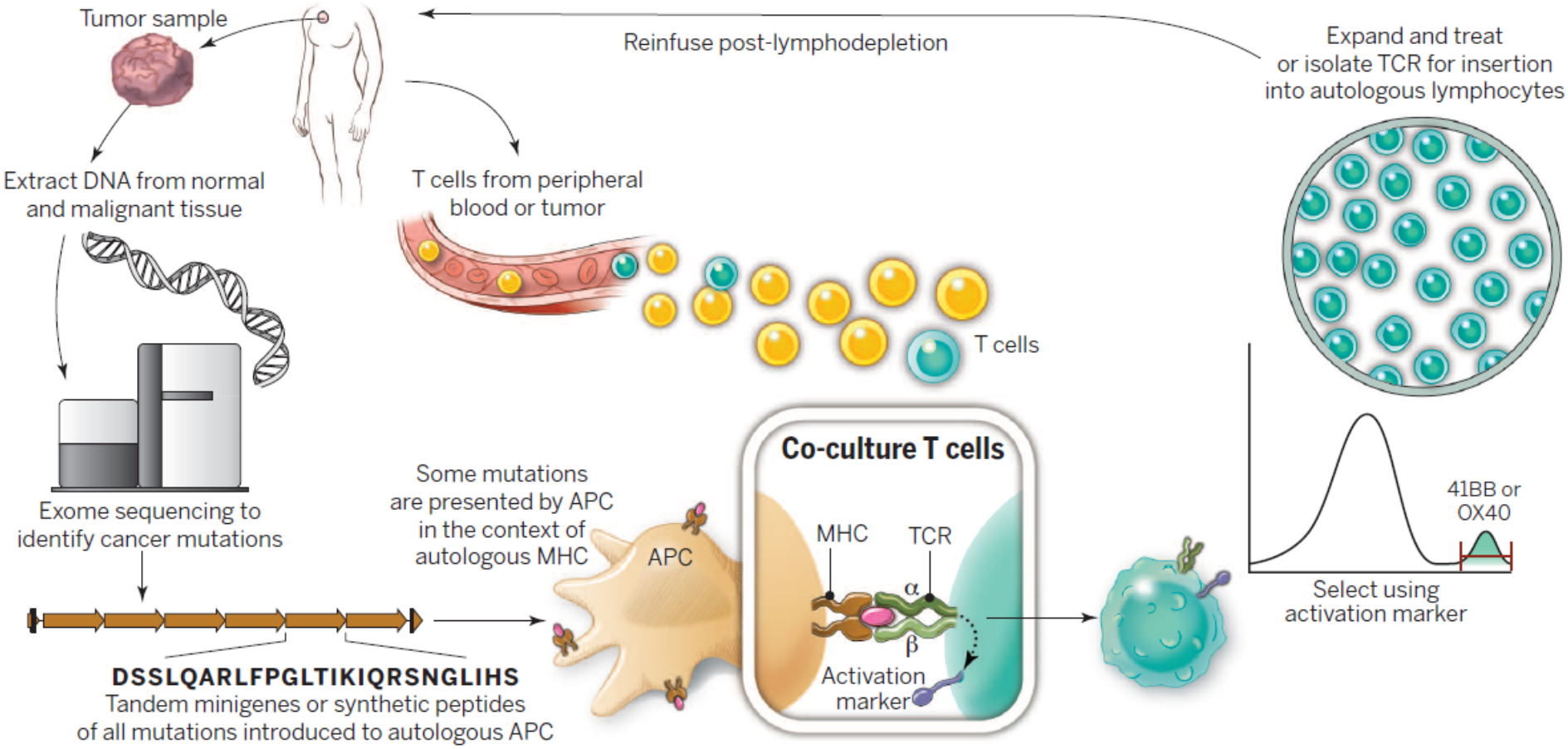


Table 1. Selected clinical trials of ACT for the treatment of human cancer. CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; CR, complete response; HPC, human papillomavirus; allo-HSCT, allogeneic hematopoietic stem cell transplantation; DLBCL, diffuse large B cell lymphoma; EBV, Epstein-Barr virus. Dashes indicate not applicable.

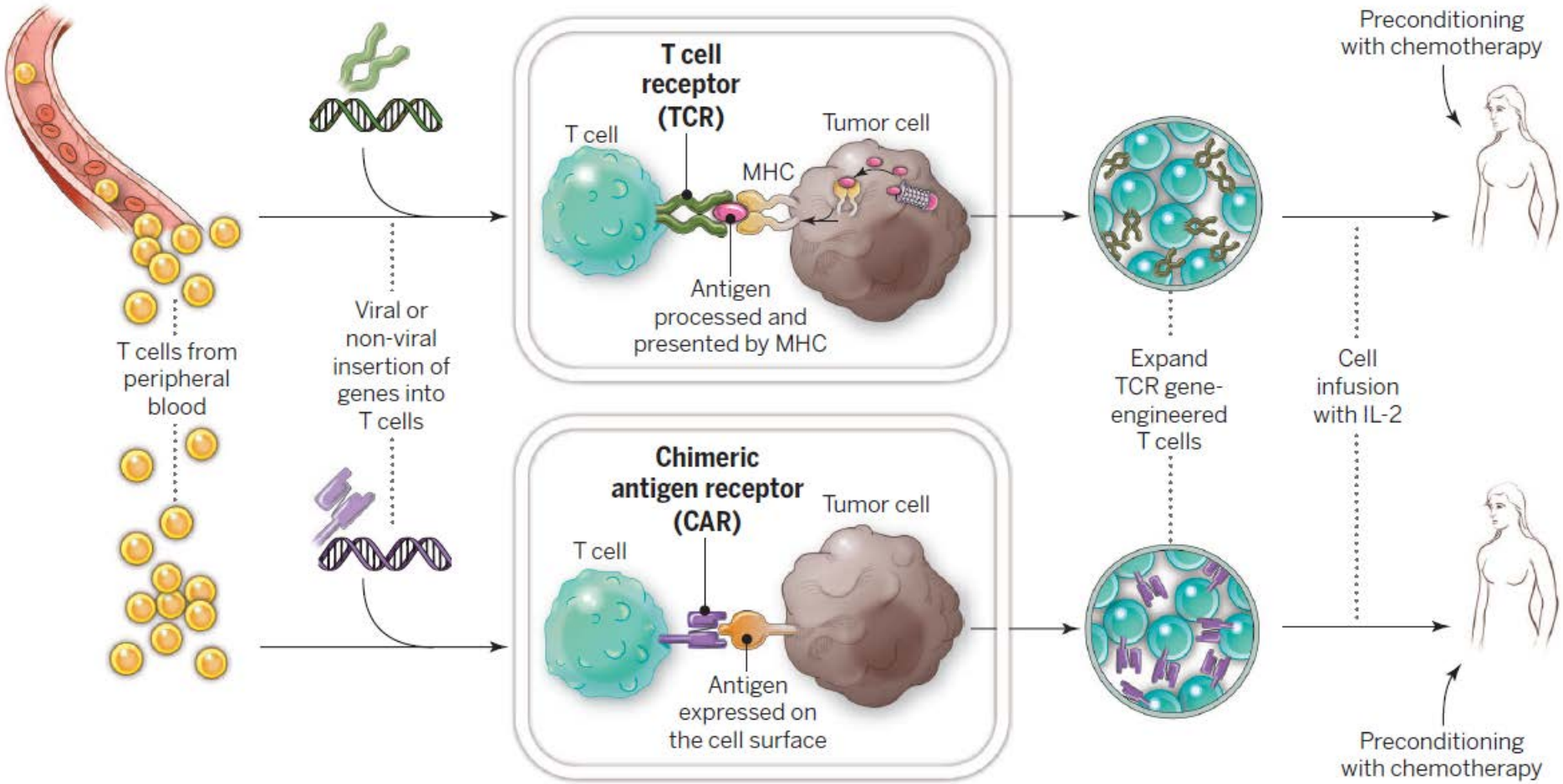
CELLS USED FOR ACT	YEAR	CANCER HISTOLOGY	MOLECULAR TARGET	PATIENTS	NUMBER OF ORS	COMMENTS
Tumor-inflitrating lymphocytes*	1998	Melanoma (*12*)		20	55%	Original use TIL ACT
	1994	Melanoma (*88*)		86	34%	
	2002	Melanoma (*13*)		13	46%	Lymphodepletion before cell transfer
	2011	Melanoma (*17*)		93	56%	20% CR beyond 5 years
	2012	Melanoma (*19*)		31	48%	
	2012	Melanoma (*18*)		13	38%	Intention to treat: 26% OR rate
	2013	Melanoma (*20*)		57	40%	Intention to treat: 29% OR rate
	2014	Cervical cancer (*89*)		9	33%	Probably targeting HPV antigens
	2014	Bile duct (*44*)	Mutated ERB2	1	–	Selected to target a somatic mutation
In vitro sensitization	2008	Melanoma (*90*)	NY-ESO-1	9	33%	Clones reactive against cancer-testes antigens
	2014	Leukemia (*91*)	WT-1	11	–	Many treated at high risk for relapse
Genetically engineered with CARs	2010	Lymphoma (*16*)	CD19	1	100%	First use of anti-CD19 CAR
	2011	CLL (*68*)	CD19	3	100%	Lentivirus used for transduction
	2013	ALL (*70*)	CD19	5	100%	Four of five then underwent allo-HSCT
	2014	ALL (*92*)	CD19	30	90%	CR in 90%
	2014	Lymphoma (*71*)	CD19	15	80%	Four of seven CR in DLBCL
	2014	ALL (*93*)	CD19	16	88%	Many moved to allo-HSCT
	2014	ALL (*94*)	CD19	21	67%	Dose-escalation study
	2011	Neuroblastoma (*78*)	GD2	11	27%	CR2 CARs into EBV-reactive cells
Genetically engineered with TCRs	2011	Synovial sarcoma (*81*)	NY-ESO-1	6	67%	First report targeting nonmelanoma solid tumor
	2006	Melanoma (*15, 32*)	MART-1	11	45%	

*Molecular targets of TIL in melanoma appear to be exomic mutations expressed by the cancer (*39, 40, 44*)

A “blueprint” for the treatment of patients with T cells recognizing tumor-specific mutations

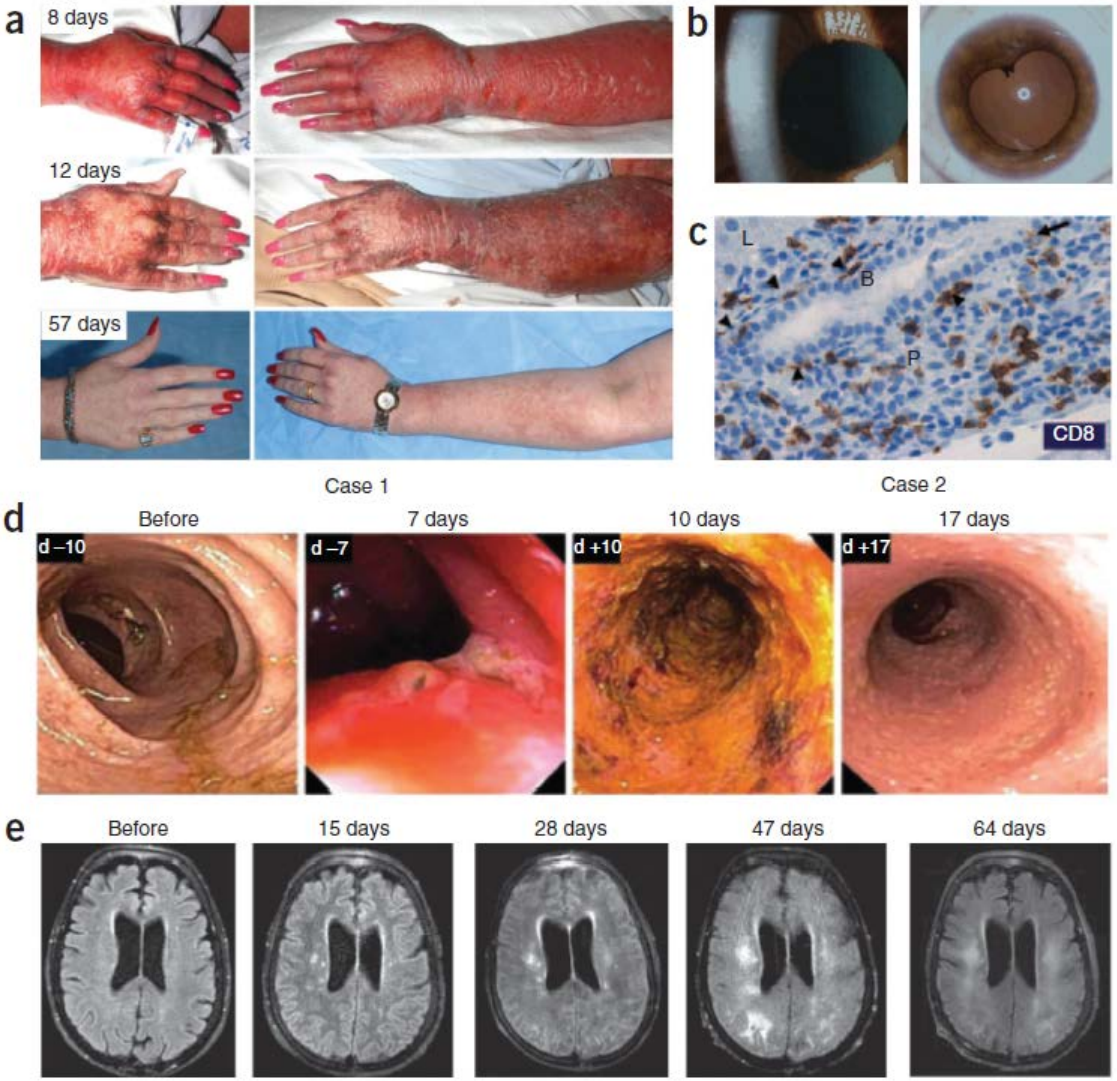


Gene-modification of peripheral blood lymphocytes



Autoimmune adverse events in ACT clinical trials

melanoma patient with T cells engineered to express a TCR with high affinity for MART1

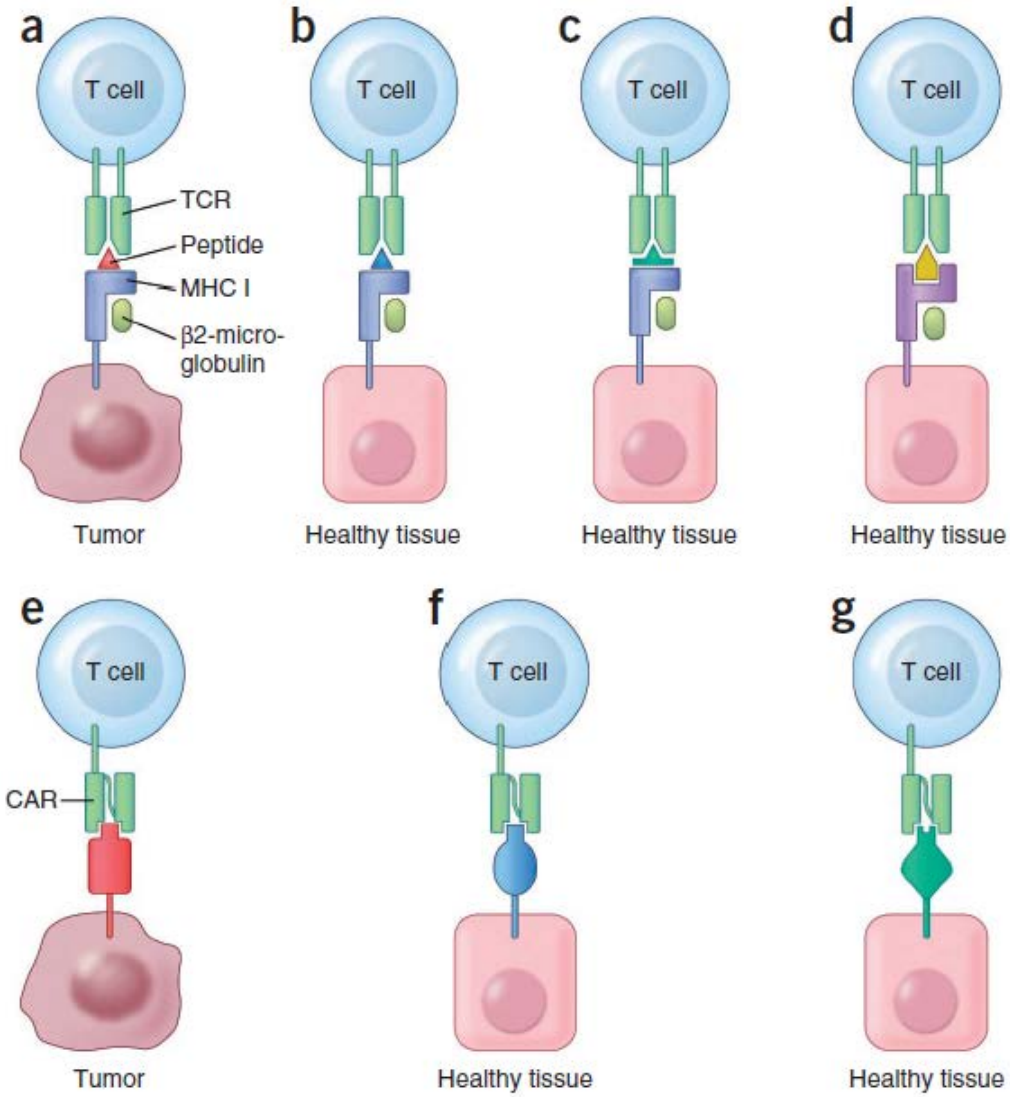


liver biopsy ,4 days after treatment of a renal cell carcinoma patient with T cells transduced with a CAR specific for carbonic anhydrase IX. CD8 T cells line the basal side of (arrowheads) and infiltrate (arrow) the bile duct epithelium. L, liver parenchyma; P, portal triangle; B, bile duct

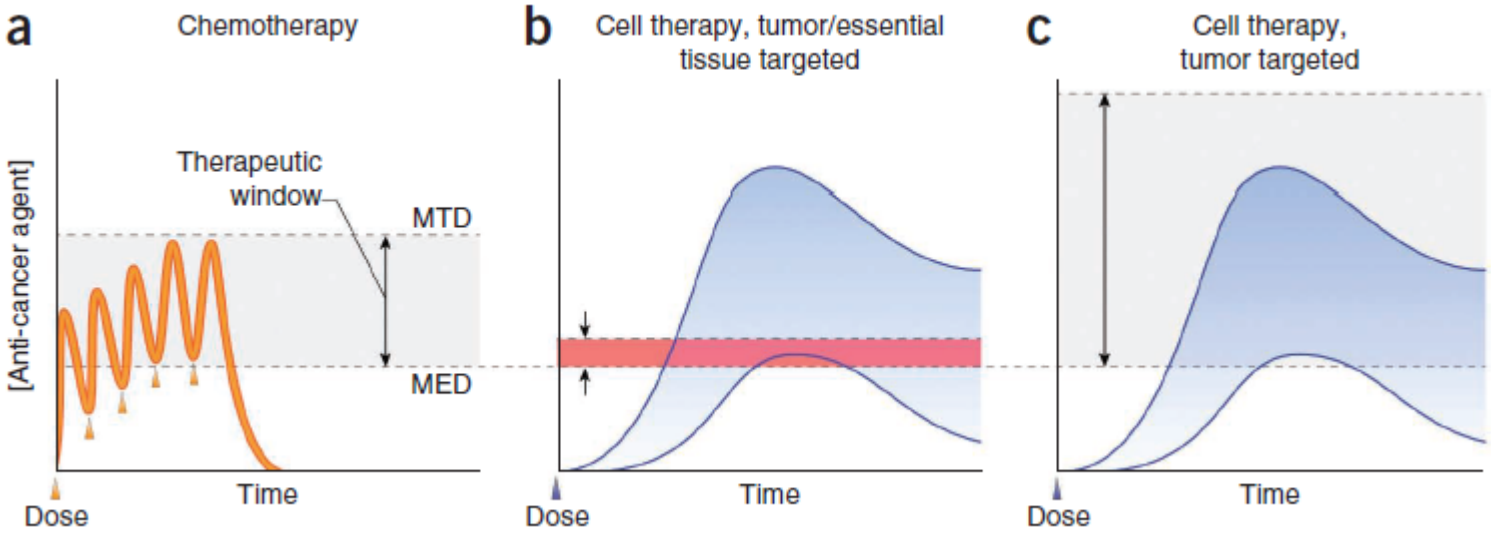
colon cancer , after administration of T cells engineered to express a TCR specific for carcinoembryonic antigen (CEA).

brain of a melanoma patient, after injection of T cells expressing a receptor that recognizes MAGEA3 but is cross-reactive with MAGEA12

Scenarios in which TCRs and CARs can recognize and cross-react with untargeted antigens



Differences in the pharmacokinetics and mechanisms of action between cytotoxic chemotherapy and ACT therapies



MED:minimum effective dose, the dose at which tumor regression occurs
MTD: maximum tolerated dose, the dose at which intolerable toxicities occur

T cells may target healthy tissues more efficiently than they target tumors, independent of the relative abundance of target antigen on each tissue

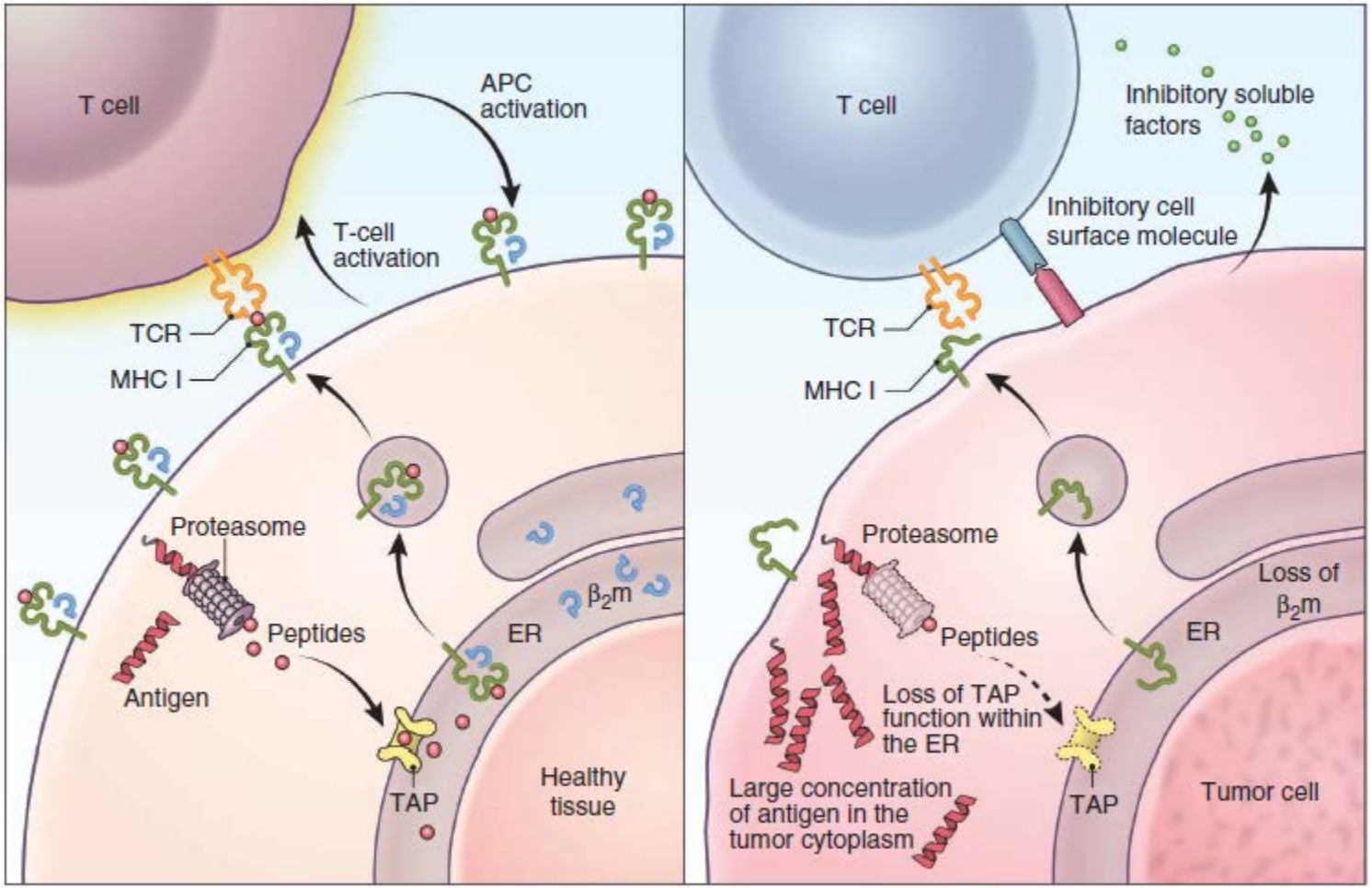


Table 1 Distribution in healthy tissue of antigens previously ranked highly by NCI as candidate ACT target antigens

Antigen	NCI priority rank	Gene	Healthy tissue expression that may cause major morbidity[a]
WT1	1	*WT1*	Kidney, hematopoietic cells[27,62–64]
MUC1	2	*MUC1*	Lung, liver, pancreas, esophagus, stomach, small bowel, colon, rectum, kidney, bone marrow, lymph node, peripheral nerve, skin, parathyroid gland, adrenal gland[26,27,66]
ERBB2	6	*ERBB2*	Heart, lung, esophagus, stomach, small bowel, colon, rectum, kidney, urinary bladder[26,27]
MAGEA3	8	*MAGEA3*	None[27,91]
p53	9	*TP53*	Bone marrow, spleen, stomach, esophagus, small bowel, colon, rectum, skin[26,27,59]
NY-ESO-1	10	*CTAG1B*	None[27,91]
PSMA	11	*FOLH1*	Brain, kidney, liver, spinal cord, nervous tissue, skin[26,27,92]
GD2	12	*N/A*	Brain, connective tissue from colon and kidney, skin, peripheral nerve, posterior pituitary[72,74,93,94]
CEA	13	*CEACAM5*	Bone marrow, liver, lung, esophagus, stomach, small bowel, colon, rectum[25–27]
MART1	14	*MLANA*	Melanocytes including skin, eye, ear[10,26]
gp100	16	*PMEL*	Melanocytes including skin, eye, ear[10]
Proteinase 3 (PR1)	18	*PRTN3*	Hematopoietic stem cells[27,95]
Tyrosinase	20	*TYR*	Melanocytes including skin, eye, ear[26,96]
Survivin	21	*BIRC5*	Bone marrow, esophagus, stomach, small bowel, colon, rectum, heart, urinary bladder[26,27,97]
PSA	22	*KLK3*	Pancreas, salivary gland[98,99]
hTERT	23	*TERT*	Hematopoietic cells, lymphocytes, skin, intestine[100–104]
EphA2	25	*EPHA2*	Skeletal muscle, liver, colon, lung, esophagus[27,105]

[a]Tissues that might be associated with tolerable toxicities, such as reproductive organs, were not included.
N/A, not applicable; NCI, National Cancer Institute.

TECHNICAL REPORTS

nature medicine

Transgenic mice with a diverse human T cell antigen receptor repertoire

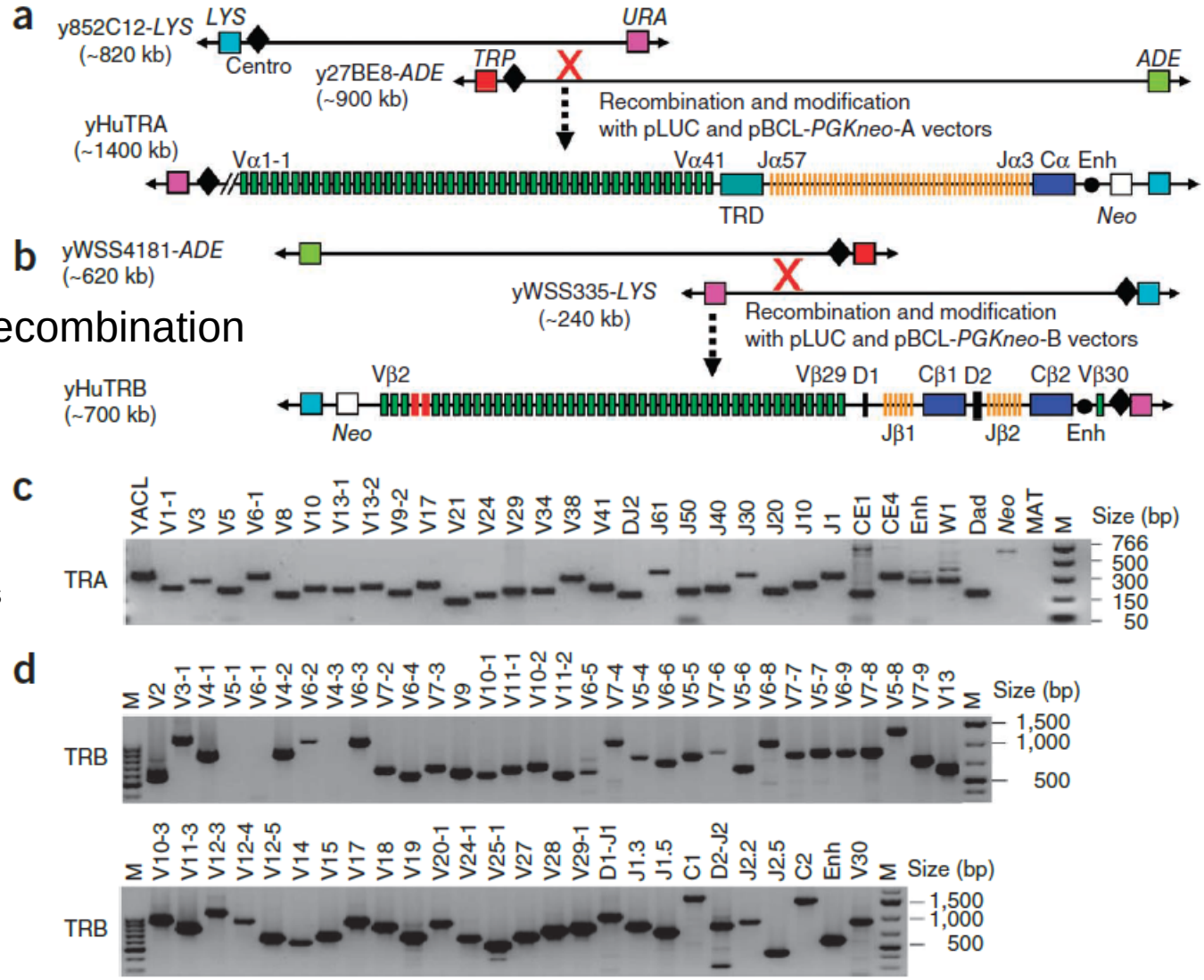
Liang-Ping Li[1,2,4], J Christoph Lampert[1,2,4], Xiaojing Chen[1,2], Catarina Leitao[1,2], Jelena Popović[1,2], Werner Müller[3] & Thomas Blankenstein[1,2]

Generation of mice transgenic for the human TCRα and TCRβ gene loci

yeast-selectable markers:
URA: Uracil
LYS: lysine
ADE: adenosine
TRP: tryptophan
Centro, yeast centromere

Meiotic homologous recombination

- fused YAC-containing yeast cells with embryonic stem cells and selected for neomycin
- injected ES cells into blastocysts to produce chimeric mice.



Mating strategy

Hu TCRα locus–transgenic (hTRATg)
x Mu TCRα–deficient (*Tcra*−/−)

↓

hTRA-Tg Tcra−/−

hu TCRβ– transgenic (hTRB-Tg) x
Mu TCRα–deficient (*Tcra*−/−)

↓

hTRB-Tg Tcrb−/−

X

↓

hTRA-Tg, hTRB-Tg Tcra−/−;Tcrb−/− (ABab)
(**human TCRs and mouse MHC I**)

X

HHDII
(**mouse TCRs and single human MHC I**)

↓

ABabDII (ABab HHDII)
(**human TCRs and single human MHC I gene**)

T cell development in human TCRαβ gene loci transgenic mice with a diverse TCR repertoire.

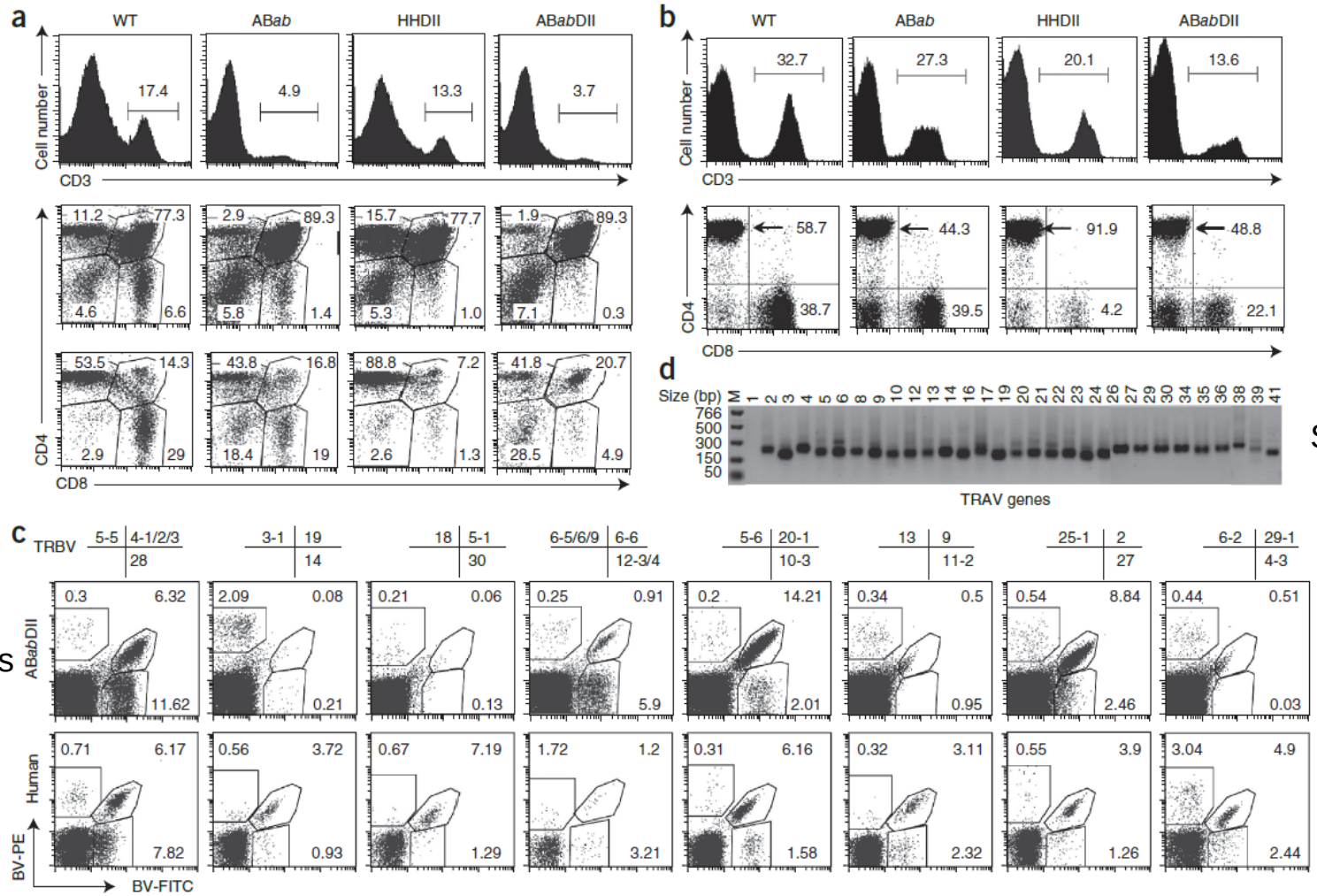
- human TCRαβ chains can compensate for mouse TCRαβ deficiency
- T cells with human TCRs were positively selected by mouse MHC molecules

Thymocytes

Blood

Splenocytes

Splenocytes



CD8+ T cells in ABabDII mice are functional and use similar TCRs as human CD8+ T cells against an immunogenic antigen

Immunized AB*ab*DII mice with ELA or, the tyrosinase 369–378 peptide (YMD), and 7–9 d later, stained CD8+ T cells with peptide-specific HLA-A2 tetramers (ELA-A2 and YMD-A2).

unique usage of AV12-2 and limited Vβ gene usage (splenocytes)

Jurkat cells transduced to express two TCRαβ combinations bound the ELA-A2 tetramers

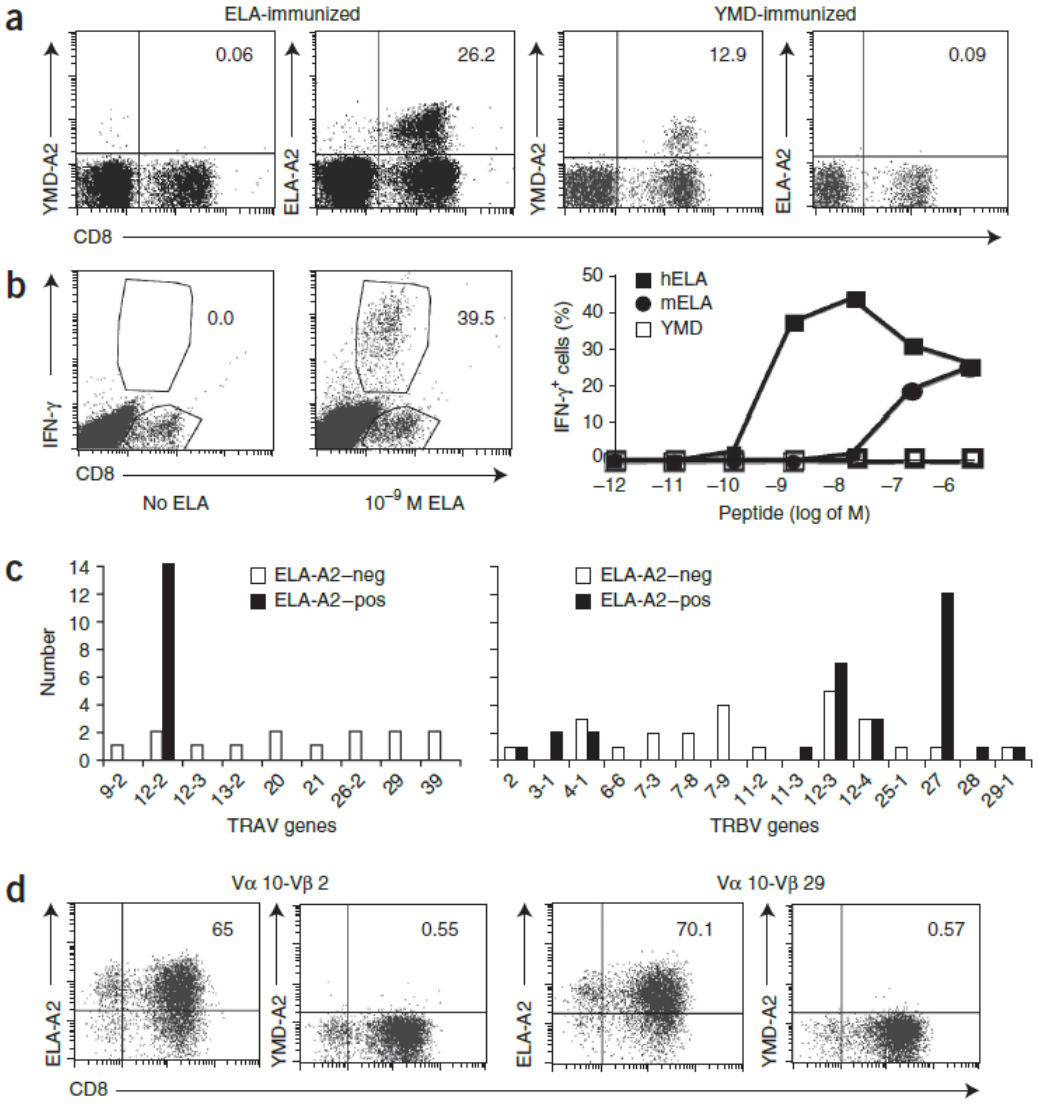
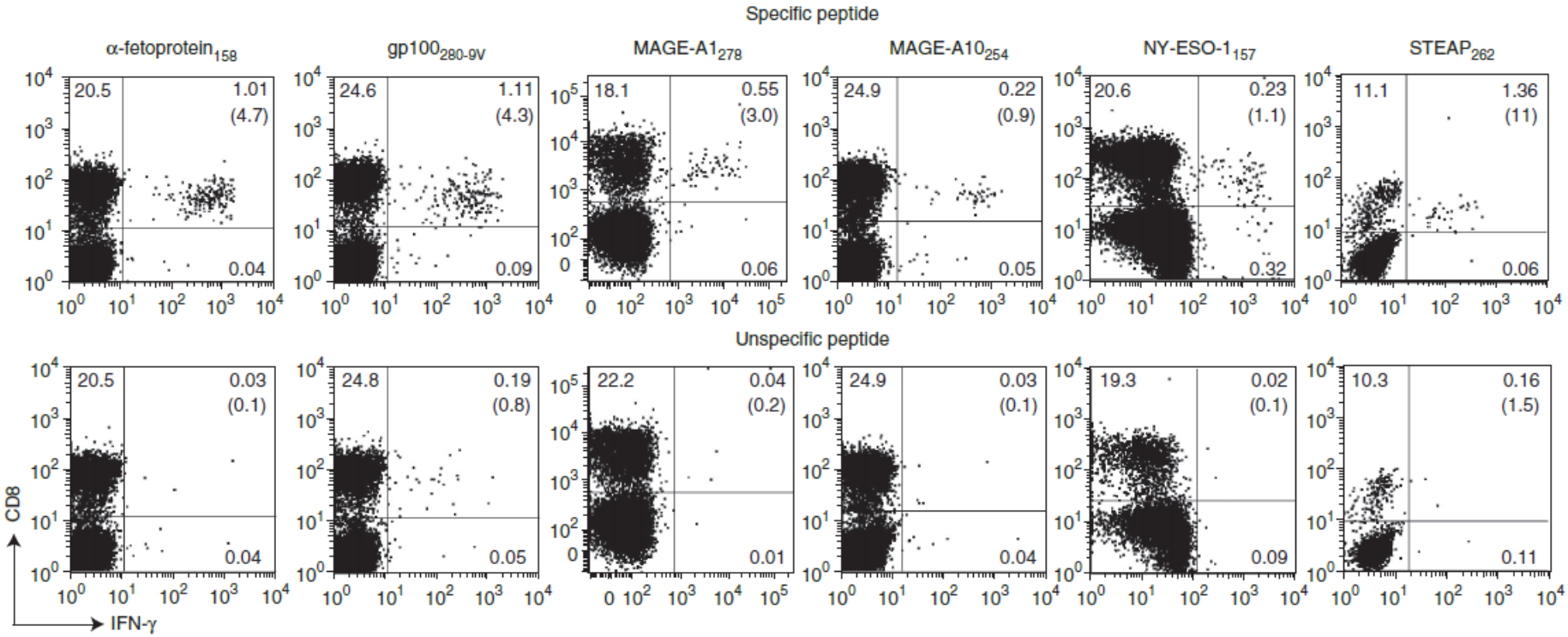


Table 1 Similar Melan-A–specific TCR usage in AB*ab*DII mice and T cell clones from individuals with vitiligo and melanoma

Source of TCR	AV		CDR3		AJ
AB*ab*DII 10	12-2	CAV	NIGFGNVL	HCG	35
Melanoma	12-2	CAV	NIGFGNVL	HCG	35
Melanoma	12-2	CAV	SIGFGNVL	HCG	35
Vitiligo	12-2	CAV	TIGFGNVL	HCG	35
Vitiligo	12-2	CAV	SRGFGNVL	HCG	35
AB*ab*DII 21	12-2	CAV	NDAGKS	TFG	27
Vitiligo	12-2	CAV	GAGKS	TFG	27
AB*ab*DII 26	12-2	CAV	NDSGAGSYQL	TFG	28
Melanoma	12-2	CAV	PDQGAGSYQL	TFG	28
Source of TCR	**BV**		**CDR3**		**BJ**
AB*ab*DII 2	27	CASS	FLGDTQ	YFG	2-3
Melanoma	27	CASS	SLGDTQ	YFG	2-3
Melanoma	27	CAS	SLGNEQ	FFG	2-1
Melanoma	27	CAS	SLGVATGEL	FFG	2-2
AB*ab*DII 29	3-1	CASS	P LAGYTGEL	FFG	2-2
AB*ab*DII 22	28	CASSQ	PGLAGYEQ	YFG	2-7
Vitiligo	3-1	CASS	PGLAYYEQ	YFG	2-7
Vitiligo	15	CATSR	APGLAVTDTQ	YFG	2-3
Melanoma	4-2	CASSQ	EGLAGASQ	YFG	2-7
AB*ab*DII 37	3-1	CASSQ	GTSGVNEQL	FFG	2-1
Melanoma	27	CASS	MTSY NEQ	FFG	2-1

CDR3 amino acid alignment of TRAV and TRBV genes isolated from the ELA-A2 tetramer$^+$ fraction (all clones are shown in **Supplementary Table 2**) and ELA-specific human T cell clones from individuals with vitiligo or melanoma[14–18]. AV, TCRα variable gene; BV, TCRβ variable gene; AJ, TCRα joining gene; BJ, TCRβ joining gene.

Specific CD8+ T cell responses against a panel of human TAAs in ABabDII mice



Mice were immunized with the indicated human TAAs (pooled splenocytes and LN cells)

Summary

- Here we generated transgenic mice with the entire human TCRab gene loci (1.1 and 0.7 Mb), whose T cells express a diverse human TCR repertoire that compensates for mouse TCR deficiency.

- A human major histocompatibility class I transgene increases the generation of CD8+ T cells with human compared to mouse TCRs.

- Functional CD8+ T cells against several human tumor antigens were induced, and those against the Melan-A melanoma antigen used similar TCRs to those that have been detected in T cell clones from individuals with autoimmune vitiligo or melanoma.

- These mice will allow researchers to identify pathogenic and therapeutic human TCRs.

LETTERS

nature biotechnology

Identification of human T-cell receptors with optimal affinity to cancer antigens using antigen-negative humanized mice

Matthias Obenaus[1], Catarina Leitão[1,7], Matthias Leisegang[1], Xiaojing Chen[1], Ioannis Gavvovidis[1], Pierre van der Bruggen[2,3], Wolfgang Uckert[1,4], Dolores J Schendel[5] & Thomas Blankenstein[1,6]

Generation of MAGE-A1-specific TCRs in ABabDII mice

a

```
         270                                300
          |                                  |
Human  –ALAETSYVKVLEYVIKVSARVRFFFPSLREA–
Mouse  –AFAETSKMKVLQFFASINKTHPRAYPEKYAE–
         * ****   ***                 *
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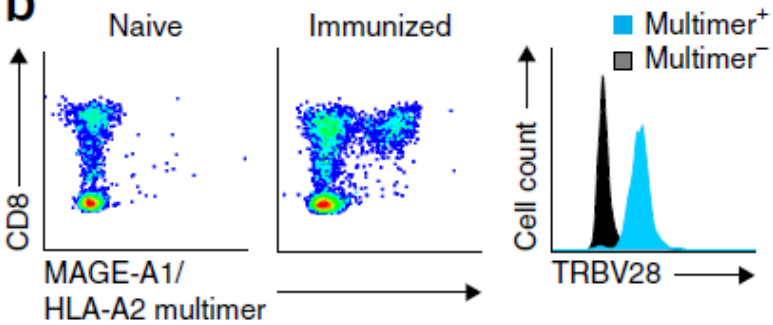
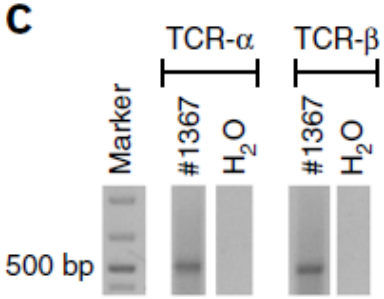
MAGE-A1, cancer/testis antigens, are expressed in a variety of tumors, but, with the exception of testis and placenta, have not been detected in healthy adult tissues.

Immunized multiple times with peptide and CpG oligonucleotides in incomplete Freund's adjuvant, that received a boost around 300 days later, gated on CD3+ cells

b



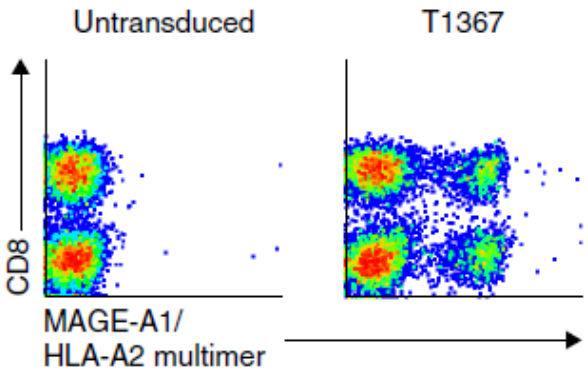
c



d

T1367	*TRAV5–CAESIGSNSGYALNF–TRAJ41* *TRBV28–CASRGLAGYEQYF–TRBJ2-7*
T1405	*TRAV13-1–CAARPNSGNTPLVF–TRAJ29* *TRBV29-1–CSVEQDTNTGELFF–TRBJ2-2*
T1705	*TRAV12-3–CAMSDTGNQFYF–TRAJ49* *TRBV13–CASSFRGGGANVLTF–TRBJ2-6*

e



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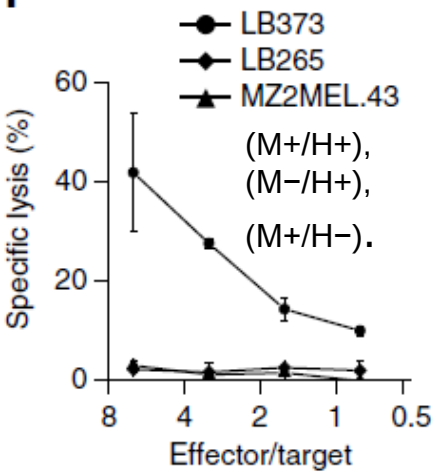
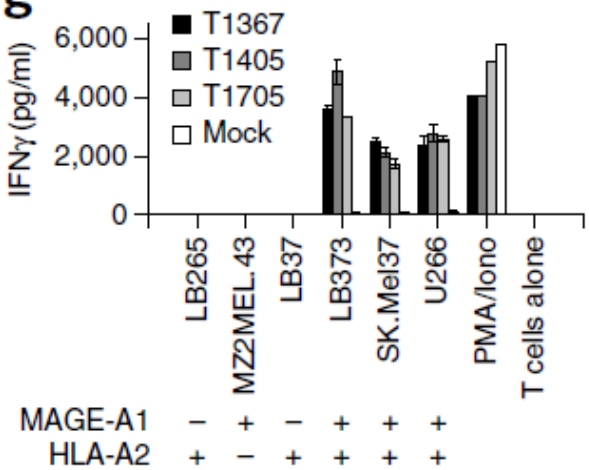
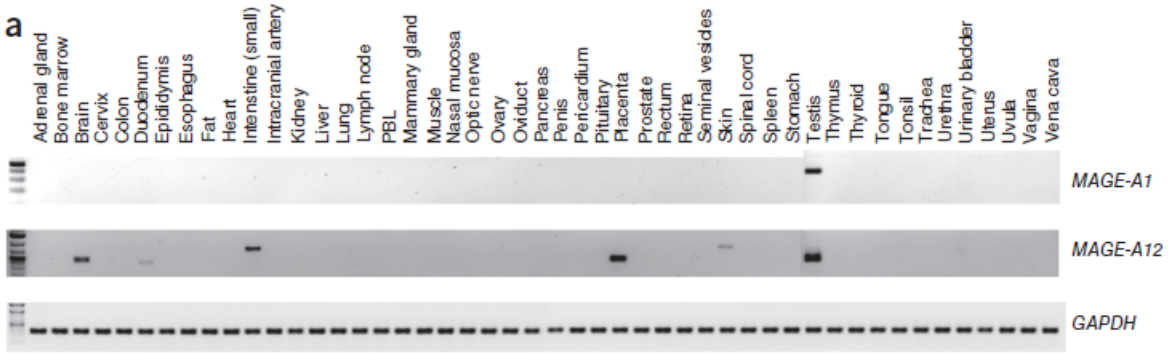


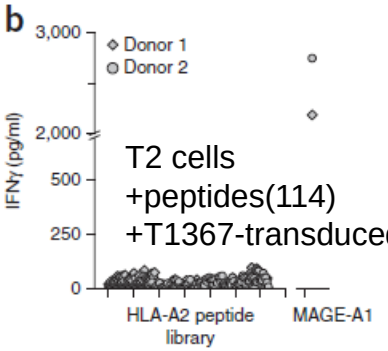
g



Specificity of ABabDII derived TCRs

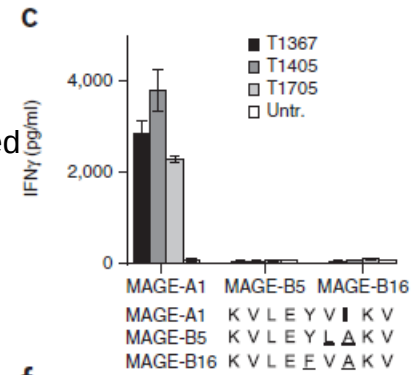
on-target toxicity

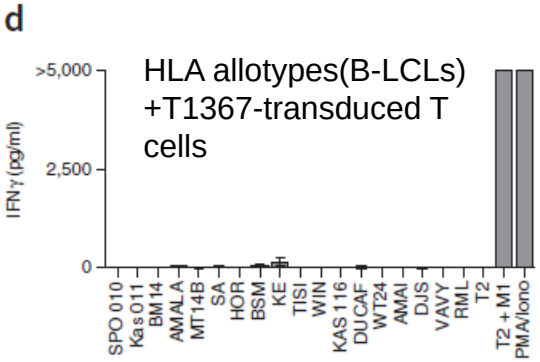




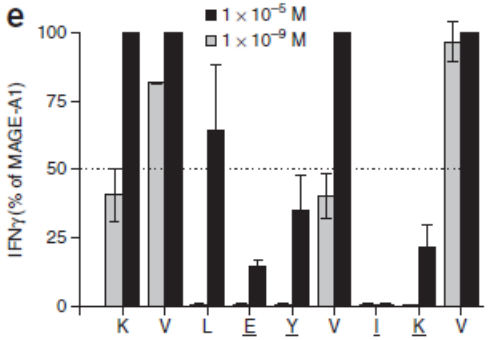
T2 cells
+peptides(114)
+T1367-transduced T cells

T2 cells
+ peptide
+transduced
T cells

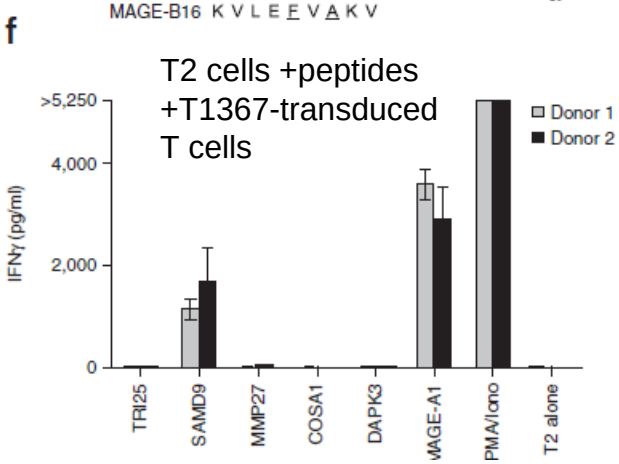


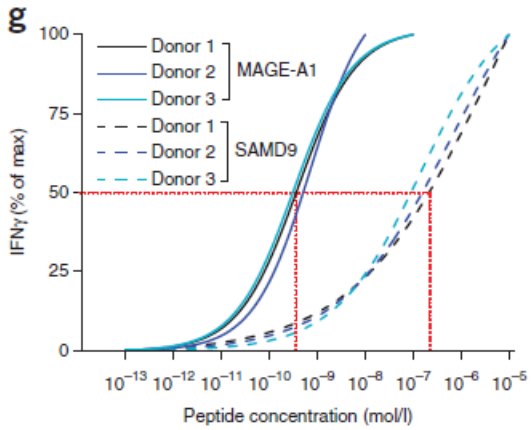


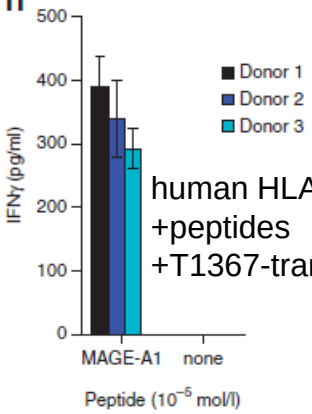
HLA allotypes(B-LCLs)
+T1367-transduced T cells



T2 cells
+peptides
+T1367-transduced T cells

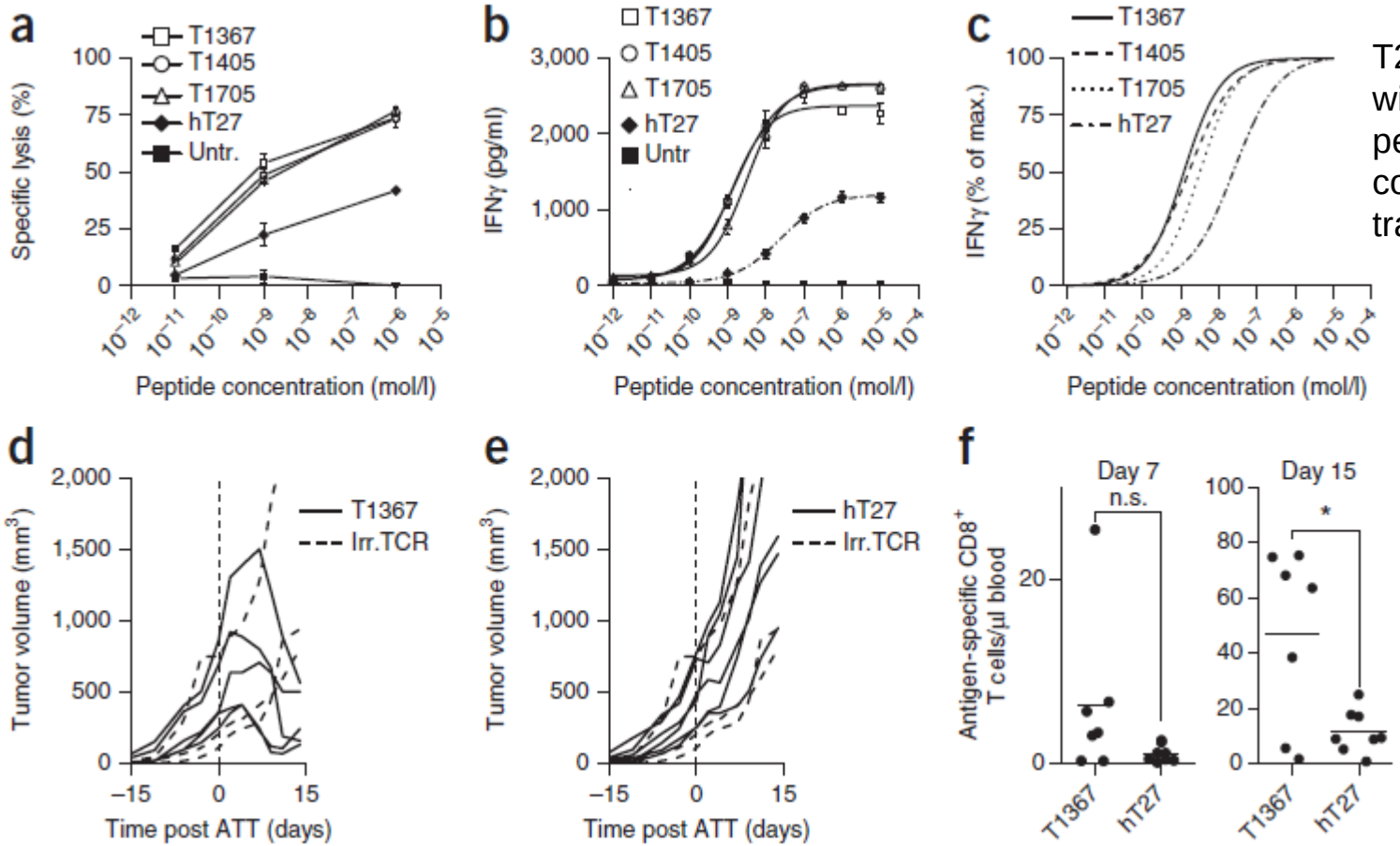






human HLA-A2+ T cells
+peptides
+T1367-transduced T cells

Functional comparison of ABabDII-derived TCRs with a human-derived TCR *in vitro* and *in vivo*

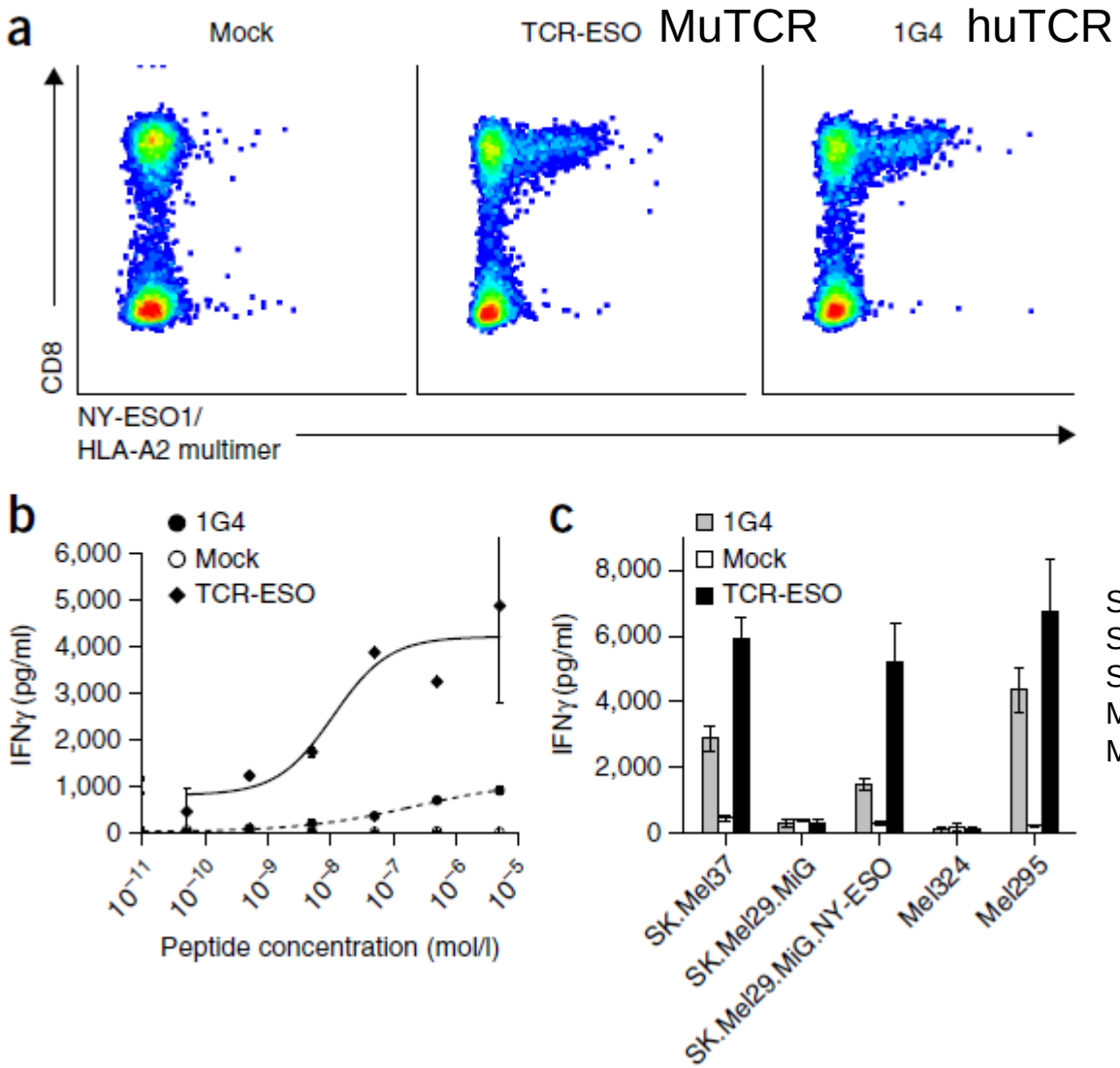


T2 cells were loaded with MAGE-A1278 peptide co-culture with TCR-transduced T cells

Post adoptive T-cell transfer

- HLA-A2+ MC703 fibrosarcoma cells, from an HLA-A2 (HHD) transgenic mouse and transduced to express MAGE-A1
- HHD×Rag−/− mice
- HHD-derived CD8+ T cells, transduced with either T1367 or hT27) TCRs.

Functional comparison of a NY-ESO157-specific TCR from ABabDII mice with a patient-derived TCR



PBMCs

SK.Mel37: HLA-A2+/NY-ESO+
SK.Mel29.MiG: HLA-A2+/NY-ESO−
SK.Mel29.MiG.NY-ESO: HLA-A2+/NY-ESO+
Mel324: HLA-A2+/NY-ESO−
Mel295: HLA-A2+/NY-ESO+)

Summary

- ABabDII mice mice were immunized with human TAAs, for which they are not tolerant, allowing induction of CD8+ T cells with optimal-affinity TCRs.
- They isolate TCRs specific for the cancer/testis (CT) antigen MAGE-A1 and show that two of them have an anti-tumor effect *in vivo*.
- By comparison, human-derived TCRs have lower affinity and do not mediate substantial therapeutic effects.
- They also identify optimal-affinity TCRs specific for the CT antigen NY-ESO.

Cell PRESS

Cancer Cell
Article

Relapse or Eradication of Cancer Is Predicted by Peptide-Major Histocompatibility Complex Affinity

Boris Engels,[1,*] Victor H. Engelhard,[2] John Sidney,[3] Alessandro Sette,[3] David C. Binder,[1] Rebecca B. Liu,[1] David M. Kranz,[4] Stephen C. Meredith,[1] Donald A. Rowley,[1] and Hans Schreiber[1]
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http://dx.doi.org/10.1016/j.ccr.2013.03.018

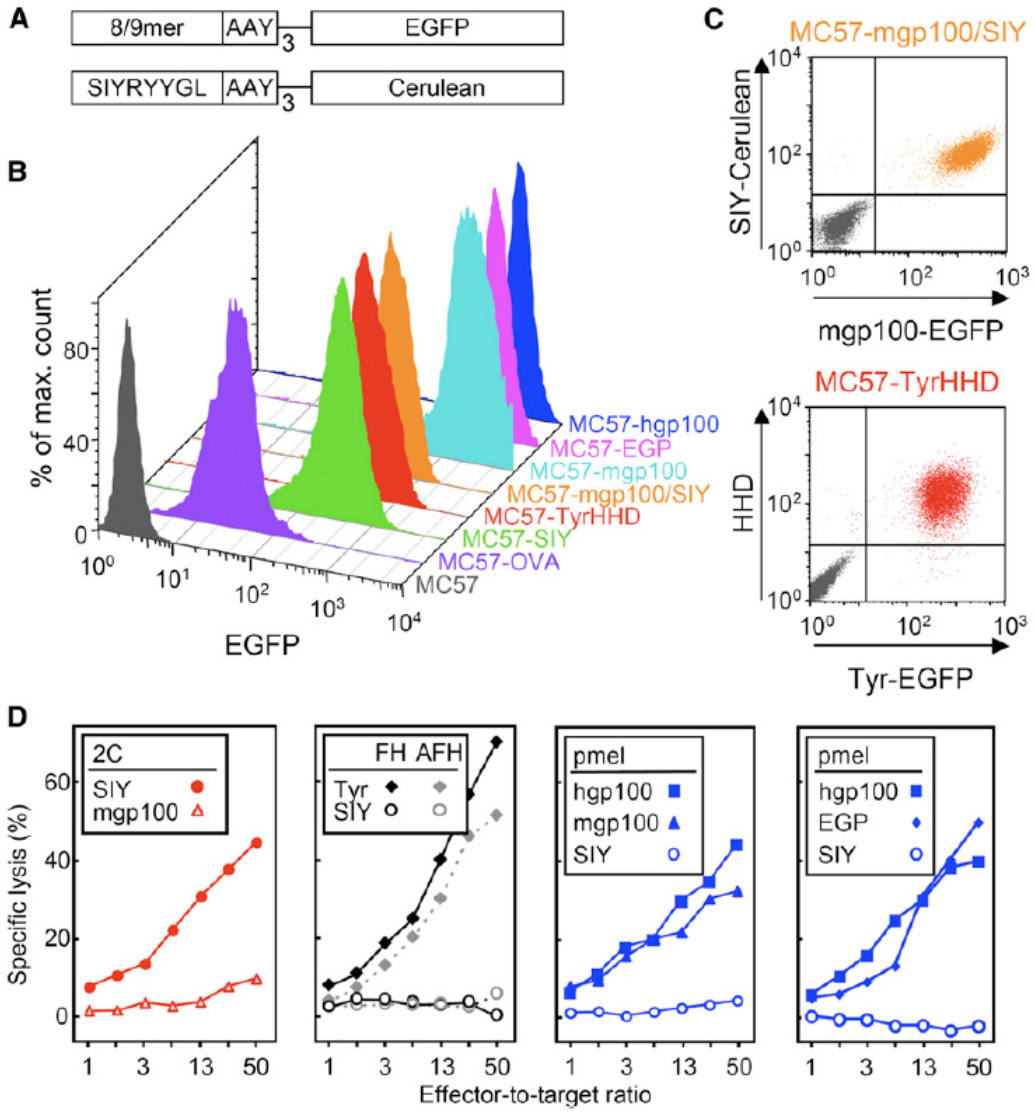
The affinities of target peptides and MHC class I

- Selected several peptides that, when targeted, caused tumor eradication and others that caused relapse.
- To reduce the influence of differences between cancers, we used two cancer cell lines that were both transduced to express the different peptides.
- To reduce differences due to expression levels, we used the same design of triple peptides fused to fluorescent proteins. Proteasomal cleavage of proteins may not generate or destroy immunogenic peptides.
- To minimize differences in proteasomal cleavage of the fusion proteins, we designed peptide triplets separated by ‘‘Ala-Ala-Tyr’’ cleavage sites.
- Targeted antigens with no known oncogenic activity to reduce the possibility that the nature of a particular targeted antigen prevented the cancer from escaping.
- To exclude the influence of other T cells helping or regulating the relevant CD8+ T cells, T cell receptor (TCR)-transgenic T cells with a single specificity were adoptively transferred into hosts, which were TCR-transgenic for an irrelevant target.
- Single adoptive T cell transfer regimen was used without providing any additional stimulation.

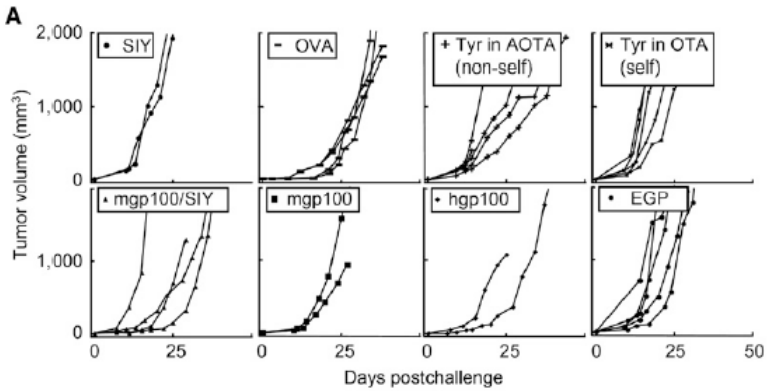
All transduced cancer cell lines that express antigens at high levels were effectively killed in vitro

MC57 fibrosarcoma cells

51Cr release assay



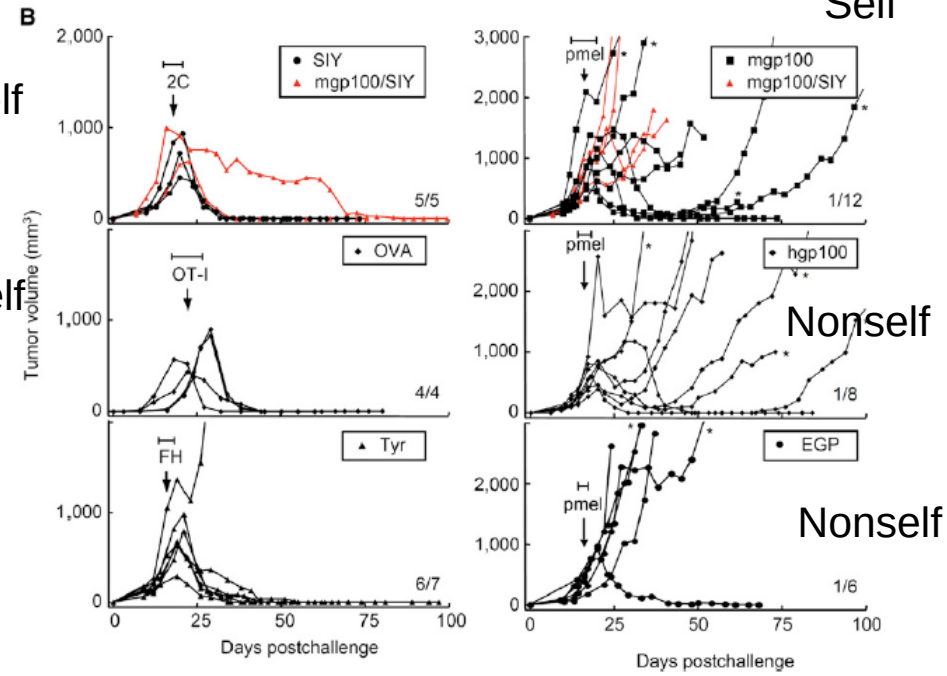
Targeting SIY, OVA257, or Tyr369 eradicated large tumors while targeting mgp10025, hgp10025, or EGP caused initial tumor regression but was followed by relapse



Nonself Nonself Nonself Self

Self Self Nonself Nonself

Self



Nonself

Nonself

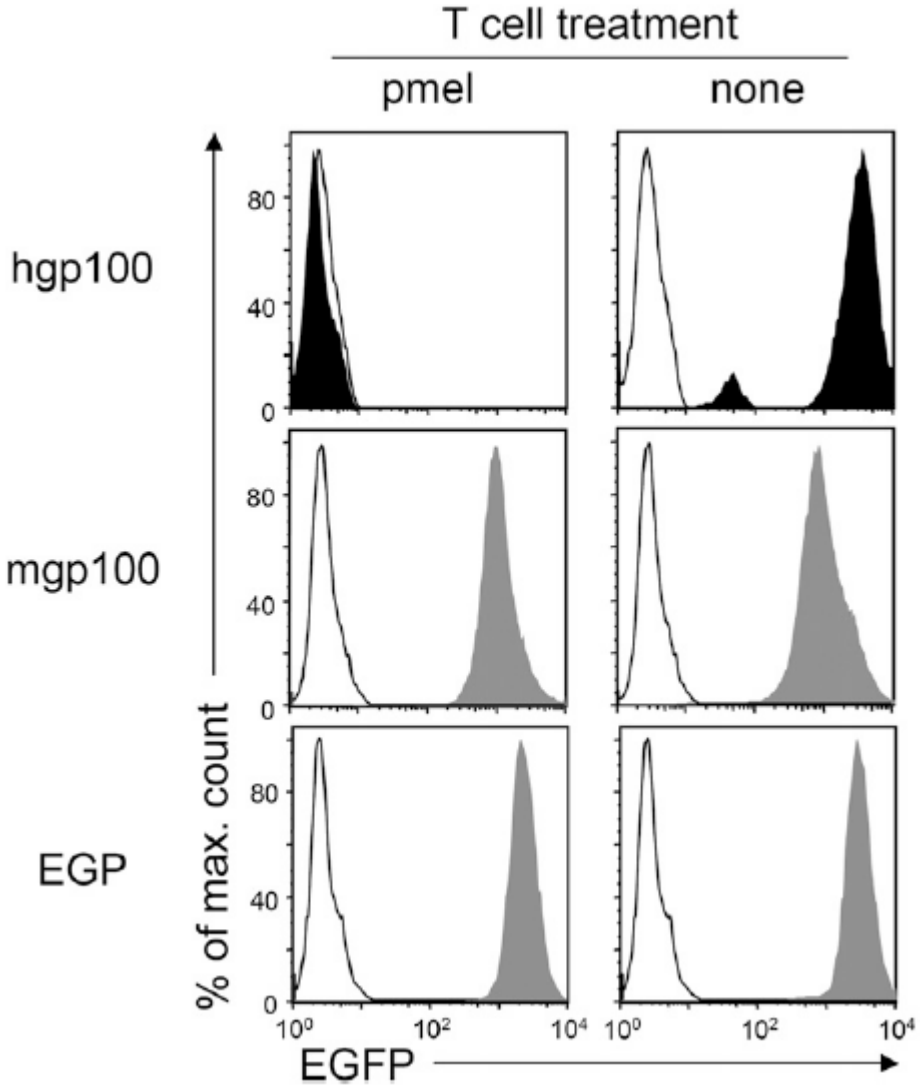
Self

Nonself

Nonself

Tumors could be rejected :
(1) whether the targeted peptide was self or nonself for the tumor-bearing host and
(2)whether the targeted peptide was self or nonself for the donor T cells

Outgrowth of antigen-loss variants after pmel T Cell treatment of cancer cells expressing hgp10025 but not of cancers expressing mgp10025 or EGP



pmel T cells showed a stronger effect when targeting hgp10025 compared to mgp10025 and EGP

T cells transferred to treat the tumors expressing the different peptides showed the same phenotype of activated T Cells

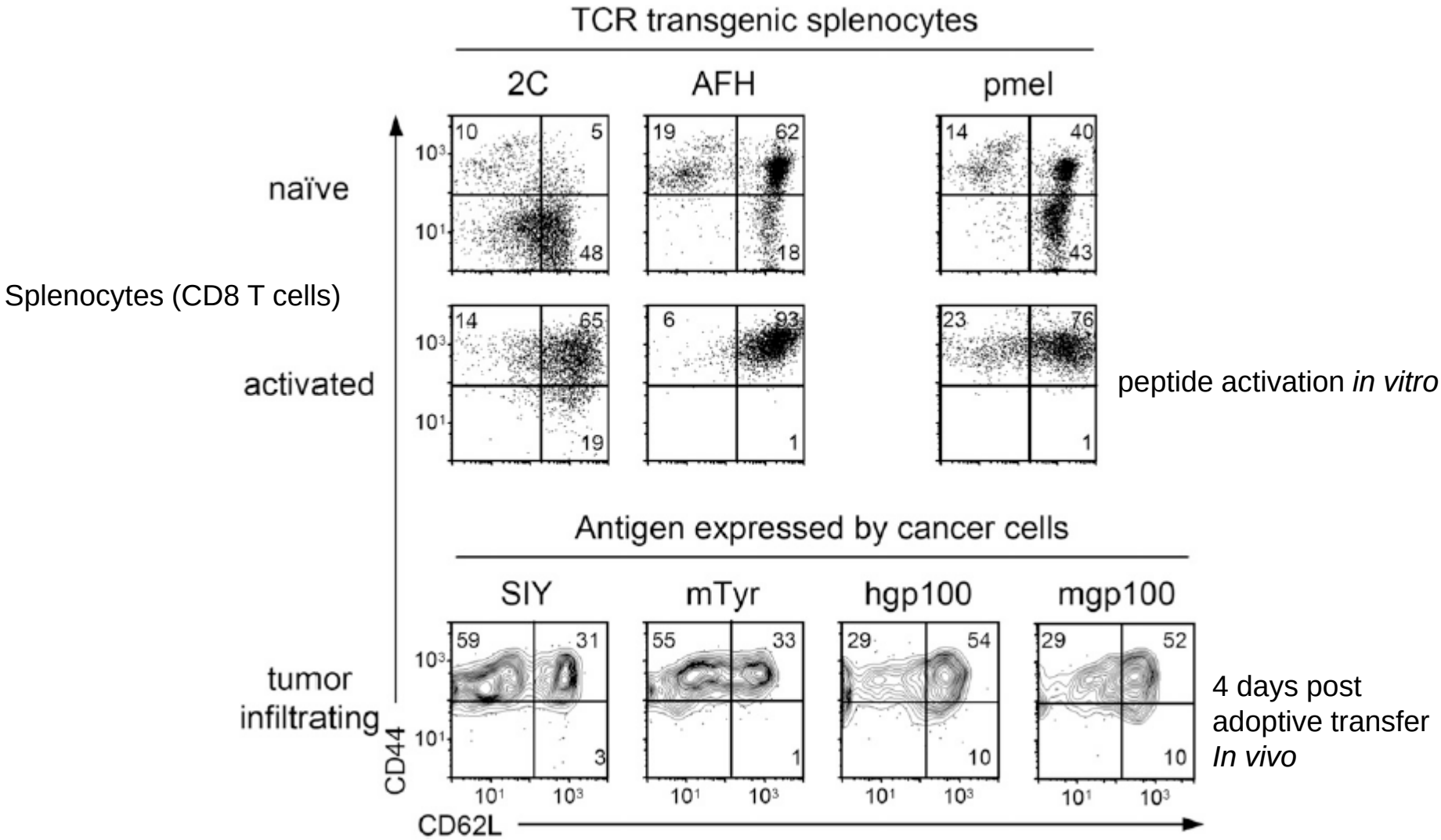


Table 1. Abbreviations, Conditions, and Summary of Results for Key Experiments

Target Peptide on Cancer Cells				Hosts		T Cells		
Designation	Sequence	MHC	Affinity of Peptide for MHC (IC_{50} [nM])[a]	Designation	Relationship of Antigen to Recipient	Designation	Relationship of Antigen to Donor	Tumor Rejection
SIY	SIYRYYGL	K^b	1.1	OT-I	nonself	2C	nonself	5/5[b,c,d,e]
						none		0/6[b]
$OVA_{257–264}$	SIINFEKL		0.9	2C	nonself	OT-I	nonself	4/4[f]
						none		0/4[f]
$Tyr_{369–377}$	FMDGTMSQV	A2	4.2[g]	OTA	self	FH	self	6/7[h]
						none		0/5[h]
$hgp100_{25–33}$	KVPRNQDWL[i]	D^b	186	OT-I	nonself	pmel	nonself	1/8[c]
						none		0/2
EGP	EGPRNQDWL		454	OT-I	nonself	pmel	nonself	1/6[d]
						none		0/5
$mgp100_{25–33}$	EGSRNQDWL		22,975	OT-I	self	pmel	self	1/12[e]
						none		0/6

See Table S1 for details.

[a] IC_{50} values represent the geometric mean of five or more experiments.

[b] $p = 0.002$.

[c] $p < 0.005$.

[d] $p = 0.015$.

[e] $p < 0.001$.

[f] $p < 0.029$.

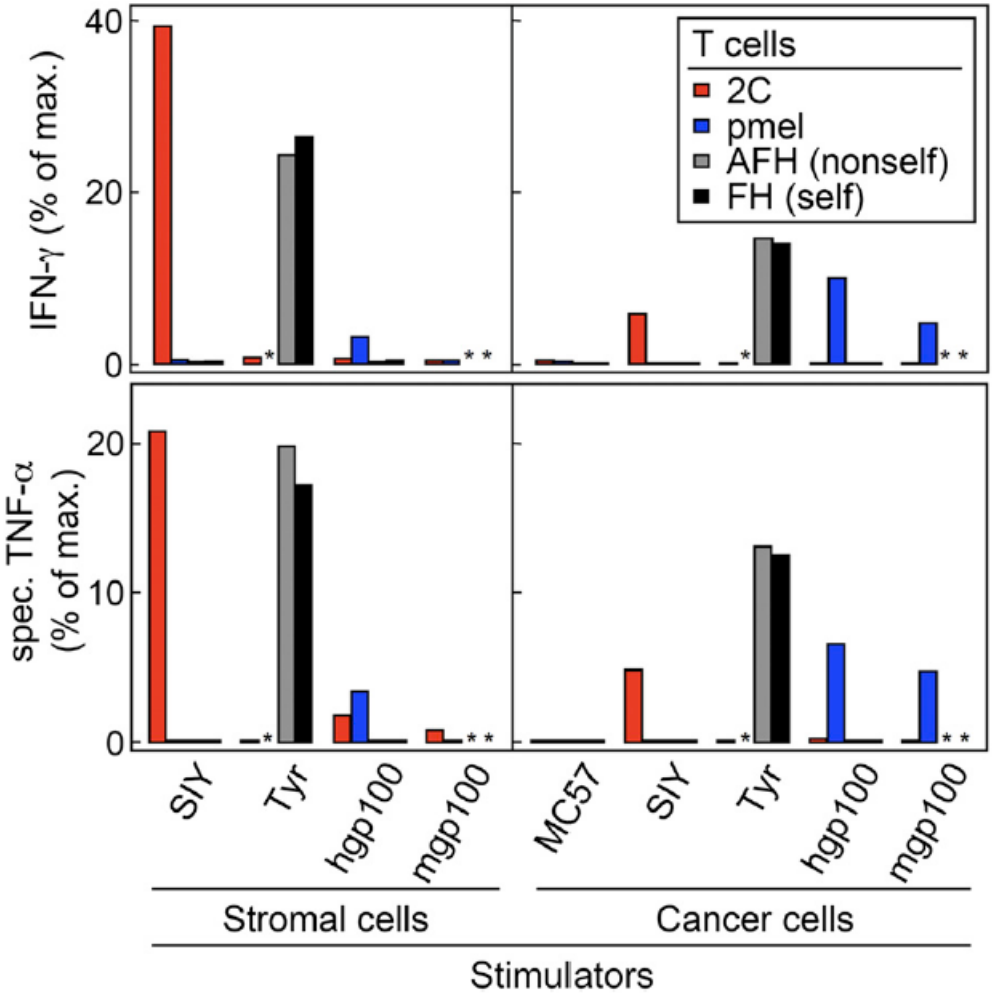
[g] A higher IC_{50} value of 65 nM was published for this peptide earlier (Colella et al., 2000). The differences in affinity measurements likely arose as a result of small differences in reagents, methodology, and procedures.

[h] $p = 0.015$.

[i] Only the underlined amino acids differ between the three gp100 peptide variants.

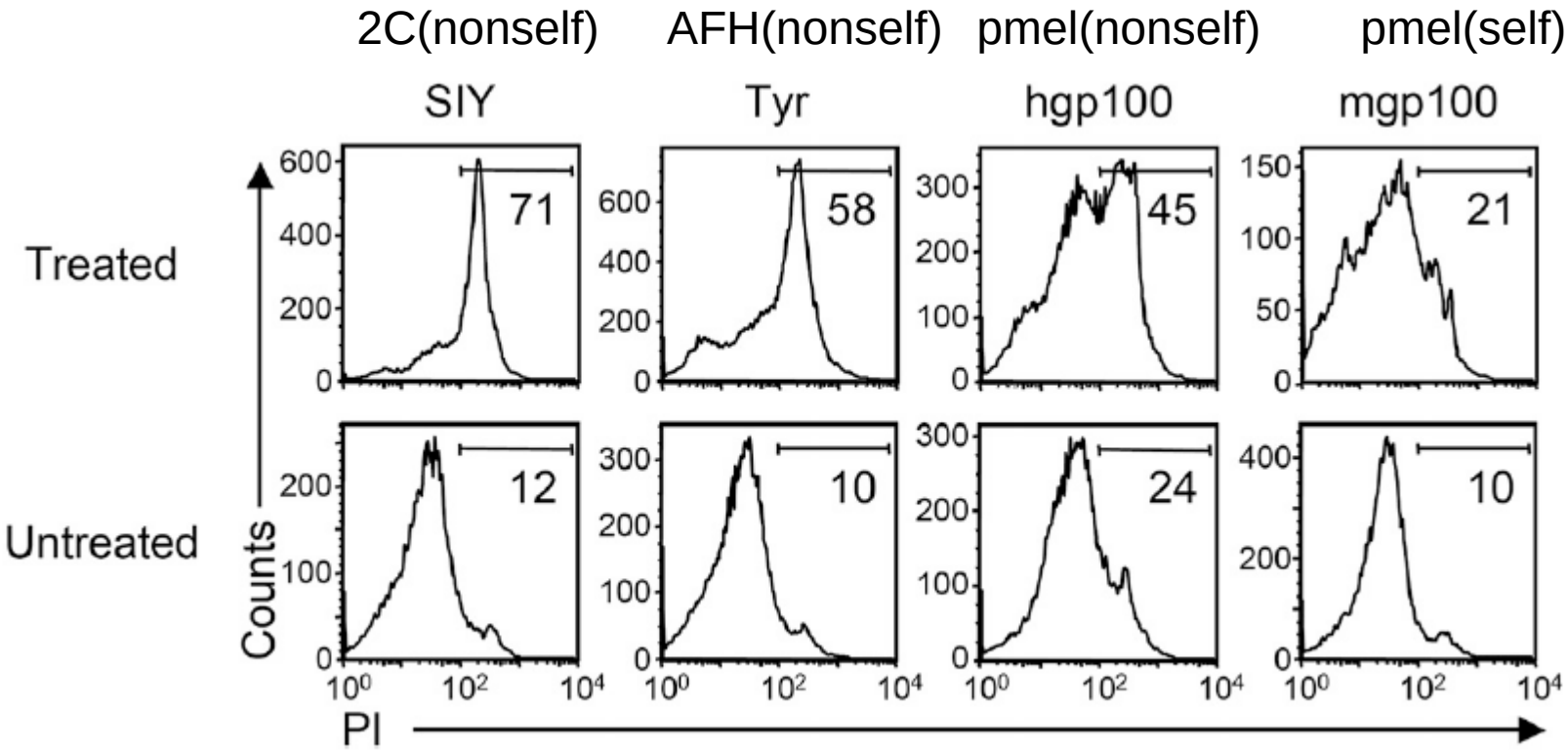
Only SIY and Tyr369 are cross- presented, as detected by cytokine secretion by T cells stimulated by stromal cells isolated from untreated tumors

Enriched stromal cells from tumors grown from MC57-SIY, MC57-hgp100, and MC57-mgp100 cells (all grown in OT-I mice) and MC57-TyrHHD (grown in AOTA mice [nonself]) were cocultured with 2C, pmel, AFH (nonself), or FH (self) TCR-transgenic T cells.



Death of stromal cells in T cell-treated SIY- and Tyr369-expressing tumors

Tumors were dissected on day 5 after adoptive T cell transfer, analyzed the viability of CD11b+ stromal cells.



Summary

- Tumor eradication by T cells required high affinities of the targeted peptides for MHC class I.
- Affinities of at least 10 nM were required for relapse-free regression.
- Only high-affinity peptide-MHC interactions led to efficient cross-presentation of antigen, thereby stimulating cognate T cells to secrete cytokines.


Thank You!