

TCR-engineered T cells targeting a shared β -catenin mutation eradicate solid tumors

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Peptide	HLA	Peptide sequence	Mass (m/z)	Charge	Retention time (min)	NCE	Transitions (1+)	Transition masses (m/z)
WT	A2	YLDSGIHSGA	510.243263	2	17.58-20.58	27	NA	NA
WT _{heavy}	A2	YLDSGI(¹³ C ₆ , ¹⁵ N)HSGA	513.751845	2	17.58-20.58	27	NA	NA
WT	A24	SYLDSGIHS	489.729989	2	21.4-24.4	27	y3, y4,y5	356.1928, 413.2143, 500.2463
WT _{heavy}	A24	SYLDSGI(¹³ C ₆ , ¹⁵ N)HS	493.238571	2	21.4-24.4	27	y3, y4,y5	363.2100, 420.2315, 507,2635
Neo	A2	YLDSGIHFGA	540.261456	2	30.2-33.2	27	y7, y8, y9	688.3413, 803.3682, 916.4523
Neo _{heavy}	A2	YLDSGI(¹³ C ₆ , ¹⁵ N)HFGA	543.770038	2	30.2-33.2	27	y7, y8, y9	695.3585, 810.3854, 923.4695
Neo	A24	SYLDSGIHF	519.748181	2	33.7-36.7	27	y5, y6. y7	560.2827, 675.3097, 788.3937
Neo _{heavy}	A24	SYLDSGI(¹³ C ₆ , ¹⁵ N)HF	523.256763	2	33.7-36.7	27	y5, y6. y7	567.2999, 682.3268, 795.4109

Supplementary Table 1. Heavy isotope-labeled peptides used for quantification of CTNNB1-encoded peptides

Information about the isotope labeled peptides and data attained during the targeted mass spectrometry analysis. NCE, Normalized Collision Energy.

Cell line name	HLA-A allele 1	HLA-A allele 2	Tissue	Disease
BV-173	30:01	02:01	Hematopoietic and lymphoid	Blast Phase Chronic Myelogenous Leukemia
HaCat	31:01	31:01	Skin	Keratinocyte
UT-7	02:01	11:01	Bone marrow	Acute myeloid leukemia
CHP-212	24:02	02:01	Brain	Neuroblastoma
OCI-M2	02:01	02:01	Blood	Acute myeloid leukemia
EBV-LCL IHW01111	02:01	02:01	EBV-bearing lymphoblastoid cell lines	
EBV-LCL IHW09028	24:02	24:02	EBV-bearing lymphoblastoid cell lines	
K562	11:01	31:01	Hematopoietic and lymphoid	Blast phase chronic myelogenous leukemia
HEL92.1.7	03:01		Bone marrow	Erythroleukemia
COLO668	01:01	11:01	Derived from metastatic site: brain	Small cell lung cancer
T2	02:01		Somatic cell hybrid	
HepG2	02:01	24:02	Liver	Hepatocellular carcinoma
HCT116	01:01	02:01	Colon	Colon carcinoma
RD	01:01	01:01	Soft tissue	Rhabdomyosarcoma
EA.hy926	24:01	25:01	Somatic cell hybrid	
Daoy	01:01	02:01	Cerebellum	Desmoplastic cerebellar medulloblastoma
HEK293	02:01	03:01	Kidney; embryo	
RS4;11	03:01	32:01	Hematopoietic and lymphoid	B acute lymphoblastic leukemia
Caco2	02:01	02:01	Colon	Colon adenocarcinoma
MV4-11	68:01	03:01	Blood	Acute biphenotypic leukemia
EOL-1	24:02	29:02	Hematopoietic and lymphoid	Acute myeloid leukemia
MOLM-13	26:01	31:01	Blood	Acute myeloid leukemia
SET2	33:03	02:01	Blood	Acute myeloid leukemia
Jurkat	03:01	03:01	Hematopoietic and lymphoid	T acute lymphoblastic leukemia
MOLT-4	01:01	25:01	Lymphoid	T-lymphoblastic leukemia
Reh	32:01	23:01	Hematopoietic and lymphoid	B acute lymphoblastic leukemia
THP-1	02:01	02:01	Hematopoietic and lymphoid	Acute myeloid leukemia
SNU-638	03:19	24:02	Stomach	Gastric carcinoma
KLE	02:01	24:02	Uterus	Adenocarcinoma
PANC-1	02:01	11:01	Pancreas	Epithelioid carcinoma
CFPAC-1	02:01	03:01	Pancreas	Ductal adenocarcinoma
Mel888	24:02	01:01	Derived from metastatic site: oral cavity; soft plate; hypodermis	Melanoma
Hutu80	02:01	30:02	Small intestine; Duodenum	Adenocarcinoma
HCM-SANG-0270-C20 organoid (CRC)			Large intestine; rectum	Adenocarcinoma
922882-316-R-organoid (EAC)	02:01	01:01	Uterus	Endometrial endometrioid adenocarcinoma
922882-316-R PDX (EAC)	02:01	01:01	Uterus	Endometrial endometrioid adenocarcinoma

Supplementary Table 2. Overview of cell lines, organoids, and PDX, their HLA-A alleles, and tissue and disease origin

A list of all cell lines, organoids and PDX used in the study and their tissue origin, disease, and HLA-A alleles. HLA-A allele expression status was obtained from Cellosaurus and Tron Cell Line Portals (<http://celllines.tron-mainz.de/>; <https://web.expasy.org/cellosaurus/>).

a

Peptide sequence	Gene name	Full name	Tissue expression
SVMAPLWACI	5-HT3B	5-Hydroxytryptamine receptor 3B	Expressed in many tissues but has highest expression in the brain.
SMMVQVWFAC	AT8B3	ATPase Phospholipid Transporting 8B3	No protein data. RNA expression in Male tissue (high) and bone marrow/lymphoid tissue (lower).
SLDSPVHAVC	PADI6	Peptidyl-Arginine Deiminase type-6	No protein data. RNA only expressed in Ovary.
SLDTSLFFGT	ZXDA	Zinc finger X-linked protein	Expressed everywhere.

b

Peptide sequence	Gene name	Full name	Tissue expression
FYYDSENQL	AGGF1	Angiogenic factor with G patch and FHA domains 1	No protein data. RNA expressed everywhere.
PYMSSSASM	ARID1B	AT-rich interactive domain-containing protein 1B	Expressed everywhere.
KYLNSCLKW	ARMH4	Armadillo-like helical domain-containing protein 4	Expressed in many tissues with highest expression in kidney.
QYADSGSHF	ATXN1	Ataxin-1 (Spinocerebellar ataxia type 1 protein)	Expressed everywhere.
PYLESGLSL	BRCA1	Breast cancer type 1 susceptibility protein	Expressed everywhere.
GYASSSLSI	C1orf216	Chromosome 1 open reading frame 216	Expressed in many tissues with highest expression in the brain.
SYLSSAAAL	C6orf15	Chromosome 6 open reading frame 15	No protein data. Expressed in certain tissues with highest expression in cervix, skin and lymphoid tissues.
QFASSGLSM	COG1	Component of oligomeric Golgi complex 1	Expressed everywhere.
AFADSGAHW	CYP17A1	Cytochrome P450 Family 17 Subfamily A Member 1	Expressed in adrenal gland and testis.
LFYESSSSI	CRISPLD2	Cysteine-rich secretory protein LCCL domain-containing 2	Expressed everywhere.
SFYTSAITF	CATSPERB	Cation channel sperm-associated auxiliary subunit beta	No protein data. RNA expressed in many tissues but has highest expression in pancreas.
VYASSSCSM	DENND5B	DENN domain-containing protein 5B	Expressed everywhere.
SFATSSTQF	DNAH6	Dynein axonemal heavy chain 6	Expressed in certain tissues with highest expression in choroid plexus and fallopian tube.
QYASSEATI	EEA1	Early endosome antigen 1	Expressed everywhere.
RFYESGSSL	HDX	Highly divergent homeobox	Expressed everywhere.
SFYSSSASM	HOXD10	Homeobox protein D10	No protein data. RNA expressed in certain tissues with highest expression in cervix, endometrium and vagina.
PFLSSSATL	IGSF10	Immunoglobulin superfamily member 10	Expressed everywhere with highest expression in gallbladder and ovary.
AYAESASHF	KIAA1549	UPF0606 protein KIAA1549	Expressed everywhere.
SYASSAITF	LAT3	Large neutral amino acids transporter small subunit 3	Expressed in many tissues with highest expression in skeletal muscle.
SYASSAVTF	SLC43A2	Large neutral amino acids transporter small subunit 4	Expressed everywhere with highest expression in skeletal muscle.
YYLSENHL	LRRC42	Leucine-rich repeat-containing protein 42	Expressed in many tissues.
EYYSSSSSL	MED22	Mediator of RNA polymerase II transcription subunit 22	Expressed everywhere.
QYLTSLLHF	MTMR3	Myotubularin-related protein 3	Expressed in most tissues with highest expression in bone marrow.
SFLDSCNSL	MYBL2	Myb-related protein B	Expressed everywhere with highest expression in bone marrow and lymphoid tissue.

b

Peptide sequence	Gene name	Full name	Tissue expression
SFYSSALAM	MYO9A	Unconventional myosin-Ixa	Expressed everywhere with highest expression in retina.
YYLTSAIQL	ART4	Ecto-ADP-ribosyltransferase 4	No protein data. RNA expressed in several tissues with highest expression in liver and lymphoid tissue.
KYLESSATF	NUP210L	Nucleoporin 210-like	Expressed in testis.
AFAESSLAL	PCDH12	Protocadherin-12	No protein data. RNA expressed everywhere with highest expression in bone marrow, lymphoid tissues and female tissues.
NYASSSTSL	PE2R3	Prostaglandin E2 receptor EP3 subtype	Protein expressed in certain tissues, RNA expressed everywhere.
EYYESATHL	PPR37	Protein phosphatase 1 regulatory subunit 37	Expressed everywhere.
TFLDSELSM	R3HCC1L	R3H domain and coiled-coil containing 1 like	Expressed in most tissues.
RYAESSSKL	RAI14	Reinoic acid induced 14	Expressed in most tissues.
AYLESEVAI	RTN4	Reticulon-4	Protein expressed in certain tissues, RNA expressed everywhere.
SFMNSALSW	SLC28A3	Solute carrier family 28 member 3	Expressed in many tissues with highest expression in choroid plexus.
CFLNSATSF	SLC6A12	Solute carrier family 6 member 12	Expressed in many tissues with highest expression in brain, kidney and liver.
SFLHSSVKM	SCYL2	SCY1-like protein 2	Expressed everywhere.
QYLSSEITL	SECISBP2	Selenocysteine insertion sequence-binding protein 2	Expressed everywhere.
CYADSGCKL	SCGB2A1	Secretetoglobulin family 2A member 1	Expressed in several tissues with highest expression in cervix and epididymis.
VFYNSEYSM	SGPP1	Sphingosine-1-phosphate phosphatase 1	Expressed in most tissues.
GFYSSASKL	TDRD6	Tudor domain-containing protein 6	Protein expressed in testis. RNA expressed everywhere.
AFLTSGTQF	LTB	Lymphotoxin-beta	Expressed in several tissues with highest expression in intestine and lymphoid tissue.
VYLNSGSSL	TRAK2	Trafficking kinesin-binding protein 2	Expressed everywhere with highest expression in brain.
EYASSGSSL	ZFP36	ZFP36 ring finger protein	Expressed everywhere.
AFANSGNAI	USP36	Ubiquitin carboxyl-terminal hydrolase 36	Protein expressed in many tissues. RNA expressed everywhere.
GYAWSESML	UBR2	Ubiquitin-protein ligase E3 component n-recognin 2	Expressed everywhere.
SYLTSASSL	UBR5	Ubiquitin-protein ligase E3 component n-recognin 5	No protein data. RNA expressed everywhere.
LFLDSSTSM	ERCC4	DNA repair endonuclease XPF	Expressed everywhere with highest expression in skeletal muscle.

Supplementary Table 3 a,b. List of TCR^{A2-2} and TCR^{A24} candidate off-target peptides

A list of all potential cross-reactive peptides in the human proteome identified by the ScanProsite algorithm based on amino acid substitutions in the peptide mimotope libraries (Fig. 3b) resulting in reactivities of a) TCR^{A2-2} and b) TCR^{A24} cells. Information includes the amino acid sequence of the peptide, the name of the gene which the peptide originates from, and the gene tissue expression. Tissue expression status was obtained from The Human Protein Atlas (<https://www.proteinatlas.org/>).

Mouse ID	ROI (from low to high)	Treatment
2-5	1.96E+09	TCR ^{A2-2}
6-2	2.28E+09	TCR ^{A24}
7-2	2.53E+09	TCR ^{1G4}
7-4	2.81E+09	Mock
3-5	2.95E+09	Tumor only
3-4	3.17E+09	TCR ^{A2-2}
5-3	3.37E+09	TCR ^{A24}
5-5	3.59E+09	TCR ^{1G4}
6-5	3.66E+09	Mock
5-4	4.20E+09	Tumor only
6-4	4.80E+09	TCR ^{A2-2}
3-3	5.11E+09	TCR ^{A24}
4-4	5.23E+09	TCR ^{1G4}
5-6	5.36E+09	Mock
3-6	5.58E+09	Tumor only
4-6	6.06E+09	TCR ^{A2-2}
5-1	6.26E+09	TCR ^{A24}
5-2	6.61E+09	TCR ^{1G4}
4-3	7.39E+09	Mock
2-1	7.84E+09	Tumor only
3-7	8.60E+09	TCR ^{A2-2}
1-3	8.61E+09	TCR ^{A24}
7-5	9.38E+09	TCR ^{1G4}
1-5	9.45E+09	Mock
4-1	9.76E+09	Tumor only
4-2	1.06E+10	TCR ^{A2-2}
3-1	1.07E+10	TCR ^{A24}
2-6	1.14E+10	TCR ^{1G4}
1-4	1.29E+10	Mock
2-2	1.41E+10	TCR ^{A2-2}
2-4	1.62E+10	TCR ^{A24}
1-2	1.94E+10	TCR ^{1G4}
7-1	1.99E+10	Mock
1-1	2.42E+10	Tumor only

Supplementary Table 4. Overview of treatment grouping of Mel888^{A24+A2+ffluc-eGFP} engrafted mice based on ROI values at day -1

ROI, Region of Interest.

Mouse ID	Days after T cell treatment (date)	Reason for euthanasia
Tumor only #1	35 (euthanized 25.04.2023)	Weight loss. Large tumor
Tumor only #2	35 (euthanized 25.04.2023)	Large tumor
Tumor only #3	35 (euthanized 25.04.2023)	Weight loss. Measurable tumor
Tumor only #4	49 (euthanized 09.05.2023)	Large tumor
Tumor only #5	37 (euthanized 27.04.2023)	Large tumor and weight loss
Tumor only #6	37 (euthanized 27.04.2023)	Large tumor
Mock #1	28 (euthanized 18.04.2023)	Weight loss. Measurable tumor
Mock #2	35 (euthanized 25.04.2023)	Large tumor and weight loss
Mock #3	35 (euthanized 25.04.2023)	Large tumor
Mock #4	50 (euthanized 10.05.2023)	Large tumor
Mock #5	44 (euthanized 04.05.2023)	Large tumor and symptoms of GvHD (in pain, small eye opening)
Mock #6	43 (euthanized 03.05.2023)	Large tumor
Mock #7	49 (euthanized 09.05.2023)	Weight loss. Large tumor
TCR ^{1G4} #1	49 (euthanized 09.05.2023)	Large tumor
TCR ^{1G4} #2	35 (euthanized 25.04.2023)	Weight loss. Measurable tumor
TCR ^{1G4} #3	35 (euthanized 25.04.2023)	Weight loss. Large tumor
TCR ^{1G4} #4	37 (euthanized 27.04.2023)	Large tumor
TCR ^{1G4} #5	43 (euthanized 03.05.2023)	Large tumor
TCR ^{1G4} #6	35 (euthanized 25.04.2023)	Weight loss. Large tumor
TCR ^{1G4} #7	49 (euthanized 09.05.2023)	Large tumor
TCR ^{A24} #1	101 (euthanized 30.06.2023)	Splinter from the bedding in the eye. No tumor
TCR ^{A24} #2	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. Relapsed, large tumor
TCR ^{A24} #3	37 (euthanized 27.04.2023)	Weight loss. Palpable tumor
TCR ^{A24} #4	45 (euthanized 05.05.2023)	Symptoms of GvHD (in pain, small eye opening). No tumor
TCR ^{A24} #5	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. No tumor
TCR ^{A24} #6	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. Relapsed, large tumor
TCR ^{A24} #7	43 (euthanized 03.05.2023)	Large tumor
TCR ^{A2-2} #1	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. No tumor
TCR ^{A2-2} #2	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. No tumor
TCR ^{A2-2} #3	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. No tumor
TCR ^{A2-2} #4	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. No tumor
TCR ^{A2-2} #5	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. No tumor
TCR ^{A2-2} #6	44 (euthanized 04.05.2023)	Symptoms of GvHD (in pain, small eye opening). No tumor
TCR ^{A2-2} #7	115 (euthanized 14.07.2023)	Euthanized at experimental endpoint. Relapsed, palpable tumor

Supplementary Table 5. Overview of reasons for euthanasia of Mel888^{A24+A2+ffluc-eGFP} engrafted mice

GvHD, Graft-versus Host Disease.

Mouse ID	Tumor size (mm ³), day -1	Treatment	Tumor size (mm ³), day 56
19-1	0,5	Tumor only	235,6
2-1	4,2	Tumor only	33,5
9-1	4,2	Tumor only	175,9
15-1	4,2	Tumor only	113,1
20-1	4,2	Tumor only	269,9
8-1	14,1	Tumor only	33,5
25-2	14,1	Tumor only	33,5
4-2	4,2	TCR ^{A2-2}	No tumor
11-2	4,2	TCR ^{A2-2}	No tumor
16-2	4,2	TCR ^{A2-2}	No tumor
21-2	4,2	TCR ^{A2-2}	33,5
27-2	4,2	TCR ^{A2-2}	No tumor
10-2	14,1	TCR ^{A2-2}	No tumor
26-2	14,1	TCR ^{A2-2}	No tumor
13-3	0,5	TCR ^{1G4}	131,9
7-3	4,2	TCR ^{1G4}	150,8
12-3	4,2	TCR ^{1G4}	33,5
17-3	4,2	TCR ^{1G4}	94,2
22-3	4,2	TCR ^{1G4}	33,5
14-3	14,1	TCR ^{1G4}	251,3
29-3	14,1	TCR ^{1G4}	14,1

Supplementary Table 6. Overview of treatment grouping of 922882-316-R PDX implanted mice based on caliper measurements at day -1, and tumor size at endpoint