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for CANCER IMMUNOTHERAPY

Cellular Therapies for Cancer

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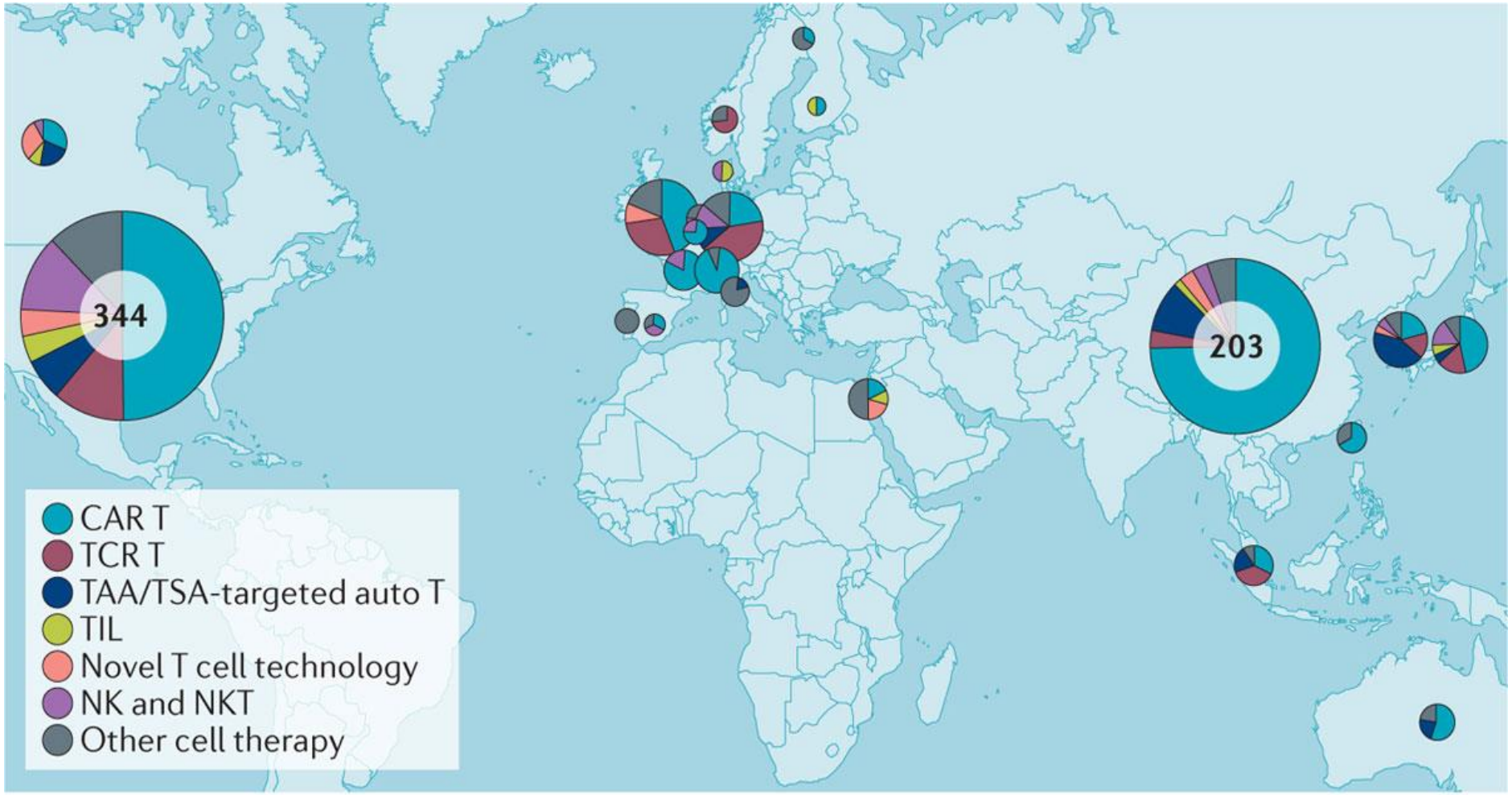
Disclosures:

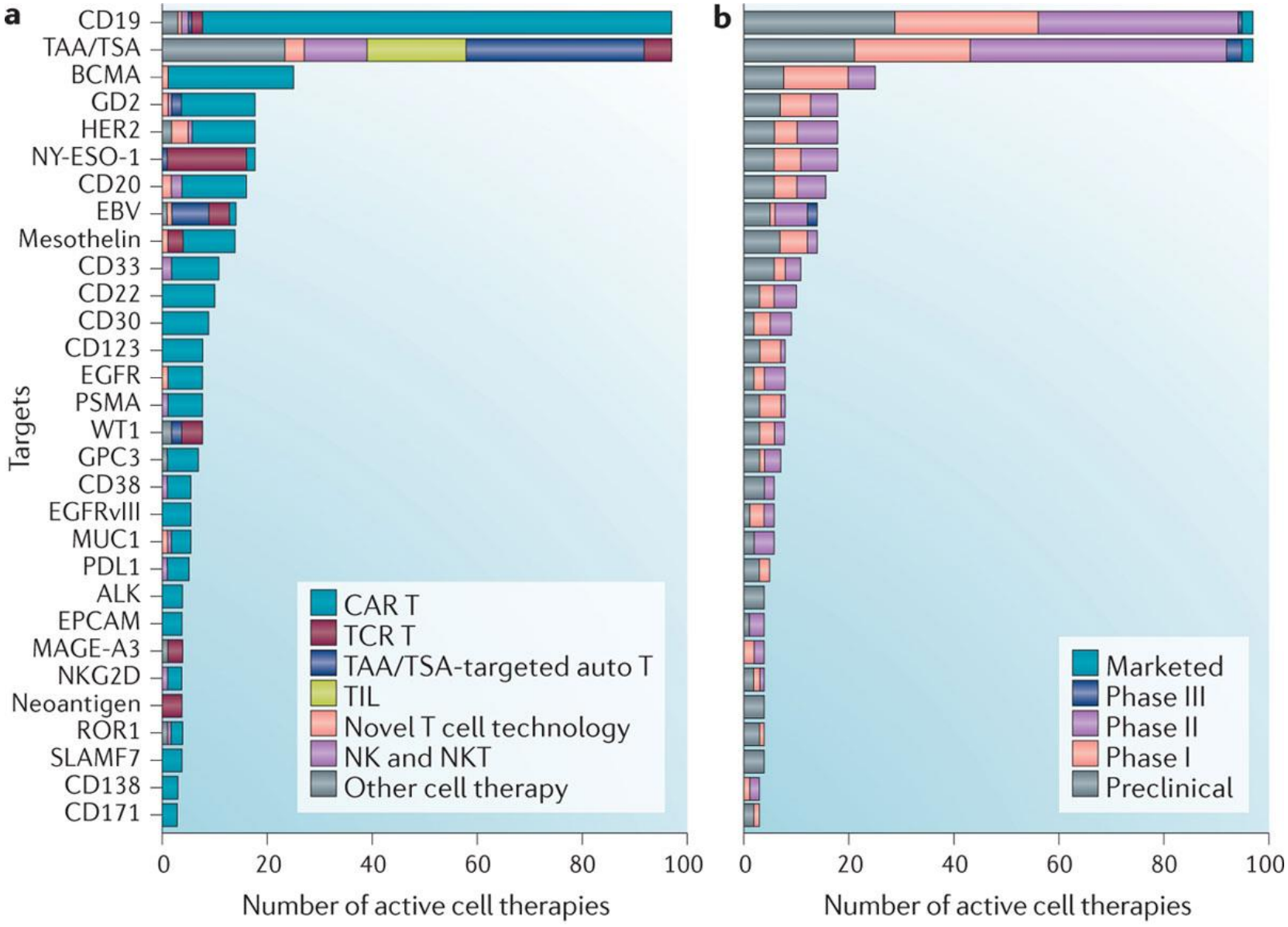
Simpatica, Scientific Advisory Board Member, Jan. 2017-present
StemImmune Scientific and Medical Advisory Board, April 6, 2017-present
SapVax Advisory Board meetings Nov. 15, 2017; Dec. 6, 2018
NextCure, Scientific Advisory Board, 2018-present
Replimmune, Scientific Advisory Board, 2018-present
Western Oncolytics, Scientific Advisory Board, 2018-present
Torque Therapeutics, Scientific Advisory Board, 2018-present

Global geographic distribution of 753 cell therapies in development.



Nature Reviews | Drug Discovery

Top 30 targets in cancer cell therapy

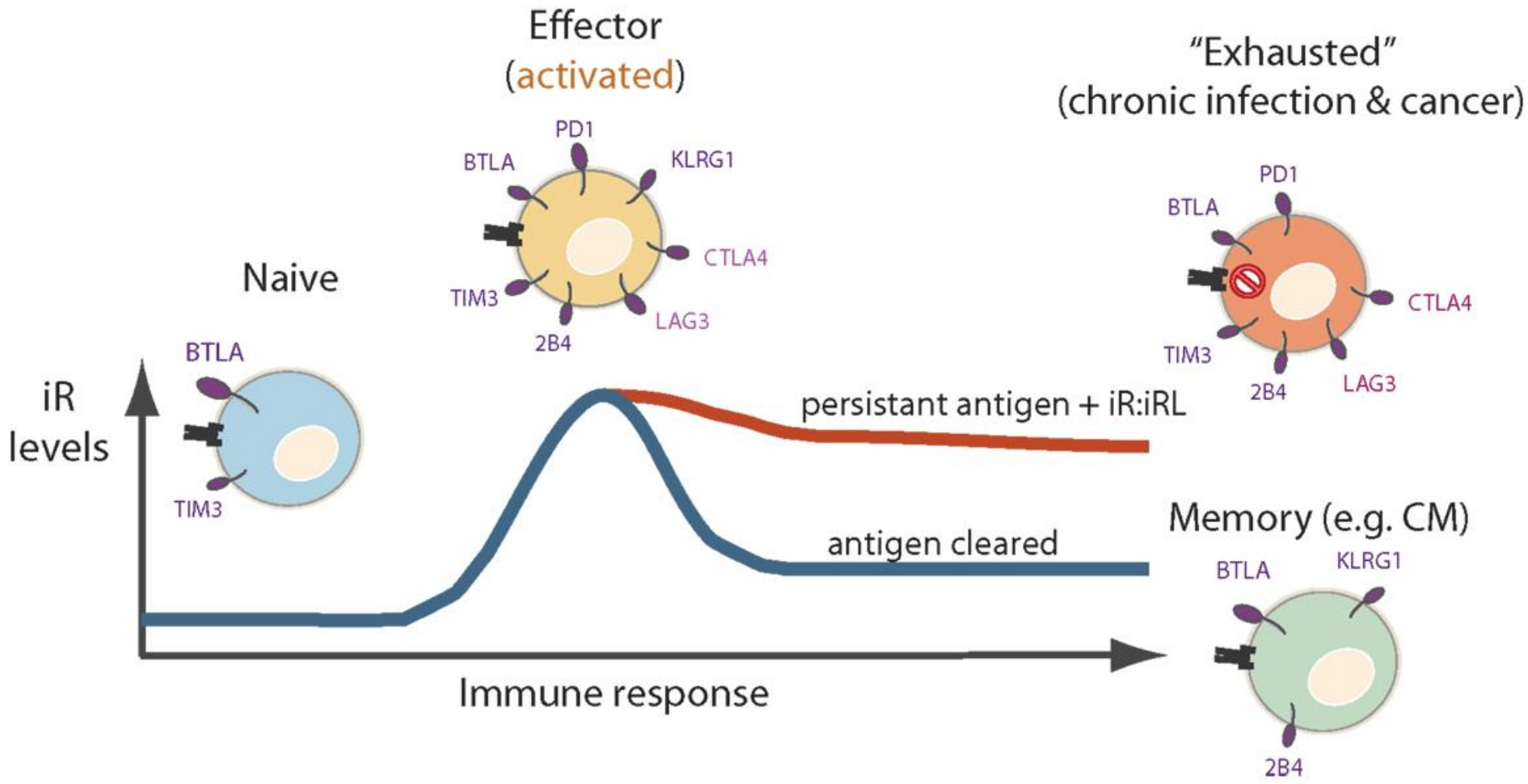


Nature Reviews | Drug Discovery

Table 1 | **Selected approved or late-stage cell therapies**

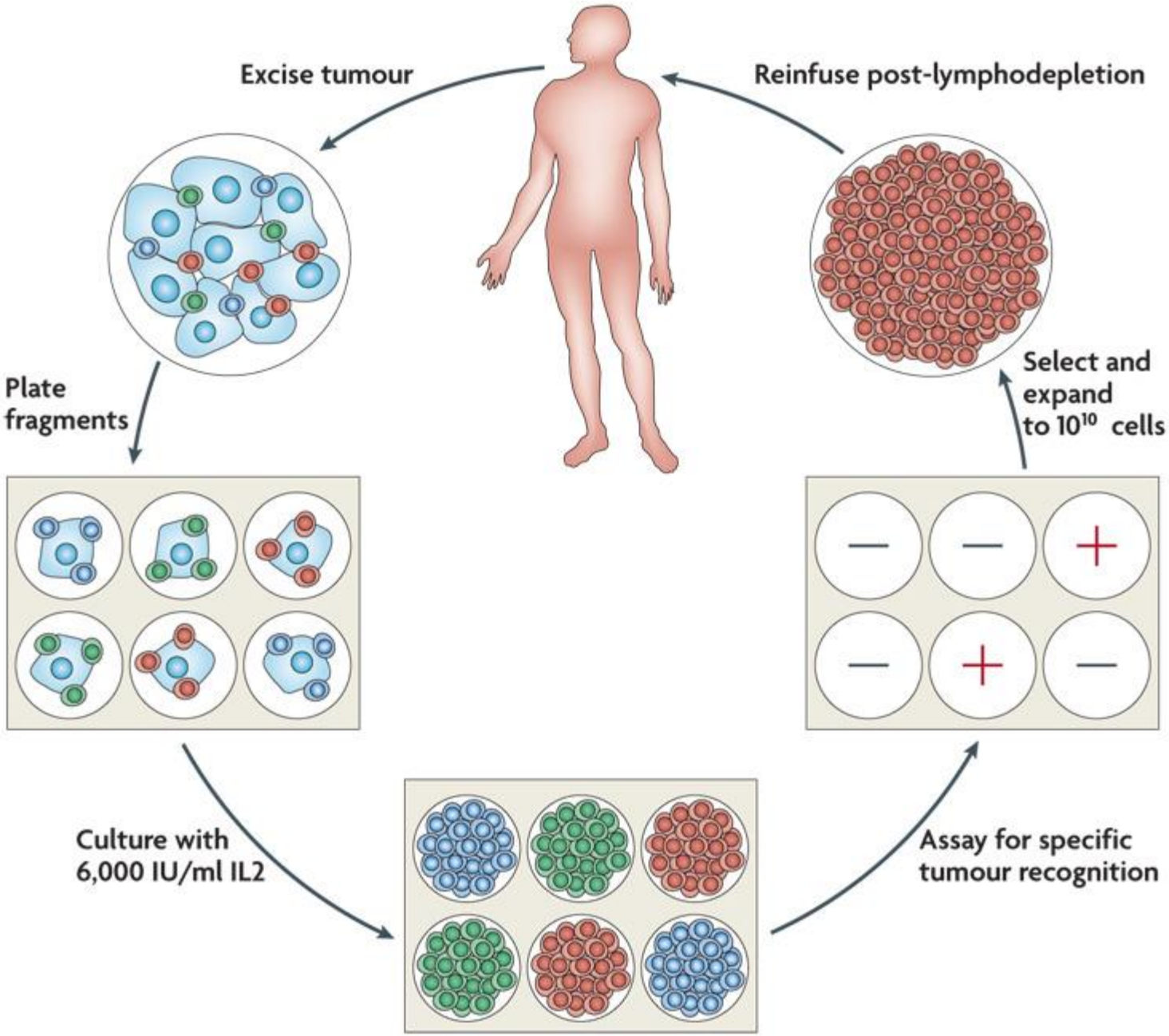
Drug name	Company	Indication	ClinicalTrials.gov identifier
Marketed			
Axicabtagene ciloleucel	Gilead Sciences	DLBCL, follicular lymphoma and PMBCL	NA
Tisagenlecleucel	Novartis	B cell ALL and LBCL	NA
Immuncell-LC	Green Cross Cell	Liver cancer	NA
Nalotimagene carmaleucel	MolMed SpA	Leukaemia	NA
Phase III			
ATA129	Atara Biotherapeutics	EBV + PTLD	NCT03394365 NCT03392142
TT-10	Tessa Therapeutics	Nasopharyngeal carcinoma	NCT02578641
NiCord	Gamida Cell	ALL, CML and MDS	NCT02730299
Phase II/III			
ImmuniCell	TC BioPharm	Melanoma, NSCLC and RCC	NCT02459067
Phase II			
Lisocabtagene maraleucel	Celgene	NHBCL	NCT03483103
bb2121	Bluebird bio/ Celgene	Multiple myeloma	NCT03361748

ALL, acute lymphocytic leukaemia; CML, chronic myelocytic leukaemia; DLBCL, diffuse large B cell lymphoma; EBV, Epstein–Barr virus; LBCL, large B cell lymphoma; MDS, myelodysplastic syndrome; NA, not applicable; NHBCL, non-Hodgkin B cell lymphoma; NSCLC, non-small-cell lung cancer; PMBCL, primary mediastinal B cell lymphoma; PTLD, post-transplant lymphoproliferative disease; RCC, renal cell carcinoma.



T Cell Exhaustion. Naïve cells express mainly BTLA and low levels of TIM3. Effector cells express a wider variety of **inhibitory receptors**. The levels of certain inhibitory receptors such as PD1, CTLA-4, LAG3, and TIM3 may peak at the effector phase. Thereafter, expression differs in chronically stimulated cells ("exhausted cells") where inhibitory receptors are relatively maintained, as opposed to memory cells after clearance of an acute infection where inhibitory receptors are down-modulated.

Front. Immunol., 26 June 2015 Fuertes, Speiser



Nat Rev Cancer. 2008. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME.

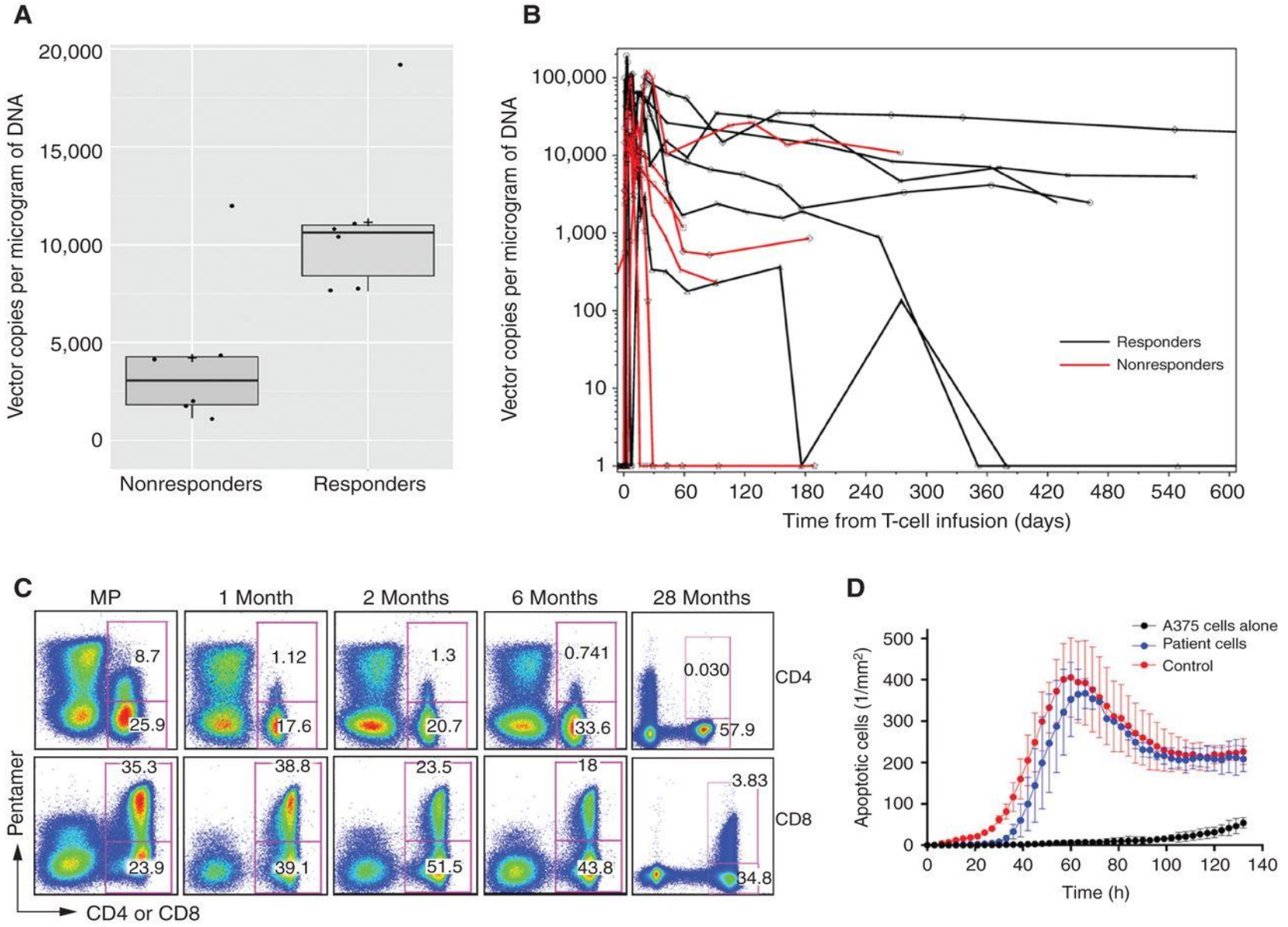
Current Cell Therapies:
NY-ESO-1-specific T cells for Sarcoma

Metastatic synovial sarcoma is incurable with standard therapy. NY-ESO-1^{c259}, an affinity-enhanced T-cell receptor (TCR) recognizes an HLA-A2-restricted NY-ESO-1/LAGE1a-derived peptide.

We employed engineered T cells targeting NY-ESO-1, and the data suggest that robust, self-regenerating pools of CD8^{+} NY-ESO-1^{c259}T cells produce a continuing supply of effector cells over several months that mediate clinically meaningful antitumor effects despite prolonged exposure to antigen.

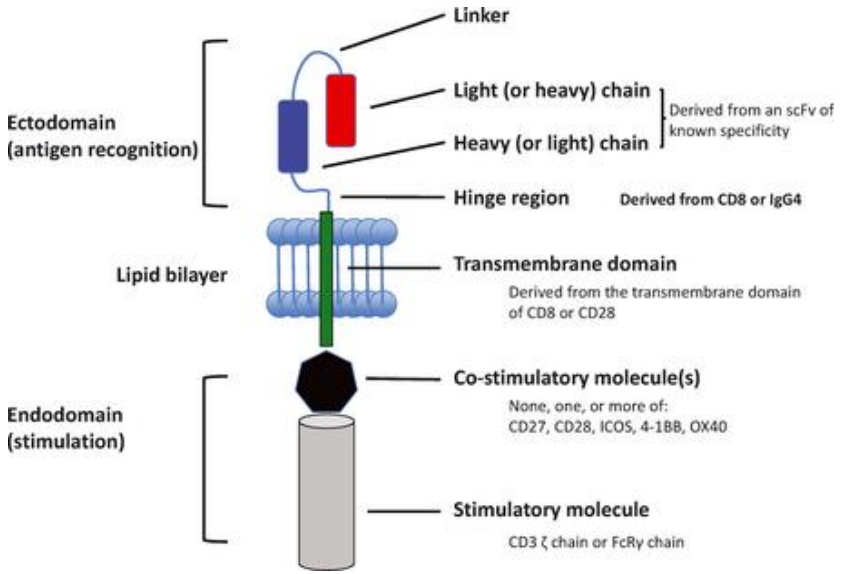
"Antitumor Activity Associated with Prolonged Persistence of Adoptively Transferred NY-ESO-1 c259T Cells in Synovial Sarcoma". Cancer Discovery, Aug. 2018, S.P. D'Angelo, L. Melchiori, M.S. Merchant, D. Bernstein, …T. Holdich, L. Pandite, R. Amado and C.L. Mackall

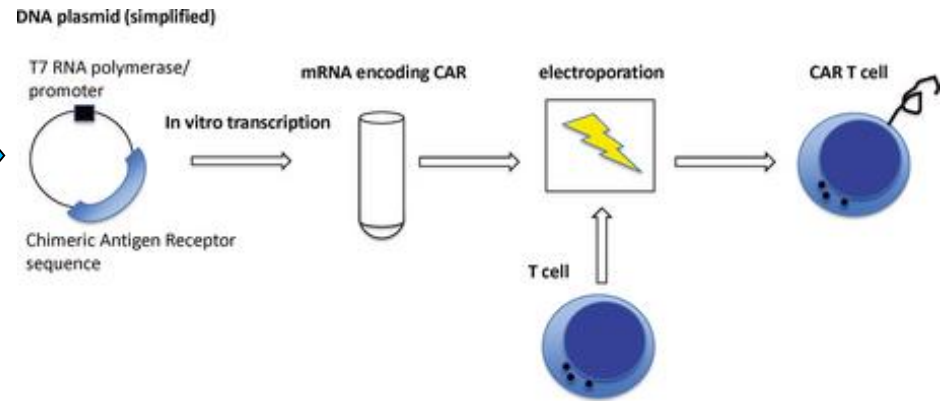
NY-ESO-1c259T cells show increased expansion in responding patients and persist with functional capacity to kill NY-ESO-1 targets for several months.



Sandra P. D'Angelo et al. Cancer Discov 2018;8:944-957

Going viral: chimeric antigen receptor (CAR)T-cell therapy for hematological malignancies





Antigen-specific immunotherapy approach to B-cell malignancies	
Technology	CART
Example	CART19 (Penn) CTL019 (Novartis) (autologous ex vivo expanded T cells transduced with an anti-CD19 scFv)
Dosing	One infusion
Complete responses (relapsed/refractory B-ALL)	**90%** [172]
Survival	78% 6 months OS
Major toxicity	Cytokine release syndrome, encephalopathy
Antigen-loss relapses noted?	Yes
Major challenges	Complex process to manufacture an individualized product

Immunological Reviews
Volume 263, Issue 1, pages 68-89, 15 DEC 2014
http://onlinelibrary.wiley.com/doi/10.1111/imr.12243/full#imr12243-fig-0001

CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells

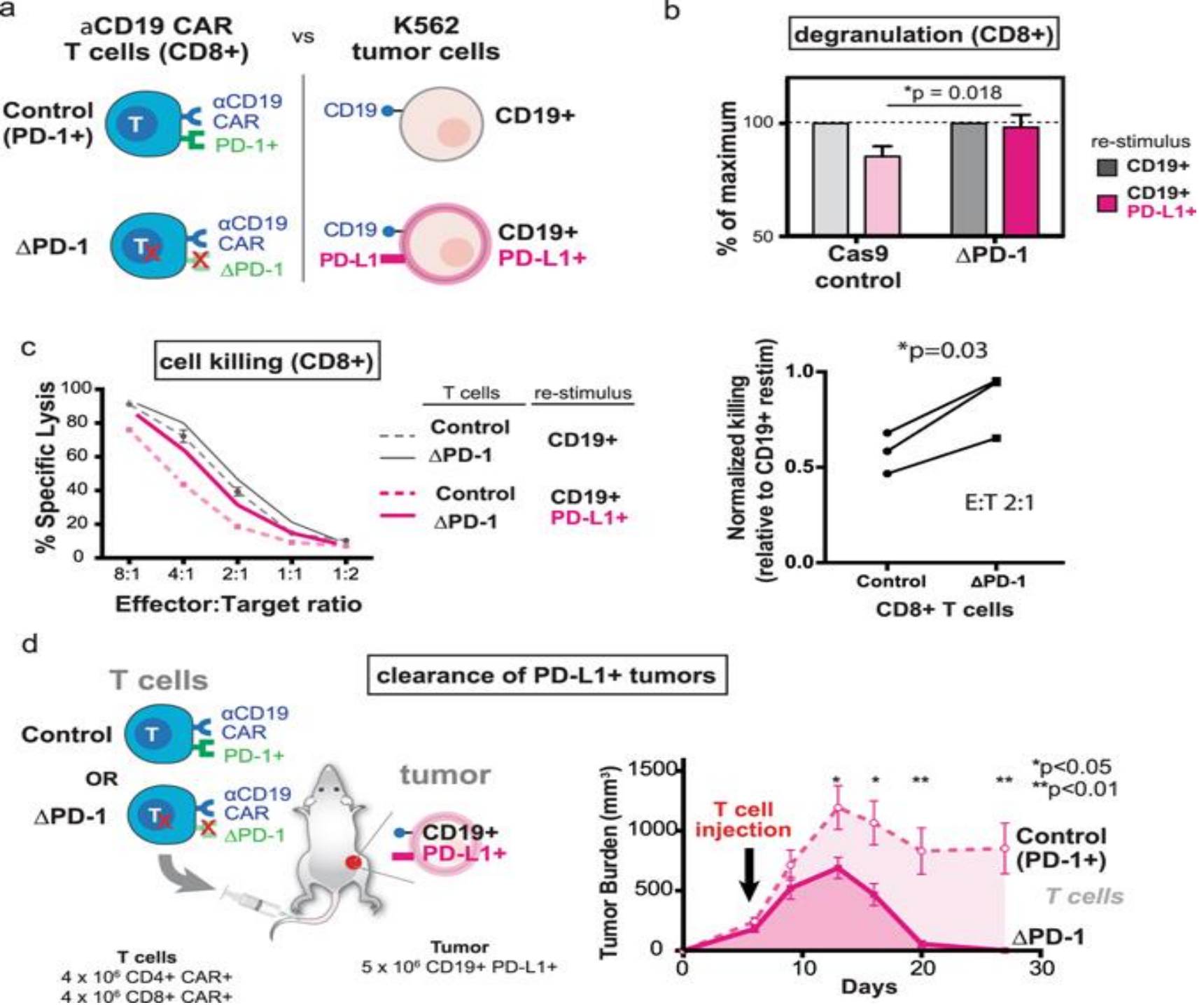
Immunotherapies with CAR T cells and checkpoint inhibitors (including PD-1 blockade) have both opened new avenues for cancer treatment.

Here we show that programmed death ligand 1 (PD-L1) expression on tumor cells can render human CAR T cells (anti-CD19 4-1BBζ) hypo-functional, resulting in impaired tumor clearance in a sub-cutaneous xenograft model.

To overcome this suppressed anti-tumor response, we developed a protocol for combined Cas9 ribonucleoprotein (Cas9 RNP)-mediated gene editing and lentiviral transduction to generate PD-1 deficient anti-CD19 CAR T cells. Pdcd1 (PD-1) disruption augmented CAR T cell mediated killing of tumor cells in vitro and enhanced clearance of PD-L1+ tumor xenografts in vivo.

This study demonstrates improved therapeutic efficacy of Cas9-edited CAR T cells and highlights the potential of precision genome engineering to enhance next-generation cell therapies.

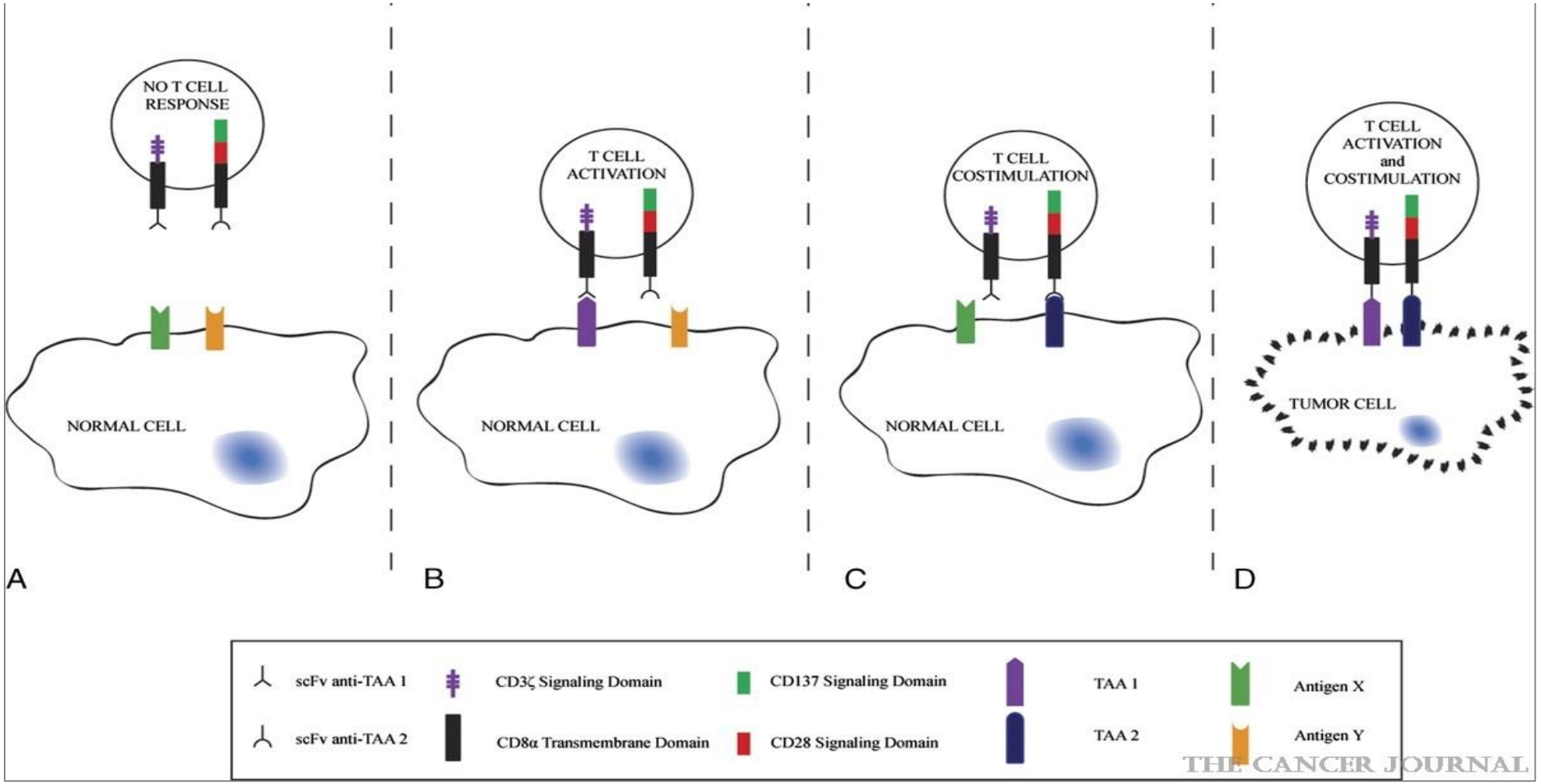
Levi J. Rupp, Kathrin Schumann, Kole T. Roybal, Rachel E. Gate, Chun J. Ye, Wendell A. Lim & Alexander Marson, Scientific Reports v.7, (2017)



CRISPR-mediated PD-1 editing rescues anti-CD19 CAR T cell function *in vitro* and enhances tumor clearance *in vivo*

Levi J. Rupp, Kathrin Schumann, Kole T. Roybal, Rachel E. Gate, Chun J. Ye, Wendell A. Lim & Alexander Marson, Scientific Reports v.7, (2017)

Novel Approaches to Enhance the Specificity and Safety of Engineered T Cells: Dual Specificity



Combinatorial antigen recognition enhances selective tumor eradication. By using a CAR to supply a CD3ζ signal (purple) upon binding respective antigen (1) and a CCR to supply CD28 and CD137 signals (green and red) upon binding respective antigen (2), engineered T cells are unresponsive to cells that do not express either antigen (A). Upon binding of the CAR alone, T cells receive an activation signal that can result in short-term cell lysis. Modulating the affinity or efficacy of the CAR interaction can limit activity against single antigen positive cells (B). Having only the CCR bind single positive cells will engage T-cell costimulation, but without activation, no response can be measured (C). **Only when T cells encounter tumor cells positive for both CAR and CCR respective antigens (D) can both T cell activation and costimulation occur, resulting in complete eradication of double-positive tumor cells.** Selective tumor eradication (D) can be accomplished provided that A-B-C also occur.

Fedorov, Victor D.; Sadelain, Michel; Kloss, Christopher C. The Cancer Journal20(2):160-165, March/April 2014.

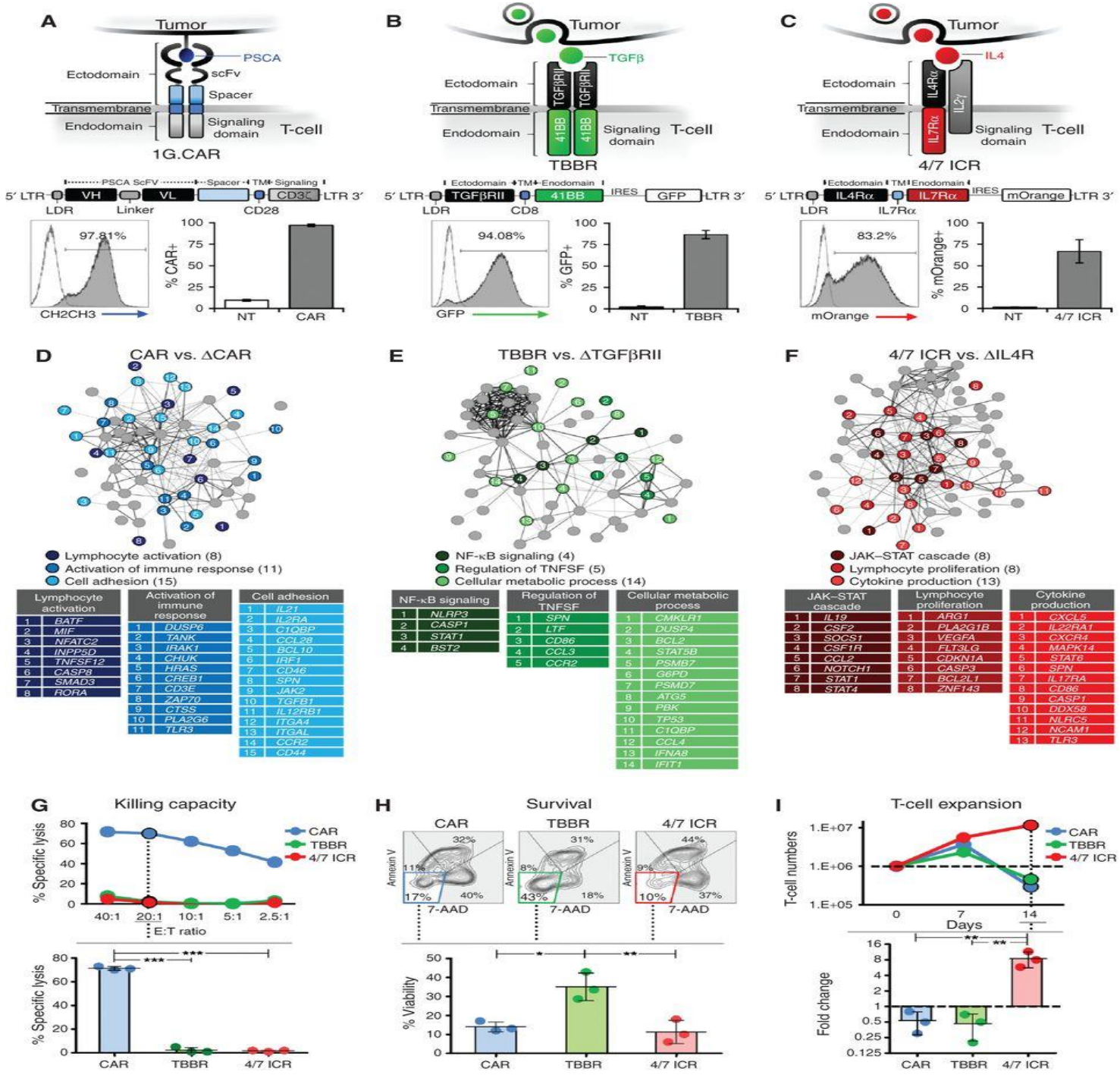
Incorporating a combination of receptors that ensure that cells are active exclusively at the tumor site

We developed an approach to render T cells responsive to an expression pattern present exclusively at the tumor by using a trio of novel chimeric receptors.

Using pancreatic cancer as a model, we demonstrate how T cells engineered with receptors that recognize **prostate stem cell antigen, TGFβ, and IL4**, and whose endodomains recapitulate physiologic T-cell signaling by providing signals for activation, costimulation, and cytokine support, produce potent antitumor effects selectively at the tumor site.

In addition, this strategy has the benefit of rendering our cells resistant to otherwise immunosuppressive cytokines (TGFβ and IL4) and can be readily extended to other inhibitory molecules present at the tumor site (e.g., PD-L1, IL10, and IL13).

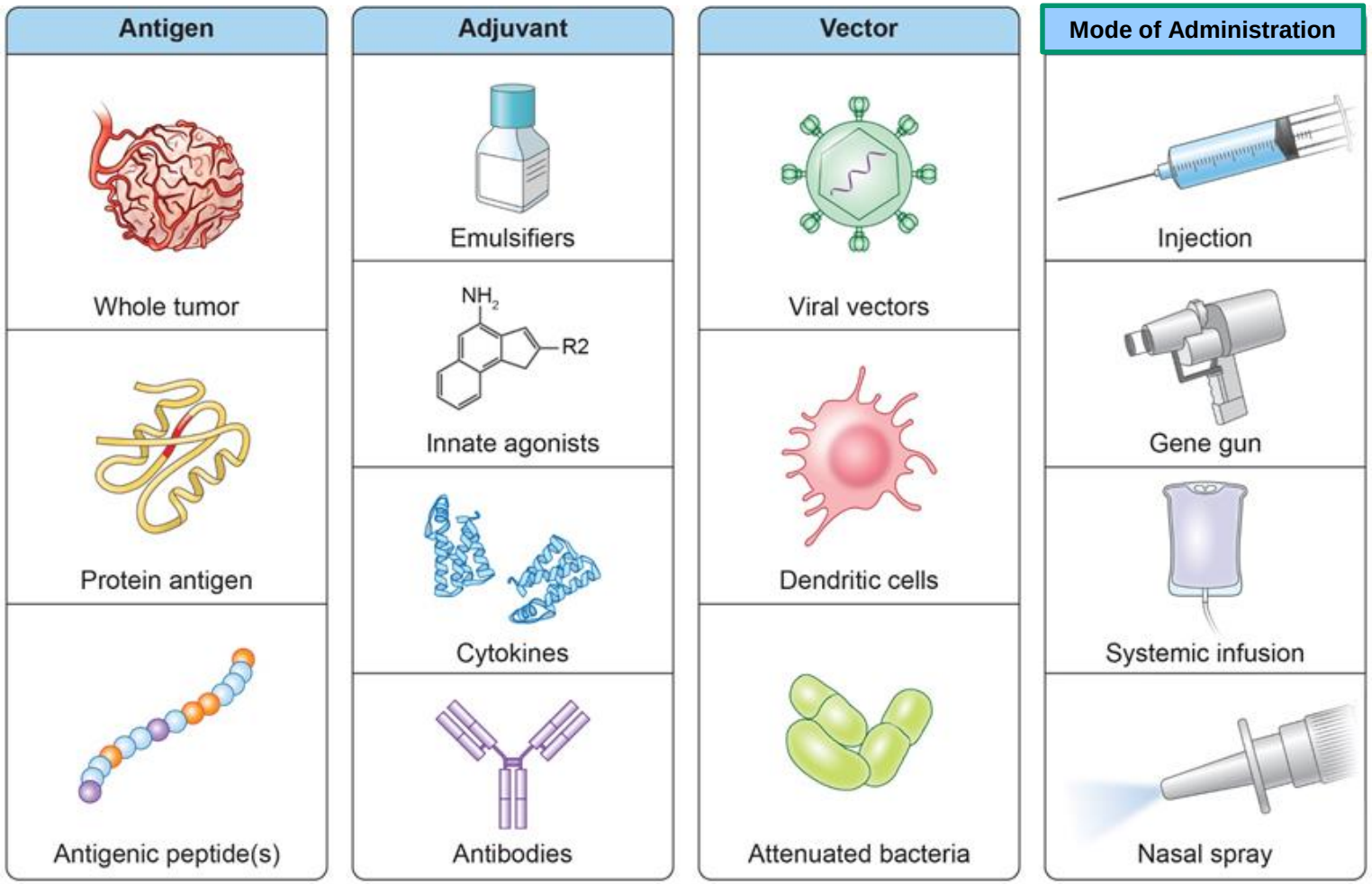
Sujita Sukumaran et al. Cancer Discov; 8(8); 972–87. ©2018 AACR.



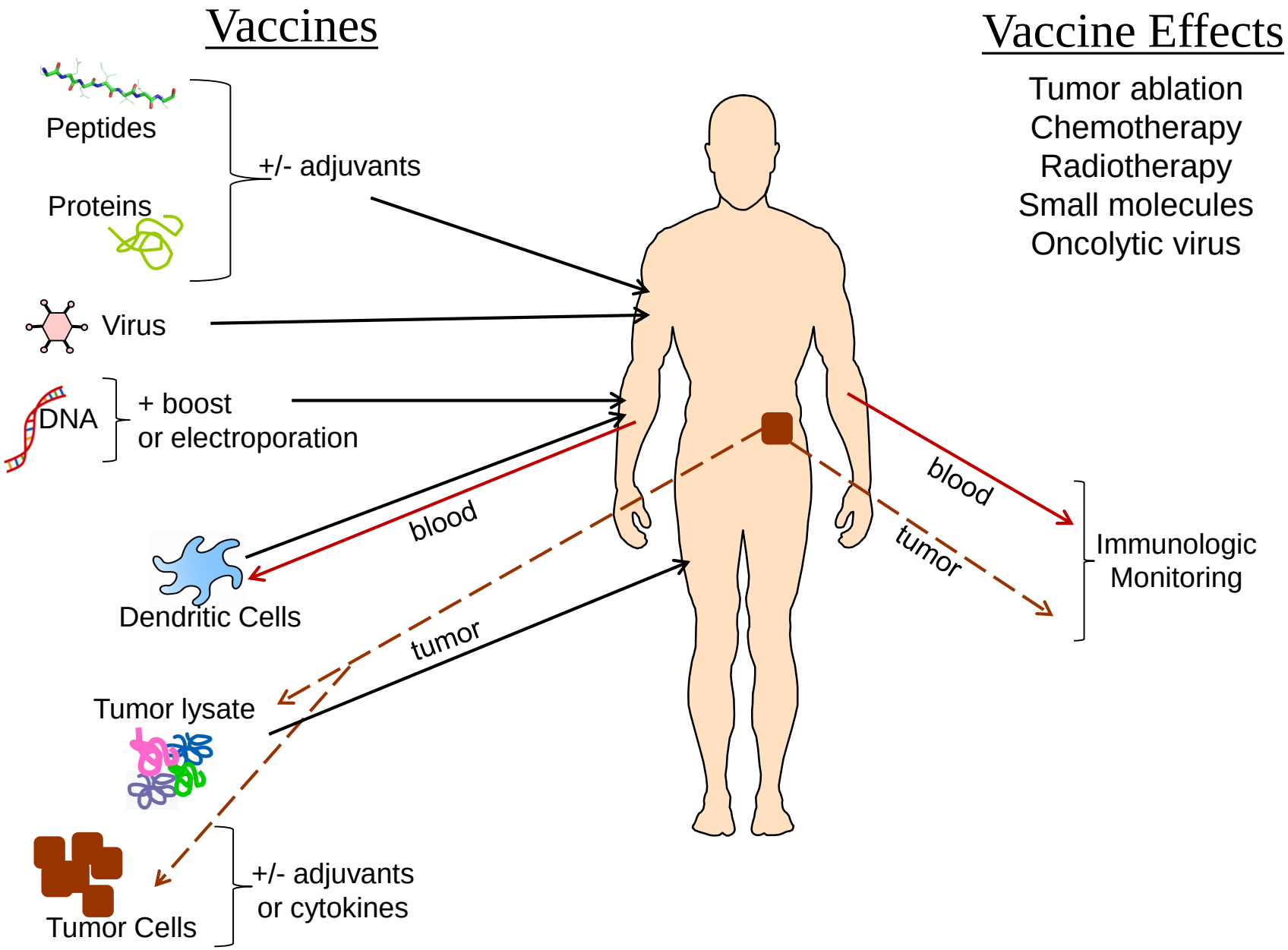
Synthetic T-cell receptors recognize the pancreatic tumor environment and deliver signals

Sujita Sukumaran et al.
Cancer Discov 2018

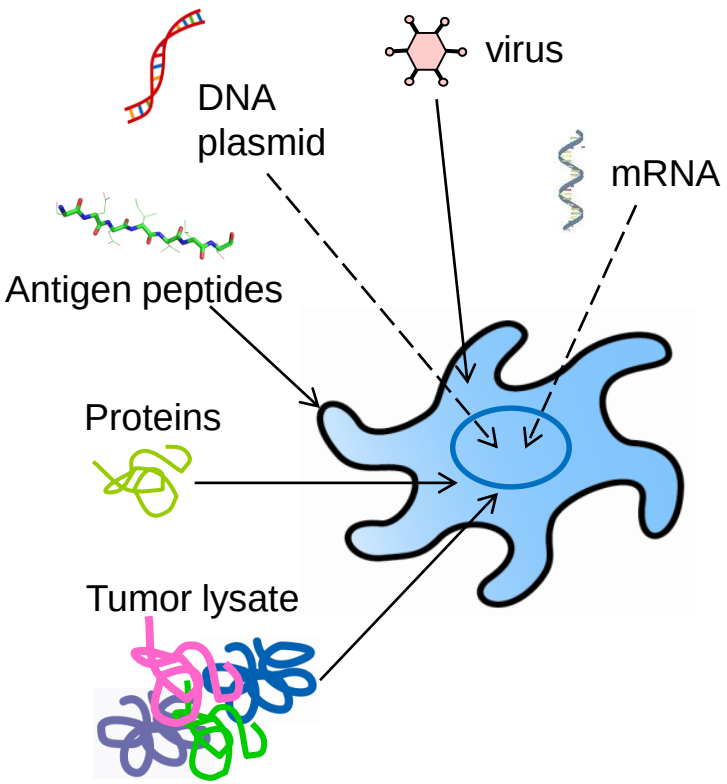
Components of a cancer vaccine



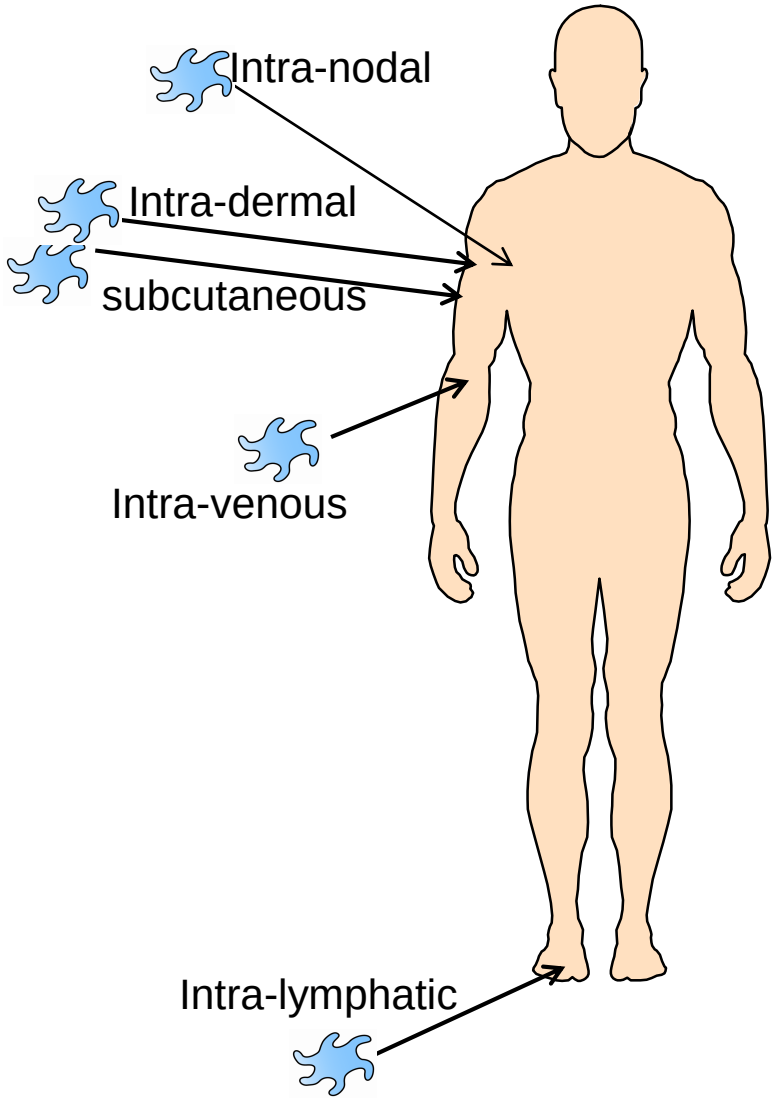
DNA/RNA


Vaccines
Peptides
Proteins
+/- adjuvants
Virus
DNA
+ boost
or electroporation
Dendritic Cells
blood
tumor
Tumor lysate
Tumor Cells
+/- adjuvants
or cytokines
Vaccine Effects
Tumor ablation
Chemotherapy
Radiotherapy
Small molecules
Oncolytic virus
blood
tumor
Immunologic
Monitoring

Antigen delivery to the DC

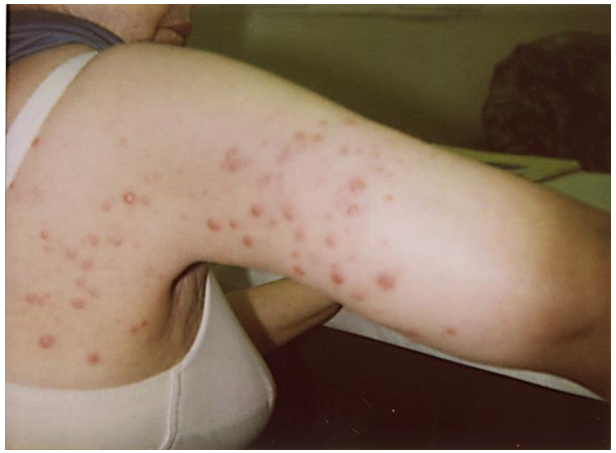


Vaccine delivery to the patient

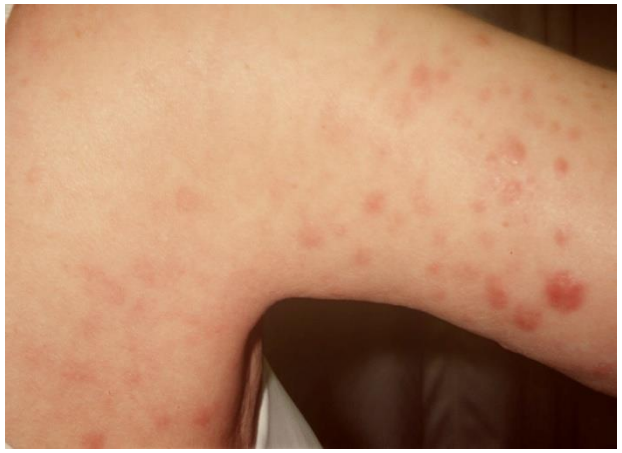


Patient E1 (10^7 DC, i.d.) post: 6 surgeries, 32 doses radiation, 6 infusions IFNα. >10 yrs NED

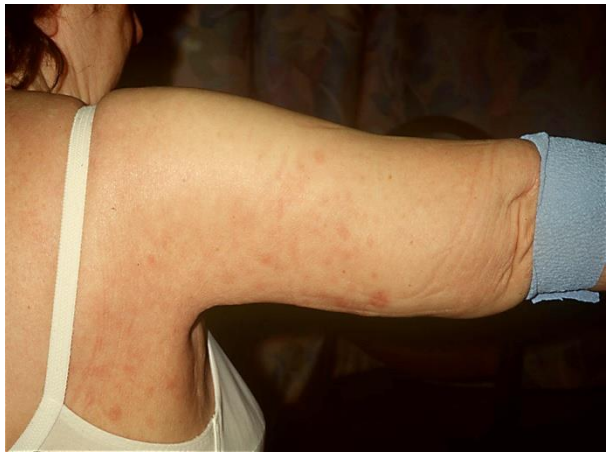
Pretreatment

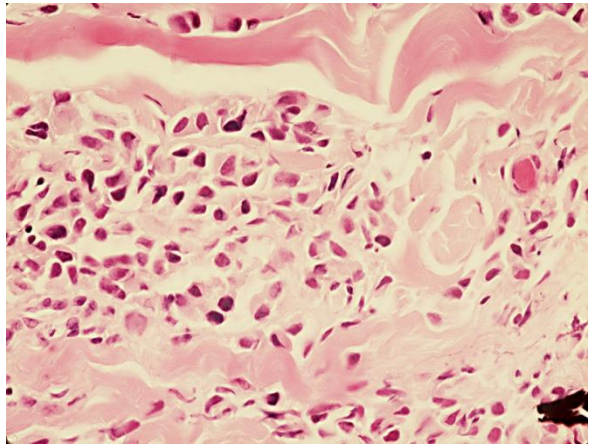


+56 days

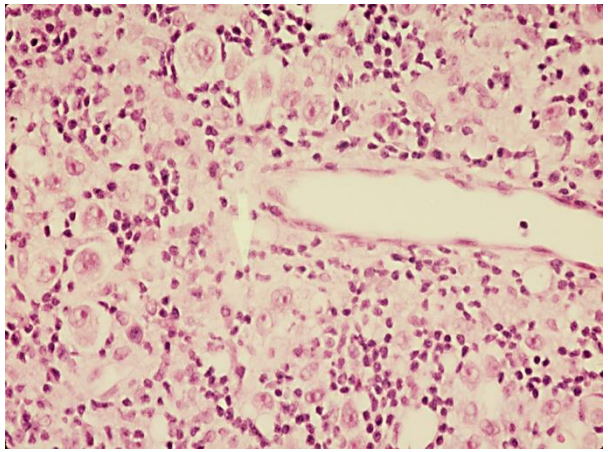


+130 days

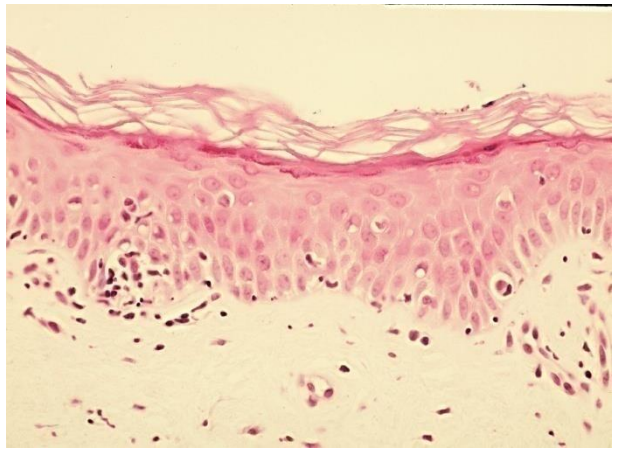




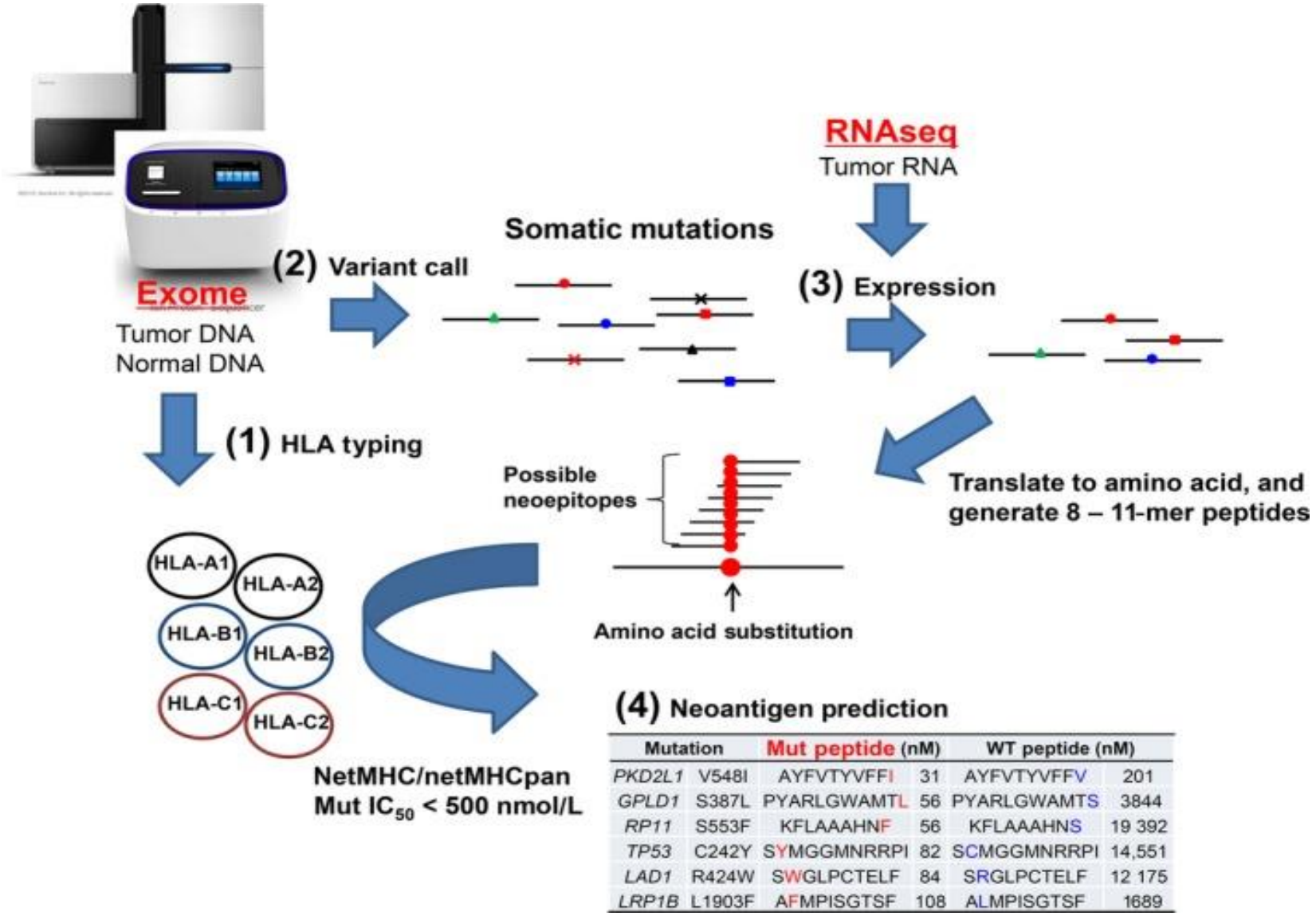
Melanoma Tumor



Lymphocytic Infiltrate
(largely CD8+, also CD4+)



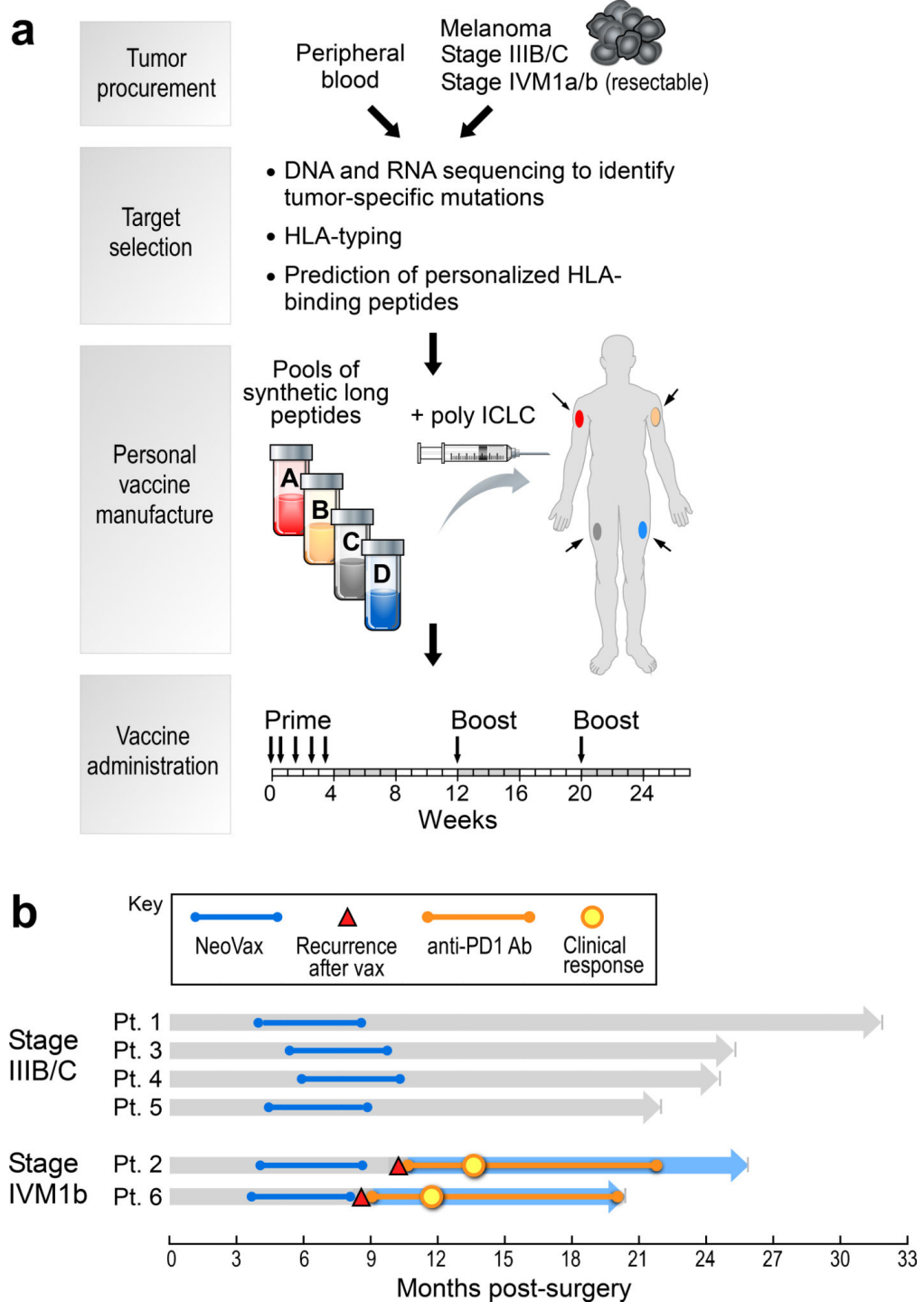
Absence of Melanoma



Mutation		Mut peptide (nM)		WT peptide (nM)	
PKD2L1	V548I	AYFVTYVFFI	31	AYFVTYVFFV	201
GPLD1	S387L	PYARLGWAMTL	56	PYARLGWAMTS	3844
RP11	S553F	KFLAAAHNF	56	KFLAAAHNS	19 392
TP53	C242Y	SYMGGMNRRPI	82	SCMGGMNRRPI	14,551
LAD1	R424W	SWGLPCTELF	84	SRGLPCTELF	12 175
LRP1B	L1903F	AFMPISGTSF	108	ALMPISGTSF	1689

Workflow of a neoantigen prediction pipeline. From whole-exome sequence data (from normal and tumor DNAs) and RNA sequencing data (RNAseq; from tumor RNA), we obtain information on (1) HLA genotypes, (2) somatic mutations, and (3) the expression levels of mutated genes. Using this information, we estimate affinities of peptides to HLA molecules, and list possible neoantigens (4).

Kazuma Kiyotani, et al. Cancer Sci. 2018 Mar;109(3):542-549.



Generation of a personal, multi-peptide neoantigen vaccine for patients with high-risk melanoma

a, Somatic mutations were identified by WES of melanoma and germline DNA and their expression confirmed by tumor RNA-sequencing. Immunizing peptides were selected based on HLA binding predictions. Each patient received up to 20 long peptides in 4 pools.

b, Clinical event timeline for 6 vaccinated patients from surgery until time of data cutoff (36 months from study initiation).

P.A.Ott, …C. J. Wu, An Immunogenic Personal Neoantigen Vaccine for Melanoma Patients, Nature 2017

Parker Institute: Next generation cell therapy

CARL JUNE, MD
The University of Pennsylvania

Novel CARs and vectors for clinical trials

NK cell evaluation and engineering

LEWIS LANIER, PhD
UCSF

CASSIAN YEE, MD
MD Anderson

Endogenous T cell priming and therapeutics

CAR-T persistence and pediatric clinical trials

CRYSTALL MACKALL, MD
Stanford Medicine

HIDEHO OKADA, MD, PhD
UCSF

T cell trafficking and glioma targeting

Non-viral methods for T cell engineering

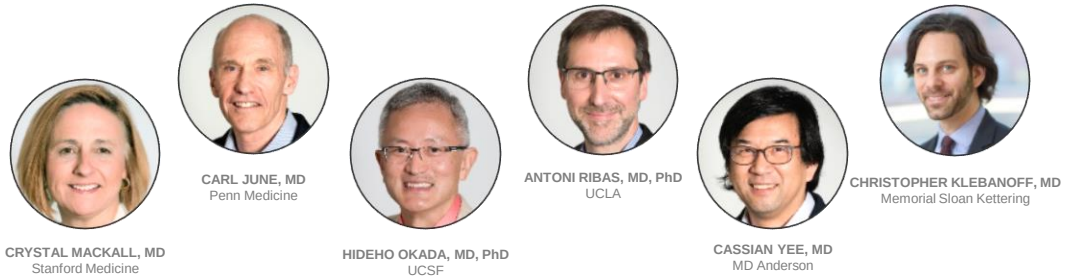
ALEXANDER MARSON, MD, PhD
UCSF

STEPHEN FORMAN, MD
City of Hope

Novel cell therapy programs in GBM

PICI T Cell Engineering Initiative

Expertise



CRYSTAL MACKALL, MD
Stanford Medicine

CARL JUNE, MD
Penn Medicine

HIDEHO OKADA, MD, PhD
UCSF

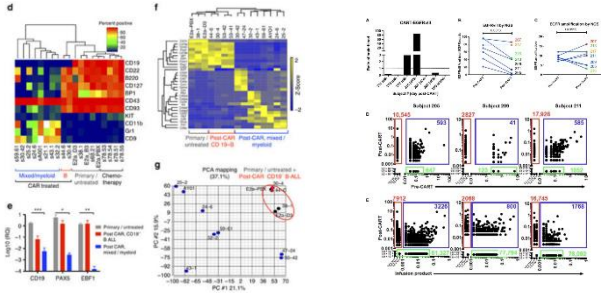
ANTONI RIBAS, MD, PhD
UCLA

CASSIAN YEE, MD
MD Anderson

CHRISTOPHER KLEBANOFF, MD
Memorial Sloan Kettering

PICI Member Researchers have world-class experience and a history of innovation in engineered adoptive cell therapy

Research



PICI Member Researchers are making fundamental advances in understanding behavior of CAR/TCR adoptive cell therapy

Collaboration

BD Biosciences

Bristol-Myers Squibb

TESSA THERAPEUTICS

PICI is enabling collaboration between a wide range of biotech and pharma companies

Clinical Trials

TMUNITY

PICI is supporting a first-of-its-kind clinical trial at Penn and other sites involving CRISPR-modified T cells

Phase I trial: NY-ESO redirected CRISPR edited T cells

Background:

NY-ESO-1 targeted cell therapy has shown safety and evidence of antitumor activity in melanoma, sarcoma and myeloma. However, lack of response and progression have been associated with T cell exhaustion and lack of T cell persistence, which may be mediated by PD-1

Study Design:

Genetically modify autologous T cells (i) with lentiviral vector to introduce high-affinity TCR directed to NY-ESO-1, and (ii) use CRISPR/Cas9 editing to delete alpha/beta chains of endogenous TCR and checkpoint inhibitor, PD-1 [i.e. four genetic modifications]

Trial Sites:

UPenn manufacturing site; UPenn, UCSF and MD Anderson clinical trial sites

Eligible Patients:

Adult patients with refractory multiple myeloma, melanoma or sarcoma that express NY-ESO-1 and are HLA A0201+

JEM

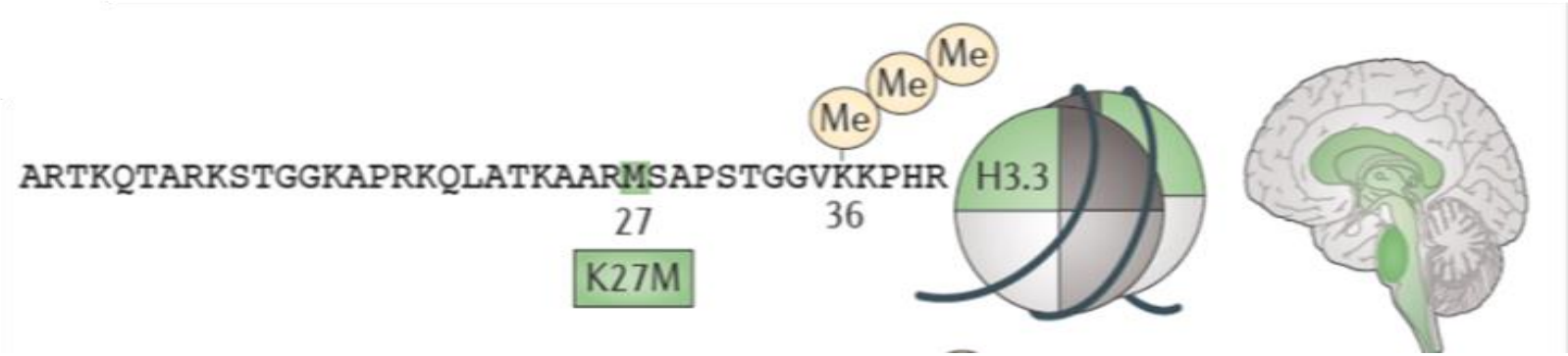
Article

Novel and shared neoantigen derived from histone 3 variant H3.3K27M mutation for glioma T cell therapy

Zinal S. Chheda,[1]* Gary Kohanbash,[1,10]* Kaori Okada,[1] Naznin Jahan,[1] John Sidney,[4] Matteo Pecoraro,[5] Xinbo Yang,[6] Diego A. Carrera,[1] Kira M. Downey,[1] Shruti Shrivastav,[1] Shuming Liu,[1] Yi Lin,[1] Chetana Lagisetti,[9] Pavlina Chuntova,[1] Payal B. Watchmaker,[1] Sabine Mueller,[1] Ian F. Pollack,[10] Raja Rajalingam,[2] Angel M. Carcaboso,[11] Matthias Mann,[5] Alessandro Sette,[4] K. Christopher Garcia,[6,7,8] Yafei Hou,[1] and Hideho Okada[1,3,12]

J Exp Med. 2018. PMID: 29203539

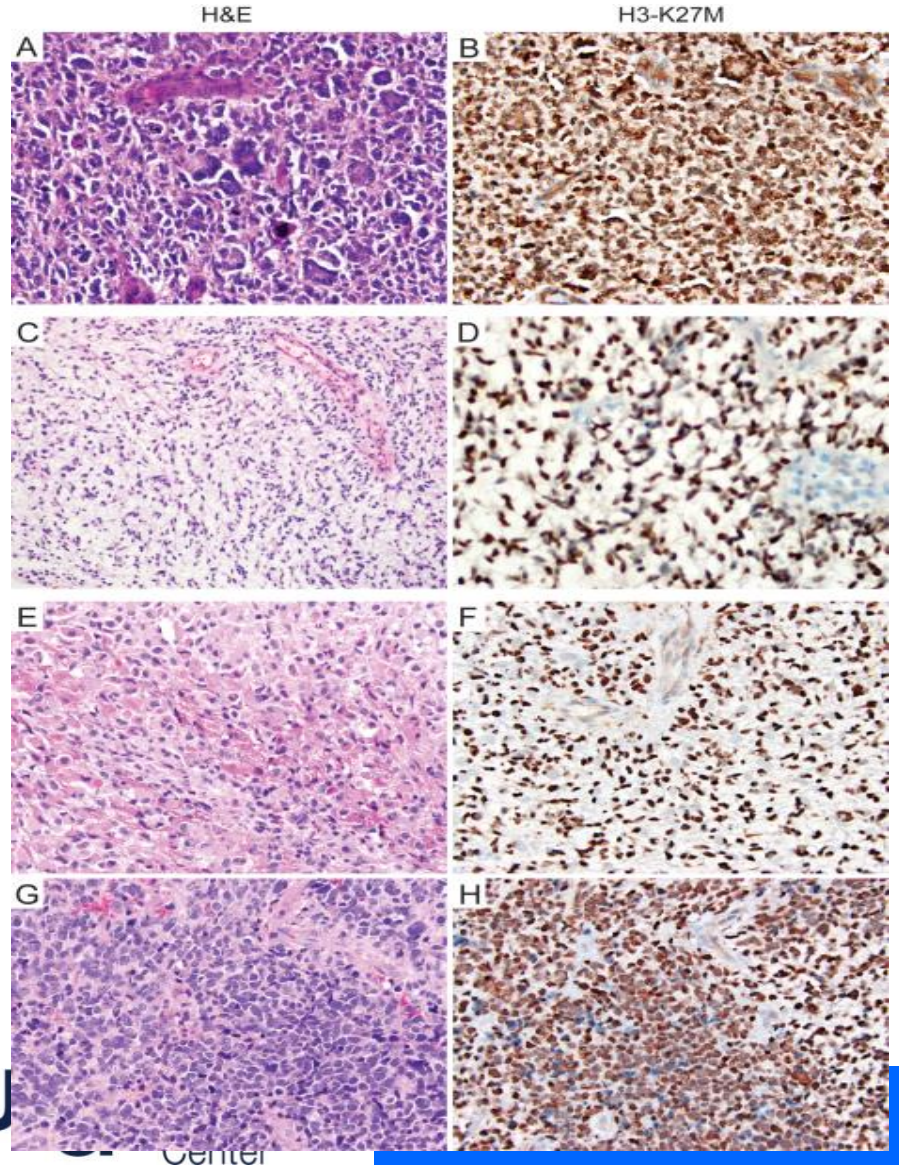
H. Okada: Histone 3 variant H3.3 K27M mutation, Diffuse Midline Gliomas



Chris Jones and Suzanne J. Baker, Nature Reviews Cancer (2014)

- Brain tumors are the leading cause of cancer-related mortality in children
- H3.3 K27M is present in a majority of diffuse midline gliomas (DMG), including 70-80% of diffuse intrinsic pontine glioma (DIPG).
- H3.3 K27M results in a global reduction of H3K27me3, leading to derepression of targets of polycomb repressive complex 2 (PRC2), thereby causing aberrant gene expression, and is universally associated with shorter survival.

Homogeneous presence of H3.3 K27M mutation in diffuse midline gliomas in young adults and children

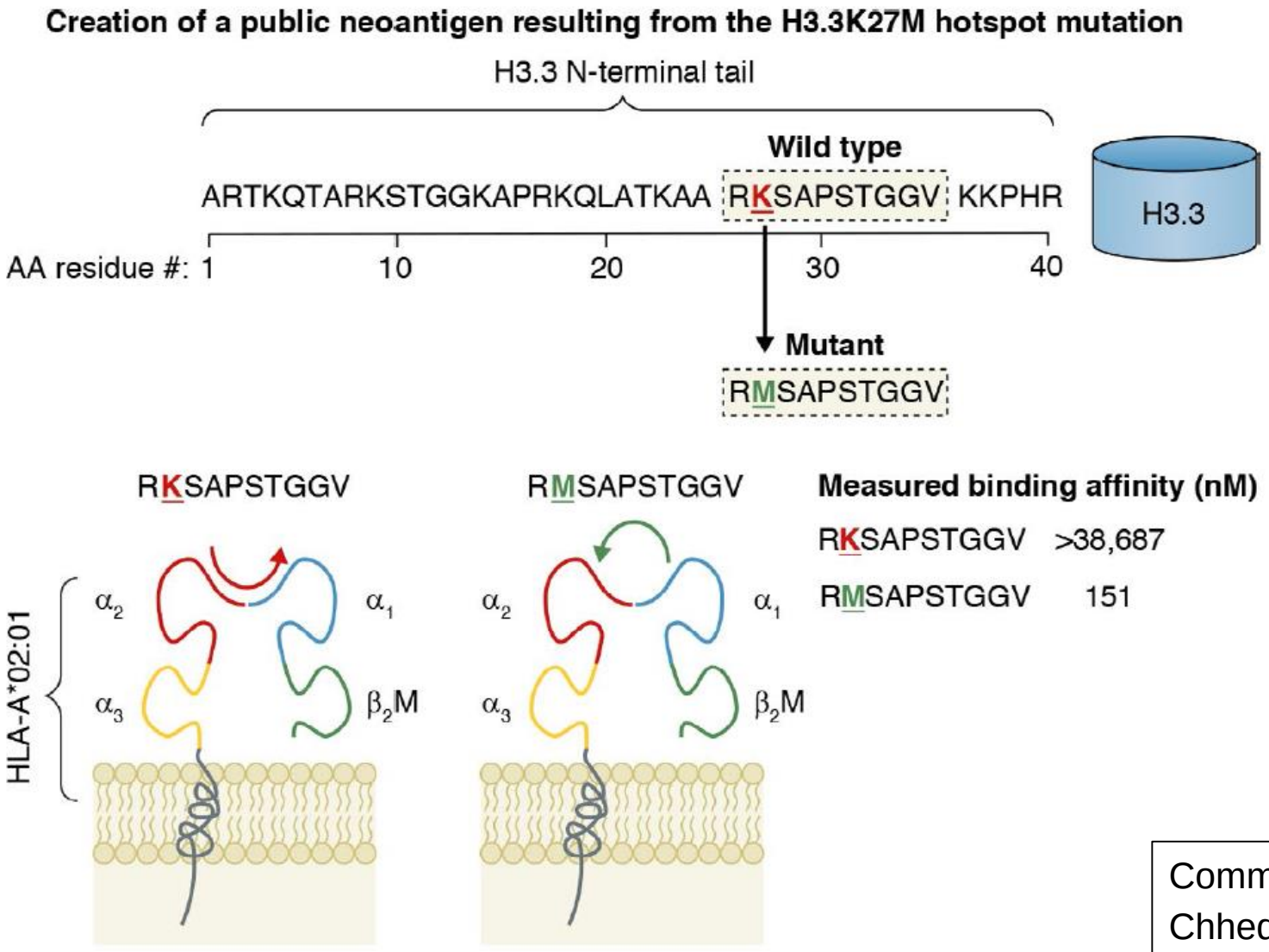


Solomon D et al. Brain Pathology 2015

IHC using a specific mAb against H.3K27M

“In each case, histone H3-K27M mutant protein staining was diffusely positive throughout all tumor nuclei, suggesting that histone H3 mutation is an early or initiating event in these diffuse midline gliomas.”

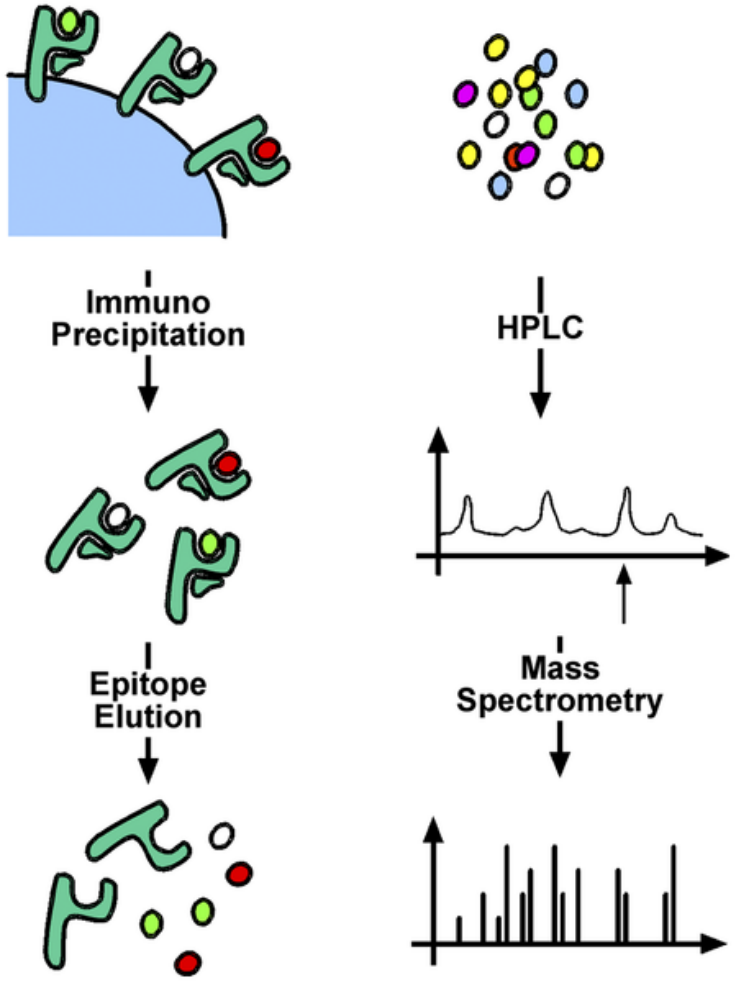
H3.3K27M peptide binds to HLA-A*02:01



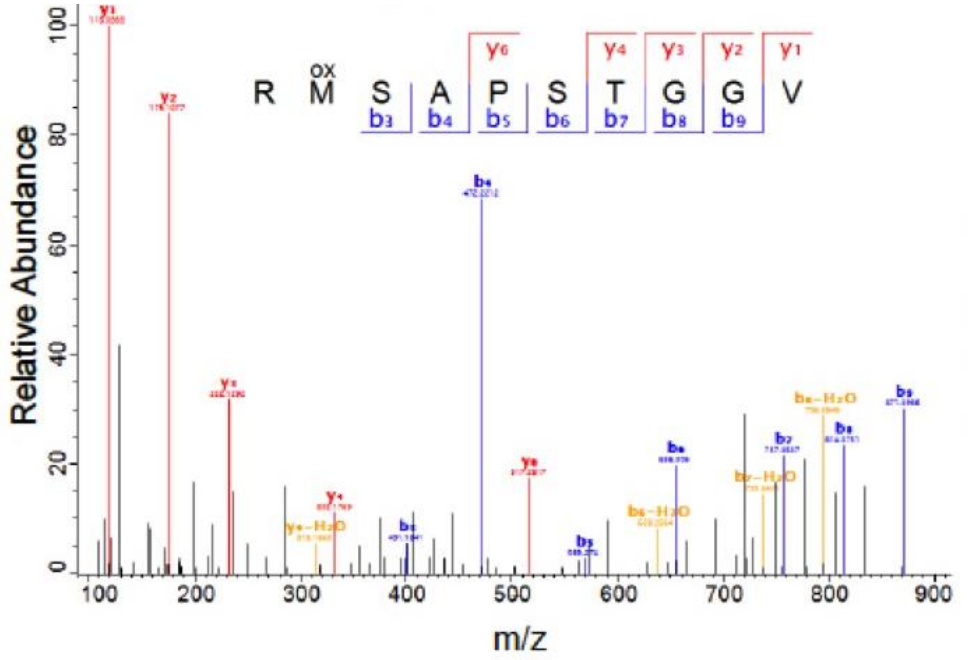
Contributed by Alex Sette and John Sidney at La Jolla Institute for Allergy and Immunology

Commentary by Klebanoff CA and Wolchok JD on Chheda Z., Kohanbash G. et al. J. Exp. Med. (2018)

H3.3K27M is processed and presented on HLA-A2 of human glioma cells



Wölk B et al. (2012) PLOS ONE 7(1): e29286.



Contributed by Matthias Mann and Matteo Pecoraro at Max Planck Institute of Biochemistry, Martinsried, Germany

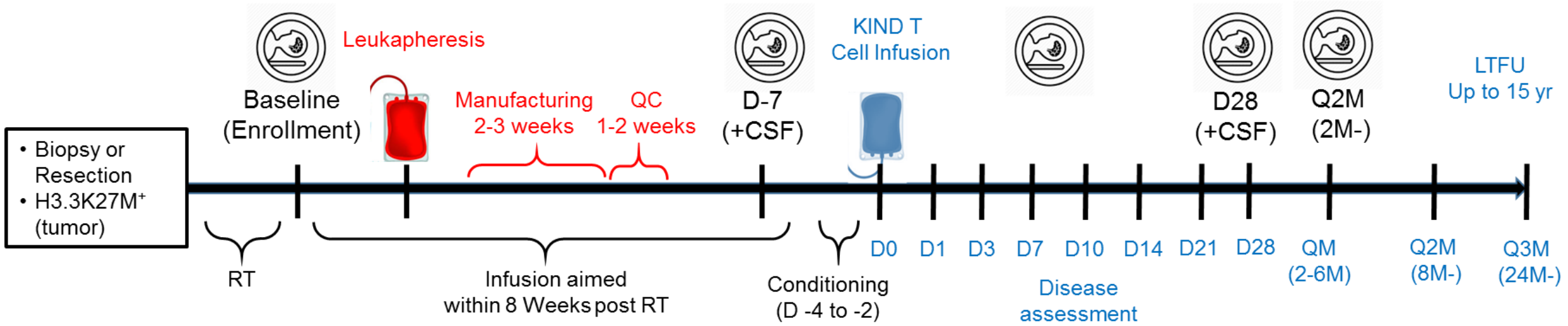
Summary and ongoing directions for the K27M project

- We identified HLA-A*0201-binding **H3.3K27M epitope**, and cloned a corresponding **TCR**.
- The H3.3.K27M epitope is naturally presented by HLA-A*0201+ H3.3K27M+ glioma cells.
- The TCR demonstrates an **outstanding affinity (2.9 μM)** and functional avidity.
- Alanine scanning suggests that **there are no cross-reactive amino acid motifs in known human proteins**.
- **TCR-T-cells specifically kill HLA-A*0201+ H3.3K27M glioma cells** *in vitro* and *in vivo.*

- These data provide a strong basis for development of: 1) **peptide-based vaccine** targeting the H3.3K27M epitope (**ongoing PNOC007 study**) and 2) **TCR-transduced T-cell therapy** approaches.

Trial: PICI0029 phase I study of KIND T cells

(T Cells transduced with H3.3K27M-specific TCR with Inhibition of Endogenous TCR)



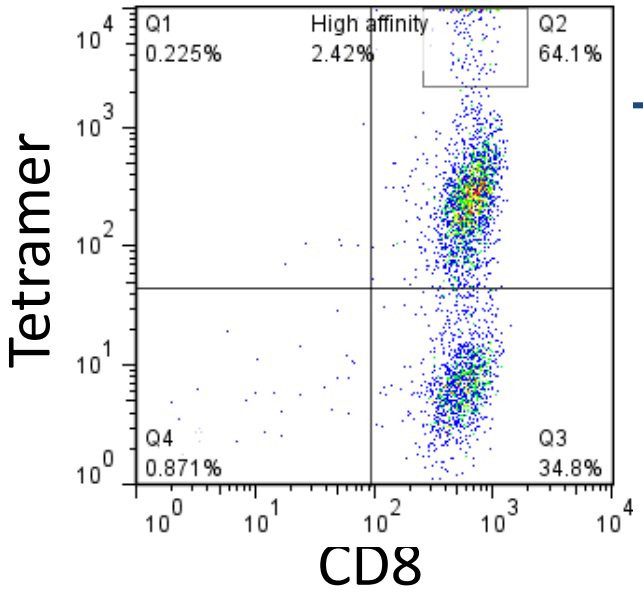
Primary endpoints: Safety and manufacturing feasibility

Secondary endpoints: T cell persistence and functions as well as preliminary efficacy

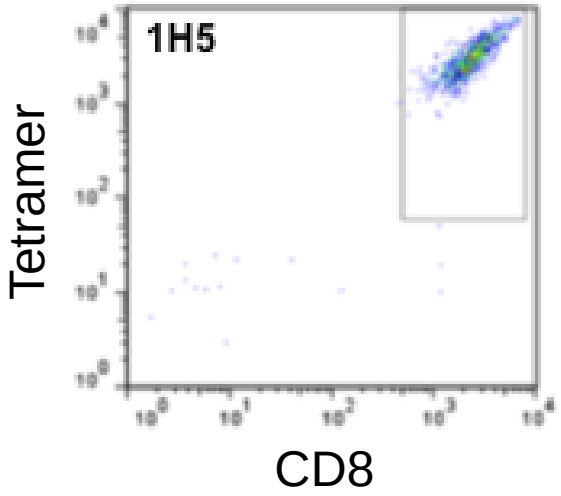
H. Okada

Repeated H3.3K27M peptide stimulation generates CTL clones that are H3.3K27M-HLA-A2 Tetramer^{high}

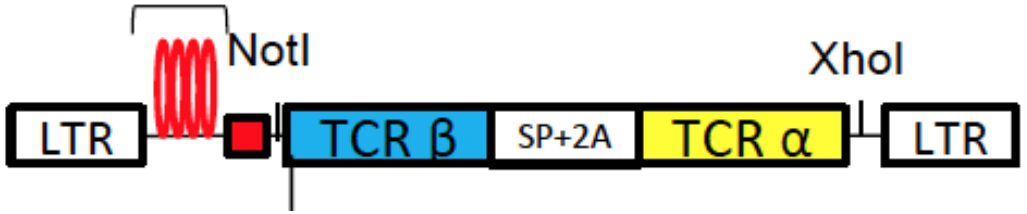
Repeated peptide stimulation of healthy donor PBMC



Limiting dilution cloning

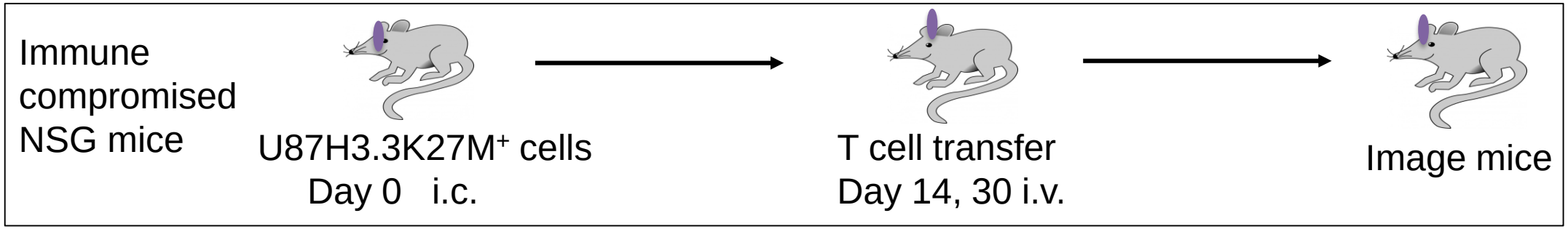


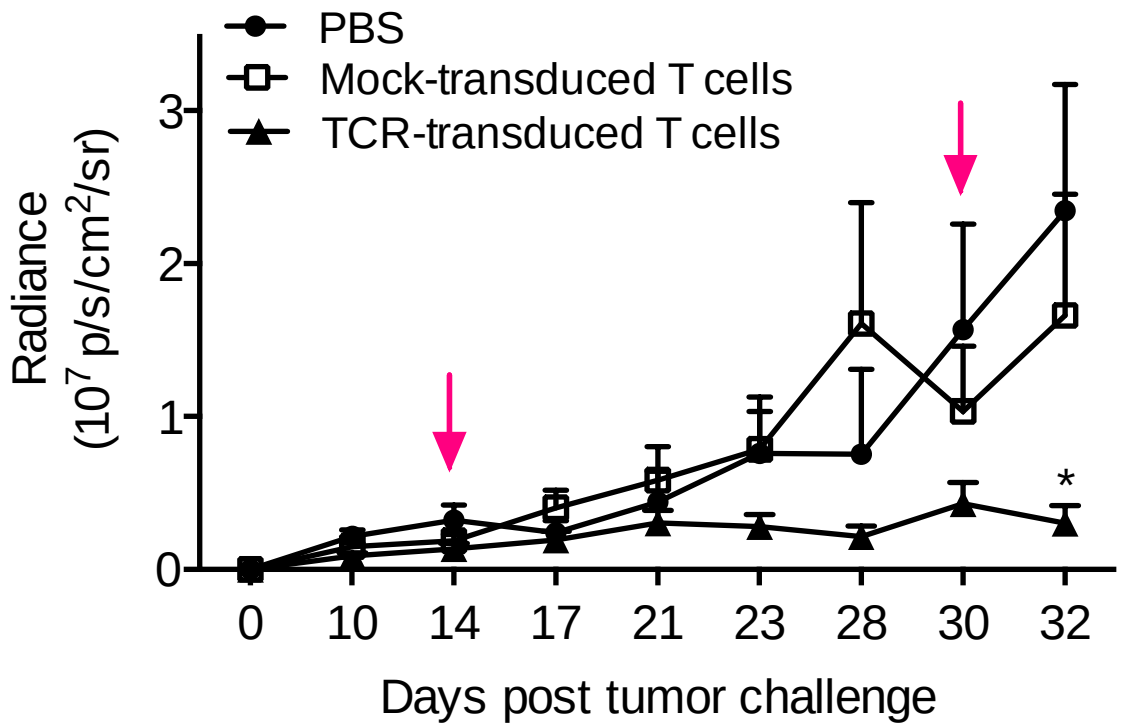
CTL clones with specificity to H3.3K27M epitope

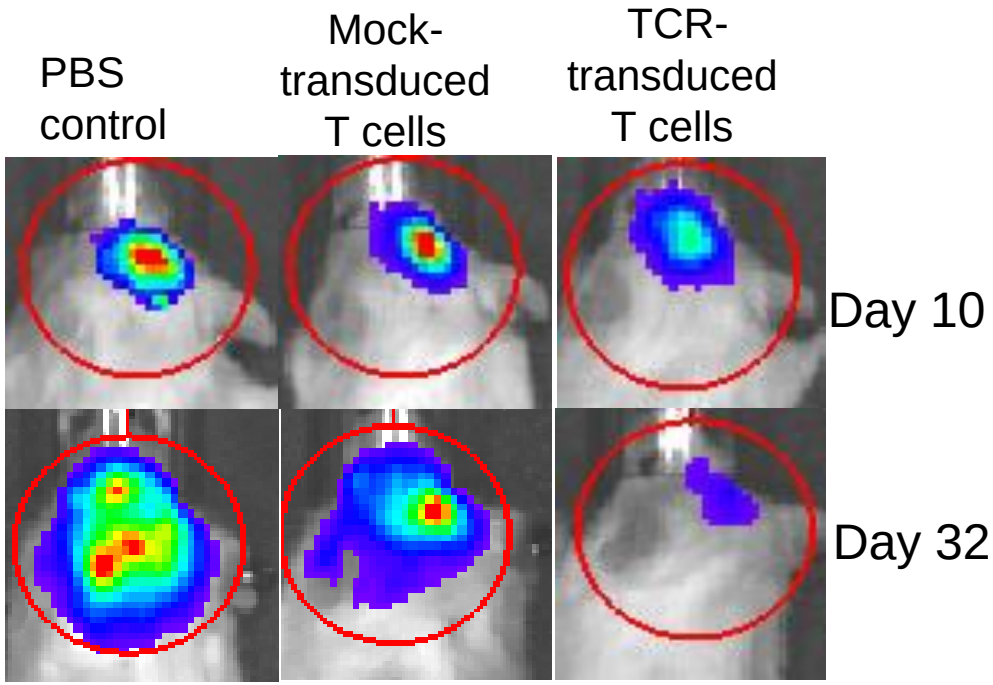


TCR chains isolated and cloned in a retroviral vector with siRNA for eTCR

TCR+T cells significantly suppress H3.3K27M+ glioma progression







Conclusions

CAR T cells targeting CD19 have shown great clinical success

TIL therapies continue to be used to treat melanoma patients and investigated in other tumor types where TIL can be expanded

Many novel T cell engineering approaches are being tested to improve the ability of T cells to be truly tumor specific and to resist multiple tumor resistance mechanisms that are encountered in the tumor microenvironment on solid tumors

Cancer vaccines can promote antitumor T cell responses, but with limited clinical efficacy. Neoantigen-specific vaccines are now being tested, and the optimal place for cancer vaccines is under investigation.