



Ziopharm
ONCOLOGY

Development of *Sleeping Beauty* transposed TCR-T cells for adoptive cell therapy of cancer

Drew C. Deniger

09-JUN-2021

Development of the non-viral *Sleeping Beauty* transposon/transposase system

Cell, Vol. 91, 501-510, November 14, 1997, Copyright ©1997 by Cell Press

Molecular Reconstruction of *Sleeping Beauty*, a *Tc1*-like Transposon from Fish, and Its Transposition in Human Cells

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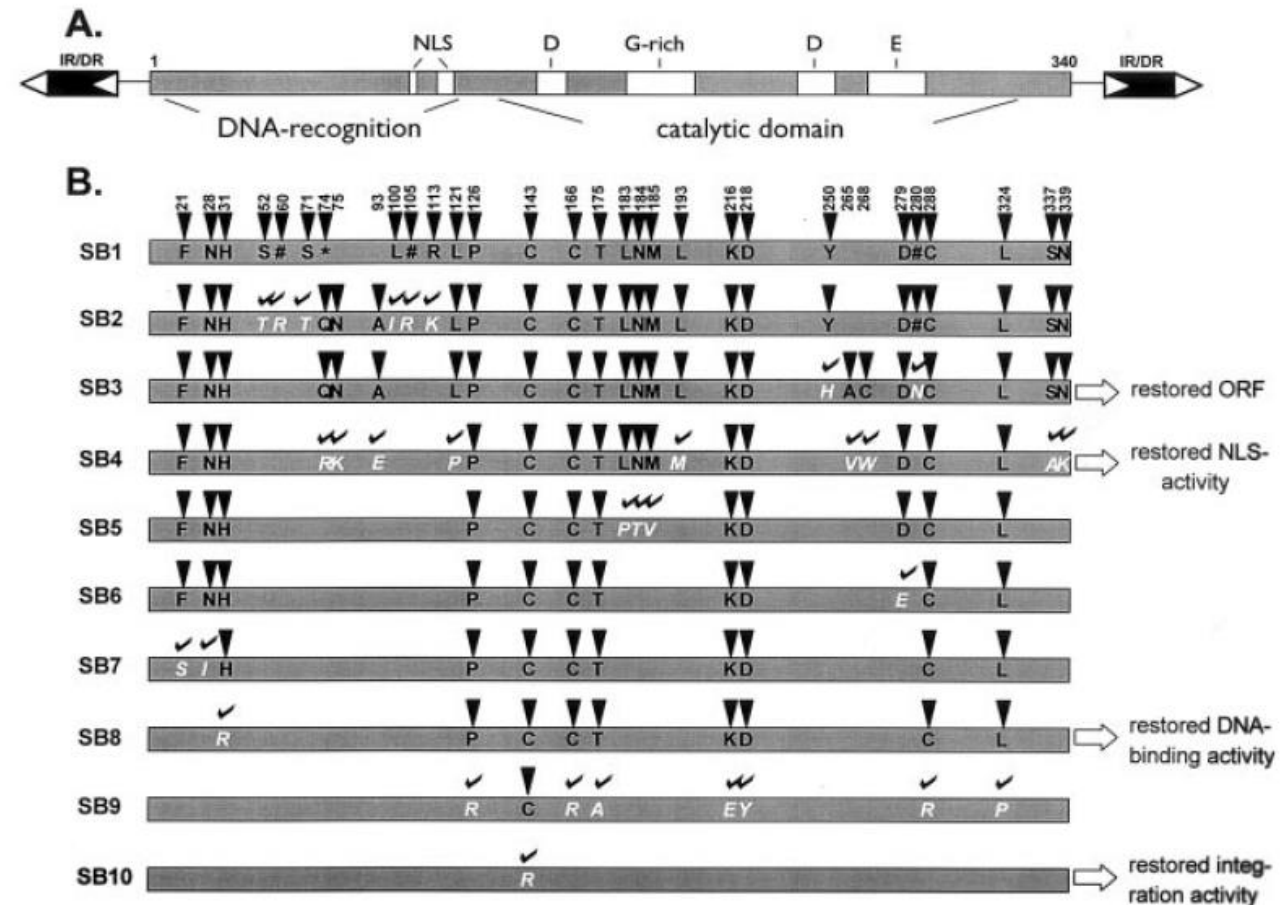
§Institute of Biochemistry

Biological Research Center of Hungarian

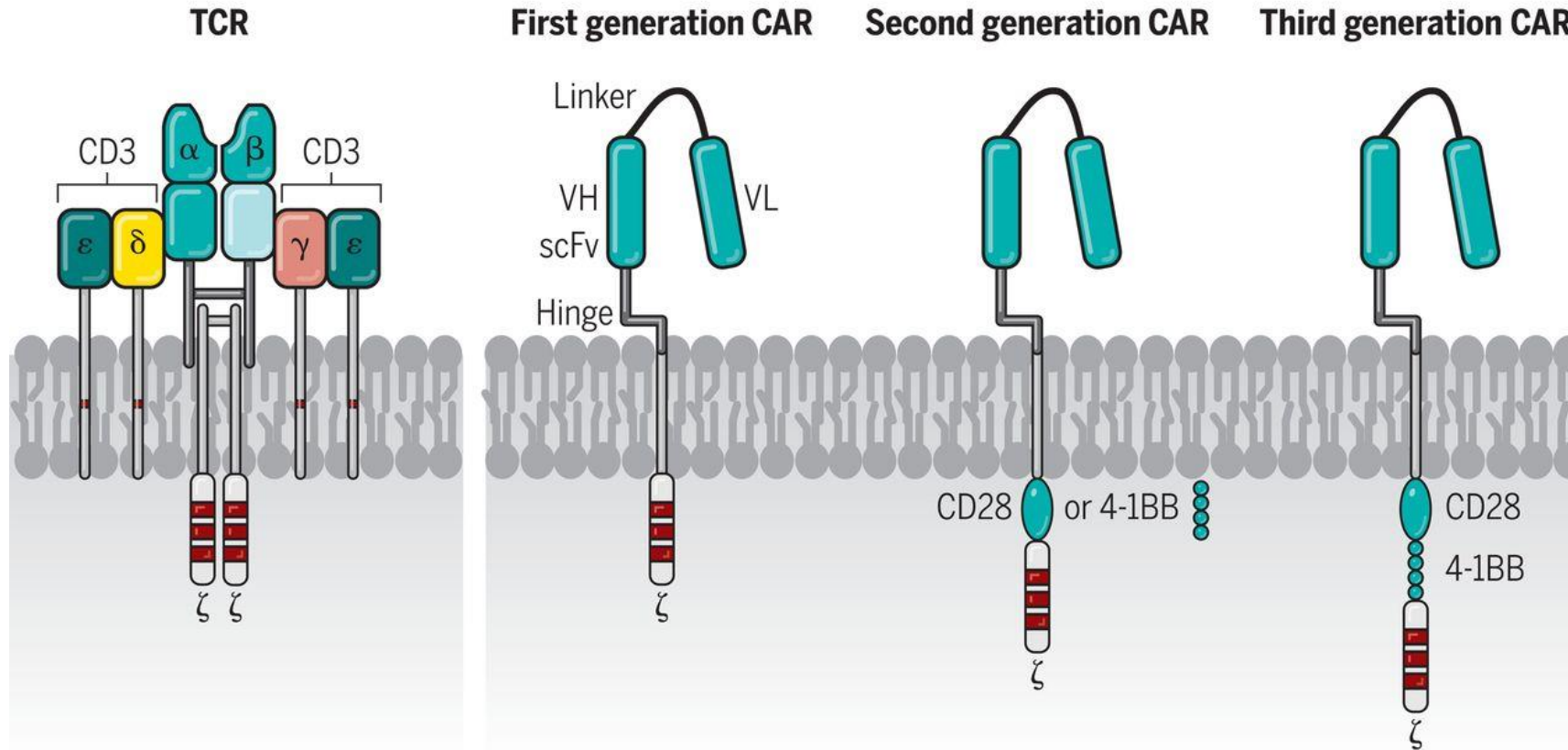
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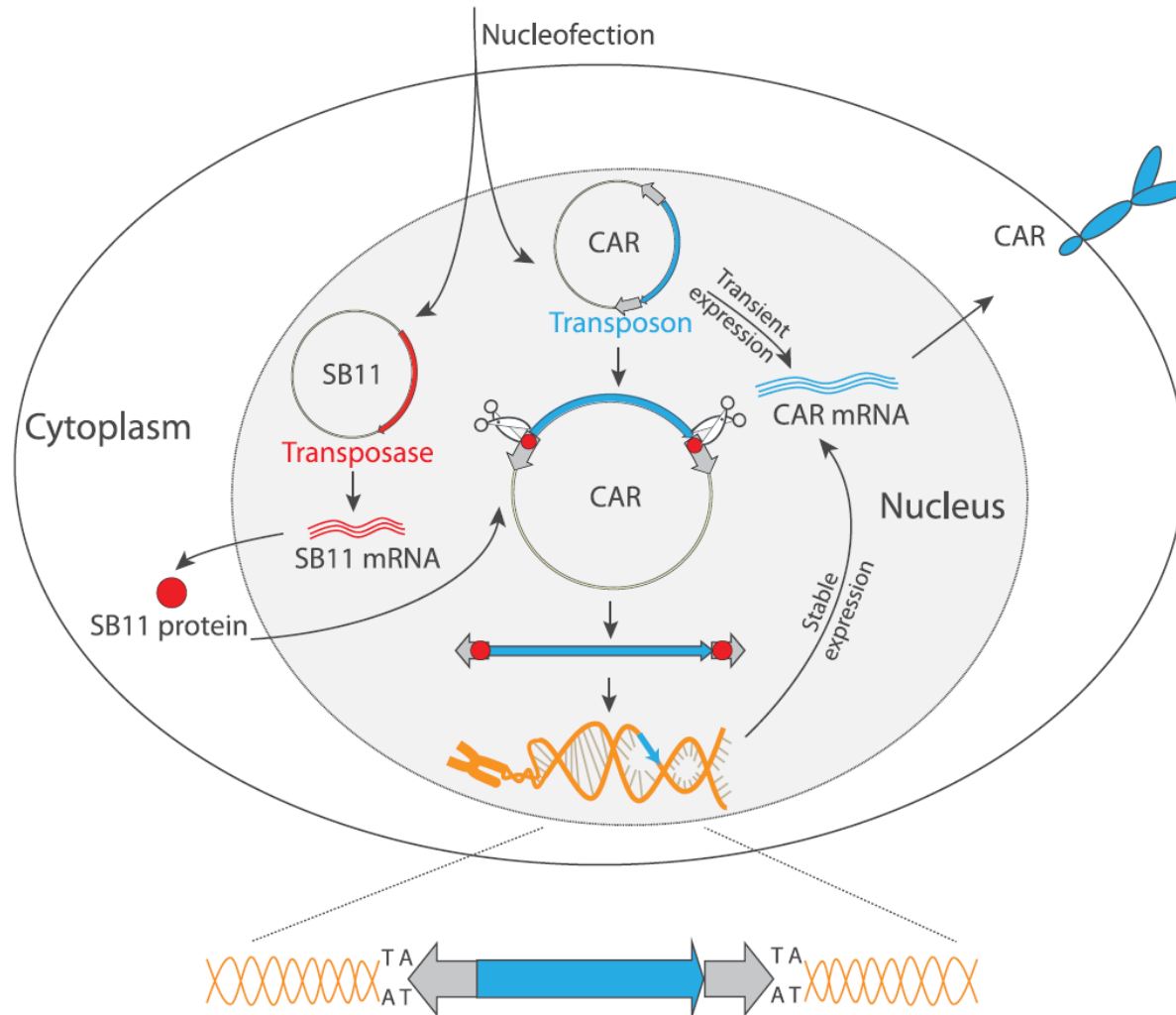


T-cell receptor (TCR) and chimeric antigen receptor (CAR)



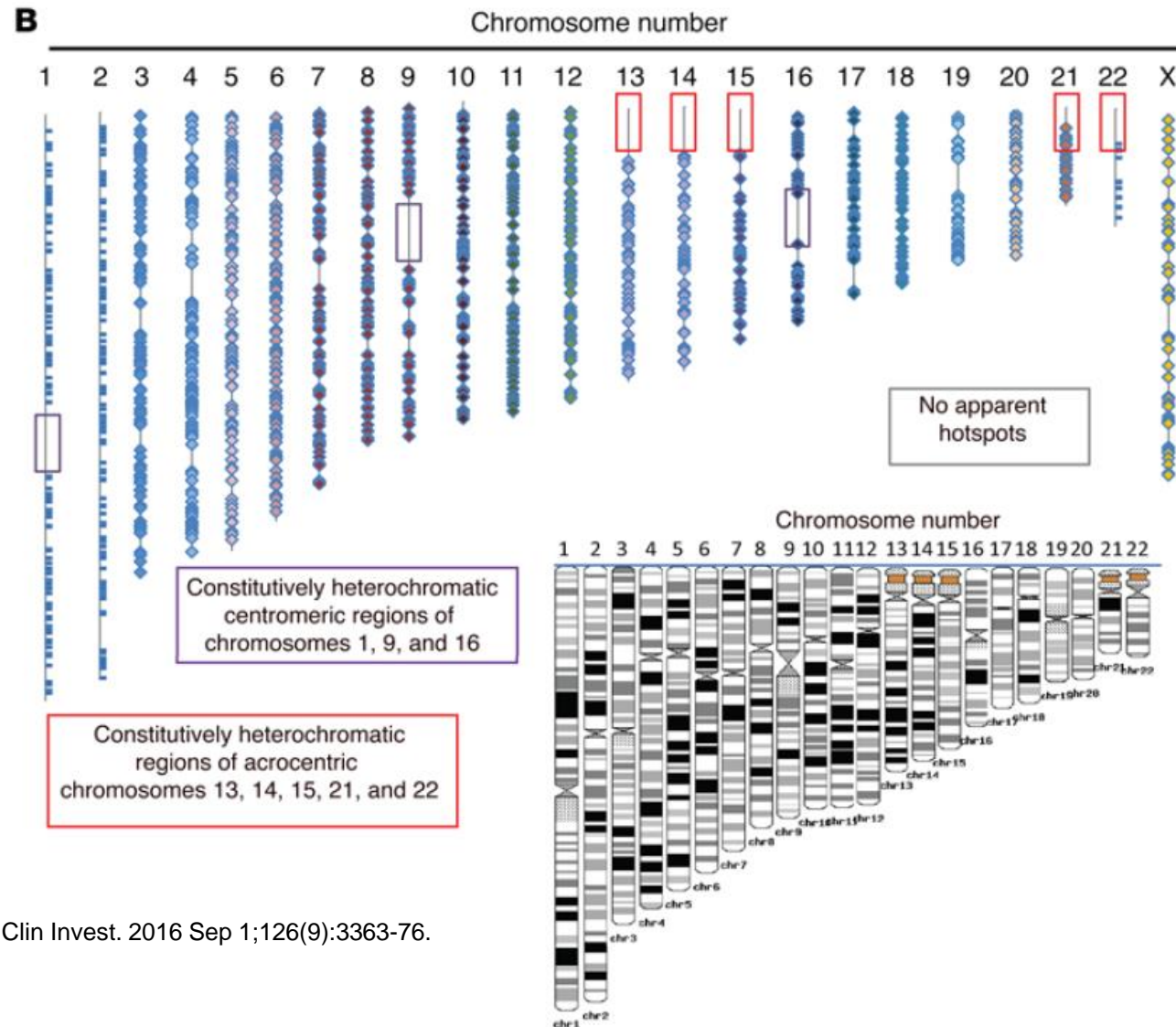
June CH, et al. Science. 2018 Mar 23;359(6382):1361-1365. doi: 10.1126/science.aar6711.

Integration of tumor-specific receptors into T cells via *Sleeping Beauty* transposition



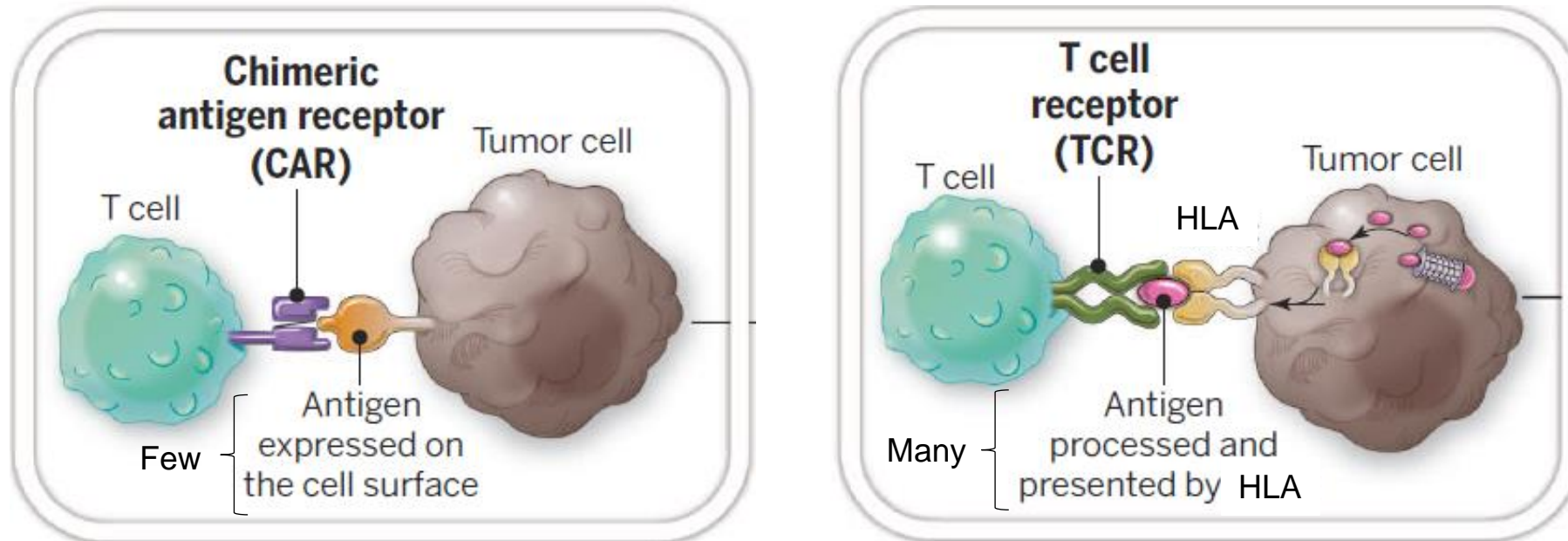
Singh H, et al. Immunol Rev 2014 Jan;257(1):181-90.

Integration profile of *Sleeping Beauty* transposition in human T cells was widely distributed



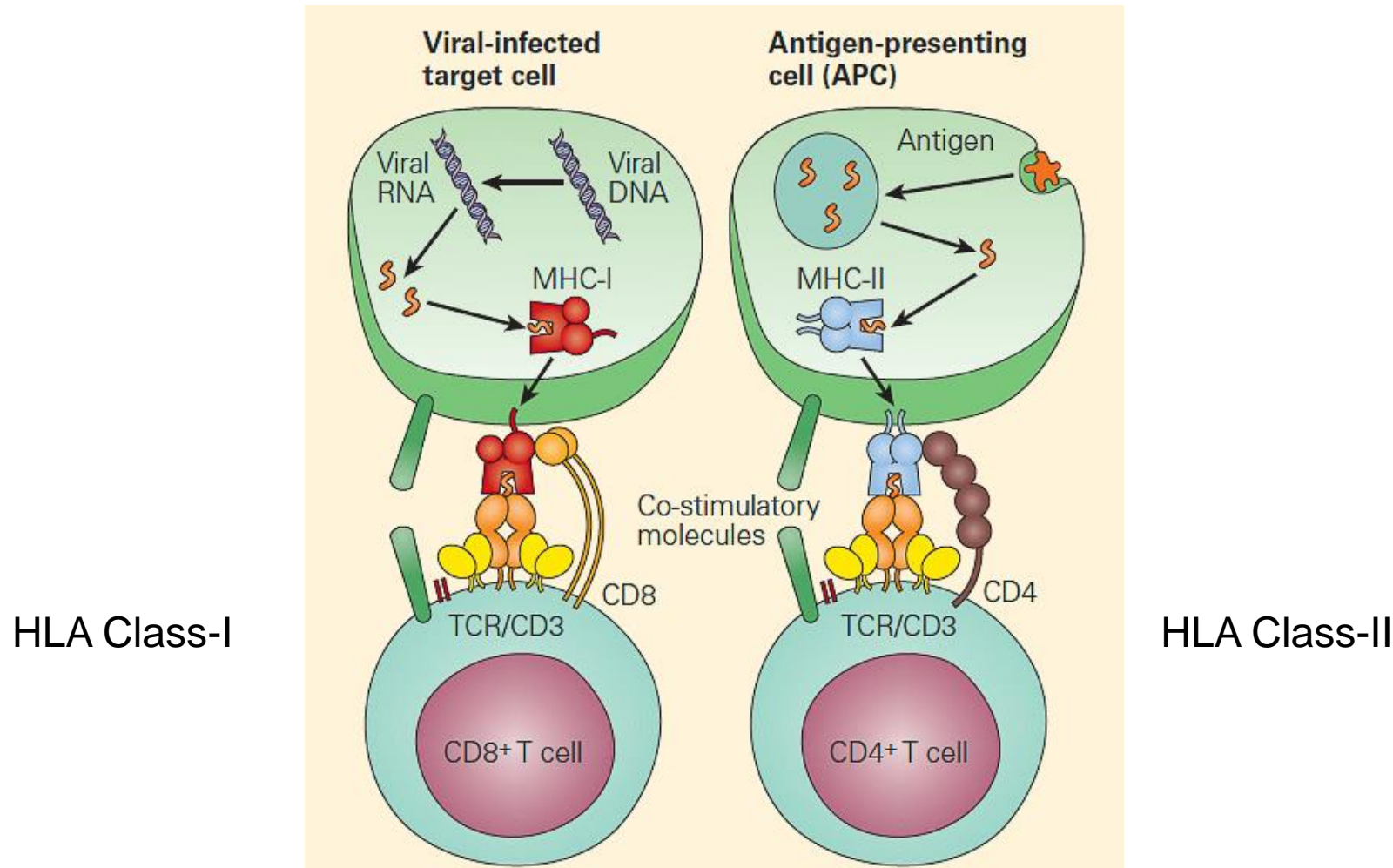
Keabriaei P et al. J Clin Invest. 2016 Sep 1;126(9):3363-76.

TCR-T cell therapy has exceptionally larger targeting capacity relative to CAR-T cell therapy

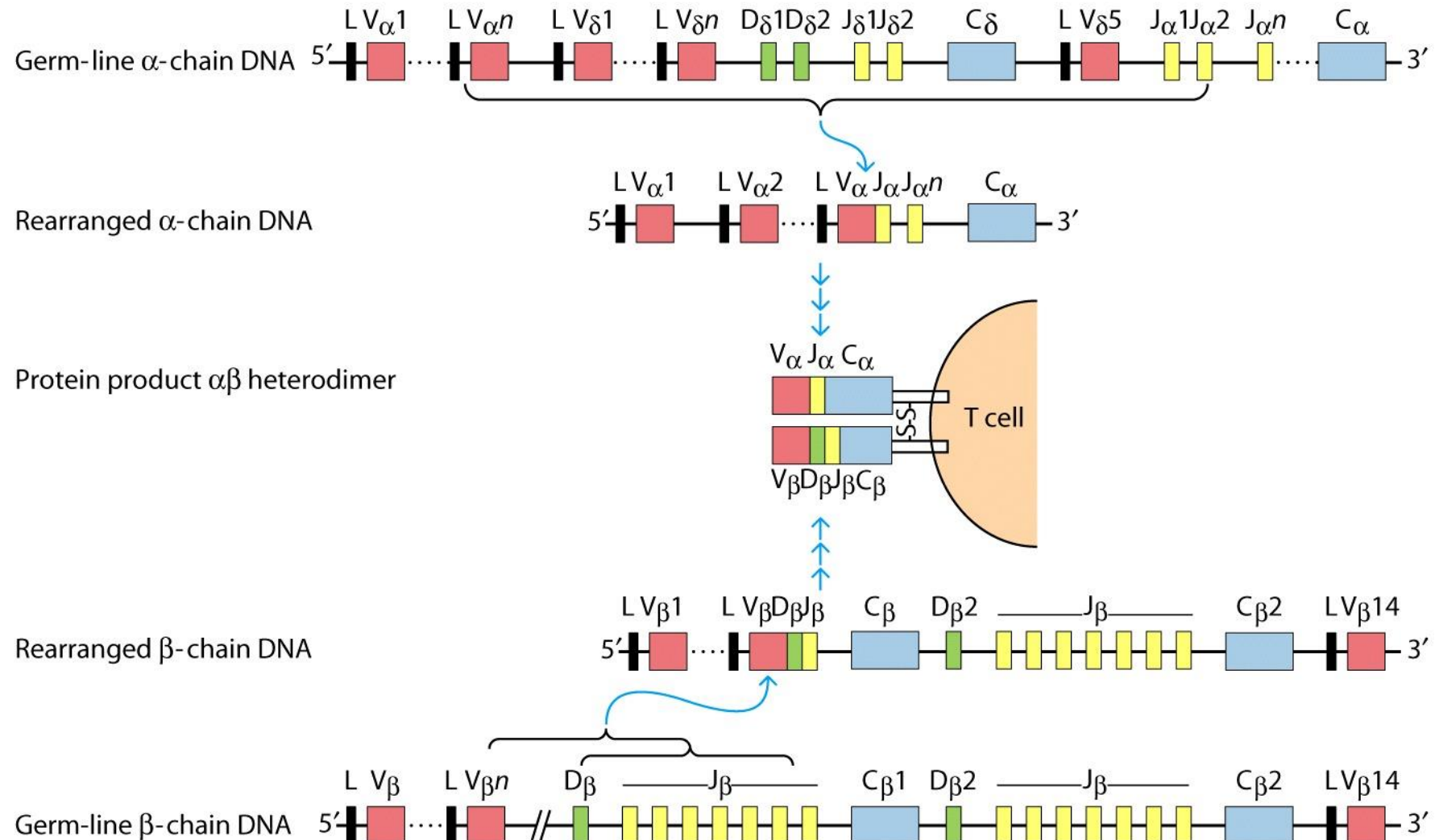


Rosenberg SA and Restifo NP. Science. 2015 Apr 3;348(6230):62-8.

Presentation of antigens on human leukocyte antigen (HLA) to T-cell receptor on the T-cell surface



VDJ recombination as genetic LEGOs in thymic development



Diversity of the TCR repertoire is complex on purpose

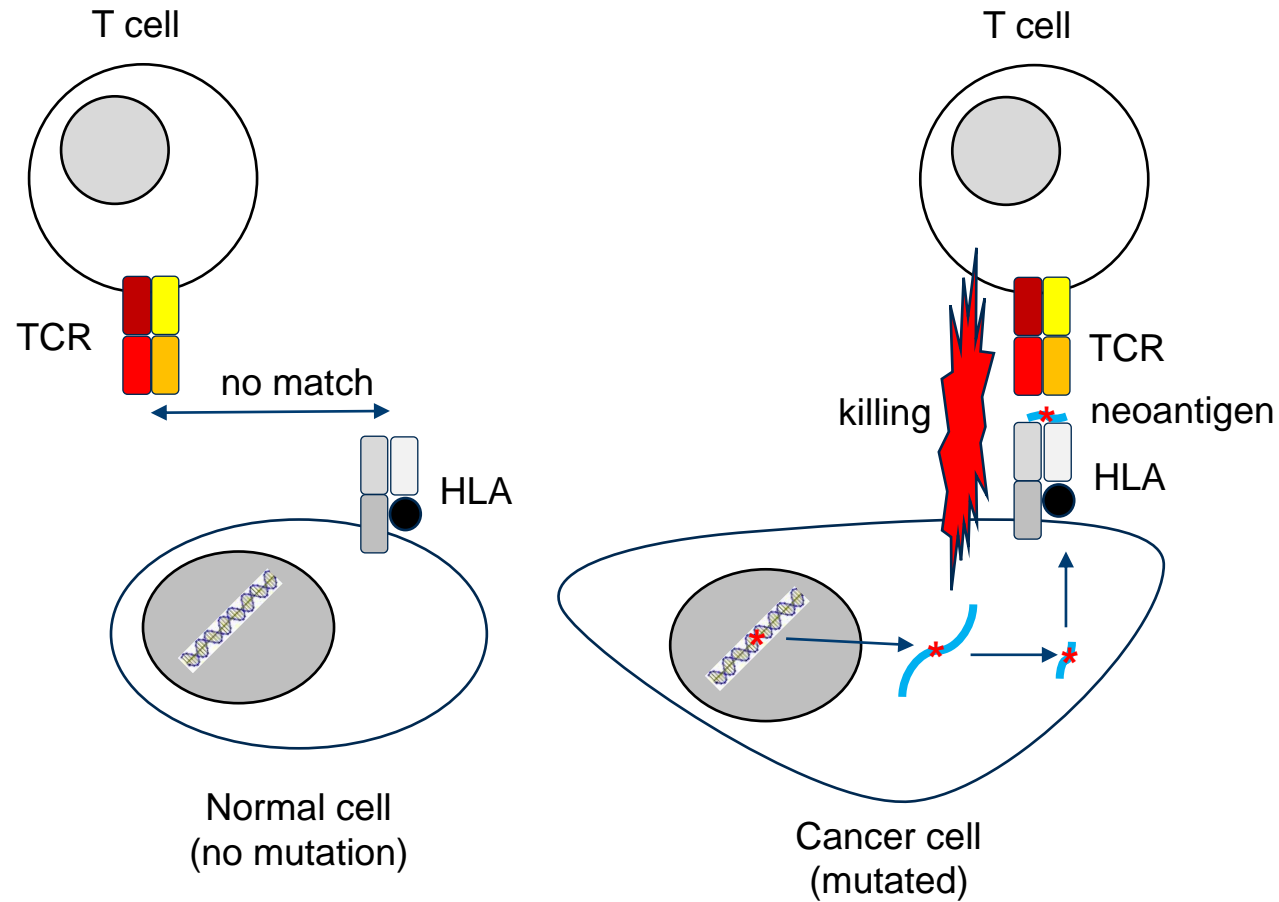
Element	Immunoglobulin		$\alpha:\beta$ T-cell receptors	
	H	$\kappa+\lambda$	β	α
Variable segments (V)	40	70	52	~70
Diversity segments (D)	25	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

Figure 5.9 The Immune System, 3ed. (© Garland Science 2009)

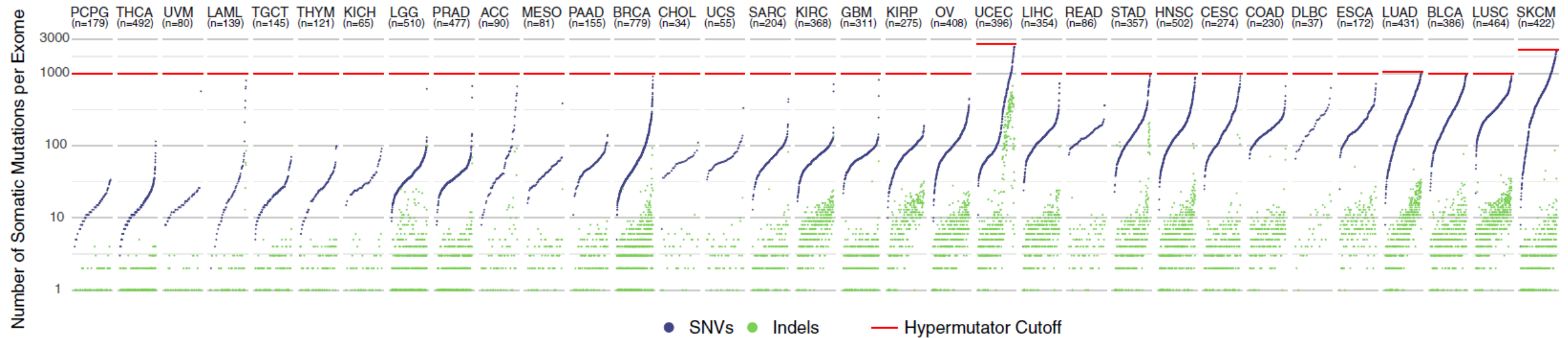
Somatic mutations are the blueprint for pan-cancer therapy

- Genomic instability is a hallmark of cancer. Somatic (non-inherited) mutations arising from this instability are largely unique to the patient, but a subset of mutations are shared in “hotspots” of critical cancer genes, *e.g.*, *KRAS* and *TP53*.
- Some somatic genetic mutations will be transcribed, translated, processed and presented on the cancer cell surface generating a “neoantigen” which is not in the normal cells.
- T cells, through their TCR, recognize neoantigens in the context of HLA and can kill the cancer cell with mutation.
- Transfer of neoantigen-specific TCRs into naïve T cells with *Sleeping Beauty* transposition would generate TCR-T cells with unique and highly tumor-specific reactivity.

Presentation of neoantigens to T cells leads to exquisite tumor-specificity of TCR-T cells



Virtually all cancers have somatic mutations which can become neoantigens if processed and presented on the tumor surface



Bailey MH, et al. Cell. 2018 Apr 5;173(2):371-385.e18.

Most epithelial cancer patients have TILs which recognize at least one autologous neoantigen

nature
medicine

Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells

Paul F Robbins¹, Yong-Chen Lu¹, Mona El-Gamil¹, Yong F Li¹, Colin Gross¹, Jared Gartner², Jimmy C Lin³, Jamie K Teer^{4,5}, Paul Clifton³, Eric Tycksen³, Yardena Samuels^{2,5} & Steven A Rosenberg¹

Robbins PF *et al.* Nat Med. 2013 Jun;19(6):747-52.

Tumor infiltrating lymphocytes (TIL) therapy in metastatic melanoma: boosting of neoantigen-specific T cell reactivity and long-term follow-up

Joost H van den Berg¹, Bianca Heemskerk², Nienke van Rooij², Raquel Gomez-Eerland², Samira Michels², Maaik van Zon¹, Renate de Boer¹, Noor A M Bakker¹, Annelies Jorritsma-Smit², Marit M van Buuren², Pia Kvistborg², Hergen Spits^{3,4}, Remko Schotte³, Henk Mallo⁵, Matthias Karger⁵, Joris A van der Hage⁶, Michel W J M Wouters^{7,8}, Loes M Pronk⁹, Marnix H Geukes Foppen⁵, Christian U Blank¹⁰, Jos H Beijnen^{11,12}, Bastiaan Nuijen¹¹, Ton N Schumacher^{2,13}, John B A G Haanen²

van den Berg JH *et al.* J Immunother Cancer 2020 Aug;8(2):e000848.

Detection of neoantigen-specific T cells following a personalized vaccine in a patient with glioblastoma

Tanner M. Johanns^{a,b}, Christopher A. Miller^c, Connor J. Liu^{b,d}, Richard J. Perrin^e, Diane Bender^b, Dale K. Kobayashi^b, Jian L. Campian^a, Michael R. Chicoine^d, Ralph G. Dacey^d, Jiayi Huang^f, Edward F. Fritsch^g, William E. Gillanders^{b,h}, Maxim N. Artyomov^{b,e}, Elaine R. Mardisⁱ, Robert D. Schreiber^{b,e}, and Gavin P. Dunn^{b,d}

Johanns TM *et al.* Oncoimmunology. 2019 Jan 25;8(4):e1561106.

CANCER IMMUNOTHERAPY

Immunogenicity of somatic mutations in human gastrointestinal cancers

Eric Tran, Mojgan Ahmadvadeh, Yong-Chen Lu, Alena Gros, Simon Turcotte,* Paul F. Robbins, Jared J. Gartner, Zhili Zheng, Yong F. Li, Satyajit Ray, John R. Wunderlich, Robert P. Somerville, Steven A. Rosenberg†

Tran E *et al.* Science. 2015 Dec 11;350(6266):1387-90.

Clinical Trials: Immunotherapy

T-cell Responses to TP53 "Hotspot" Mutations and Unique Neoantigens Expressed by Human Ovarian Cancers

Drew C. Deniger, Anna Pasetto, Paul F. Robbins, Jared J. Gartner, Todd D. Prickett, Biman C. Paria, Parisa Malekzadeh, Li Jia, Rami Yossef, Michelle M. Langhan, John R. Wunderlich, David N. Danforth, Robert P.T. Somerville, and Steven A. Rosenberg

Deniger DC *et al.* Clin Cancer Res. 2018 Nov 15;24(22):5562-5573.

ARTICLE

DOI: 10.1038/s41467-018-03301-0

OPEN

Sensitive and frequent identification of high avidity neo-epitope specific CD8⁺ T cells in immunotherapy-naïve ovarian cancer

Sara Bobisse¹, Raphael Genolet¹, Annalisa Roberti², Janos L. Tanyi², Julien Racle^{1,3}, Brian J. Stevenson³, Christian Iseli³, Alexandra Michel¹, Marie-Aude Le Bitoux¹, Philippe Guillaume¹, Julien Schmidt¹, Valentina Bianchi¹, Denarda Dangaj¹, Craig Fenwick⁴, Laurent Derré⁵, Ioannis Xenarios³, Olivier Michielin^{1,3}, Pedro Romero¹, Dimitri S. Monos⁶, Vincent Zoete^{1,3}, David Gfeller^{1,3}, Lana E. Kandalaft^{1,2}, George Coukos¹ & Alexandre Harari¹

Bobisse S *et al.* Nat Commun. 2018 Mar 15;9(1):1092.

Unique Neoantigens Arise from Somatic Mutations in Patients with Gastrointestinal Cancers

Maria R. Parkhurst¹, Paul F. Robbins¹, Eric Tran², Todd D. Prickett¹, Jared J. Gartner¹, Li Jia¹, Gabriel Ivey¹, Yong F. Li¹, Mona El-Gamil¹, Almin Lalani¹, Jessica S. Crystal¹, Abraham Sachs¹, Eric Groh¹, Satyajit Ray¹, Lien T. Ngo¹, Scott Kivitz¹, Anna Pasetto¹, Rami Yossef¹, Frank J. Lowery¹, Stephanie L. Goff¹, Winifred Lo¹, Gal Cafri¹, Drew C. Deniger¹, Parisa Malekzadeh¹, Mojgan Ahmadvadeh¹, John R. Wunderlich¹, Robert P.T. Somerville¹, and Steven A. Rosenberg¹

Parkhurst MR *et al.* Cancer Discov. 2019 Aug;9(8):1022-1035.

Landscape of immunogenic tumor antigens in successful immunotherapy of virally induced epithelial cancer

Sanja Stevanović^{1,*}, Anna Pasetto², Sarah R. Helman¹, Jared J. Gartner², Todd D. Prickett², Bryan Howie³, Harlan S. Robins^{3,4}, Paul F. Robbins², Christopher A. Klebanoff^{5,6}, Steven A. Rosenberg², Christian S. Hinrichs^{1,*}

Stevanović S *et al.* Science 2017 Apr 14;356(6334):200-205.

The Journal of Immunology

Identification of Neoantigen-Reactive Tumor-Infiltrating Lymphocytes in Primary Bladder Cancer

Vid Leko^{*,†}, Lucas A. McDuffie^{*,†}, Zhili Zheng^{*}, Jared J. Gartner^{*}, Todd D. Prickett^{*}, Andrea B. Apolo[‡], Piyush K. Agarwal[§], Steven A. Rosenberg^{*}, and Yong-Chen Lu^{*}

Leko V *et al.* J Immunol. 2019 Jun 15;202(12):3458-3467.

Shared “hotspot” mutations in *KRAS* and *TP53* genes are immunogenic and can be used for off-the-shelf TCR-T cells

The Journal of Clinical Investigation

CLINICAL MEDICINE

The Journal of Clinical Investigation

CONCISE COMMUNICATION

mRNA vaccine–induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer

Gal Cafri,^{1,2} Jared J. Gartner,¹ Tal Zaks,³ Kristen Hopson,³ Noam Levin,¹ Biman C. Paria,¹ Maria R. Parkhurst,¹ Rami Yossef,¹ Frank J. Lowery,¹ Mohammad S. Jafferji,¹ Todd D. Prickett,¹ Stephanie L. Goff,¹ Christine T. McGowan,¹ Samantha Seitter,¹ Mackenzie L. Shindorf,¹ Anup Parikh,¹ Praveen D. Chatani,¹ Paul F. Robbins,¹ and Steven A. Rosenberg¹

¹Surgery Branch, National Cancer Institute (NCI), NIH, Bethesda, Maryland, USA. ²Sheba Medical Center, Ramat Gan, Israel. ³Moderna Inc., Cambridge, Massachusetts, USA.

Cafri G *et al.* J Clin Invest. 2020 Nov 2;130(11):5976-5988.

ARTICLE

<https://doi.org/10.1038/s41467-019-08304-z>

OPEN

Memory T cells targeting oncogenic mutations detected in peripheral blood of epithelial cancer patients

Gal Cafri¹, Rami Yossef¹, Anna Pasetto¹, Drew C. Deniger¹, Yong-Chen Lu¹, Maria Parkhurst¹, Jared J. Gartner¹, Li Jia¹, Satyajit Ray¹, Lien T. Ngo¹, Mohammad Jafferji¹, Abraham Sachs¹, Todd Prickett¹, Paul F. Robbins¹ & Steven A. Rosenberg¹

Cafri G *et al.* Nat Commun. 2019 Jan 25;10(1):449.

Enhanced detection of neoantigen-reactive T cells targeting unique and shared oncogenes for personalized cancer immunotherapy

Rami Yossef,¹ Eric Tran,^{1,2} Drew C. Deniger,¹ Alena Gros,^{1,3} Anna Pasetto,¹ Maria R. Parkhurst,¹ Jared J. Gartner,¹ Todd D. Prickett,¹ Gal Cafri,¹ Paul F. Robbins,¹ and Steven A. Rosenberg¹

Yossef R *et al.* J Clin Invest. 2019 Mar 1;129(3):1109-1114.

Neoantigen screening identifies broad *TP53* mutant immunogenicity in patients with epithelial cancers

Parisa Malekzadeh,¹ Anna Pasetto,¹ Paul F. Robbins,¹ Maria R. Parkhurst,¹ Biman C. Paria,¹ Li Jia,¹ Jared J. Gartner,¹ Victoria Hill,¹ Zhiya Yu,¹ Nicholas P. Restifo,¹ Abraham Sachs,¹ Eric Tran,^{1,2} Winifred Lo,¹ Robert P.T. Somerville,¹ Steven A. Rosenberg,¹ and Drew C. Deniger¹

¹Surgery Branch, National Cancer Institute, Bethesda, Maryland, USA. ²Earle A. Chiles Research Institute, Providence Cancer Institute, Portland, Oregon, USA.

Malekzadeh P *et al.* J Clin Invest. 2019 Mar 1;129(3):1109-1114.

Cancer Immunology Miniature

Cancer Immunology Research

Immunologic Recognition of a Shared p53 Mutated Neoantigen in a Patient with Metastatic Colorectal Cancer

Winifred Lo^{1,2}, Maria Parkhurst², Paul F. Robbins², Eric Tran³, Yong-Chen Lu², Li Jia², Jared J. Gartner², Anna Pasetto², Drew Deniger², Parisa Malekzadeh², Thomas E. Shelton², Todd Prickett², Satyajit Ray², Scott Kivitz², Biman C. Paria², Isaac Kriley^{1,2}, David S. Schrupp¹, and Steven A. Rosenberg²

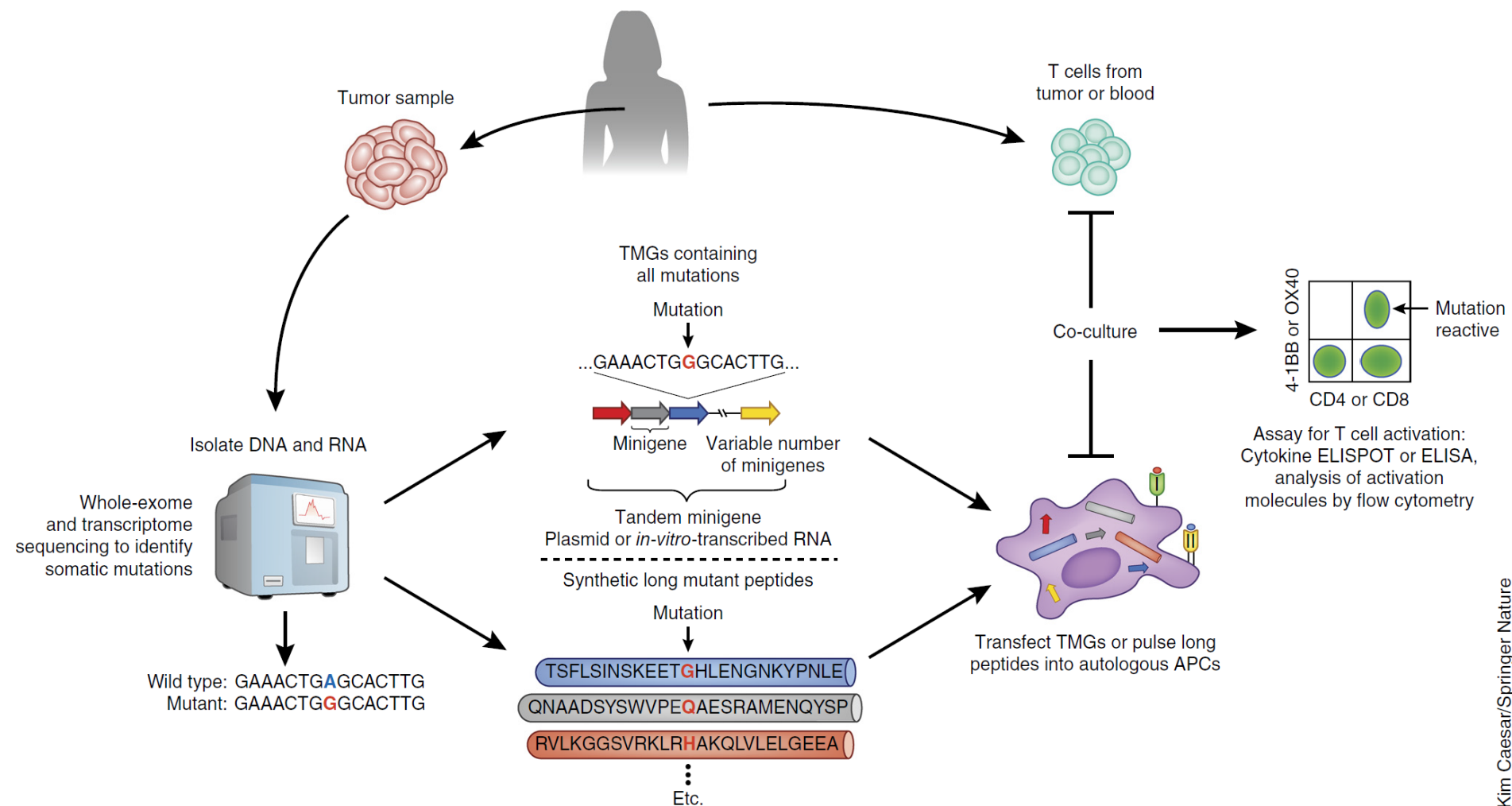
Lo W *et al.* Cancer Immunol Res. 2019 Apr;7(4):534-543.

Antigen Experienced T Cells from Peripheral Blood Recognize p53 Neoantigens

Parisa Malekzadeh¹, Rami Yossef¹, Gal Cafri¹, Biman C. Paria¹, Frank J. Lowery¹, Mohammad Jafferji¹, Meghan L. Good¹, Abraham Sachs¹, Amy R. Copeland¹, Sanghyun P. Kim¹, Scott Kivitz¹, Maria R. Parkhurst¹, Paul F. Robbins¹, Satyajit Ray¹, Liqiang Xi², Mark Raffeld², Zhiya Yu¹, Nicholas P. Restifo¹, Robert P.T. Somerville¹, Steven A. Rosenberg¹, and Drew C. Deniger¹

Malekzadeh P *et al.* Clin Cancer Res. 2020 Mar 15;26(6):1267-1276.

Unbiased identification of neoantigen-reactive T cells



Kim Caesar/Springer Nature

Tran E, Robbins PF and Rosenberg SA. Nat Immunol. 2017 Feb 15;18(3):255-262. doi: 10.1038/ni.3682.

Neoantigen-reactive T cells (non-gene modified) resulted in objective regressions of metastatic epithelial cancers



Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Eric Tran,¹ Simon Turcotte,^{1*} Alena Gros,¹ Paul F. Robbins,¹ Yong-Chen Lu,¹ Mark E. Dudley,^{1†} John R. Wunderlich,¹ Robert P. Somerville,¹ Katherine Hogan,¹ Christian S. Hinrichs,¹ Maria R. Parkhurst,¹ James C. Yang,¹ Steven A. Rosenberg^{1‡}

Tran E *et al.* Science. 2014 May 9;344(6184):641-5.

1 unique driver
neoantigen from
26 total mutations



The NEW ENGLAND
JOURNAL of MEDICINE

ORIGINAL ARTICLE BRIEF REPORT

T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer

Eric Tran, Ph.D., Paul F. Robbins, Ph.D., Yong-Chen Lu, Ph.D., Todd D. Prickett, Ph.D., Jared J. Gartner, M.Sc., Li Jia, M.Sc., Anna Pasetto, Ph.D., Zhili Zheng, Ph.D., Satyajit Ray, Ph.D., Eric M. Groh, M.D., Isaac R. Kriley, M.D., and Steven A. Rosenberg, M.D., Ph.D.

Tran E *et al.* N Engl J Med. 2016 Dec 8;375(23):2255-2262 **Driver gene**

nature
medicine

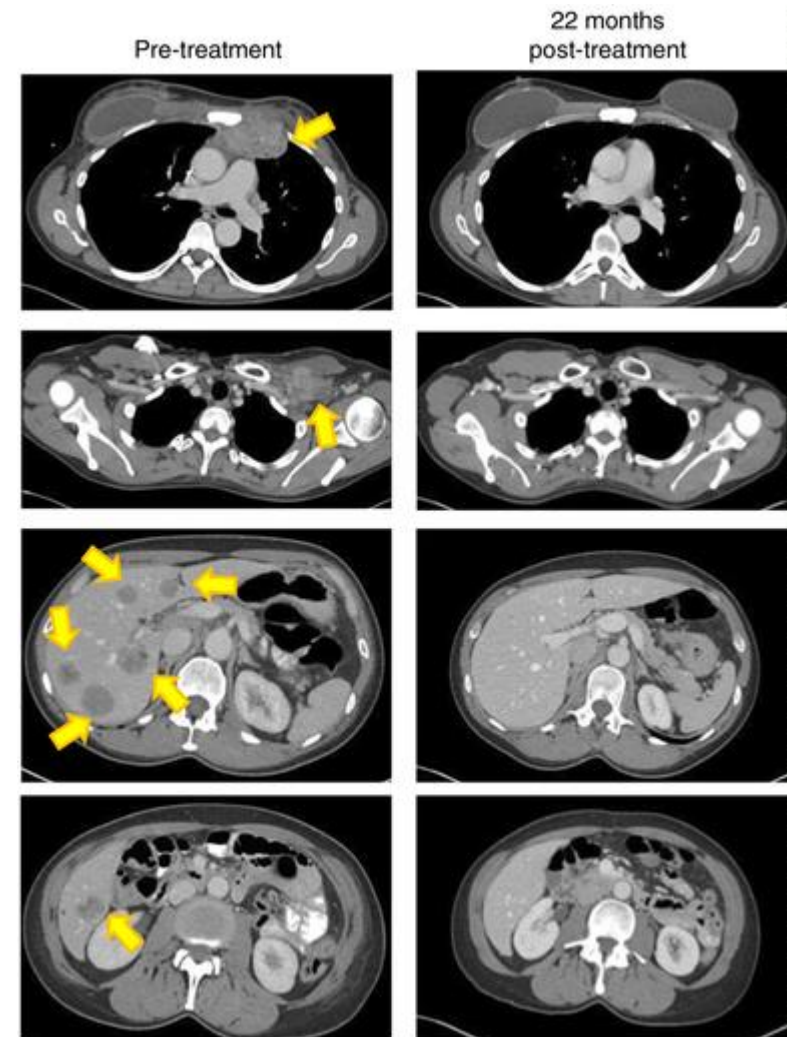
Letter | Published: 04 June 2018

Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer

Nikolaos Zacharakis, Harshini Chinnasamy, Mary Black, Hui Xu, Yong-Chen Lu, Zhili Zheng, Anna Pasetto, Michelle Langhan, Thomas Shelton, Todd Prickett, Jared Gartner, Li Jia, Katarzyna Trebska-McGowan, Robert P. Somerville, Paul F. Robbins, Steven A. Rosenberg, Stephanie L. Goff & Steven A. Feldman

Zacharakis N *et al.* Nat Med. 2018 Jun;24(6):724-730.

Multiple unique
neoantigen
specificities



Application of neoantigen-specific TCR-T cell therapy

- Prior TCR-T cell therapy established that metastatic cancers can be effectively treated in some patients when targeting non-neoantigen targets, but the application was limited by target expression and cancer type.
- Non-gene modified adoptive cell therapy (TIL) resulted in objective clinical regressions in some patients but was ineffective for most people likely due to infrequent and/or terminal differentiation of neoantigen-specific T cells.
- The ability to translate a library or personalized neoantigen-specific TCR-T cell approach is complex and will likely require a rapid, mobile and cost-effective solution.

***Sleeping Beauty* transposition is an ideal candidate for this because it uses plasmid DNA, which is inexpensive to manufacture and allows for rapid personalization.**

Sleeping Beauty transposition has been established for TCR-T cell and translated for CAR-T cell to the clinic

The Journal of Clinical Investigation

CLINICAL MEDICINE

Phase I trials using *Sleeping Beauty* to generate CD19-specific CAR T cells

Partow Kebriaei,¹ Harjeet Singh,² M. Helen Huls,² Matthew J. Figliola,² Roland Bassett,² Simon Olivares,² Bipulendu Jena,² Margaret J. Dawson,² Pappanaicken R. Kumaresan,² Shihuang Su,² Sourindra Maiti,² Jianliang Dai,³ Branden Moriarity,⁴ Marie-Andrée Forget,^{2,5} Vladimir Senyukov,² Aaron Orozco,² Tingting Liu,¹ Jessica McCarty,¹ Rineka N. Jackson,² Judy S. Moyes,² Gabriela Rondon,¹ Muzaffar Qazilbash,¹ Stefan Ciurea,¹ Amin Alousi,¹ Yago Nieto,¹ Katy Rezvani,¹ David Marin,¹ Uday Popat,¹ Chitra Hosing,¹ Elizabeth J. Shpall,¹ Hagop Kantarjian,⁶ Michael Keating,⁶ William Wierda,⁶ Kim Anh Do,³ David A. Largaespada,⁴ Dean A. Lee,^{2,7} Perry B. Hackett,⁴ Richard E. Champlin,¹ and Laurence J.N. Cooper^{2,7}

Kebriaei P et al. J Clin Invest. 2016 Sep 1;126(9):3363-76.



TO THE EDITOR:

Long-term outcomes of *Sleeping Beauty*-generated CD19-specific CAR T-cell therapy for relapsed-refractory B-cell lymphomas

S. A. Srour,¹ H. Singh,² J. McCarty,¹ E. de Groot,³ H. Huls,² G. Rondon,¹ M. Qazilbash,¹ S. Ciurea,¹ G. Bardelli,³ J. Buck,³ A. Alousi,¹ Y. Nieto,¹ K. Rezvani,¹ D. Marin,¹ U. Popat,¹ C. Hosing,¹ E. J. Shpall,¹ W. G. Wierda,⁴ H. Kantarjian,⁴ R. E. Champlin,¹ L. J. Cooper,³ and P. Kebriaei¹

Srour SA et al. Blood. 2020 Mar 12;135(11):862-865.

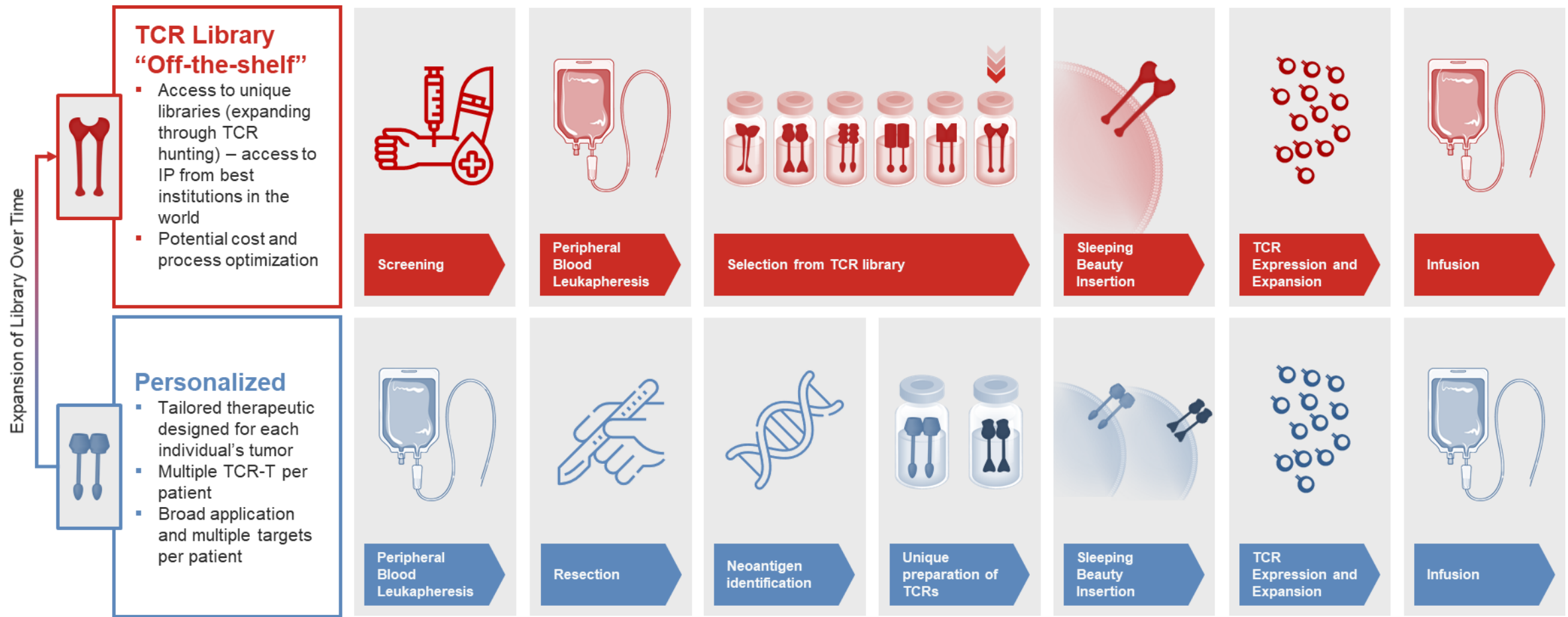
Stable, Nonviral Expression of Mutated Tumor Neoantigen-specific T-cell Receptors Using the *Sleeping Beauty* Transposon/Transposase System

Drew C Deniger¹, Anna Pasetto¹, Eric Tran¹, Maria R Parkhurst¹, Cyrille J Cohen², Paul F Robbins¹, Laurence JN Cooper^{3,4} and Steven A Rosenberg¹

¹Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; ²Tumor Immunology and Immunotherapy, Bar-Ilan University, Ramat Gan, Israel; ³Division of Pediatrics, University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA; ⁴ZIOPHARM Oncology, Inc., Boston, Massachusetts, USA

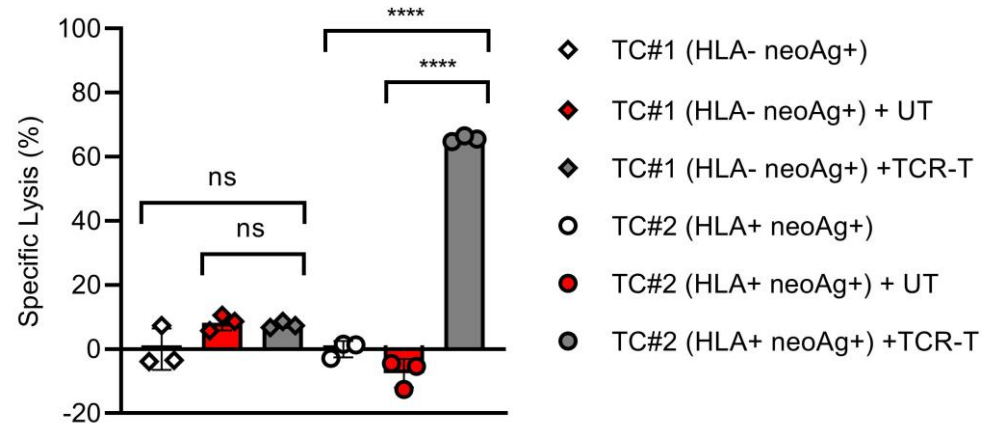
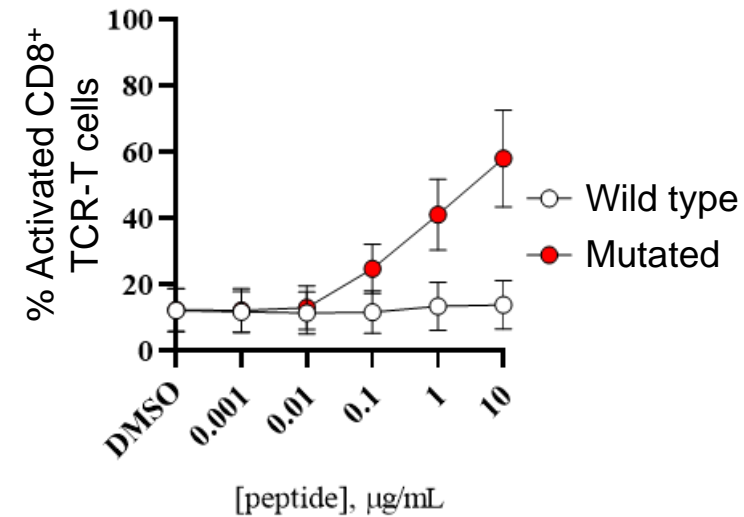
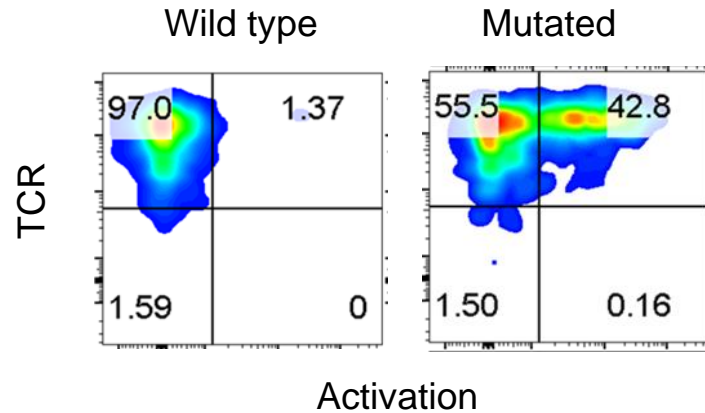
Deniger DC et al. Mol Ther. 2016 Jun;24(6):1078-1089.

Two cutting-edge Ziopharm programs for TCR-T cell therapy in the treatment of epithelial cancers



Ziopharm is the first and currently only commercial group evaluating library TCR-T cells targeting shared “hotspot” neoantigens in the non-viral setting.

Ziopharm's *Sleeping Beauty* library TCR-T cells were neoantigen-specific and led to tumor cell lysis



Conclusions

- Neoantigens are the blueprint and “Achilles heel” for effective targeting of all cancers.
- TCR-T cell therapy is the answer to targeting neoantigens, which will require a rapid, flexible, cost-effective gene transfer platform, of which *Sleeping Beauty* transposition is the most advanced and commercially appealing.
- Ziopharm is the world leader of *Sleeping Beauty*-transposed TCR-T cell therapy and is positioned for clinical and commercial success treating solid tumors.