T Cell Stemness: An Emerging Principle of Successful Adoptive Cell Therapy

Nicholas P Restifo, MD

NCI/UCSF Meeting on: Cell-Based Immunotherapy: From Bench to Bedside and Beyond
January 22, 2019

No financial conflicts of interest

All reproducibly effective cancer immunotherapies involve T cells

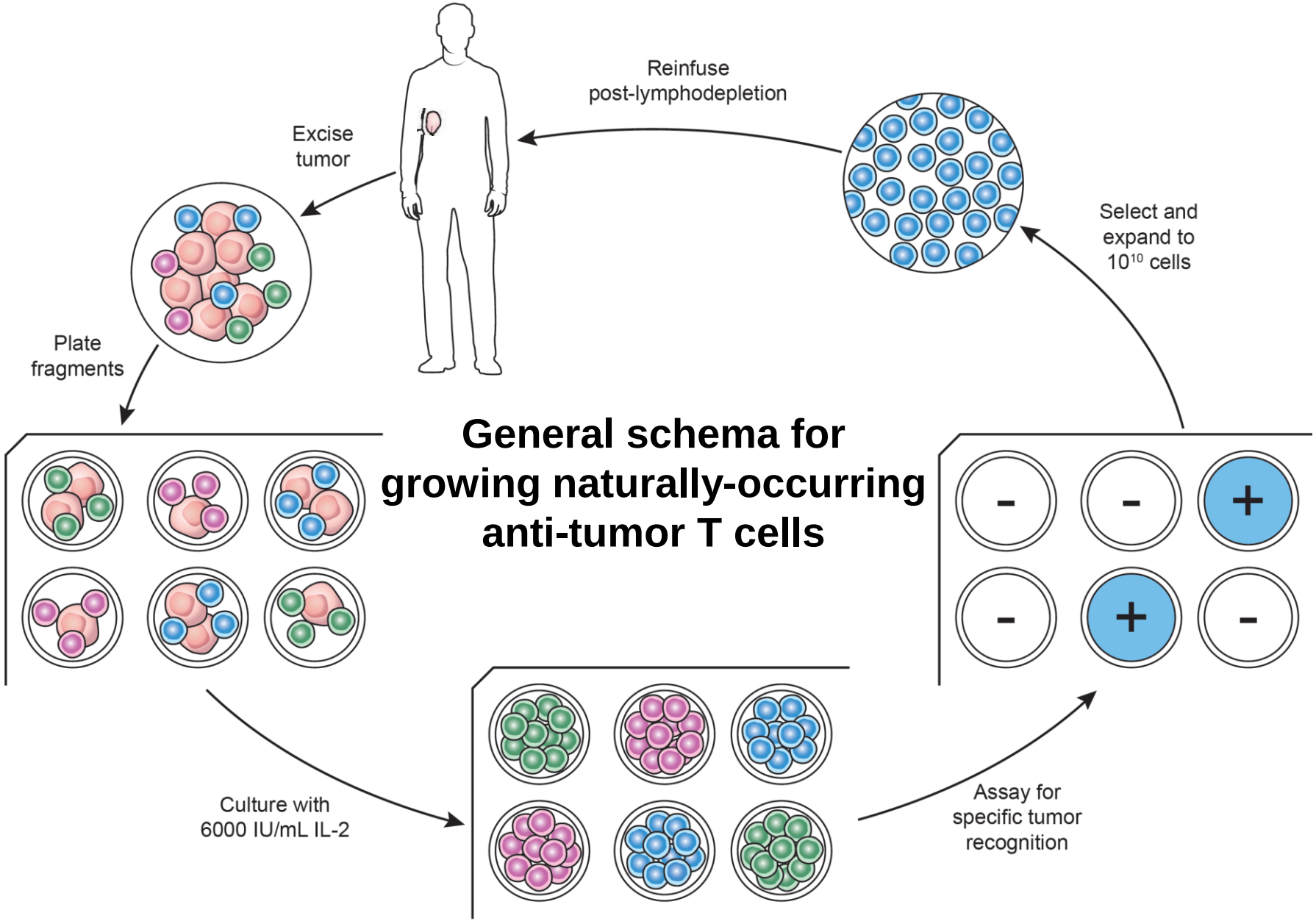
Cancer Vaccines

T cells

Tumor cells

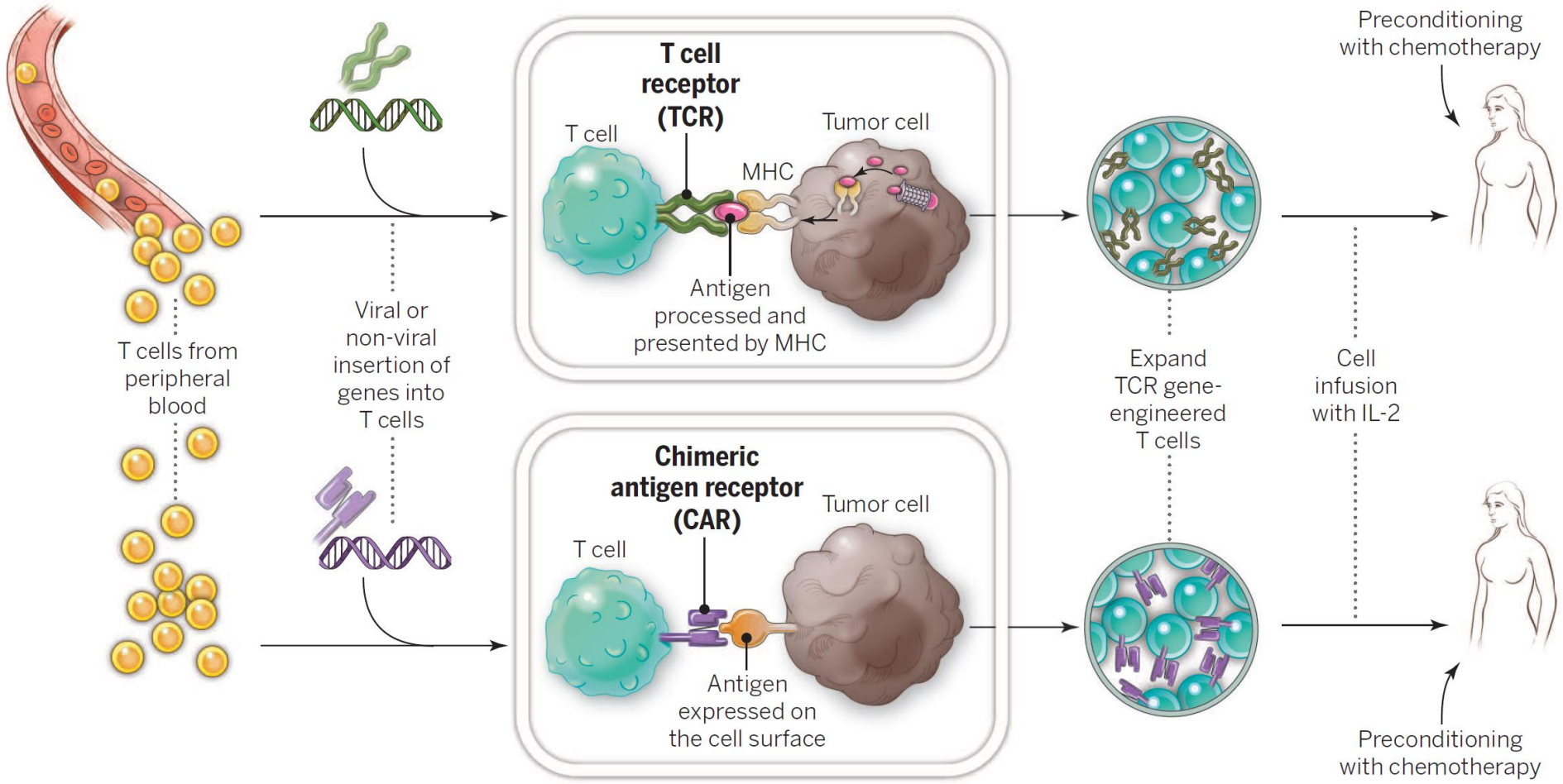
Checkpoint blockade anti-PD-(L)1, anti-CTLA-4

CAR/TCR/TIL-based treatments

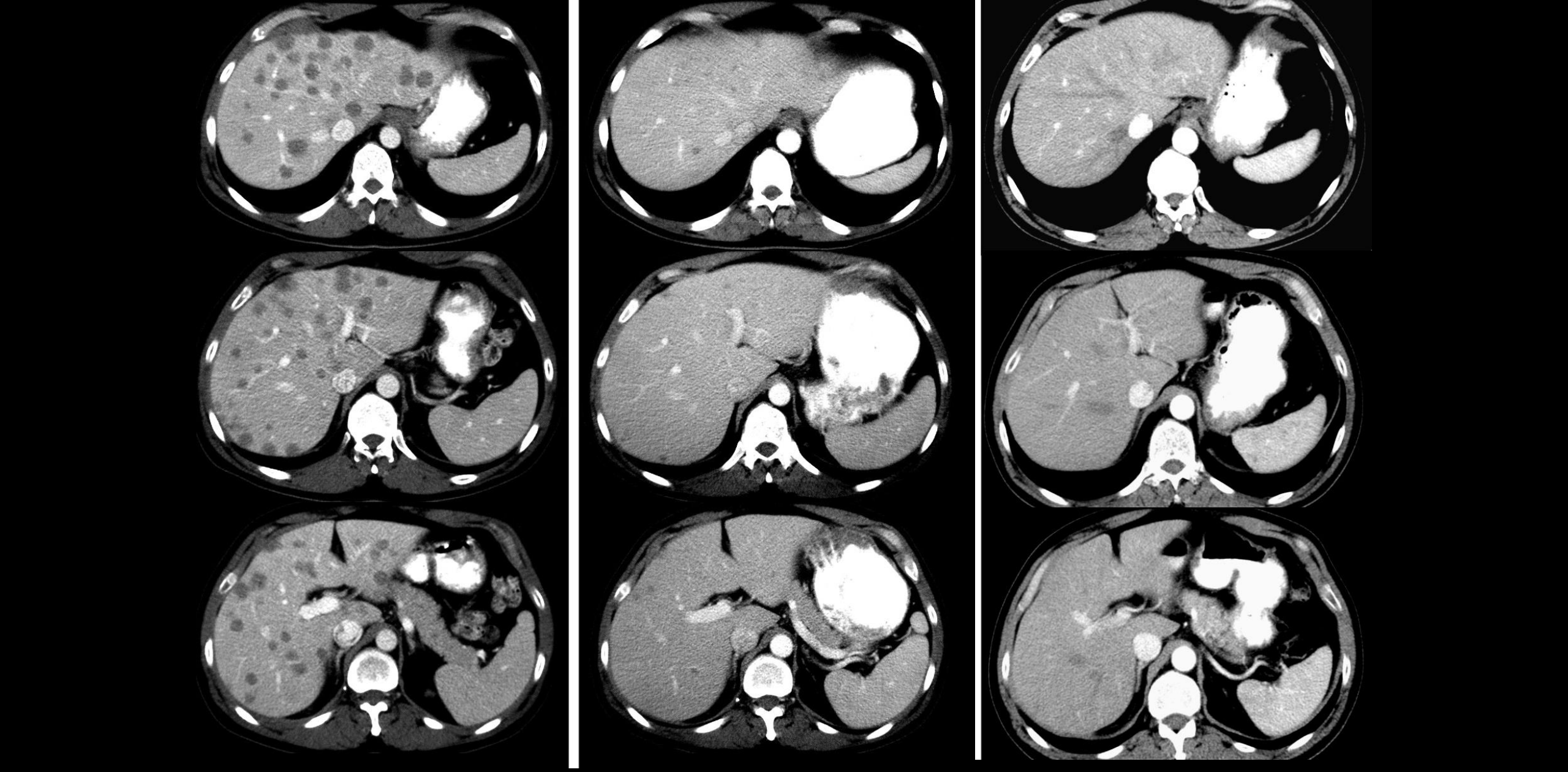


Rosenberg & Restifo
Science 2015

Gene-modification of T cells



Kochenderfer, et al, Blood 2010; Rosenberg & Restifo, Science 2015

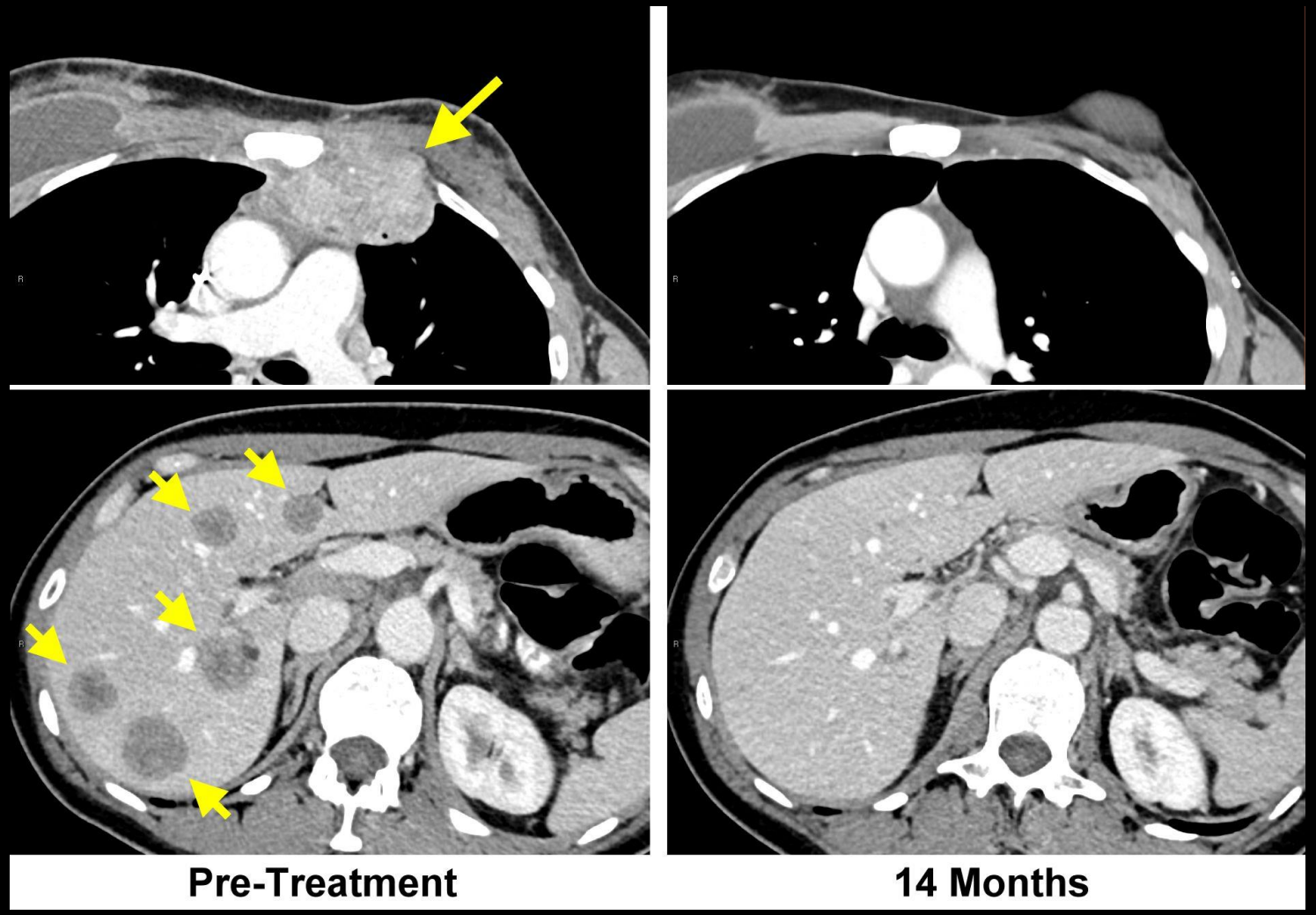


Pre-Tx

Day +34

7 years later

Complete regression of metastatic breast cancer



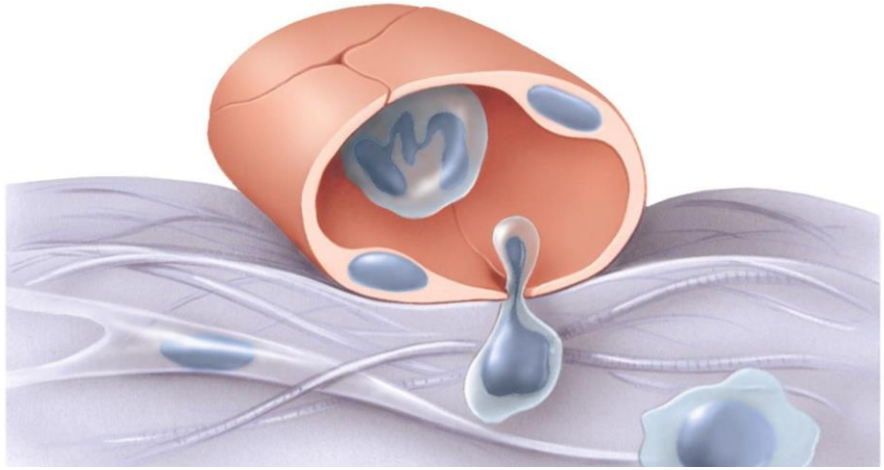
Adapted from Zacharakis et al, *Nat Med* 2018

Why do our efforts focus on adoptive cell transfer (ACT) therapy for treating metastatic cancer?

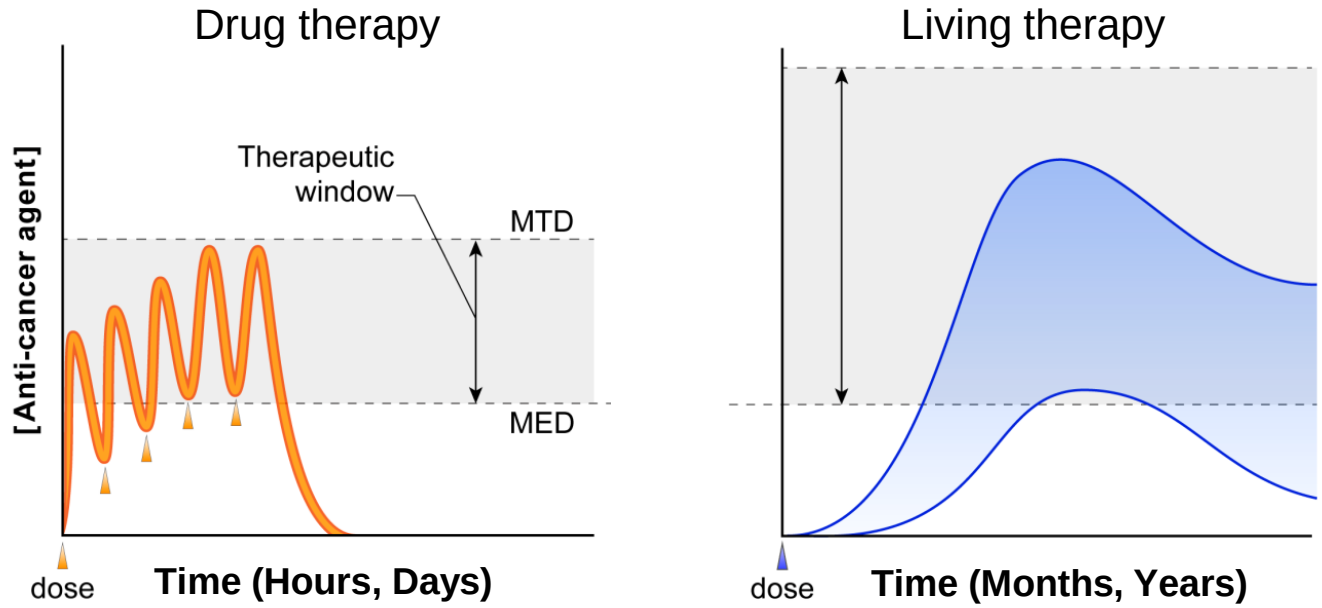
1. **Identify and enrich** for qualities associated with anti-tumor efficacy.
2. **Pharmacologically or genetically modify** T cells to enhance their therapeutic efficacy.
3. **Confer new specificity** in transferred T cells (eg CAR, TCR).
4. **Administer large numbers** of tumor antigen-specific cells.
5. **‘Lymphodeplete’** host prior to cell transfer, reducing immunosuppressive cells in the tumor microenvironment.

CELLS AS DRUGS

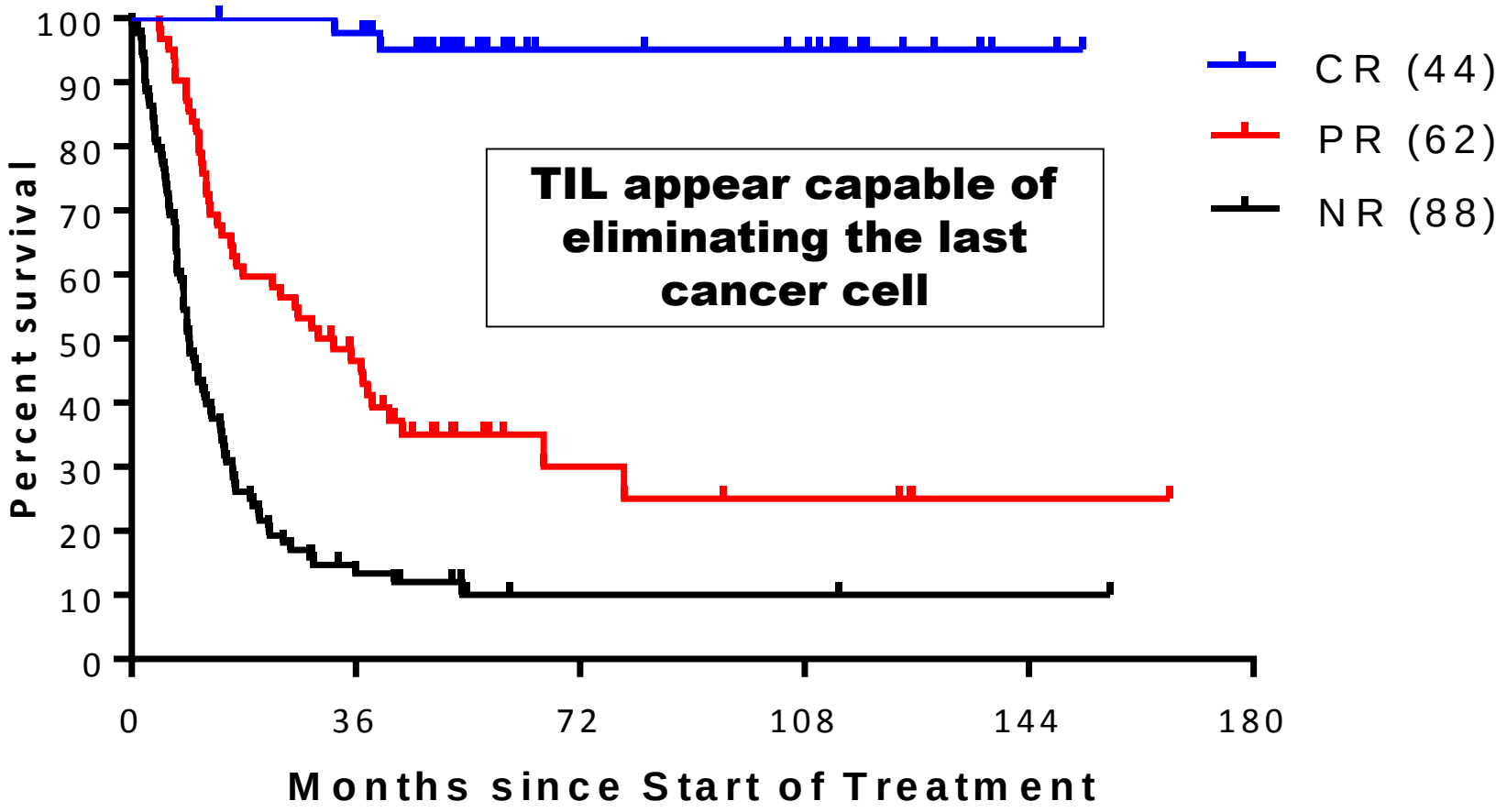
New paradigms in tissue distribution and pharmacokinetics



Living cells can move 'against' concentration gradients and exhibit conditional function after integrating micro-environmental information

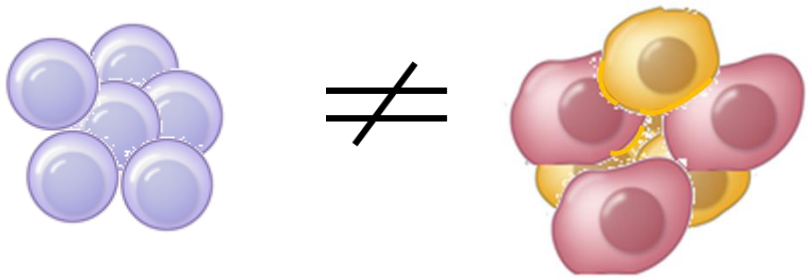


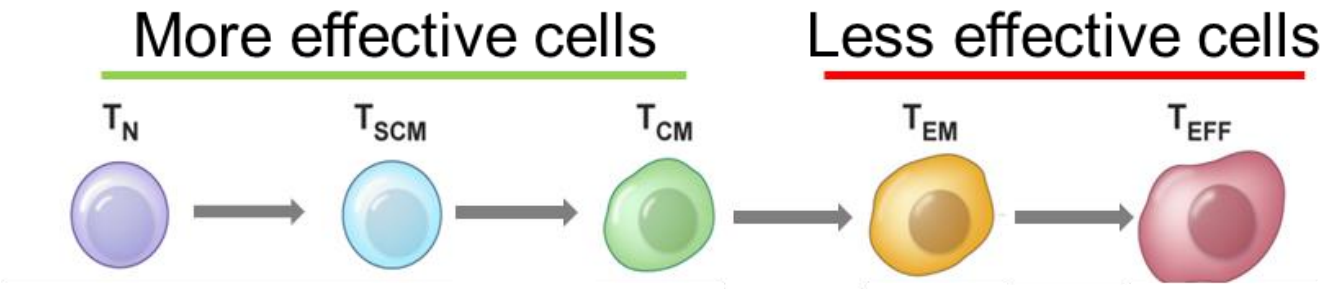
Melanoma-specific survival in patients treated with autologous tumor infiltrating lymphocytes (n=194)



Nov 2016

Not all T cells are equal…

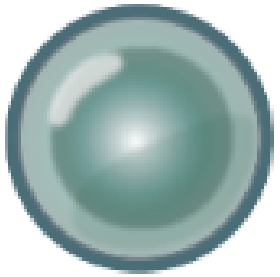




T cells don’t live forever

- Cancer cells are generally immortal, but the T cells used to kill tumor cells are themselves programmed to experience aging, senescence and death.

Senescence



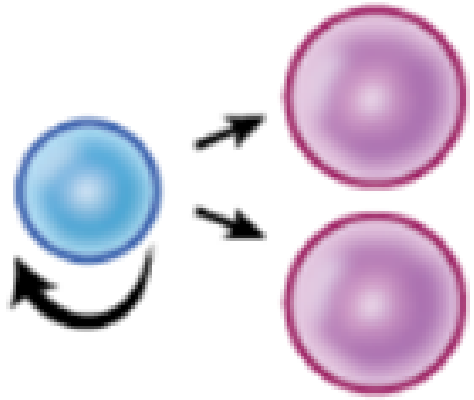
Apoptosis

What is T cell stemness and why does it matter?

Stemness: Noun; Etymology stem + -ness

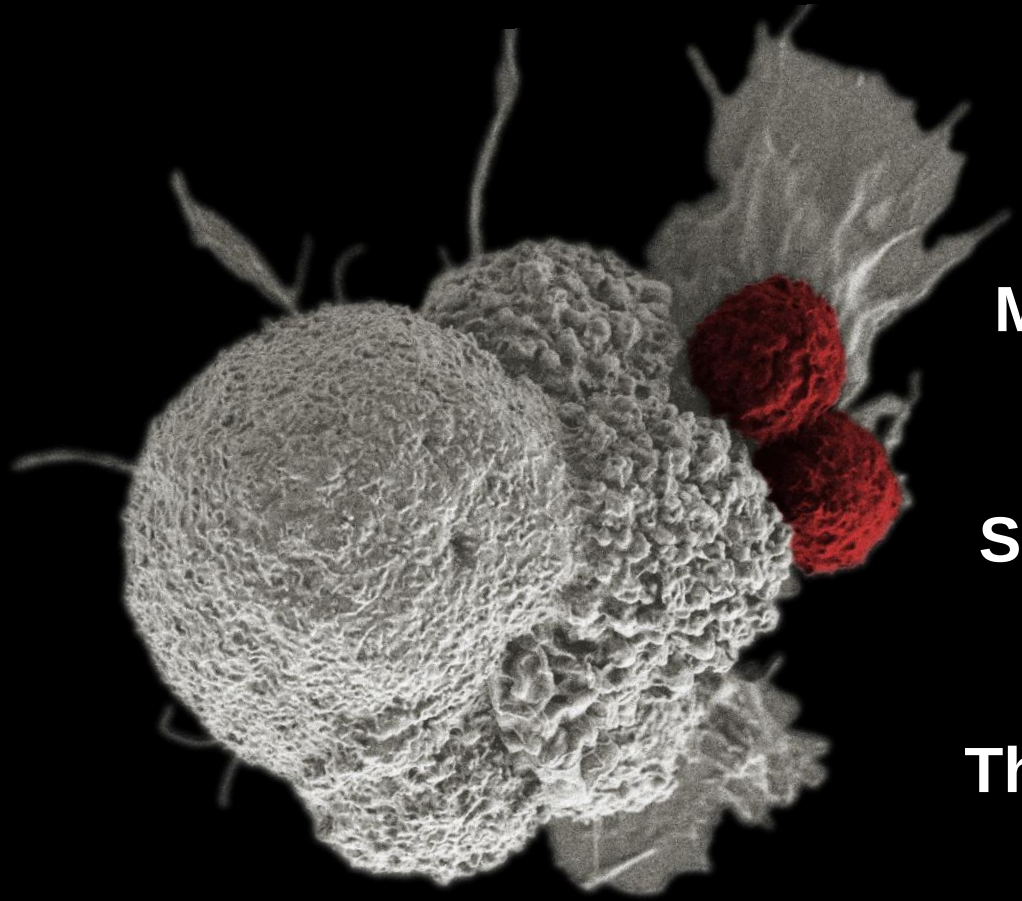
"An essential characteristic of a stem cell"

1. The capacity to **self-new**
2. **Multipotentcy** (can generate differentiated T cell subsets)
3. **Persistence** and **proliferative potential.**



Gattinoni, Klebanoff & Restifo,
Nat Rev Cancer, 2012

Individual T cells (clonotypes) are capable of ‘stemness’



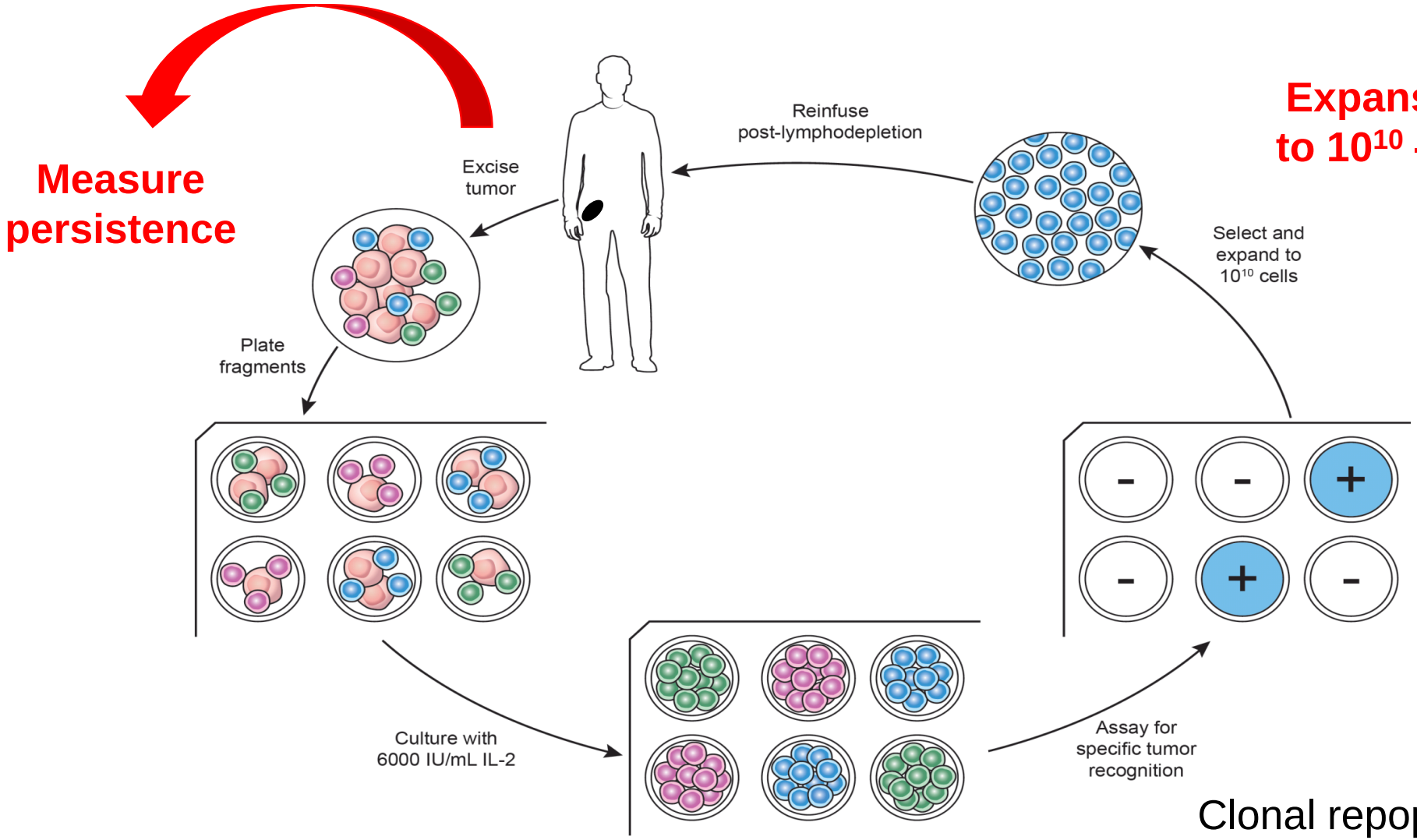
Many organ systems in adult metazoans involve stem cells

Stem cell-like T cells have been identified in mice and in humans

The TCF7 transcription factor is centrally involved in T cell stemness

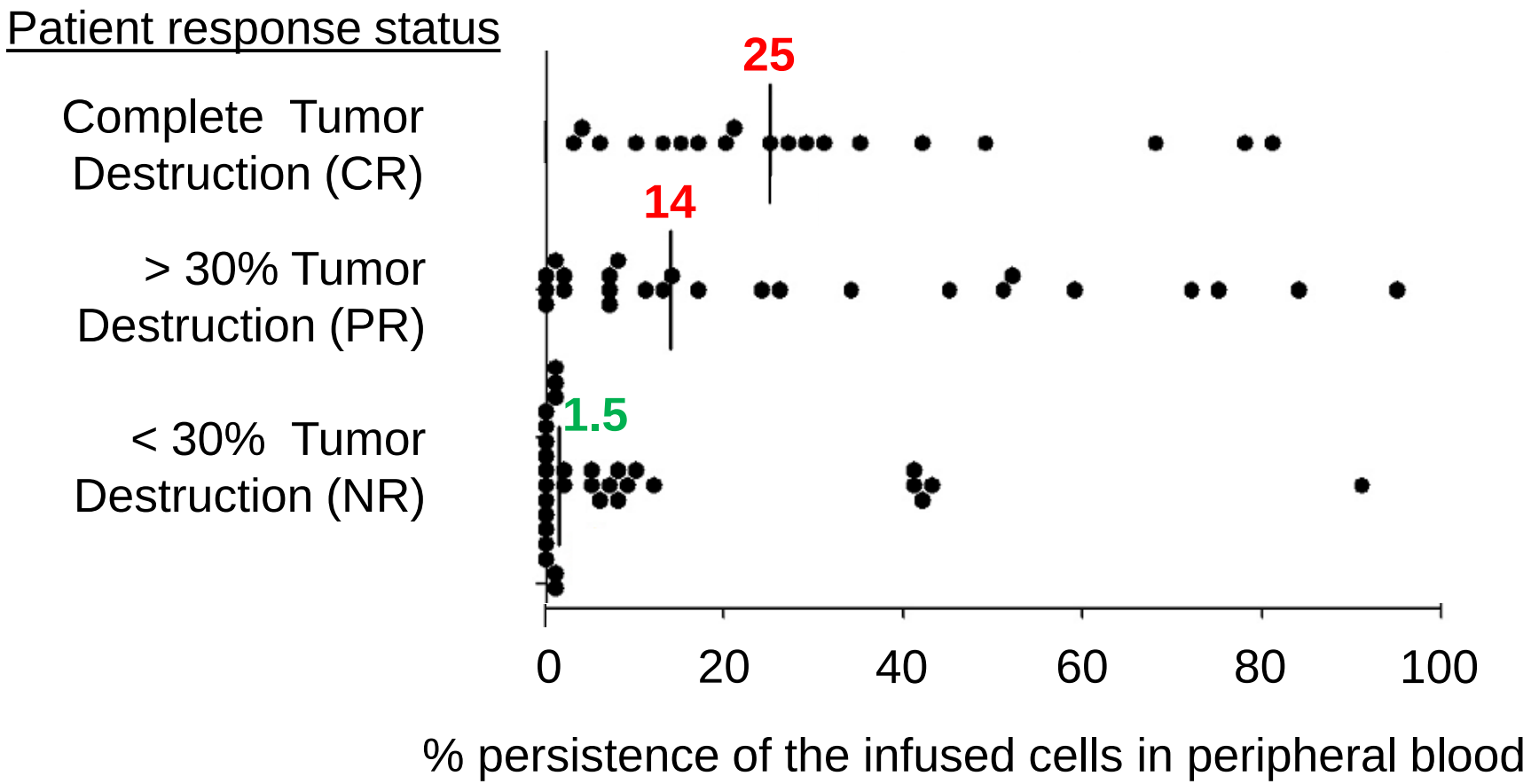
Stem-like behavior:

"Lymphodepleted" patients can experience clonal repopulation of anti-tumor T cells



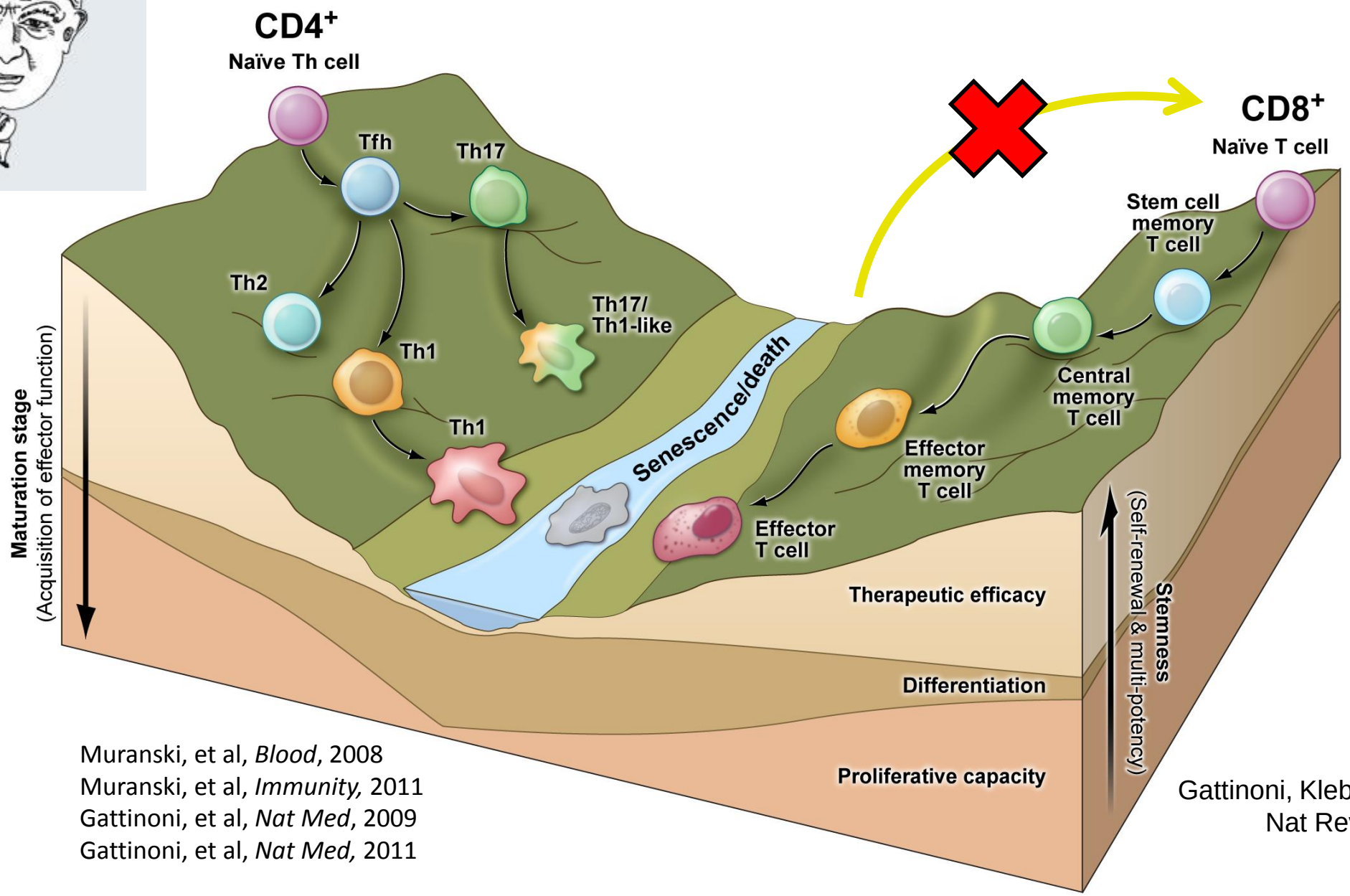
Clonal repopulation, Dudley, et al Science 2002

T cell survival at 1 month is highly correlated with objective clinical response



* CR + PR (>30% reduction) vs. NR (<30% reduction) < 0.001

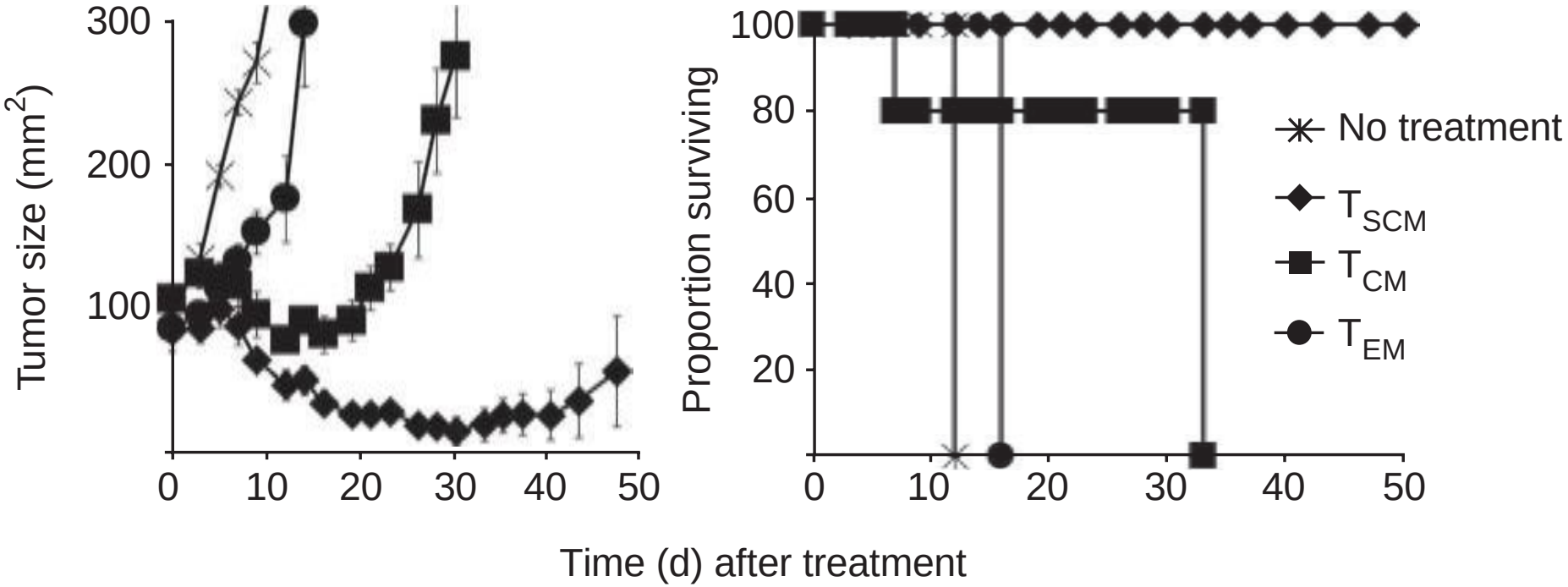
A Waddington model of T cell differentiation



Muranski, et al, *Blood*, 2008
Muranski, et al, *Immunity,* 2011
Gattinoni, et al, *Nat Med*, 2009
Gattinoni, et al, *Nat Med,* 2011

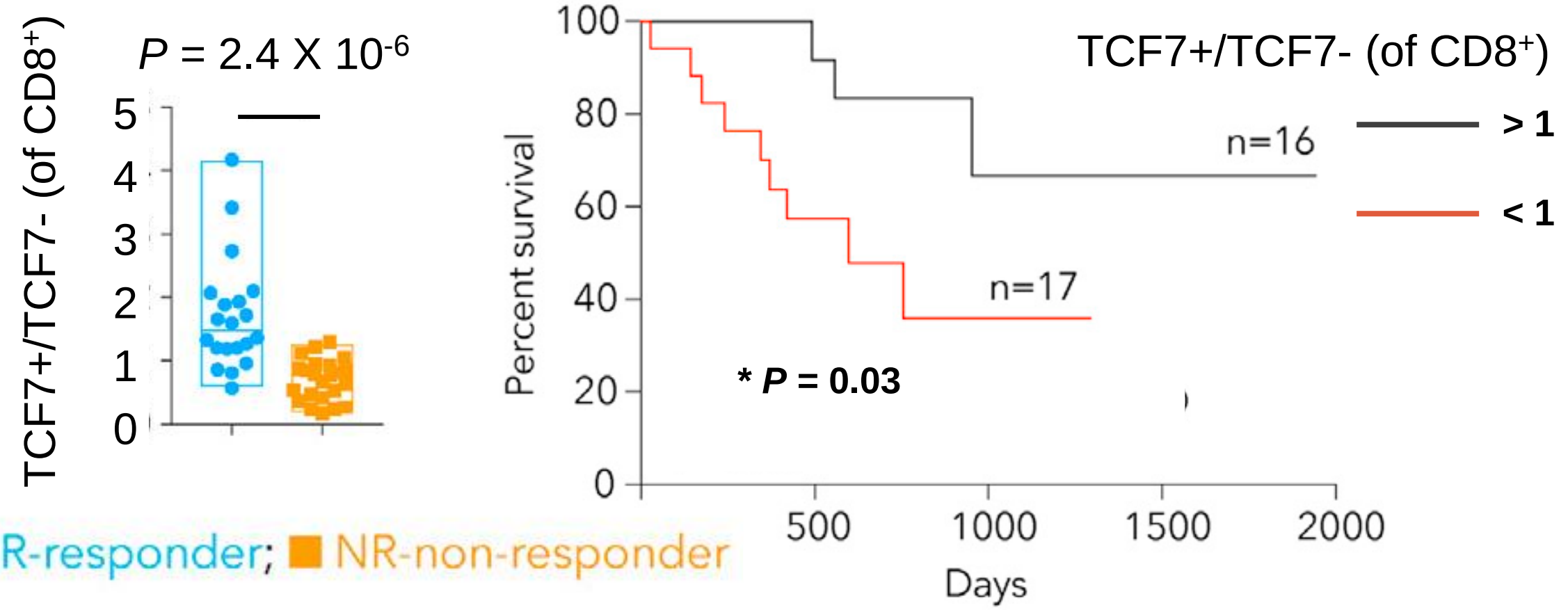
Gattinoni, Klebanoff & Restifo
Nat Rev Cancer, 2012

Tcf7 high T memory stem cells (T_{scm}) cells are more effective and can be used to treat large established tumors at lower doses



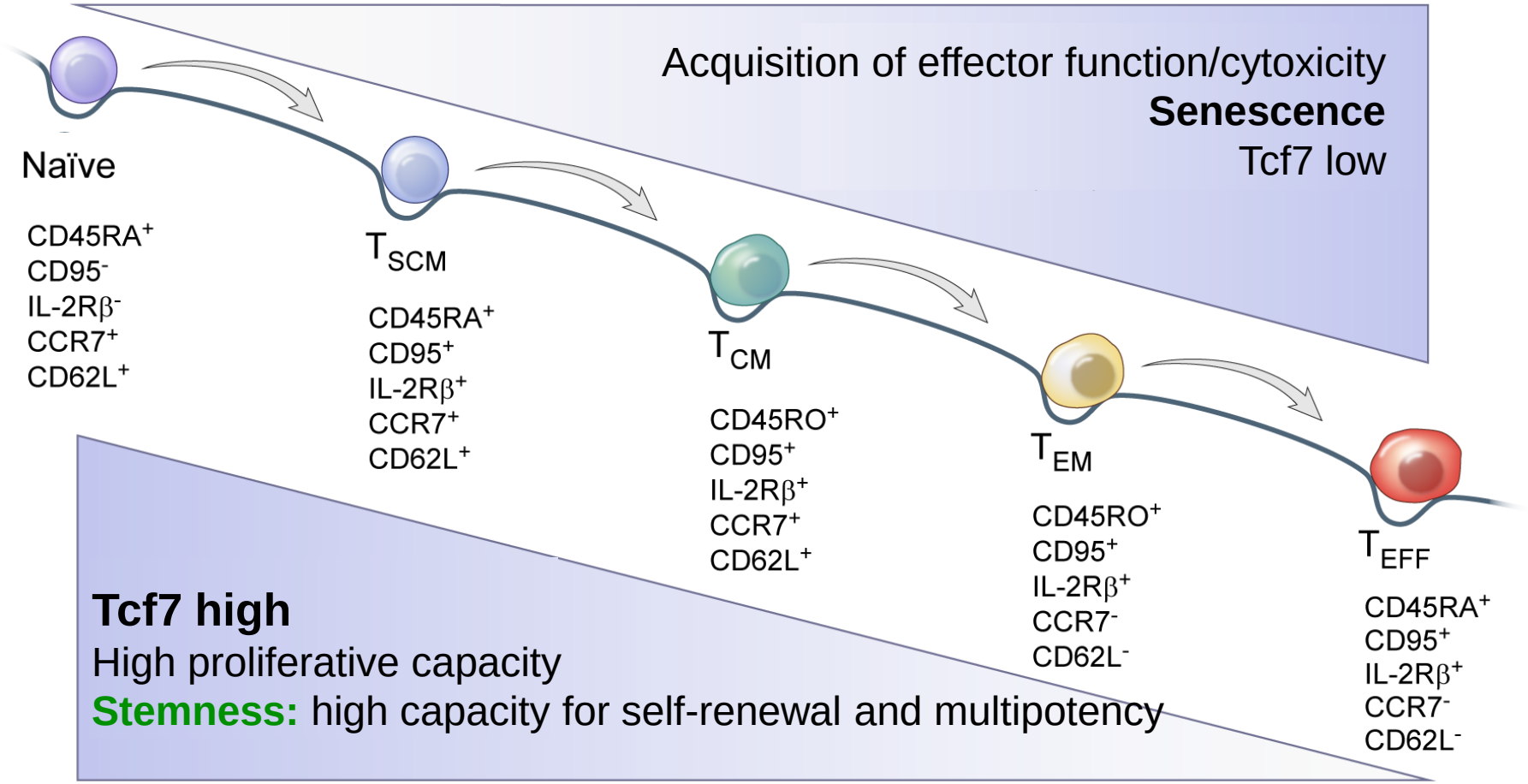
Gattinoni, et al, Nat Med, 2009; 2010; and Human Tscm Nat Med 2011

TCF7^{+}CD8^{+} T cell frequency in tumor tissue predicts response and better patient survival after checkpoint blockade



Sade-Feldman, et al, Cell, 2018

Each T cell clonotype is a stem cell system



~ 900 Genes are dynamically regulated during post-thymic T cell differentiation

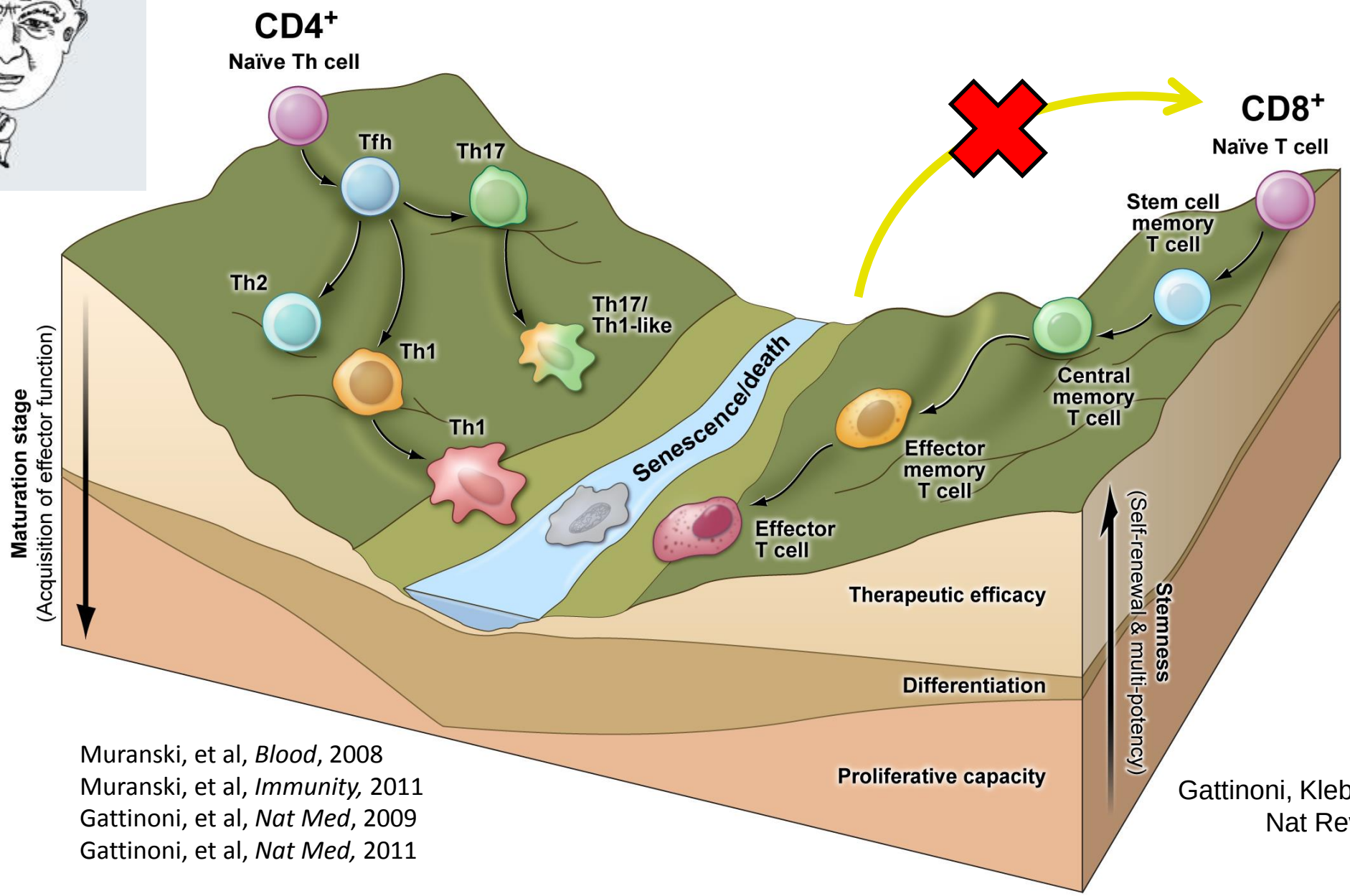
How do we consolidate these advances to make cancer immunotherapy more effective?

- Disrupt the cell death program to keep T cells alive longer (eg use CRISPR to remove mediators of senescence and apoptosis).
- Use drugs to uncouple T cell proliferation from differentiation (eg inhibitors of cell signaling cascades like PI3K → Akt → mTOR pathway
- Metabolically or epigenetically alter T cells to improve their longevity

Senescence

Apoptosis

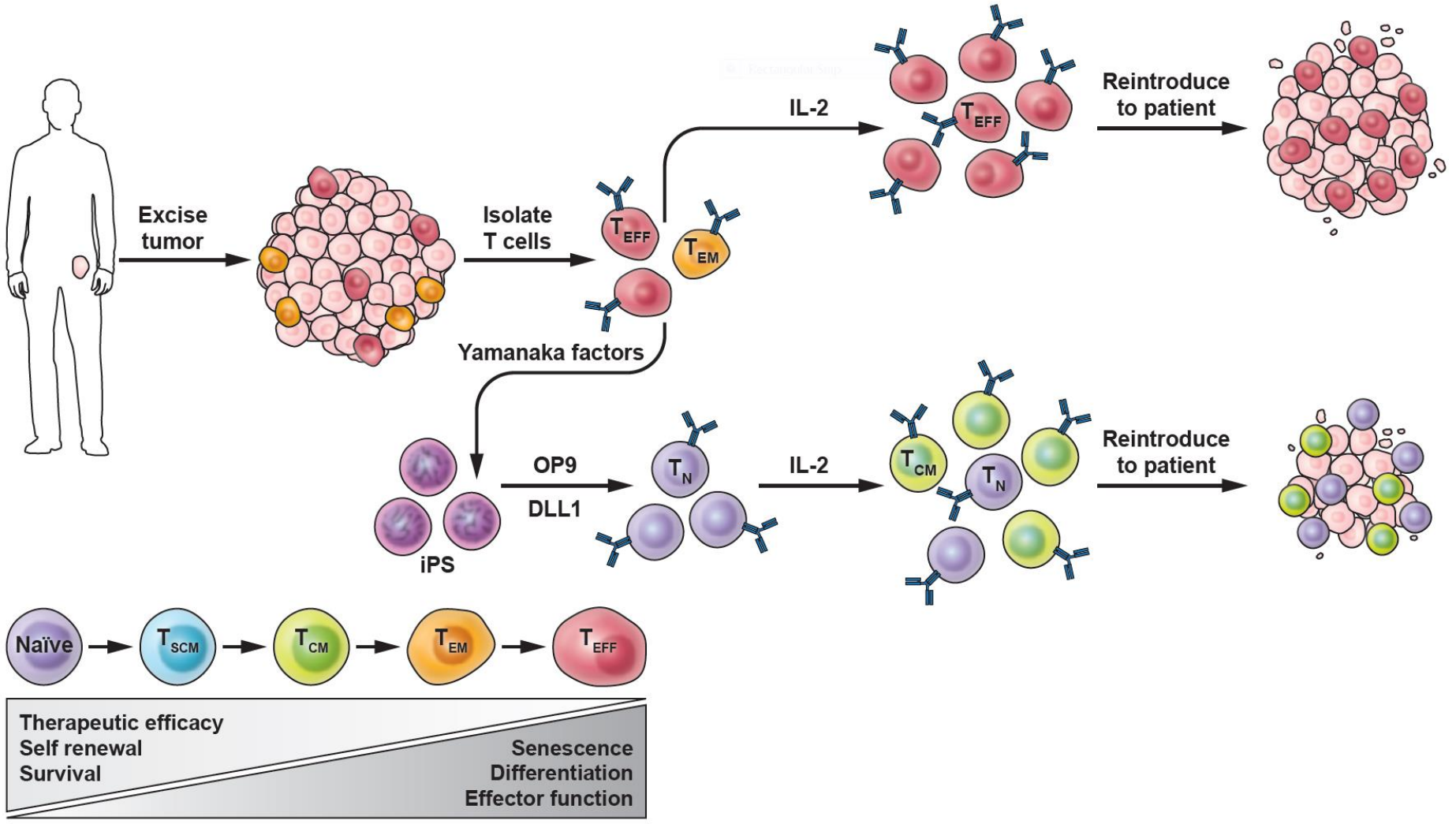
A Waddington model of T cell differentiation



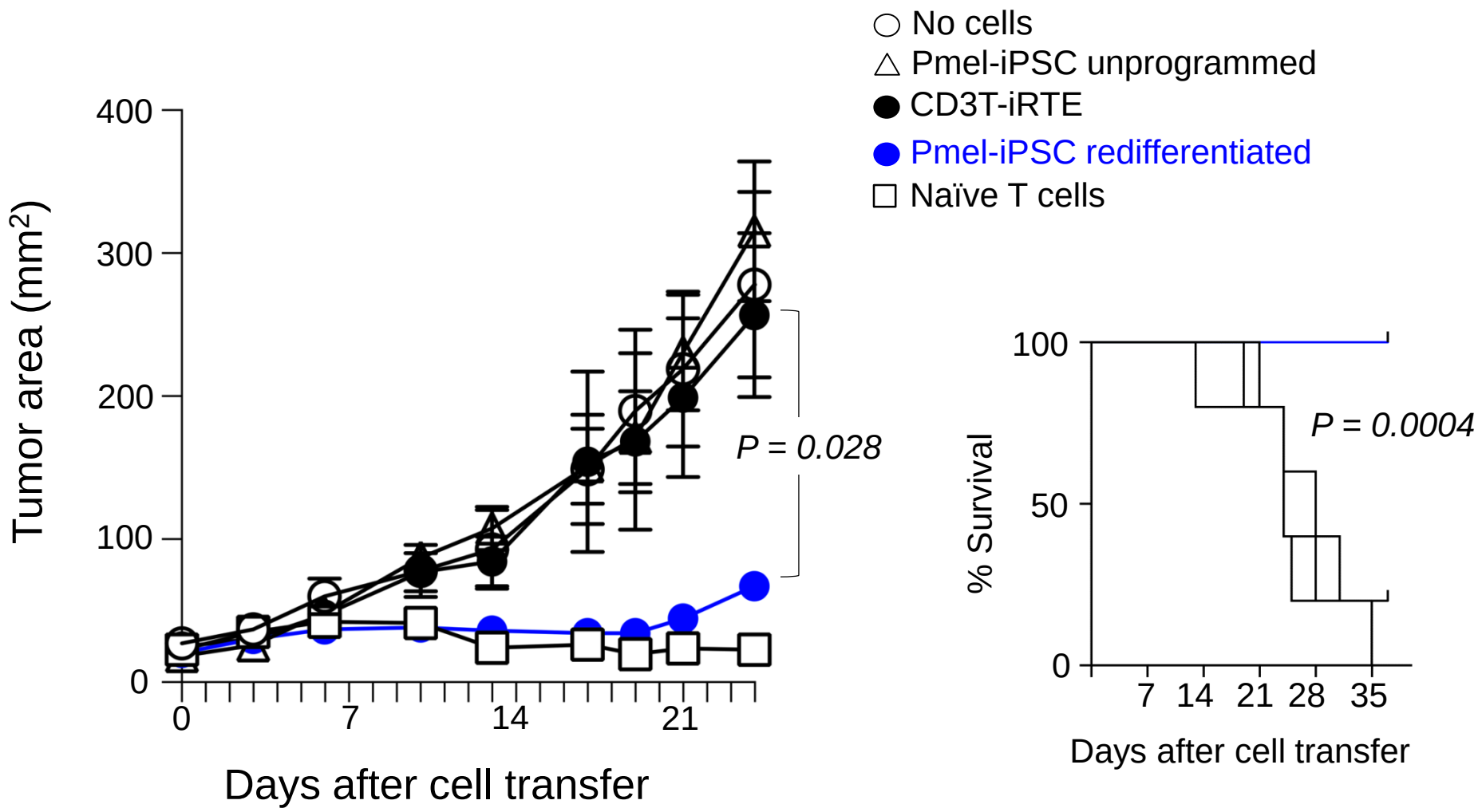
Muranski, et al, *Blood*, 2008
Muranski, et al, *Immunity,* 2011
Gattinoni, et al, *Nat Med*, 2009
Gattinoni, et al, *Nat Med,* 2011

Gattinoni, Klebanoff & Restifo
Nat Rev Cancer, 2012

Fundamentally reprogram the T cells to make them young and vibrant again



iPSC-derived T cells treat established solid tumors and prolong survival

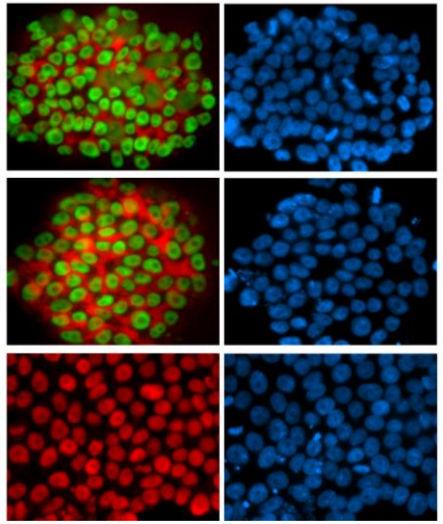


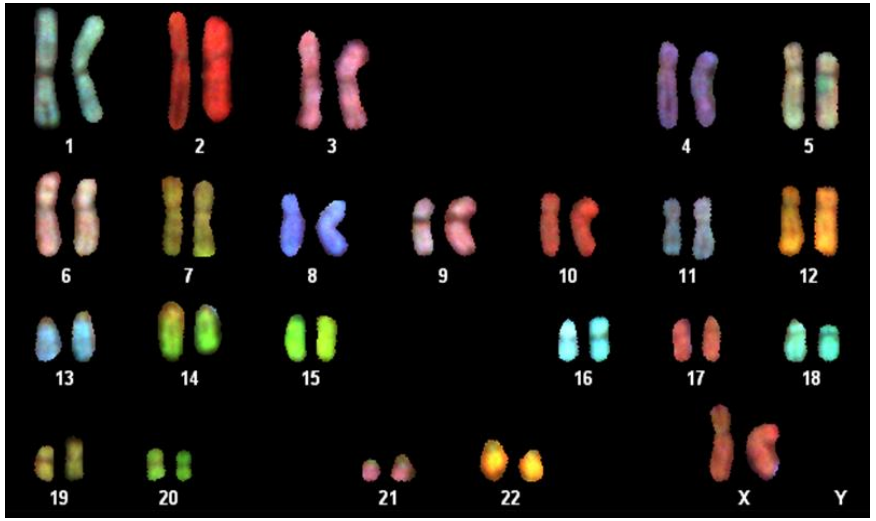
Characterization of human TIL-iPSC

OCT-4
TRA-1-60

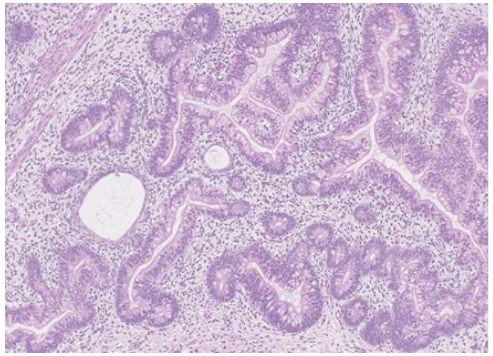
NANOG
TRA-1-81

SOX2

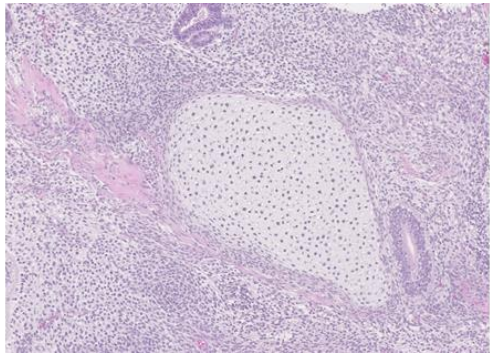




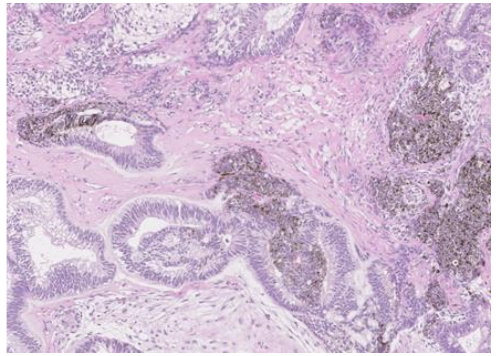
Endoderm



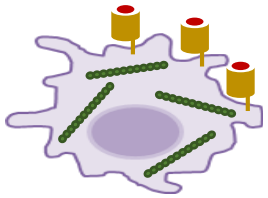
Mesoderm



Ectoderm

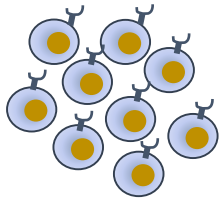


iPSC derived from human T cells are mutation specific



Mutated peptide pulsed autologous B cells

+

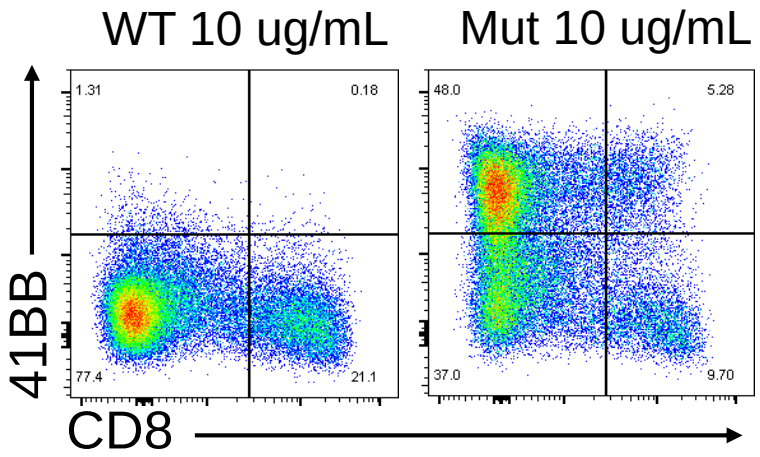


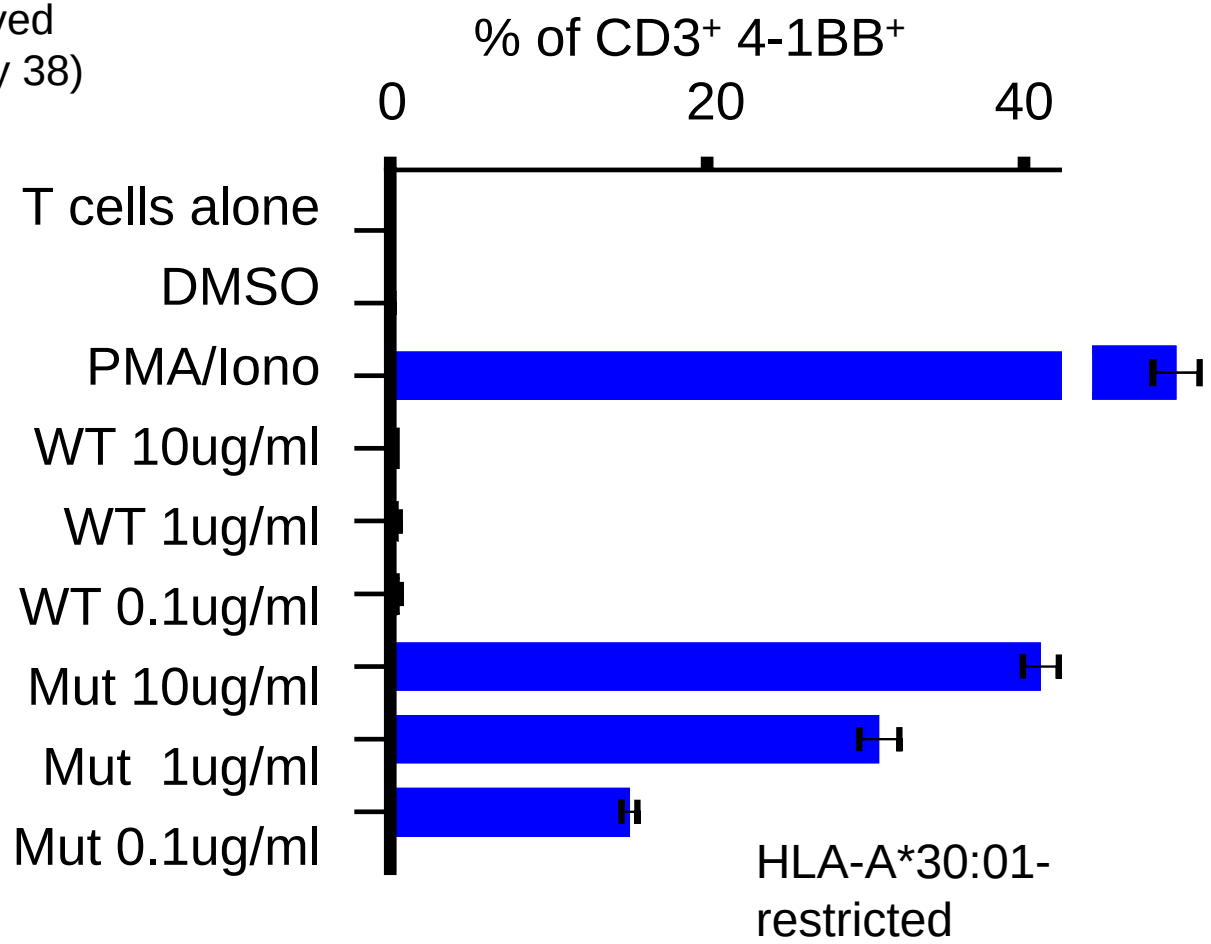
Bulk TIL-iPSC-derived immature T cells (day 38)

Co-culture 18 hrs → FACS

WT: AYRDLQTRE

Mut: AYRDLQTR**K**





> 800 individual iPSC lines derived from mutation-reactive T cells from a variety of cancer histologies

Patient	Tumor type	Cell sources	Clonality	Efficiency of iPSC generation	TIL-iPSC lines established
A	Colon	Infusion bag	Oligoclonal	1 in 2000	28
B	Colon	Infusion bag	Oligoclonal	1 in 2500	13
C	Gastric	Limiting dilution	Clonal	1 in 2500	23
D	Pancreatic	Infusion bag	Oligoclonal	1 in 627	87
E	Melanoma	Tumor Fragment	Polyclonal	1 in 288	178
F	Melanoma	Tumor Fragment	Polyclonal	1 in 250	353

Summary

- Stemness, the capacity of T cells to self renew, proliferate, persist and form large numbers of more differentiated progeny, may be an emerging concept of cell-based therapy.

- The transcription factor, **Tcf7**, is central to the stemness phenotype.

- iPSC-derived T cells are clonal (specific for one antigen) and retain a great degree of proliferative capacity and specificity for tumor neo-antigens.

What we need to bring cell-based therapies to the many patients who need them:

1. Concerted and continued commitment to **basic science**.
2. **Clean space** for at or near scale experimental pre-clinical work (eg CRISPR, iPSC).
3. A **robust vector production** laboratory.
4. Large, GMP-quality **cell production laboratories** to produce cells for **patients**.

Restifo Lab:
Past and present

Suman Vodnala
Raul Vizcardo

Rafiqul Islam
Zhiya Yu
Tori Yamamoto
Devikala Gurusamy
Amanda Henning
Ping-Hsien Lee
Doug Palmer
Yogin Patel
Rigel Kishton
Madhusudhanan Sukmar
Yin Huang
Meghan Good
Takuya Maeda
Naritaka Tamaoki
Marta Bosch-Marce

Restifo Lab:
Past and present

Robert Eil
David Clever
Christopher Klebanoff
Joe Crompton
Nick Klemen
Jenny Pan
Shashank Patel
Gautam Mehta
Yun Ji

Luca Gattinoni
Chrystal Paulos
Pawel Muranski
Rahul Roychoudhuri
Enzo Bronte
Willem Overwijk
Paul Antony
Christian Hinrichs

Restifo Lab:
Past and present

Marc Theoret
Dale Chappell
Barbara J McCabe
Howard Kaufman
Hung Khong
Han Ying
Steve Finkelstein
Nicolas Acqavella
Sid Kerkar
Andrew Kaiser
Lydie Cassard
Pauline Chen
Leroy Hwang

Collaborators
Warren Leonard
Mario Roederer
Enrico Lugli
Golnaz Vahedi
Tal Zacks

Collaborators
David Stroncek
John O'Shea
Jon Yewdell
Jiajie Wei
Jim Kochenderfer
Giorgio Trinchieri

Rosenberg Lab Current and Former Members
Gal Cafri
Eric Tran
Alena Gross

Clinical Team:
James Yang
Rachel Beyer
Rick Sherry
Stephanie Goff
Paul Robbins
Steve Feldman
Robert Somerville
Steve Rosenberg