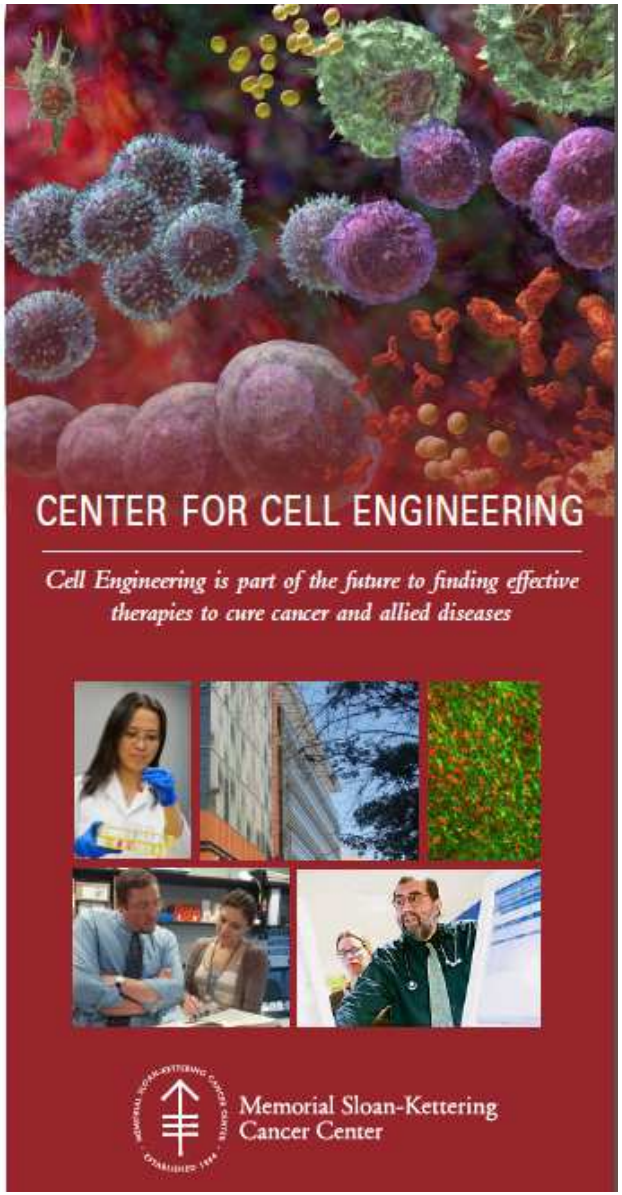
From Adoptive Cell Therapy to CAR Therapy

SITC, November 5, 12015

Michel Sadelain, MD, PhD
Director, Center for Cell Engineering
Immunology Program, Sloan-Kettering Institute
Departments of Medicine and Pediatrics
Memorial Sloan Kettering Cancer Center
New York, NY



Disclosures

- Consultant and co-founder, Juno Therapeutics

Adoptive cell therapy (ACT)

- **Passive immunotherapy** refers to approaches in which immunologic reagents, such as serum, cells, or cell products (e.g. antibodies, cytokines) that are thought to have antitumor activity are administered to a tumor-bearing host.

- **Active immunotherapy** refers to interventions in which the host is stimulated to produce an immune response that directly or indirectly causes tumor-cell death.

From ACT to CAR Therapy

The quest for specificity and potency

- Adoptive transfer of immunity (mice)

- LAK therapy (lymphokine-activated killer cells)

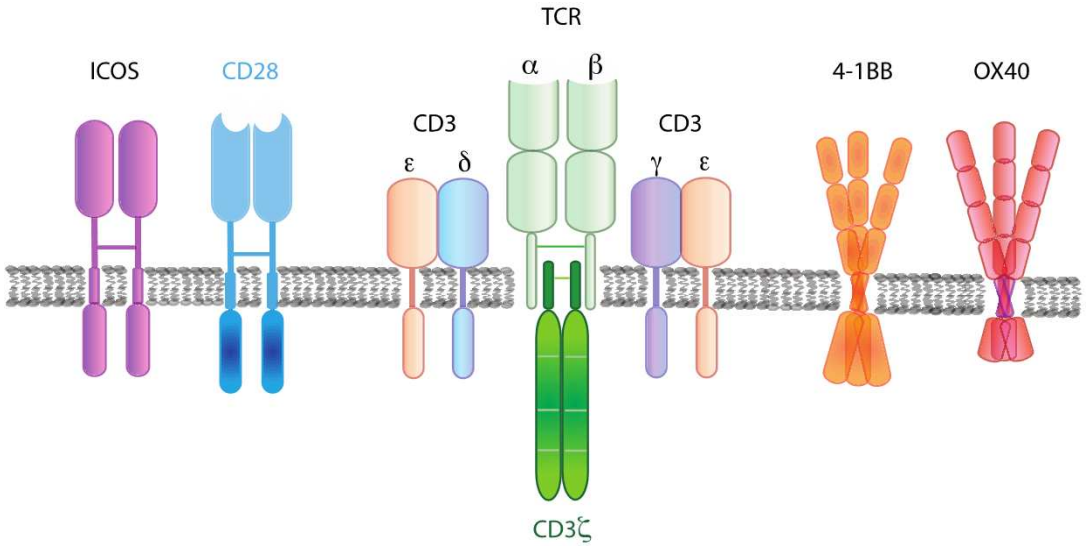
- TIL therapy (tumor-infiltrating lymphocytes)

- Emergence of T cell engineering

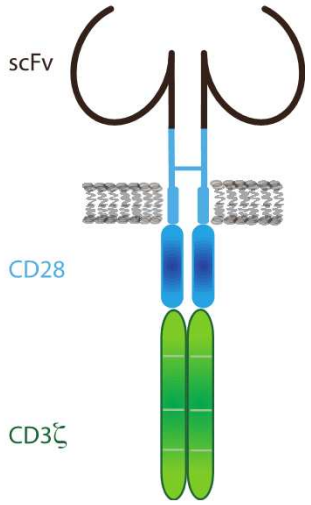
- CAR therapy

Physiological and synthetic receptors for T cell engineering

The TCR/CD3 complex and costimulatory constellation



CARs



Sadelain, Riviere & Brentjens, *Nat Rev Cancer*, 2003
Sadelain, *AACR Education Program*, 2014

Goals of T cell Engineering

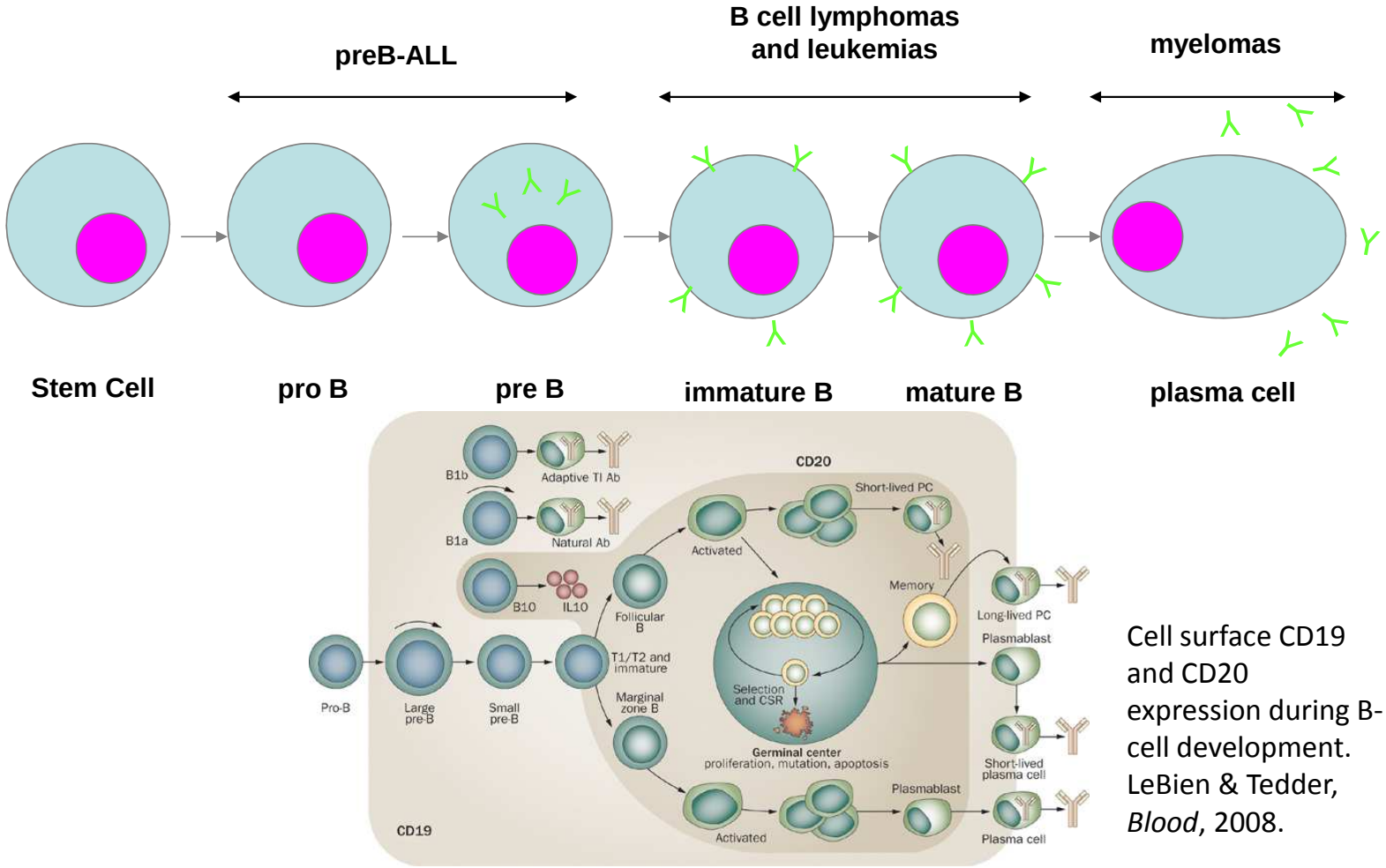
Core hypothesis: T cells can be genetically targeted to any antigen and enhanced to overcome immune escape mechanisms to achieve tumor eradication

Table 1. Rationale for T cell engineering in oncology

Goal	Rationale
To overcome central immune tolerance	CAR T cells can be genetically targeted to any antigen, overcoming clonal deletion and repertoire gaps
To circumvent HLA downregulation	CARs enable HLA-independent antigen recognition, thereby overcoming irreversible defects in HLA expression or antigen presentation
To target both $CD4^+$ and $CD8^+$ T cells to the tumor	$CD4^+$ T cell help can be provided in the absence of HLA class II expression, using a CAR (HLA-independent) or a high-affinity HLA class I–restricted TCR (HLA class I–dependent)
To broaden T cell reactivity to carbohydrates and glycolipids	CAR recognition is not limited to proteins and HLA-peptide complexes
To target cancer stem cells	CAR T cells can be directed to tumor-initiating cells when such cells have been defined and target antigens identified therein
To augment T cell potency	CARs enable increased antitumor activity by over-riding T cell inhibitory mechanisms, reprogramming the tumor microenvironment, or recruiting/boosting endogenous T cell responses
To control T cell longevity	CARs can modulate T cell longevity through the use of different costimulatory signals, different T cell subsets, and/or suicide genes
To exploit alternative (nonautologous) T cell sources	T cell engineering may facilitate the utilization of T cells harvested from healthy donors or induced in culture from stem/progenitor cells

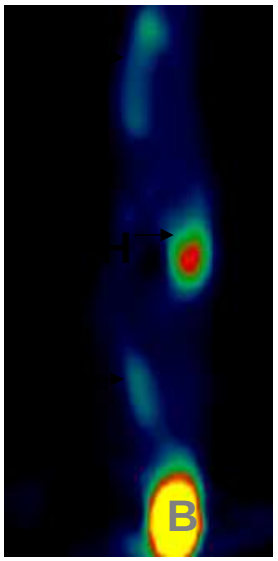
Sadelain, *J Clin Invest*, 2015

Selecting CD19 as a target for CAR therapy

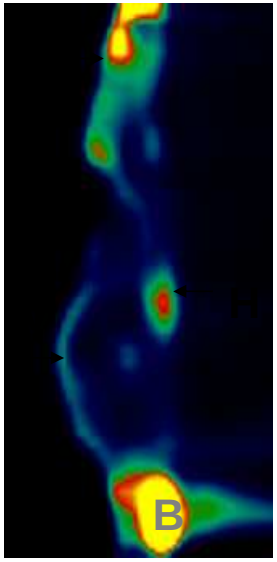


Cell surface CD19 and CD20 expression during B-cell development. LeBien & Tedder, *Blood*, 2008.

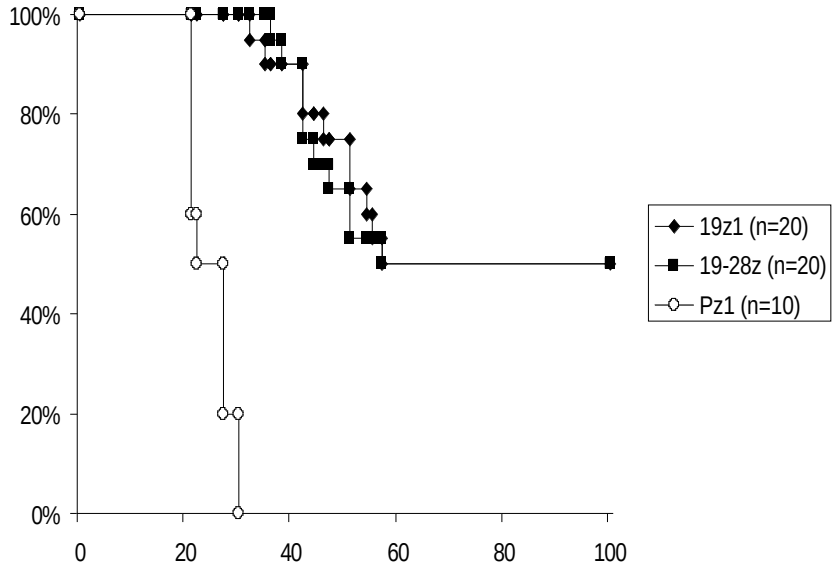
19z1 CART cells expanded on CD19+CD80+IL15+ AAPCs eradicate established systemic Raji in SCID-beige mice



Tumor Free



Untreated
4 weeks

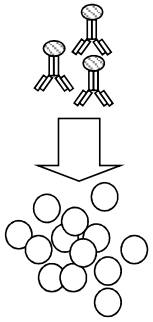


Brentjens et al, *Nat Med*, 2003

CAR T cell Manufacturing Flow

Day 0
Apheresis
Thaw/Wash
(Cell Saver5)

Incubation
Dynabeads CD3/28



Apheresis
Patient T cells

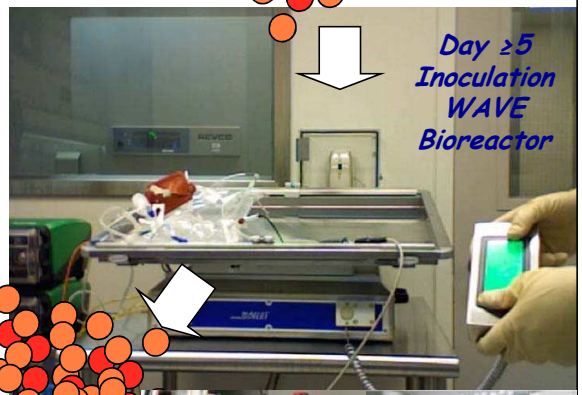
Selection
ClinExVivo MPC

Day 3-4
Transduction
SFG-CAR
Vector

CD3+ enriched
activated
T cells

Przybylowski et al, *Gene Ther*, 2006

CAR
transduced
T cells



Day ≥5
Inoculation
WAVE
Bioreactor

CAR+
expanded T cells

Isabelle Riviere
Xiuyan Wang

DAY ≥ 10
Debeading
ClinExVivo MPC

Wash
(Cell Saver5)

Cryopreservation

Biosafety/
QC release tests

Patient Infusion

Validation
In vivo antitumor activity
in SCID mice, CTL

Hollyman et al, *J. Immunother*, 2009

A Phase I trial of precursor B cell Acute Lymphoblastic Leukemia (B-ALL) treated with autologous T cells genetically targeted to the B cell specific antigen CD19
(PI: J. Park; Past PI : M. Davila; co-PIs: R. Brentjens, M. Sadelain, I. Rivière)

- **Enrollment Criteria:**
  Patients with relapsed B-ALL initially treated with re-induction chemotherapy followed by consolidation with cyclophosphamide and 1928z+ T cells

- **Protocol Design:**
  Escalating T cell dose (3 x10^6 19-28z+T cells/kg,10^7 19-28z+T cells/kg, 3x10^7 19-28z+ T cells/kg) in combination with cyclophosphamide (3.0g/m^2)

- **Primary Endpoint:**
  To assess the toxicity of adoptively transferred 1928z+ T cells

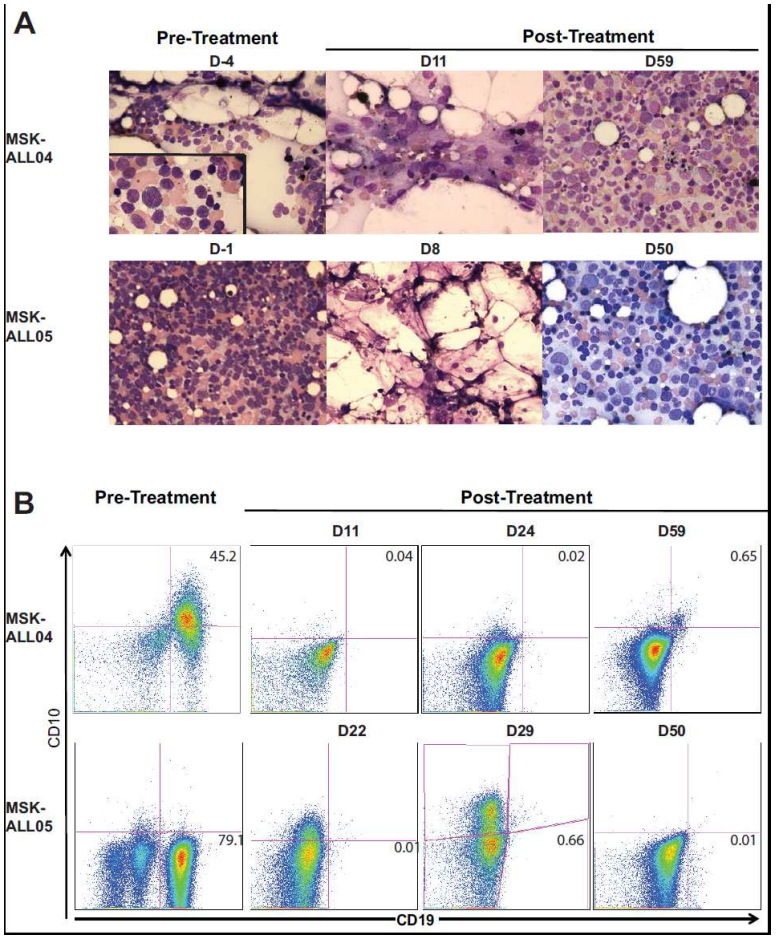
- **Secondary Endpoints:**
  1928z+ T cell survival
  Role of CY T cell survival
  1928z+ T cell homing
  B cell aplasias


Jae Park


Marco Davila


Renier Brentjens

Rapid tumor elimination mediated by 19-28z T cells



A. BM aspirates pre- and post-treatment with 19-28z T cells in 2 patients with morphologic chemotherapy-refractory B-ALL. Cyclophosphamide was given at Day -1 and CD19 CAR-targeted T cells were infused on Days 1 and 2. Left panels. BM prior to CAR modified T cell therapy demonstrated predominant blast cells with an absence of normal BM precursors. For MSK-ALL04 the left panel includes an inset with 100x magnification. Middle panels are BM aspirates done shortly after 19-28z T cell infusion and is hypocellular with normal stromal elements, histiocytes, and no evidence of blasts. Right panels. By 1 to 2 months after CAR modified T cell therapy there is BM recovery with normal hematopoiesis and no evidence of abnormal blasts.

B. B. Flow cytometry for CD19 and CD10 expression in BM pre- and post-treatment. Cells were gated on CD45+7AAD- cells.

Brentjens, Davila, Riviere et al, *Science Transl Med*, 2013

Deep Sequencing for IgH rearrangements before and after CD19 CAR-targeted T cell therapy

Patient ID	Day of Treatment	Total # IgH rearrangements	Total # malignant IgH rearrangements
MSK-ALL01*	-1: 55:	57,480 15,925	58 0
MSK-ALL03*	-5: 30:	1,084 0	0 0
MSK-ALL04	-4: 11: 24: 59:	2,430,058 2,407 1,144 995,563	2,426,898 1,316 637 0
MSK-ALL05*	-1: 8: 29:	3,307,494 1,880 8,270	3,300,732 0 0
MSK-ALL06	-34: 18: 39:	255,301 5,429 1,866,851	174,698 4 0

Adaptive Biotechnologies performed multiplex PCR and Deep Sequencing on genomic DNA prepared from BM aspirated on the noted day. Malignant IgH rearrangement refers to IgH rearrangements associated with the B-ALL tumor cells. Total # of IgH rearrangements are derived from both malignant and non-malignant B cells. * Patient has gone to allo-SCT and is off-study.


Science
20 December 2013 | $10
Breakthrough of the Year
Cancer Immunotherapy
T cells on the attack
AAAS

Cancer Immunotherapy

This year marks a turning point in cancer, as long-sought efforts to unleash the immune system against tumors are paying off—even if the future remains a question mark

History's path is unchartable when it's not yet past but present, when we are still standing in the middle of it. That's what made *Science*'s selection of this year's Breakthrough of the Year such a topic of internal debate, even anxiety. In celebrating cancer immunotherapy—harnessing the immune system to battle tumors—did we risk hyping an approach whose ultimate impact remains unknown? Were we irresponsible to label as a breakthrough a strategy that has touched a tiny fraction of cancer patients and helped only some of them? What do we mean when we call something a breakthrough, anyway?

Ultimately, we concluded, cancer immunotherapy passes the test. It does so because this year, clinical trials have cemented its potential in patients and swayed even the skeptics. The field hums with stories of lives extended: the woman with a grapefruit-size tumor in her lung from melanoma, alive and healthy 13 years later; the 6-year-old near death from leukemia, now in third grade and in remission; the man with metastatic kidney cancer whose disease continued fading away even after treatment stopped.

As the anecdotes coalesce into data, there's another layer, too, a sense of paradigms shifting. Immunotherapy marks an entirely different way of treating cancer—by targeting the immune system, not the tumor itself. Oncologists, a grounded-in-reality bunch, say a corner has been turned and we won't be going back.

With much pressure these days to transform biological insights into lifesaving drugs, there's a lesson to be learned from immunotherapy's successes: They emerged from a careful decoding of basic biology that spanned many years. The early steps were taken by cancer immunologist James Allison, now at the University of Texas MD Anderson Cancer Center in Houston. In the late 1980s, French researchers who weren't thinking about cancer at all identified a new protein receptor on the surface of T cells, called cytotoxic T-lymphocyte antigen 4, or CTLA-4. Allison found that CTLA-4 puts the brakes on T cells, preventing them from launching full-out immune attacks. He wondered whether blocking the blocker—the CTLA-4 molecule—would set the immune system free to destroy cancer.

Online
sciencemag.org
Podcasts, videos, and other extras (http://scim.ag/med_6165).

Allison's rationale was untested. He and his colleagues changed the conversation, in the words of one cancer researcher, "to consider immunosuppression as the focal point, and manipulation of immunosuppression as the target."

Doing so took time. CTLA-4 was discovered in 1987. In 1996, Allison published a paper in *Science* showing that antibodies against CTLA-4 erased tumors in mice.

Seek and destroy. Instead of targeting tumors directly, cancer immunotherapy enlists the immune system to attack them. Here, an antibody (pink) blocks a receptor (purple) found on T cells (gray), setting off a chain reaction that leads to an assault on cancer cells (brown).

CREDIT: V. ALTOUNIAN/SCIENCE

Pharmaceutical companies shied away from cancer immunotherapy, wary of past flops but also of a strategy very unlike the standard zapping of a tumor. So the job of getting anti–CTLA-4 into people fell to a small biotechnology company, Medarex, in Princeton, New Jersey. In 1999, it acquired rights to the antibody, taking the leap from biology to drug.

Crucial results
Bristol-Myers Sc
more than $2 billi
melanoma lived a
compared with 6
any treatment had
a randomized trial
at least 2 years.

The numbers f
and the side effec

Memorial Sloan-Kettering Cancer Center in New York reported that the T cell therapy in their studies put 45 of 75 adults and children with leukemia into complete remission, although some later relapsed. CAR therapy is now the focus of numerous clinical trials. Researchers hope that it, like the antibodies, can target an assortment of cancers. Engineered T cells are still experimental.

in Japan discovered a molecule expressed in dying T cells, which he called programmed death 1, or PD-1, and which he recognized as another brake on T cells. He wasn't thinking of cancer, but others did. One, oncologist Drew Pardoll at Johns Hopkins University, met with a leader of Medarex at a Baltimore coffee shop. He urged the company to test an anti–PD-1 antibody in people.

The first trial, with 39 patients and five different cancers, began in 2006. By 2008, doctors were jolted by what they saw: In five of the volunteers, all of them with refractory disease, tumors were shrinking. Survival in a few stretched beyond what was imagined possible.

Still, understanding what these therapies were doing inside the body was a challenge. Other cancer treatments either work or they don't, and the answer is nearly instantaneous. With both anti–CTLA-4 and anti–PD-1, physicians saw some tumors grow before vanishing months later. Some patients kept responding even after the antibody had been discontinued, suggesting their immune system had been fundamentally changed. Some, particularly those on anti–CTLA-4, developed unnerving side effects, inflammation of the colon, for example, or of the pituitary gland. All of these were the fine points of a new template, one whose vagaries physicians were just beginning to understand. The learning curve would be steep.

It was steep in another area of immunotherapy as well. For years, Steven Rosenberg at the National Cancer Institute had harvested T cells that had migrated into tumors, expanded them in the lab, and reinfused them into patients, saving some with dire prognoses. The technique worked only when doctors could access tumor tissue, though, limiting its application.

Then in 2010, Rosenberg published encouraging results from so-called chimeric antigen receptor therapy, or CAR therapy—a personalized treatment that involves genetically modifying a patient's T cells to make them target tumor cells. One group, led by Carl June at the University of Pennsylvania, began reporting eye-catching responses to CAR therapy: patients with pounds of leukemia that melted away. At a meeting in New Orleans this month, June's

developing antibodies such as anti–PD-1. In 2011, the U.S. Food and Drug Administration approved Bristol-Myers Squibb's anti–CTLA-4 treatment, called ipilimumab, for metastatic melanoma. The cost is high: The company charges $120,000 for a course of therapy. In 2012, Suzanne Topalian of Hopkins, Mario Sznol of Yale University, and their colleagues reported results for anti–PD-1 therapy in nearly 300 people, and they provided an update earlier this year. Tumors shrunk by about half or more in 31% of those with melanoma, 29% with kidney cancer, and 17% with lung cancer.

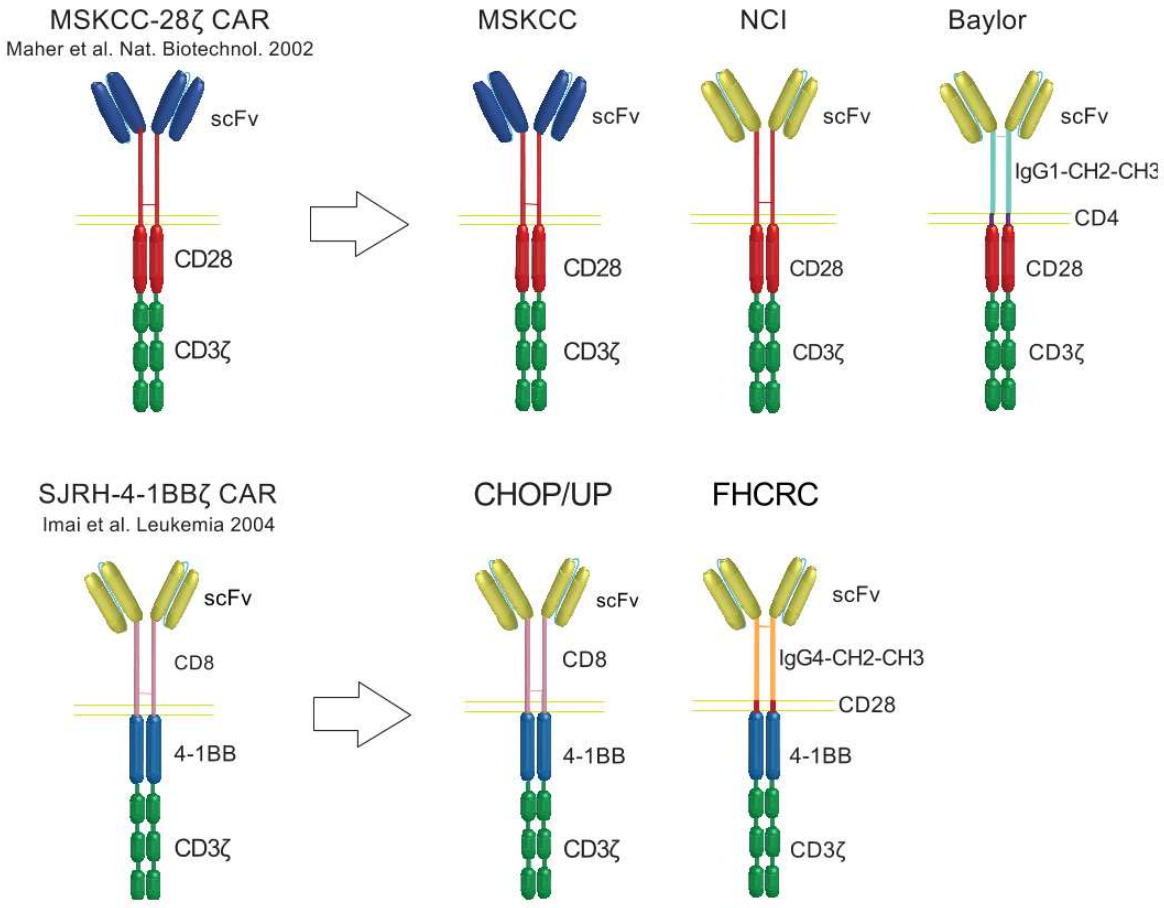
This year brought even more encouragement. Bristol-Myers Squibb reported this fall that of 1800 melanoma patients treated with ipilimumab, 22% were alive 3 years later. In June, researchers reported that combining ipilimumab and anti–PD-1 led to "deep and rapid tumor regression" in almost a third of melanoma patients. Drugs blocking the PD-1 pathway have not yet been proven to extend life, although survival rates so far have doctors optimistic that they do.

For physicians accustomed to losing every patient with advanced disease, the numbers bring a hope they couldn't have fathomed a few years ago. For those with metastatic cancer, the odds remain long. Today's immunotherapies don't help everyone, and researchers are largely clueless as to why more don't benefit. They are racing to identify biomarkers that might offer answers and experimenting with ways to make therapies more potent. It's likely that some cancers will not yield to immunotherapy for many years, if ever.

Even in the fluid state oncology now finds itself, this much is certain: One book has closed, and a new one has opened. How it will end is anyone's guess.

–JENNIFER COUZIN-FRANKEL

CD19CARs: origi



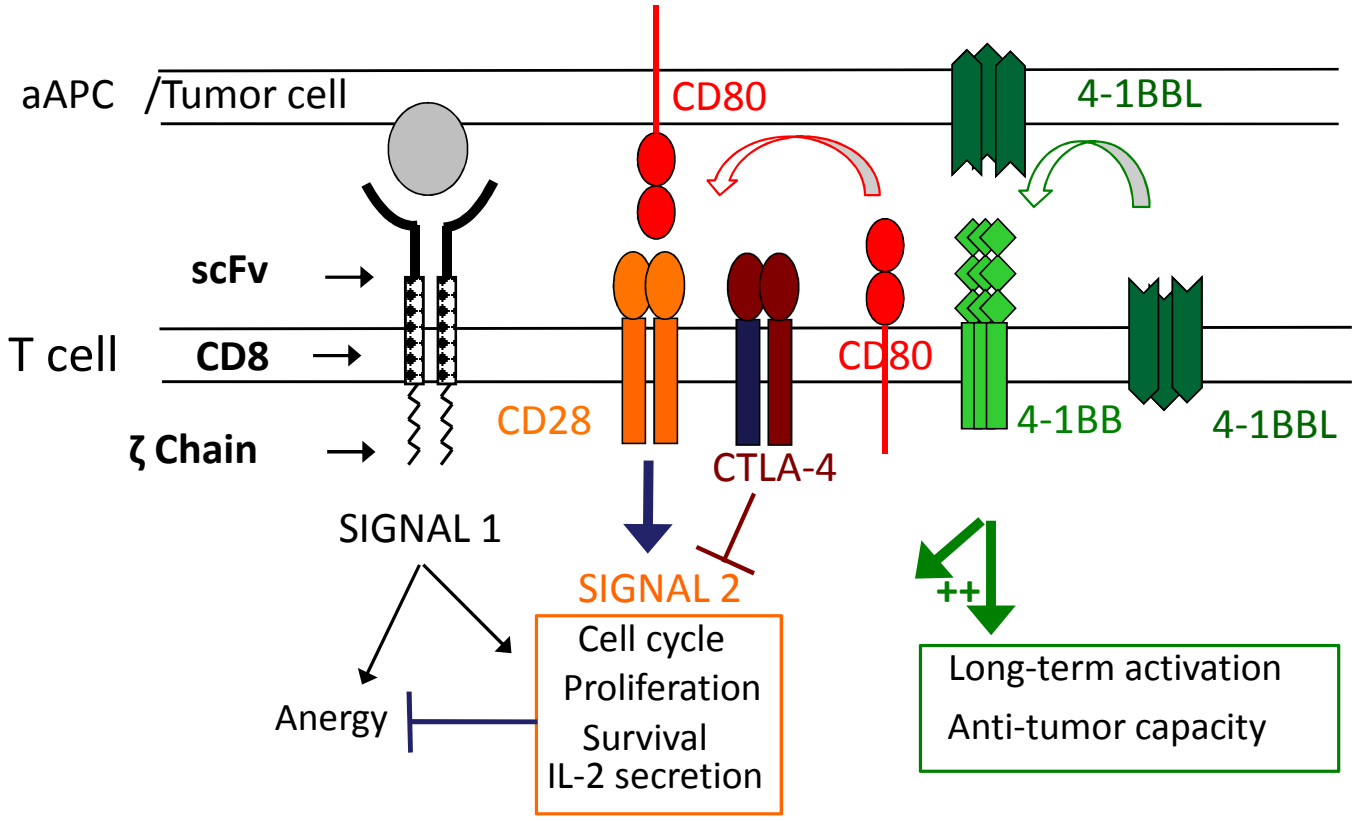
Sadelain, *J Clin Invest*, 2015

Patient numbers/outcomes with CD19 CAR therapy for ALL

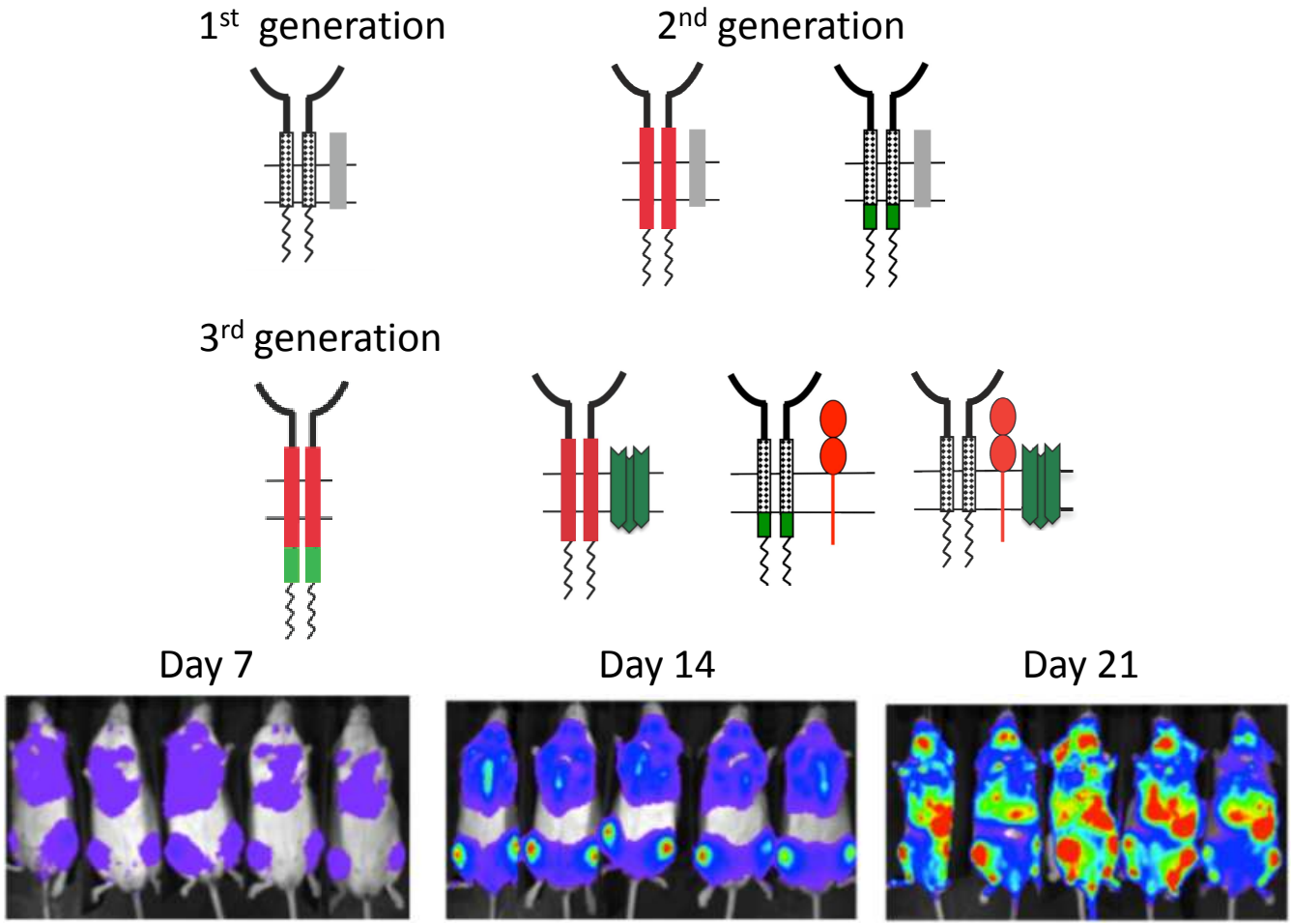
Publication/meeting date	Number/age of subjects	Complete remission rate
Brentjens, *Sci Transl Med*, March 21, 2013	5 adults	100%
Grupp, *New Engl J Med*, April 18, 2013	2 children	100 %
Davila, *Sci Transl Med*, February 19, 2014	16 adults	88%
Lee, *Lancet*, AOL, October 13, 2014	20 children	70%
Maude, *N Engl J Med*, October 16, 2014	25 children, 5 adults	90% 100%
Park, *ASH 2014*, December 6, 2014	27 adults	89%
Frey, *ASH 2014*, December 6, 2014	12 adults	89%

CD28/CTLA4/CD80 and 4-1BB/4-1BBL in T cell activation

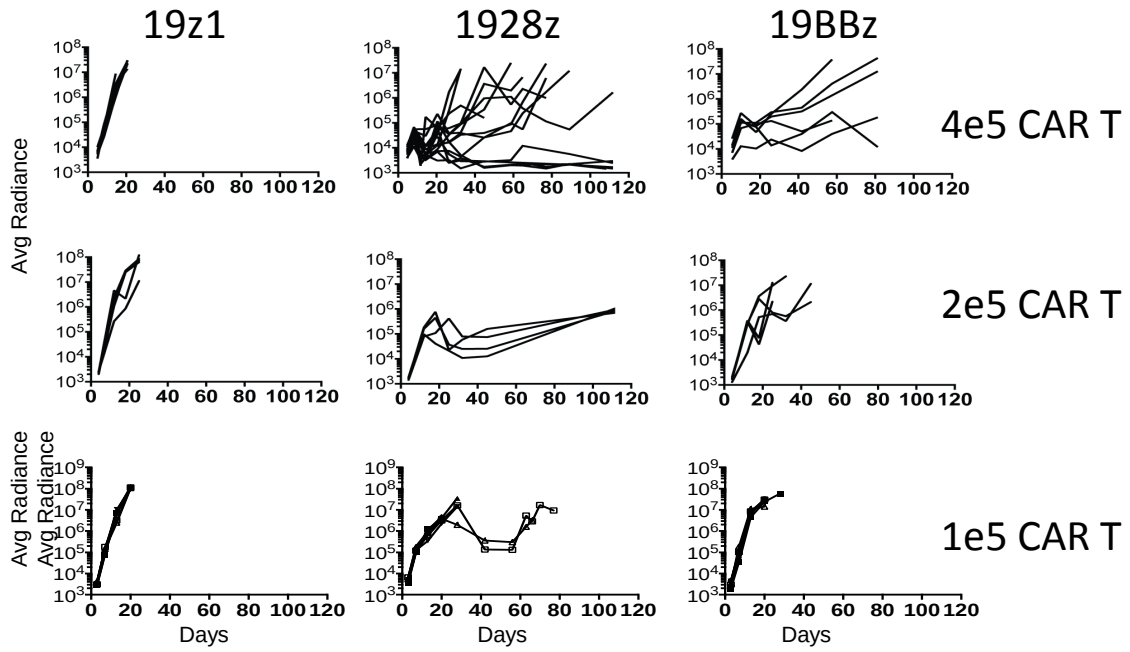
T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection. Stephan et al., *Nat Med*, 13(12):1440-9, 2007.

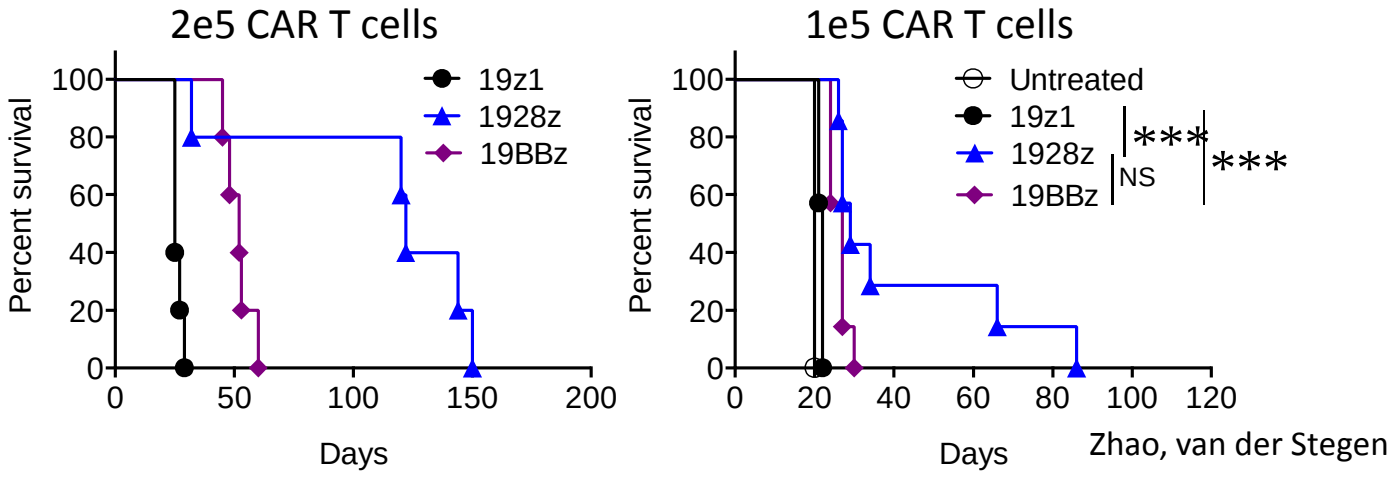


Assays for T cell potency: the ***CAR stress test***



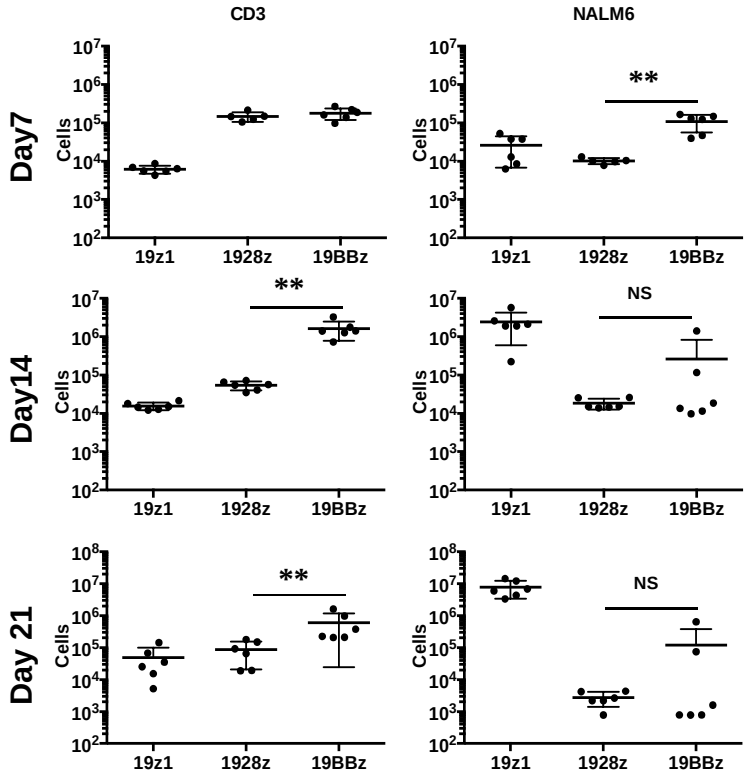
The NALM/6 B-ALL NSG model


19z1
1928z
19BBz
4e5 CAR T
2e5 CAR T
1e5 CAR T
Avg Radiance
Days

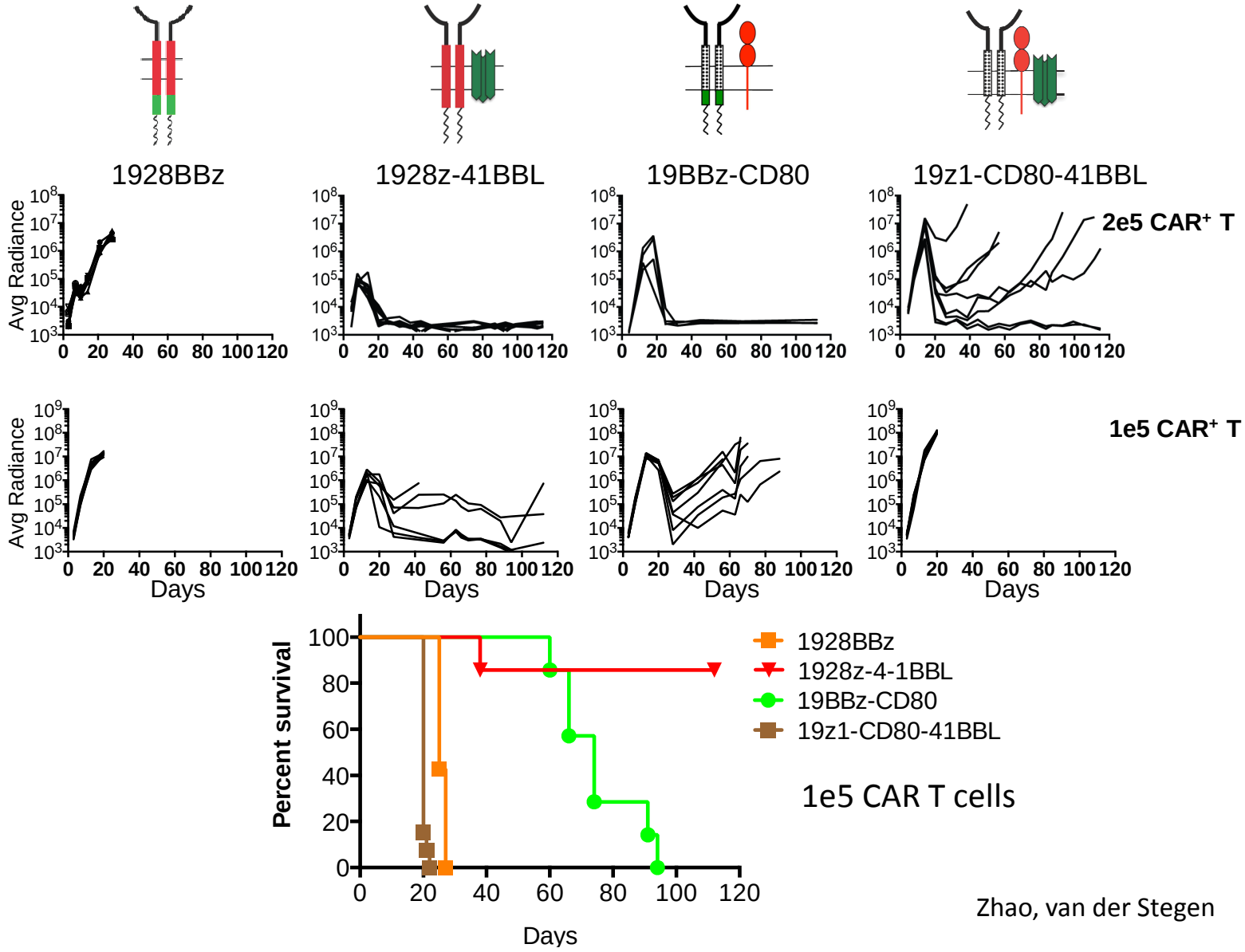

2e5 CAR T cells
1e5 CAR T cells
Percent survival
Days
19z1
1928z
19BBz
Untreated
***
NS
***
Zhao, van der Stegen

T cell accumulation and tumor burden in bone marrow

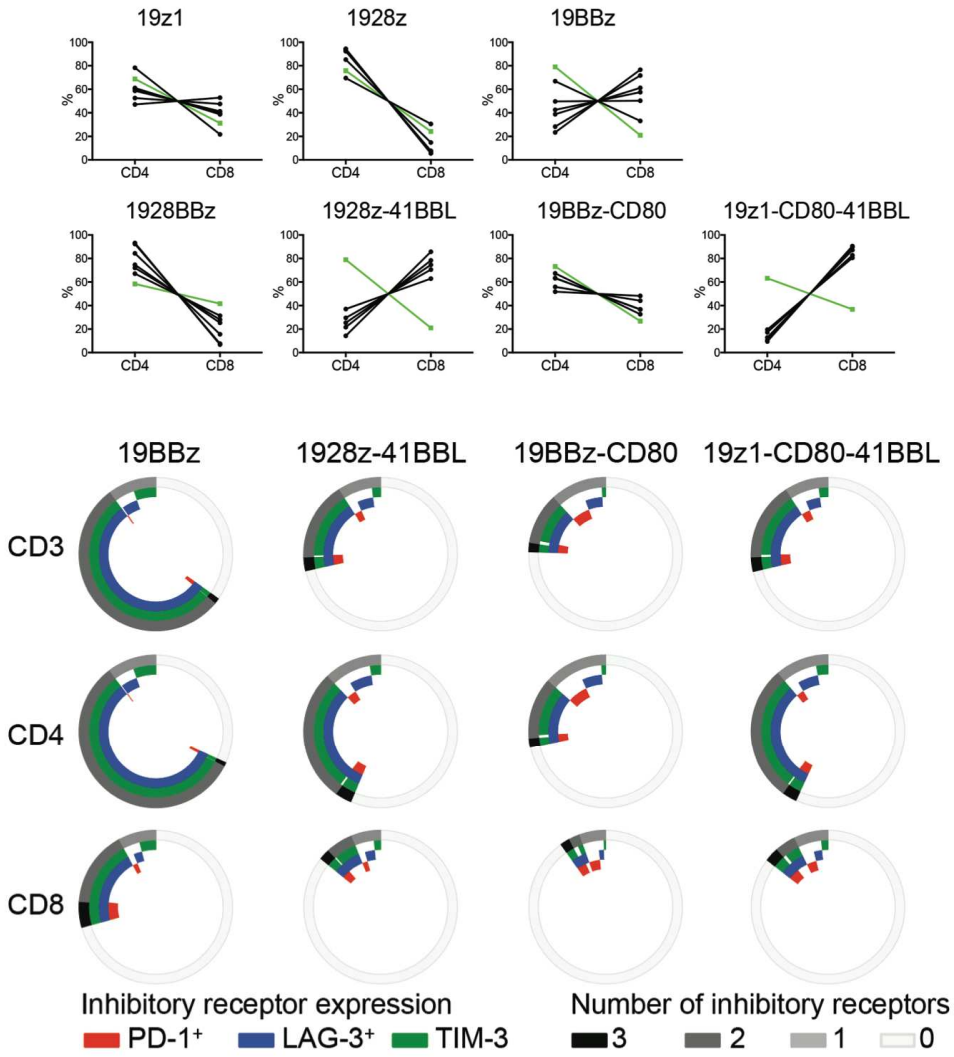
19z1 vs 19-28z vs 19-BBz



2e5 CAR T cells

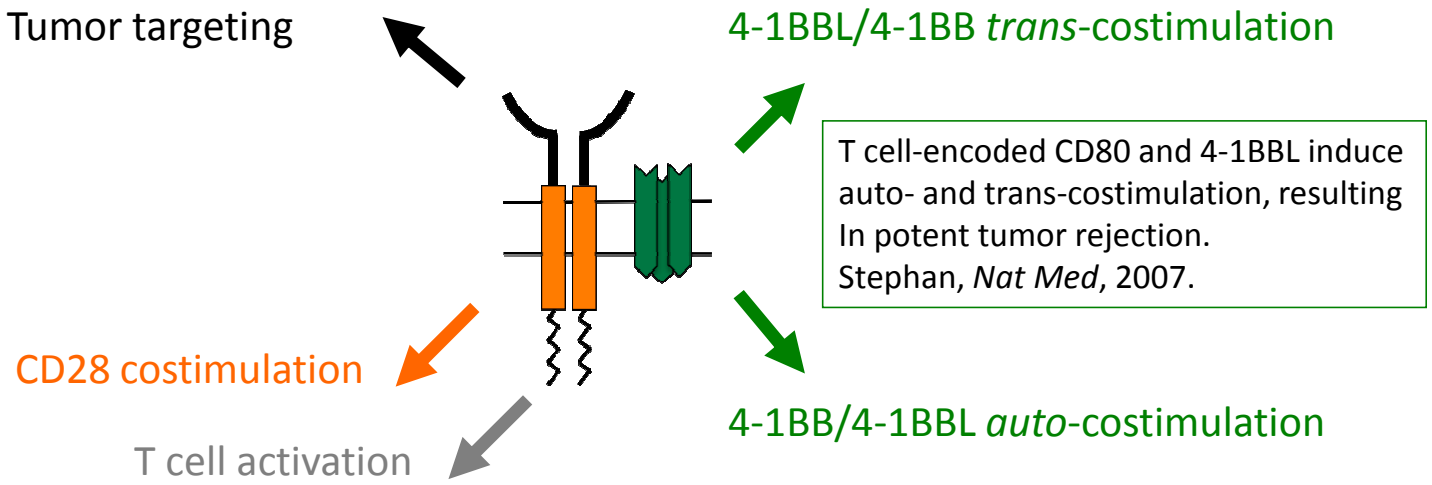


Zhao, van der Stegen


19z1
1928z
19BBz
1928BBz
1928z-41BBL
19BBz-CD80
19z1-CD80-41BBL
%
CD4
CD8
19BBz
1928z-41BBL
19BBz-CD80
19z1-CD80-41BBL
CD3
CD4
CD8
Inhibitory receptor expression
PD-1+
LAG-3+
TIM-3
Number of inhibitory receptors
3
2
1
0

Altering the tumor microenvironment by *trans*-costimulation

The "28z + 4-1BBL" CAR model

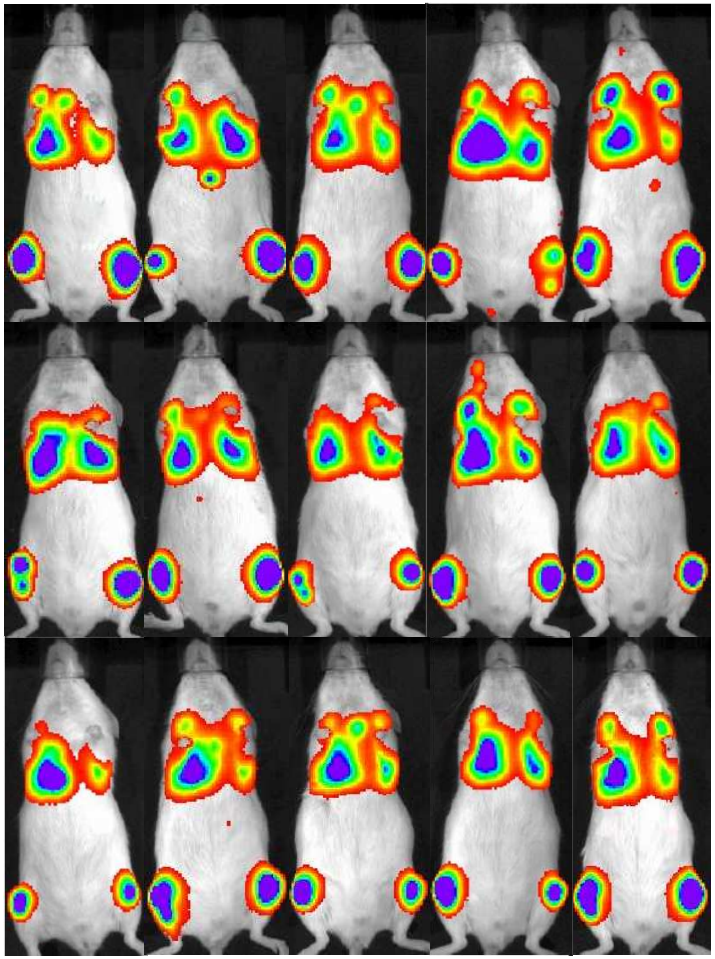


DAY 0

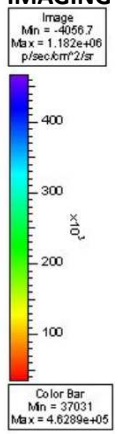
PZ1 click
+19Z click
+PZ1

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

PZ1 click
+19Z click
+19Z-4-1BBL-CD80



TUMOR
IMAGING

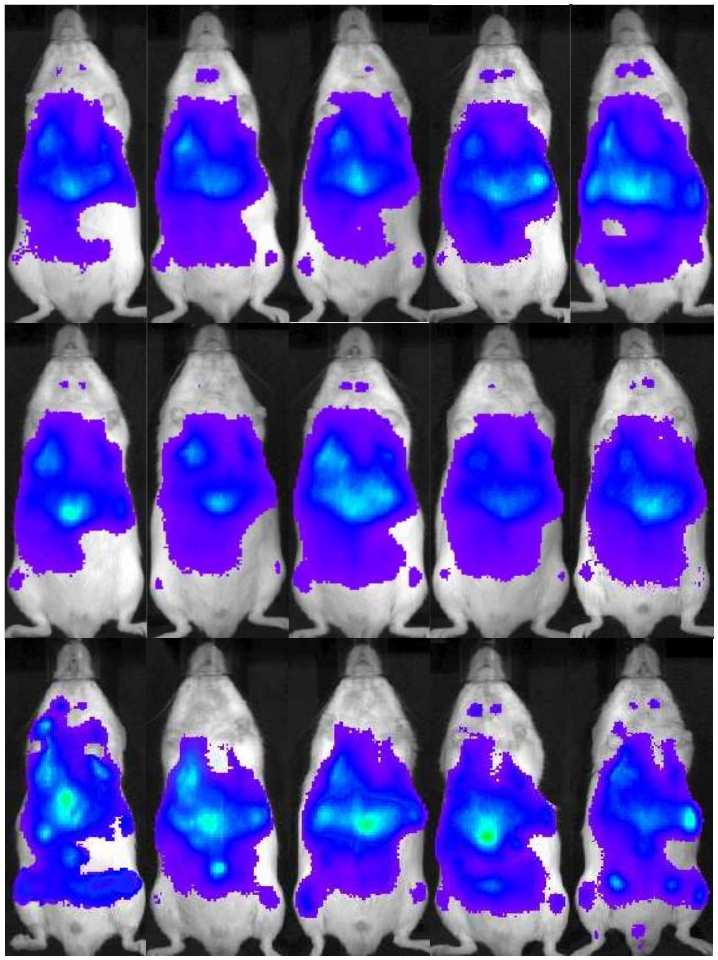


DAY 1

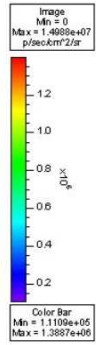
PZ1 click
+19Z click
+PZ1

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

PZ1 click
+19Z click
+19Z-4-1BBL-CD80



T CELL IMAGING

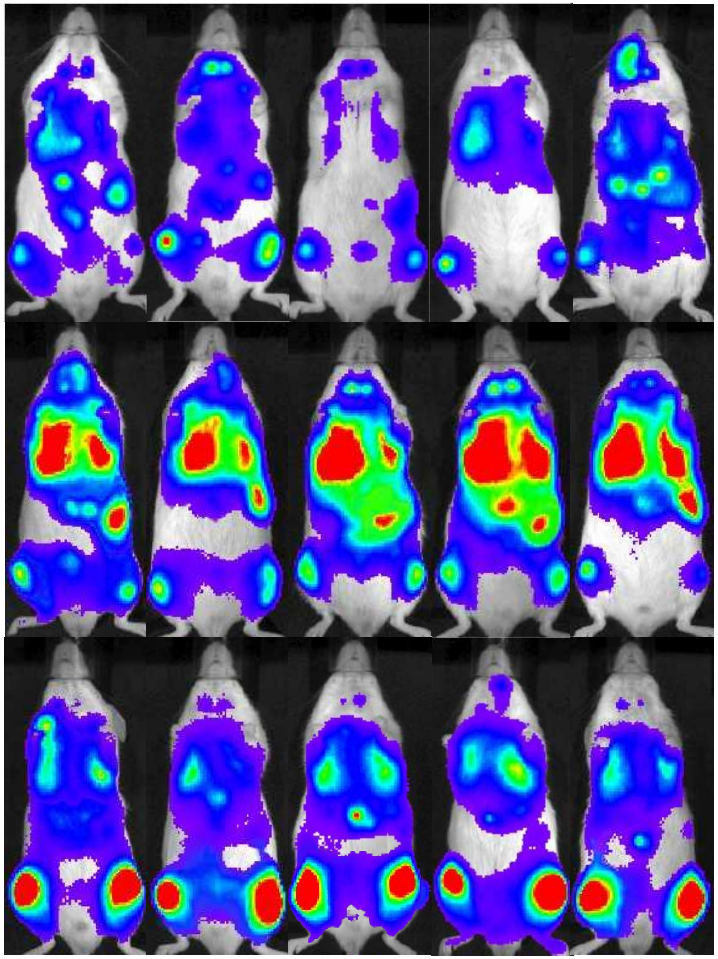


DAY 5

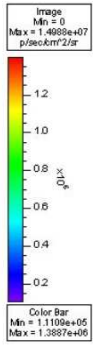
PZ1 click
+19Z click
+PZ1

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

PZ1 click
+19Z click
+19Z-4-1BBL-CD80

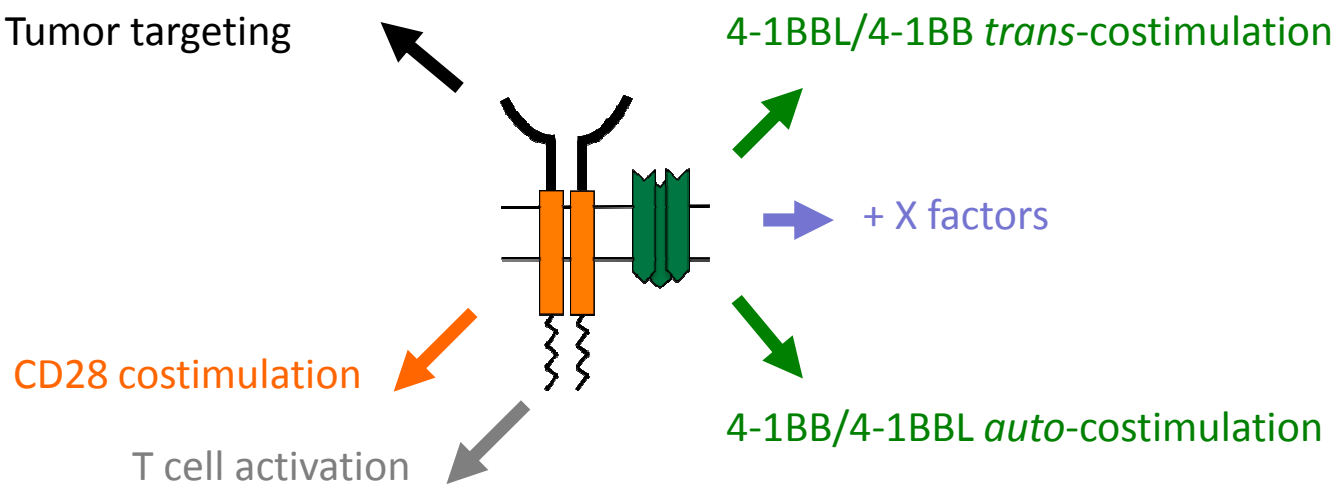


T CELL IMAGING

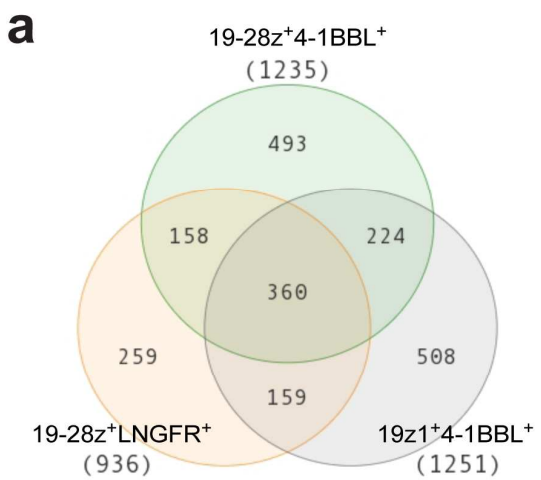


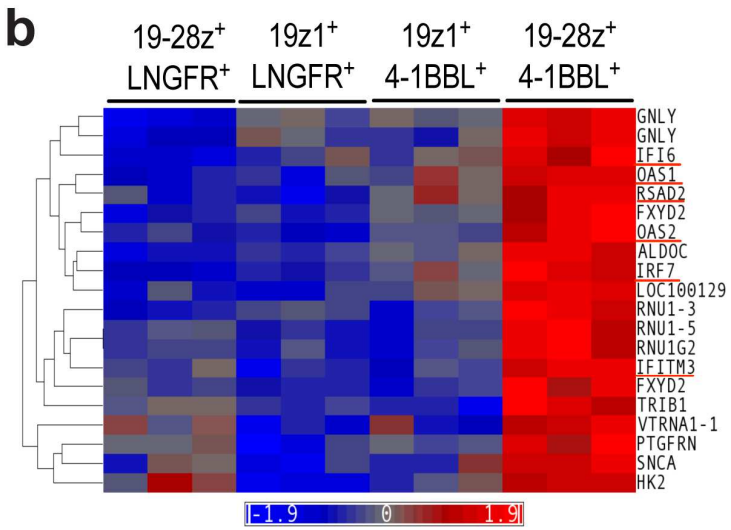
Altering the tumor microenvironment by *trans*-costimulation… and more

The “28z + 4-1BBL” CAR model

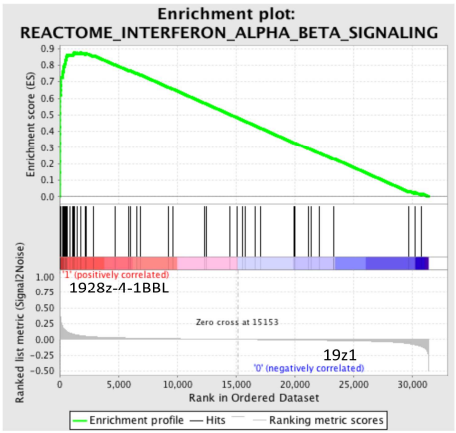


Microarray studies reveal strong induction of IRF7/IFNß in 19-28z/4-1BBL T cells

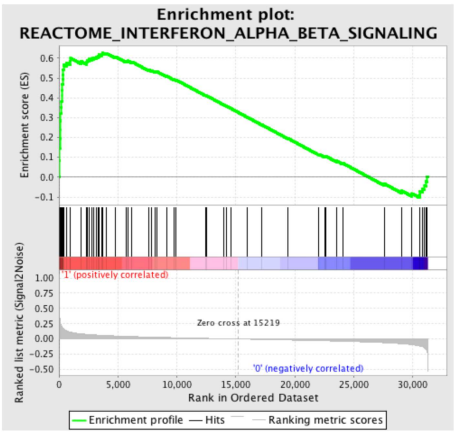




CD4

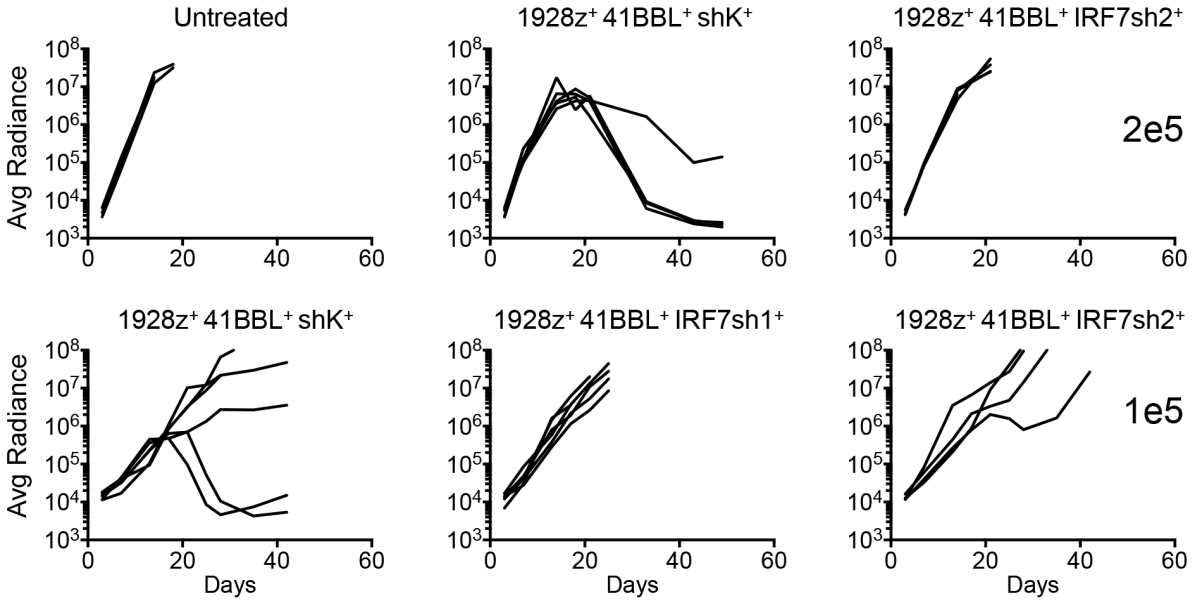


CD8

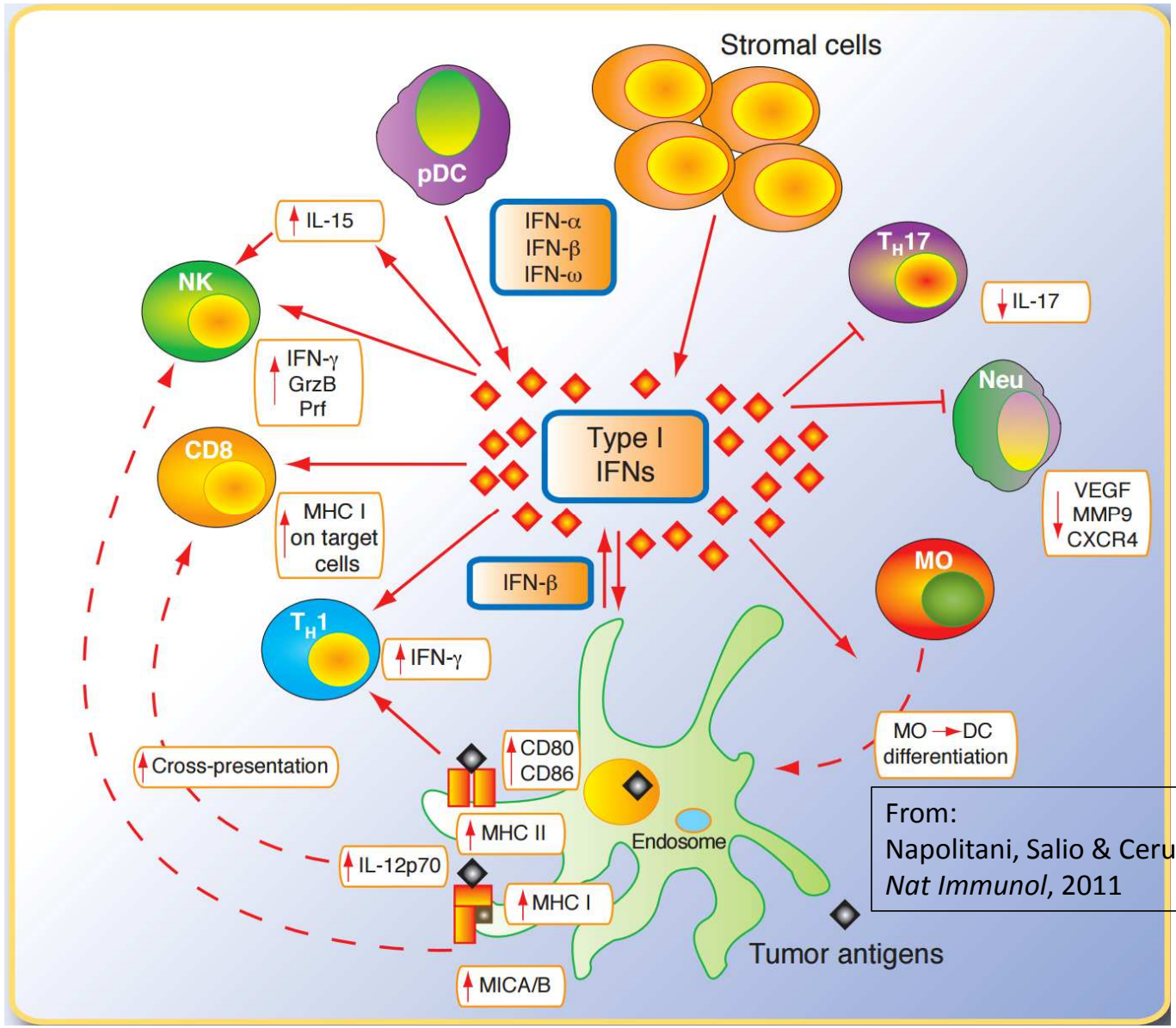


Maud Condomines,
Fabiana Perna

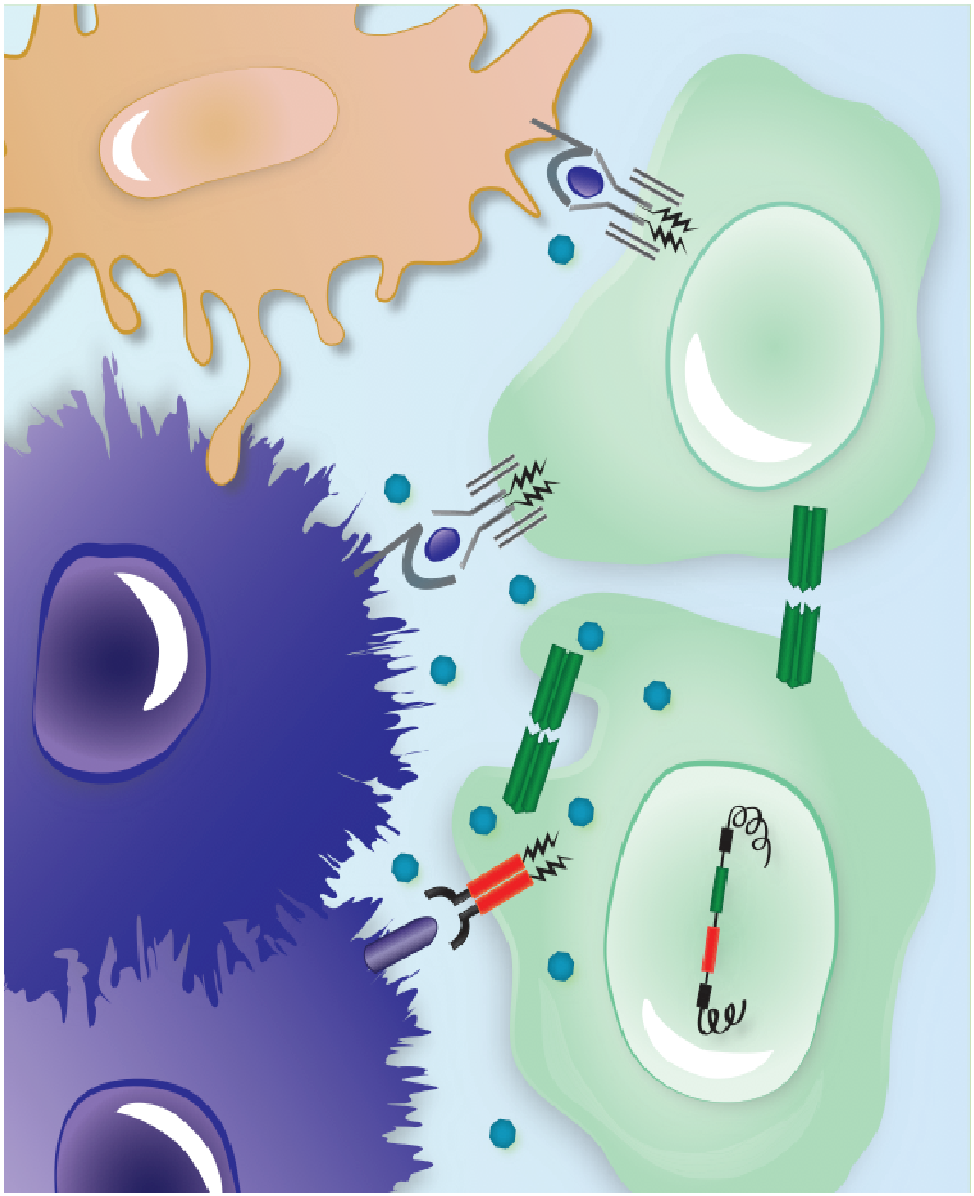
IRF7 knock-down impairs the therapeutic efficacy of CD19 CAR T cells



Maud Condomines, Zeguo Zhao

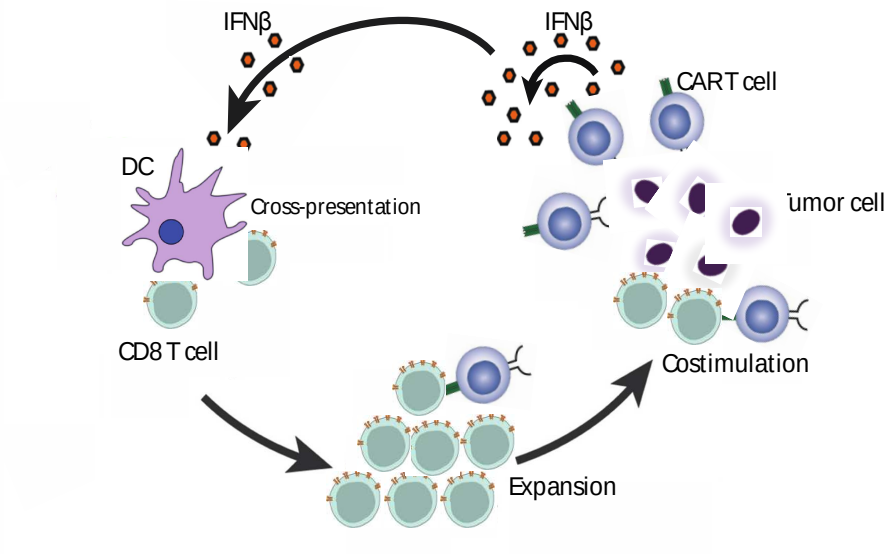


From:
Napolitani, Salio & Cerundolo
Nat Immunol, 2011



Summary

- 2nd-gen CARs retarget and functionally enhance T cells
- CAR T cells induce CRs where chemotherapy drugs have failed ("the CD19 paradigm")
- CAR T cells can be engineered to graded potency levels
- CAR T cells are not just "tumor killers" and can be harnessed to reprogram the TME (trans-costimulation, IFN-ß)



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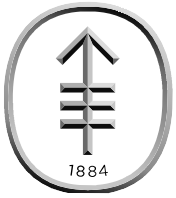
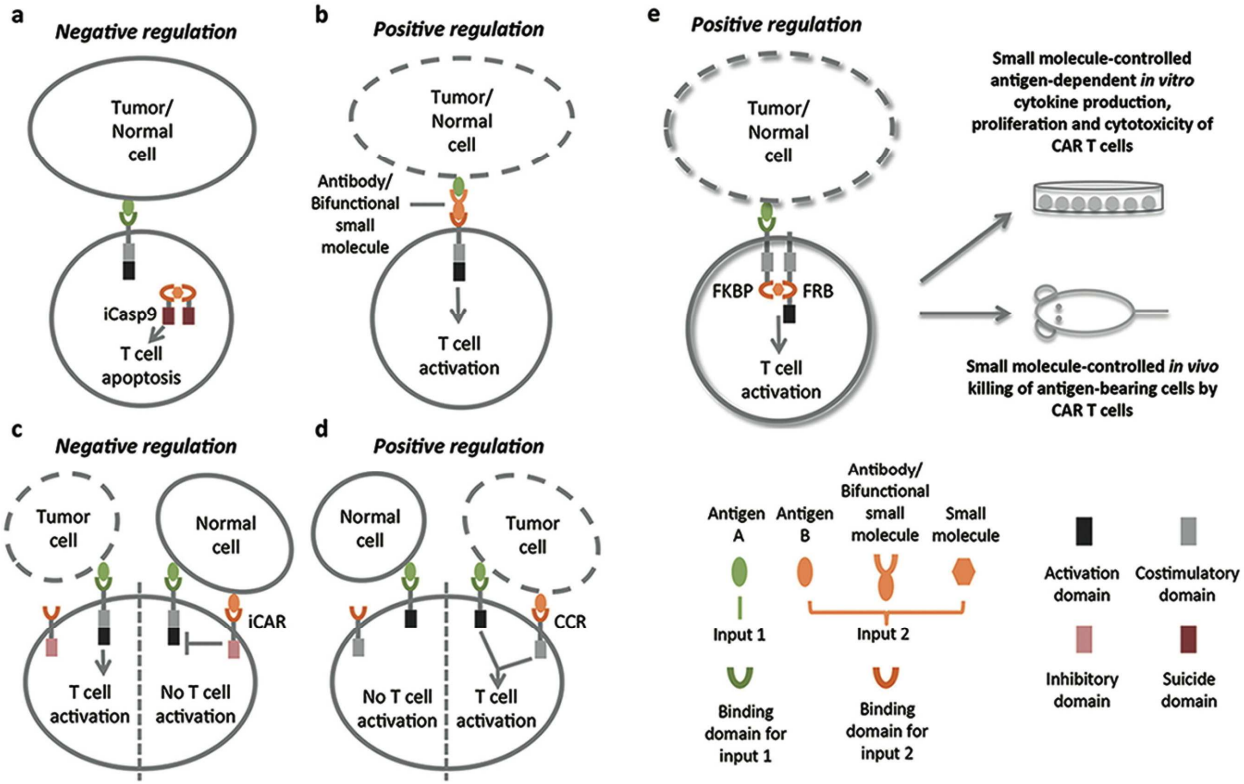


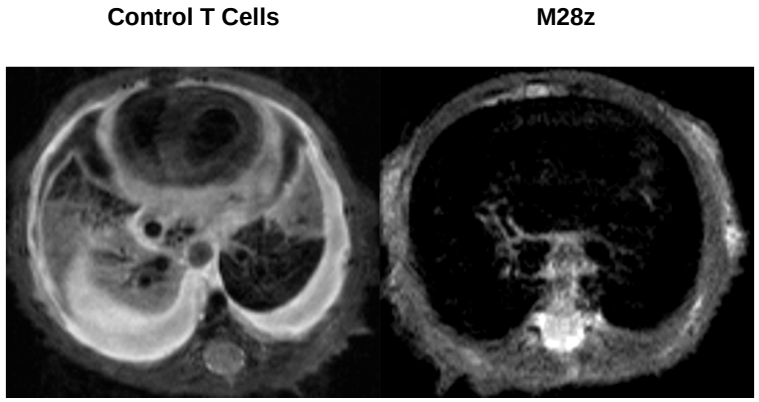
Memorial Sloan Kettering Cancer Center



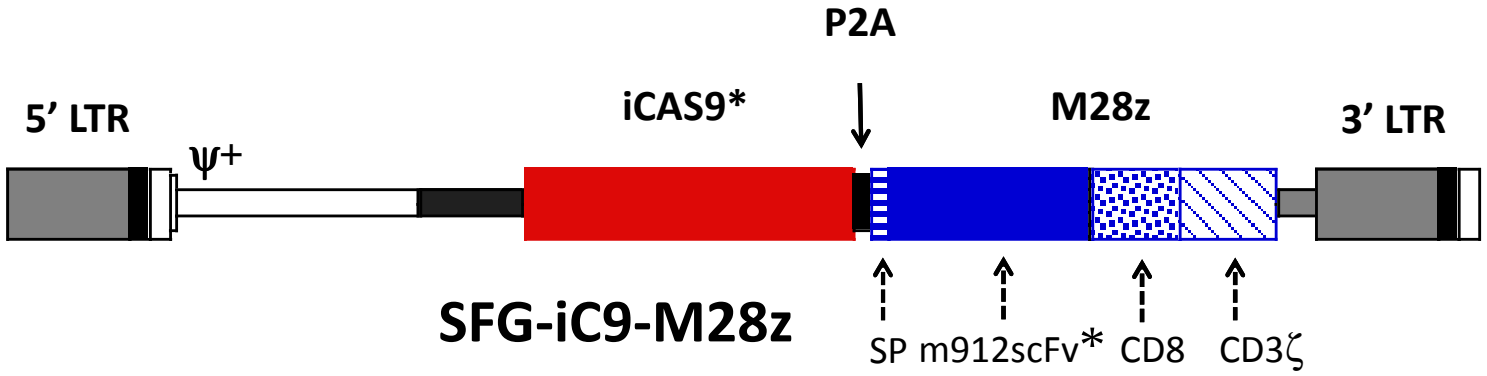
Building controls into engineered T cells. (a) The small molecule AP1903 can dimerize the suicide switch iCasp9 to induce T cell apoptosis. (b) Bifunctional small molecule bridging the binding between antigen and CAR or antibody mediating the interaction between antigen and synthetic Fc receptor can be remote controls of CAR T cells. (c) iCAR can inhibit CAR function in the presence of an antigen present in normal cells but not tumor cells. (d) CCR binding to a second antigen in tumor cells is required for full T cell activation. (e) The small molecule AP21976 can dimerize two independent signaling entities through an FKBP-FRB module to control T cell activation. (a, b, e) Strategies employing one remote control (antibody or small molecule) in addition to one autonomous control (antigen A). (c, d) Strategies with two autonomous controls (antigen A and antigen B). Negative regulation involves inducing apoptosis (a) or turning off T cell activation (c) by input 2 while positive regulation (b, d, e) results in T cell activation by input 2. Jie and Sadelain, *Cell Res*, 2015

Mesothelin CAR trial at MSKCC - NCT02414269

A Phase I Clinical Trial of Malignant Pleural Disease Treated with Autologous T Cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin
PI: Prasad Adusumilli

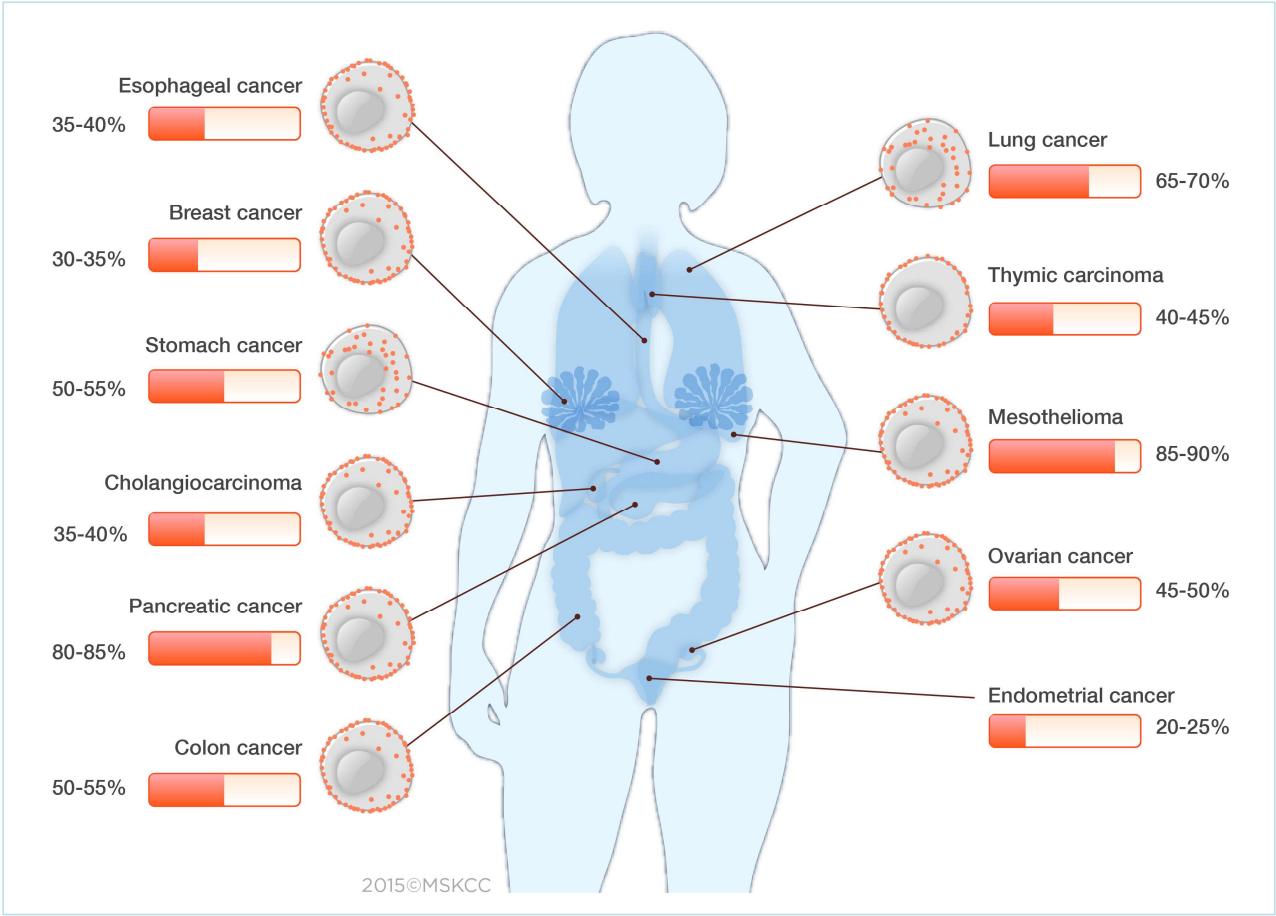


MRI Scan in Representative Mice Administered with Control or Mesothelin-Targeted M28z CAR T Cells



*confers sensitivity to AP1903 (Bellicum); human scFv

Mesothelin expression in solid tumors



Morello, Sadelain and Adusumilli, *Can Discov*, in press