CAR T-cell therapy of solid tumors

- Interdisciplinary Technical Journal Club: special series on Laboratory Animal Science -

Silvia Sorce

7th November 2017

Outline

✧ Introduction

✧ Specific challenges of CAR T-cell therapy of solid tumors:

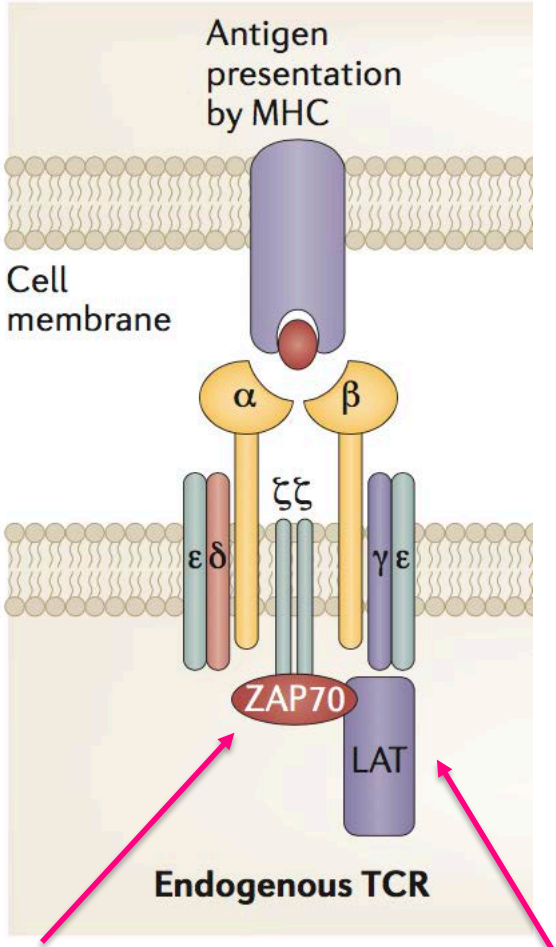
- antigen selection

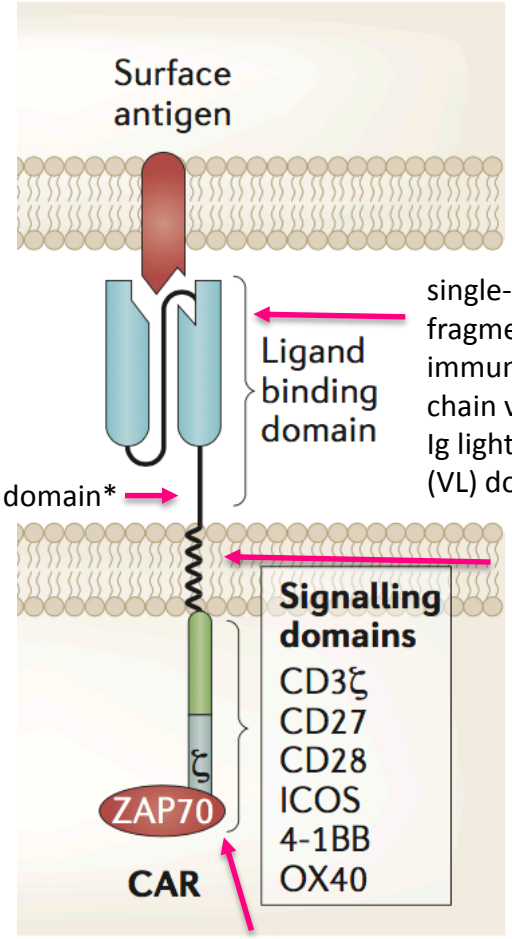
- trafficking and penetration

- immunosoppressive environment

✧ Clinical applications

Chimeric antigen receptors (CARs)





single-chain variable fragments derived from immunoglobulin heavy chain variable (VH) and Ig light chain variable (VL) domains

ζ-associated protein of 70 kDa (ZAP70)

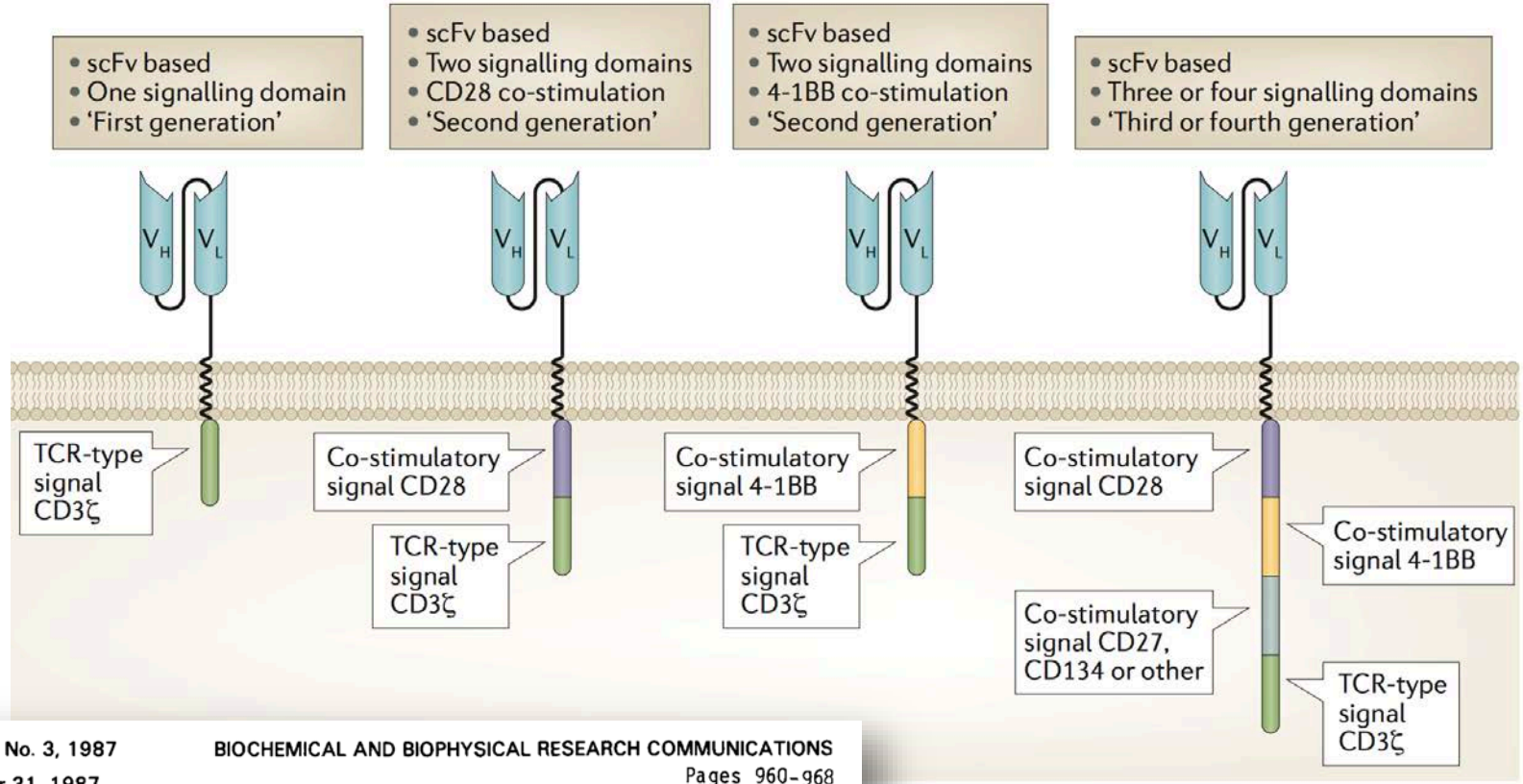
linker for activation of T cell family member 1

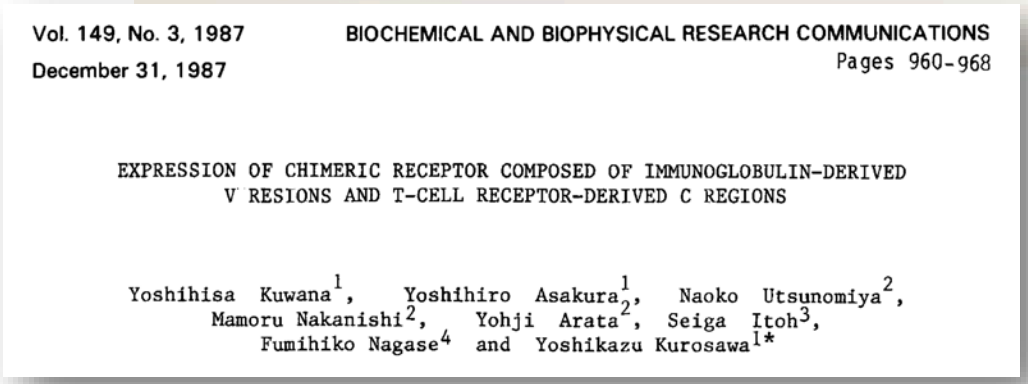
intracellular co-stimulatory domain and an intracellular CD3ζ chain domain

*CARs frequently incorporate a spacer/linker region based on the constant region of either IgG1 or IgG4 to connect extracellular ligand-binding with intracellular signaling domains.

Fesnak et al., 2016

Chimeric antigen receptor design and evolution





Vol. 149, No. 3, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 31, 1987 Pages 960-968

EXPRESSION OF CHIMERIC RECEPTOR COMPOSED OF IMMUNOGLOBULIN-DERIVED V REGIONS AND T-CELL RECEPTOR-DERIVED C REGIONS

Yoshihisa Kuwana[1], Yoshihiro Asakura[1], Naoko Utsunomiya[2], Mamoru Nakanishi[2], Yohji Arata[2], Seiga Itoh[3], Fumihiko Nagase[4] and Yoshikazu Kurosawa[1]*

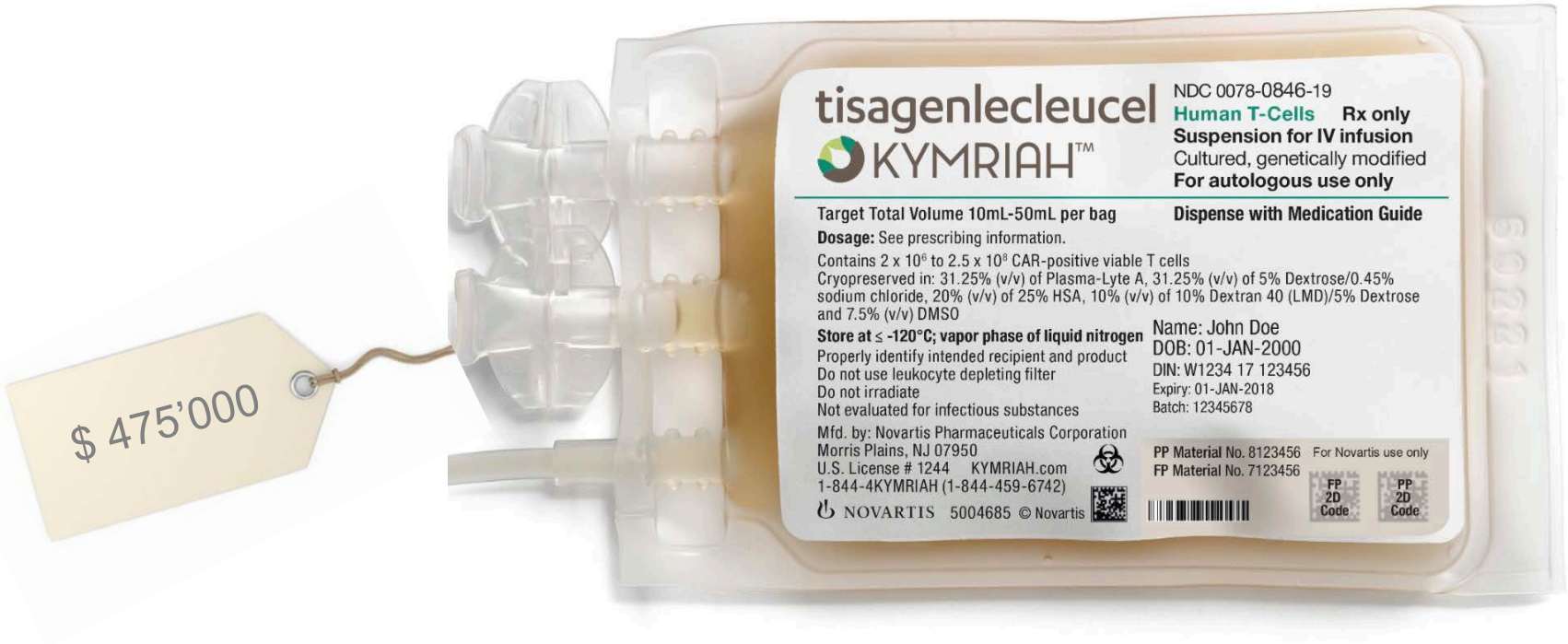
Fesnak et al., 2016

1st FDA approval for a CAR-T cell therapy: Kymriah™

- for children and young adults with **B-cell acute lymphoblastic leukemia** that is refractory or has relapsed at least twice

- first-in-class therapy showed an **83% (52/63) overall remission rate** in this patient population with limited treatment options and historically poor outcomes

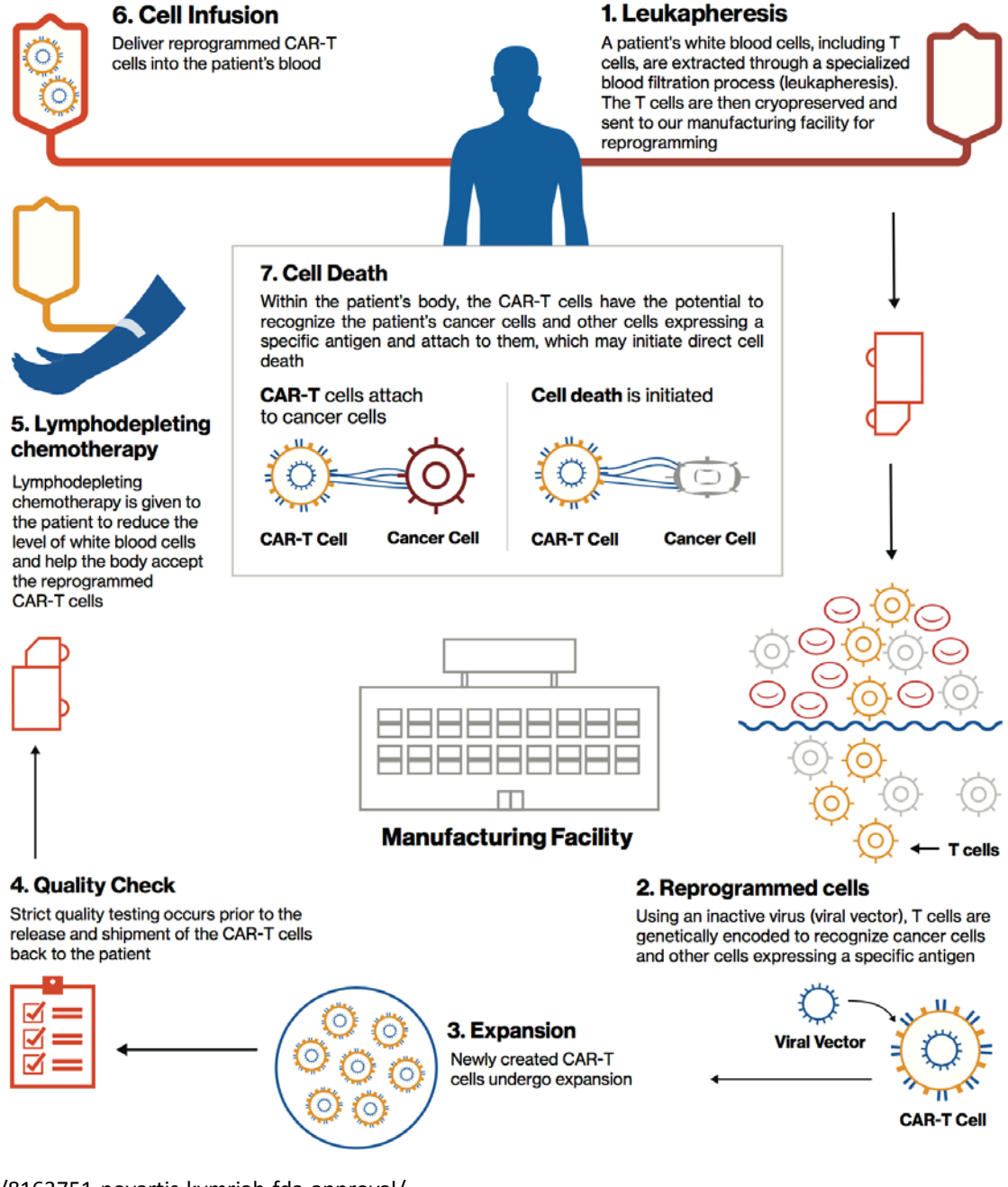
- in contrast to typical small molecule or biologic products, autologous CAR-T therapies are **specifically manufactured for each individual patient** and require a paradigm shift in the approach to manufacturing, logistics and administration



https://www.multivu.com/players/English/8163751-novartis-kymriah-fda-approval/

http://www.fiercepharma.com/pharma/at-475-000-per-treatment-novartis-kymriah-a-bargain-or-just-another-example-skyrocketing

How CAR-T Therapy Works



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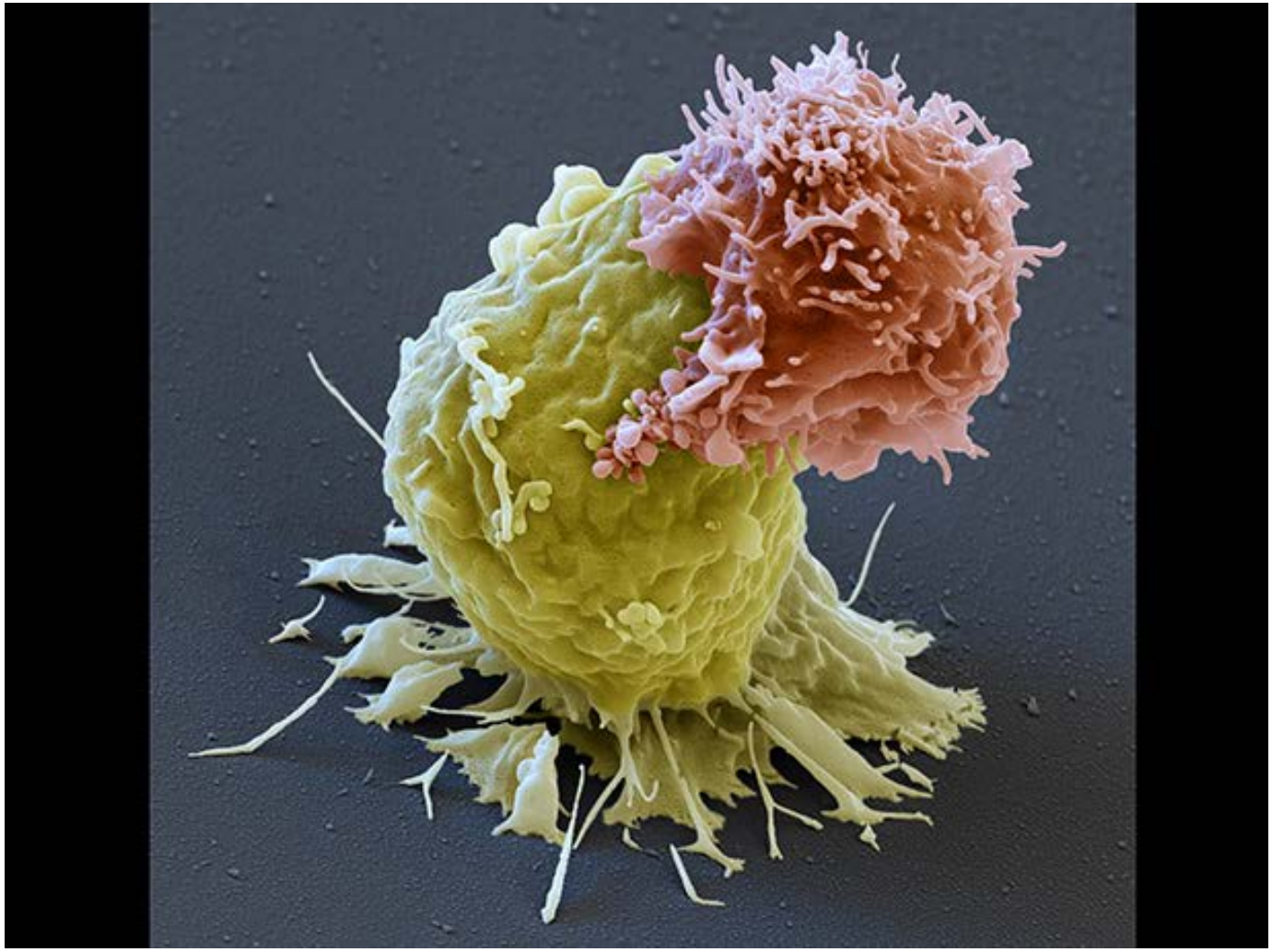
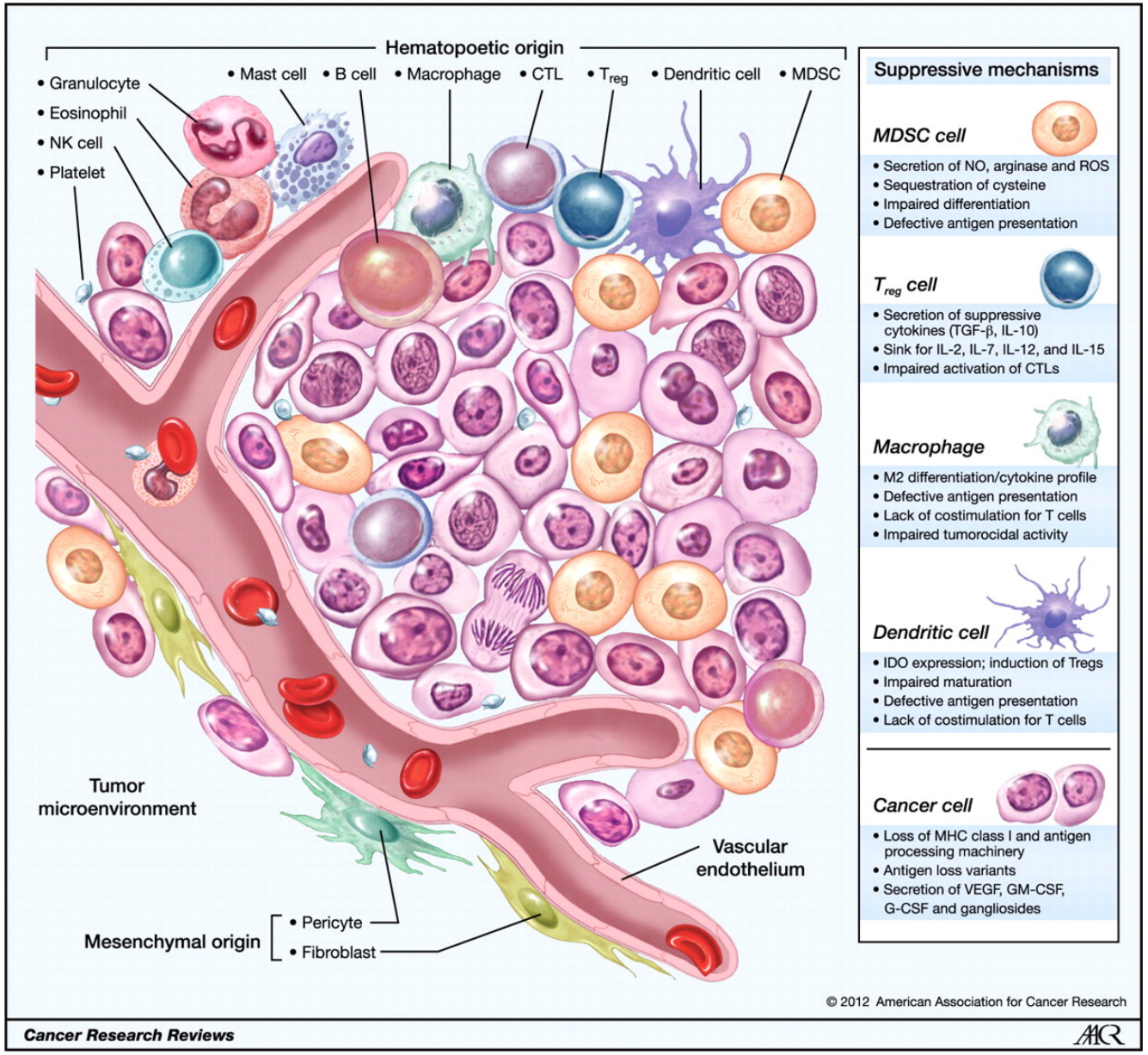


A CAR-T cell (red) attacks a leukemia cell (yellow)

https://www.sciencenews.org/article/engineered-immune-cells-boost-leukemia-survival-some



-Established tumors are complex masses that contain **not only neoplastic cells** but also nontransformed cellular elements such as stromal cells, the neovasculature, and the full gamut of immune cells

-these cells foster neovascularization and provide optimal cytokine and inflammatory support to drive the proliferation of transformed cells into solid masses

-immune cells that reside within tumors are dysregulated and functionally impaired

Cancer Res; 72(13); 3125–30

CARs for solid tumors: challenges

The use of CAR T cells for the treatment of solid tumors involves a unique set of challenges, including:

- **antigen selection**: target antigens on solid tumors are often heterogenous, differing not just within one tumor but also between both primary and metastatic tumors
- **tumor trafficking and infiltration**: CAR T cells must encounter the correct chemotactic signals to traffic to the tumor in sufficient numbers. Abnormal vasculature impedes efficient infiltration, and physical barriers from both surrounding stroma and infiltrating pro-tumor immune cells prevent adequate penetration
- **an immunosuppressive microenvironment:** multitude of immunosuppressive factors such as checkpoint pathways, cytokines and by-products from an altered metabolism all accumulate into what seems to be an almost impossible challenge for CAR T cells

Yong et al., 2017

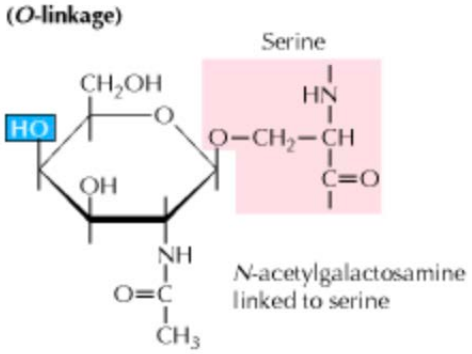
ANTIGEN SELECTION

Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma

Avery D. Posey, Jr.,[1,*] Robert D. Schwab,[1] Alina C. Boesteanu,[1] Catharina Steentoft,[2] Ulla Mandel,[2] Boris Engels,[3,7] Jennifer D. Stone,[4] Thomas D. Madsen,[2] Karin Schreiber,[3] Kathleen M. Haines,[1] Alexandria P. Cogdill,[1] Taylor J. Chen,[1] Decheng Song,[1] John Scholler,[1] David M. Kranz,[4] Michael D. Feldman,[5] Regina Young,[1] Brian Keith,[1] Hans Schreiber,[3] Henrik Clausen,[2] Laura A. Johnson,[1,5,6] and Carl H. June[1,5,6,*]

- One well-characterized cellular process involved in differential processing following **malignant transformation** is **protein glycosylation**
- The most prevalent aberrant glycoforms found in cancer are the **Tn** (GalNAcα1-O-Ser/Thr) and **sialyl-Tn** (STn) (NeuAcα2-6-GalNAca1-O-Ser/Thr) **glycoforms**
- **Aberrant expression of Tn and STn glycoforms** have in particular been found **on the cell membrane mucin MUC1**, which is a large protein with tandem repeated sequences carrying O-glycans overexpressed in most adenocarcinomas

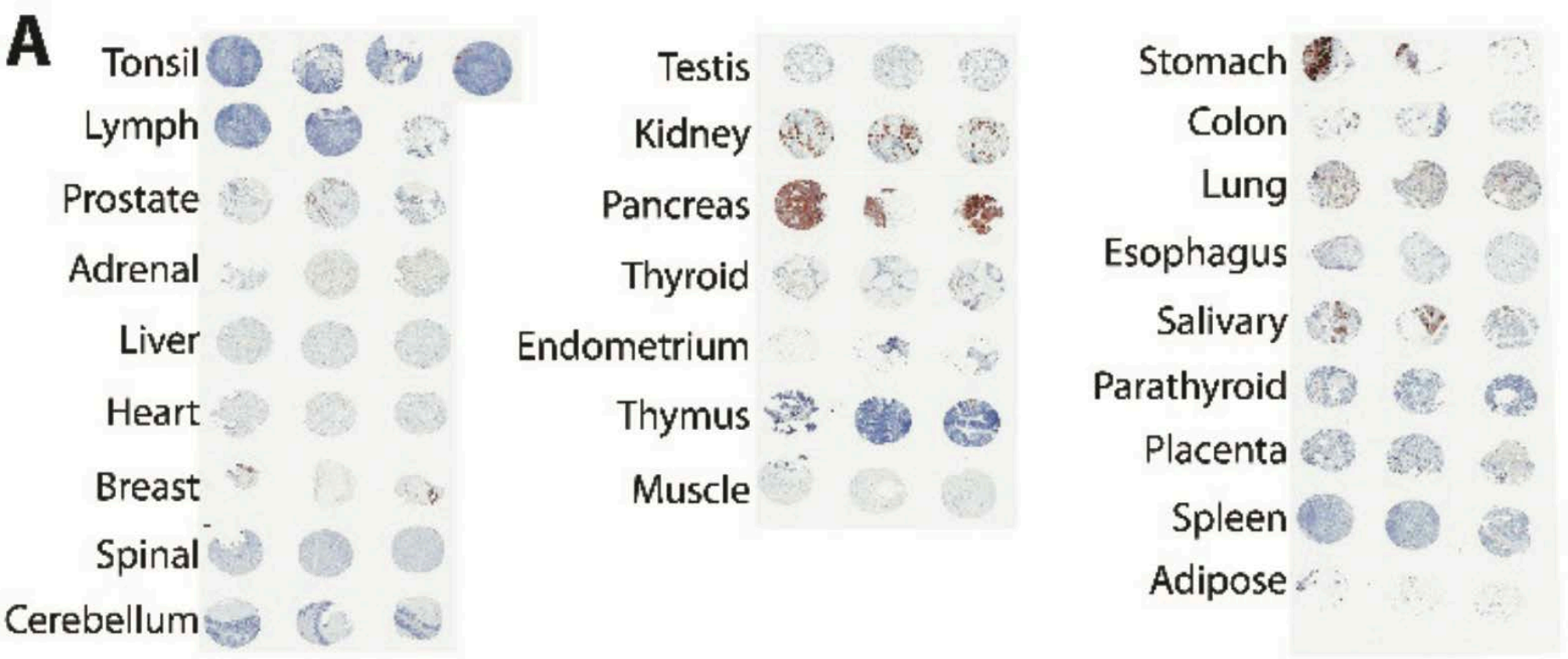
→ **developed and characterized a novel CAR based on a monoclonal antibody (5E5) specific to a Tn-MUC1 glycopeptide epitope widely expressed by adenocarcinomas**



In normal cells GalNAc residues attached to the protein back-bone are further elongated by the T synthase, which require the chaperone COSMC

Posey et al., 2016, Immunity *44*, 1444–1454
June 21, 2016
http://dx.doi.org/10.1016/j.immuni.2016.05.014

5E5 staining of normal human tissue



Immunostaining of normal human tissues with 5E5 mAb demonstrates no bind- ing to most tissues, including tonsil, lymph node, prostate, adrenal, liver, heart, breast, spinal cord, cerebellum, cervix, testes, thyroid, endometrium, thymus, muscle, colon, esoph- agus, parathyroid, placenta, spleen, and adipose

In contrast, the tissue microarray for stomach, lung, pancreas, and kidney did stain with 5E5 mAb. However, with confocal microscopy, the 5E5 staining pattern was found to be largely intracellular

5E5 mAb has intense binding to the plasma membranes of human breast cancer

Posey et al., 2016, Immunity *44*, 1444–1454
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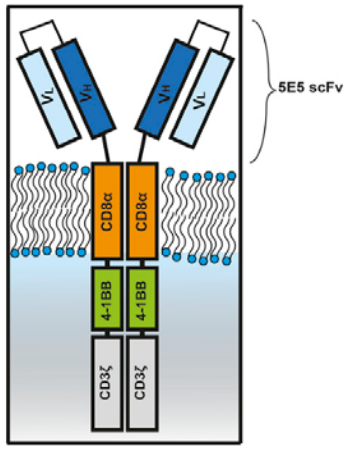
http://dx.doi.org/10.1016/j.immuni.2016.05.014

5E5 CAR T cell reactivity vs normal cell lines



Jurkat leukemia cell lines

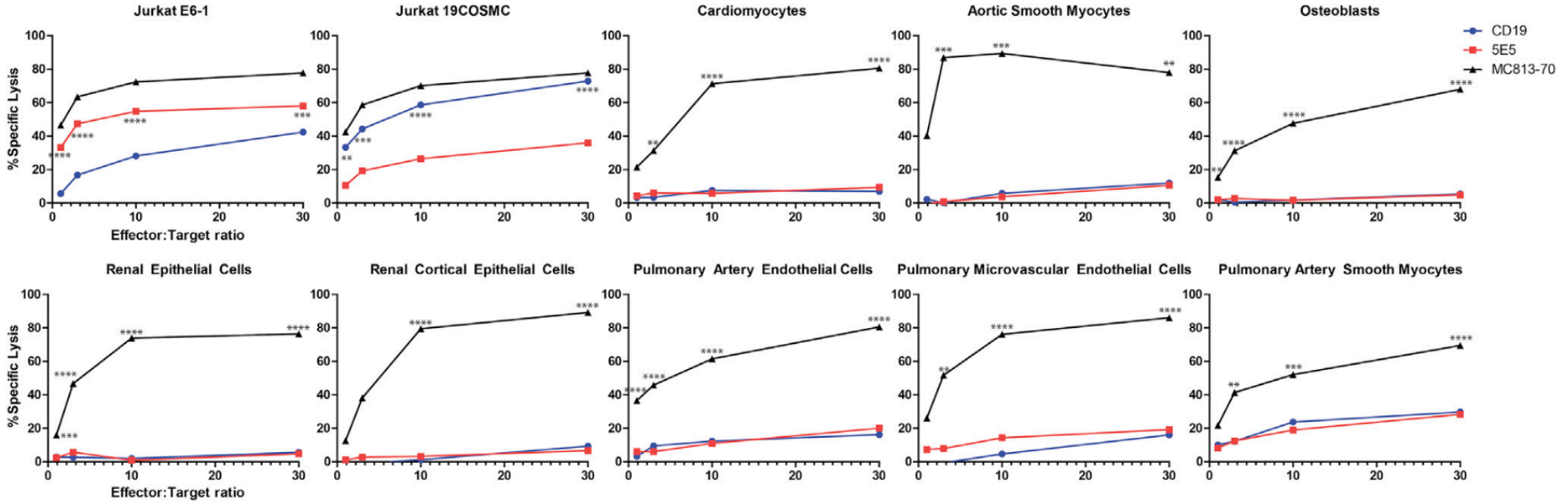


Figure 2. Evaluation of 5E5 CAR T Cells Reactivity to a Panel of Human Primary Cells

5E5 and CD19 CAR T cells were tested in a chromium release lysis assays at effector:target ratios of 1:1 to 30:1. MC813-70 CAR T cells known to exhibit normal tissue toxicity were used as a positive control. In addition the various CAR T cells were tested on wild-type Jurkat and Jurkat with reconstituted COSMC. Statistical comparisons are between 5E5 CAR and CD19 CAR in the positive controls Jurkat E6-1 and Jurkat 19COSMC. All other comparisons are made between MC813-70 and 5E5. ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

Posey et al., 2016, Immunity *44*, 1444–1454
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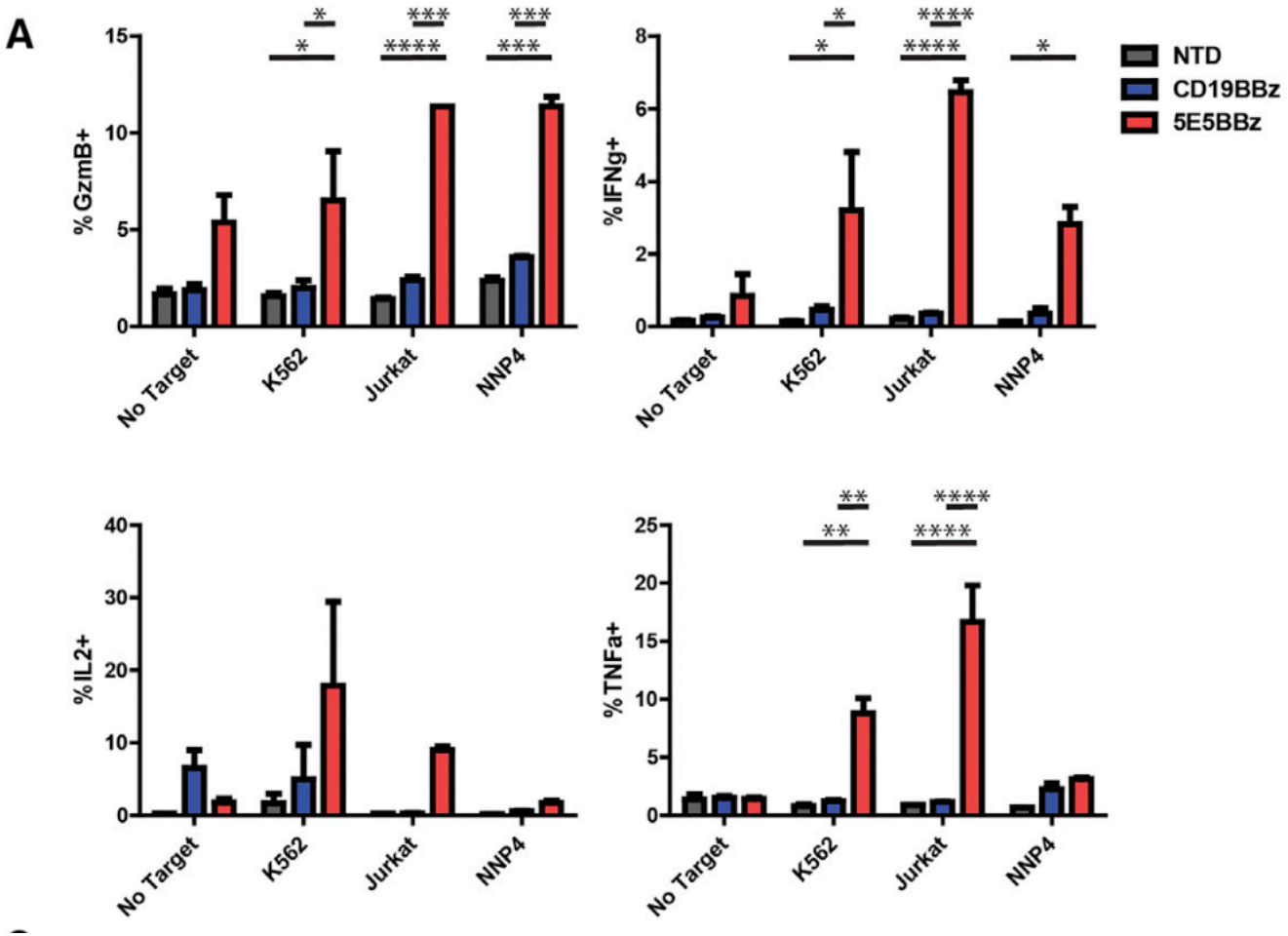
5E5 CAR T cell reactivity vs cancer cell lines *in vitro*



cultured overnight with K562 leukemia cell line, Jurkat leukemia cell line, or NNP4 primary ovarian cancer cells collected from a malignant pleural effusion, and analyzed for intracellular cytokine production and degranulation via flow cytometry

Posey et al., 2016, Immunity *44*, 1444–1454
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5E5 CAR T cell reactivity *in vivo:* leukemia model

Jurkat cells were then transduced with lentiviral vector encoding GFP and Click Beetle Green (CBG) luciferase, connected by a T2A signal peptide (GFP-T2A-CBG), for bioluminescence assays

A xenograft model of T cell leukemia was established in immune-compromised NOD-SCID-Gamma (NSG) mice by intravenously injecting 5 x 10^6 Jurkat GFP-T2A-CBG cells

8 days after tumor injection, mice were injected intravenously with non-transduced normal human donor T cells, CD19 CAR T cells, or 5E5 CAR T cells, where all cell populations were 50% CD4 and 50% CD8, and, in transduced groups (CD19 and 5E5), CD4+ T cells were 60% CAR+ and CD8+ T cells were 25% CAR+.

Posey et al., 2016, Immunity *44*, 1444–1454
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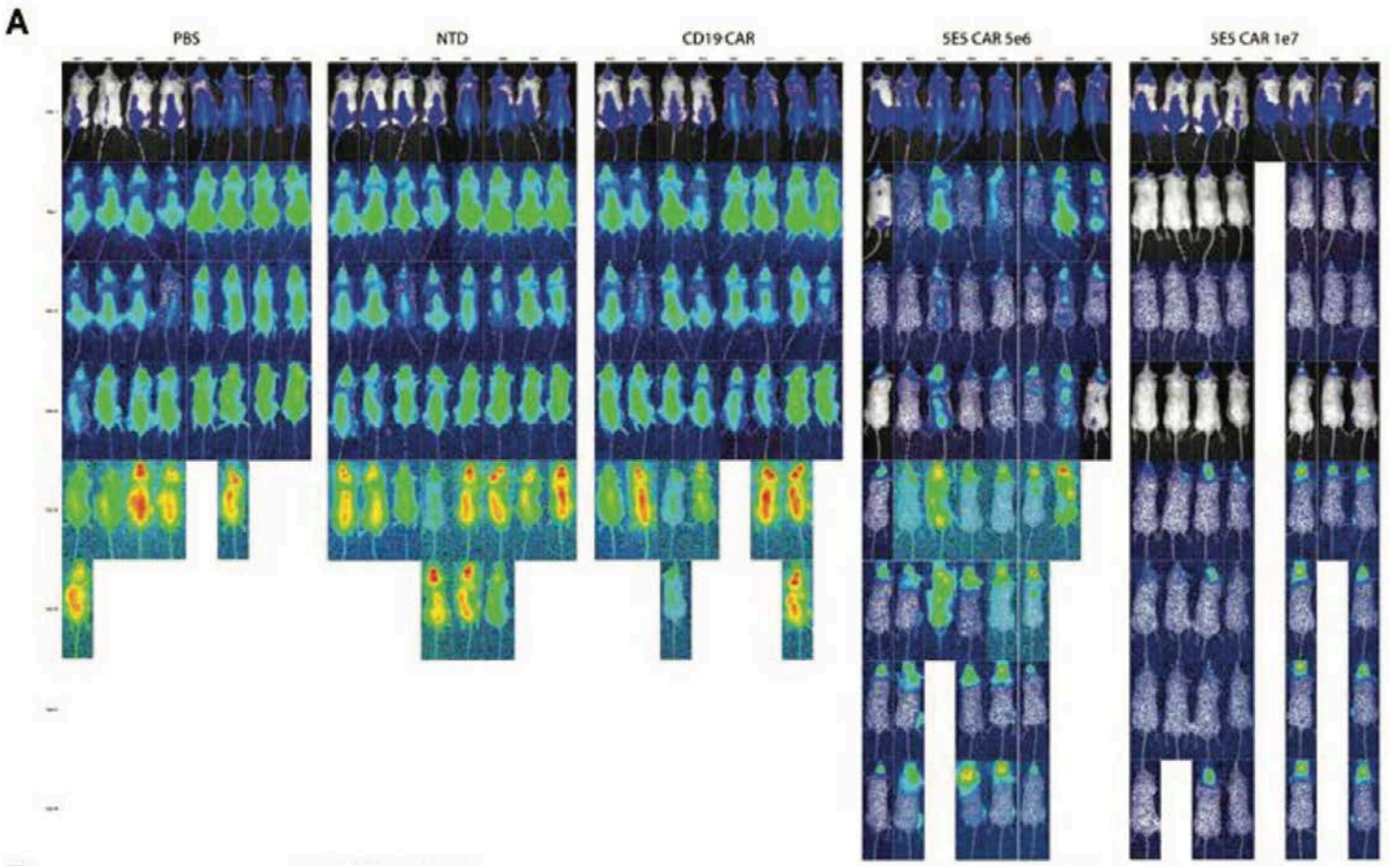
5E5 CAR T cell reactivity *in vivo:* leukemia model



Treated mice were imaged weekly for tumor bioluminescence until all control mice had reached pre-approved morbidity endpoints

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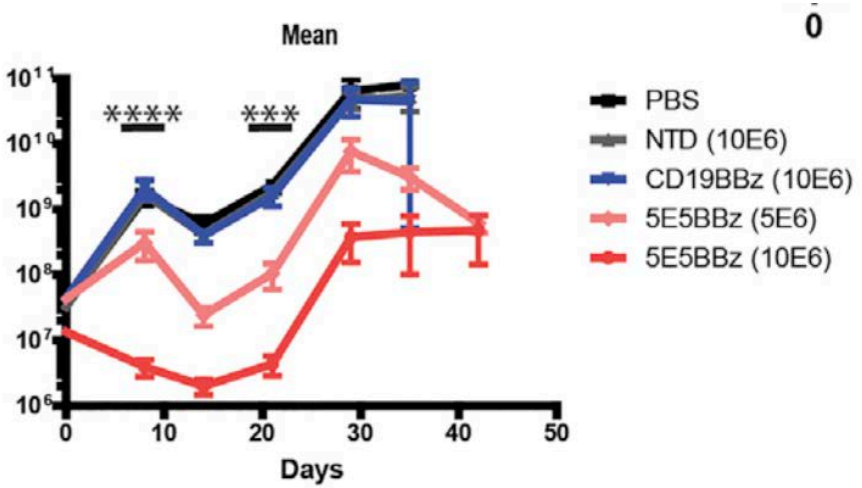
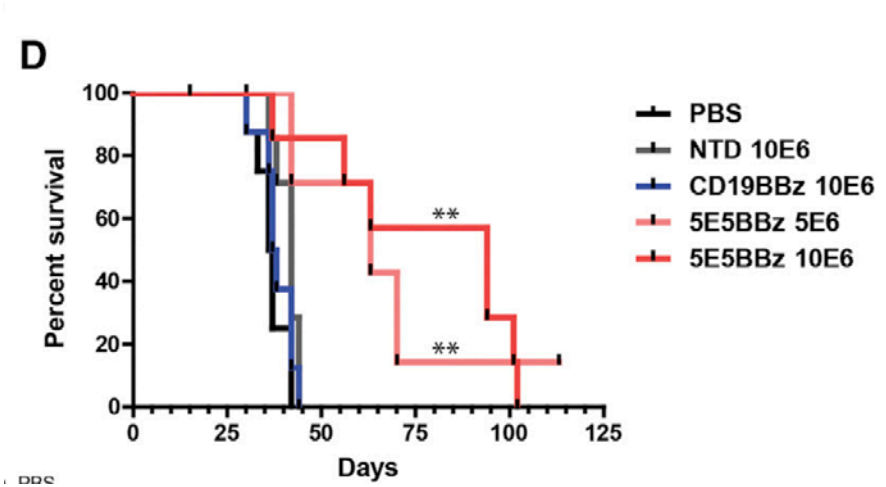
5E5 CAR T cell reactivity *in vivo:* leukemia model





5E5 CAR T cell treated mice demonstrated a reduction in tumor growth over all other groups throughout the experiment

Median survival of mice treated with non-transduced and CD19 CAR T cells was 42 and 37.5 days, respectively, with all control-treated mice dead within 36 days post T cell infusion.

In contrast, mice treated with 5E5 CAR T cells had a dose dependent increase in median survival of 63 and 94 days.

Posey et al., 2016, Immunity *44*, 1444–1454
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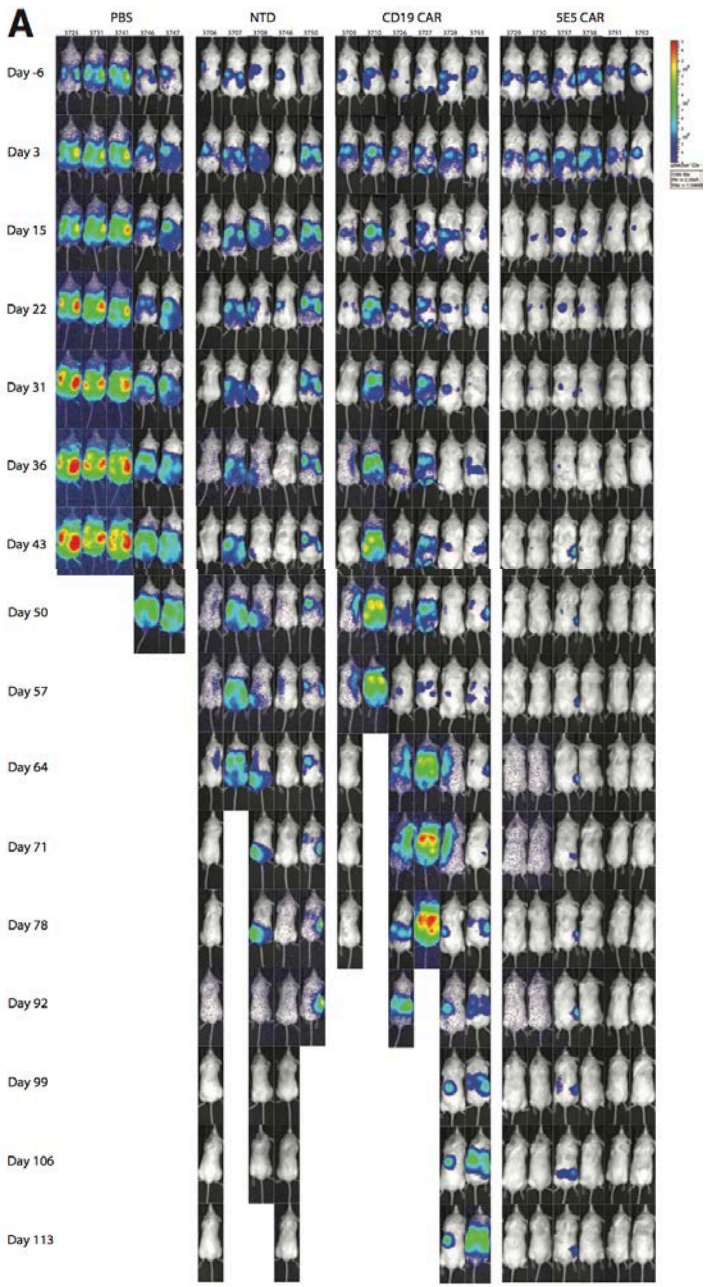
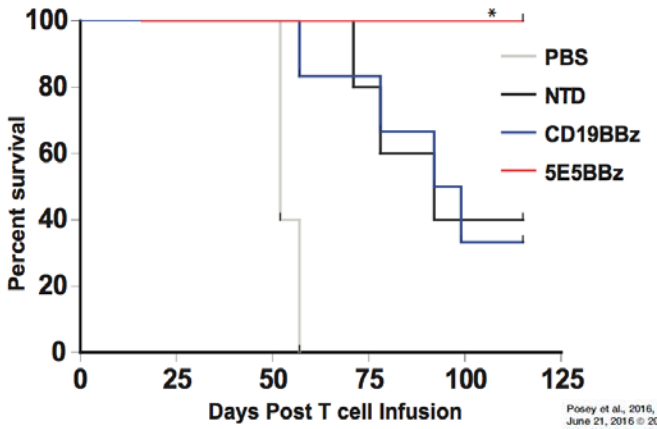
5E5 CAR T cell reactivity *in vivo:* Disseminated Pancreatic Cancer



- disseminated tumor xenograft model by injecting mice intraperitoneally with 1 x 105 **luciferase-expressing pancreatic cancer cell line Hs766T**
- Tumor engrafted in many areas of the peritoneal cavity, including in the mouse pancreas.
- Three weeks post tumor engraftment, when the mean tumor bioluminescence reached approximately $5 x 107 photons/second, mice were treated intravenously with PBS or non-transduced T cells, CD19 CAR or 5E5 CAR T cells.
- The 5E5 CAR T cells had a potent antitumor effect as **mice treated with 5E5 CAR T cells survived for 113 days post T cell infusion, at the termination of the experiment**
- **The 5E5 CAR treated mice had 100% survival**, compared with 40% and 33% survival of mice treated with non-transduced and CD19 CAR T cells, respectively ($p < 0.02$).



Posey et al., 2016, Immunity 44, 1444–1454
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http://dx.doi.org/10.1016/j.immuni.2016.05.014

Driving CARs into Sweet Roads: Targeting Glycosylated Antigens in Cancer

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*Correspondence: gabyrabi@gmail.com
http://dx.doi.org/10.1016/j.immuni.2016.06.010

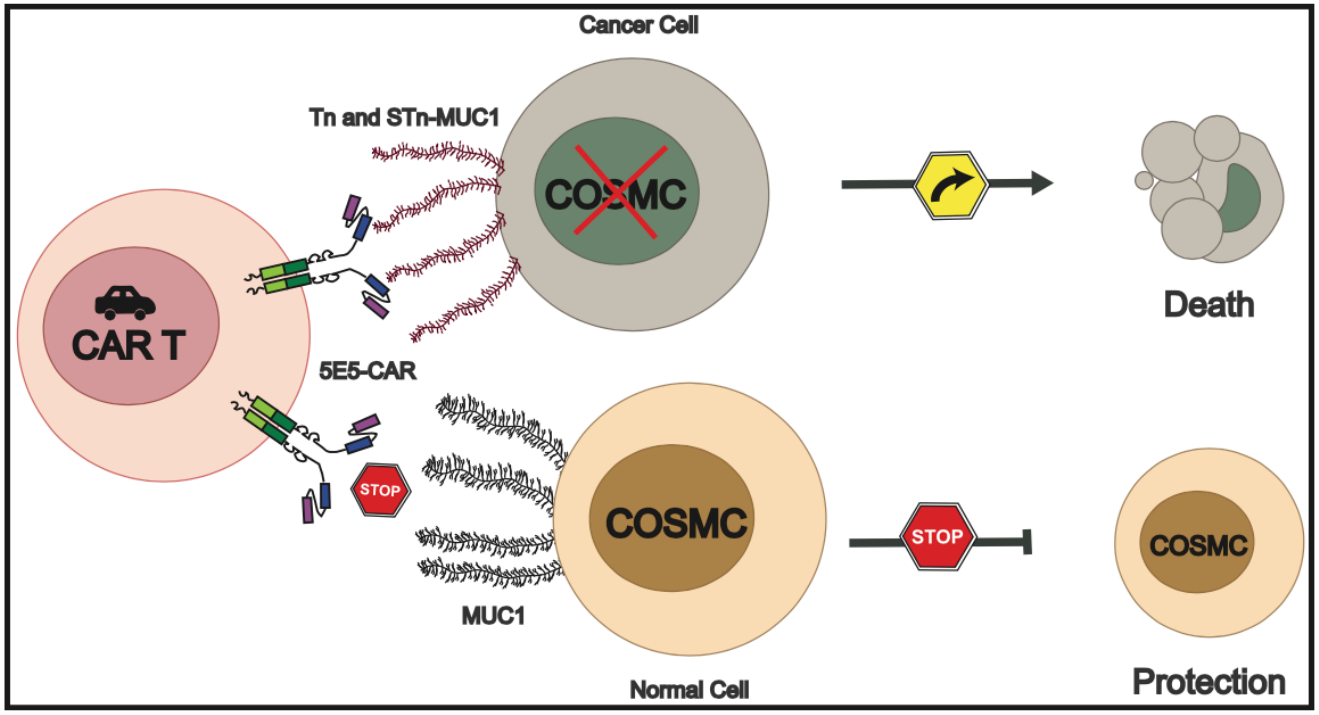


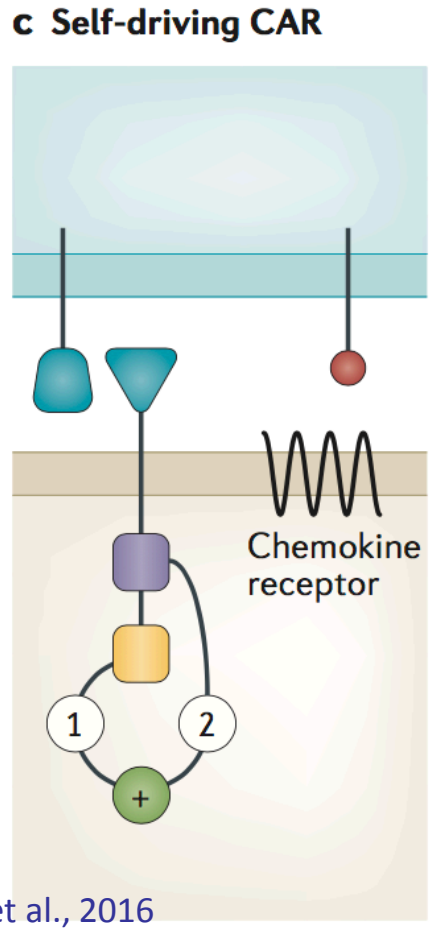
Figure 1. Selective Recognition of the Tumor-Associated Hypoglycosylated Forms Tn-STn on MUC-1 by T Cells Engineered to Express the Chimeric Antigen Receptor 5E5
In the absence of the molecular chaperone COSMC (required for stability of the C1GalT1 glycosyltransferase), exposure of the Tn-STn glycoepitope on MUC-1 is favored, leading to selective recognition of tumor cells by 5E5 CAR-T cells. In contrast, sustained expression of COSMC inhibits exposure of these hypoglycosylated epitopes, preventing cytotoxicity induced by engineered 5E5 CAR-T cells.

TRAFFICKING AND PENETRATION

TRAFFICKING AND PENETRATION: CHEMOTACTIC MANIPULATION (I)

Problem: solid tumors are known to secrete chemokines, such as CXCL12 and CXCL5 which inhibit T-cell migration

→ genetically modifying CAR T cells to express the appropriate chemokine receptor(s) may allow a greater proportion of cells to home to the tumor



Fesnak et al., 2016

Enhanced Tumor Trafficking of GD2 Chimeric Antigen Receptor T Cells by Expression of the Chemokine Receptor CCR2b

John A. Craddock, An Lu, Adham Bear, Martin Pule, Malcolm K. Brenner, Cliona M. Rooney, and Aaron E. Foster

TRAFFICKING AND PENETRATION: CHEMOTACTIC MANIPULATION (I)

GD2 is a disialoganglioside expressed on tumors of neuroectodermal origin, including human neuroblastoma and melanoma, with highly restricted expression on normal tissues, principally to the cerebellum and peripheral nerves in humans

GD2-specific CAR, incorporating the transmembrane signaling domains of CD28, OX40, and the T-cell receptor z-chain

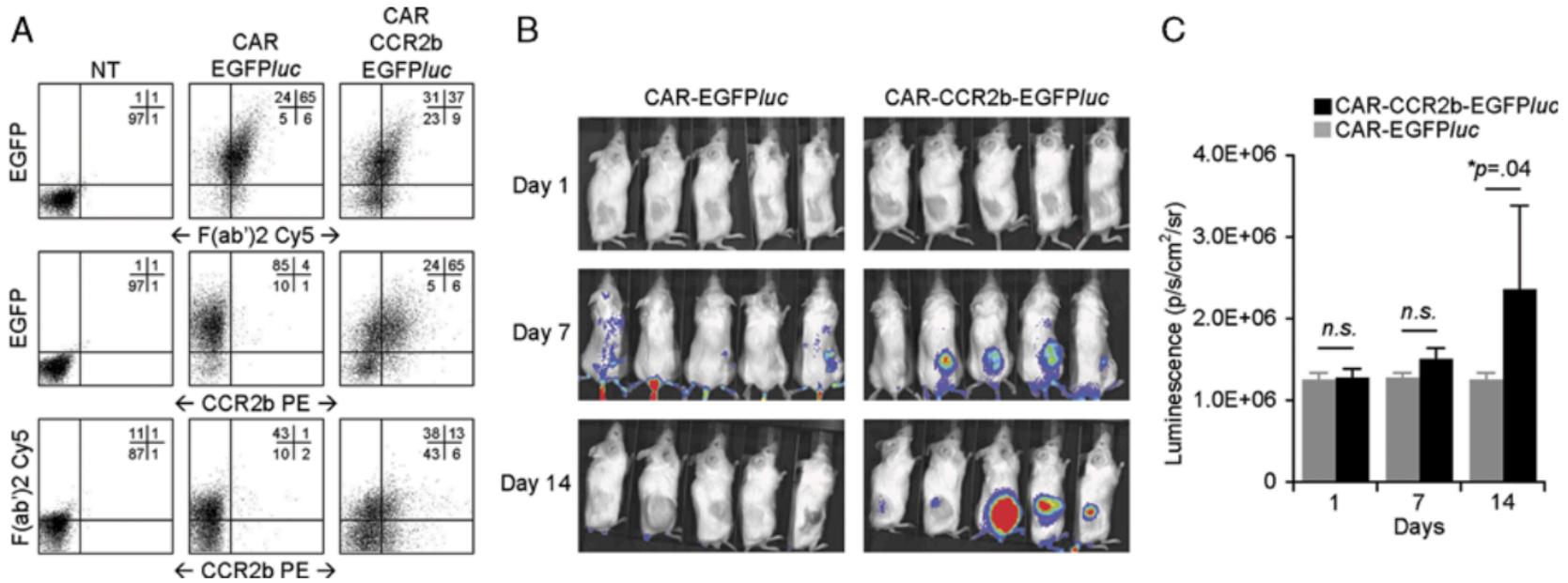


FIGURE 5. CAR-CCR2b ATCs show enhanced homing and expansion at the tumor site. A, ATCs were transduced with EGFPluc and either CAR (middle panel) or CAR and CCR2b (right panel) and analyzed by fluorescence-activated cell sorter for EGFP, CAR [F(ab')2 cyanin 5 (Cy5) antibody], and CCR2b [CCR2 phycoerythrin (PE)]. B, Severe combined immune-deficient mice received SK-N-AS tumor cells subcutaneously in the right flank followed by intravenous injection of CAR-EGFPluc or CAR-CCR2b-EGFPluc gene-modified ATCs 7 days later. C, Mice were imaged for bioluminescence using tumor region of interest on days 1, 7, and 14 showing enhanced homing and expansion of CAR-CCR2b-EGFPluc modified ATCs compared with ATCs modified only with CAR-EGFPluc. ATCs indicates activated T cells; CAR, chimeric antigen receptor; NS, not significant; NT, nontransduced.

Craddock et al. 2010

TRAFFICKING AND PENETRATION: CHEMOTACTIC MANIPULATION (I)

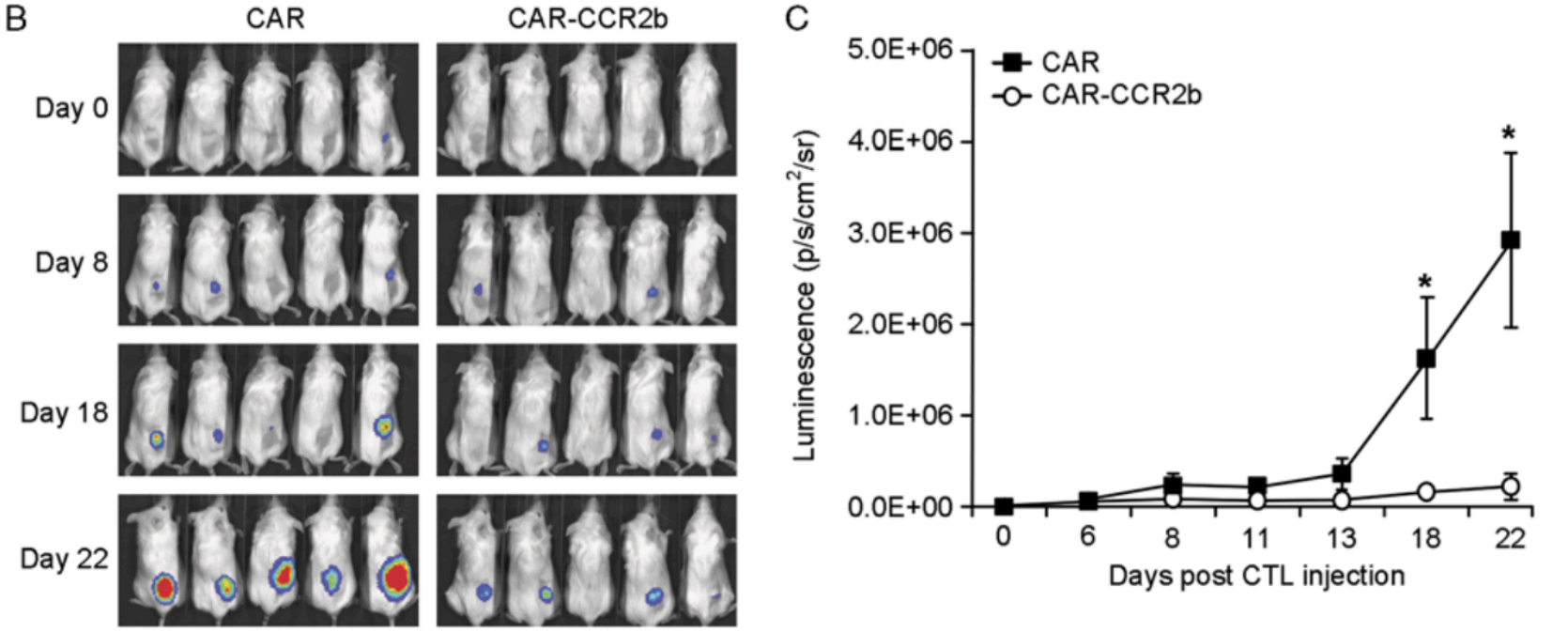


FIGURE 6. CAR-CCR2b ATCs show enhanced antitumor effect. A, ATCs cells were transduced with either CAR (middle panel) or CAR and CCR2b (right panel) and analyzed by fluorescence-activated cell sorter for expression of CAR [by F(ab′)2 fluorescein isothiocyanate (FITC) antibody; y-axis] and CCR2 [CCR2 phycoerythrin (PE); x-axis] indicating that both T-cell populations express equivalent CAR but only CCR2b gene-modified ATCs express CCR2. B and C, Severe combined immune-deficient mice received an subcutaneous injection right flank of SK-N-AS modified to express EGFPluc followed by IV injection of either CAR (left panel) or CAR-CCR2b (right panel) and measured for tumor growth by bioluminescence on days 0, 8, 18, and 22. CAR-CCR2b ATCs showed significant ($P < 0.05$) inhibition of tumor growth compared with ATCs modified with CAR alone. ATCs indicates activated T cells; CAR, chimeric antigen receptor; CTL, cytotoxic T lymphocytes.

Craddock et al. 2010

TRAFFICKING AND PENETRATION: CHEMOTACTIC MANIPULATION (II)

Problem: chemokine receptors present on T cells do not adequately match the chemokine signature of the tumors

→ chemokine secretion from tumors can be modulated to correlate with the chemokine receptors that are naturally present on CAR T cells

Therapeutics, Targets, and Chemical Biology

Cancer Research

Armed Oncolytic Virus Enhances Immune Functions of Chimeric Antigen Receptor–Modified T Cells in Solid Tumors

Nobuhiro Nishio[1], Iulia Diaconu[1], Hao Liu[2], Vincenzo Cerullo[3], Ignazio Caruana[1], Valentina Hoyos[1], Lisa Bouchier-Hayes[4], Barbara Savoldo[1,4], and Gianpietro Dotti[1,5,6]

→ oncolytic virus armed with the chemokine RANTES and the cytokine IL15

→adenovirus Ad5D24 exerted a potent, dose-dependent, cytotoxic effect on tumor cells

→whereas the intratumoral release of both RANTES and IL15 attracted CAR-T cells and promoted their local survival

TRAFFICKING AND PENETRATION: CHEMOTACTIC MANIPULATION (II)

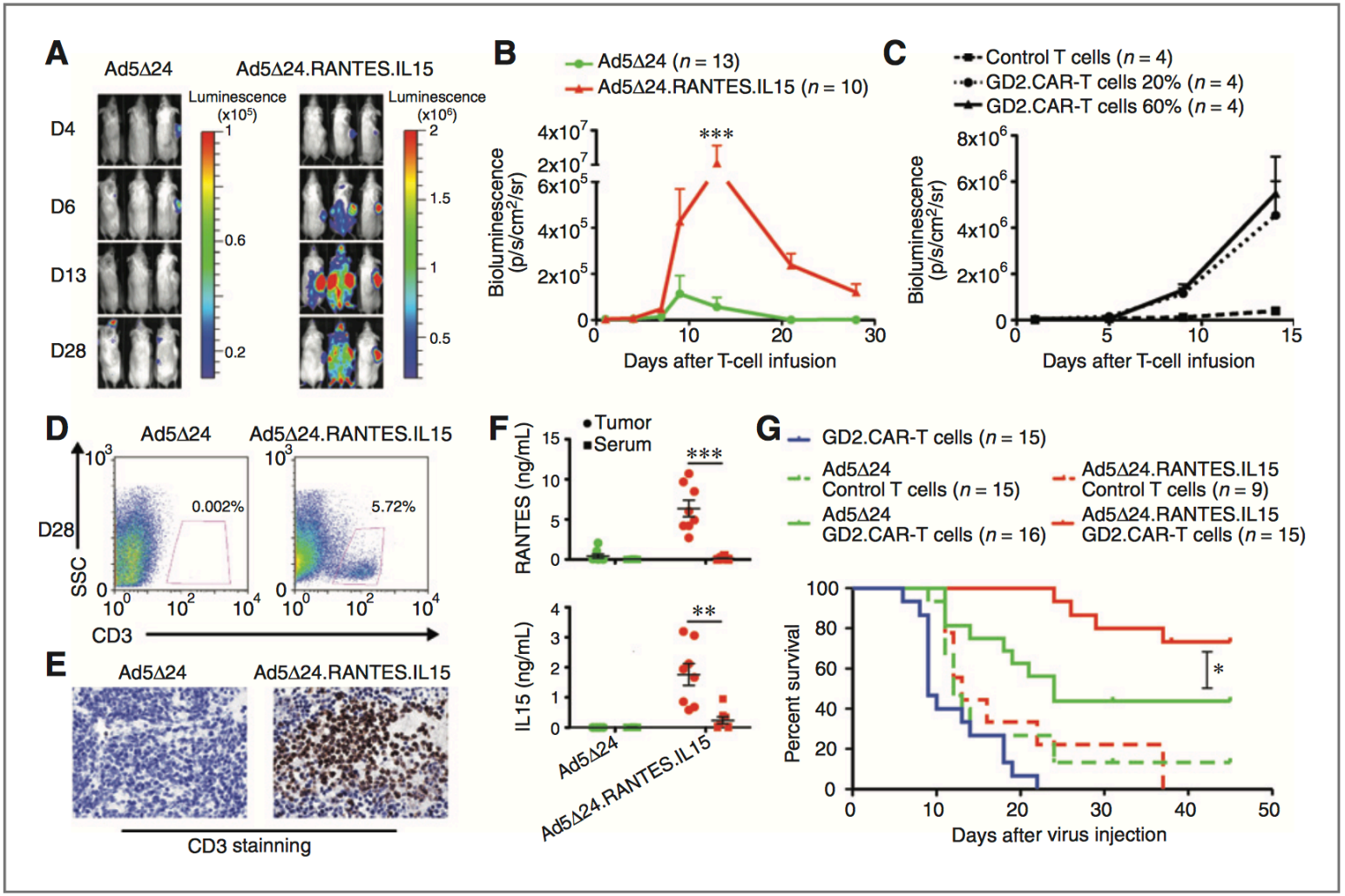


Figure 5. Ad5Δ24.RANTES.IL15 improves persistence of GD2.CAR-T cells. A and B, NSG mice engrafted subcutaneously with CHLA-255 cells were inoculated intratumorally with oncolytic viruses (10^6–10^8 vp) by days 10 to 14. Four days later, mice were infused intravenously with FFluc labeled GD2. CAR-T cells. T-cell bioluminescence was then measured. Data represent mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by Student *t* test. C, NSG mice engrafted subcutaneously with CHLA-255 cells were inoculated intratumorally with oncolytic viruses (10^8 vp) by day 10. Four days later, mice were infused intravenously with FFluc-labeled control T cells or FFluc-labeled GD2.CAR-T cells diluted with control T cells at 6:10 or 2:10 ratios. T-cell bioluminescence was then measured. Data represent mean ± SEM. D and E, T cells infiltrating the tumors were assessed by FACS and IHC. F, detection of RANTES and IL15 by ELISA in serum and tumor homogenates collected from mice 14 to 18 days after inoculations of oncolytic viruses. Data represent mean ± SEM in 8 mice for each virus. **, $P < 0.01$; ***, $P < 0.001$ by Student *t* test. G, survival curves of NSG mice bearing CHLA-255 cells and treated with single and combined agents. *, $P < 0.05$ by log-rank test.

Nishio et al. 2010

TRAFFICKING AND PENETRATION: BREAKING DOWN BARRIERS (I)

Problem: tumor microenvironment is a physical barrier prohibiting efficient T-cell infiltration into the tumor

→ degradation of extracellular matrix, formed by myeloid cells and tumor fibroblasts

Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes

Ignazio Caruana[1], Barbara Savoldo[1,2], Valentina Hoyos[1], Gerrit Weber[1], Hao Liu[3], Eugene S Kim[4], Michael M Ittmann[5–7], Dario Marchetti[5] & Gianpietro Dotti[1,5,8]

- Heparanase (HPSE) is an enzyme integral for degradation of heparin sulfate proteoglycans, which constitute a majority of the extracellular matrix
- Loss of HPSE has been observed in T cells post in vitro culture
- Overexpression of HPSE in CAR T cells, or alternatively targeting the surrounding non-malignant stroma using CAR T cells directed against the 'fibroblast activation protein' can overcome these physical barriers, enhancing T-cell infiltration into the TME.

VOLUME 21 | NUMBER 5 | MAY 2015 NATURE MEDICINE

TRAFFICKING AND PENETRATION: BREAKING DOWN BARRIERS (I)

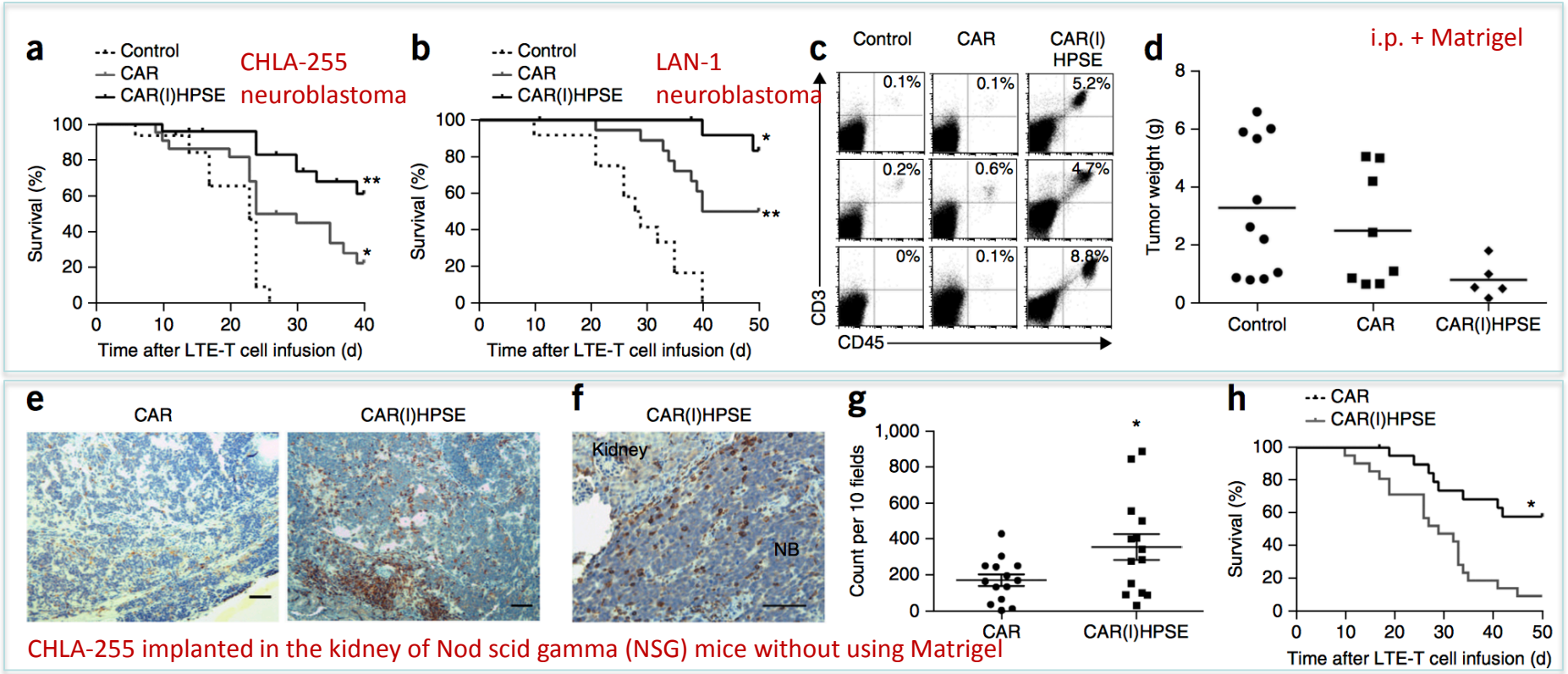


Figure 4 CAR-GD2$^+$ LTE-T cells co-expressing *HPSE* show enhanced tumor infiltration and improve overall survival in xenograft tumor models. (**a**) Kaplan–Meier analysis of mice engrafted i.p. with the tumor cell line CHLA-255 and treated i.p. with control, CAR$^+$ and CAR(I)HPSE$^+$ LTE-T cells. Shown are data from three independent experiments using LTE-T cells generated from three donors. Control, $n = 16$; CAR, $n = 22$; CAR(I)HPSE, $n = 26$ mice; $^*P < 0.007$, $^{**}P < 0.0001$. (**b**) Kaplan–Meier analysis of mice engrafted i.p. with the tumor cell line LAN-1 and treated i.p. with control, CAR$^+$ and CAR(I)HPSE$^+$ LTE-T cells. For these experiments, we generated LTE-T cells from two donors. Control, $n = 12$; CAR, $n = 18$; CAR(I)HPSE, $n = 14$ mice; $^*P = 0.039$, $^{**}P < 0.0001$. (**c**) Flow cytometry analysis of CD3$^+$ T cells detected within the tumor samples. Dot plots are representative of three mice per group from mice infused with LTE-T cells generated from the same donor. (**d**) Weight of the tumors collected from euthanized mice engrafted with LAN-1 tumor cells. (**e**,**f**) Immunohistochemical analysis showing CD3$^+$ T cell infiltration in NB tumor CHLA-255 cells implanted in the kidney of mice infused with either CAR$^+$ or CAR(I)HPSE$^+$ LTE-T cells. 100× magnification (**e**) and 200× magnification (**f**); scale bars, 100 µm. (**g**) The graph shows the numbers of infiltrating CD3$^+$ T cells per ten high-power fields in tumors collected from mice treated with either CAR$^+$ or CAR(I)HPSE$^+$ LTE-T cells (cell numbers 357 ± 72 and 173 ± 32, respectively), $^*P = 0.028$. (**h**) Kaplan-Meier analysis of mice surgically implanted under the renal capsule with CHLA-255 NB cells and infused i.v. with either CAR$^+$ or CAR(I)HPSE$^+$ LTE-T cells. For these experiments, we generated LTE-T cells from two donors, CAR, $n = 21$; CAR(I)HPSE, $n = 21$ mice; $^*P = 0.0006$.

Caruana et al. 2015

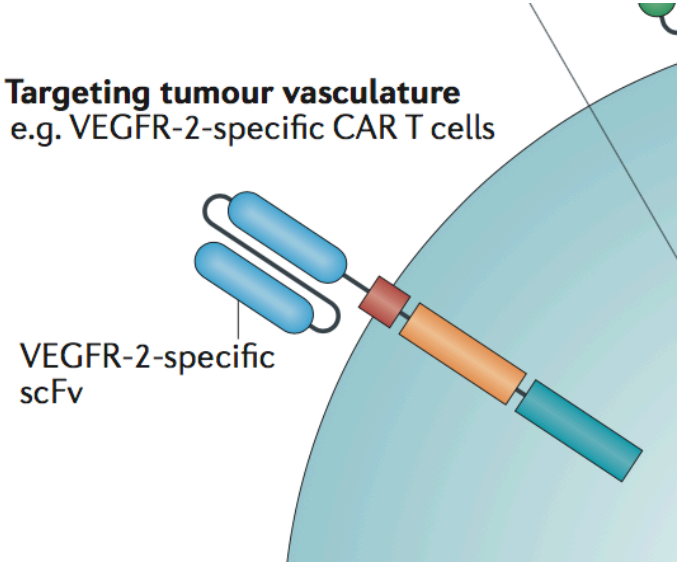
TRAFFICKING AND PENETRATION: BREAKING DOWN BARRIERS (II)

Problem: tumor microenvironment is a physical barrier prohibiting efficient T-cell infiltration into the tumor

→ Targeting and disrupting the vasculature can restrict blood flow and nutrient supplies to the tumor, impeding its development, whereas at the same time enhancing T-cell infiltration

VEGFR-2 : vascular endothelial growth factor receptor 2, expressed on angiogenic endothelial cells and myeloid suppressor cells

αvβ3 : CARs incorporating ligands for angiogenic vessel- associated molecules such as αvβ3, an integrin commonly expressed on tumor vascular endothelium



PSMA : Prostate-specific membrane antigen is found on malignant prostate cells and the endothelium of some tumor vasculature but not on normal vasculature, making it an ideal target for immunotherapy.

Yong et al., 2017; Jackson et al., 2016

Therapeutics, Targets, and Chemical Biology

Cancer Research

Simultaneous Targeting of Tumor Antigens and the Tumor Vasculature Using T Lymphocyte Transfer Synergize to Induce Regression of Established Tumors in Mice

Dhanalakshmi Chinnasamy, Eric Tran, Zhiya Yu, Richard A. Morgan, Nicholas P. Restifo, and Steven A. Rosenberg

anti-VEGFR2 CAR: DC101 CAR T cells + T-cells specific for the tumor antigens gp100 (PMEL)

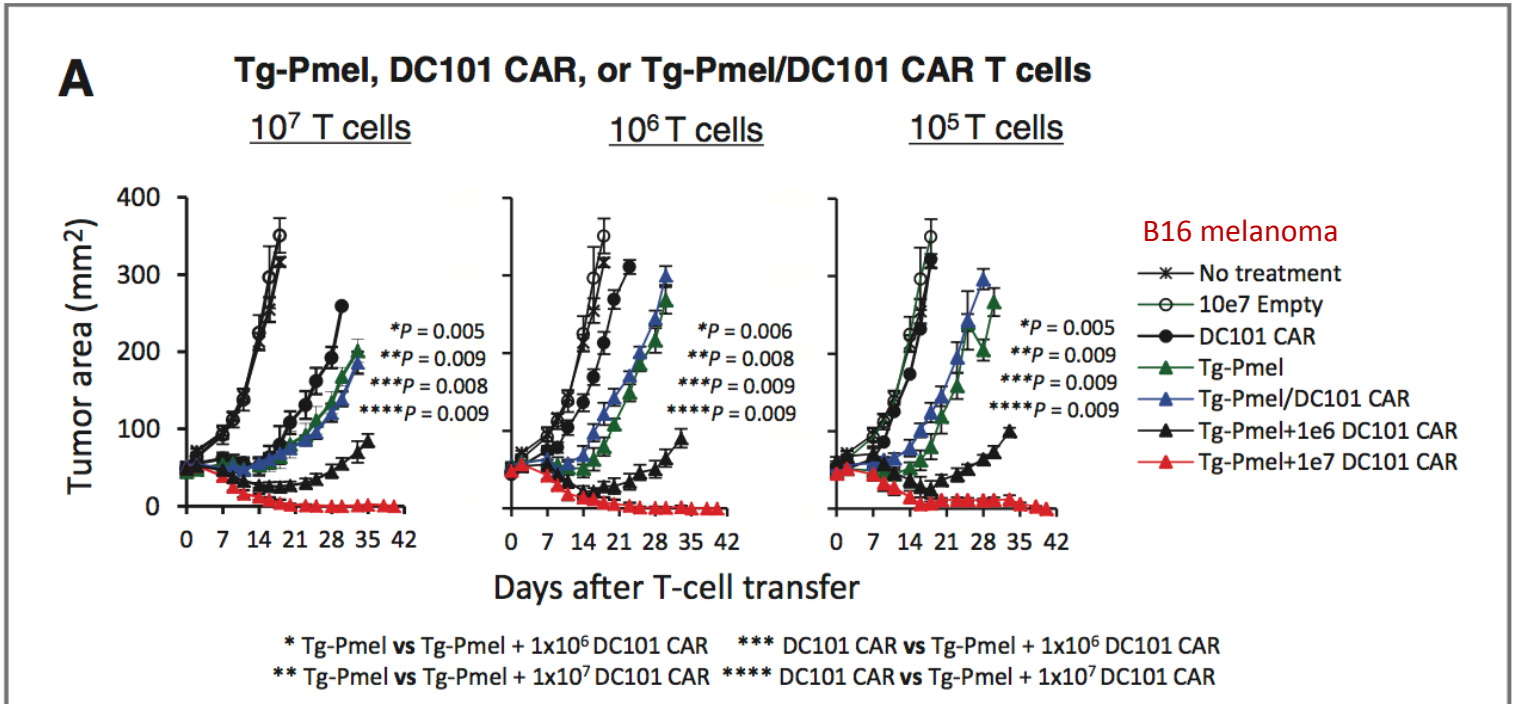


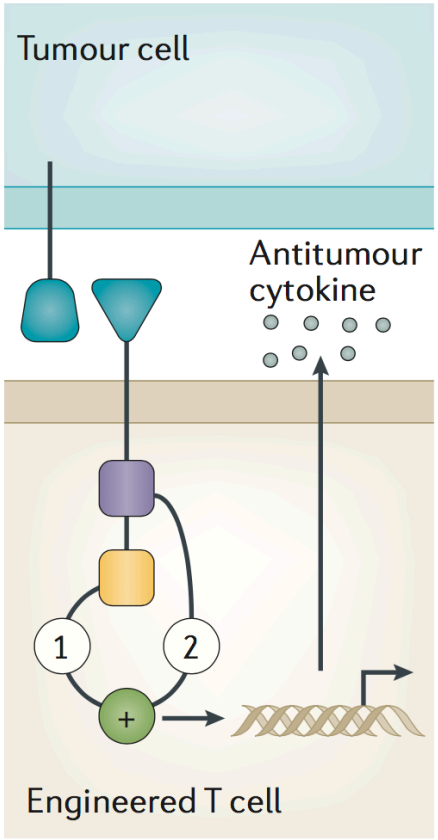
Figure 2. Cotransfer of anti-VEGFR2 CAR–transduced open repertoire T cells and Tg-Pmel T cells induced durable tumor regression compared with Tg-Pmel transduced with anti-VEGFR2 CAR. A, groups of 5 C57BL/6 mice bearing B16 tumors were sublethally irradiated with 5-Gy TBI and treated with 10^5, 10^6, or 10^7 Tg-Pmel T cells, open repertoire T cells from Wt mice transduced with an empty vector, or an anti-VEGFR CAR, or Tg-Pmel T cells transduced with an anti-VEGFR2 CAR. Some groups received a combination of Tg-Pmel T cells and anti-VEGFR2 CAR–transduced open repertoire T cells. Control groups received neither T cells nor vaccine nor rhIL-2. All treatment groups received a single dose of 2×10^7 pfu vaccinia virus expressing hgp100 antigen and 2 daily doses of 2.2×10^5 IU rhIL-2 per dose for 3 consecutive days. Serial, blinded tumor measurements were obtained and the products of perpendicular diameters were plotted ±SEM. The data shown are representative of 3 independent experiments. B, groups of 5 C57BL/6 mice bearing B16 tumors were sublethally

COMBATING IMMUNOSUPPRESSION

COMBATING IMMUNOSUPPRESSION: TRUCKs

Problem: tumor microenvironment is comprised of multiple cellular and molecular components that reduce efficient anti-tumor immune function

→inhibition of immunosuppression or altering the immunosuppressive TME may rescue hypofunctional CAR T cells



TRUCKs: T cells redirected for universal cytokine killing

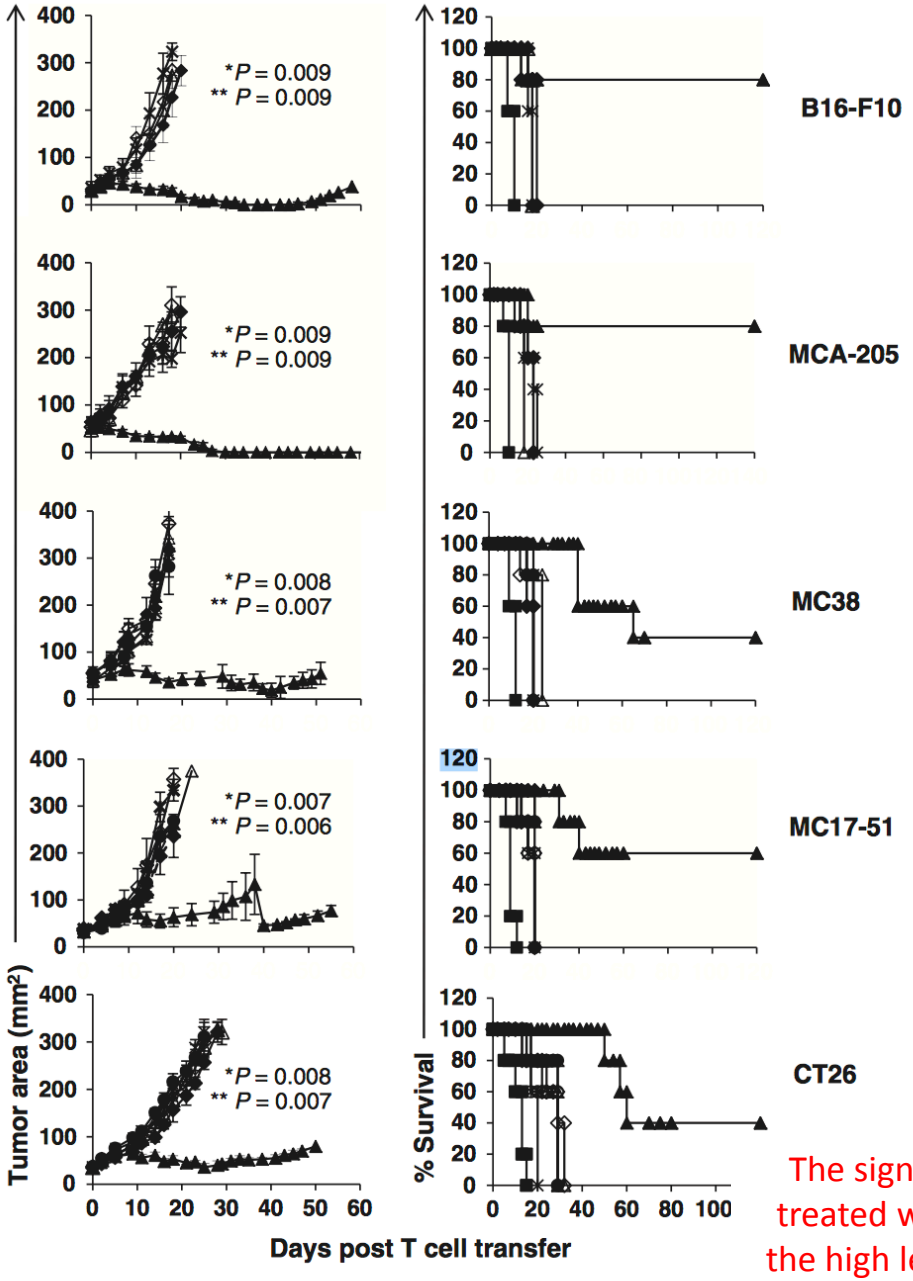
Cancer Therapy: Preclinical

Clinical Cancer Research

Local Delivery of Interleukin-12 Using T Cells Targeting VEGF Receptor-2 Eradicates Multiple Vascularized Tumors in Mice

Dhanalakshmi Chinnasamy, Zhiya Yu, Sid P. Kerkar, Ling Zhang, Richard A. Morgan, Nicholas P. Restifo, and Steven A. Rosenberg

Yong et al., 2017; Fensak et al., 2016



10 to 12 days old B16 (melanoma), MCA-205 (sarcoma), MC38 (colorectal adenocarcinoma), or MC17-51 (sarcoma) tumor-bearing C57BL/6 mice and 12 to 14 days old CT26 colon tumor-bearing BALB/c mice were sublethally irradiated at 5 Gy TBI and treated with 1 10^6 or 5 10^5 syngeneic T cells transduced with various retroviral vectors as indicated in the figure

1 10^6 anti–VEGFR-2 CAR (DC101 CAR)-transduced cells had no impact on tumor growth
1 10^6 cells cotransduced with the anti– VEGFR-2 CAR and with the gene for Flexi-IL12 were lethal.

T cells engineered to express either the anti–VEGFR-2 CAR or single- chain IL-12 alone or given in a 1:1 mixture of these single gene transduced cells did not alter tumor growth → both the anti–VEGFR-2 CAR and IL-12 had to be expressed in the same cell to mediate the antitumor effect

T cells cotransduced with an empty vector and IL-12 were unable to control tumor growth

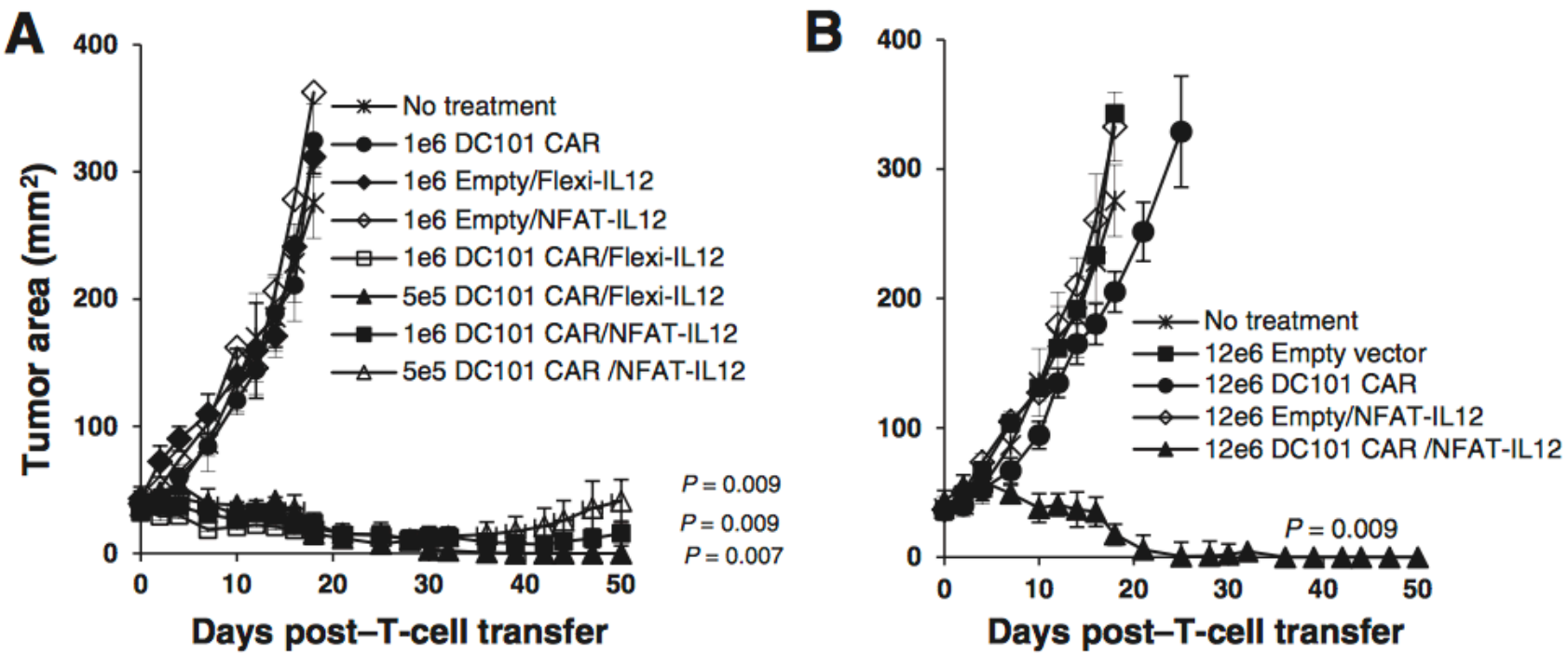
5 10^5 cells cotransduced with anti–VEGFR-2 CAR and Flexi-IL12 prolonged the survival and mediated cures

rent

The significant treatment related mortality seen in mice treated with more than 5 10^5 cells was likely attributed to the high levels of systemic IL-12 constitutively produced by the proliferating anti–VEGFR-2 CAR and Flexi-IL12–cotransduced T cells

Restricting IL-12 expression and accumulation locally to the tumor site

new vector whereby expression of IL-12 was controlled by an **Nuclear Factor of Activated T-cells (NFAT)-responsive elements**, which could drive IL-12 secretion only upon T cell activation



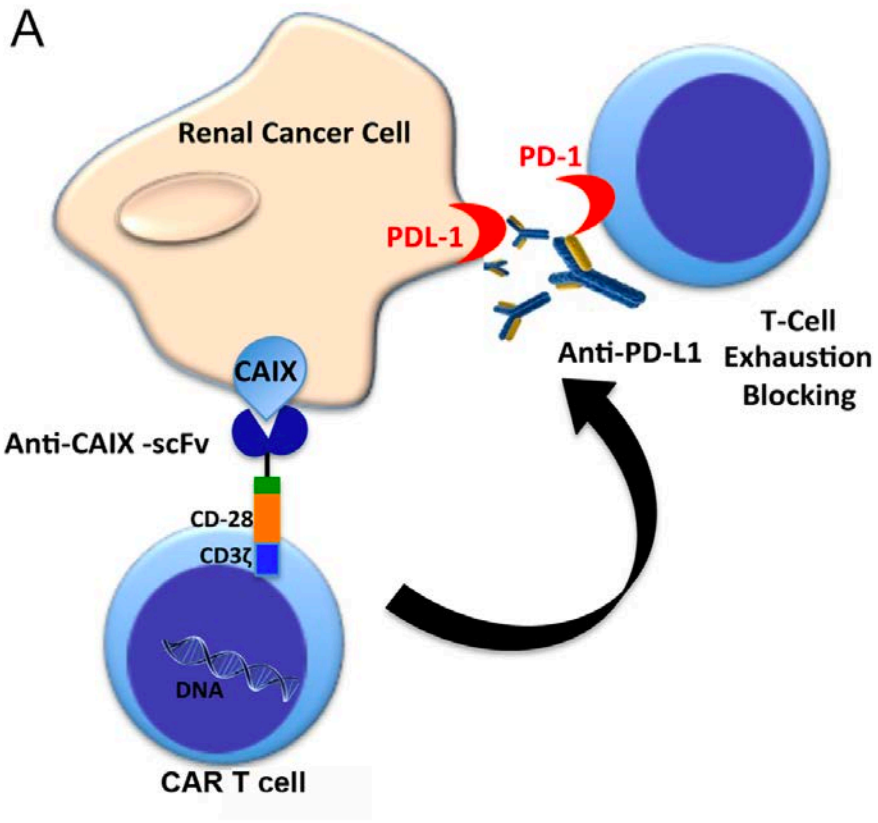
Flexi→ mortality of mice was again seen if cell numbers were increased to 1 106

cells expressing the anti–VEGFR-2 CAR and the inducible NFAT-IL12 coexpressing T cells induced long-term tumor regression at all doses from 5 105 to 1 106 in the absence of any apparent toxicity as measured by body weight loss and pathologic examination of tissues from treated mice (data not shown)

COMBATING IMMUNOSUPPRESSION

Problem: tumor microenvironment is comprised of multiple cellular and molecular components that reduce efficient anti-tumor immune function

→inhibition of immunosuppression or altering the immunosuppressive TME may rescue hypofunctional CAR T cells



Armored CAR: secrete antibodies

Checkpoint inhibitory proteins, such as PD-L1, which normally function to regulate the immune response are often upregulated on tumors.

On interaction of PD-L1 with its receptor PD-1, which is upregulated on exhausted T cells, T lymphocytes become hypofunctional.

Suarez ER, et al., Oncotarget 2016; 7: 34341–34355.

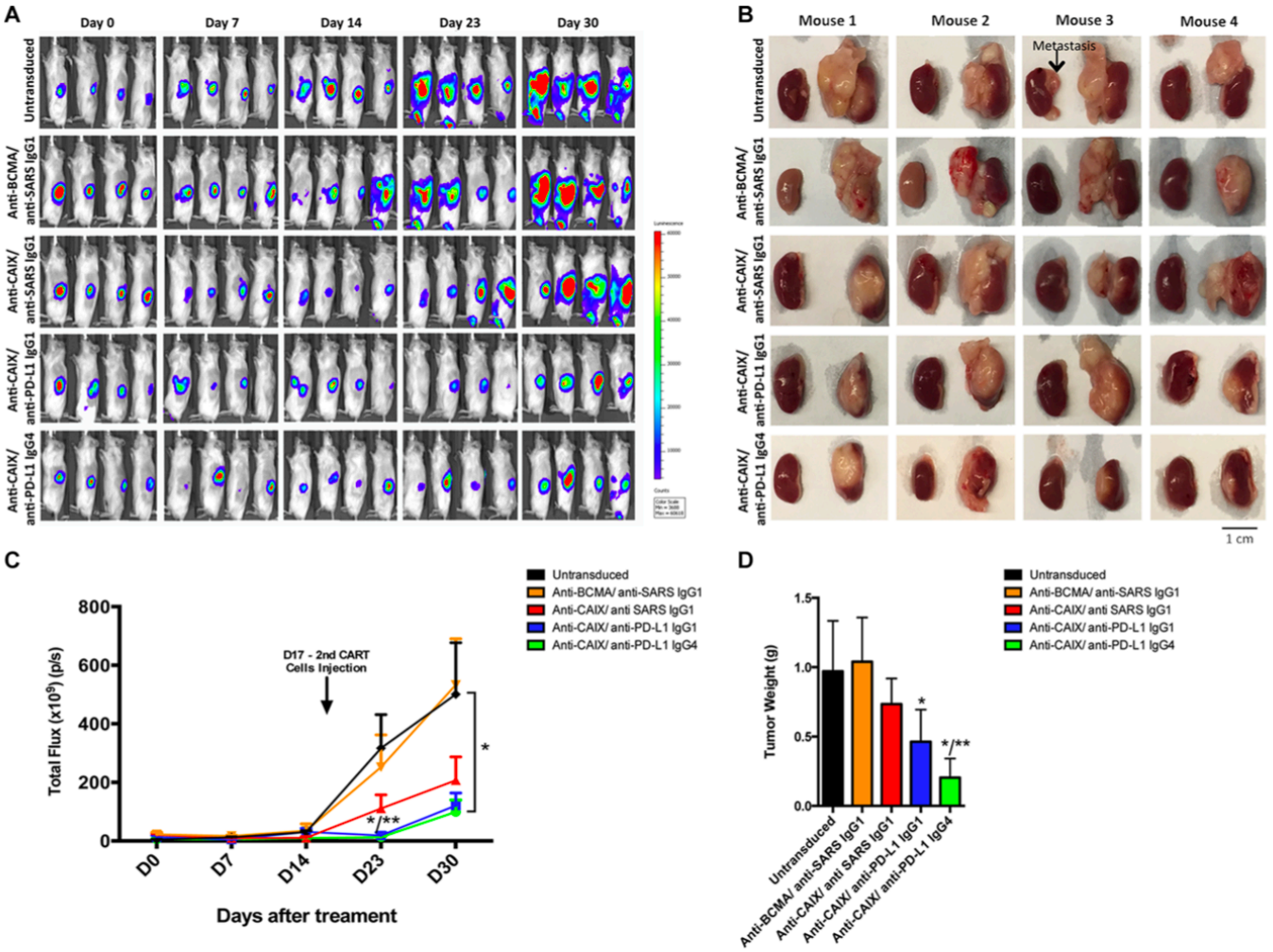
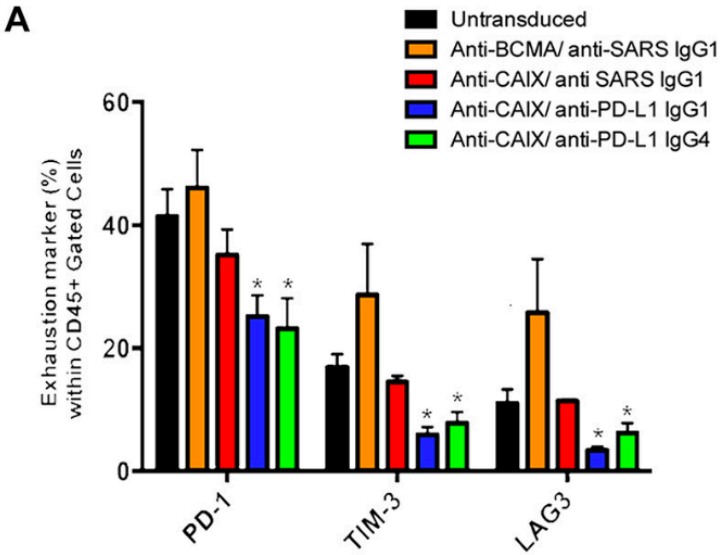
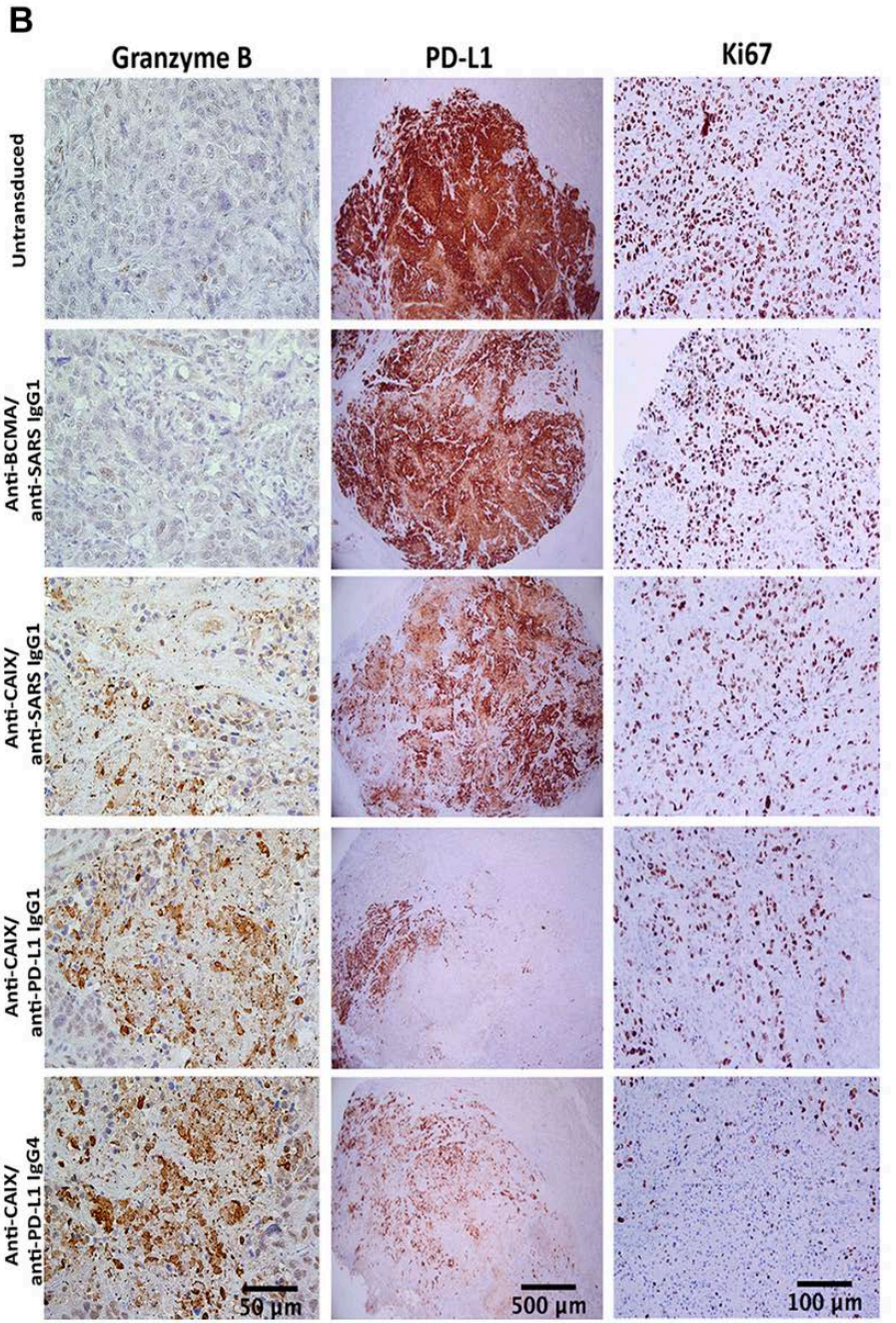


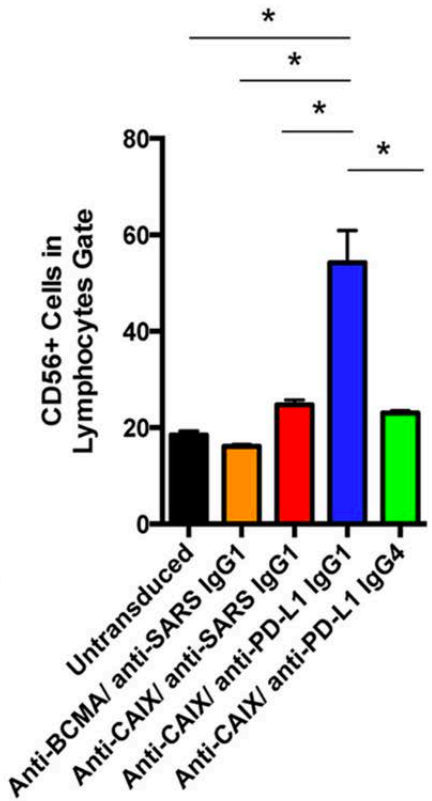
Figure 4: Effects of the CAR T cells in an orthotopic model of human ccRCC. (**A**) NSG Mice (N = 30) were injected with 5.0×10^4 skrc59 CAIX+/PD-L1+/luciferase+RCC cells. After a week, the mice were injected by i.v. with 1.0×10^7 CAR T or untransduced T cells (Day 0). The CAR T cells were generated by transduction with the lentiviral vectors encoding: anti-CAIX CAR/anti-PD-L1 IgG1, anti-CAIX CAR/anti-PD-L1 IgG4, anti-CAIX CAR/anti-SARS IgG1, or anti-BCMA CAR/anti-SARS IgG1 (N = 6 mice per group). Tumor growth was quantified by bioluminescence imaging after 5 minutes of luciferin IP injection using IVIS on Day 0 before, and on Days 7, 14, 23 and 30 after the first CAR T cells injection. A second injection of 2.5×10^6 cells was made on Day 17. (**B**) Imaging of the tumors after excision on Day 30 with RCC-implanted kidney on the right side of each image. Scale bar = 1 cm. (**C**) Tumor growth curve. *$P < 0.05$ when anti-PD-L1 IgG1 and IgG4 groups were compared to anti-BCMA CAR/anti-SARS IgG1 and **$P < 0.05$ when anti-PD-L1 IgG1 and IgG4 groups were compared to anti-CAIX CAR/anti-SARS IgG1. (**D**) Average tumor weight after 30 days of treatment. *$P < 0.05$ compared with anti-BCMA CAR/anti-SARS IgG1 CAR, **$P < 0.05$ compared with anti-CAIX CAR/anti-SARS IgG1. Animal experiments were performed in accordance with the guidelines of the DFCI Animal Care Committee.

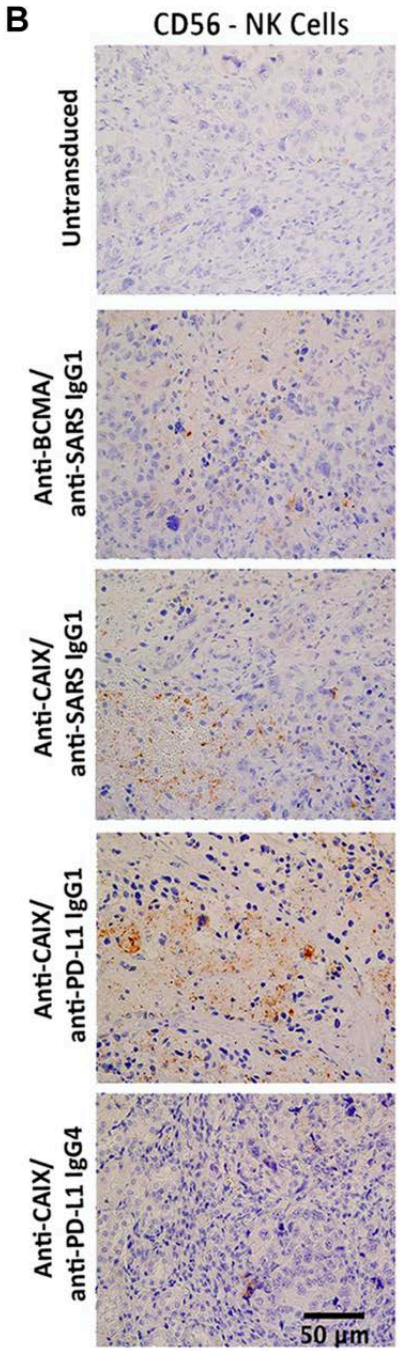


→ **Reduction of exhaustion markers**



Suarez ER, et al., Oncotarget 2016; 7: 34341–34355.





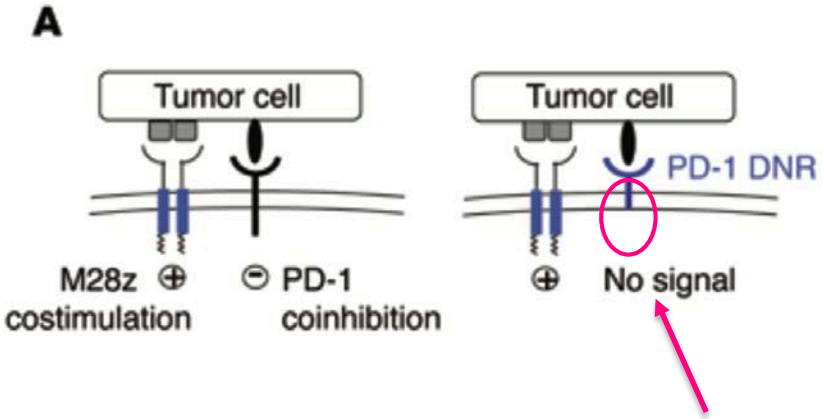
In addition to significantly reducing tumor growth in a humanized renal cell carcinoma mouse model, local secretion of anti-PD-L1 antibodies from CAR T cells can **mediate antibody-dependent cell-mediated cytotoxicity (ADCC)** and **increased migration of adoptively transferred human NKs into the tumor**

Suarez ER, et al., Oncotarget 2016; 7: 34341–34355.

COMBATING IMMUNOSUPPRESSION: dominant negative receptors (DNRs)

Problem: tumor microenvironment is comprised of multiple cellular and molecular components that reduce efficient anti-tumor immune function

→inhibition of immunosuppression or altering the immunosuppressive TME may rescue hypofunctional CAR T cells



RESEARCH ARTICLE

The Journal of Clinical Investigation

Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition

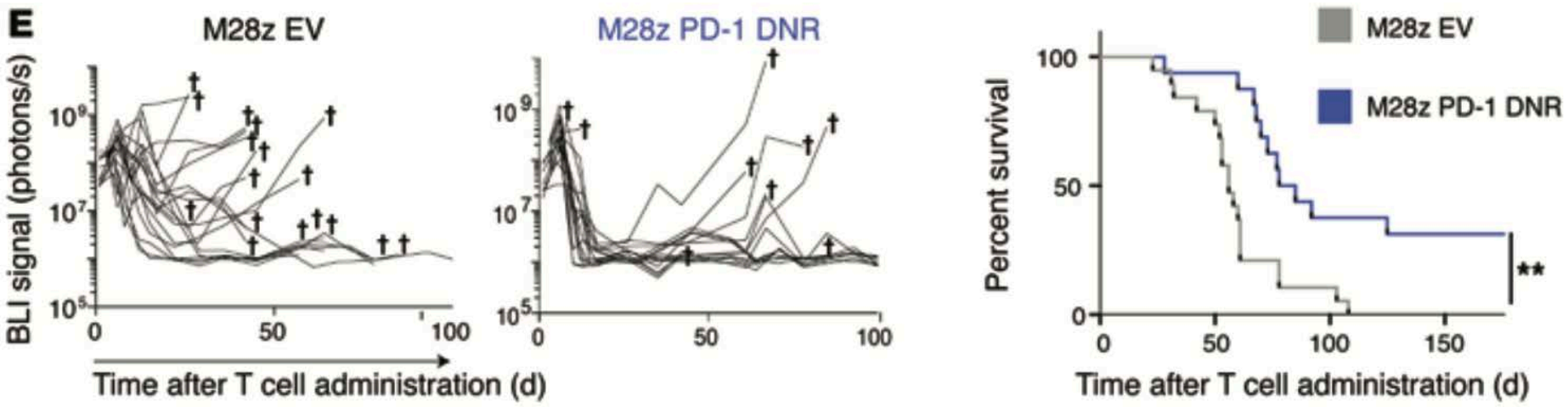
Leonid Cherkassky,[1,2] Aurore Morello,[1,2] Jonathan Villena-Vargas,[1,2] Yang Feng,[3] Dimiter S. Dimitrov,[3] David R. Jones,[2] Michel Sadelain,[1] and Prasad S. Adusumilli[1,2]

[1]Center for Cell Engineering and [2]Thoracic Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York, USA. [3]Protein Interactions Section, Laboratory of Experimental Immunology, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, NIH, Frederick, Maryland, USA.

DNRs maintain the extracellular region of a membrane receptor but generally harbor a mutation in the intracellular chain, resulting in an absence of downstream signal transduction and subsequent loss of function.37 As such, DNRs are often able to compete with their endogenous receptors for target ligands, thus prohibiting the full effect of target/receptor binding.

COMBATING IMMUNOSUPPRESSION: dominant negative PD-1 receptor

orthotopic mouse model of pleural mesothelioma



Serial bioluminescence imaging (BLI) of firefly-luciferase–transduced (ffLuc-trans- duced) MSTO-211H tumor cells (left) and Kaplan-Meier survival analysis (right) comparing the in vivo efficacy of a single dose of 5×10^4 M28z EV (n = 19; grey) or M28z PD-1 DNR (n = 16; blue) pleurally administered.

Data shown are a combination of 2 independent experiments. Daggers indicate deaths. Median survival is shown in days following T cell administration. The survival curve was analyzed using the log-rank test (P = 0.001). The log-rank test for each independent experiment was significant at the $P < 0.05$ level; 2 experiments are combined for illustration

→ As PD-1/PD-L1 blockade is normally achieved through antibody blockade, which due to its broad target range can lead to autoimmune effects, the use of PD-1 'insensitive' DNR T cells may overcome this issue.

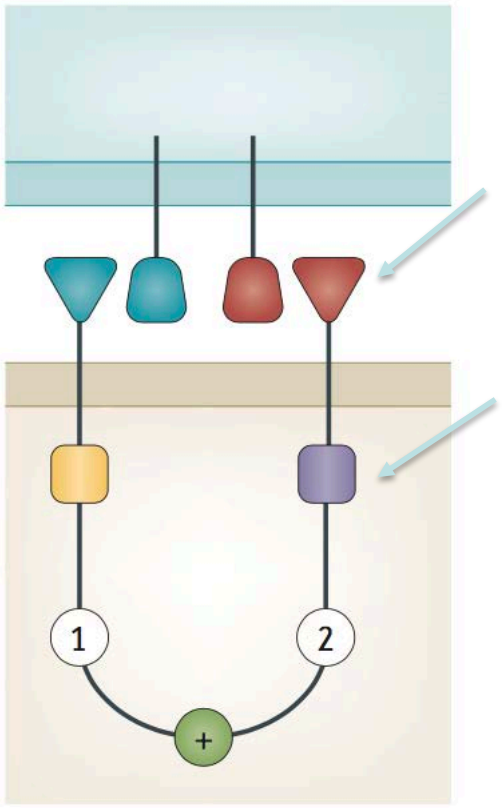
Mesothelin specific CAR, mesothelioma model

Cherkassky, et al., JCI 2016

COMBATING IMMUNOSUPPRESSION: switch receptors

Problem: tumor microenvironment is comprised of multiple cellular and molecular components that reduce efficient anti-tumor immune function

→inhibition of immunosuppression or altering the immunosuppressive TME may rescue hypofunctional CAR T cells



extracellular portion of an antibody specific for an immunosuppressive molecule, such as PD-1 or CTLA-4

intracellular activating signaling molecule, such as CD28

Fesnak et al., 2016

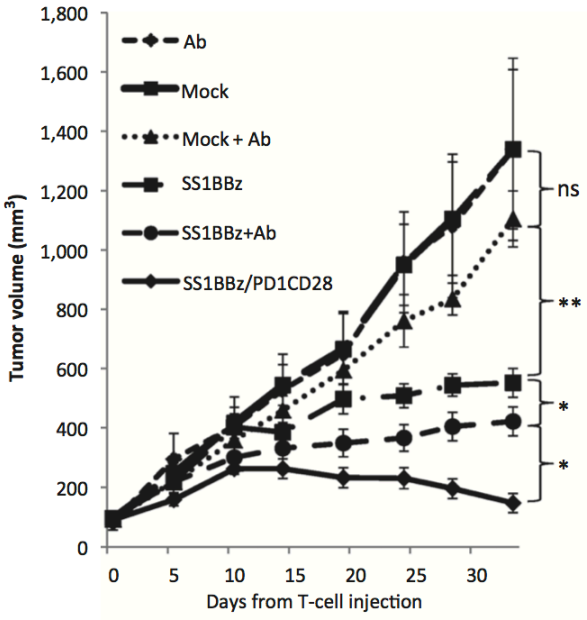
Therapeutics, Targets, and Chemical Biology

Cancer Research

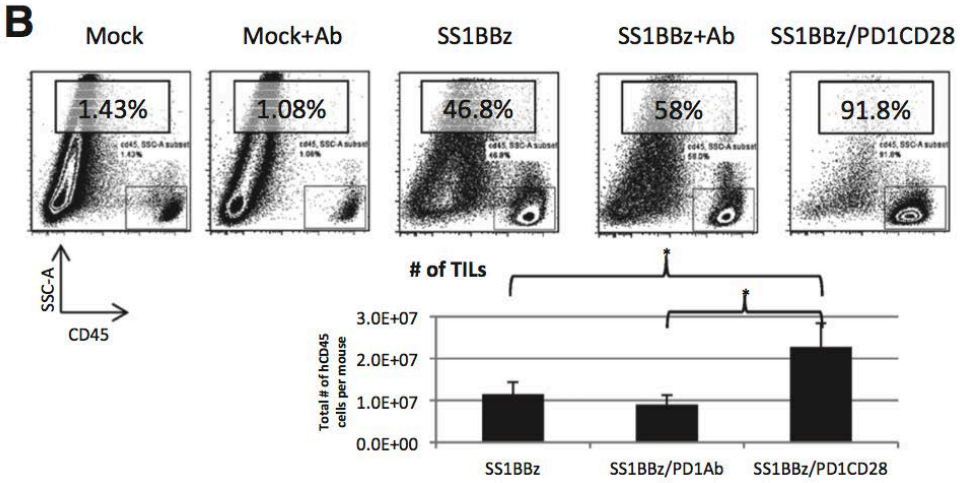
A Chimeric Switch-Receptor Targeting PD1 Augments the Efficacy of Second-Generation CAR T Cells in Advanced Solid Tumors

Xiaojun Liu[1], Raghuveer Ranganathan[2], Shuguang Jiang[1], Chongyun Fang[1], Jing Sun[2], Soyeon Kim[2], Kheng Newick[2], Albert Lo[3], Carl H. June[1], Yangbing Zhao[1], and Edmund K. Moon[2]

anti-PD1 Ab alone (Ab),
1 107 mock transduced T cells (mock),
1 107 mock T cells and anti-PD1 Ab (mock þ Ab),
1 107 SS1BBz T cells (SS1BBz),
1 107 SS1BBz T cells + anti-PD1 Ab (SS1BBz þ Ab),
1 107 SS1BBz T cells modified with PD1-CD28 switch-receptor (SS1BBz/PD1CD28).



A portion of the cells was stained with CD45 antibody to assess T-cell infiltration via FACS analysis.



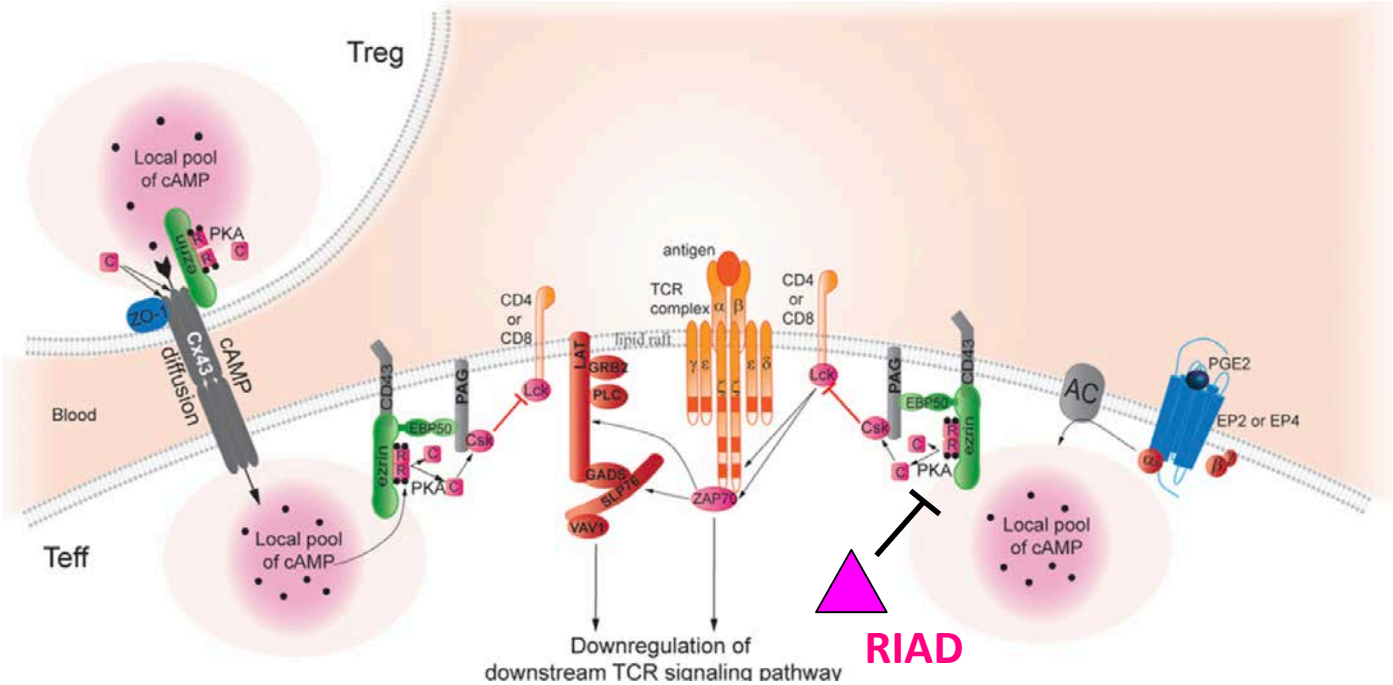
→ reduction in checkpoint inhibitors, namely LAG3 and
→ increase in IL-2 signaling, perhaps suggesting the gain in function may result from an overall 'younger', less exhausted population

CAR specific for mesothelin (SS1BBz with CD3z signaling and 41BB costimulation)

COMBATING IMMUNOSUPPRESSION: RIAD peptide

Problem: tumor microenvironment is comprised of multiple cellular and molecular components that reduce efficient anti-tumor immune function

→inhibition of immunosuppression or altering the immunosuppressive TME may rescue hypofunctional CAR T cells



PGE2 and adenosine activate protein kinase A (PKA), which then inhibits T-cell receptor (TCR) activation. This inhibition process requires PKA to localize to the immune synapse via binding to the membrane protein ezrin.

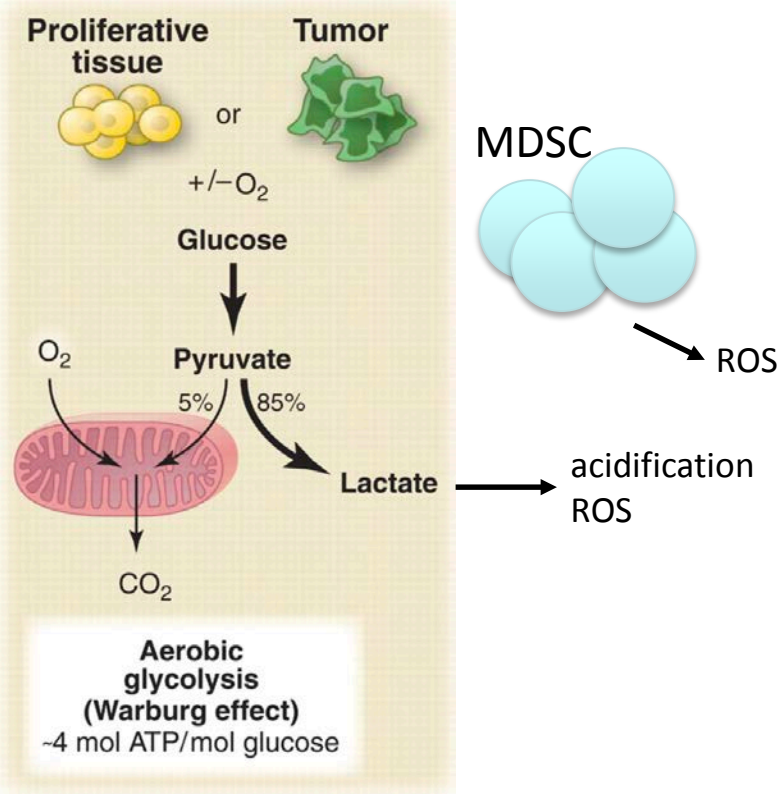
→ **CAR T cells that expressed a small peptide called the "regulatory subunit I anchoring disruptor" (RIAD) that inhibits the association of PKA with ezrin, thus blunting the negative effects of PKA on TCR activation**

Newick et al., Cancer Immunol Res; 2016

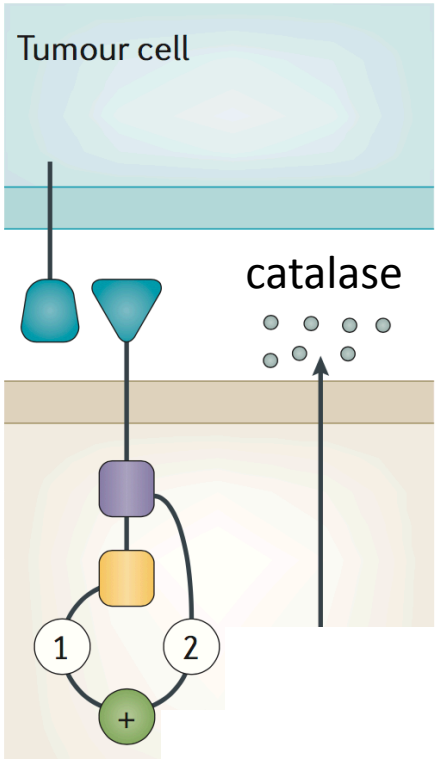
COMBATING IMMUNOSUPPRESSION: catalase vs ROS

Problem: tumor microenvironment is comprised of multiple cellular and molecular components that reduce efficient anti-tumor immune function

→inhibition of immunosuppression or altering the immunosuppressive TME may rescue hypofunctional CAR T cells

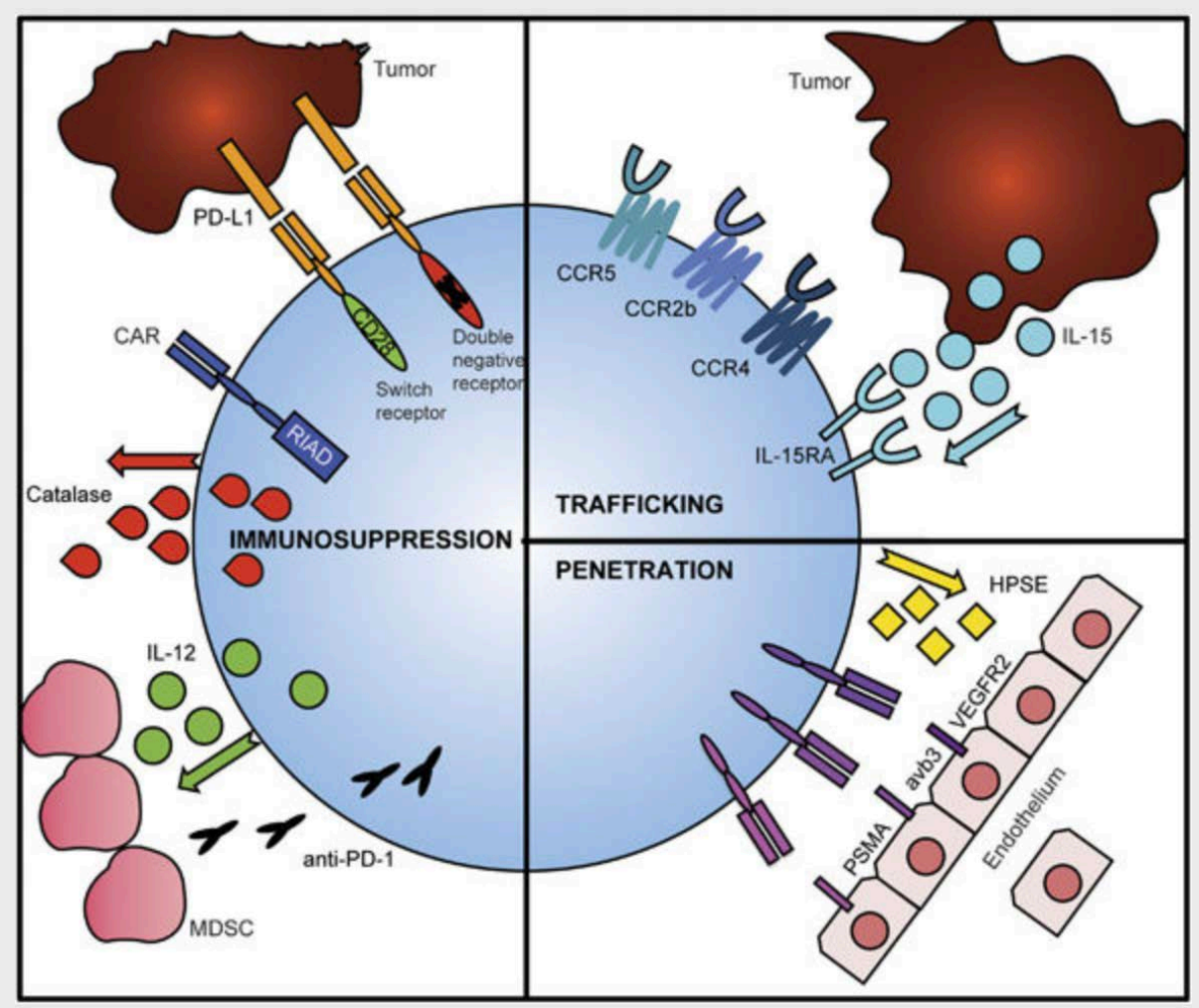


CAR T cells easily succumb to the immunosuppres- sion of oxidative stress, where both proliferation and cytotoxicity are greatly impaired. However, when engineered to secrete catalase (CAT), an anti-oxidant enzyme, into the local environment, CAR-CAT T cells retained their anti-tumor functions



Fesnak et al., 2016

Summary



Yong et al., 2017

ENHANCING SPECIFICITY AND SAFETY OF CAR T-CELL THERAPY

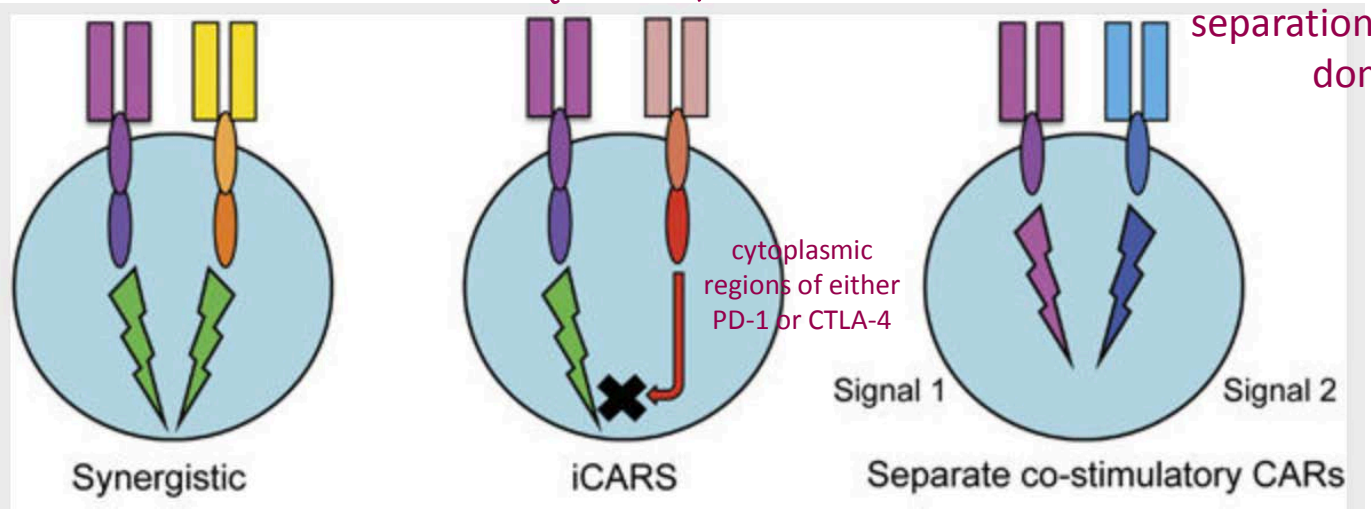
Novel CAR designs to reduce off-tumor effects

two target antigens

tumor target antigen

normal tissue target antigen

two target antigens + separation of signaling domains



Yong et al., 2017

CAR for solid tumors in the clinic

→ The success of CAR T cells in treating hematological malignancies has been impressive

→ the clinical efficacy of CAR T cells in solid tumors has been much less rewarding, with multiple cases of toxic side effects and/or a lack of therapeutic response

→the majority of completed clinical trials on solid tumors have utilized first-generation CARs, and their limited therapeutic efficacies are perhaps unsurprising in retrospect

→ 81 active or planned clinical trials using CAR T cells against hematological cancers and only 51 trials for solid tumors (Table 1)

Yong et al., 2017

CAR for solid tumors in the clinic

Table 1 List of clinical trials involving CAR T cells directed against solid cancers

Antigen	Type of cancer	Pre-conditioning regimen	Additional information	Phase	ID	Cited in (PMID)
CD133	Liver, brain, breast, AML, ALL	Unknown	Comparing CD3ζ to CD3ζ-CD137	1	NCT02541370	27009301
CD138	Multiple myeloma	Unknown	CD3ζ and CD3ζ-CD137	1 and 2	NCT01886976	26574053
CD171	Neuroblastoma, ganglioneuroblastoma	Chemotherapy	2nd and 3rd generation CARs	1	NCT02311621	26451319
CD70	Renal and other CD70 expressing cancer	C, F	IL-2 at 720 000 IU kg^{-1}	1 and 2	NCT02830724	27803044
CEA	Lung, colorectal, gastric, pancreatic	Unknown	Unknown	1	NCT02349724	27000958 27550819 26574053
CEA	Colorectal adenocarcinoma	Unknown	Minor responses in 2 of 7 patients	1	NCT00004178	23880905
EGFR	Lung, colorectal, ovary, pancreatic	Unknown	CD3ζ-CD137 CAR	1 and 2	NCT01869166	26968708 26574053
EGFR	Advanced glioma	C, F, IL-2	Lentiviral vector, +IL-2	1	NCT02331693	27000958 26574053
EGFRvIII	Glioblastoma	R	Lentiviral vector, CD3ζ-CD137, +TMZ	1	NCT02209376	25696001 25829274
EGFRvIII	Glioblastoma	R	TMZ	1	NCT02664363	
EGFRvIII	Malignant glioma, glioblastoma	C, F	IL-2, CD28-CD137-CD3ζ	1 and 2	NCT01454596	22780919
EPCAM	Liver neoplasms+stomach neoplasms	Lymphodepletion		1 and 2	NCT02725125	
EphA2	Malignant glioma	Unknown		1 and 2	NCT02575261	27009301
FAP	Malignant pleural mesothelioma	Palliative chemotherapy	1×10^6 CAR T cells into pleural effusion	1	NCT01722149	23259649 26574053
GD2	Neuroblastoma	C, F	3rd generation CAR. iCASP9 gene. Autologous NKs	1	NCT02439788	26390167
GD2	Sarcoma, osteosarcoma, neuroblastoma, melanoma	C, AP1903		1	NCT02107963	26425336 26574053
GD2	Neuroblastoma	C, F	4th generation lentivirus	2	NCT02765243	
GD2	Neuroblastoma	C, F, P	iC9-GD2-CD28-OX40	1	NCT01822652	26574053
GD2	Relapsed/refractory Neuroblastoma	C, F	1RG-CART	1	NCT02761915	
GD2	Osteosarcoma	Unknown	iC9-GD2-CAR-VZV-CTls plus vaccine for VZV	1	NCT01953900	26110321 26574053
GD2	Metastatic melanoma	Vemurafenib concurrently	Patients with BRAF V600E+ or V600K+ tumors	1	ACTRN12613000 198729	
GD2	Neuroblastoma	Submyeloablative	Completed. Viral-specific CTLs used.	1	NCT01460901	24333408 25734008
GD2	Neuroblastoma	No lymphodepletion	EBV-specific CTLs	1	NCT00085930	21984804
GPC3	HCC			1 and 2	NCT02723942	27669301
GPC3	HCC	C, F		1	NCT02905188	
GPC3	Lung squamous cell carcinoma	C, F		1	NCT02876978	
GPC3	HCC	C	41BB included	1 and 2	NCT02715362	
GPC3	HCC	Unknown		1	NCT02395250	27000958
HER2	Glioblastoma	Unknown	Up to 1×10^8 CAR T intratumoral	1	NCT02442297	27411023
HER2	Breast cancer	Lymphodepletion	CD28-CD3ζ	1 and 2	NCT02547961	27009301
HER2	Glioblastoma multiforme	Unknown	CMV T cells, CD28-CD3ζ	1	NCT01109095	26574053
HER2	Her+ cancers	Unknown	TGFβ-resistant HER2/EBV-CTLs	1	NCT00889954	25425467 26574053
HER2	Breast, ovarian, lung, pancreatic	Unknown		1 and 2	NCT02713984	
HER2	Breast, gastric, HCC, endometrial, refractory to chemotherapy and Her2 antibody	Unknown		1 and 2	NCT01935843	25050207 26968709
HER2	Advanced sarcoma	C, F	Up to 1×10^8 CAR T cells, repeat infusions	1	NCT00902044	
IL13Ra2	Glioma		CD137-CD3ζ, truncated CD19 marker	1	NCT02208362	
Lewis-Y	AML	F	Completed, two minor responses	1	NCT01716364	23831595
Mesothelin	Pancreatic adenocarcinoma, ovarian cancer, malignant epithelial pleural mesothelioma	C	CD137-CD3ζ CAR	1	NCT02159716	27000958 26574053
Mesothelin	Pancreatic cancer	C	Transcatheter arterial infusion CD137 included in CAR	1	NCT02706782	
Mesothelin	Malignant mesothelioma	Unknown	CD137-CD3ζ CAR	1	NCT02580747	27550819
Mesothelin	Metastatic Her2- breast	C	+ iCASP9	1	NCT02792114	
MG7	Liver metastasis	C	Intratumoral delivery	1 and 2	NCT02862704	
MUC1	HCC, NSCLC, pancreatic carcinoma	Unknown	CAR NK cells	1 and 2	NCT02839954	
MUC1	Glioma, colorectal carcinoma, gastric carcinoma	Unknown		1 and 2	NCT02617134	27550819
MUC1	HCC, NSCLC, pancreatic	Unknown		1 and 2	NCT02587689	27550819
PSCA	Non-resectable pancreatic cancer	AP1903		1	NCT02744287	
PSMA	Prostate cancer	C, F	Completed, two partial responses	1	NCT01929239	27324746
T4	SCCHN	None	Locoregional disease	1	NCT01818323	26738472 24099518
ALPHA FR	Metastatic ovarian	C, F	Completed, no responses, anti-CAR responses	1	NCT00019136	17062687 25505964
CAIX	RCC	None	Completed	1	DDHK97-29	25505964
CEA	Liver metastases	Unknown	Completed, hepatic artery delivery, some CEA decrease	1	NCT01373047	25850950
GD2	Neuroblastoma	Lymphodepletion	Completed. Viral-specific CTLs used	1	NCT01460901	25734008

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AP1903, rimiducid; C, cyclophosphamide; CAIX, carboxy-anhydrase-IX; CEA, carcinoembryonic antigen; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; EBV, Epstein–Barr virus; EGFR, epidermal growth factor receptor; EPCAM, epithelial cell adhesion molecule; F, fludarabine; FAP, fibroblast activation protein; FR, folate receptor; GPC3, glypican-3; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; iCASP9, inducible caspase-9; MG7, glycosylated protein of CEA; MM, multiple myeloma; MUC1, mucin1; NK, natural killer cell; NSCLC, non-small cell lung cancer; P, pembrolizumab (anti-PD-1); PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; R, radiation; RCC, renal cell carcinoma; SSCHN, squamous cell cancer of the head and neck; TGFβ, transformation growth factor beta; TMZ, temozolomide; Vemurafenib, BRAF inhibitor; VZV, Varicella zoster virus.
Trial information can be located using trial ID at https://clinicaltrials.gov.

Targets:

CD133
CD138
CD171
CD70
CEA (3)
EGFR (2) and EGFRvIII (3)
EPCAM
EphA2
FAP
GD2 (10)
GPC3 (5)
HER2 (7)
IL13Ra2
Lewis-Y
Mesothelin (4)
MG7
MUC1 (3)
PSCA (2)
T4
ALPHA FR
CAIX

Tumors:

Liver (9)
breast (5)
renal
lung
colorectal (4)
gastric
pancreatic (8)
neuroblastoma (9)
glioma (5)
glioblastoma (5)
metastatic melanoma
etc etc…

CARs:

- different generations
- different co-stimulatory domains
- combination with drugs
etc etc

Yong et al., 2017

See page 666

Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing *ERBB2*

Richard A Morgan[1], James C Yang[1], Mio Kitano[1], Mark E Dudley[1], Carolyn M Laurencot[1] and Steven A Rosenberg[1]

[1]*Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*

In a Phase I trial, a patient with metastatic colon cancer was treated with 1 10^{11} CD8+ T cells expressing a third-generation HER2 CAR containing both CD28 and 4-1BB.

The patient experienced severe respiratory distress within 15 minutes of T- cell infusion and died of cardiac arrest 5 days later .

Postmortem analysis confirmed massive T -cell infiltration into the lung, and it was speculated that T-cell activation by low levels of HER2 expression on lung epithelial cells triggered severe CRS and contributed to patient mortality.

Cancer Immunology Miniatures

Cancer Immunology Research

T Cells Expressing Chimeric Antigen Receptors Can Cause Anaphylaxis in Humans

Marcela V. Maus[1,2], Andrew R. Haas[1,2], Gregory L. Beatty[1,2], Steven M. Albelda[1,2], Bruce L. Levine[1,3], Xiaojun Liu[3], Yangbing Zhao[1,3], Michael Kalos[1,3], and Carl H. June[1,3]

Published July 2013

Cancer Immunology Miniatures

Cancer Immunology Research

Mesothelin-Specific Chimeric Antigen Receptor mRNA-Engineered T Cells Induce Antitumor Activity in Solid Malignancies

Gregory L. Beatty[1,3], Andrew R. Haas[1,2], Marcela V. Maus[1,3], Drew A. Torigian[1,5], Michael C. Soulen[1,5], Gabriela Plesa[1], Anne Chew[1], Yangbing Zhao[1,4], Bruce L. Levine[1,4,6], Steven M. Albelda[1,2], Michael Kalos[1,4], and Carl H. June[1,4,6]

Published February 2014

→ Transient, partial response was observed in one patient, but the same patient eventually developed anaphylaxis attributed to the generation of IgE antibodies specific to the CAR, which contained a murine scFv. Human anti-mouse antibodies (HAMAs) were detected in a second patient.

CAR for solid tumors in the clinic

BRIEF REPORT

Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy

Christine E. Brown, Ph.D., Darya Alizadeh, Ph.D., Renate Starr, M.S., Lihong Weng, M.D., Jamie R. Wagner, B.A., Araceli Naranjo, B.A., Julie R. Ostberg, Ph.D., M. Suzette Blanchard, Ph.D., Julie Kilpatrick, M.S.N., Jennifer Simpson, B.A., Anita Kurien, M.B.S., Saul J. Priceman, Ph.D., Xiuli Wang, M.D., Ph.D., Todd L. Harshbarger, M.D., Massimo D'Apuzzo, M.D., Julie A. Ressler, M.D., Michael C. Jensen, M.D., Michael E. Barish, Ph.D., Mike Chen, M.D., Ph.D., Jana Portnow, M.D., Stephen J. Forman, M.D., and Behnam Badie, M.D.

City of Hope Beckman Research Institute and Medical Center, Duarte, CA;

SUMMARY

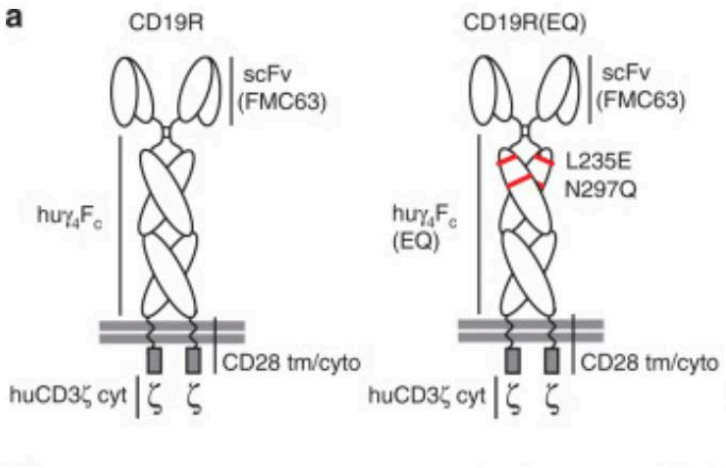
A patient with recurrent multifocal glioblastoma received chimeric antigen receptor (CAR)–engineered T cells targeting the tumor-associated antigen interleukin-13 receptor alpha 2 (IL13Rα2). Multiple infusions of CAR T cells were administered over 220 days through two intracranial delivery routes — infusions into the resected tumor cavity followed by infusions into the ventricular system. Intracranial infusions of IL13Rα2-targeted CAR T cells were not associated with any toxic effects of grade 3 or higher. After CAR T-cell treatment, regression of all intracranial and spinal tumors was observed, along with corresponding increases in levels of cytokines and immune cells in the cerebrospinal fluid. This clinical response continued for 7.5 months after the initiation of CAR T-cell therapy. (Funded by Gateway for Cancer Research and others; ClinicalTrials.gov number, NCT02208362.)

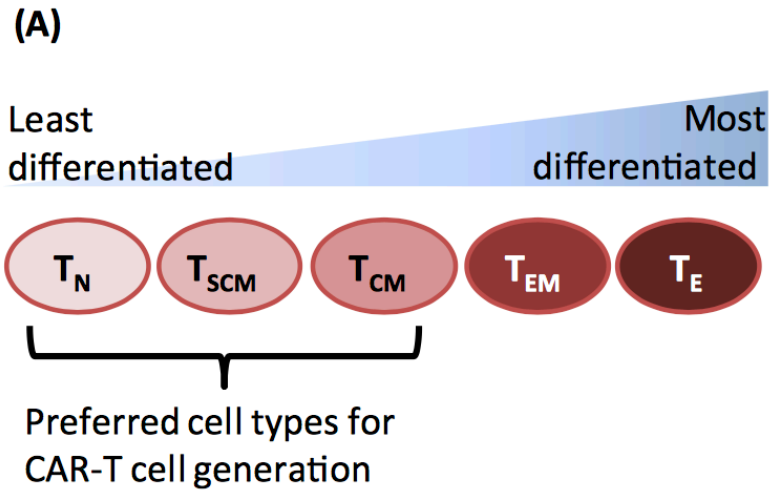
CAR for glioblastoma: case report

First clinical study evaluating intracranial administration of CD8 T cells expressing a first-generation IL13Rα2-targeted CAR in patients with glioblastoma showed transient antiglioma responses with no high-grade therapy-related side effects

→ we modified the IL13Rα2-targeted CAR T cells to improve antitumor potency and T-cell persistence by incorporating into the CAR:
→ **4-1BB (CD137) costimulation**
→ **mutated IgG4-Fc linker** to reduce off-target Fc-receptor interactions
+ genetically engineering enriched central memory T cells

N ENGL J MED 375;26 NEJM.ORG DECEMBER 29, 2016





Molecular Therapy, Volume 23, Issue 4, April 2015, Pages 757-768

Trends in Molecular Medicine, May 2017, Vol. 23, No. 5

CAR for glioblastoma: case report

50-year-old man presented with glioblastoma in the right temporal lobe

IL13Rα2 H score (range 0-300) of 100: with no staining in 30% of cells, weak-intensity staining in 30%, moderate-intensity staining in 20%, and high- intensity staining in 10%

The patient received standard-of-care therapy consisting of tumor resection, radiation therapy, and temozolomide

Six months after the diagnosis: evidence of disease recurrence

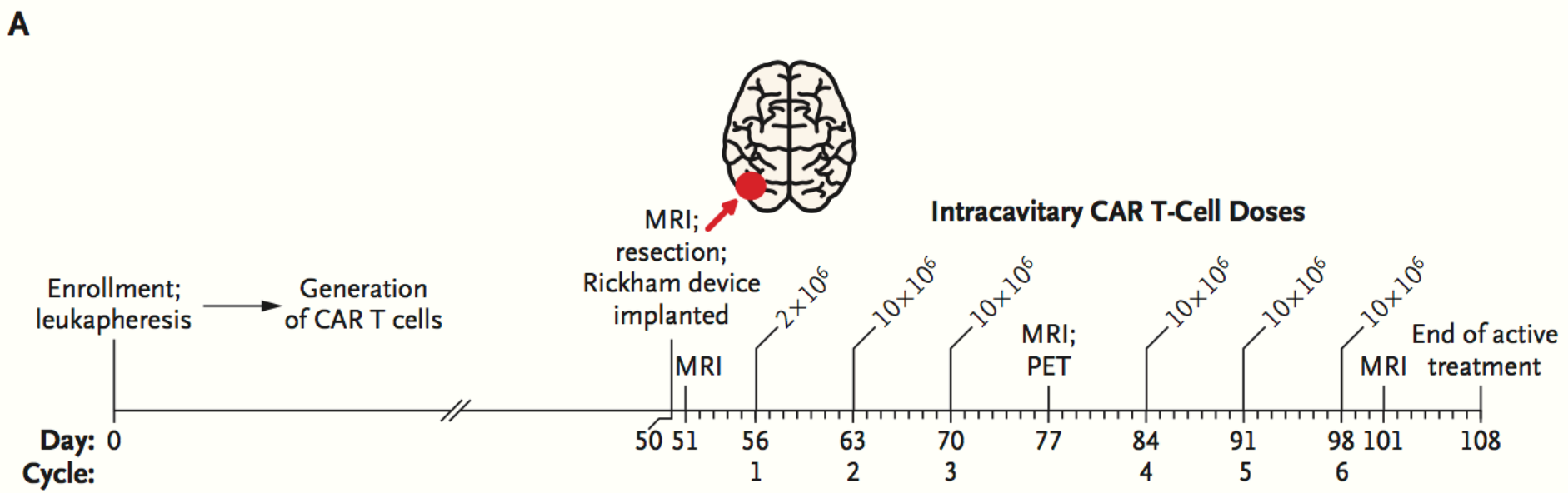
patient was then enrolled in this clinical study of IL13Rα2-targeted CAR T cells

the patient participated in an investigational clinical trial (ClinicalTrials.gov num- ber, NCT01975701) at a different institution: disease progressed rapidly during treatment, with the development of multifocal leptomeningeal glioblastoma involving both cerebral hemi- spheres

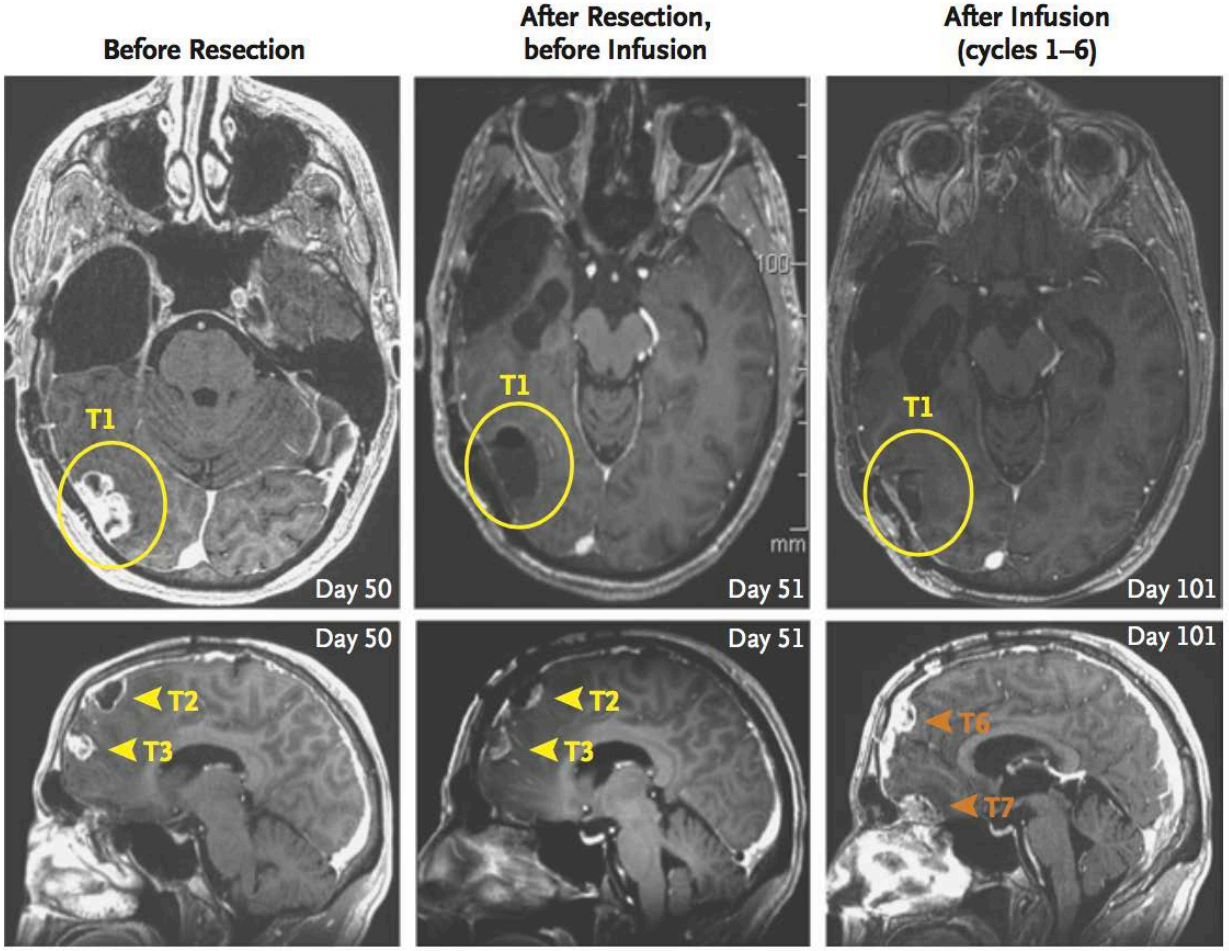
CAR for glioblastoma: case report

The patient then began to receive CAR T cell treatment and underwent
resection of three of five progressing intracranial tumors
+
IL13BBζ–CAR T cells administration according to dose schedule 1 (an initial infusion of 2×106 CAR+ T cells followed by five infusions of 10 × 106 CAR+ T cells), weekly into the resected cavity of tumor 1 through a catheter device

Treatment was paused for assessment of safety and disease after the third and sixth infusions



CAR for glioblastoma: case report

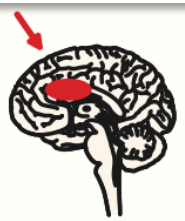


Panel B shows axial MRI (T1-weighted with gadolinium enhancement) of the brain highlighting the site of the resected tumor at which the catheter was placed for delivery of CAR T cells (tumor 1 [T1]; yellow circles), as well as the resected-only tumor sites in the frontal lobe (tumors 2 and 3 [T2 and T3]; yellow arrowheads) and the sites of tumors that developed during the intracavitary treatment period (tumors 6 and 7 [T6 and T7]; orange arrowheads).

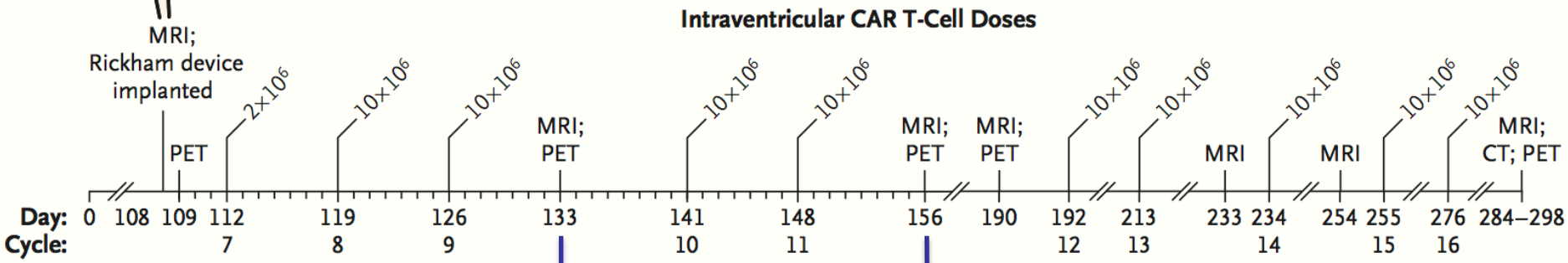
→ The **CAR T-cell injection site (T1) remained stable** without evidence of disease recurrence, **whereas other disease foci, distant from the CAR T-cell injection site, continued to progress + new metastatic lesions in the spine were detected**

→ delivery of cells into the cerebrospinal fluid would improve their trafficking to sites of multifocal disease, a second catheter device was placed in the right lateral ventricle

CAR for glioblastoma: case report



10 additional intraventricular treatment cycles into the right lateral ventricle at 1- to 3-week intervals, with a 6-week break between the fifth and sixth infusions

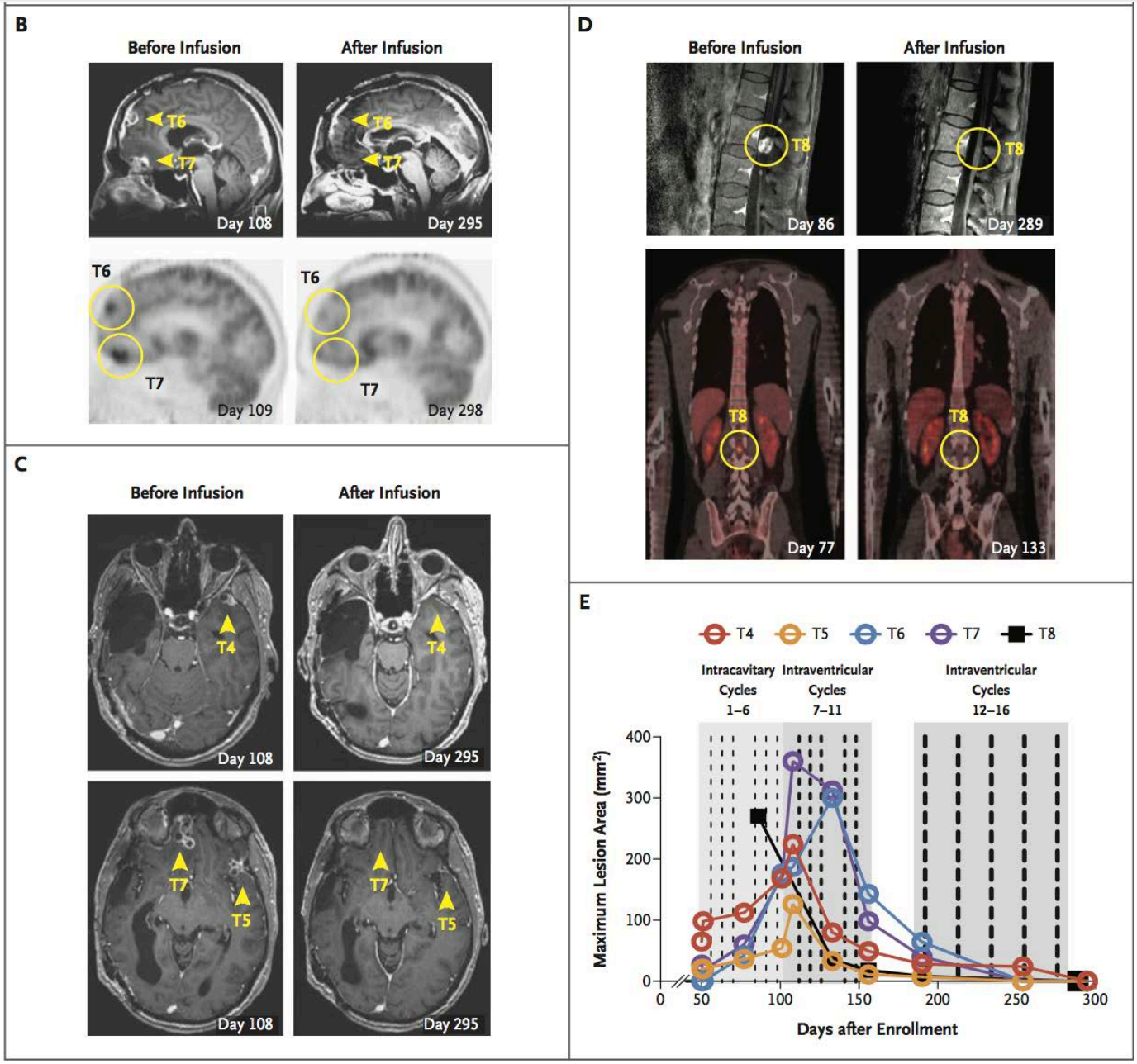


after the first three intraventricular infusions (on day 133), we observed a dramatic reduction in the size of all intracranial and spinal tumors,

after the fifth intraventricular infusion (on day 190), all tumors had decreased by 77 to 100%

Five additional intraventricular infusions were administered (cycles 12 through 16), and during this consolidation phase, all lesions continued to resolve. The tumors were not measurable by means of MRI and remained undetectable by means of PET

CAR for glioblastoma: case report



This dramatic clinical response was **sustained for 7.5 months** after the initiation of CAR T-cell therapy, and none of these initial tumors (tumors 1 through 7 and spinal tumors) recurred.

Unfortunately, this patient's disease eventually recurred after cycle 16 (228 days after the first CAR T-cell treatment) at four new locations that were distinct and non-adjacent to tumors 1 through 7 and the spinal tu- mors.

The cause of this tumor recurrence is currently under investigation, with preliminary results suggesting decreased expression of IL13Rα2

Conclusions

- The potential power of CAR therapy has been validated in hematological malignancies, yet the rate of success in solid tumors is currently low
- Novel cutting edge designs for CAR T cells to overcome many of the challenges presented by solid tumors are currently being tested
- growing wealth of knowledge about the tumor microenvironment and the speed of technological advances will promote the development of CAR T cells that are adequately modified to deliver a lethal hit to solid tumors

Yong et al., 2017

…Thank you!