The Rise of the T-cell chauvinists

Prologue: "shooting rubber bands at the stars"

1. The occasional miracle

"Laudable pus"

"The man who does the most work does the best work"

Bleak house

The method of choice

The Coley phenomenon

2. The patron saint of cytokines

In search of an interferon

Lymphodrek

"The cloning of interferon and other mistakes"

"One of my best known accidents"

3. The rise of the T cell chauvinists

The silk purse years

The rise of the T cell chauvinists

To be in motion

The end of the beginning?

"There's just so much you can learn from a mouse"

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Vice Chair Research, Dept. of Surgery
Director, Center for Damage Associated Molecular Pattern Molecule Laboratories
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Disclosures-Consultant

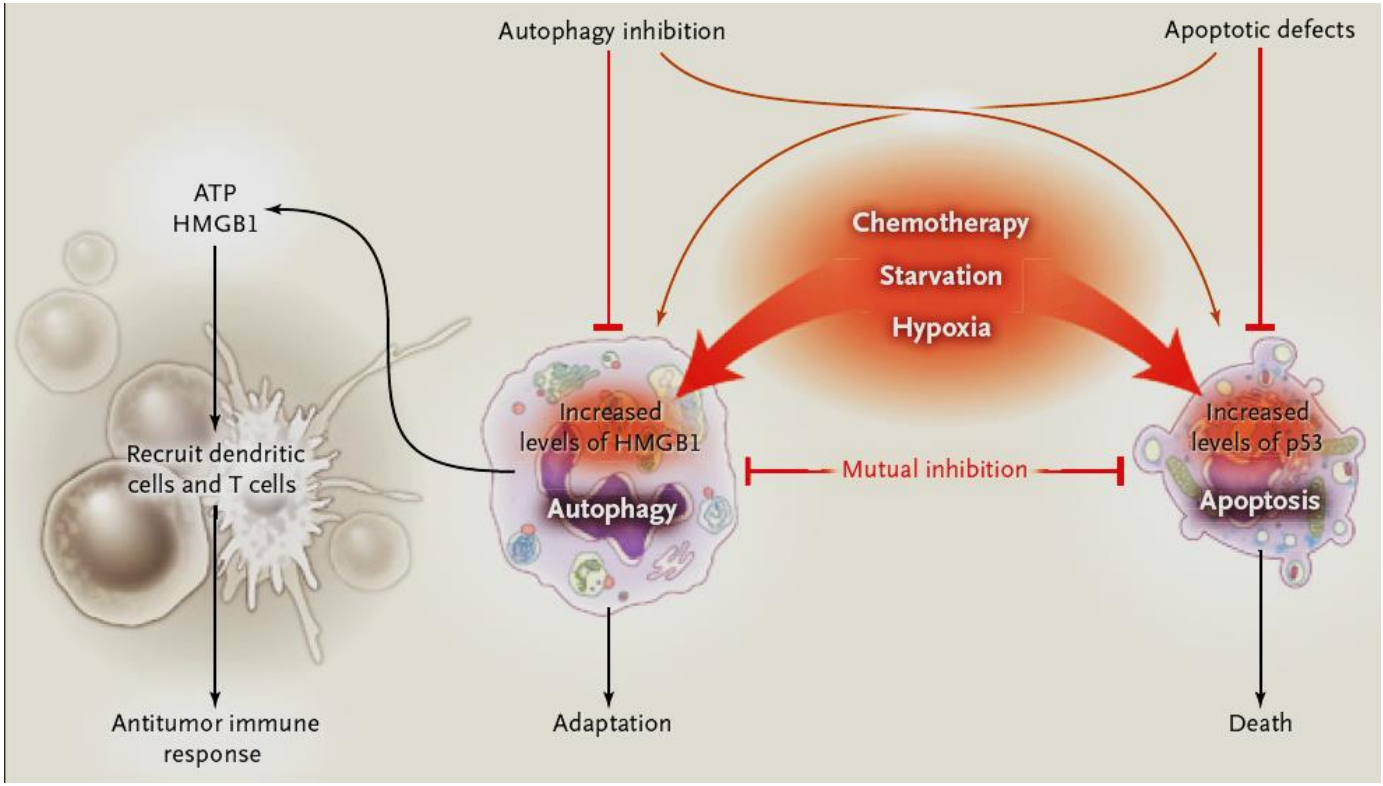
- **Prometheus**
- **Celgene Cellular Therapeutics**
- **NeuMedicine**
- **Chairman of the Advisory Board, Immunocellular Therapeutics, Ltd.**
- **Intezyne**
- **VeraStem**
- **Checkmate, Inc.**
- **Pieris, Inc.**
- **Lion/Iovance CSO**
- **iRepertoire, Inc. (Hudson Alpha Institute)**
- **Torque, Inc.**
- **Adicet, Inc.**

The NEW ENGLAND JOURNAL of MEDICINE
366:1156-1158; March 22, 2012

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Tumor-Cell Death, Autophagy, and Immunity

Louis M. Weiner, M.D., and Michael T. Lotze, M.D.



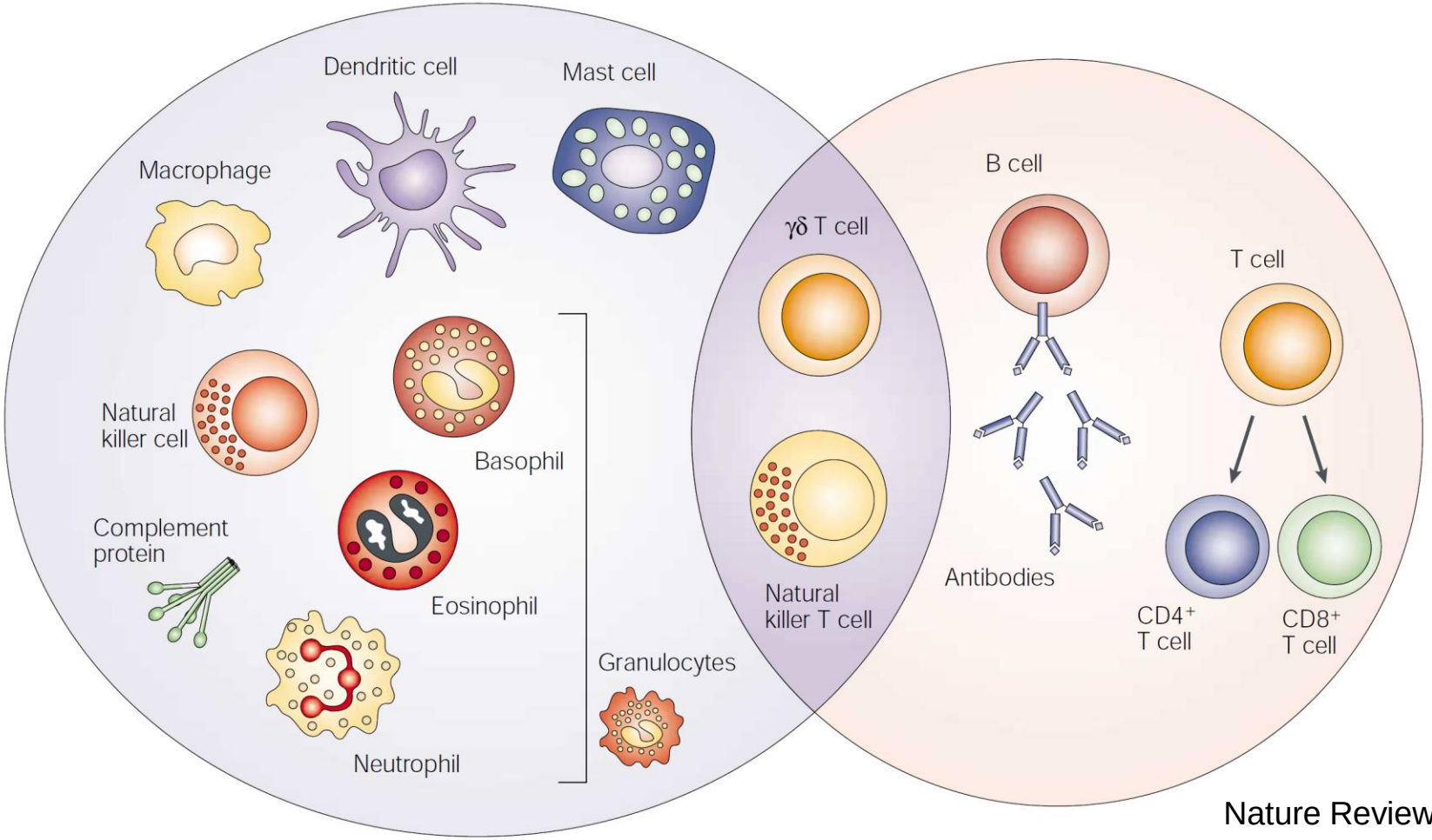
Components of the Immune System

Innate Immunity

- Rapid Response
- Non-specific
- No Memory

Adaptive Immunity

- Slow Response
- Highly Specific
- Develop Memory



Nature Reviews 2004

Goal of Adoptive T Cell Transfer

Physical repopulation of the host immune system with antigen specific T cells that:

1. Mediate potent effector function (i.e. destroy tumor)

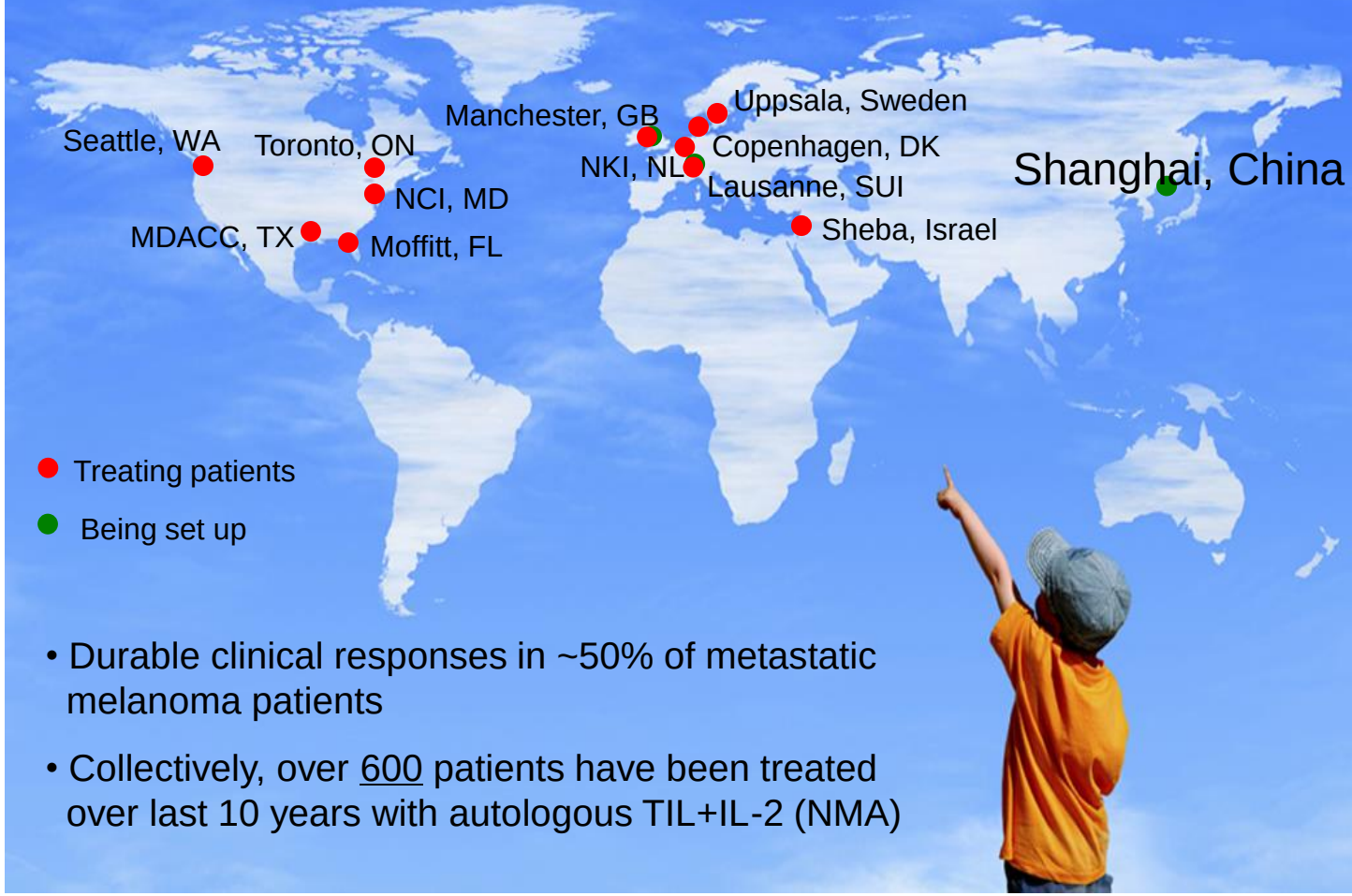
2. Persist and establish memory

Cheever, Greenberg, Fefer

Early Solid Tumor CART Cell studies

- Zelig Eshar First Concept (Israel and Surgery Branch, NCI)
- Advanced epithelial ovarian cancer - folate receptor (Patrick Hwu)
- Metastatic renal cell carcinoma carbonic anhydrase IX (CAIX)
- L1-cell adhesion molecule-specific (CD171) CAR T cells for the treatment of metastatic neuroblastoma
- First-generation GD2-targeted CAR T cells administered to children with advanced neuroblastoma (3 of 11 CRs)
- A third-generation CAR specific to the tumor antigen Her2 and integrating CD28, 4-1BB, and CD3z signaling moieties resulted in death of a patient with metastatic colon cancer (normal lung and/or cardiac tissue.

Growing Network of TIL Therapy Centers



- Durable clinical responses in ~50% of metastatic melanoma patients
- Collectively, over 600 patients have been treated over last 10 years with autologous TIL+IL-2 (NMA)

GE Cell Processing Equipment and Reagents

Apheresis → Isolation → Activation → Gene Transfer → Expansion → Harvest → Formulation → Cryopreservation → Reformulation


VIA Freeze


VIA Thaw


Ficoll

STEMCELL™ TECHNOLOGIES
EasySep™ Release CD3 Positive Selection Kit


Sepax 2

STEMCELL™ TECHNOLOGIES
ImmunoCult™ Human T cell activators CD3/CD28 & CD3/CD28/CD2


ReadyKart


Xuri W25 and Cellbags


Xuri™ Growth Factor (IL-2, IL-15, IL-21)


Xuri T-Cell Expansion Media

STEMCELL™ TECHNOLOGIES
ImmunoCult™-XFT T-Cell Expansion Media


Sepax 2


Sefia


Sepax 2


Sefia


VIA Freeze


Sepax 2

reversed Stirling engi

Launched

In development

Cell Therapy Enterprise Solutions

Other Original Equipment Manufacturers (OEM)

MACS
Miltenyi Biotec

Wilson Wolf
Superior Cell Culture Devices

G-REX

Global Transportation Partnerships

Cryoport's solutions have global reach through shipping agreements with FedEx, UPS, DHL, and World Courier

FedEx

FedEx® Deep Frozen Shipping Solution

powered by Cryoport®

ups

Cryogenic shipping from UPS

UPS Temperature True® Cryo, powered by Cryoport℠

DHL

THERMONET

Chain of Compliance™ Logistics Management

CHAIN of CUSTODY · CHAIN of CONDITION · CHAIN of IDENTITY · CHAIN of COMPLIANCE™

Traceability of the Custody of each Client's or Patient's Therapy

Traceability of the Condition of each Client's or Patient's Therapy

Traceability of the Identity of each Client's or Patient's Therapy

Traceability of the Equipment and Processes Supporting each Client's or Patient's Therapy

Supports regulatory compliance requests from the EMA/FDA and other regulatory agencies

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

FDA U.S. FOOD & DRUG ADMINISTRATION

Point of care manufacturing will be a major critical factor for the success of cell therapy products

ASH December 2018:

4553 Point-of-Care Manufacturing of CD20.19 Bi-Specific Chimeric Antigen Receptor T (CAR-T) Cells in a Standard Academic Cell Processing Facility for a Phase I Clinical Trial in Relapsed, Refractory NHL

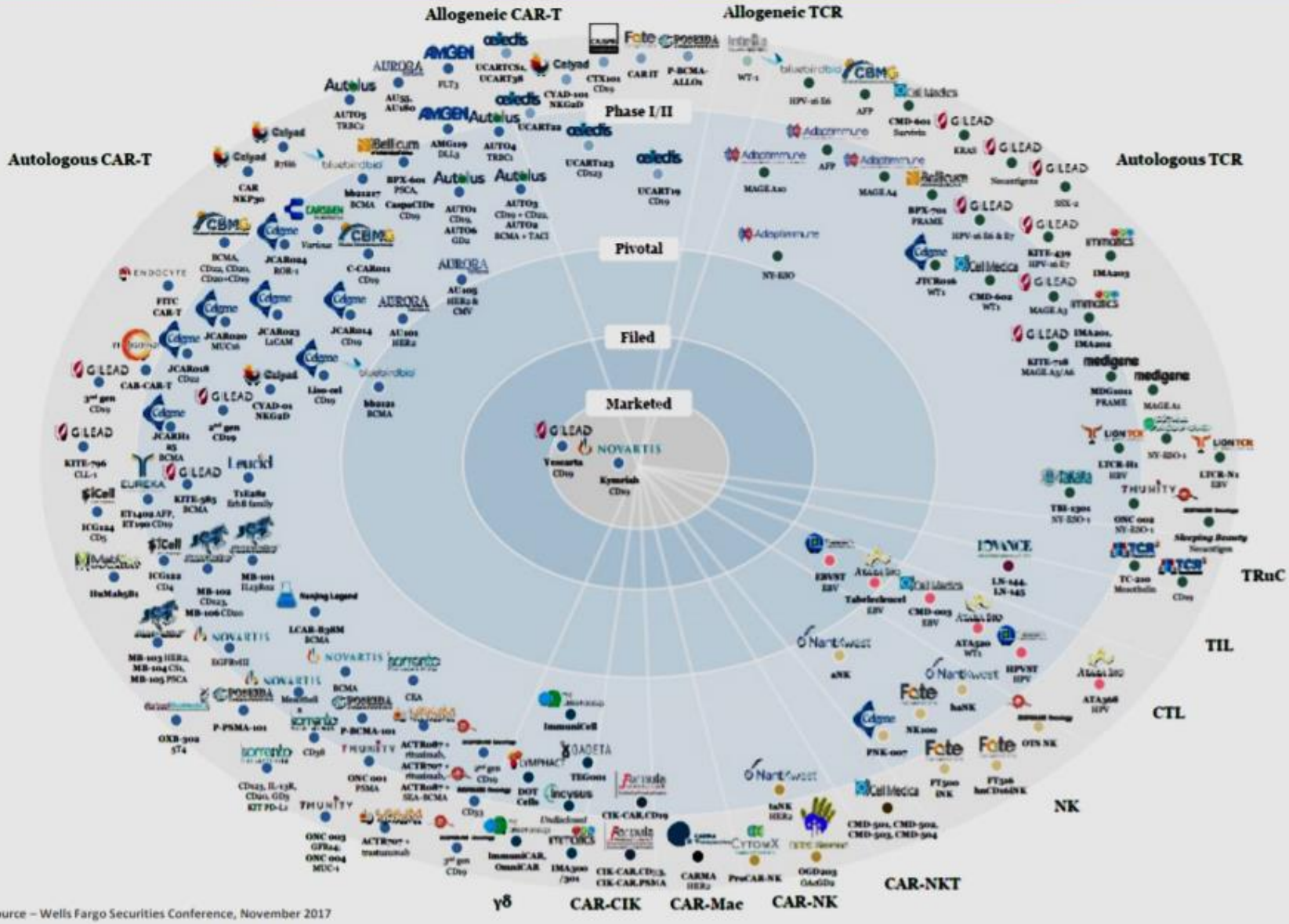
Fenlu Zhu, PhD[1], Nirav N Shah, MD[2], Dina Schneider, PhD[3*], Huiqing Xu, MD[4*], Katherine Chaney, MS[4*], Lawrence Luib, MS[5*], Carolyn A. Keever-Taylor, DPhil[6], Boro Dropulic, PhD[7*], Rimas Orentas, PhD[3*], Parameswaran Hari, MBBS, MD[8] and Bryon Johnson, PhD[6]*

[1]Medical College of Wisconsin, Milwaukee, WI

- Cell therapy will be supported by clinical centers as this bed side manufacturing model will allow them to capture value
- This is the model that will be established in a regulatory-compliant fashion
- New GMP rules already under discussions for autologous " *more than minimally manipulated cell products**" already under consideration with the FDA

** Per FDA classification*

Adoptive Cellular Therapy: Immuno-Oncology Landscape



Source – Wells Fargo Securities Conference, November 2017

CAR-T Partnerships

Company	Notable partnerships (location)	Lead CAR-T-cell asset(s)	CAR T-cell and other T cell receptor technology platforms
Novartis	University of Pennsylvania	CTL019, phase 1/2 in CD19-positive ALL	Undisclosed
Juno Therapeutics Celgene/BMS	Fred Hutchinson Cancer Research Center (Seattle); Memorial Sloan Kettering Cancer Center (New York); Seattle Children's Research Institute (Seattle); Fate Therapeutics (San Diego)	JCAR015, phase 1/2 in CD19-positive hematological cancers	Bispecific CAR technology; armored CAR technology
Kite Pharma Gilead	National Cancer Institute; Amgen	KTE-C19 CAR, phase 1 in multiple hematological cancers	eACT (engineered autologous cell therapy)
Cellectis (Paris)	Servier (Suresnes, France); Pfizer; Ohio State University (Columbus, Ohio)	UCART19, preclinical in CD19 positive ALL and CLL	UCART allogeneic CAR T-cell platform
Bellicum Pharmaceuticals	ARIAD Pharmaceuticals (Cambridge, Massachusetts); Leiden University Medical Center (Leiden, the Netherlands)	BPX-401, preclinical for CD19-positive hematological cancers; BPX-601, preclinical for PSCA-overexpressing solid tumors	CIDeCAR molecular safety switch; GoCAR-T and own small-molecule rimiducid switch
Unum Therapeutics	St. Jude Children's Research Hospital (Memphis, Tennessee); National University of Singapore	ATTCK20 (plus Rituxan (rituximab)), phase 1 for CLL and non-Hodgkin's lymphoma	ACTR (antibody-coupled T-cell receptor)
Intrexon Corp.	ZilOPHARM Oncology; MD Anderson Cancer Center; Merck Serono	Undisclosed, preclinical	RheoSwitch Therapeutic System
bluebird bio (Cambridge, Massachusetts)	Celgene; Baylor College of Medicine (Houston)	Undisclosed, preclinical	Undisclosed
Adaptimmune (Abingdon, UK)	GlaxoSmithKline	NY-ESO TCR, phase 1/2 in synovial sarcoma and multiple myeloma	Affinity-enhanced TCRs
Celyad (formerly Cardio3 BioSciences) (Mont-Saint-Guibert, Belgium)	Celdara Medical (Lebanon, New Hampshire)	CAR-NKG2D, phase 1 in multiple myeloma and acute myeloid leukemia	CAR-T using human NK cell receptors; allogeneic T cells
Mustang Therapeutics (Shanghai)	Fortress Biotech (Burlington, Massachusetts)	Undisclosed	Undisclosed

TCR, T-cell receptor; NK, natural killer; PSCA, Prostate stem cell antigen.

http://www.ascopost.com/issues/may-25-2018/treatment-centers-authorized-to-administer-car-t-cell-therapy/

MIDWEST

Illinois

Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago; www.luriechildrens.org; **T**

Northwestern Medicine Robert H. Lurie Comprehensive Cancer Center of Northwestern, Chicago; http://cancer.northwestern.edu; **A**

University of Chicago Medicine, Chicago; www.uchospitals.edu/index.shtml; **T/A**

Kansas

University of Kansas Cancer Center, Westwood; www.kucancercenter.org; **T**

Michigan

Michigan Medicine, University of Michigan Medical Center, Ann Arbor; www.uofmhealth.org; **T**

Barbara Ann Karmanos Cancer Institute, Detroit; www.karmanos.org/home; **A**

University of Michigan Comprehensive Cancer Center, Ann Arbor; www.karmanos.org; **A**

Minnesota

University of Minnesota Masonic Children's Hospital, Minneapolis; www.mhealth.org/locations/buildings/university-of-minnesota-masonic-childrens-hospital; **T**

Missouri

Children's Mercy Hospital, Kansas City; www.childrensmercy.org; **T**

The University of Kansas Cancer Center, Kansas City; www.kucancercenter.org; **A**

Siteman Cancer Center at Barnes-Jewish Hospital at Washington University Medical Center, St. Louis; www.barnesjewish.org/Medical-Services/Cancer-Center; **A**

Washington University School of Medicine Siteman Kids at St. Louis Children's Hospital, St. Louis; siteman.wustl.edu/visiting/kids/; **T**

Nebraska

Nebraska Medicine, Omaha; www.nebraskamed.com; **A**

University of Nebraska Medical Center, Omaha; www.unmc.edu; **A**

Ohio

Cincinnati Children's Hospital Medical Center, Cincinnati; www.cincinnatichildrens.org; **T**

Cleveland Clinic Cancer Center, Cleveland; my.clevelandclinic.org/departments/cancer; **A**

The Ohio State University Comprehensive Cancer Center; Columbus; cancer.osu.edu; **T/A**

Wisconsin

Froedtert & the Medical College of Wisconsin Cancer Network, Milwaukee; www.froedtert.com/cancer/network; **A**

UWHealth/American Family Children's Hospital, Madison; www.uwhealthkids.org; **T**

http://www.ascopost.com/issues/may-25-2018/treatment-centers-authorized-to-administer-car-t-cell-therapy/

NORTHEAST

Maryland

Johns Hopkins Children's Center, The Charlotte R. Bloomberg Children's Center, Baltimore; www.hopkinsmedicine.org/johns-hopkins-childrens-center/patients-and-families/bloomberg-childrens-center/index.html; **T**

University of Maryland Marlene and Stewart Greenbaum Comprehensive Cancer Center, Baltimore; www.umms.org/umgccc; **A**

Massachusetts

Dana-Farber Boston Children's Cancer & Blood Disorders Center, Boston; www.danafarberbostonchildrens.org; **T**

Dana-Farber/Brigham and Women's Cancer Center, Boston; www.brighamandwomens.org/cancer-center; **A**

Massachusetts General Hospital Cancer Center, Boston; www.massgeneral.org/cancer/; **A**

New Jersey

Hackensack University Medical Center–John Theurer Cancer Center, Hackensack; www.hackensackumc.org/services/cancer-care/; **A**

Joseph M. Sanzari Children's Hospital at Hackensack Meridian Health, Hackensack; www.hackensackumc.org/locations/joseph-m-sanzari-childrens-hospital/; **T**

New York

Memorial Sloan Kettering Cancer Center, New York; www.mskcc.org; **T/A**

Roswell Park Comprehensive Cancer Center, Buffalo; www.roswellpark.org; **A**

UR Medicine Wilmot Cancer Institute, Rochester; www.urmc.rochester.edu/cancer-institute.aspx; **A**

Pennsylvania

The Children's Hospital of Philadelphia, Philadelphia; www.chop.edu; **T**

Penn Medicine Abramson Cancer Center, Philadelphia; www.pennmedicine.org/cancer; **T**

UPMC Hillman Cancer Center, Pittsburgh; hillman.upmc.com/find/locations/hillman-cancer-center-pittsburgh-pa; **A**

SOUTHEAST

Florida

Moffitt Cancer Center, Tampa; moffitt.org; **T/A;** Sylvester Comprehensive Cancer Center, Miami; sylvester.org; **A**

Georgia

Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta; www.choa.org/medical-services/cancer-and-blood-disorders; **T**

Winship Cancer Institute of Emory University, Atlanta; winshipcancer.emory.edu; **A**

Tennessee

Vanderbilt University Medical Center, Nashville; ww2.mc.vanderbilt.edu; **T/A**

http://www.ascopost.com/issues/may-25-2018/treatment-centers-authorized-to-administer-car-t-cell-therapy/

SOUTHWEST

Arizona

Banner Health, Gilbert; www.bannerhealth.com; **A**

Phoenix Children's Hospital, Phoenix; www.phoenixchildrens.org; **T**

Texas

Baylor Charles A. Sammons Cancer Center at Dallas–Texas Oncology, Dallas, Texas; www.texasoncology.com/-cancer-centers/dallas/baylor-charles-a-sammons/medical-oncology; **A**

Children's Medical Center Dallas, Pauline Allen Gill Center for Cancer and Blood Disorders, Dallas; www.childrens.com; **T**

Houston Methodist Hospital, Houston; www.houstonmethodist.org; **T**

Texas Children's Hospital, Houston; www.texaschildrens.org; **T**

Texas Transplant Institute–Methodist Healthcare, San Antonio; sahealth.com/service/transplant-services; **A;**

The University of Texas MD Anderson Cancer Center, Houston; www.mdanderson.org; **T/A**

WEST

California

Children's Hospital Los Angeles, Los Angeles; www.chla.org; **T;** City of Hope, Duarte; www.cityofhope.org/homepage; **T/A**

Lucile Packard Children's Hospital Stanford, Palo Alto; www.stanfordchildrens.org; **T**

Stanford Health Care, Palo Alto; stanfordhealthcare.org; **A**

UCLA Health, Los Angeles; www.uclahealth.org; **A**

UCSF Benioff Children's Hospital, San Francisco; www.ucsfbenioffchildrens.org; **T**

UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco; cancer.ucsf.edu; **A**

Colorado

Children's Hospital Colorado, Aurora; www.childrenscolorado.org; **T;** Colorado Blood Cancer Institute, Denver; bloodcancerinstitute.com; **A**

Oregon

Oregon Health & Science University, Portland; bloodcancerinstitute.com; **T;** OHSU Knight Cancer Institute, Portland; www.ohsu.edu/health/cancer/index.html; **A**

Utah

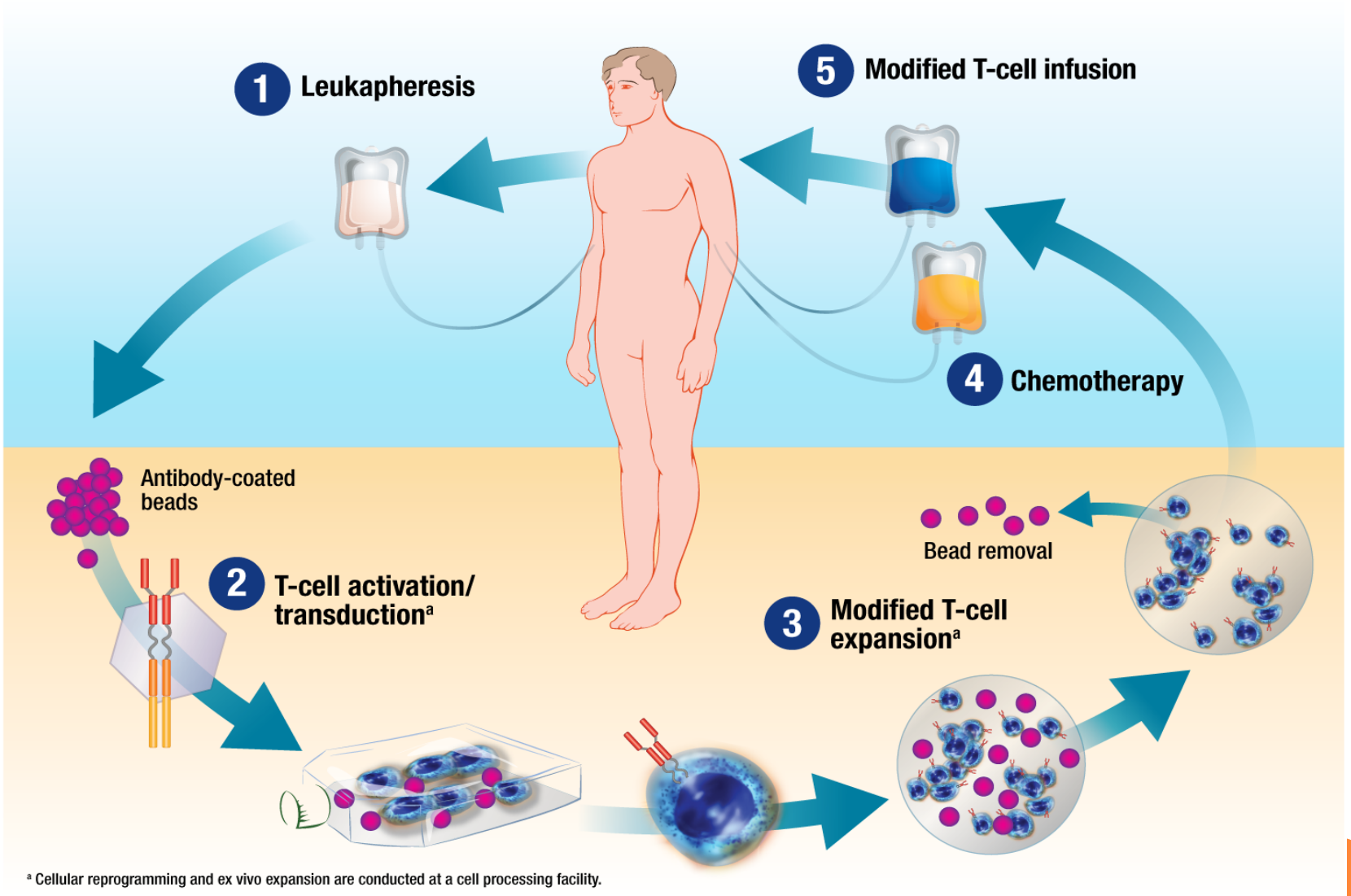
Huntsman Cancer Hospital Institute at the University of Utah, Salt Lake City, Utah; healthcare.utah.edu/huntsmancancerinstitute/; **T/A**

Primary Children's Hospital, Salt Lake City; intermountainhealthcare.org/locations/primary-childrens-hospital/; **T**

Washington

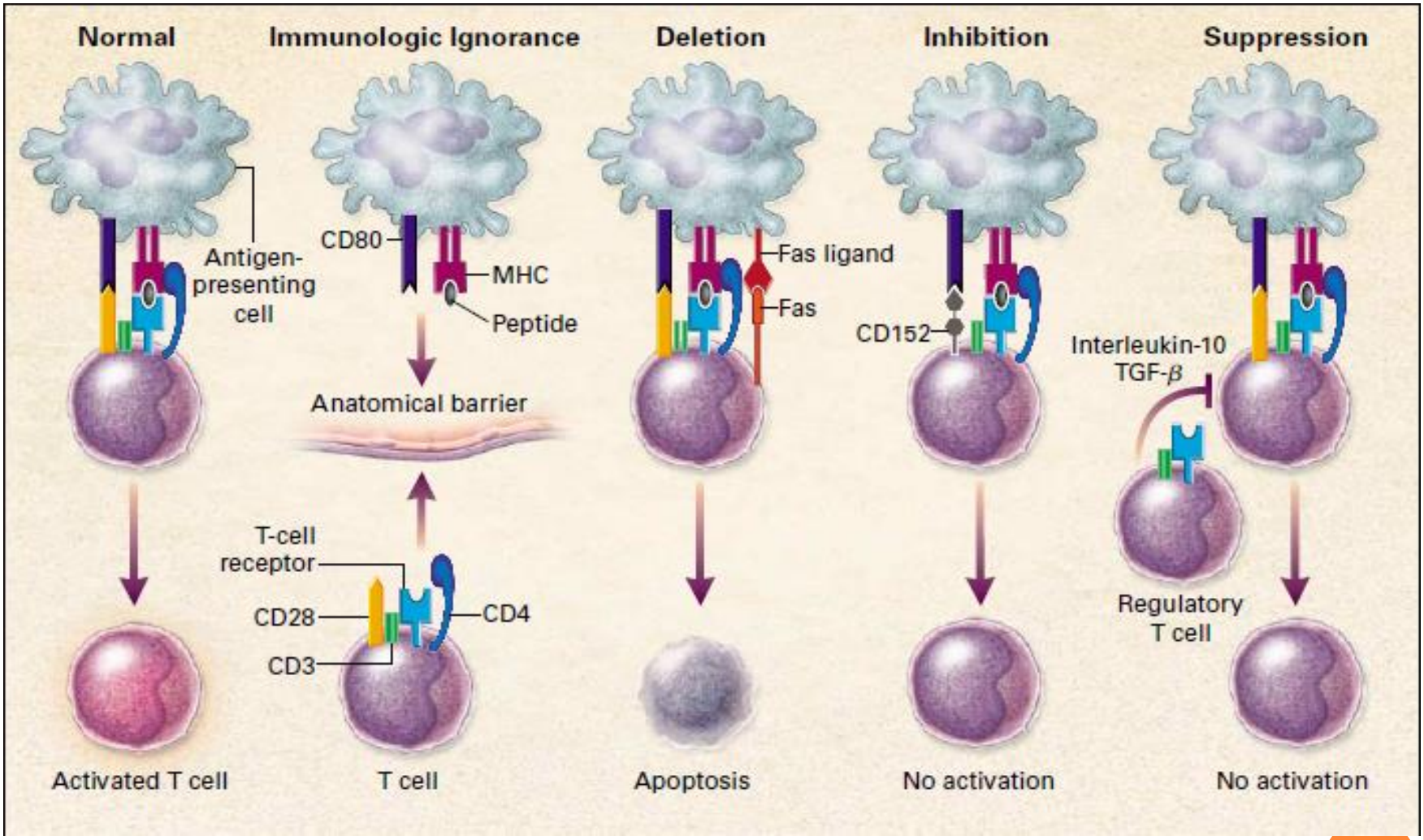
Seattle Cancer Care Alliance, Seattle; www.seattlecca.org

Overview of anti-CD19 CAR T Cell (CTL019) Therapy



Fraietta, J.A.

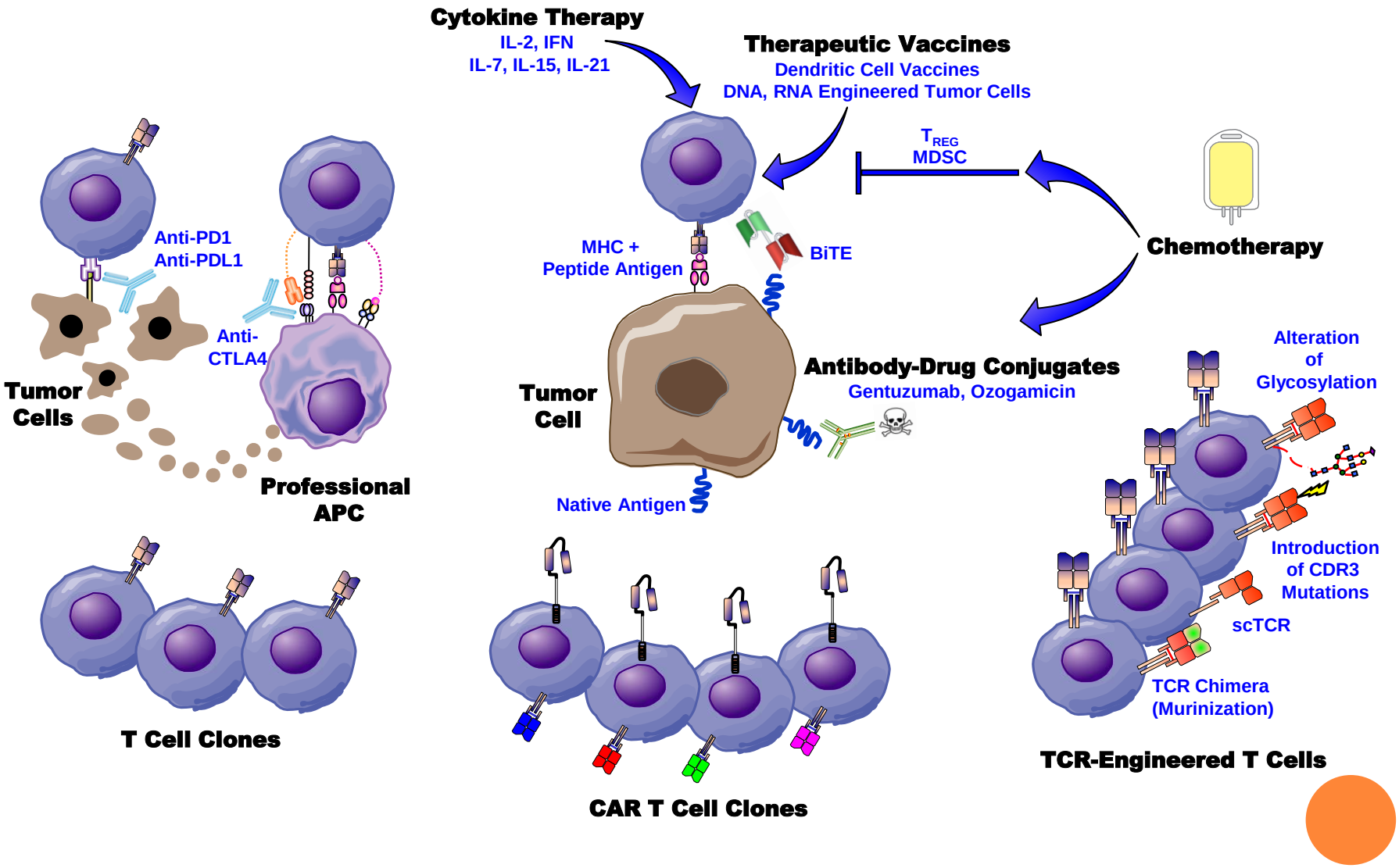
Tolerance: A Major Issue for Anti-tumor Immune Responses



NEJM 2001; 344(9): 655–664

Fraietta, J.A.

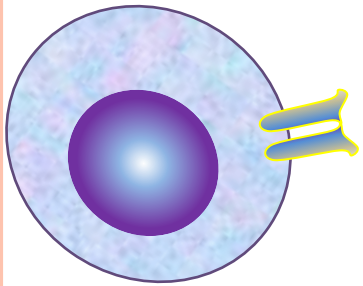
Breaking Tolerance for Immunotherapy

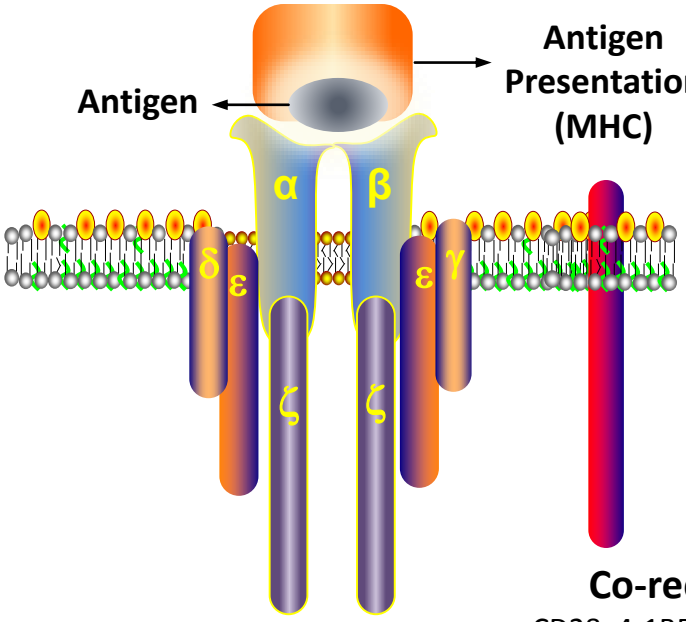


Fraietta, J.A.

A New Approach to Overcoming Tolerance: The Nuts and Bolts of CARs

T cells





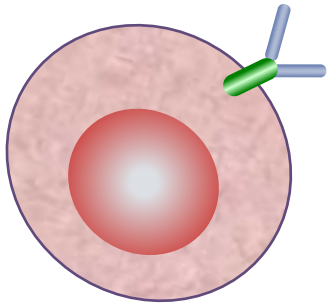
TCR complex

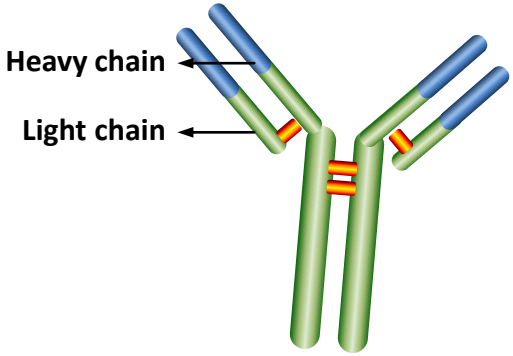
Co-receptor

CD28, 4-1BB, ICOS, OX40

- MHC-restricted antigen recognition
- Co-stimulation for complete activation and function

B cells



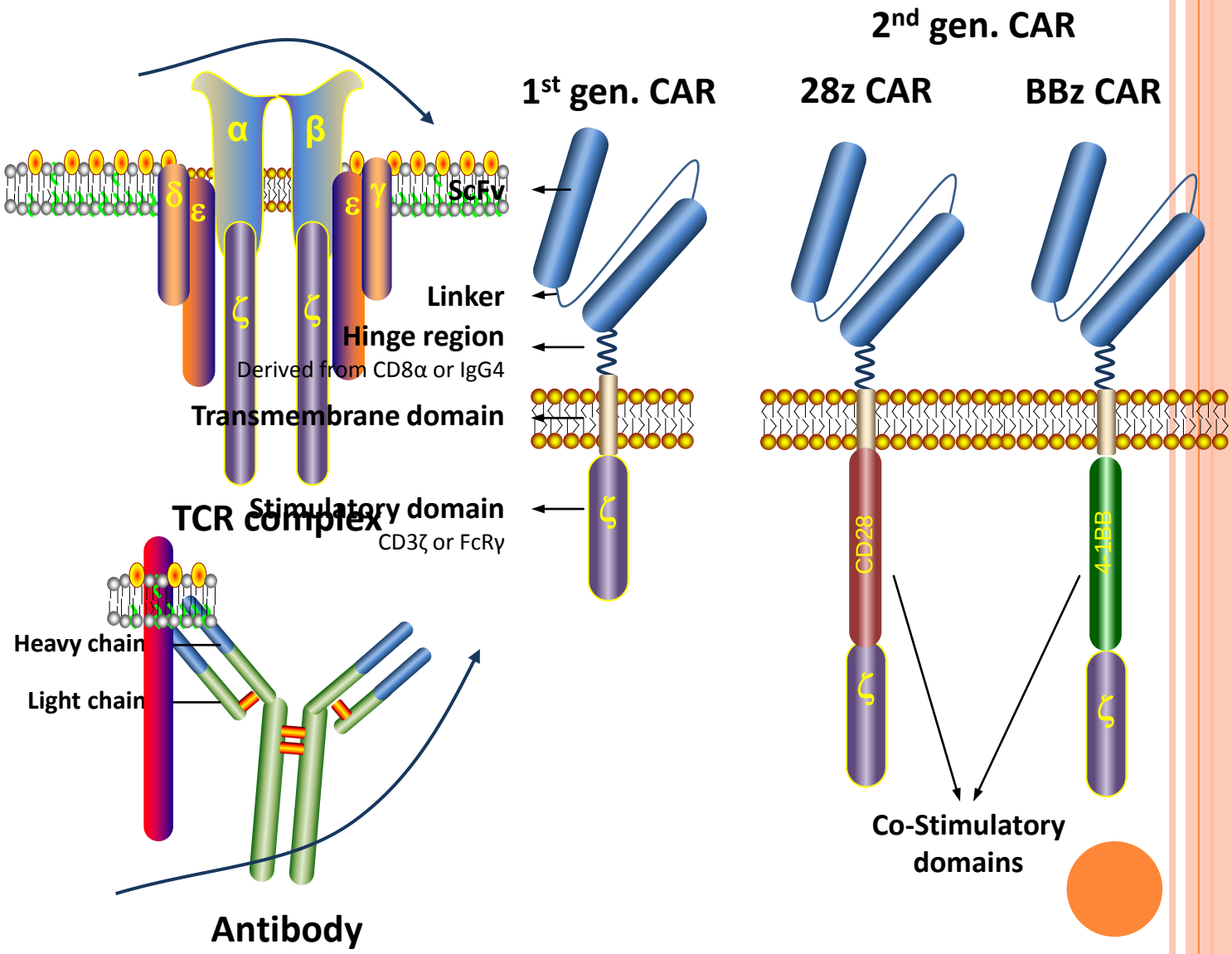


Antibody

- High specificity
- MHC-independent recognition

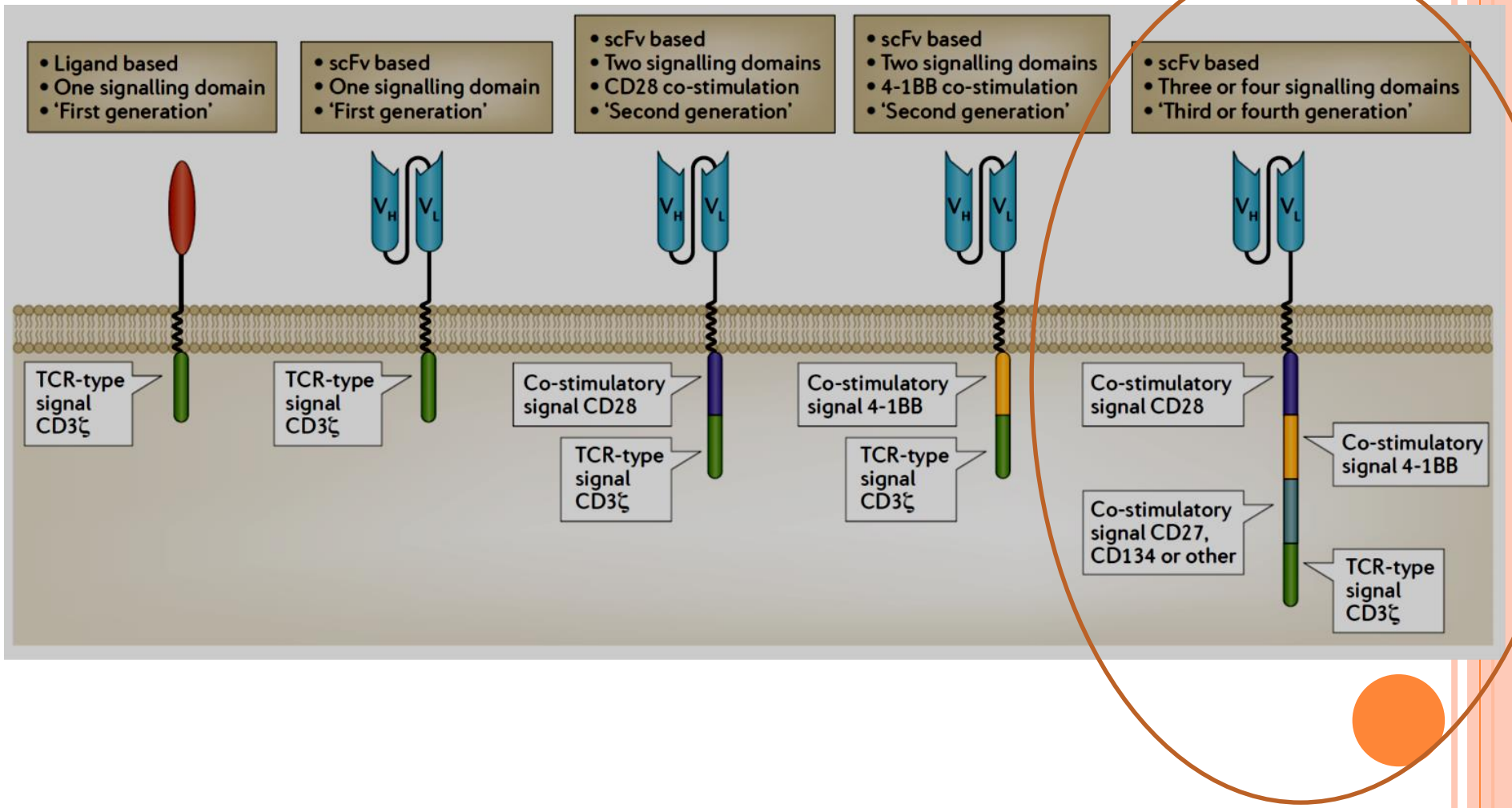
Fraietta, J.A.

A New Approach to Overcoming Tolerance: The Nuts and Bolts of CARs



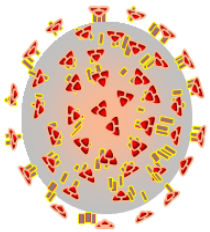
Fraietta, J.A.

Evolution of CAR-T



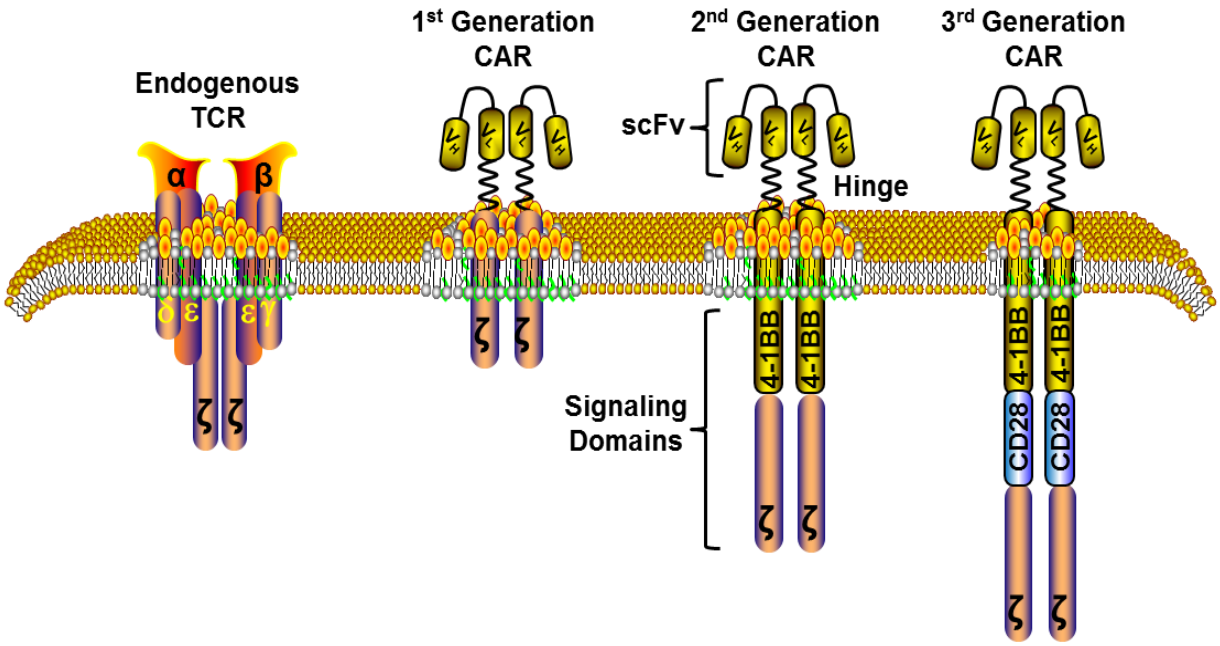
To Engineer a T Cell, You Need…

1



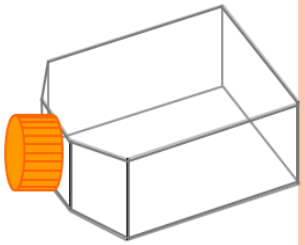
Vector to Deliver Construct

2



An Antigen Receptor (Natural Ligand, TCR or CAR)

3



Ex Vivo Culture System (anti-CD3/CD28 Beads)

Fraietta, J.A.

CAR-T Targets for Solid Tumors

Antigen	Malignancy*	CAR ectodomain, antigen class	Clinical Trial
B7H3 (60)	Sarcoma, glioma	scFv, protein	
CAIX (61, 62)	Kidney	scFv, protein	Published
CD44 v6/v7 (63, 64)	Cervical	scFv, protein	
CD171 (65)	Neuroblastoma	scFv, protein	Published
CEA (66)	Colon	scFv, protein	Ongoing
EGFRvIII (67, 68)	Glioma	scFv, protein	Ongoing
EGP2 (69, 70)	Carcinomas	scFv, protein	
EGP40 (71)	Colon	scFv, protein	
EphA2 (14)	Glioma, lung	scFv, protein	
ErbB2(HER2) (72–79)	Breast, lung, prostate, glioma	scFv, protein	Published
ErbB receptor family (22)	Breast, lung, prostate, glioma	Ligand, protein	
ErbB3/4 (80, 81)	Breast, ovarian	scFv, protein	
HLA-A1/MAGE1 (82, 83)	Melanoma	scFV, peptide/protein complex	
HLA-A2/NY-ESO-1 (84)	Sarcoma, melanoma	scFV, peptide/protein complex	
FR-α (85–88)	Ovarian	scFv, protein	Published
FAP† (89)	Cancer associated fibroblasts	scFv, protein	
FAR (90)	Rhabdomyosarcoma	scFv, protein	
GD2 (91–93)	Neuroblastoma, sarcoma, melanoma	scFv, ganglioside	Published
GD3 (94)	Melanoma, lung cancer	scFv, ganglioside	
HMW-MAA (95)	Melanoma	scFv, proteoglycan	
IL11Rα (96)	Osteosarcoma	Ligand, protein	
IL13Rα2 (16–18, 25)	Glioma	Ligand, protein	Ongoing
Lewis Y (55, 97, 98)	Breast/ovarian/pancreatic	scFv, carbohydrate	
Mesothelin (15, 99)	Mesothelioma, breast, pancreas	scFv, protein	Ongoing
Muc1 (100)	Ovarian, breast, prostate	scFv, glycosylated protein	
NCAM (101)	Neuroblastoma, colorectal	scFv, protein	
NKG2D ligands (20, 57–59)	Ovarian, sacoma	Native receptor, protein	
PSCA (102, 103)	Prostate, pancreatic	scFv, protein	
PSMA (104, 105)	Prostate	scFv, protein	
TAG72 (106, 107)	Colon	scFv, carbohydrate	
VEGFR-2† (108, 109)	Tumor vasculature	Ligand/scFv, protein	Ongoing

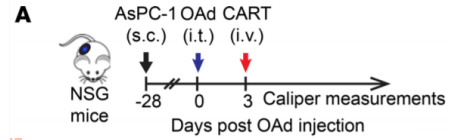
JCI insight

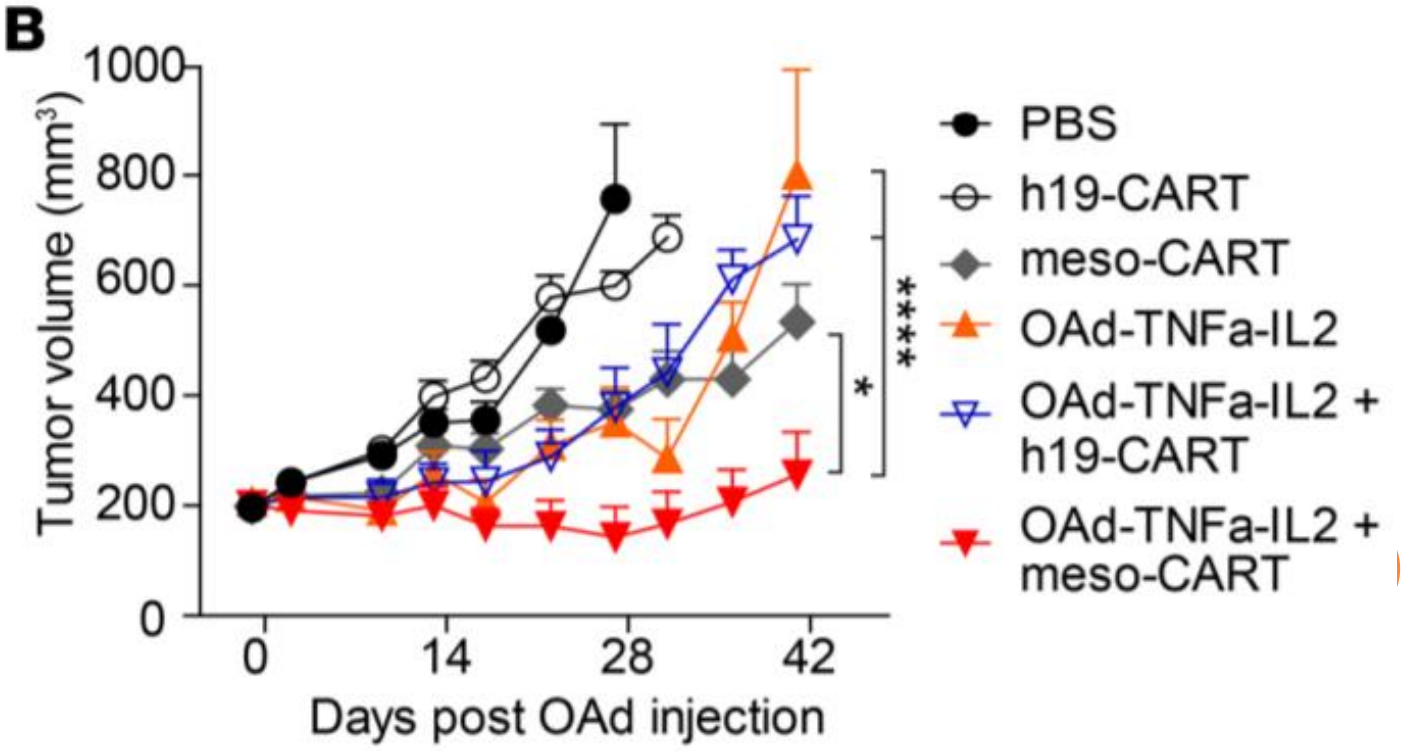
RESEARCH ARTICLE

JCI Insight. 2018;3(7):e99573. https://doi.org/10.1172/jci.insight.99573.

Pancreatic cancer therapy with combined mesothelin-redirected chimeric antigen receptor T cells and cytokine-armed oncolytic adenoviruses

Keisuke Watanabe,[1] Yanping Luo,[1] Tong Da,[1] Sonia Guedan,[1,2] Marco Ruella,[1,2] John Scholler,[1] Brian Keith,[1,3] Regina M. Young,[1] Boris Engels,[4] Suvi Sorsa,[5,6] Mikko Siurala,[5,6] Riikka Havunen,[5,6] Siri Tähtinen,[5] Akseli Hemminki,[5,6,7] and Carl H. June[1,2,8]





The NEW ENGLAND JOURNAL of MEDICINE

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Steering CAR T Cells into Solid Tumors

Marion H. Brown, Ph.D., and Michael L. Dustin, Ph.D.

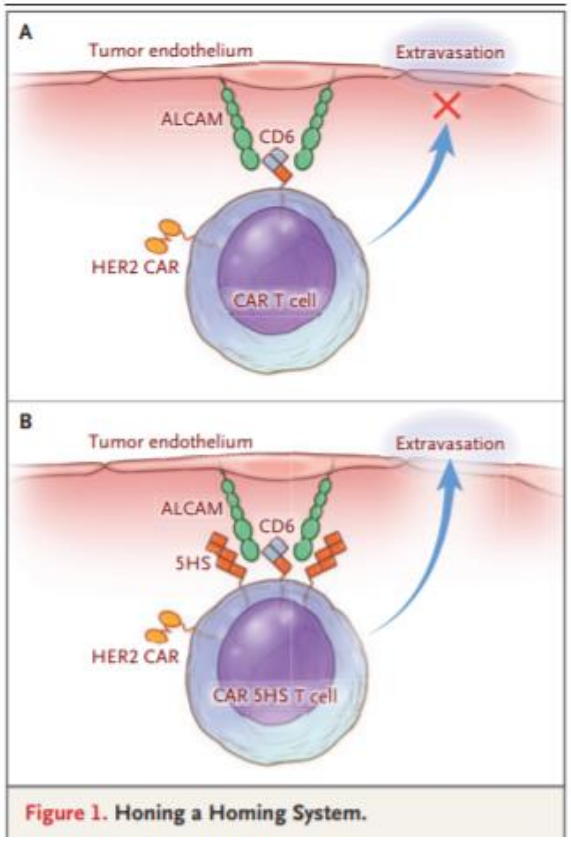


Figure 1. Honing a Homing System.

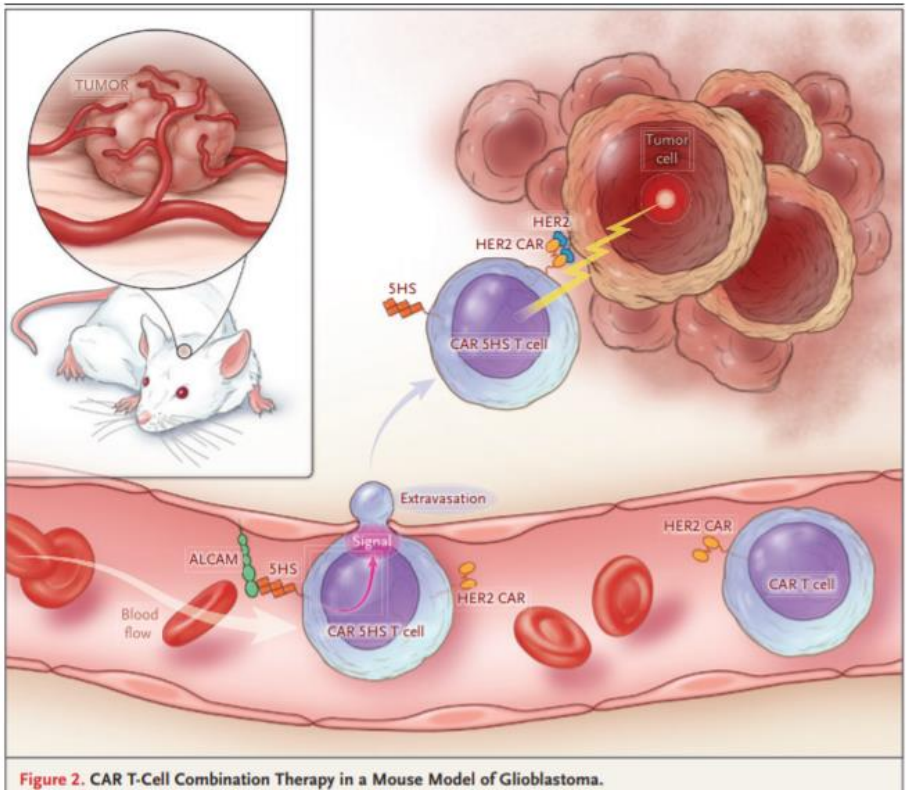
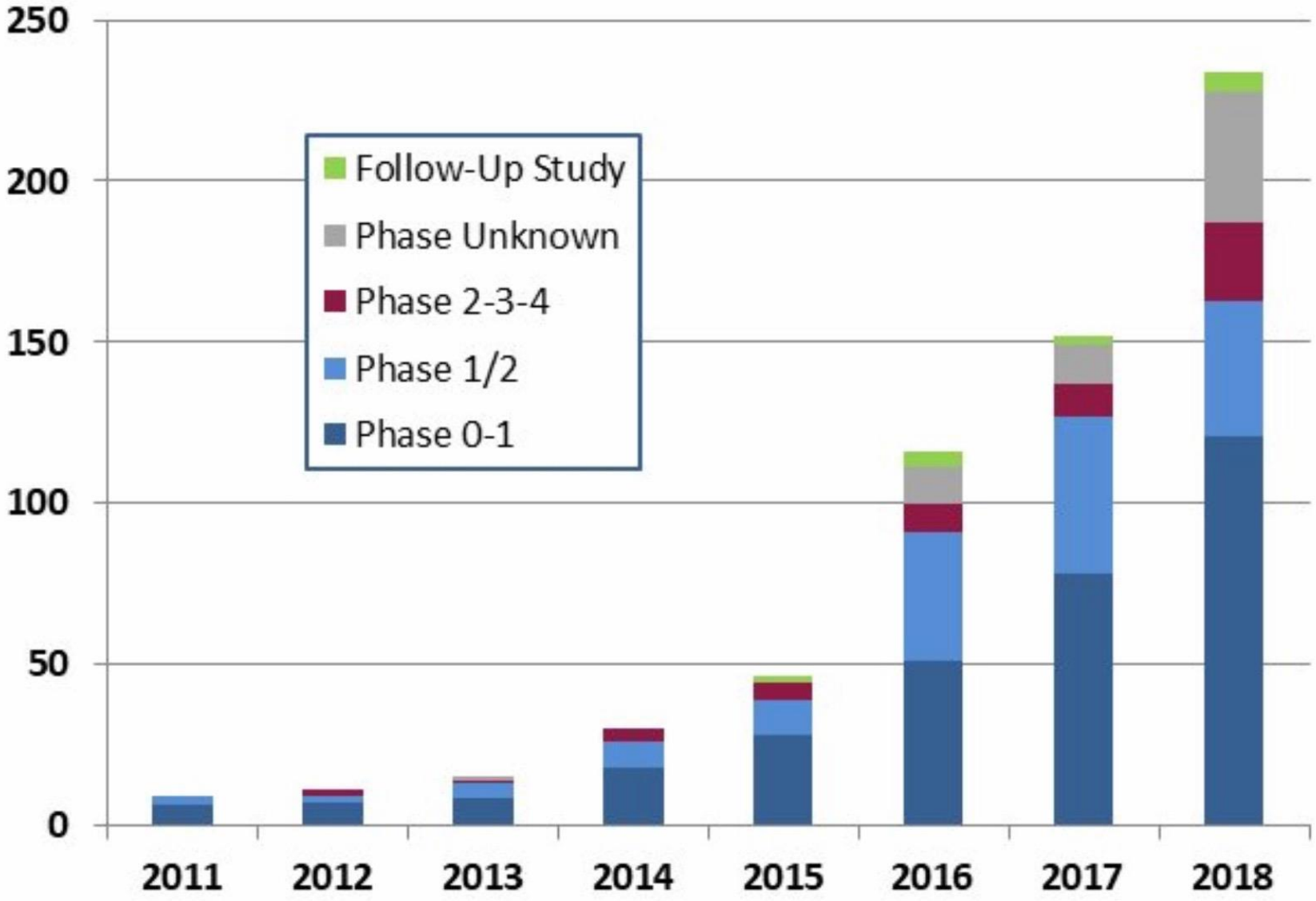


Figure 2. CAR T-Cell Combination Therapy in a Mouse Model of Glioblastoma.

n engl j med 380;3 nejm.org January 17, 2019

Samaha H, Pignata A, Fousek K, et al. A homing system targets therapeutic T cells to brain cancer. Nature 2018:561:331-7

CellTrials.org CAR-Immunotherapy Trials



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]

Cancer Regression and Neurological Toxicity Following Anti-MAGE-A3 TCR Gene Therapy

Richard A. Morgan, Nachimuthu Chinnasamy,* Daniel Abate-Daga,* Alena Gros,* Paul F. Robbins,* Zhili Zheng,* Mark E. Dudley,* Steven A. Feldman,* James C. Yang,* Richard M. Sherry,* Giao Q. Phan,* Marybeth S. Hughes,* Udai S. Kammula,* Akemi D. Miller,* Crystal J. Hessman,* Ashley A. Stewart,* Nicholas P. Restifo,* Martha M. Quezado,† Meghna Alimchandani,† Avi Z. Rosenberg,† Avindra Nath,‡ Tongguang Wang,‡ Bibiana Bielekova,‡ Simone C. Wuest,‡ Nirmala Akula,§ Francis J. McMahon,§ Susanne Wilde,‖ Barbara Mosetter,‖ Dolores J. Schendel,‖¶ Carolyn M. Laurencot,* and Steven A. Rosenberg**

Case Report of a Fatal Serious Adverse Event Upon Administration of T Cells Transduced With a MART-1-specific T-cell Receptor

Joost H van den Berg[1,2], Raquel Gomez-Eerland[1], Bart van de Wiel[3], Lenie Hulshoff[4], Daan van den Broek[5], Adriaan Bins[6], Hanno L Tan[7], Jane V Harper[8], Namir J Hassan[8], Bent K Jakobsen[8], Annelies Jorritsma[1], Christian U Blank[1,6], Ton NM Schumacher[1] and John BAG Haanen[1,6]

U Penn CAR-T: Functional Characterization Cytokine/Killing

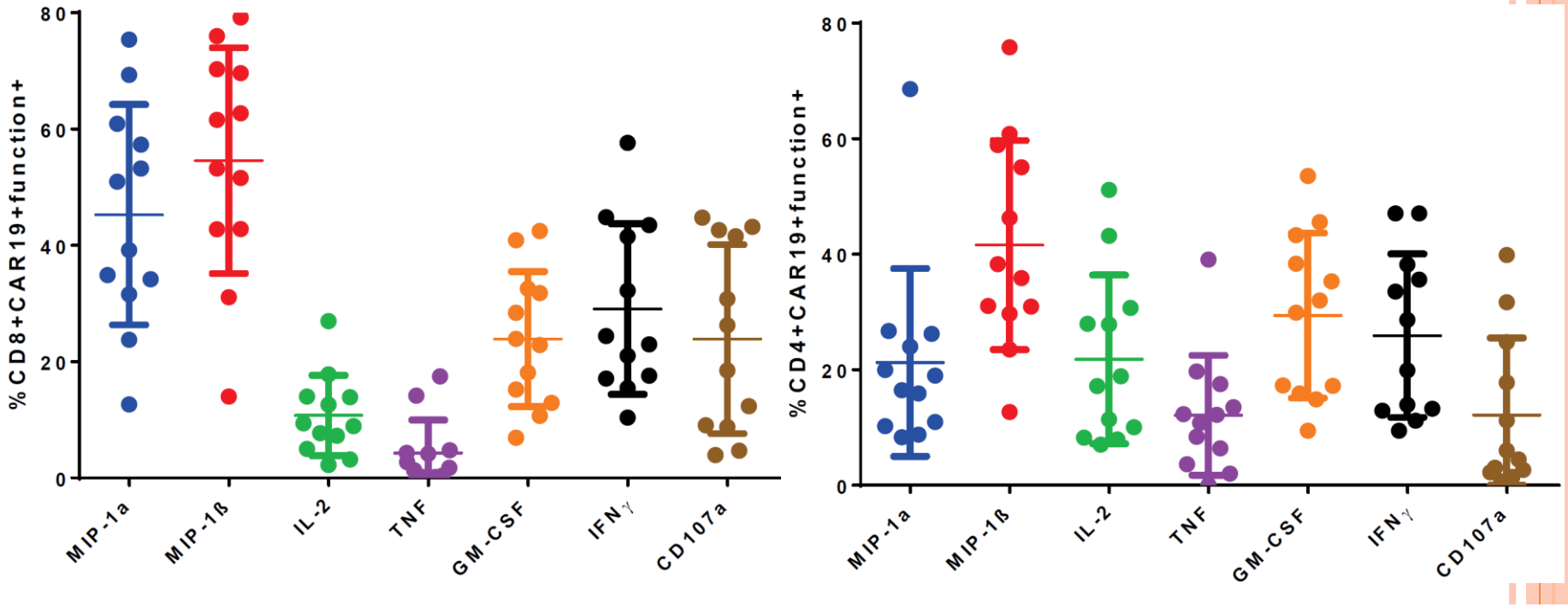


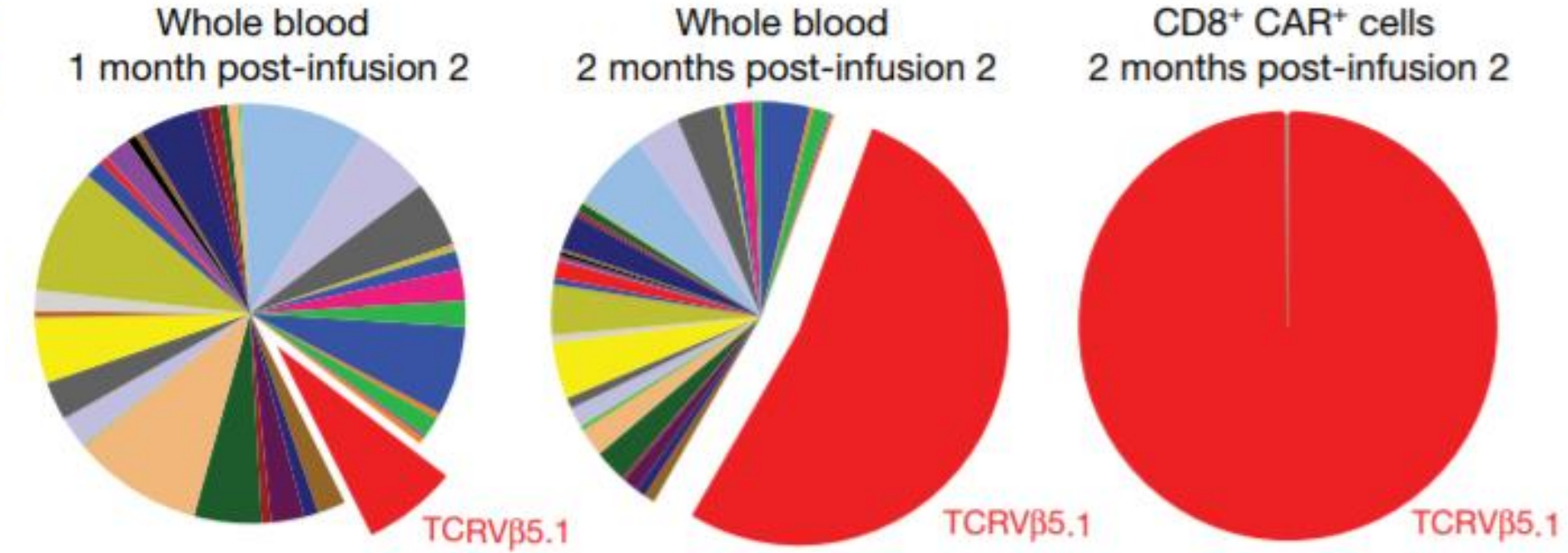
Figure S2: CTL019 cells are polyfunctional.
Manufactured CTL019 cells from 12 subjects (cells were not available for subjects 02 and 03) were stimulated for 6 hours with CD19-expressing target cells in the presence of cytokine secretion blockers plus fluorescently conjugated anti-CD107a antibody after which the cells were harvested, stained, and analyzed for intracellular cytokine expression. Shown here are the frequencies of CD4+ (**A**) or CD8+ (**B**) CAR19+ T cells expressing the indicated cytokines or degranulating upon stimulation.

LETTER

https://doi.org/10.1038/s41586-018-0178-z

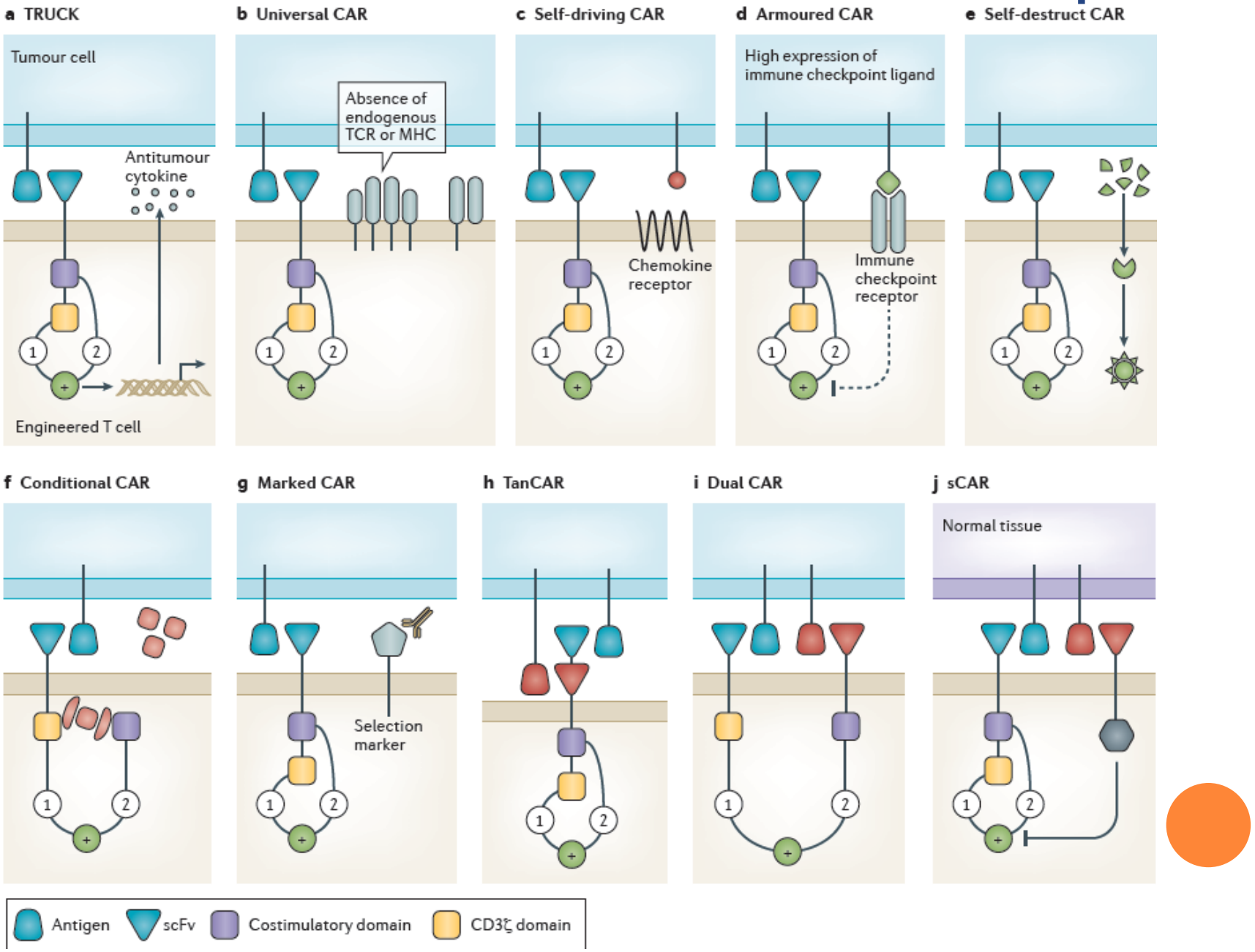
Disruption of *TET2* promotes the therapeutic efficacy of CD19–targeted T cells

Joseph A. Fraietta[1,2,3,4], Christopher L. Nobles[5], Morgan A. Sammons[6,10], Stefan Lundh[1,2], Shannon A. Carty[2,11], Tyler J. Reich[1,2], Alexandria P. Cogdill[1,2], Jennifer J. D. Morrissette[3], Jamie E. DeNizio[7,8], Shantan Reddy[5], Young Hwang[5], Mercy Gohil[1,2], Irina Kulikovskaya[1,2], Farzana Nazimuddin[1,2], Minnal Gupta[1,2], Fang Chen[1,2], John K. Everett[5], Katherine A. Alexander[6],



29 Population Doublings From a Single Clone

New CAR Models And Concepts



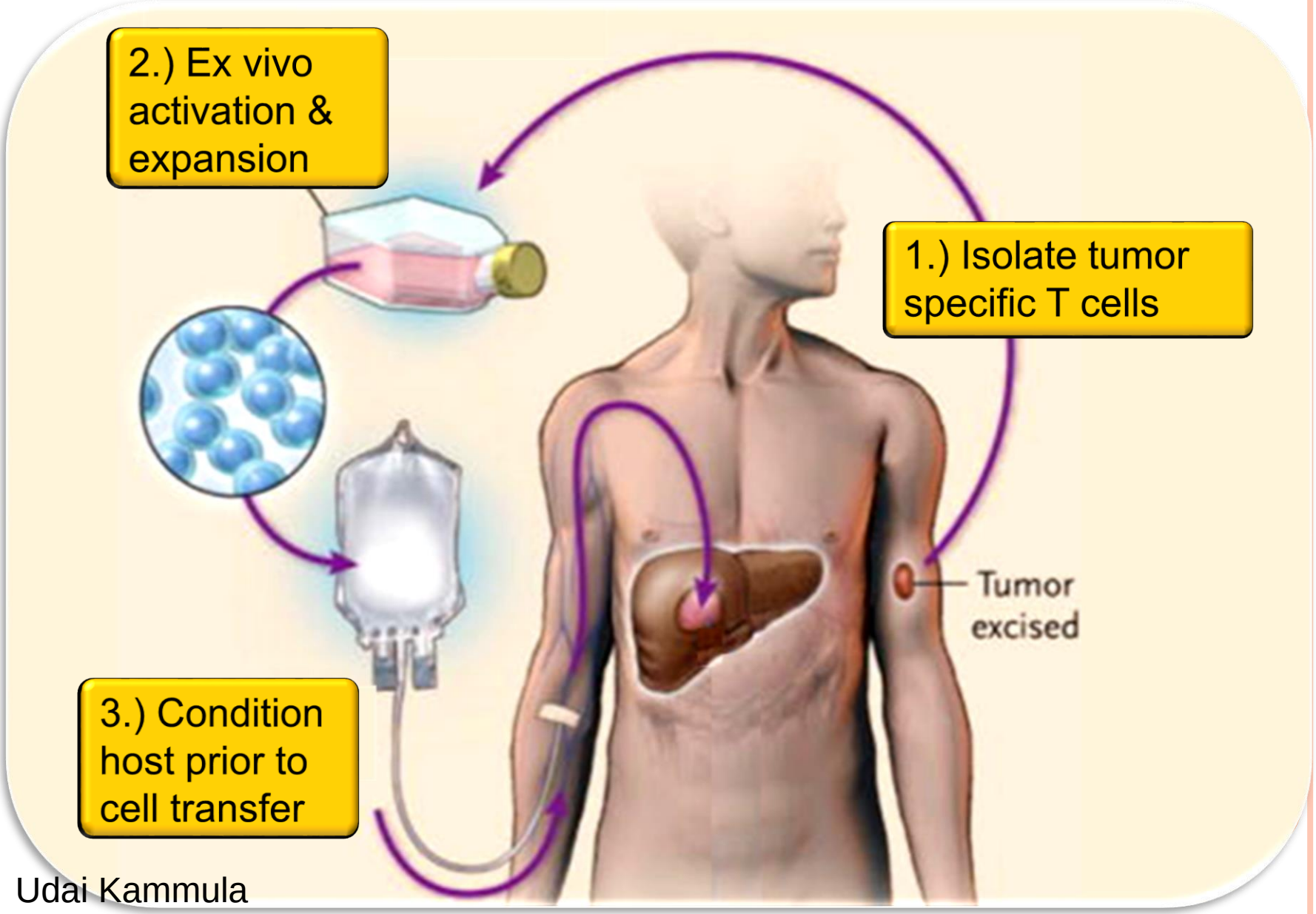
Effective Immunotherapies for Metastatic Cutaneous Melanoma (CM)

Proliferation: Cytokines

Activate (release inhibition): Checkpoint Blockade

Adoptive transfer: Autologous TIL

Udai Kammula



Udai Kammula

Why Lymphodeplete Prior to Cell Transfer?

1. Eliminate suppressive cell populations (i.e. Tregs, MDSC, inhibitory macrophages)

2. Provide “space” for homeostatic expansion

3. Provide exposure to homeostatic cytokines (i.e. IL-15 and IL-7)

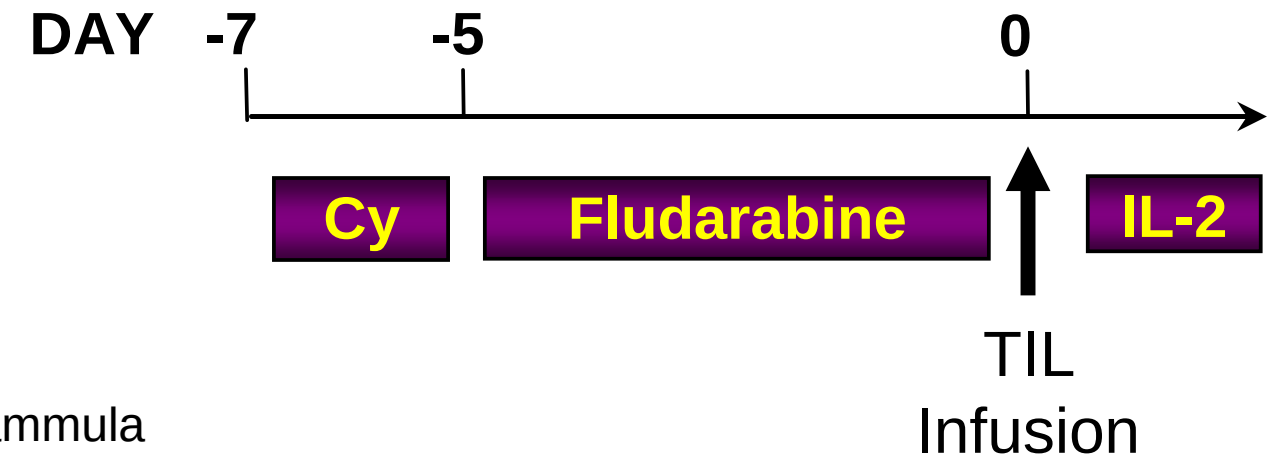
Surgery Branch/NIH
Adoptive TIL Transfer Therapy

- Non-myeloablative (NMA) lymphocyte depleting preparative regimen:

  Cyclophosphamide (60 mg/kg/day X 2 days IV)
  Fludarabine (25 mg/m2/day IV X 5 days)

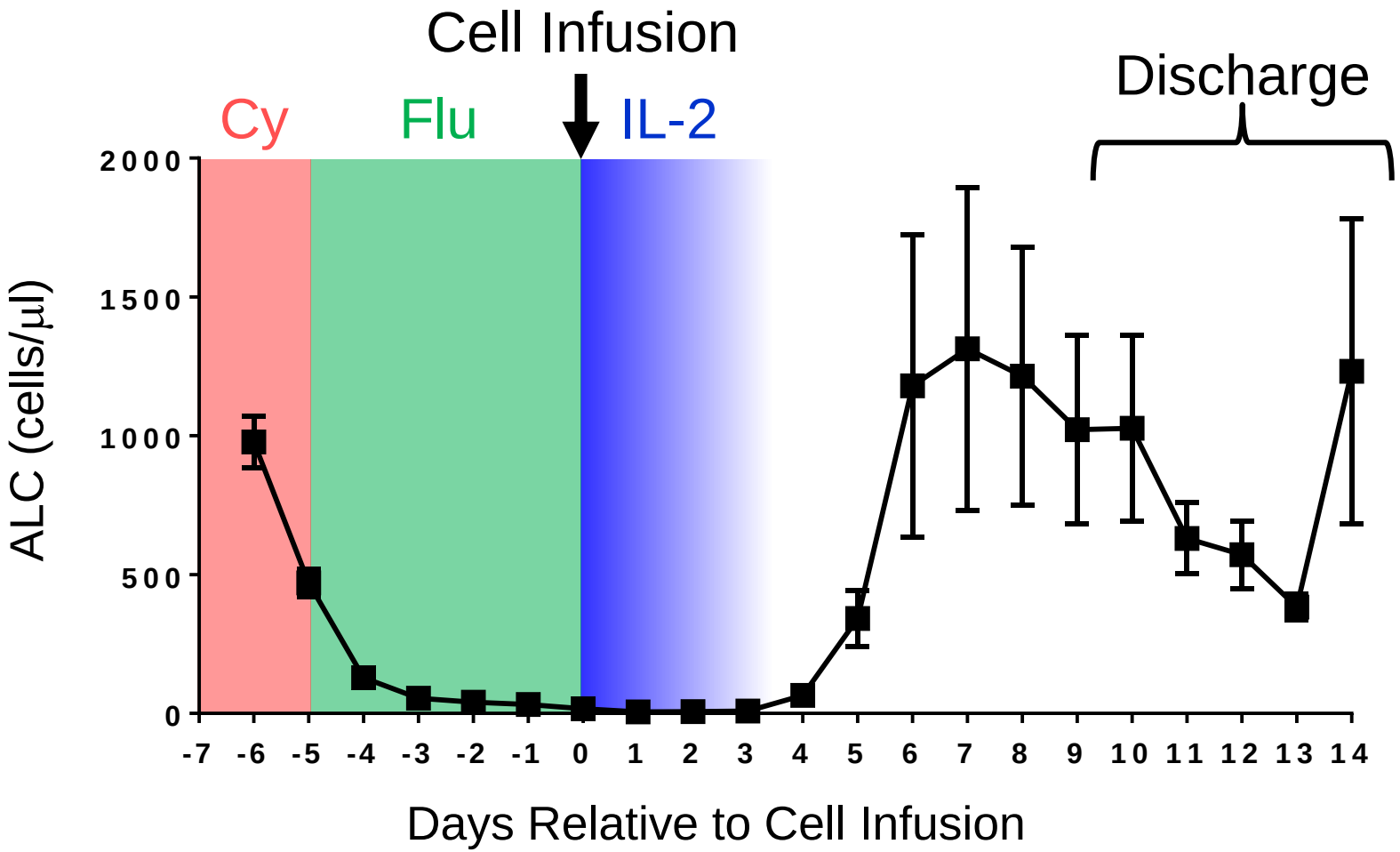
- Intravenous infusion of TIL

- High-dose intravenous (IV) IL-2



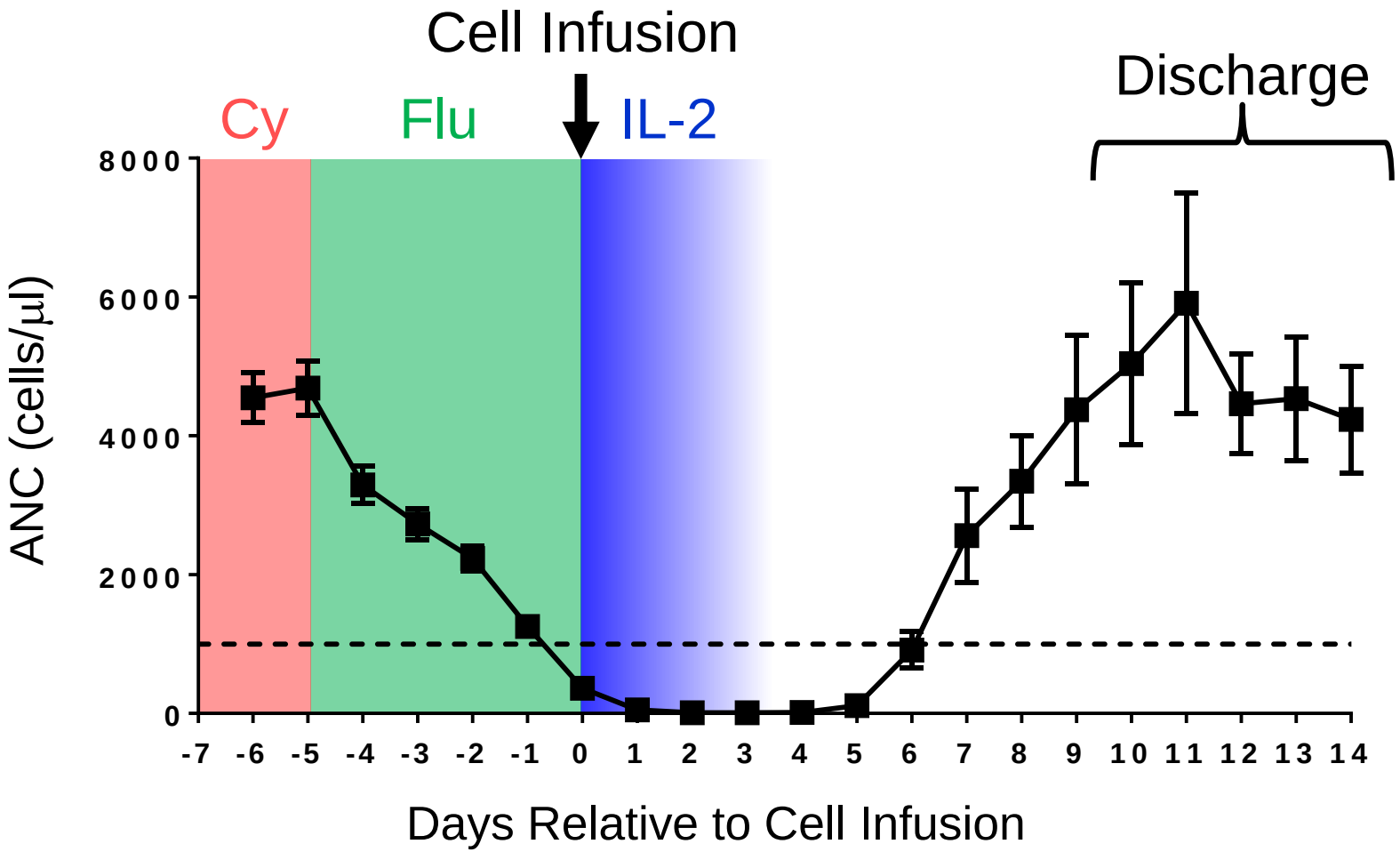
Kinetics of Lymphopenia During TIL Therapy

(n=40 metastatic melanoma patients)



Kinetics of Neutropenia During TIL Therapy

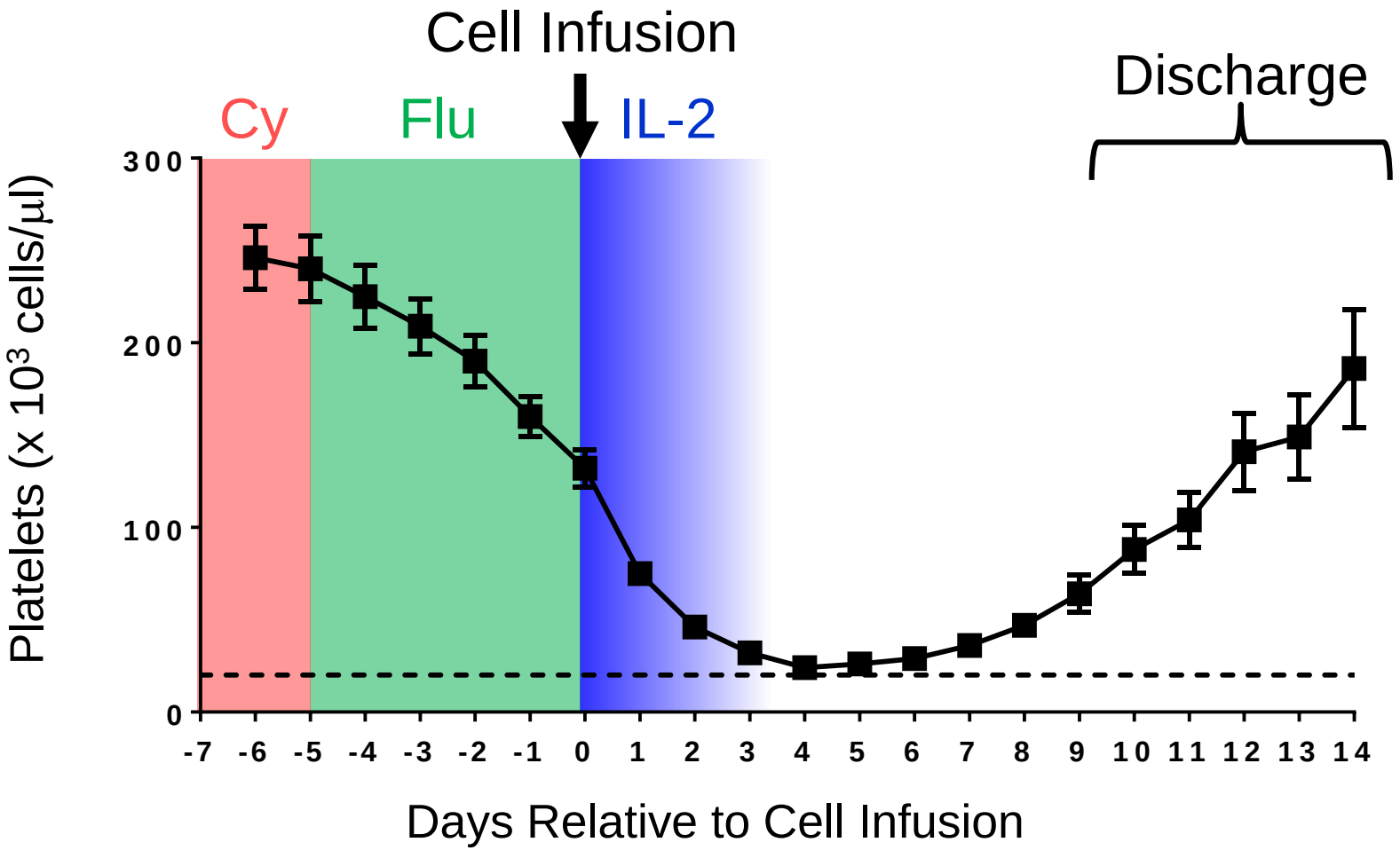
(n=40 metastatic melanoma patients)



Udai Kammula

Kinetics of Thrombocytopenia During TIL Therapy

(n=40 metastatic melanoma patients)



Common TIL Adverse Events (Grade >3)

Event	n	%
Lymphopenia	21	100
Neutropenia	21	100
Thrombocytopenia	21	100
Anemia	14	67
Infection	6	29
Treatment related death	1	5

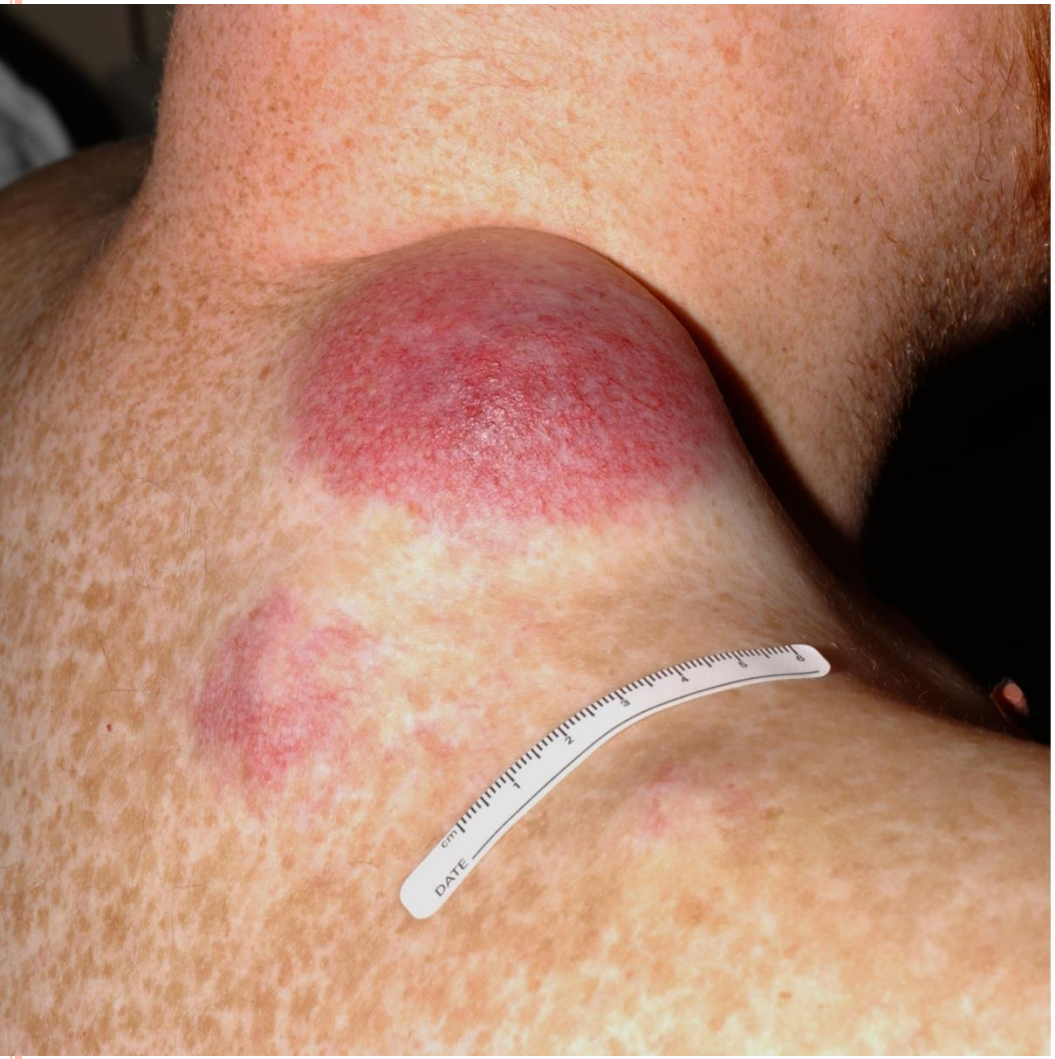
Nearly all AEs were chemotherapy related
No significant autoimmune related adverse events

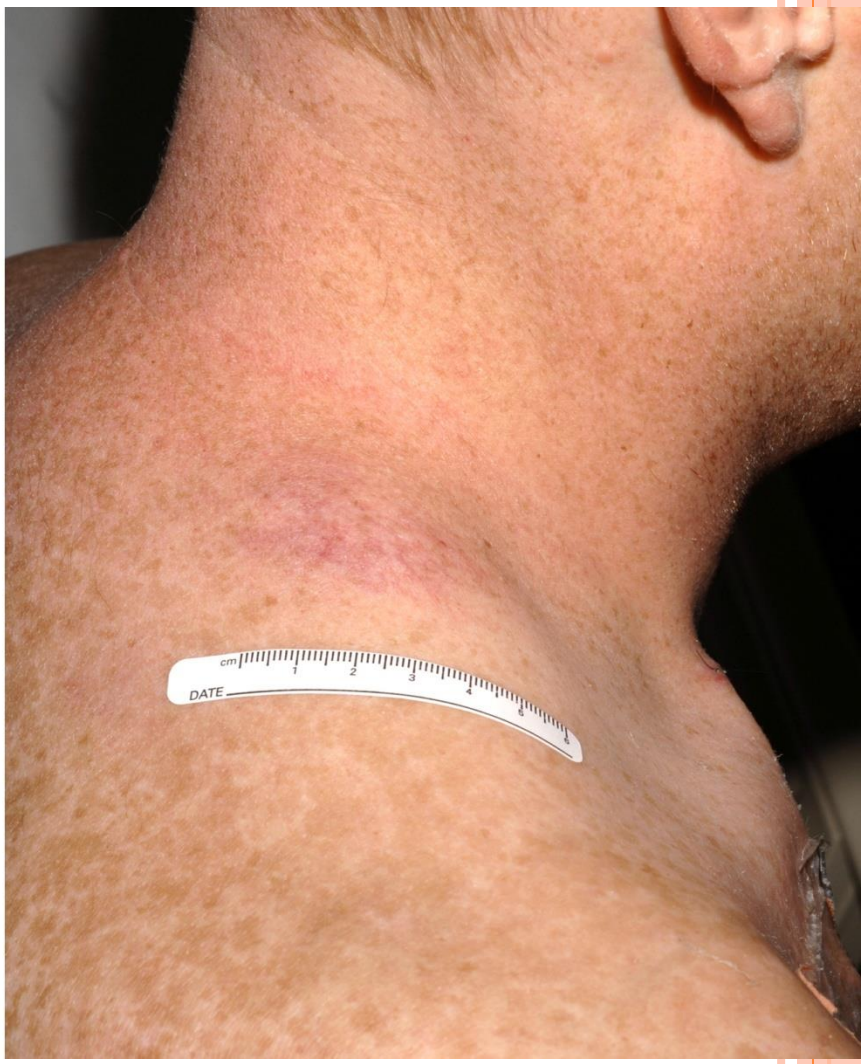
Lancet Oncol. 2017 Jun;18(6):792-802

Rapid Tumor Response after TIL Transfer Therapy: Cutaneous Melanoma

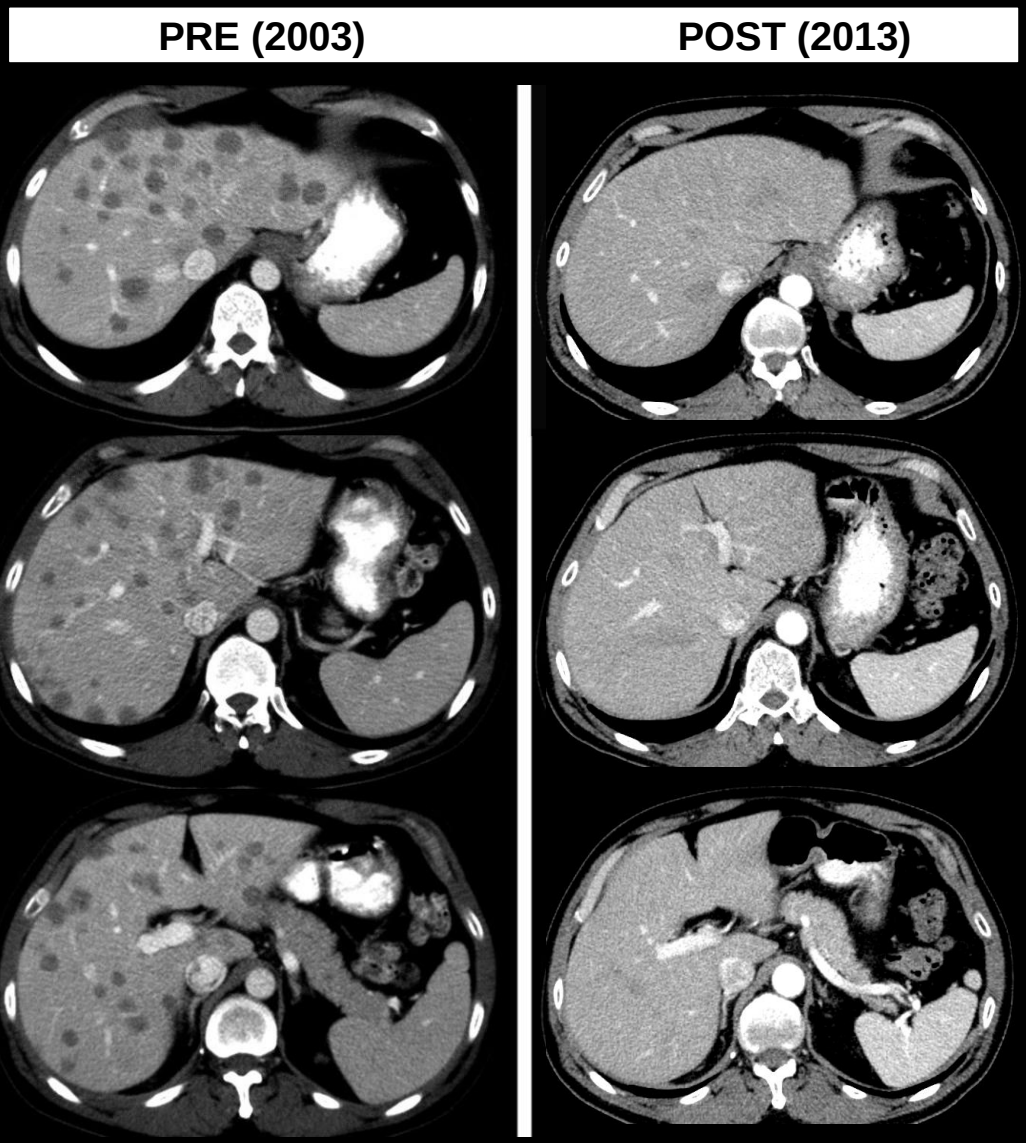
Pre

12 days

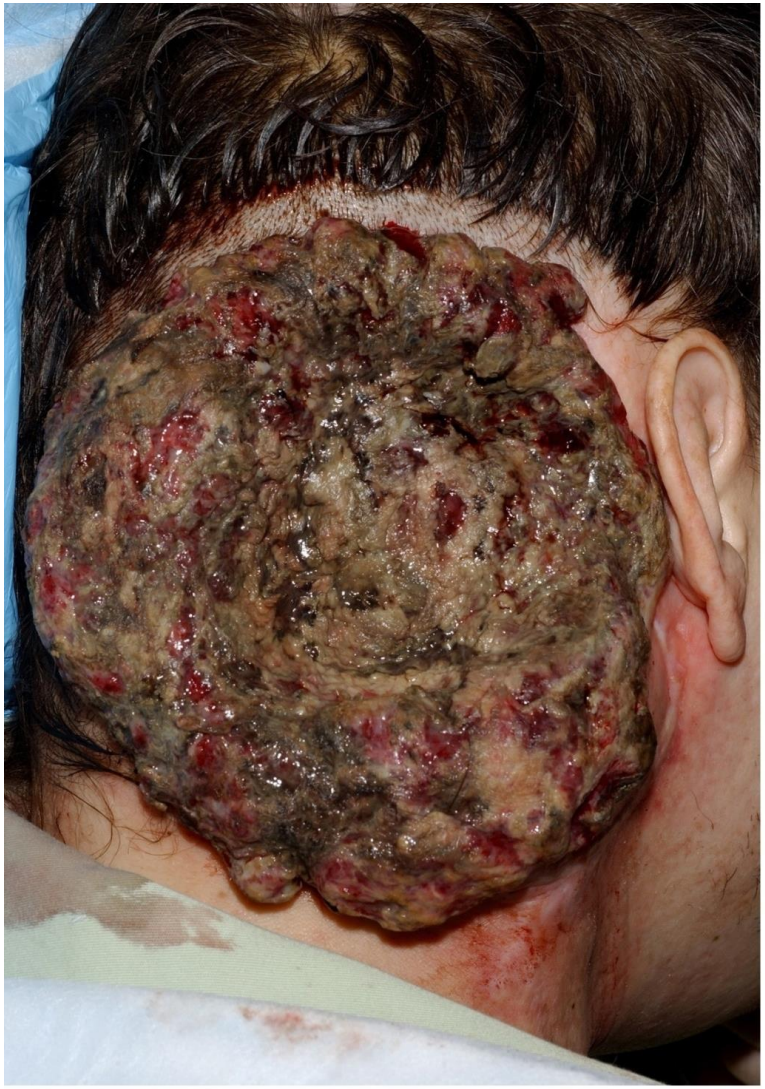




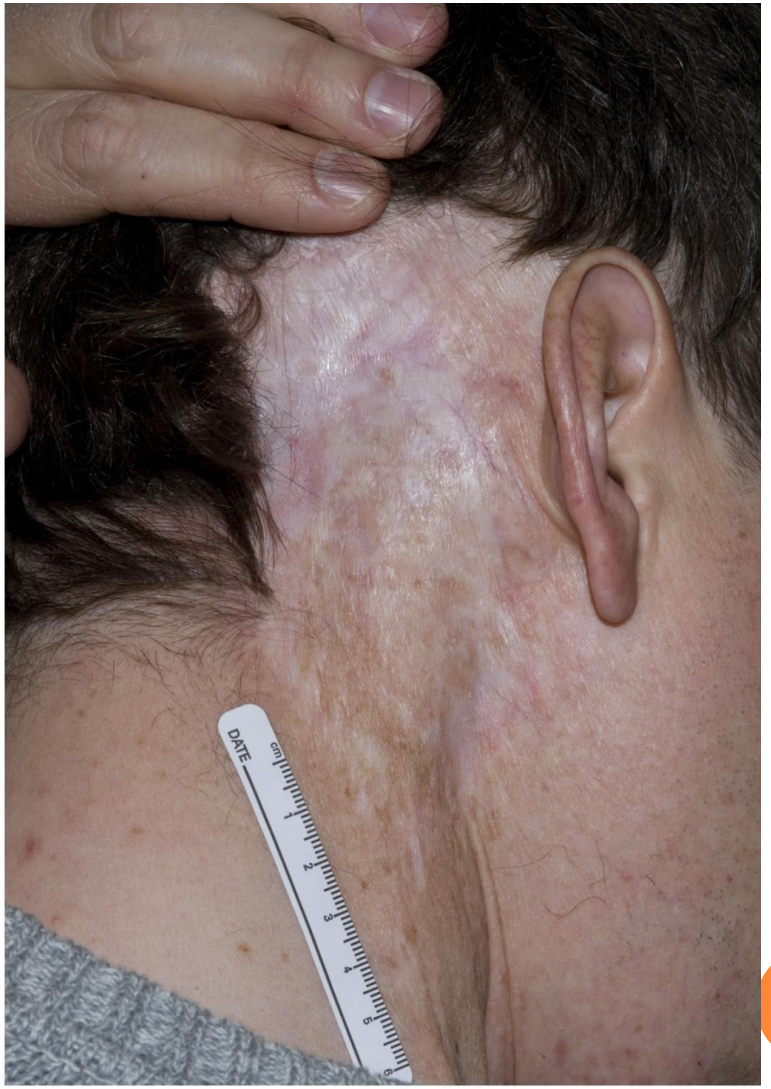
Complete and Durable Tumor Response after TIL Transfer Therapy: Cutaneous Melanoma



Specific Tumor Response after TIL Transfer Therapy: Cutaneous Melanoma

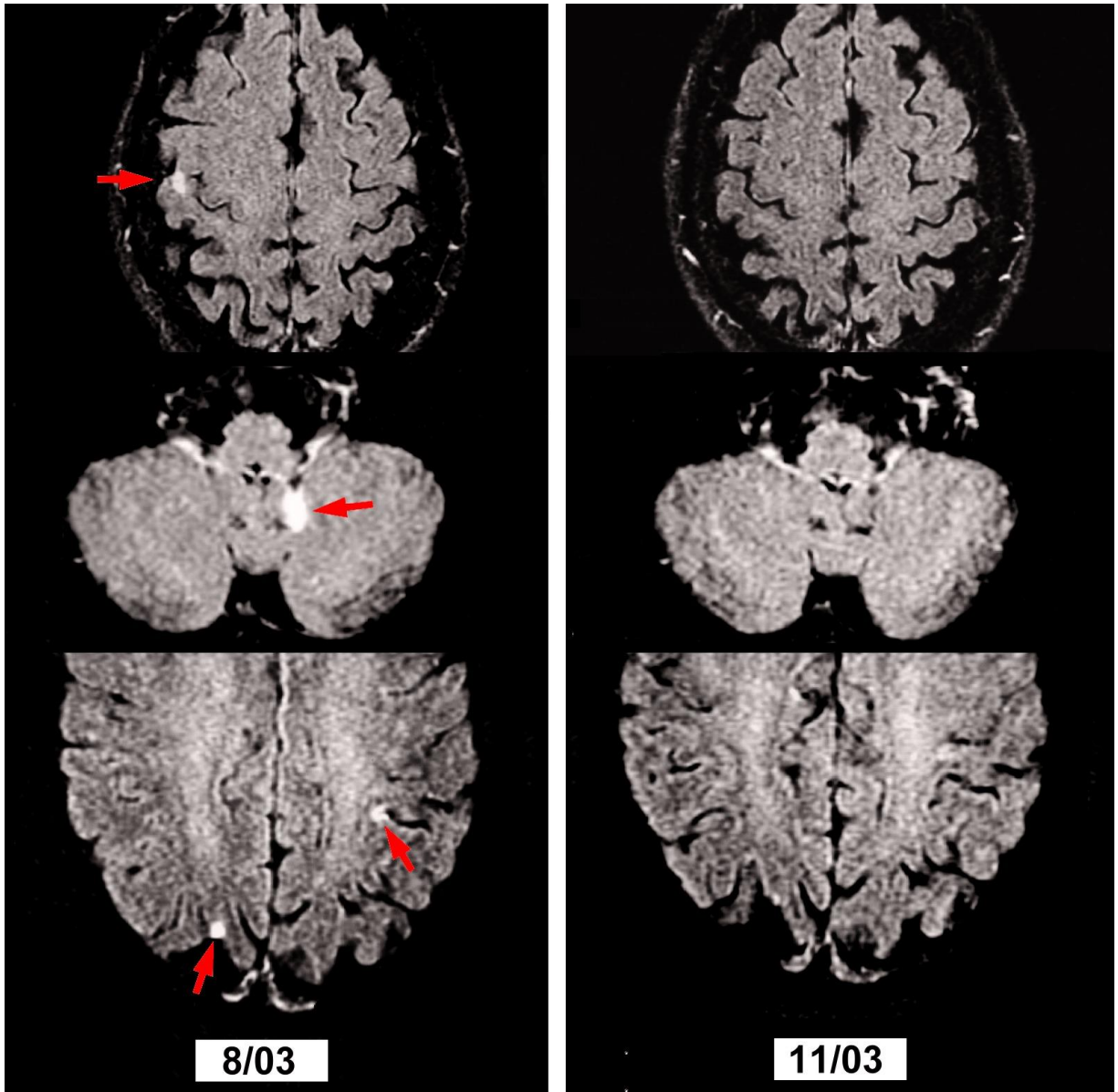


March 21, 2005



Current

Brain Metastasis Response after TIL Transfer Therapy: Cutaneous Melanoma

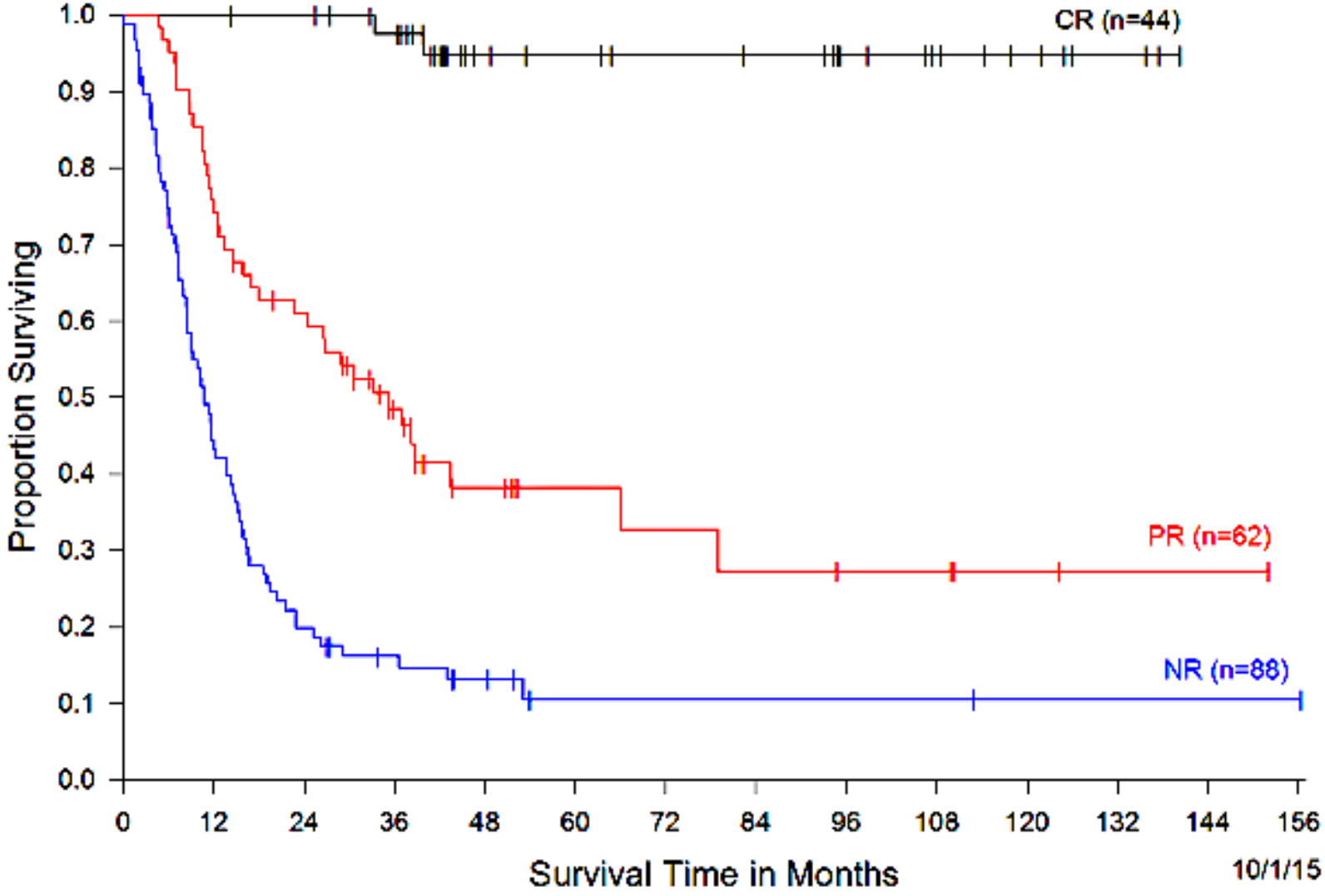


Adoptive TIL Transfer Therapy for Metastatic Cutaneous Melanoma: Surgery Branch/NIH

n	PR (%)	CR (%)	ORR (%)
194	**62 (32%)**	**44 (23%)**	**106 (55%)**

- *J Clin Oncol.* 2005 Apr 1;23(10):2346-57
- *J Clin Oncol.* 2008 Nov 10;26(32):5233-9
- *J Clin Oncol.* 2016 Jul 10;34(20):2389-97

Survival of Metastatic Melanoma Patients After TIL Therapy



TILs RECOGNIZE MUTATED NEOANTIGENS

Mutated antigens recognized by T cells from patients with epithelial cancers

Patient	Histology	Antigen	HLA RE
3737	cholangio.	ERBB2IP	DQβ1*06:01
3978	cholangio.	ITGB4	Unknown class II
3569	colon	CSMD2	Unknown class I
3971	colon	CASP8	Unknown class I
3971	colon	MARK1	B*08:01
3971	colon	XYLT1	Unknown class I
3971	colon	HIST1H3B	A*02:01
3995	colon	KRAS	C*08:02
3995	colon	TUBGCP2	Unknown class I
3995	colon	RNF213	Unknown class I
4007	colon	SKIV2L	A*03:01
4007	colon	H3F3B	Unknown class I
4032	colon	API5	Unknown class I
4032	colon	RNF10	Unknown class I
4032	colon	PHLPP1	Unknown class I
4060	colon	SMC2A	Unknown class II
4071	colon	QSOX2	Unknown class I
4071	colon	MRPS28	Unknown class I
4071	colon	POR	Unknown class I
4077	colon	CPSF6	Unknown class I
4077	colon	HIST1H2BE	Unknown class I
4077	colon	FLII	A*02:01
4090	colon	USP8	Unknown class I
4090	colon	MRPL39	Unknown class II
4095	colon	KRAS	C*08:02
3942	rectal	NUP98	Unknown class I
3942	rectal	KARS	Unknown class I
3942	rectal	GPD2	Unknown class II
4081	rectal	ALDOC	Unknown class I
4081	rectal	RPL12	Unknown class I

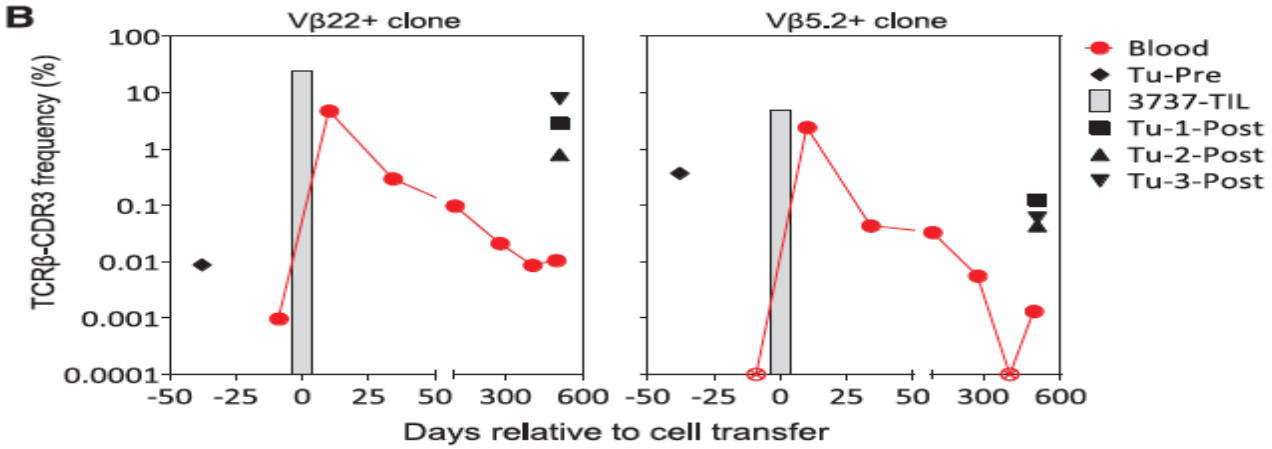
Patient	Histology	Antigen	HLA RE
4069	pancreatic	ZFYVE27	Unknown class I
3948	esophageal	PLEC	Unknown class II
3948	esophageal	XPO7	Unknown class II
3948	esophageal	AKAP2	Unknown class II
4014	NSCLC	TGFBRAP1	Unknown class I
4014	NSCLC	USP11	B*57:01
4014	NSCLC	FDT1	Unknown class I
4014	NSCLC	HYAL2	Unknown class I
4014	NSCLC	ATRIP	Unknown class II
4014	NSCLC	CSNK2A1	Unknown class II
4037	NSCLC	NPM1	Unknown class I
4037	NSCLC	NPM1	Unknown class II
4037	NSCLC	ACOT7	Unknown class II
4037	NSCLC	RAD50	Unknown class II
4037	NSCLC	TNK1	Unknown class II
4037	NSCLC	KIAA1432	Unknown class II
4073	NSCLC	FAM83A	Unknown class I
4097	ovarian	HIST1H1B	Unknown class II
4097	ovarian	FLOT1	Unknown class II
4097	ovarian	DEF6	Unknown class II
4097	ovarian	GAK	Unknown class II
4097	ovarian	INPP5K	Unknown class II
4046	ovarian	USP9X	Unknown class I
4062	breast	RPBJ	Unknown class II
3775	cervical	SETDB1	Unknown class I
3775	cervical	METTL17	Unknown class I
3775	cervical	ALDH1A1	Unknown class I

Rosenberg, SITC 2015

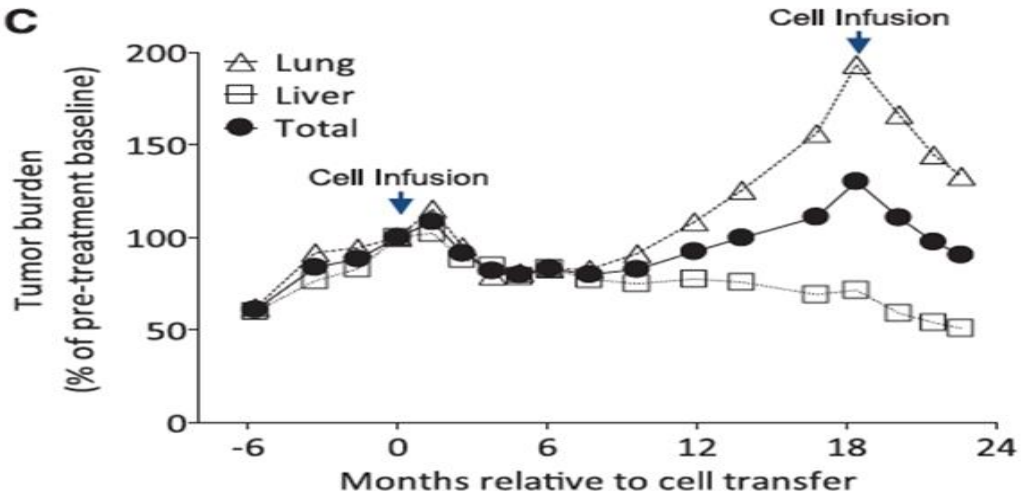
57 somatic mutations from 22 patients with epithelial cancers recognized by autologous TIL.
All were unique (except one).

Identification of TILs Recognizing Neoepitopes

Level of ERBB2IP mutation reactive T-cell clones in patient



Tumor regression observed after treatment with a highly pure population of Vβ22+ ERBB2IP mutation-reactive Th1 cells



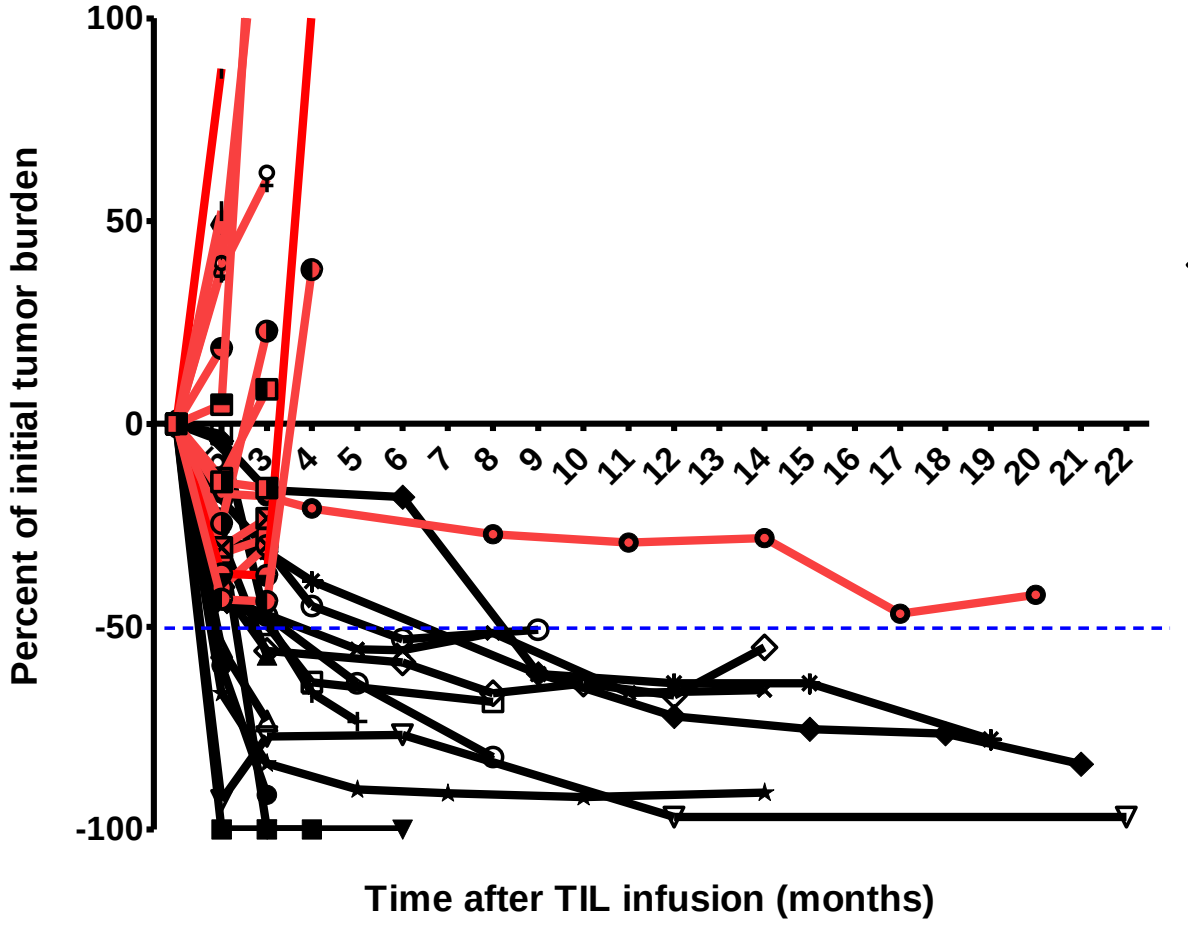
MELANOMA EFFICACY AT NON-NCI SITES

- Sheba in Israel: has harvested 80 patients
  - ORR 29% and median OS 9.8 months as harvested (29% rate of drop-outs due to PD)
  - All CRs still in remission
  - Rapid expansion and high CD8 T cell numbers were associated with benefit
- MD Anderson Cancer Center
  - 15 of 31 (48.4%) patients had an objective clinical response.
  - Higher # TIL, higher # of CD8+ TIL, and BTLA expressing TIL were associated with response

J. Weber, Miami 2016

TUMOR RESPONSES TO TIL: MELANOMA

Change in tumor burden over time after TIL infusion



Radvanyi et al., Clin. Cancer Res., 2012

PILOT TRIAL OF IPILIMUMAB AND TIL

- Treatment regimen:

- N = 12 (consented)
  - N = 11 (92%) successfully completed TIL regimen
  - N = 1 (8%) drop out due to progressive disease prior to TIL treatment
- 45% overall response:
  - CR = 9% (ongoing)
  - PR = 35% (ongoing)

S. Prabhakaran*, D.M. Woods, E.B. Royster, J.S. Zager, V.K. Sondak, S. Pilon-Thomas, A.A. Sarnaik, SSO 2015 Abstract # 50

OTHER TIL INVESTIGATORS

- In Nantes, France: Khammani, ….Dreno et al CII 2015
  - Treated 13 patients with TIL and intralesional adenoviral gamma interferon with a 38% ORR
- In Manchester, UK, Hawkins, R et al
- In Amsterdam, NL, Haanen, J et al
- In Copenhagen, Svane, I et al
  - Treated 6 pts with low dose IL-2 + TIL with 2 CR, 2 SD, 2 PD in Ellebaek, et al JTM 2012

CERVICAL CANCER AND TIL TREATMENT

- Patients with metastatic refractory or recurrent cervical cancer
  - N = 9
  - Widely metastatic disease
  - 8 out of 9 patients had prior radiotherapy and all patients had cisplatin
- Results:
  - Objective responses N = 3
    - CR: N = 2 (duration 15+ and 22+ months)
    - PR: N = 1 (duration 3 months)
    - No acute toxicities related to cell infusion
    - No autoimmune adverse events

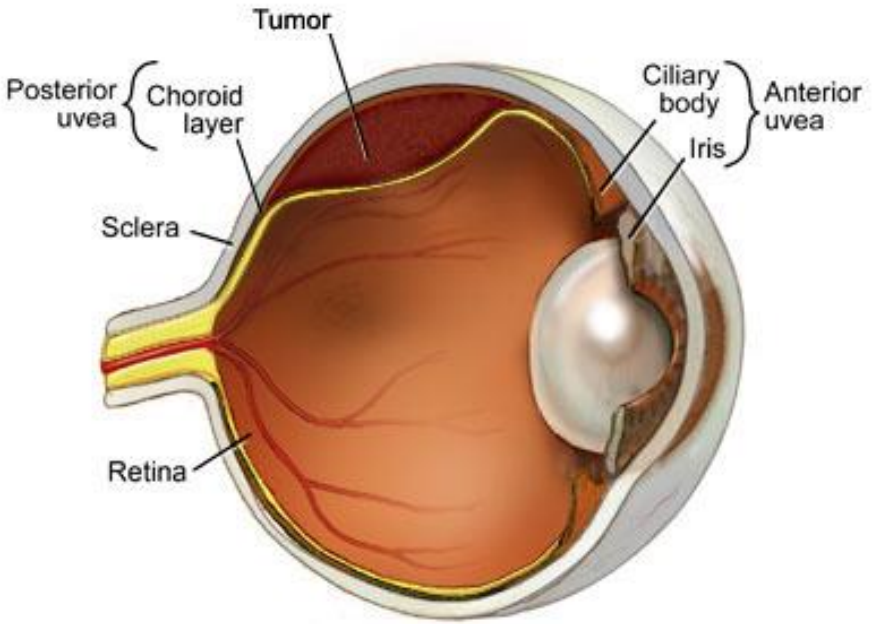
Christian S. Hinrichs et al. J Clin Oncol 2015, 33 (15)

Uveal Melanoma: A Model Cancer to Study Cellular Immunotherapy

Most frequent tumor of the eye. Arises from the **pigmented uveal tract** of the eye in 97% of the cases (choroid, ciliary body, iris) **"Uveal Melanoma (UM)"**

"Rare/orphan disease"

Annual incidence is 5.1 per million; ~1600 new cases/year in the U.S. (vs. 76,100 new CM cases)



Uveal Melanoma Natural History

~50% of patients diagnosed with primary UM will develop metastatic disease (vs. 4-9% of CM patients).

Predilection to metastasize to liver

The most common sites of UM metastases are:

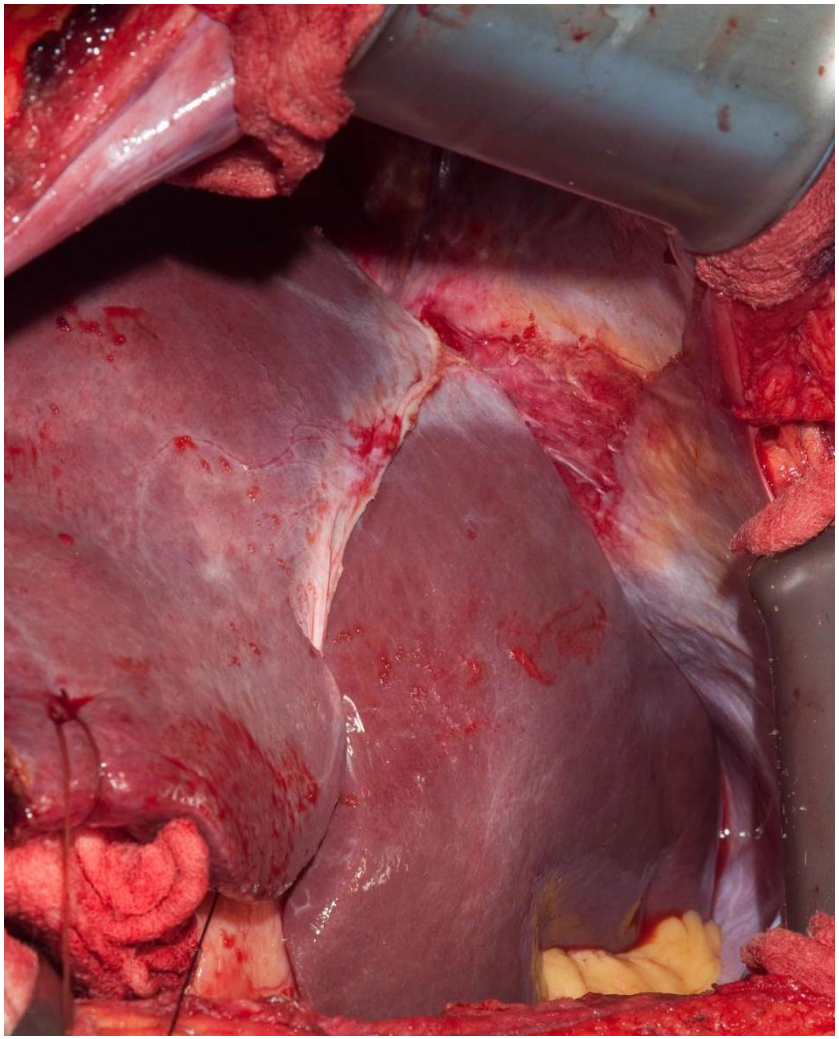
- **Liver (95%)**
- **Lungs (24%)**
- **Bone (16%)**
- **Soft tissue (11%)**

The median survival with liver metastases: 4-6 months

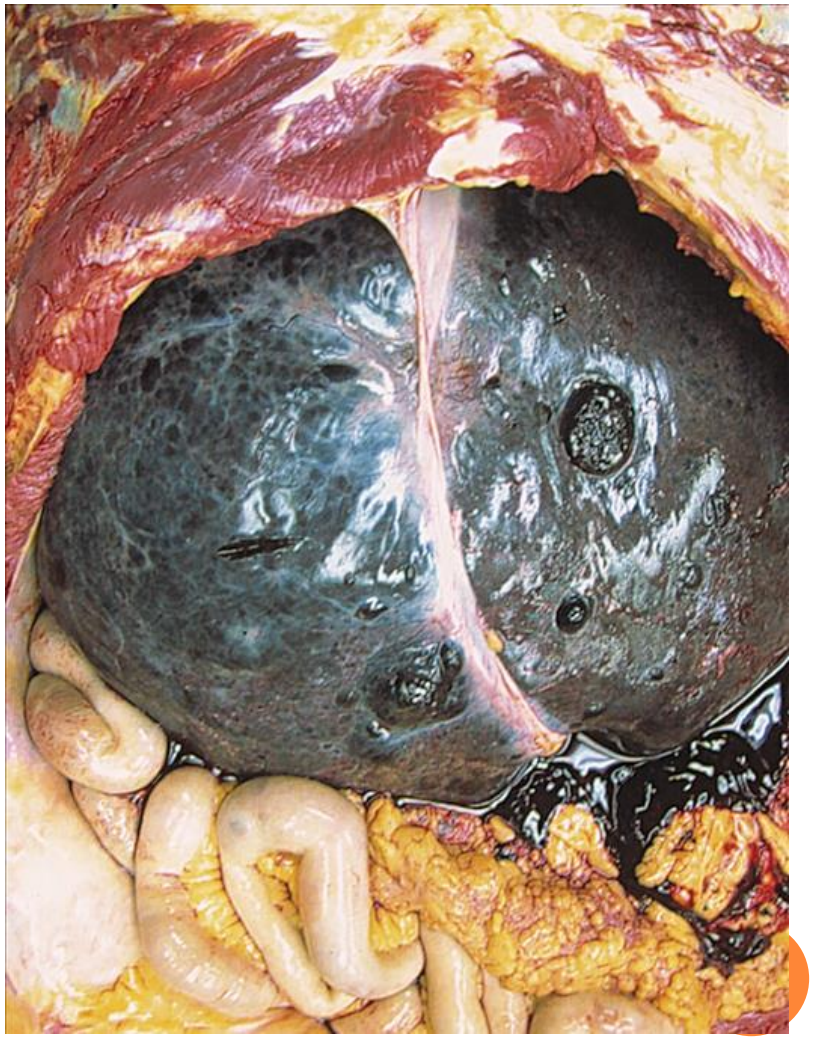
1-year survival: 10% to 15%

Uveal Melanoma Liver Metastases

Normal Liver



UM Liver Metastases



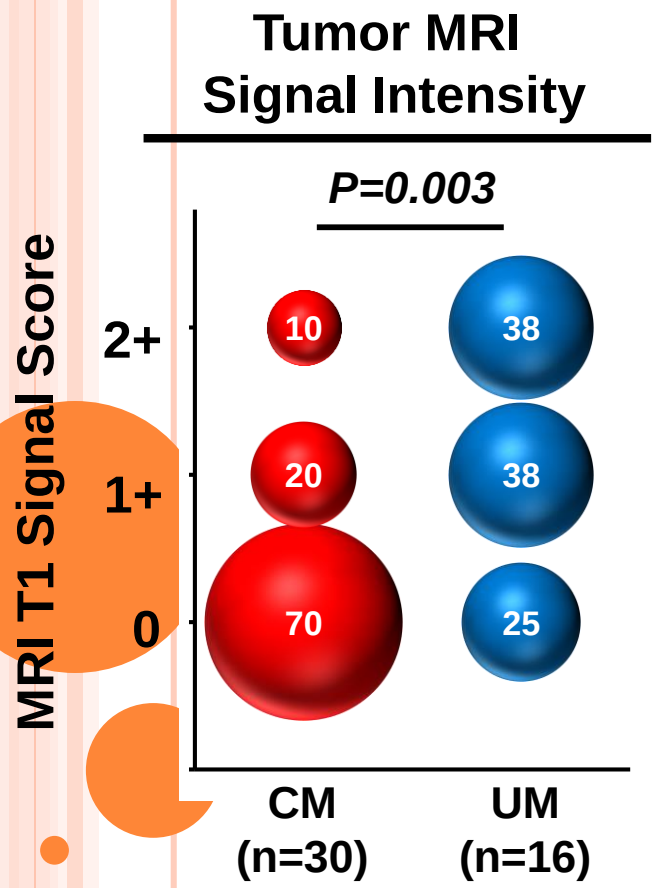
Uveal Melanoma Systemic Therapies

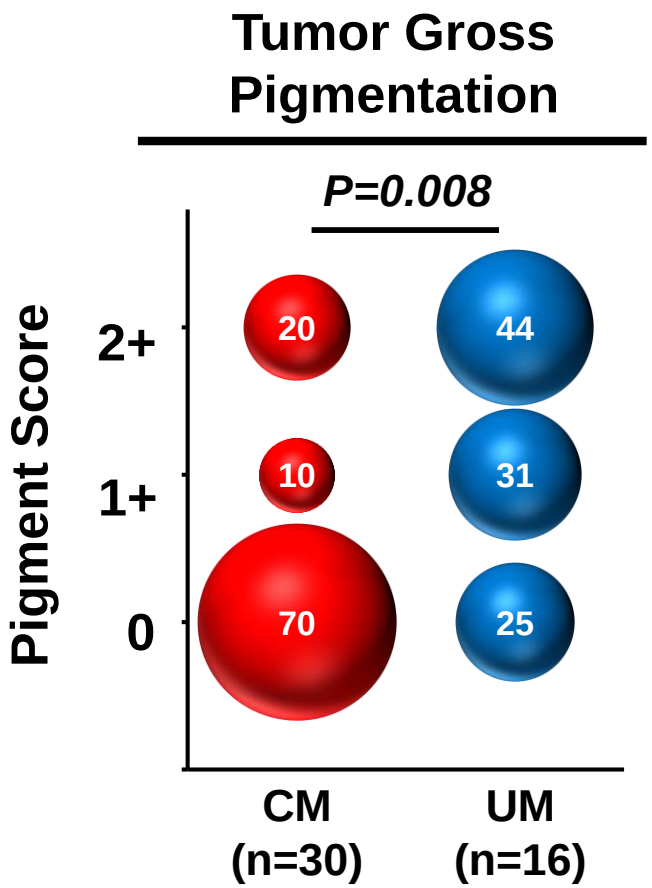
No “standard of care” systemic therapies

Checkpoint Blockade in Uveal Melanoma

Year	Author	Trial Type	Therapy	Criteria	*n*	PR	CR	ORR (%)
2011	Tarhini	Phase II	Tremelimumab 15mg/kg	RECIST	8	0	1	13
2012	Danielli	EAP	Ipilimumab 10mg/kg	mod WHO	9	0	0	0
2013	Luke	EAP	Ipilimumab 3 or 10mg/kg	irRC and mod WHO	35	1	1	6
2013	Kelderman	EAP	Ipilimumab 3mg/kg	RECIST and irRC	14	1	0	7
2013	Khattak	EAP	Ipilimumab 3mg/kg	RECIST	5	0	0	0
2013	Maio	EAP	Ipilimumab 3mg/kg	irRC	82	4	0	5
2015	Joshua	Phase II	Tremelimumab 15mg/kg	RECIST	11	0	0	0
2015	Zimmer	Phase II	Ipilimumab 3mg/kg	RECIST	34	0	0	0
2014	Herbst	NA	MPDL-3280A (Anti-PD-L1)	RECIST	4	0	0	0
2016	Kottschade	EAP	Pembrolizumab 2mg/kg	irRC	8	2	1	38
2016	Karydis	EAP	Pembrolizumab 2mg/kg	RECIST and irRC	25	2	0	8
2016	Algazi	NA	Anti-PD-1 or Anti-PD-L1 (various doses)	RECIST	56	2	0	4
	TOTAL				291	12	3	5%

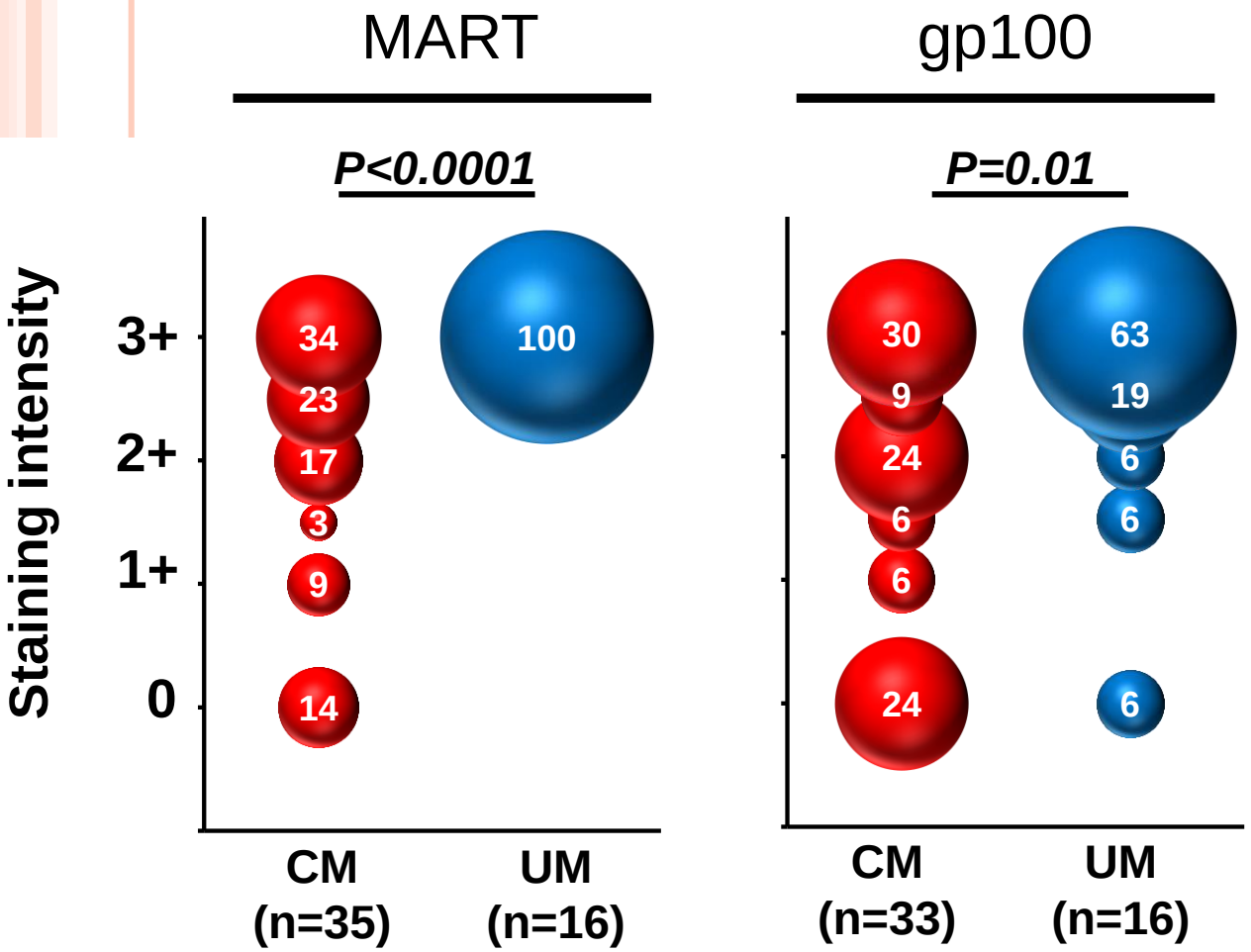
UM vs. CM Liver Metastases: Melanin Content





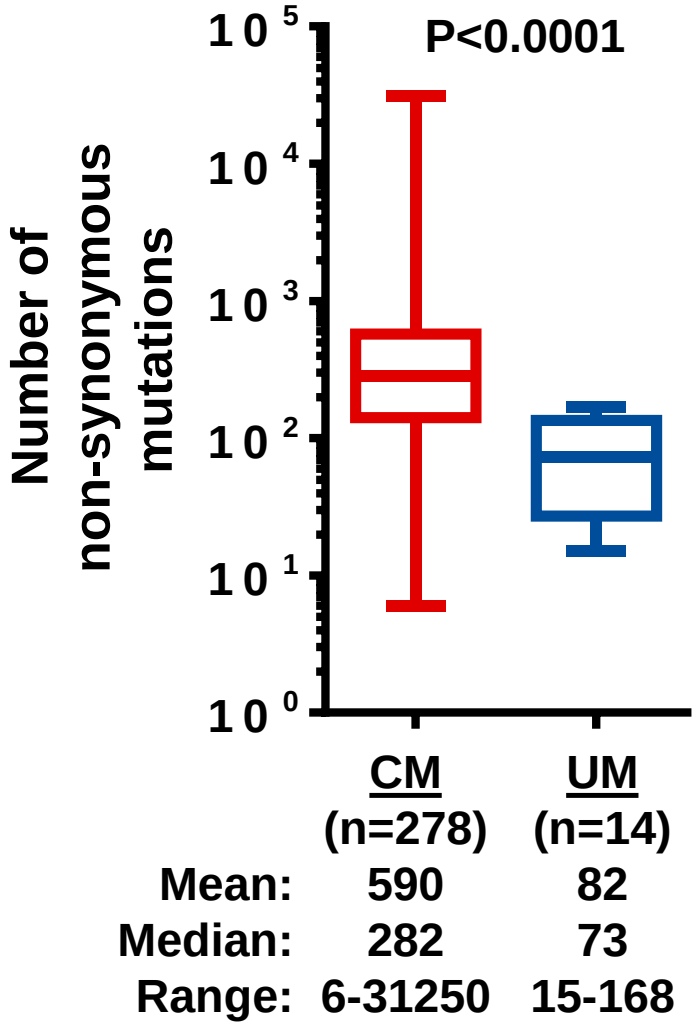
UM liver metastases are more pigmented

UM vs. CM Liver Metastases: MDA Expression



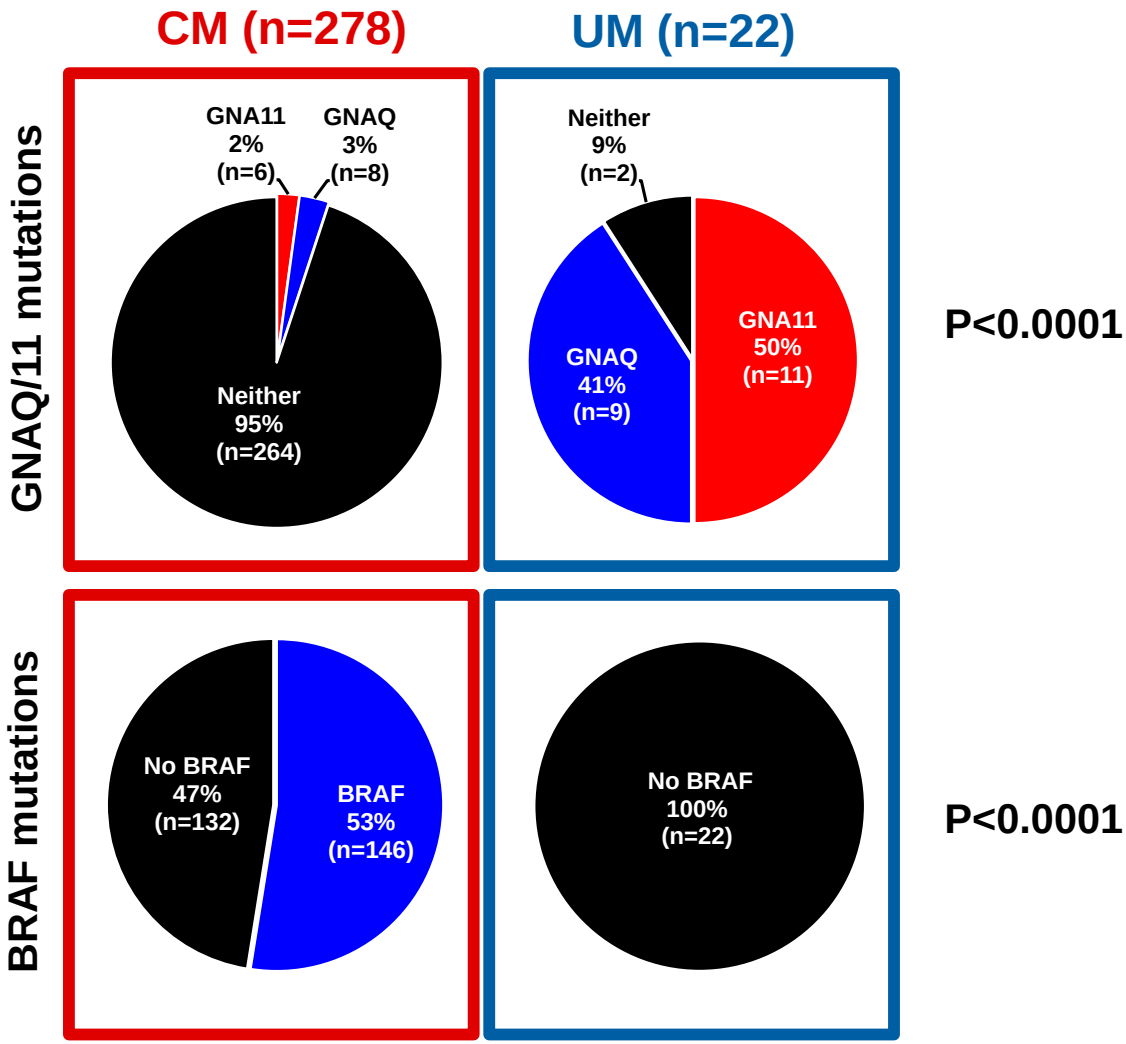
UM liver metastases have greater MDA expression

UM Metastases Have Fewer Somatic Mutations Compared to CM Metastases



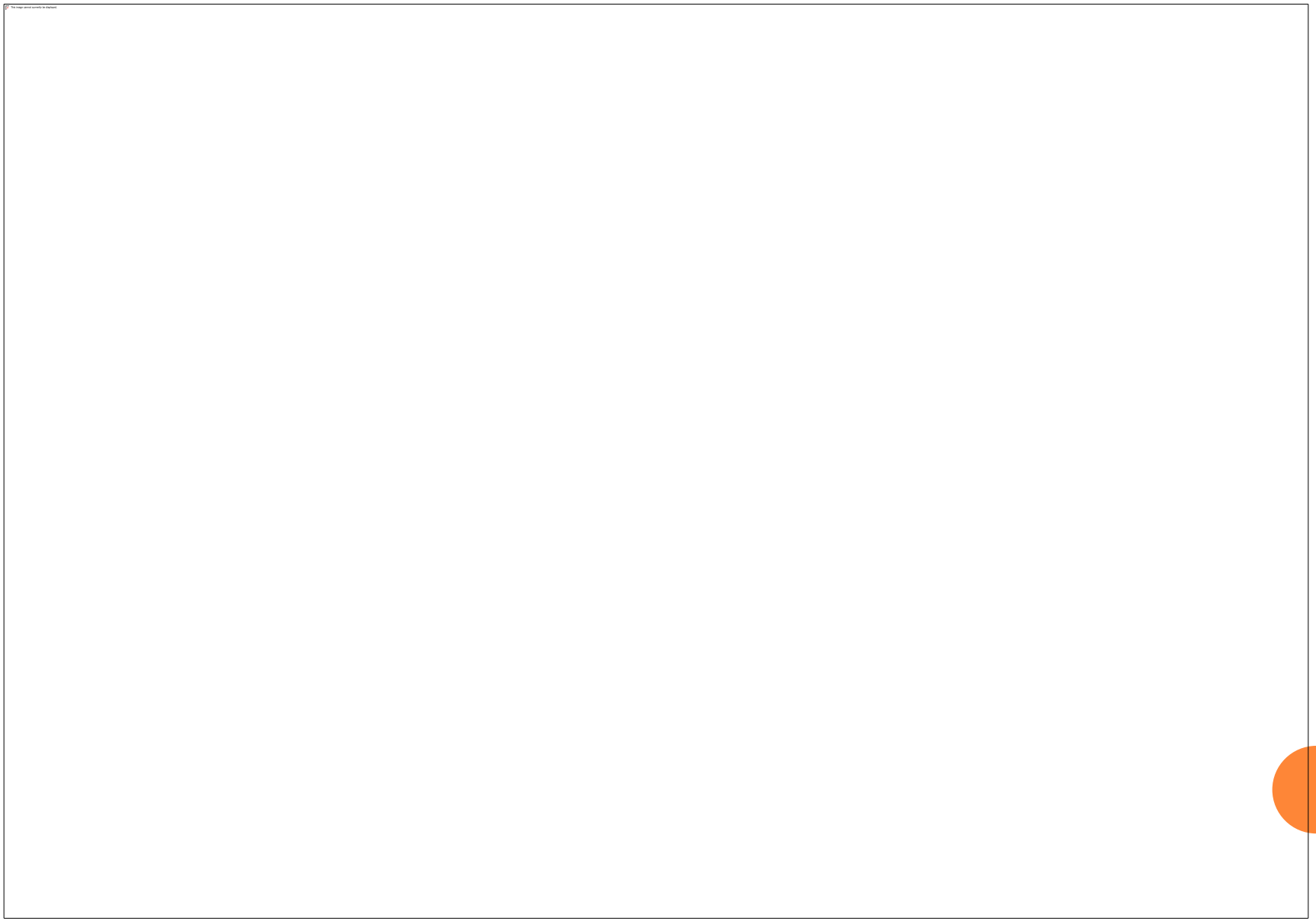
Rothermel et al., Clinical Cancer Research 2015

UM vs. CM Liver Metastases: Driver Mutations

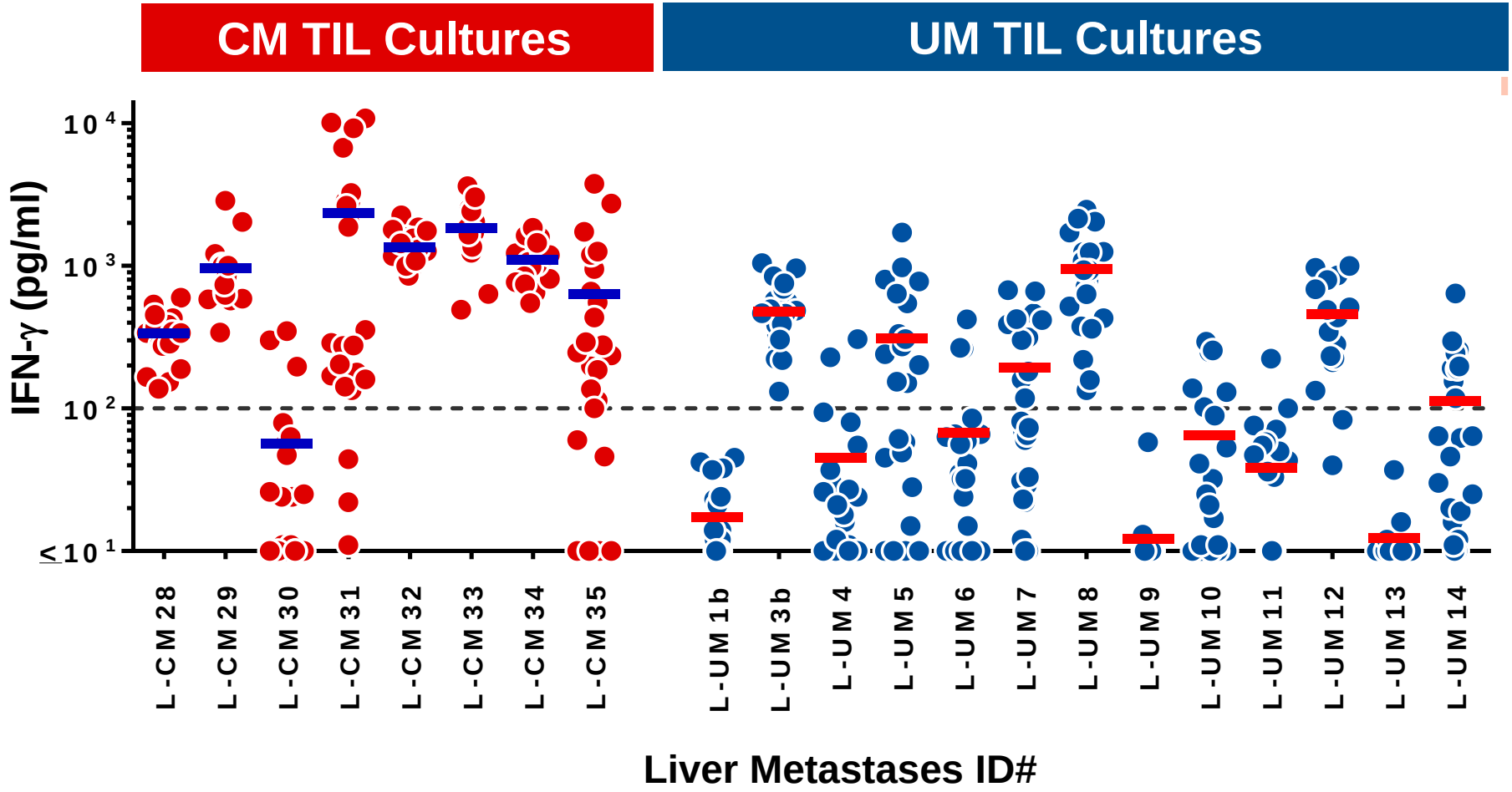


Rothermel et al., Clinical Cancer Research 2015

UM vs. CM Liver Metastases: Immunophenotype

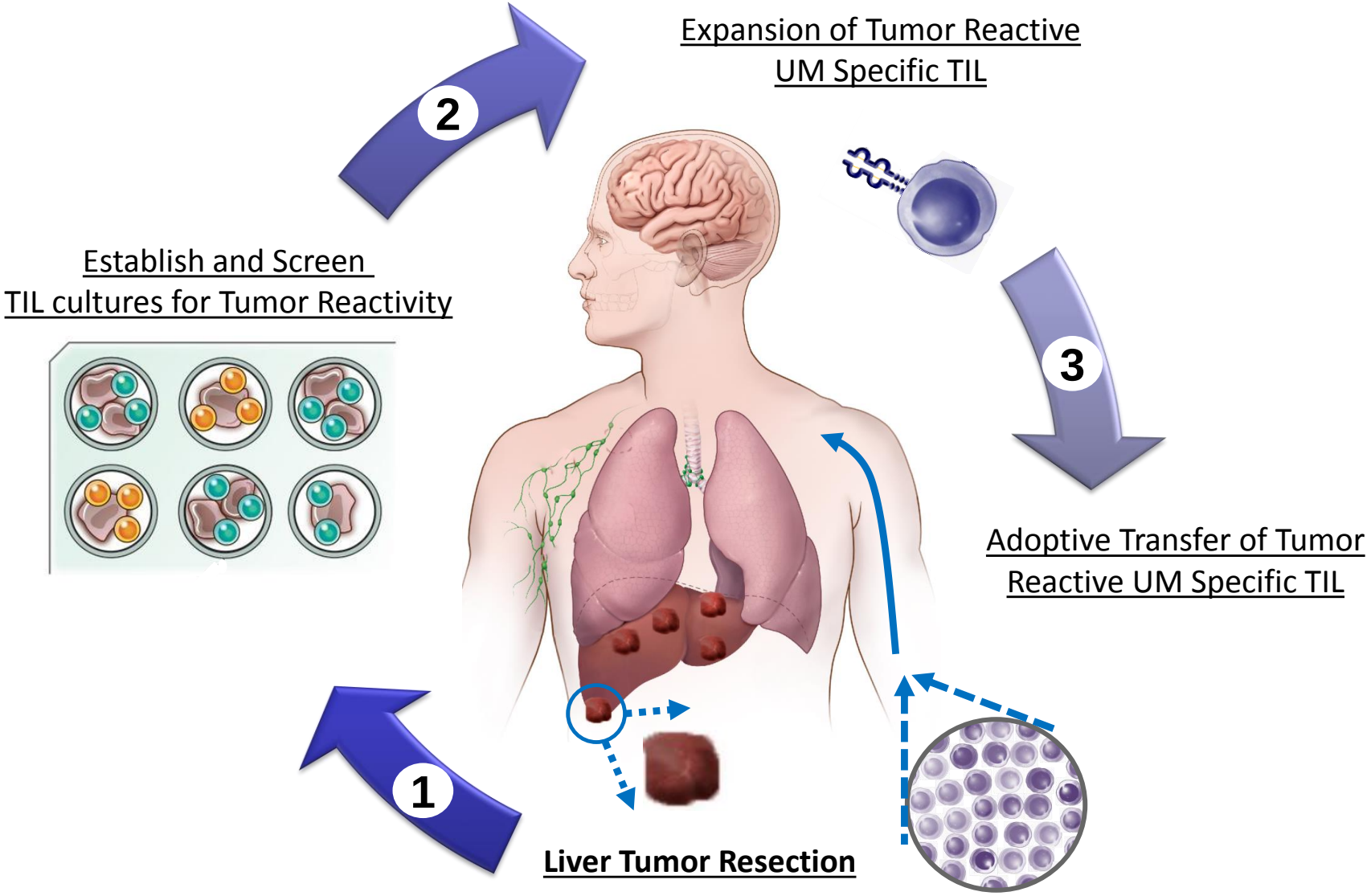


UM vs. CM Liver Metastases: Autologous Tumor Reactivity

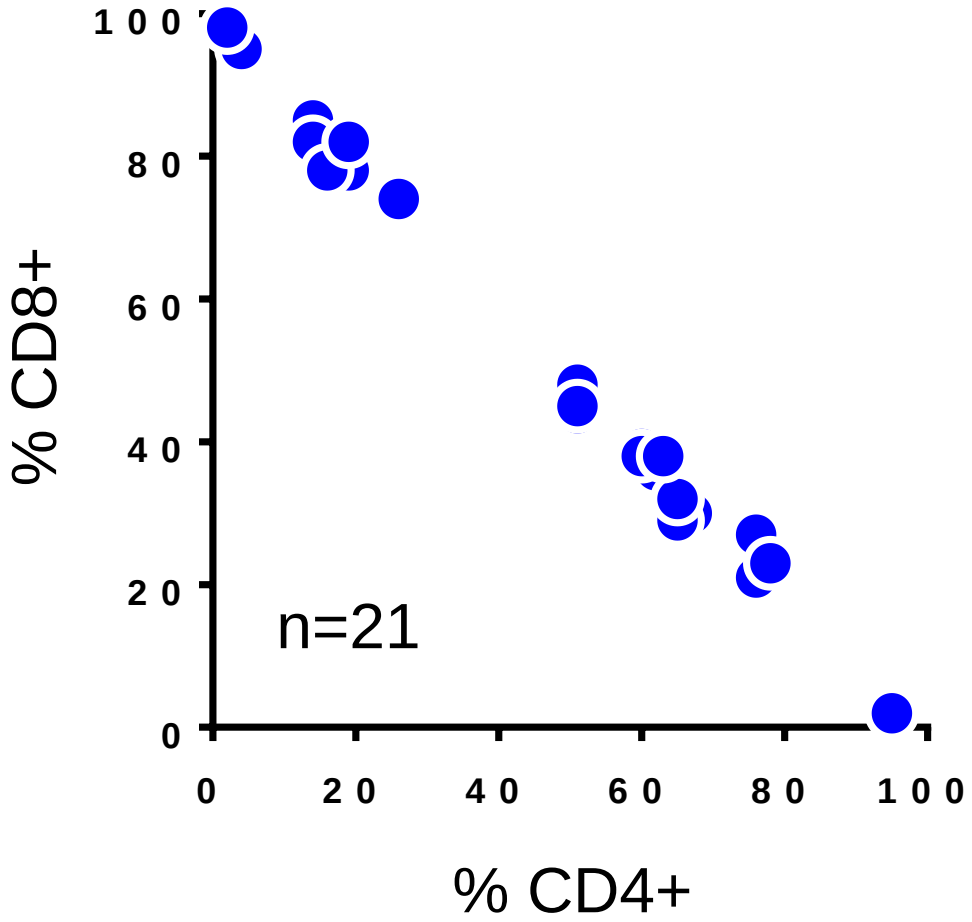


Rothermel et al., Clinical Cancer Research 2015

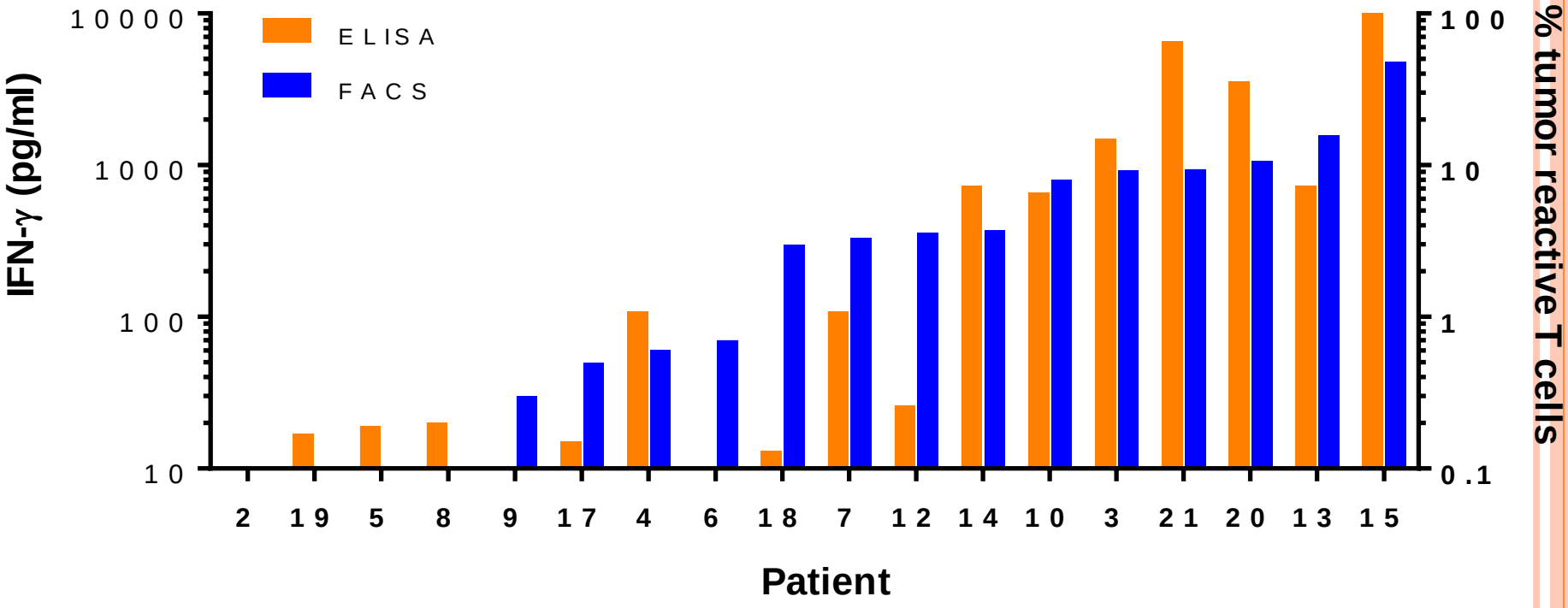
NCT01814046: Adoptive Immunotherapy for Metastatic Uveal Melanoma--Trial Design



NCT01814046: Phenotype of Infused TIL Products

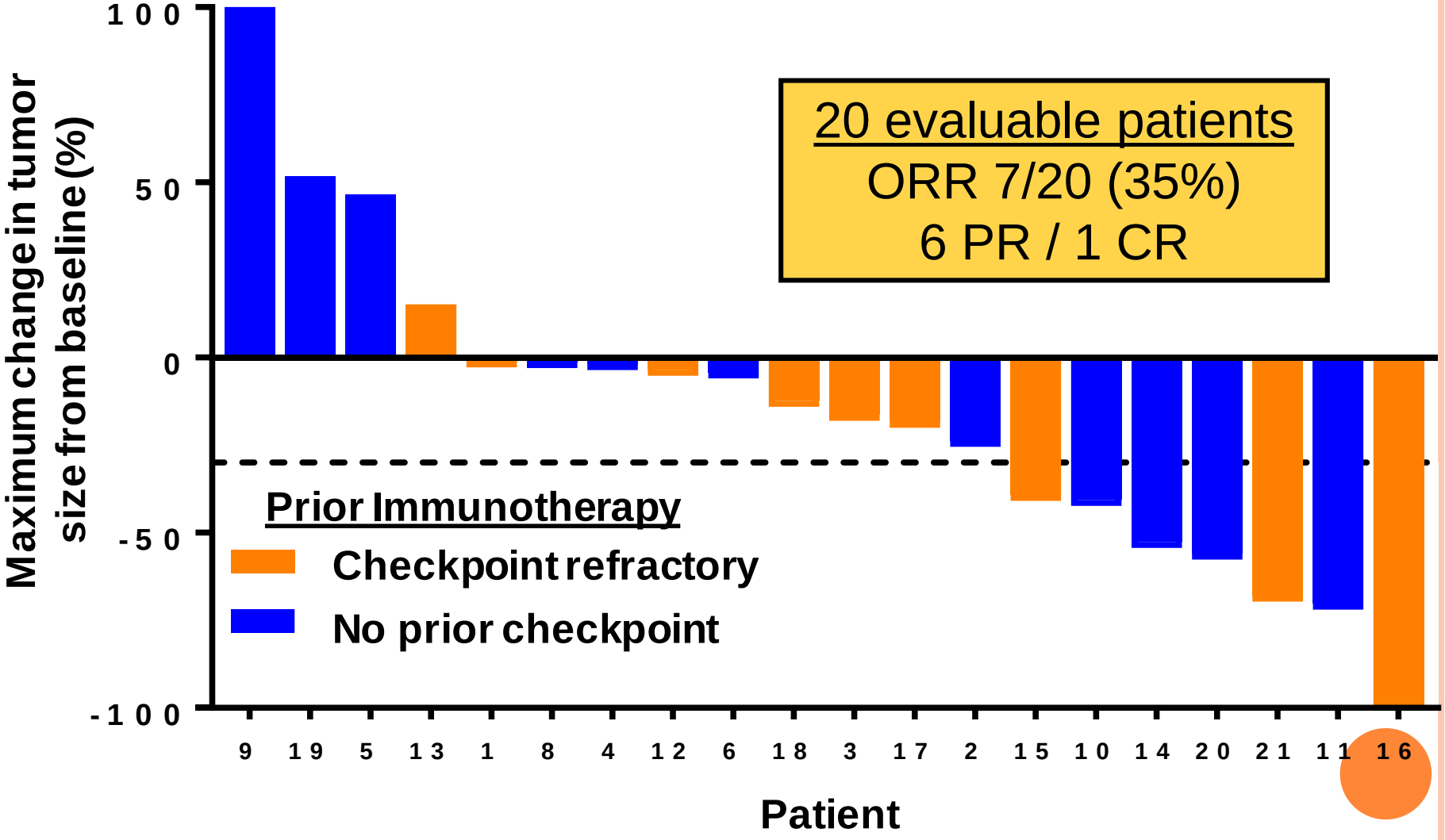


NCT01814046: Autologous Tumor Reactivity of Infused TIL Products



Excludes patients 1,11, and 16 due to insufficient tumor targets

Best Overall Response to TIL Therapy in Metastatic Uveal Melanoma

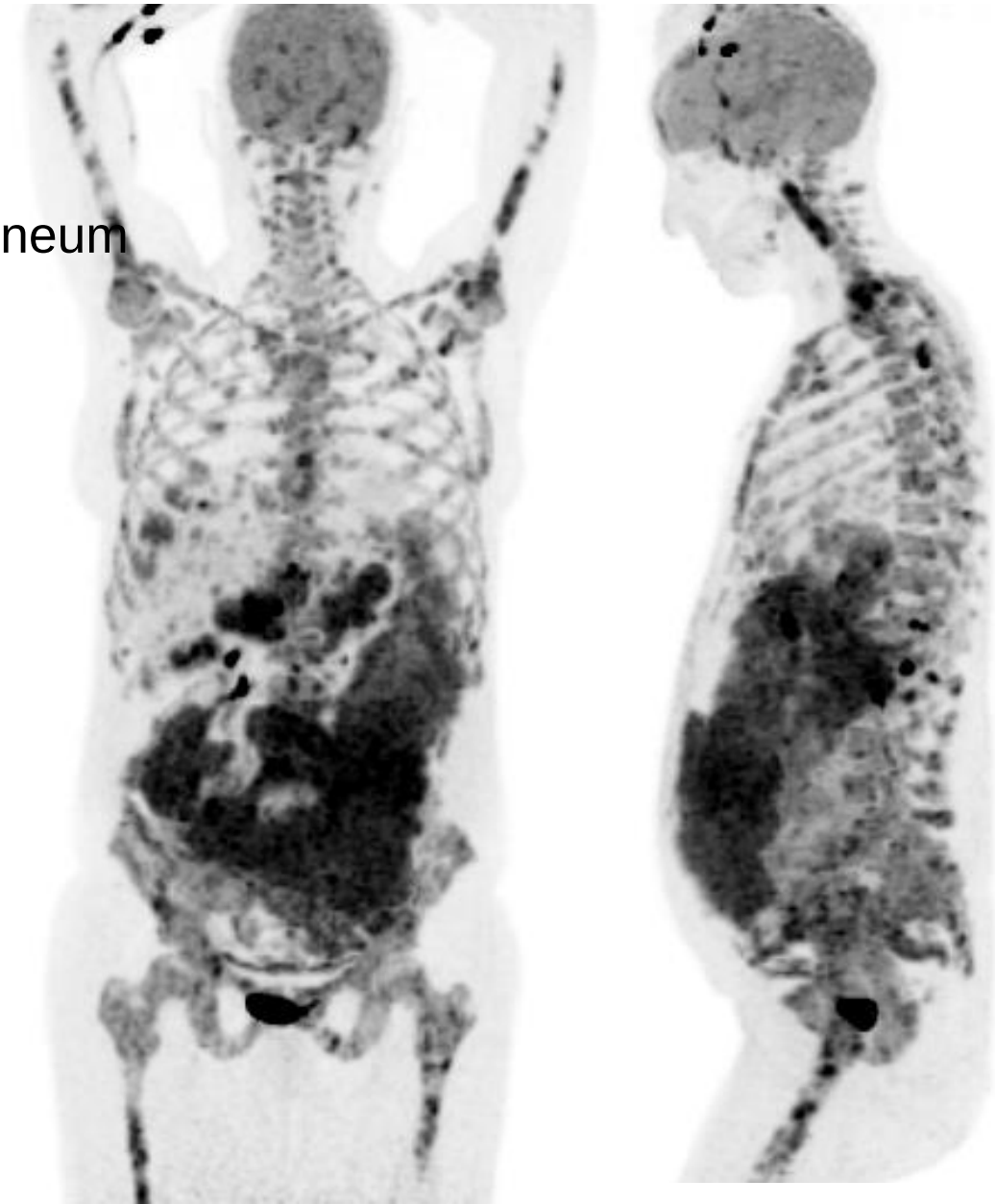


Chandran et al., Lancet Oncology 2017

UM Patient #10

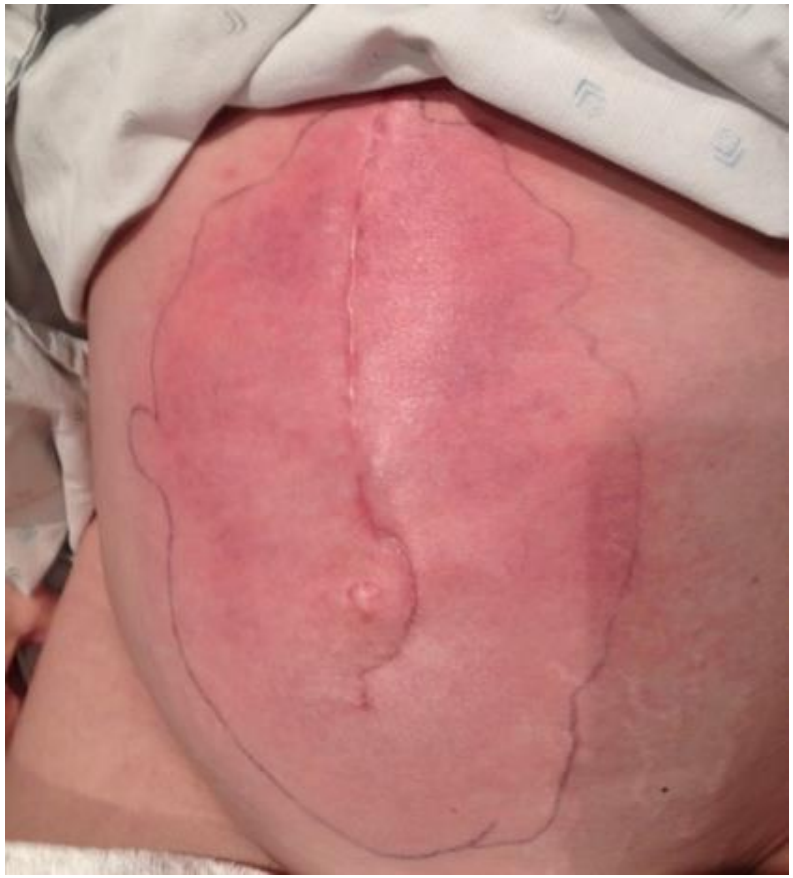
52 F with metastatic uveal melanoma to liver, bone, peritoneum

- Presented with rapidly deteriorating performance
- Abdominal pain
- Early satiety
- Ascites
- Weight loss
- Bone pain
- Narcotic use



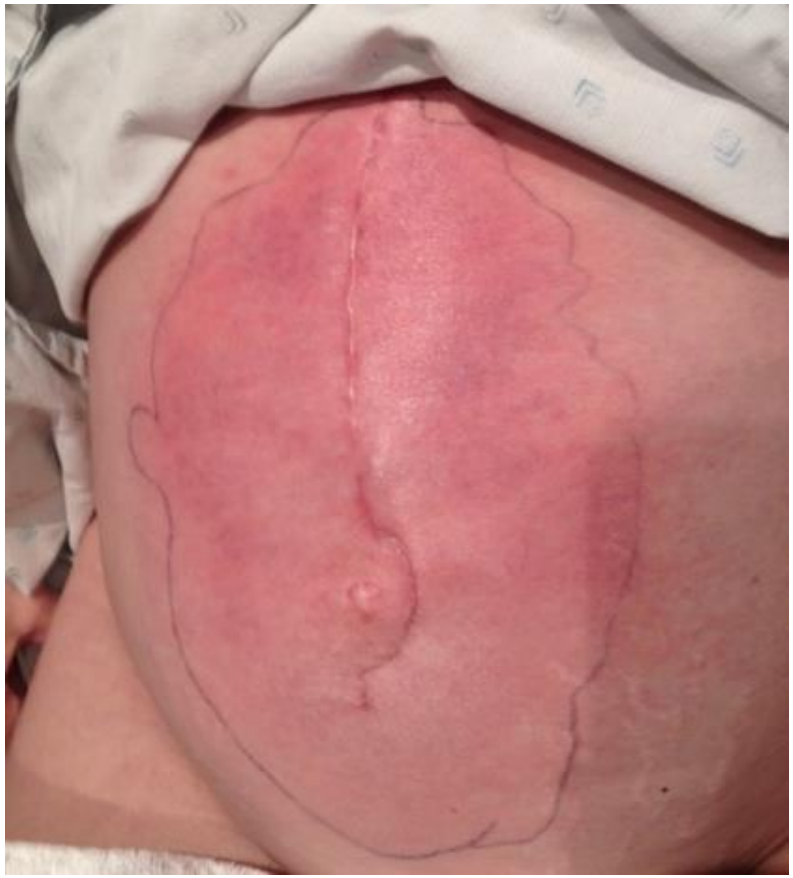
UM Patient #10

Baseline

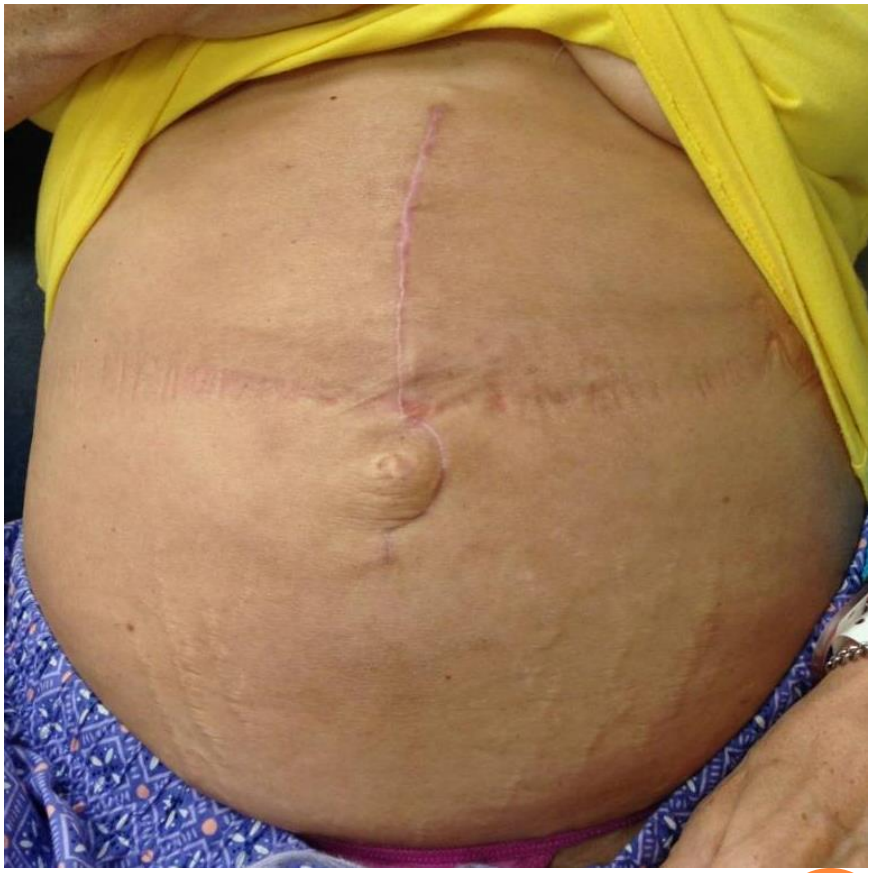


UM Patient #10

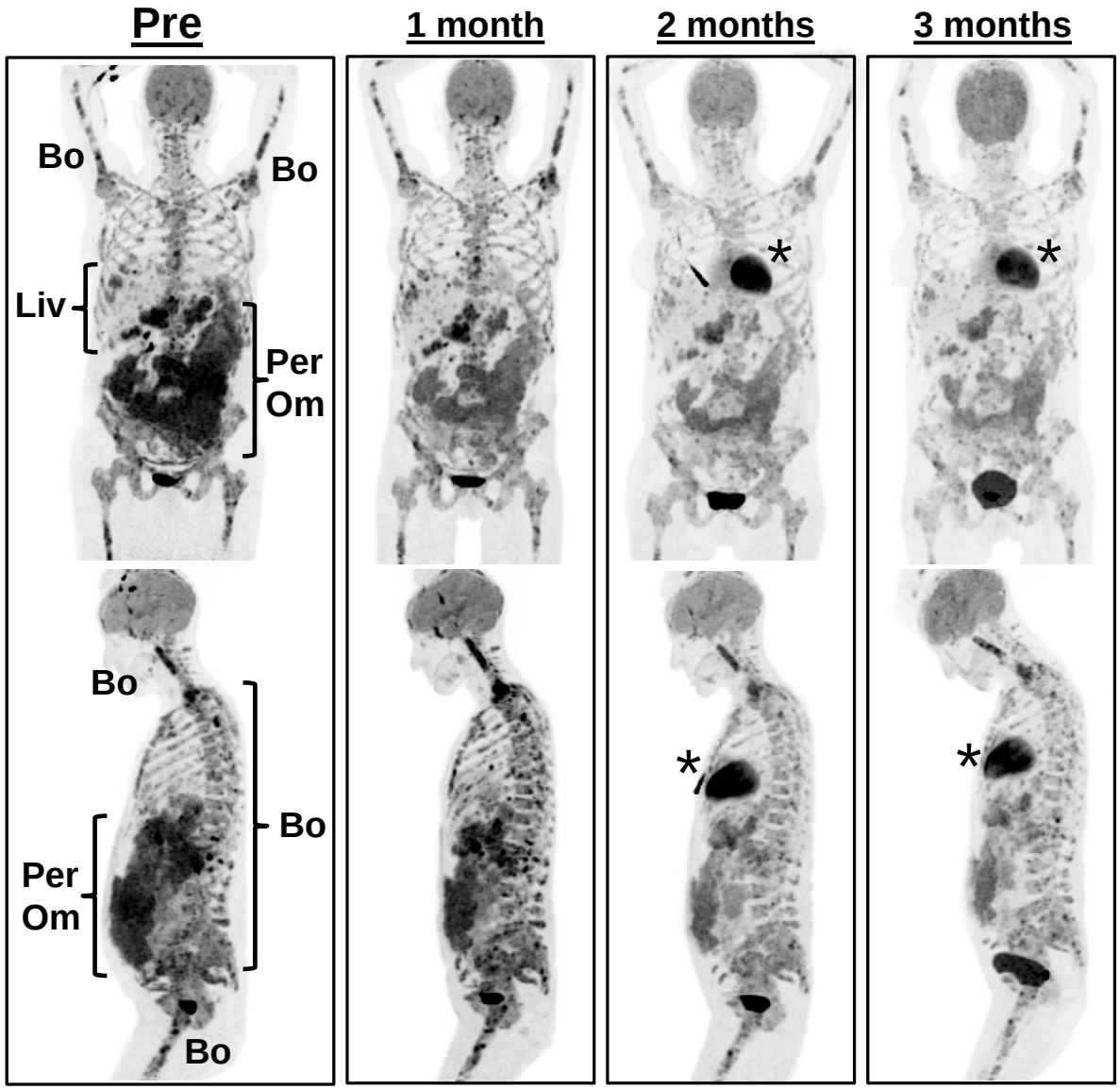
Baseline



Post ACT +1 month



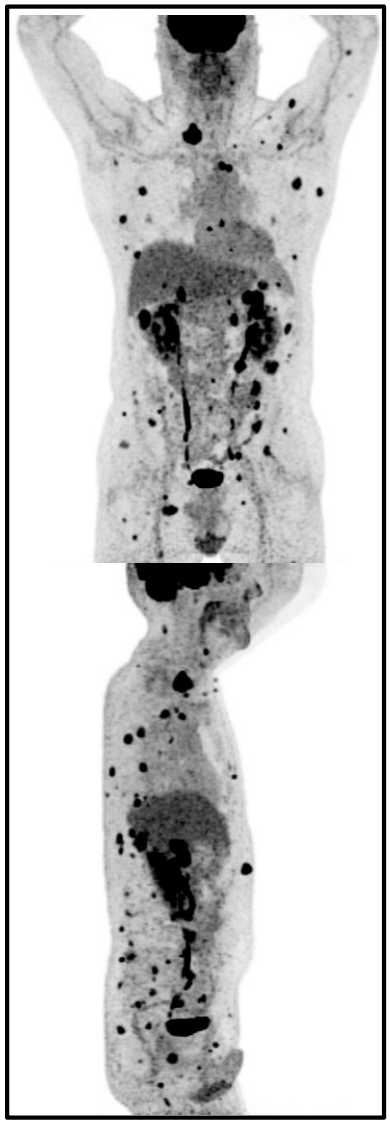
Tumor Regression in UM Patient #10 After TIL Therapy



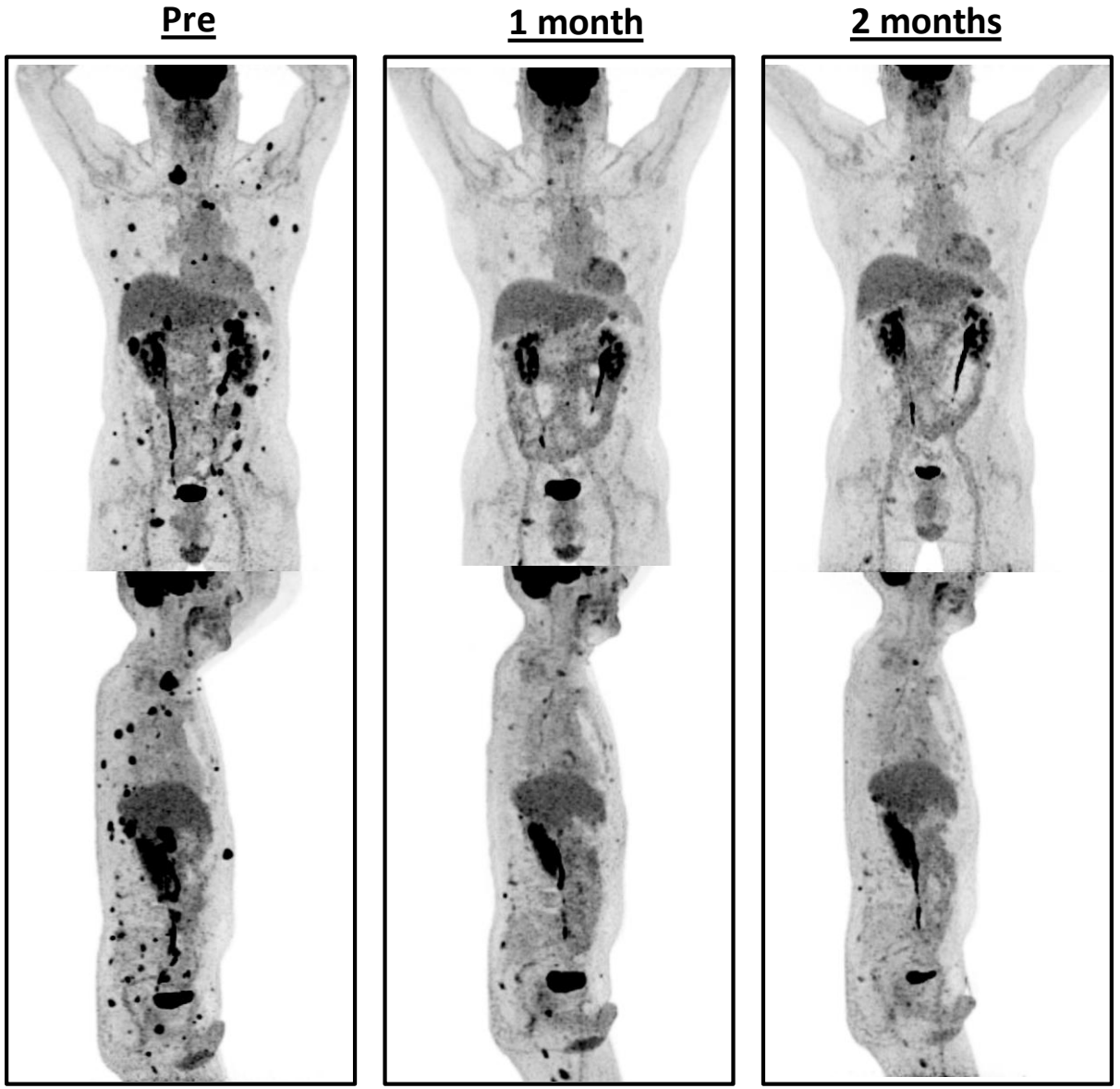
* Normal cardiac uptake

Tumor Regression in UM Patient #21 After TIL Therapy (checkpoint refractory)

Pre

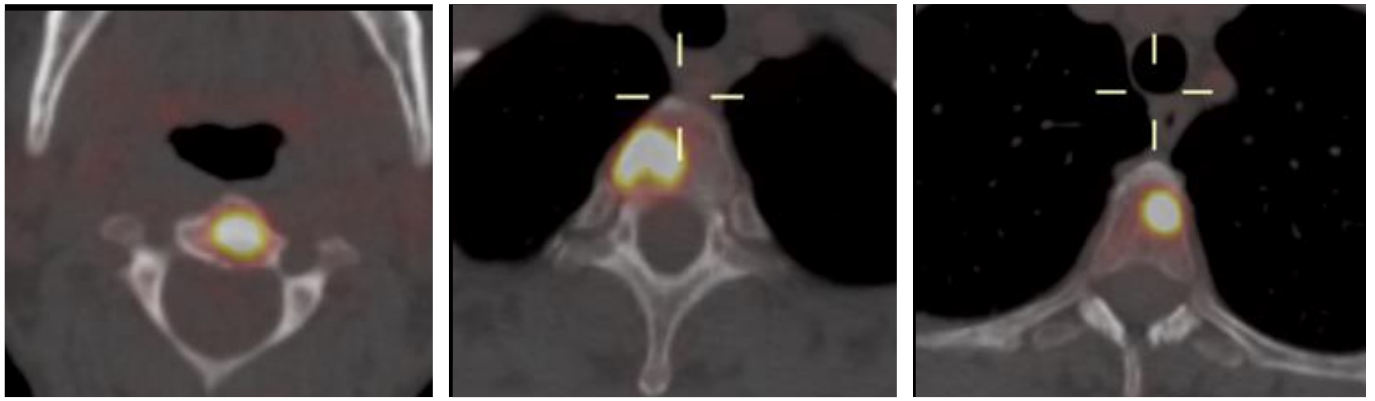


Tumor Regression in UM Patient #21 After TIL Therapy (checkpoint refractory)

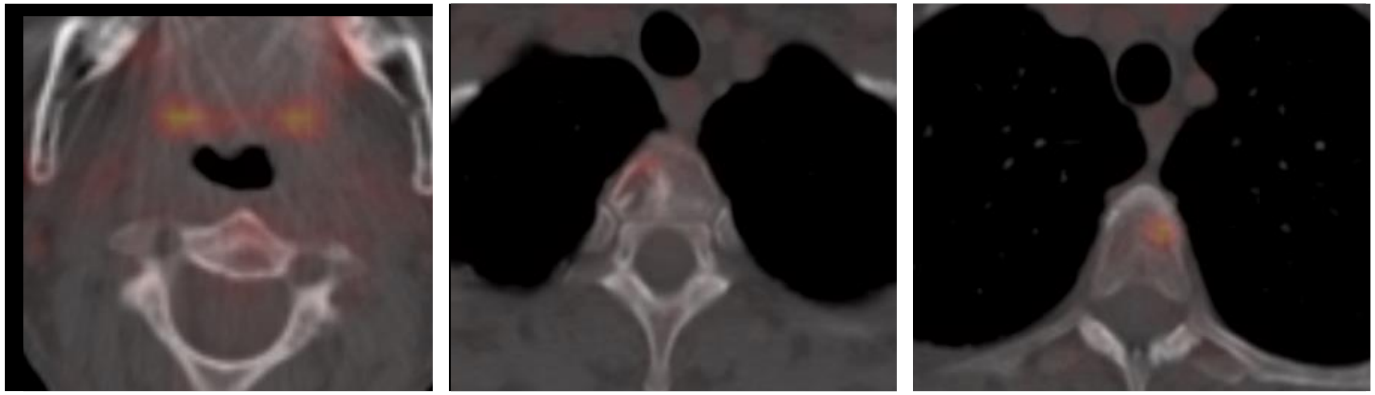


Tumor Regression in UM Patient #1 After TIL Therapy (checkpoint refractory)

Patient 1 Pre TIL

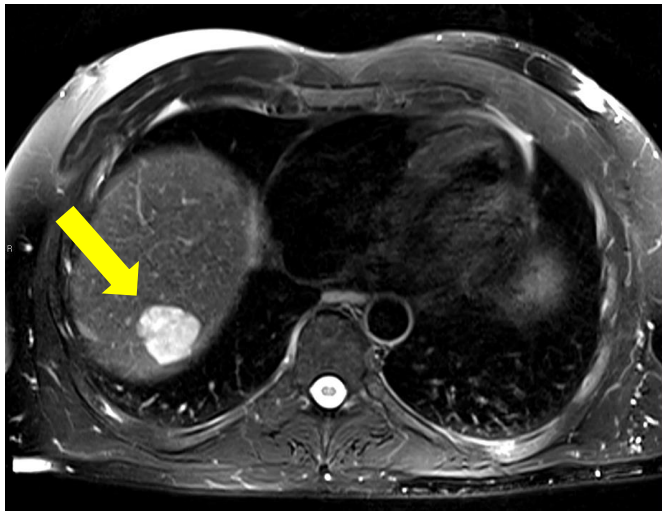


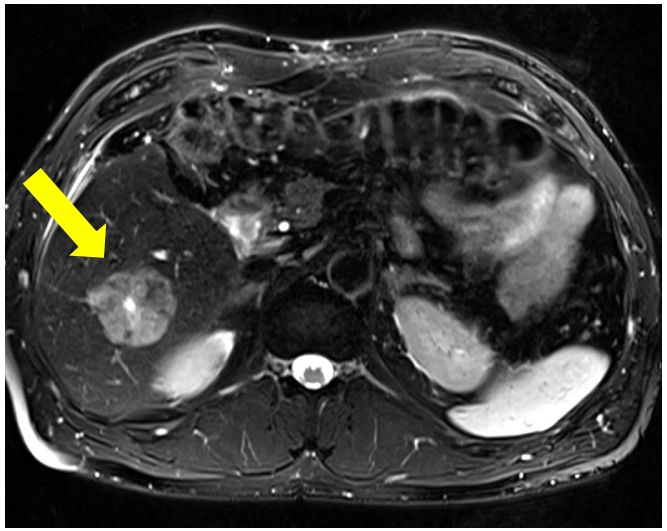
5 months



Complete Regression in UM Patient #16 After TIL Therapy (checkpoint refractory)

Pre TIL

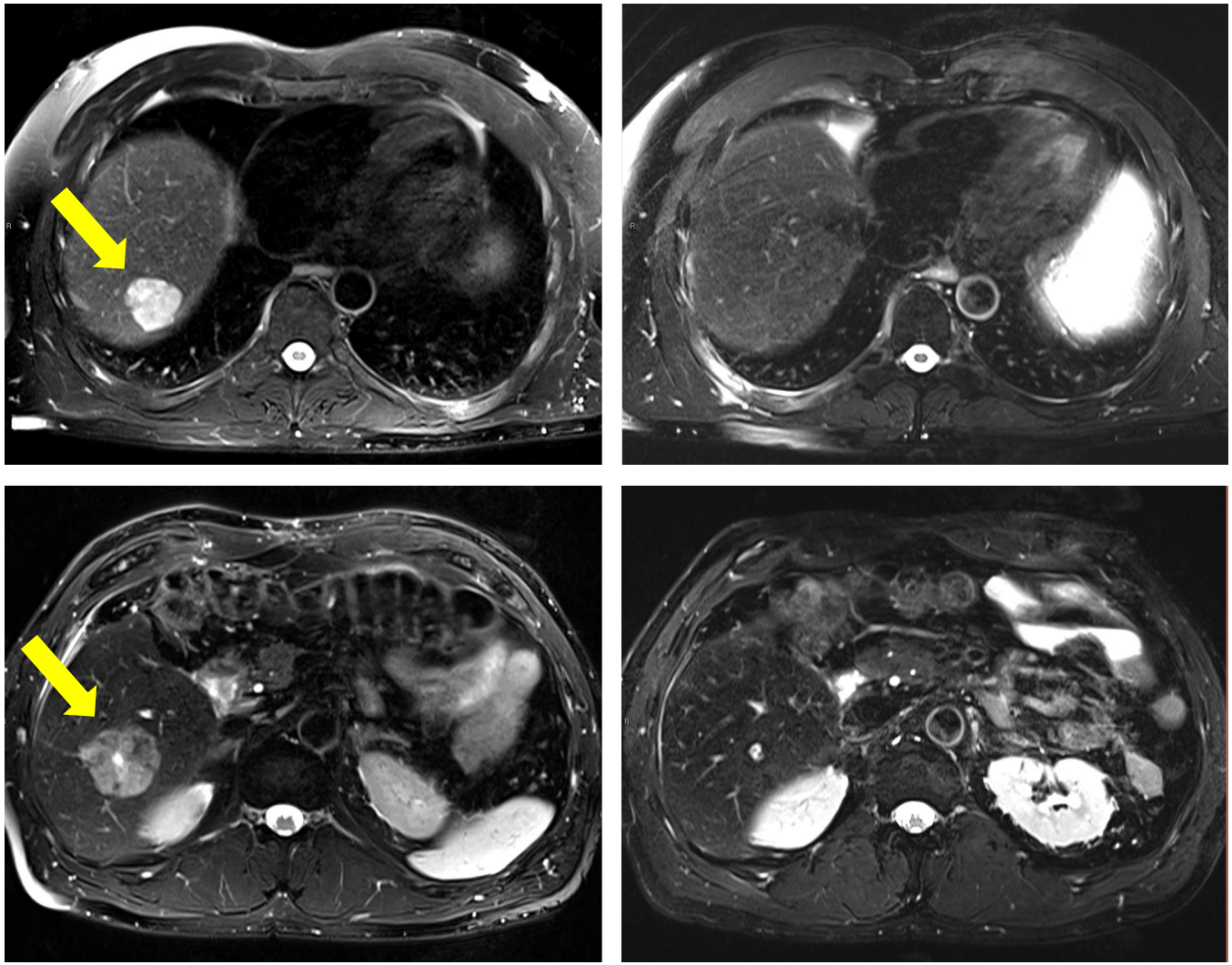




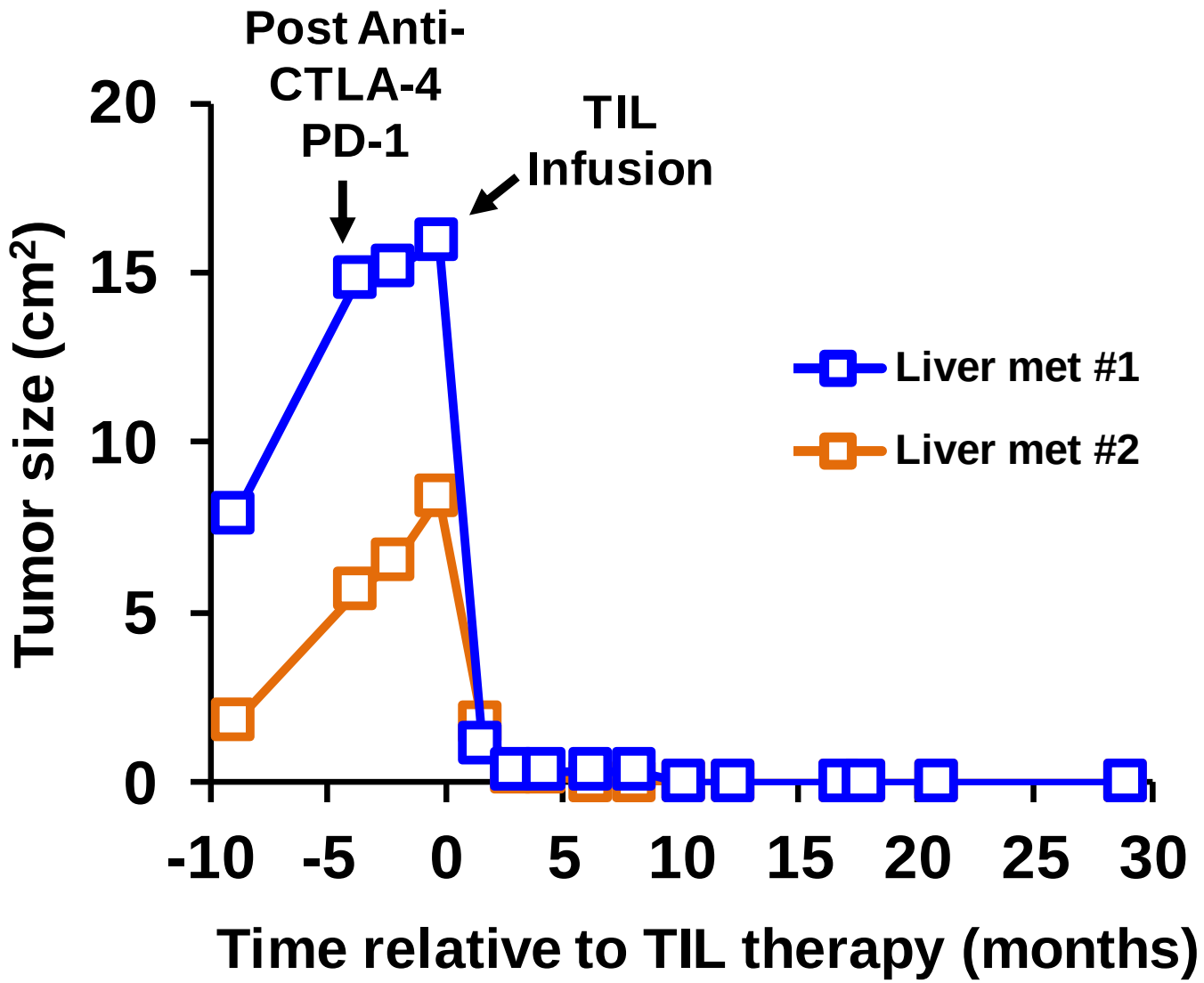
Complete Regression in UM Patient #16 After TIL Therapy (checkpoint refractory)

Pre TIL

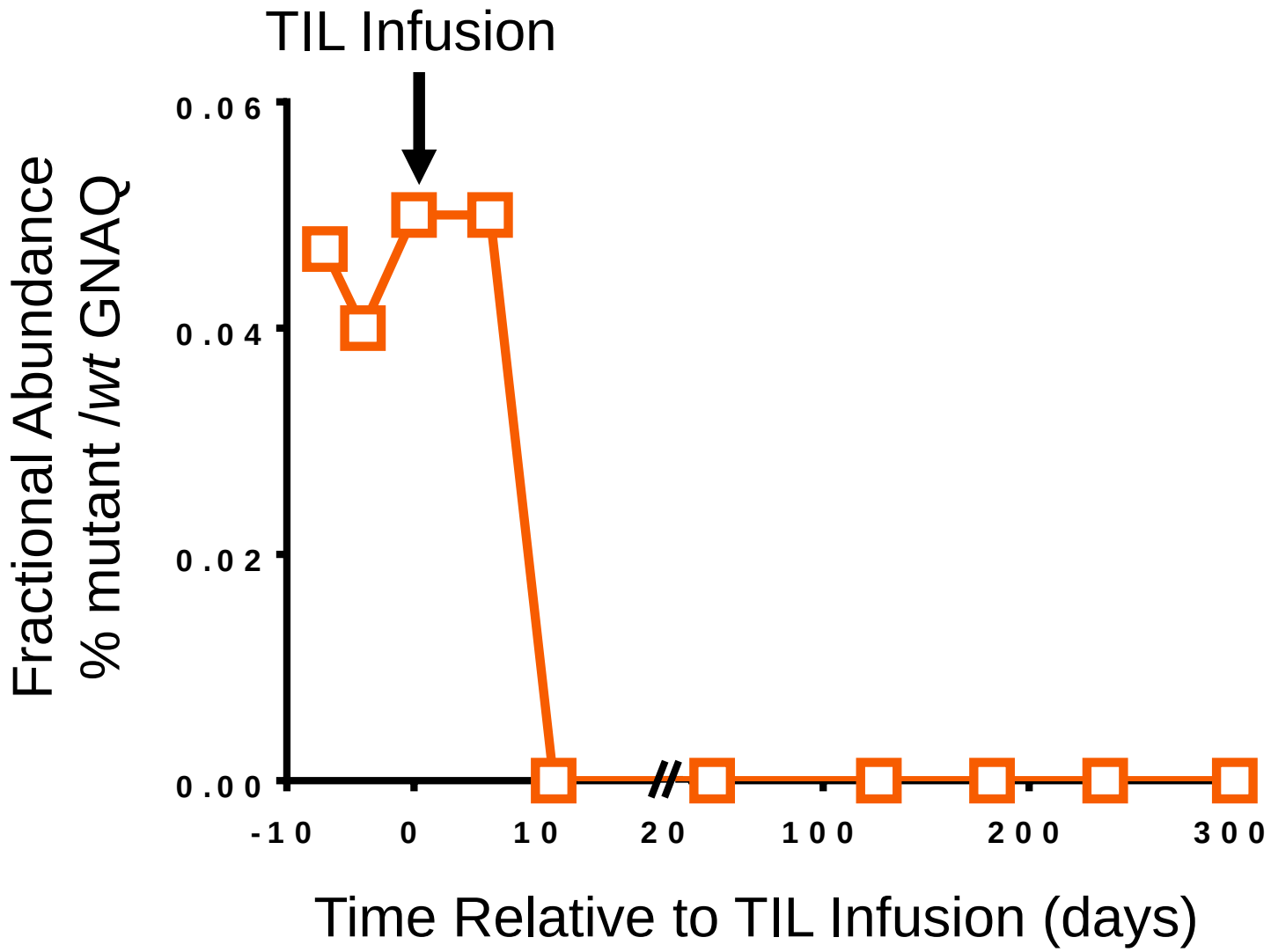
21 months



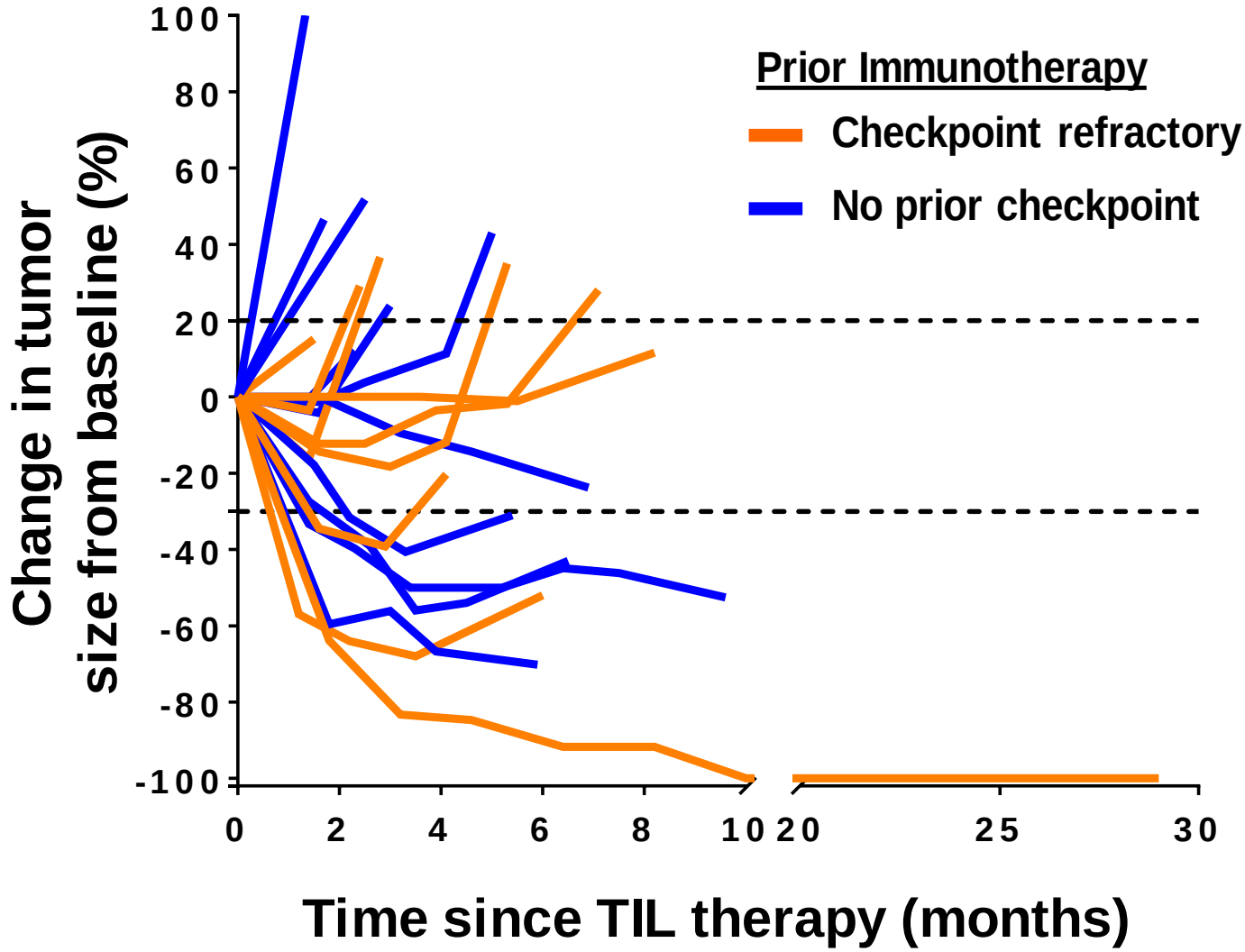
Complete Regression in UM Patient #16 After TIL Therapy (checkpoint refractory)



Complete Molecular Regression of Circulating Mutant GNAQ DNA in UM Patient #16

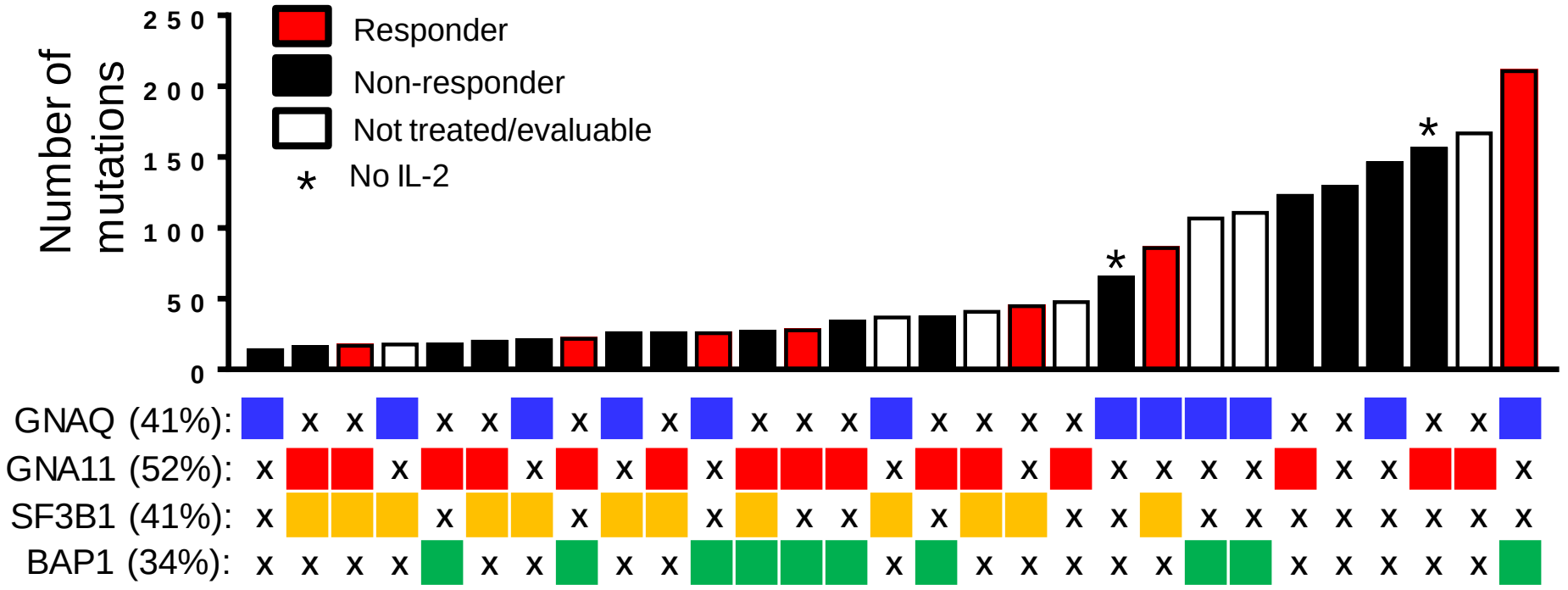


Kinetics of Tumor Response in Uveal Melanoma Patients After TIL Therapy



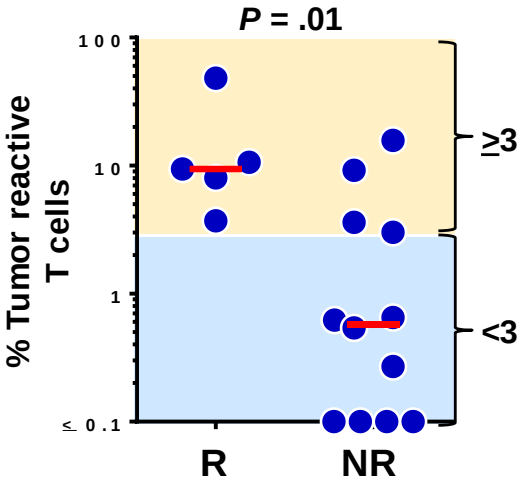
Chandran et al., Lancet Oncology 2017

No Relationship Between TIL Response and Number/Character of Somatic Mutations

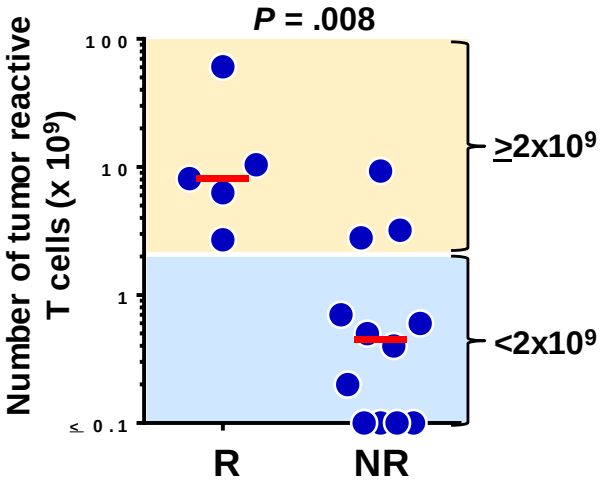


Association Between Clinical Response and Pre-treatment TIL Reactivity

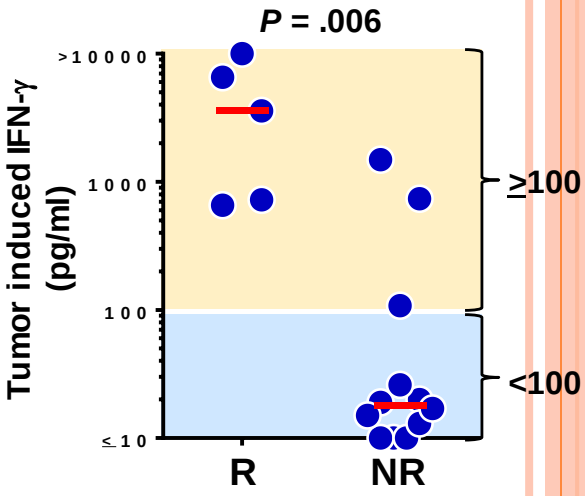
Frequency of Tumor Reactive TIL



Number of Tumor Reactive TIL



IFN-γ Release from Tumor Reactive TIL

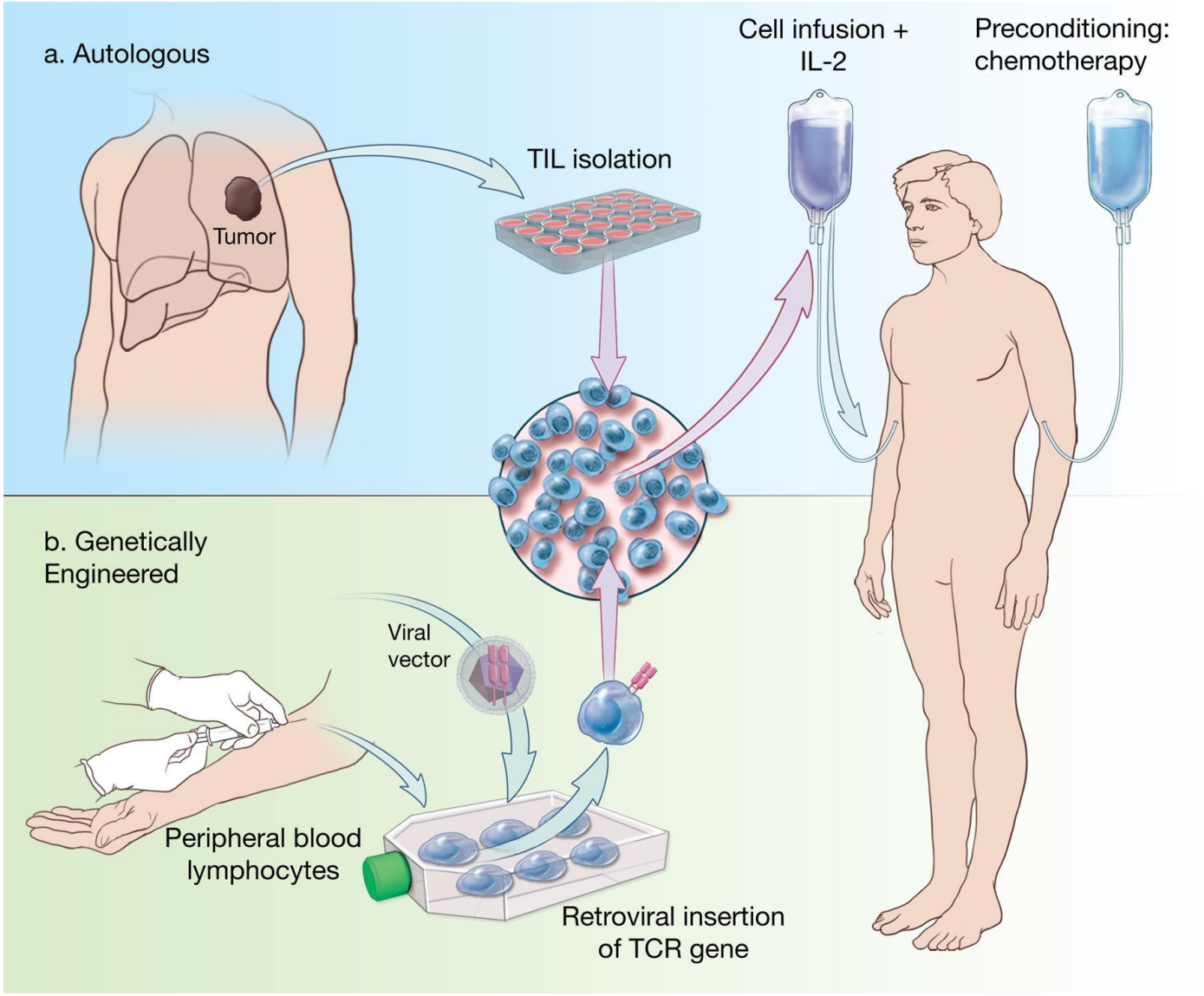


Pre-treatment In Vitro Tumor Reactivity Criteria

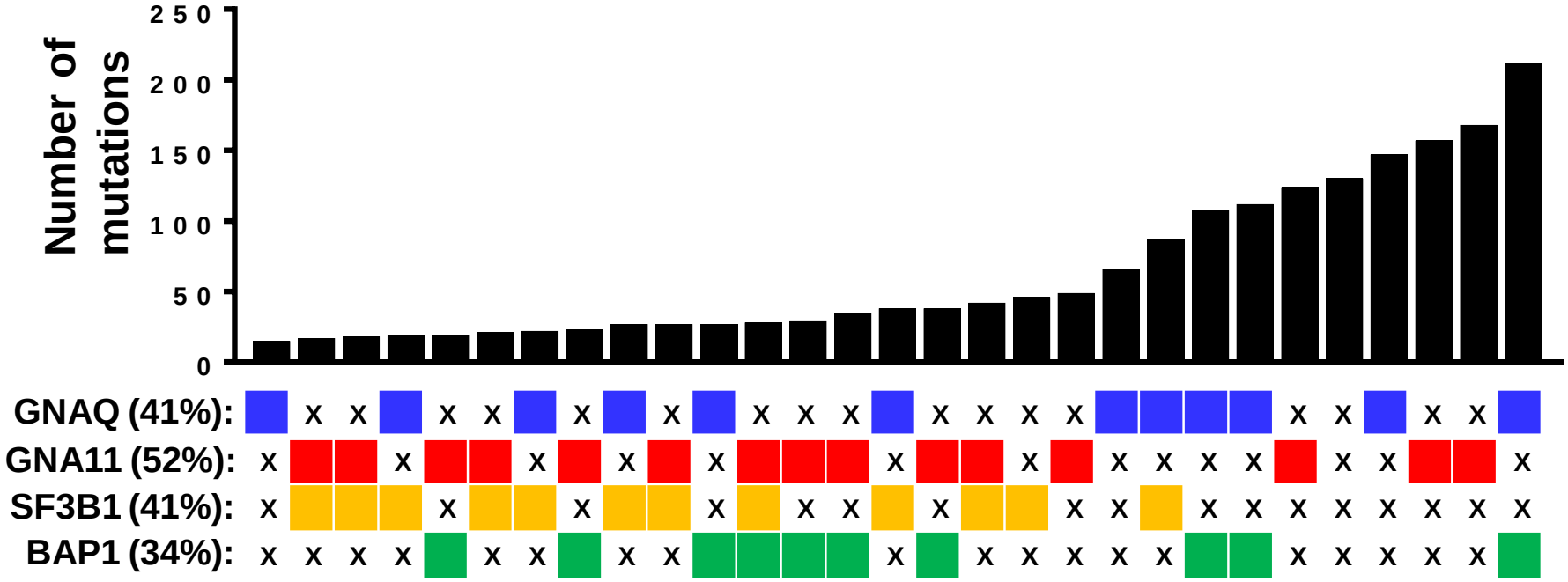
≥ 3% frequency ≥ $2x10^9$ cells ≥ 100 pg/ml IFN-γ	< 3% frequency < $2x10^9$ cells < 100 pg/ml IFN-γ

P = 0.003

Genetic Engineering of T Cells to Target Uveal Melanoma



Mining TIL to Develop a Portfolio of TCRs Specific for Somatic Mutations



GNAQ/11
c.A626C:p.Q209P
c.A626T:p.Q209L

SF3B1
c.G1874A:p.R625H
c.C1873T:p.R625C

Adoptive TIL Transfer for Additional Metastatic Solid Tumors

Cholangiocarcinoma

Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer.

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

Cervical Cancer

Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells.

Stevanović et al., *J Clin Oncol*. 2015 May 10;33(14):1543-50.

Colorectal Cancer

T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer.

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

TIL ADVANTAGES

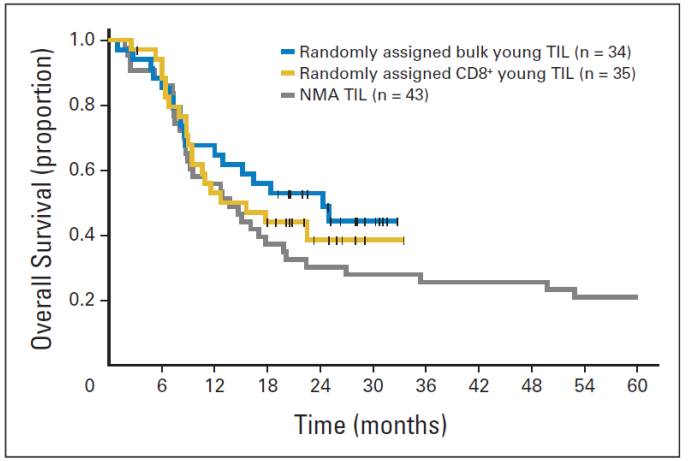
- High degree of efficacy
  - High PR and CR rates with long durations
  - Patients with prior immunotherapy
  - Patients with brain metastases
  - Patients with advanced, high bulk disease
- One treatment
  - No ancillary therapies needed after TIL and IL-2
- Less chance for long term autoimmune effects
- TIL can now be successfully prepared from > 90% of melanoma patients (NCI, Moffitt)
- Response rates reproduced at multiple sites and in multiple countries

TIL CHALLENGES

- Requires GMP manufacturing facility
- Special skills required for manufacture
- Production is expensive (labor, cytokines, plasticware)
- Length of time from tumor resection to treatment
  - Some patients may progress in the interim
- Preconditioning with cy/flu required
- High dose IL-2 used
  - Inpatient treatment to monitor toxicities
  - Centers need to be comfortable administering high dose IL-2
  - IL-2 is expensive

ENHANCING TIL ACTIVITY

- Selection
  - CD8 selection not effective
  - PD-1 selection under study
- "Young" TIL
  - Trade off with lower infused numbers
- Other approaches under study
  - Pre-treatment (oncolytic virus, CpG injection)
  - Novel cytokine culture cocktails
  - Checkpoint molecules in culture medium
  - CD137 activation
  - Genetic modification approaches



J Clin Oncol 31:2152-2159. © 2013