The Immune Rejection of Human Cancers: Cytokines, Vaccines and T-Cells

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Surgery Branch, NCI
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Disclosures

There are no conflicts of interest or commercial/ non-commercial sponsorship for this program

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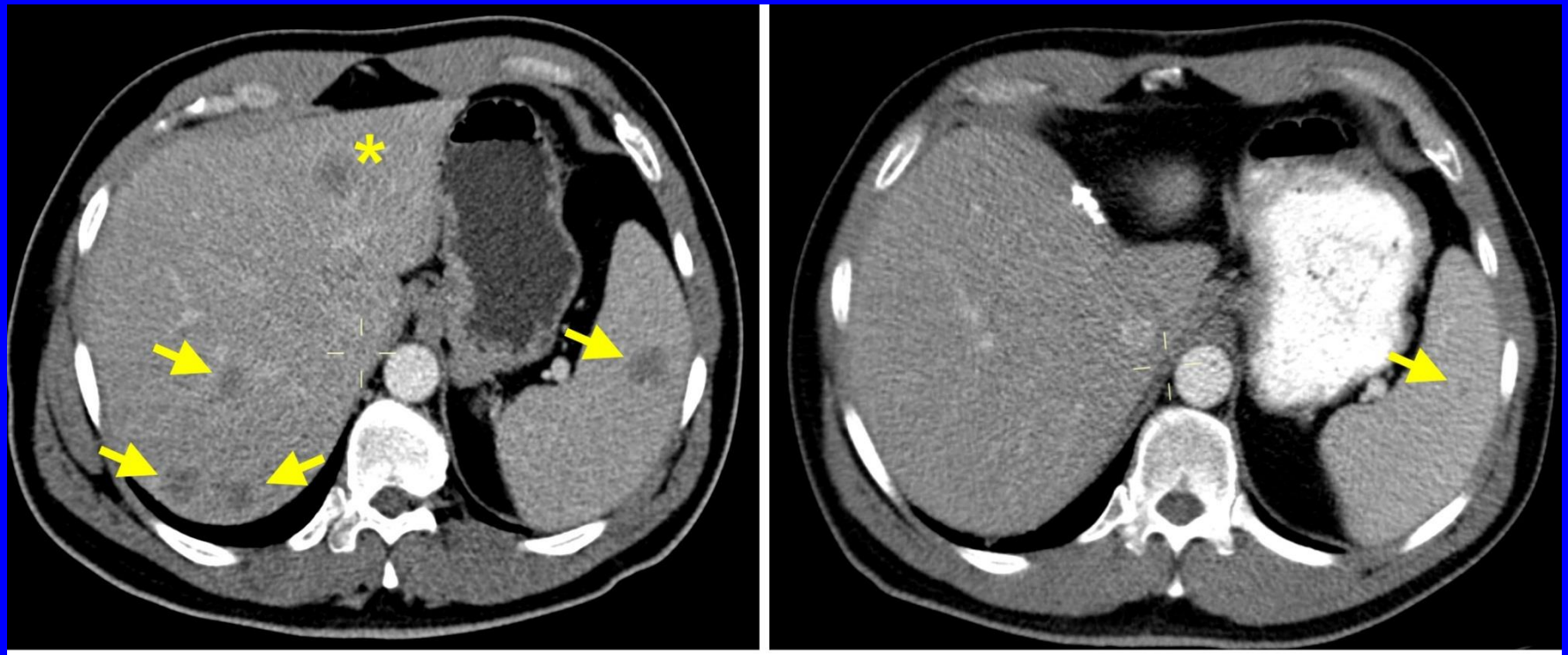
- Clinical Fellows and Nursing Staff

"Natural" Immunotherapy of Cancer

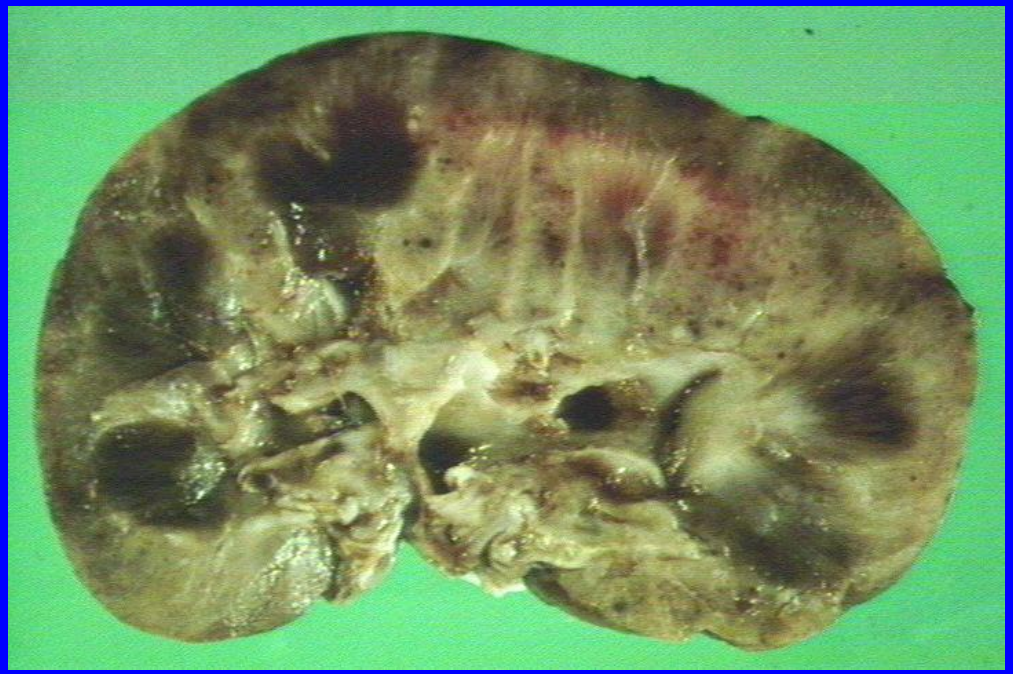
Rarely, human tumors can spontaneously regress, often after surgery or infection



The Role of T-Cells

T-lymphocytes were found to be responsible for rejection of transplanted tissue

They can kill cells that they immunologically recognize or they can secrete cytokines



Immunotherapy for Human Cancers ("The Dark Ages")

"It would be as difficult to reject the right ear and leave the left ear intact as it is to immunize against cancer."

W. H. Woglom

Interleukin-2 (IL-2)

"The Dawn"

15,500 m.w. glycoprotein made by CD4 and CD8 lymphocytes

T-cell growth factor

Activates T-cells and NK cells

Essential to the survival and action of regulatory T-cells

Has no direct effects on tumor cells

Interleukin-2

Discovered by Morgan, Ruscetti and Gallo (1976)

Gene for IL-2 cloned by Taniguchi (1983)

Recombinant IL-2 made by the Cetus Corporation and tested in the Surgery Branch (1984)

First response in a patient with cancer (1984)

History of High-Dose IL2

IL-2 was dose escalated to high levels (with significant toxicity) with no responses seen against multiple tumor types

Lymphokine Activated Killer cells (LAK) were added to HD IL2 based on results in mice

In the next 25 patients, there was 1 CR and 3 PR in 7 pts with melanoma and 3 PR in 3 patients with RCC

(Result of selecting tumor types, not LAK)

NCI SOLID — EXTENT OF DISEASE — Protocol: 88-C-20a

LK5 — Case # — Patient Name (Last, First): T, L — Course # 1 — Pt# ___

Evaluation: Baseline AND FU # 1-5 — Date: 11-22-84 (m d y)

		Site	Pri/LR/Met	Test*	Cut #	Size (cm)	Area
11/27/1984	1 R (L)	Back	P L (M)	PE		12.0 x 8.5 =	102.0
	2 (R) L	Back	P L (M)	↓		10.5 x 8.5 =	89.3
	3 R (L)	Thigh	P L (M)	↓		12.0 x 12.0 =	144.0 / 335.3
	4 R L		P L M			. x . =	.
12/5/84	1 R (L)	Back	P L (M)	PE		12.5 x 11.0 =	137.5
	2 (R) L	↓	P L (M)	↓		10.0 x 8.5 =	85.0
	3 R (L)	Thigh	P L (M)	↓		12.0 x 12.0 =	144.0 / 366.5
	5 R L		P L M			. x . =	.
12/21/84	1 R (L)	Back	P L (M)	PE		9.0 x 7.0 =	63.0
	2 (R) L	↓	P L (M)	↓		9.0 x 7.0 =	63.0
	3 R (L)	Thigh	P L (M)	↓		12.0 x 10.0 =	120.0 / 846.0
	R L		P L M			. x . =	.
1/9/1985	1 R (L)	Back	P L (M)	PE		8.0 x 7.0 =	56.0
	2 R (L)	↓	P L (M)	↓		6.0 x 6.5 =	39.0
	3 R (L)	Thigh	P L (M)	↓		11.5 x 12.0 =	138.0 / 233
	10 R L		P L M			. x . =	.
2/13/85	1 R (L)	Back	P L (M)	PE		1.0 x 1.0 =	1.0
	2 (R) L	↓	P L (M)	↓		2.0 x 2.0 =	4.0
	3 R (L)	Thigh	P L (M)	↓		6.5 x 6.5 =	42.3 / 47.3
	20 R L		P L M			. x . =	.
3/20/85	1 R (L)	Back	P L (M)	PE		0 x 0 =	0
	2 (R) L	↓	P L (M)	↓		0 x 0 =	0
	3 R (L)	Thigh	P L (M)	↓		0 x 0 =	0
	24 R L		P L M			. x . =	.

*Test Codes: ACT CCT CXR FLT BAE BS LS IVP EOE MRI US BE | TOTAL = ___ | % of Baseline: ___

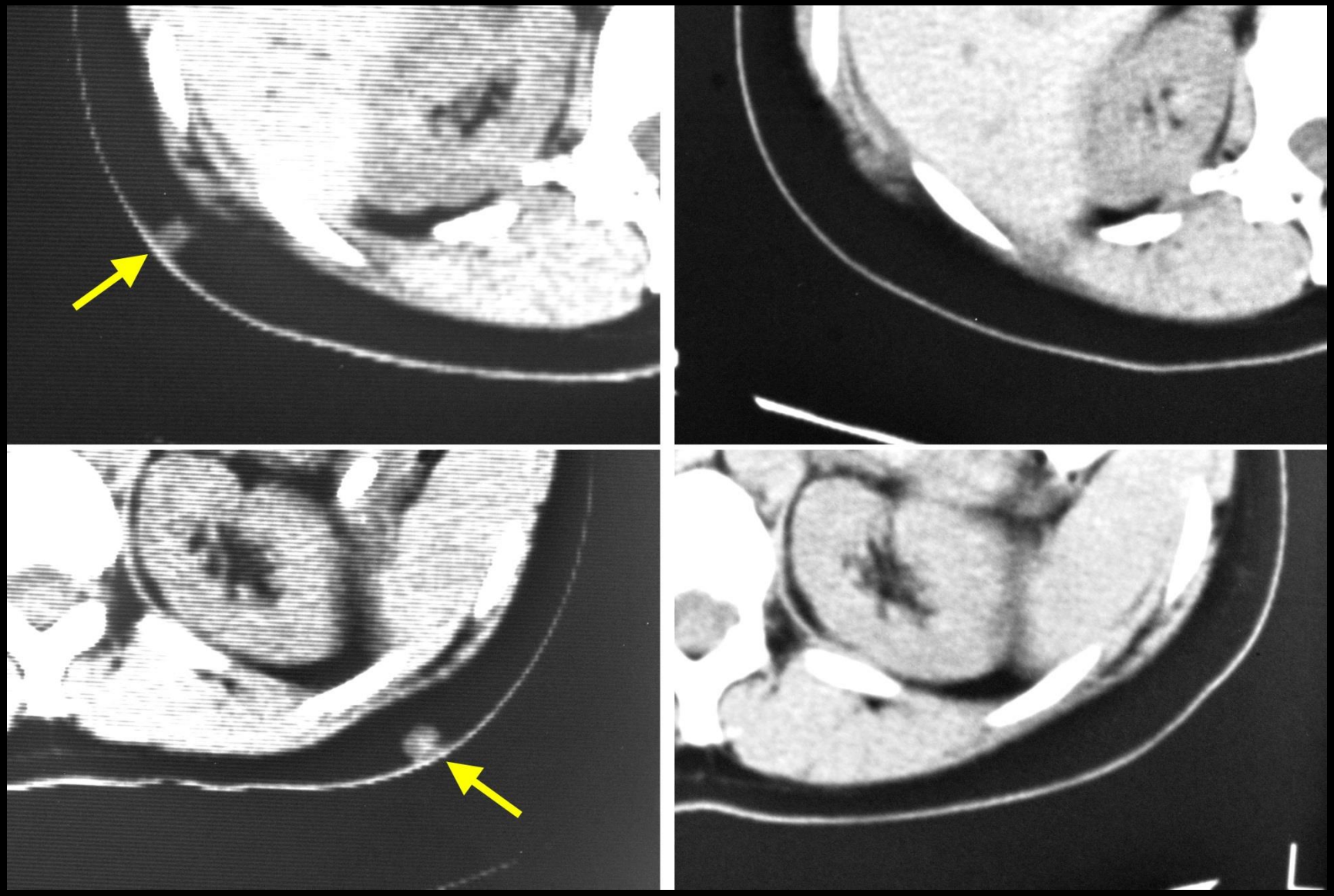
SOLID-XT 08/01/86 Completed: SAK Entered: ___

LK 5 : 11-26-84 to 12-17-84

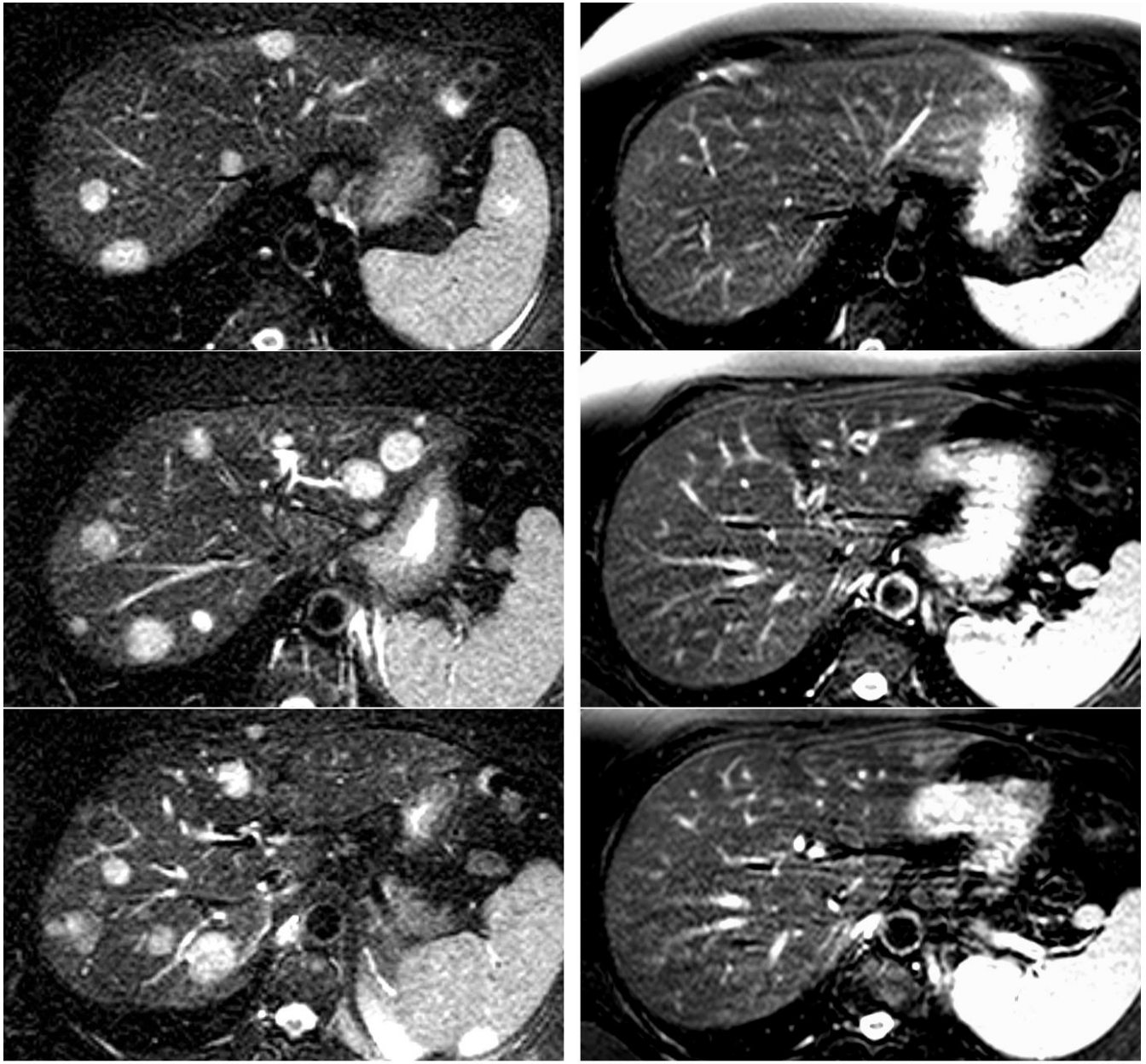
APR 15 1987

MAY 01 1987

Metastatic Melanoma



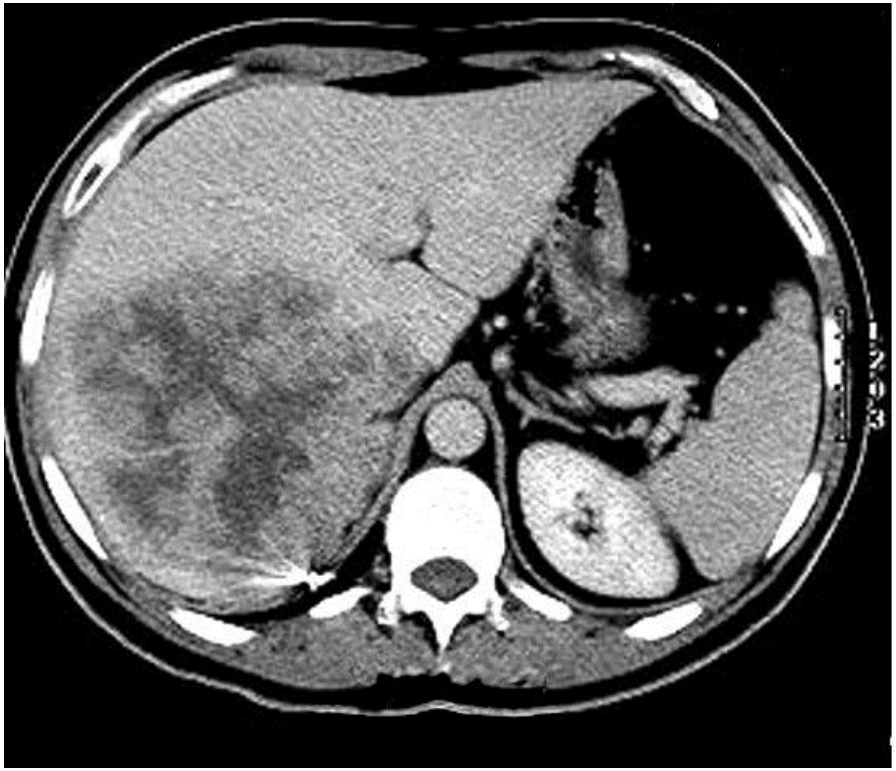
Metastatic Melanoma



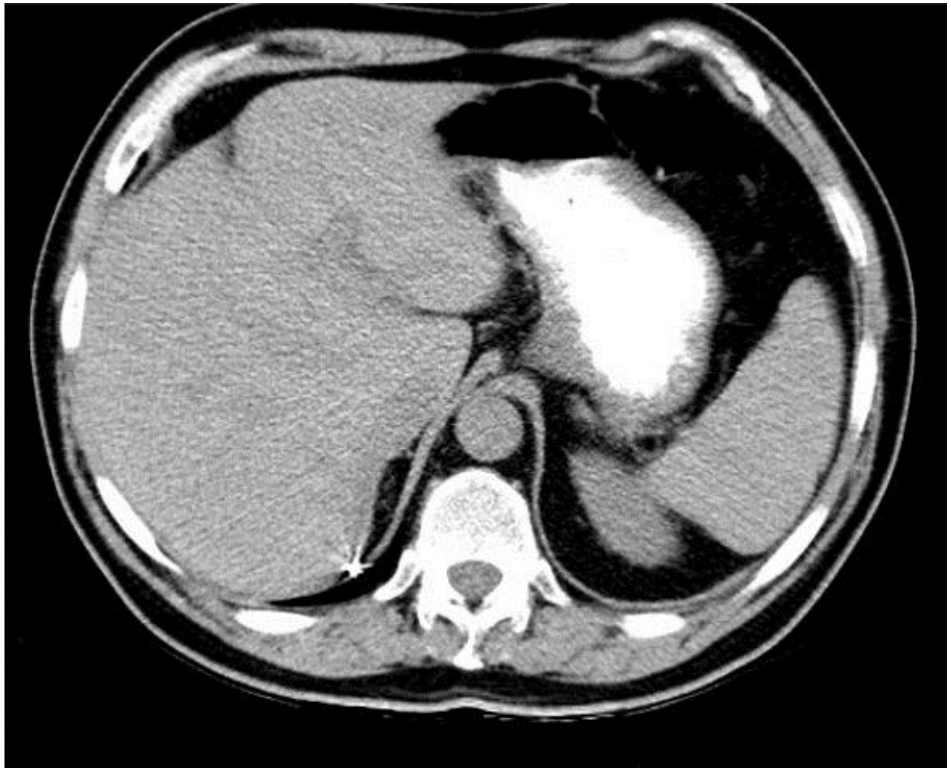
Pre-Treatment

12 Months

Metastatic Renal Cancer

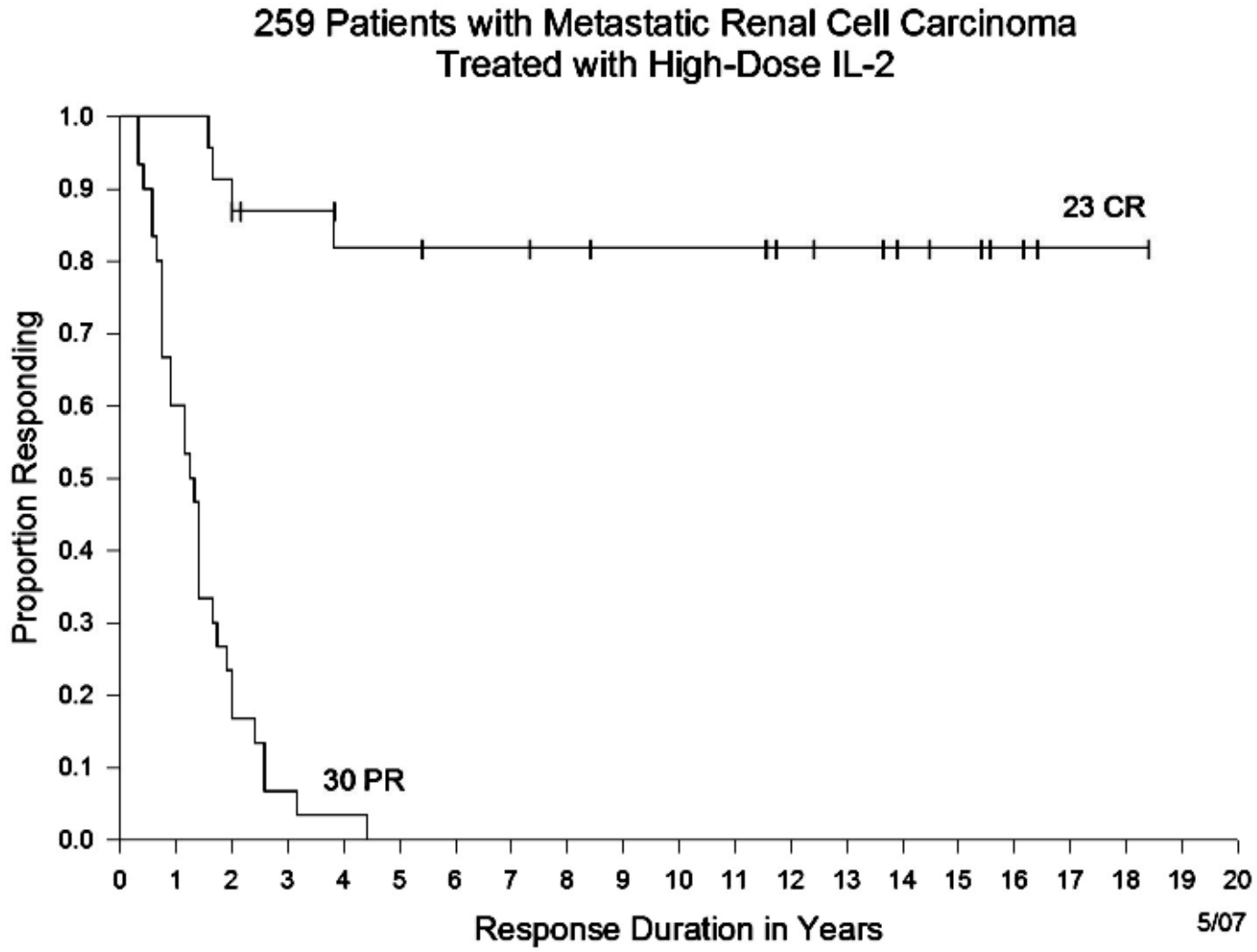


1993



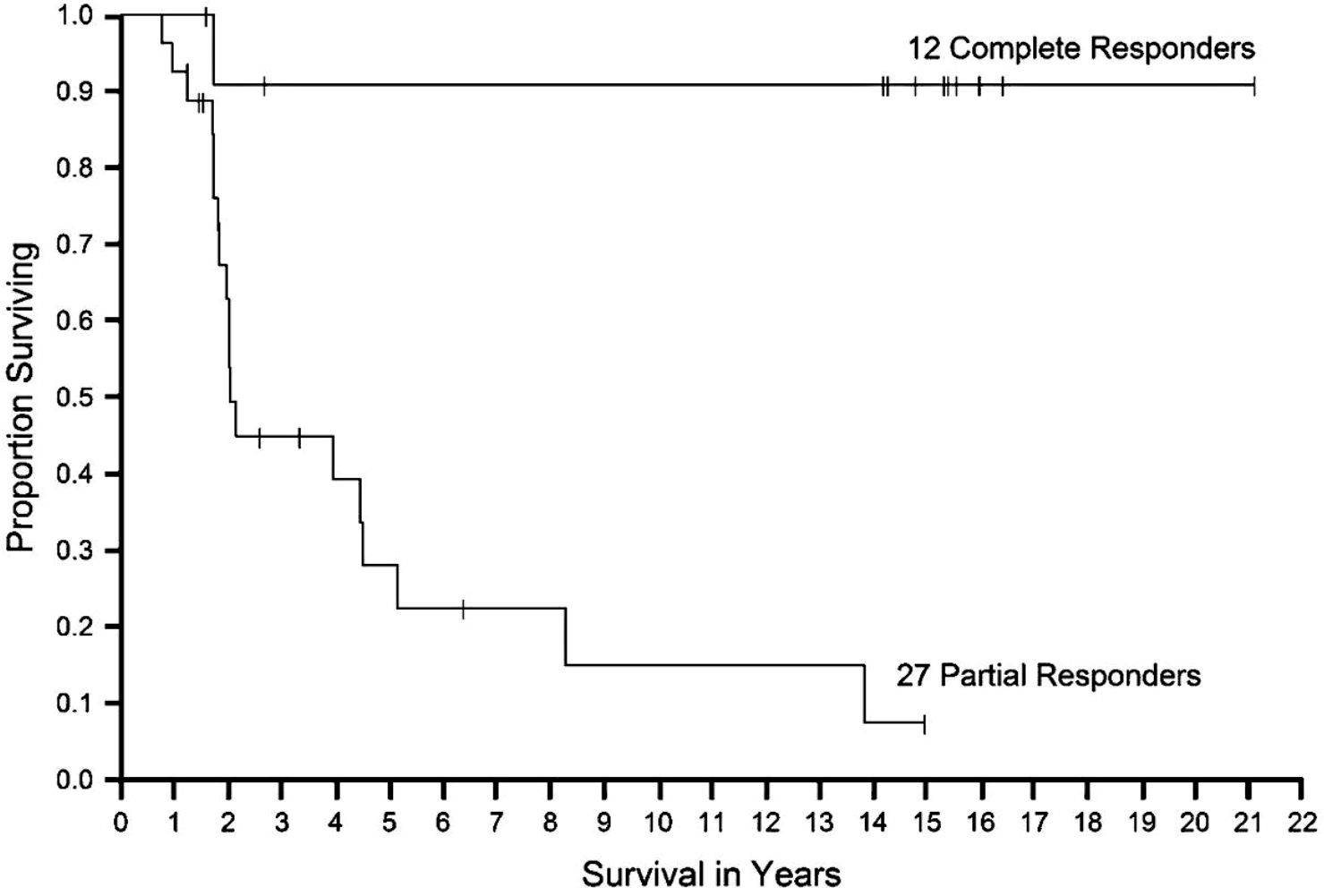
2008

Interleukin-2 for Metastatic RCC



Interleukin-2 for Metastatic Melanoma

305 Patients Treated with High-Dose IL-2



Initial Approaches to Improving IL-2

- Understand the T-cells mediating these responses
- Vaccinate patients to generate more tumor-reactive T-cells
- Grow tumor-reactive T-cells in vitro and administer them

Initial Approaches to Improving IL-2

- Understand the T-cells mediating these responses

- Vaccinate patients to generate more tumor-reactive T-cells

- Grow tumor-reactive T-cells in vitro and administer them

"The Age of Enlightenment"

December 1991

A Gene Encoding an Antigen Recognized by Cytolytic T Lymphocytes on a Human Melanoma

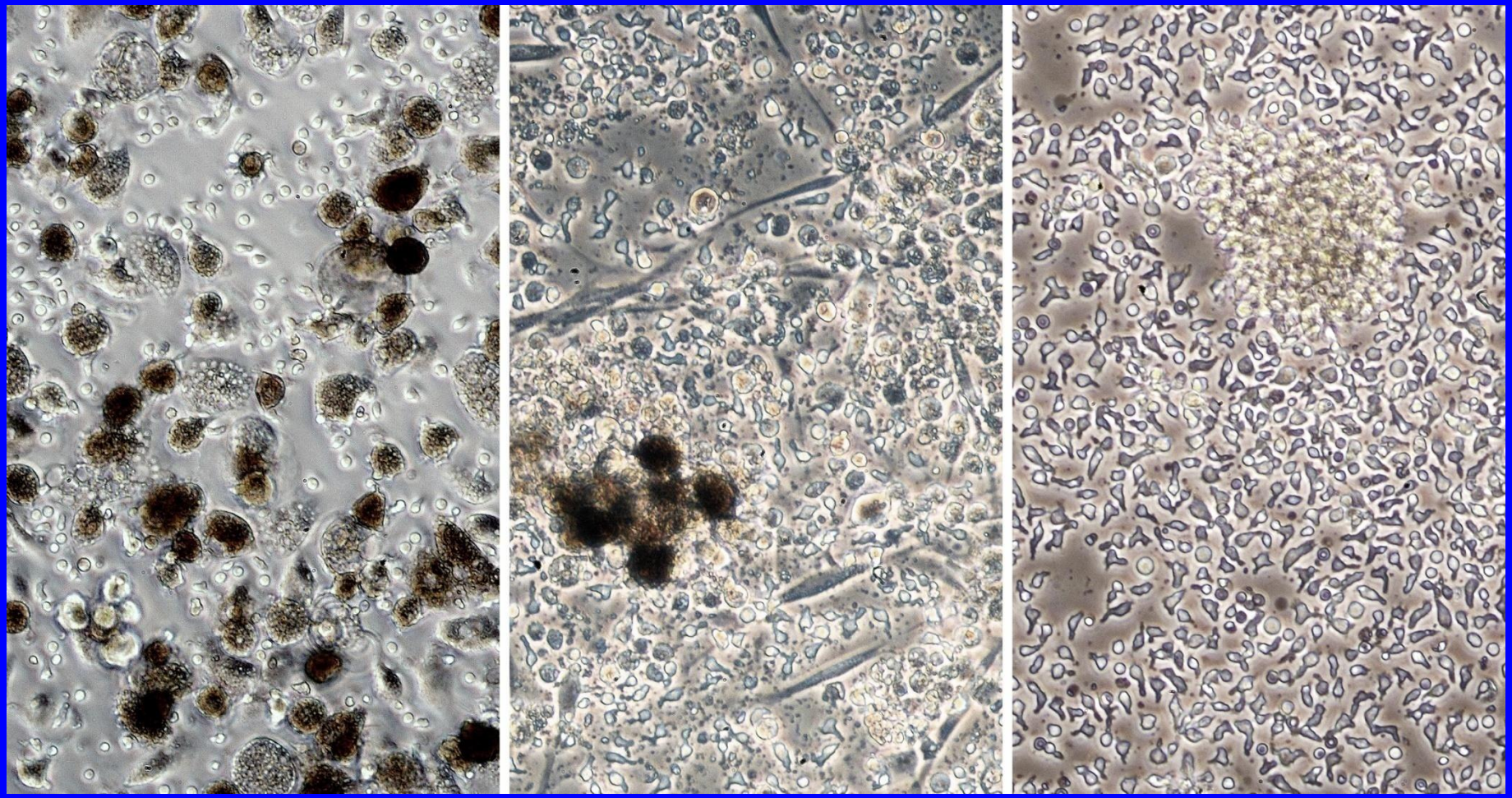
P. VAN DER BRUGGEN, C. TRAVERSARI,* P. CHOMEZ, C. LURQUIN, E. DE PLAEN, B. VAN DEN EYNDE, A. KNUTH, T. BOON†

The MAGE-1 antigen was the basis of the recognition of a patient's melanoma by T-cells which had been generated by repeated stimulation with that tumor

Tumor Infiltrating Lymphocytes (TIL)

- Almost all tumors contain lymphocytes that have infiltrated into them from the host
- Placing the entire tumor into culture with IL-2 (T-cell growth factor) will allow the TIL to expand while the tumor cells grow poorly
- TIL grown with IL-2 from human melanomas often show the ability to recognize and kill the tumor they were grown from (other TIL do not)

Melanoma TIL
(Tumor Infiltrating Lymphocytes)



Fresh digest One week Two weeks

Figuring Out What Tumor-Reactive Melanoma TIL Are Recognizing

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 3515–3519, April 1994
Immunology

Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor

(tumor antigen/immunotherapy/HLA-A2/melanocyte/MART-1)

YUTAKA KAWAKAMI*†, SIONA ELIYAHU*, CYNTHIA H. DELGADO*, PAUL F. ROBBINS*, LICIA RIVOLTINI*, SUZANNE L. TOPALIAN*, TORU MIKI‡, AND STEVEN A. ROSENBERG*

*Surgery Branch and ‡Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

MART-1 (Melanoma Antigen Recognized by T-cells), a protein involved in pigment production, was recognized by tumor-reactive melanoma TIL

Melanoma-Associated Antigens Found Using TIL

- Tissue differentiation antigens (pigment production)*
- Tumor-germline (previously tumor-testis) antigens*
- Tumor-specific mutations

*Normal proteins shared by multiple melanomas

Vaccinations Against Defined Melanoma Antigens

- MART-1, gp100, tyrosinase, NY-ESO1, MAGE family, TRP-2, Her-2 and telomerase were targeted with vaccine protocols
- Peptides, DNA, proteins, dendritic cells and recombinant viruses were used as modes of vaccination

nature medicine 2004

Cancer immunotherapy: moving beyond current vaccines

Steven A Rosenberg, James C Yang & Nicholas P Restifo

- 440 Patients were given 541 vaccines
- 96% had metastatic melanoma
- 765 patients in 35 other vaccine trials were also reviewed
- The overall response rate in the 440 Surgery Branch patients was 2.6% with only 3 patients reaching CR (0.5%) and only 3 responders had visceral involvement
- The 765 reviewed patients had an overall response rate (PR+CR) of 3.8%

Conclusions

- Cancer vaccines alone do not treat patients with metastatic cancer effectively
- The few anecdotal responses are rarely complete and are often against cutaneous or nodal disease
- Better ways to augment the anti-tumor T-cell repertoire were needed

Adoptive Cellular Therapy

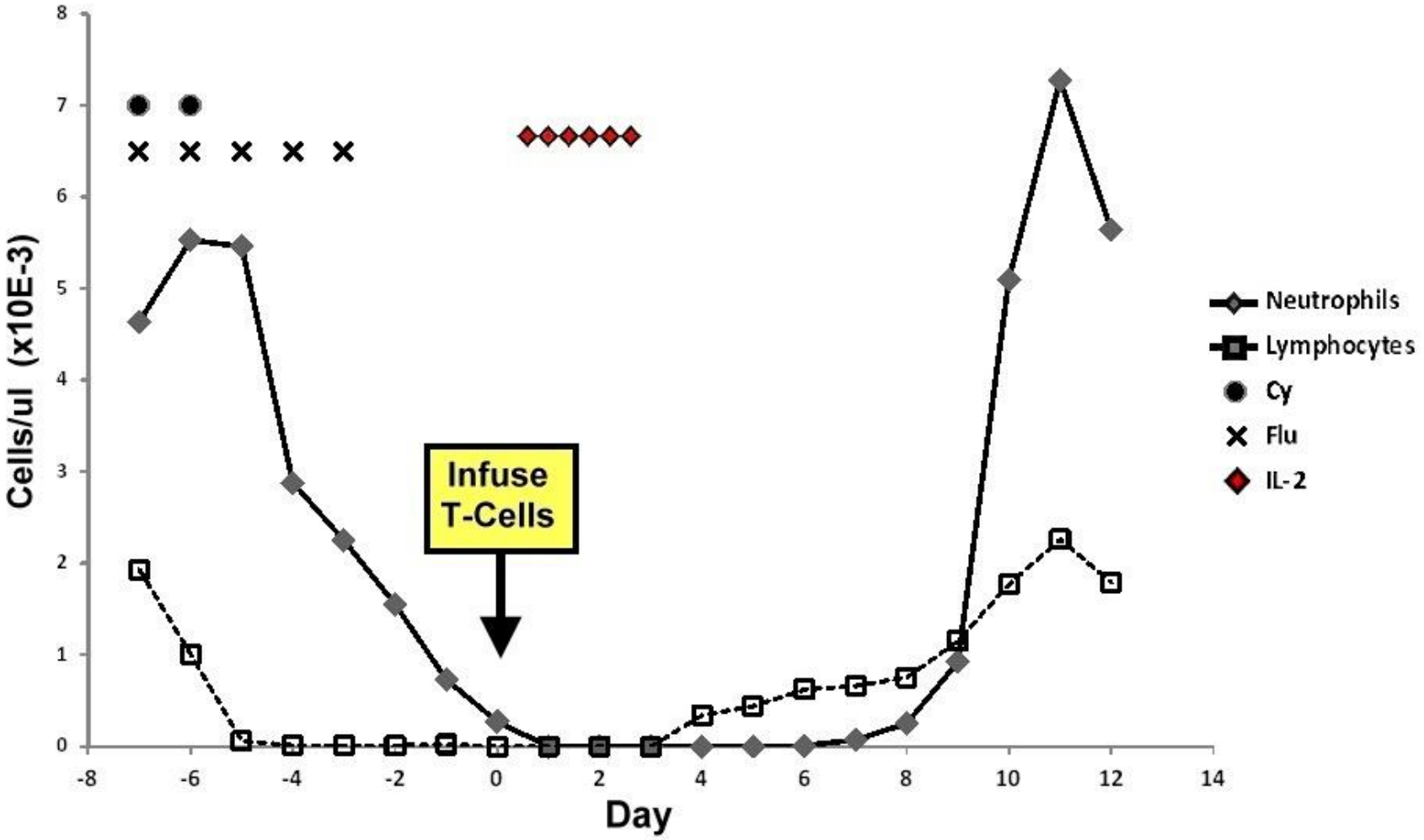
- Could cultured T-cells be infused in sufficient numbers to induce tumor rejection?
- What conditions will optimize the effectiveness of these T-cells?
- Where would one consistently obtain T-cells which recognize tumors?

TIL from melanoma frequently have anti-tumor activity when expanded in IL-2

Principles of Adoptive Cellular Therapy

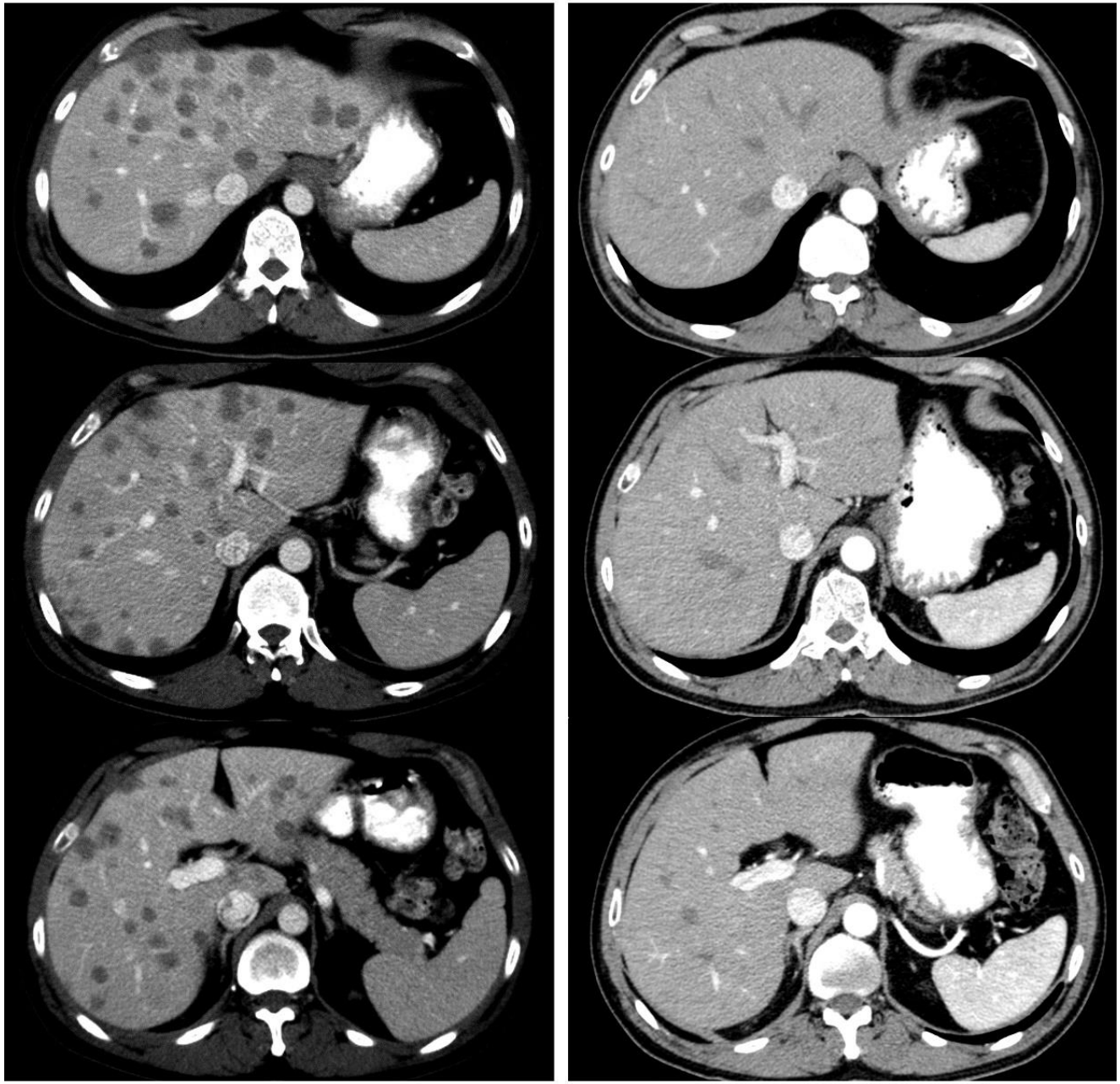
- T-cell transfer is enhanced when the recipient is temporarily immuno-suppressed prior to transfer
  - Deletes host regulatory T-cells
  - Stimulates host T-cell growth factors
- Giving systemic IL-2 with cells may support in vivo expansion and function

Cyclophosphamide + Fludarabine Preparative Chemotherapy



Other Sites: Lung

CR 99+ months

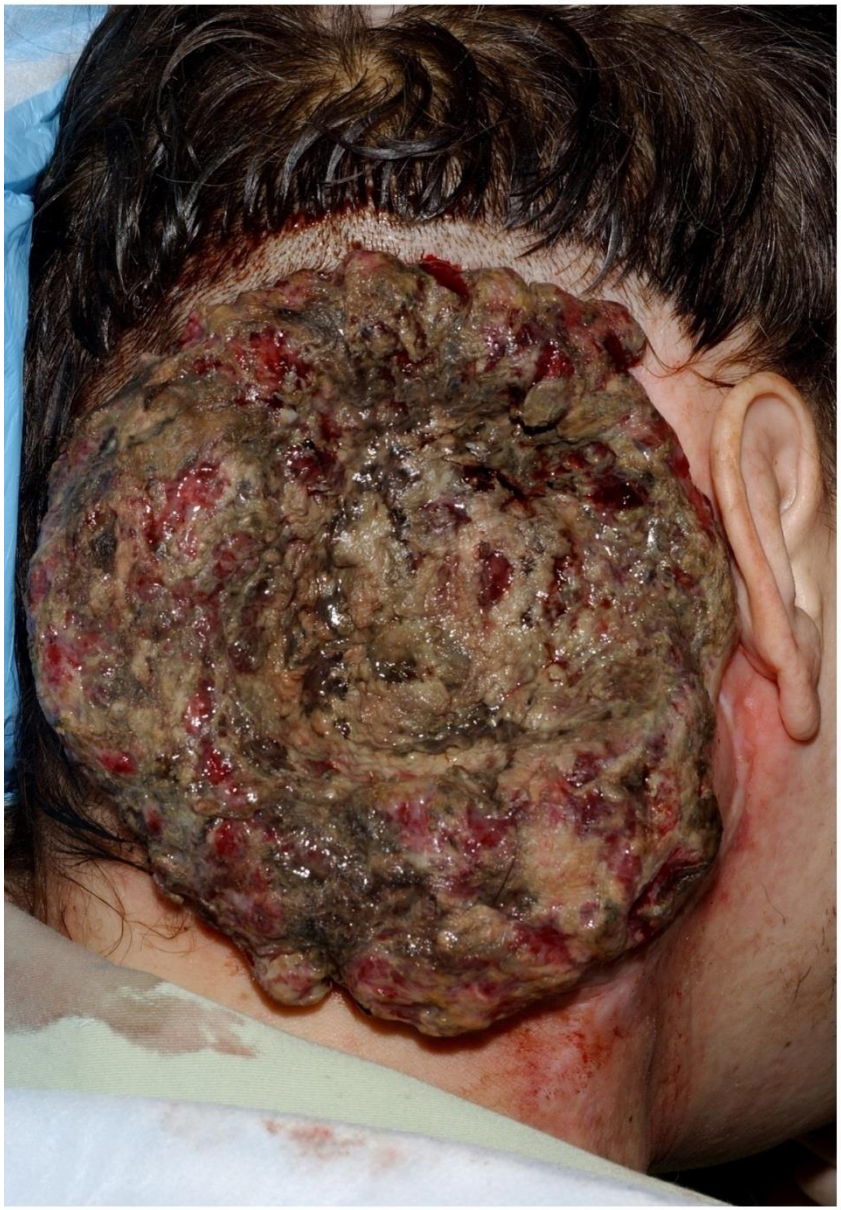


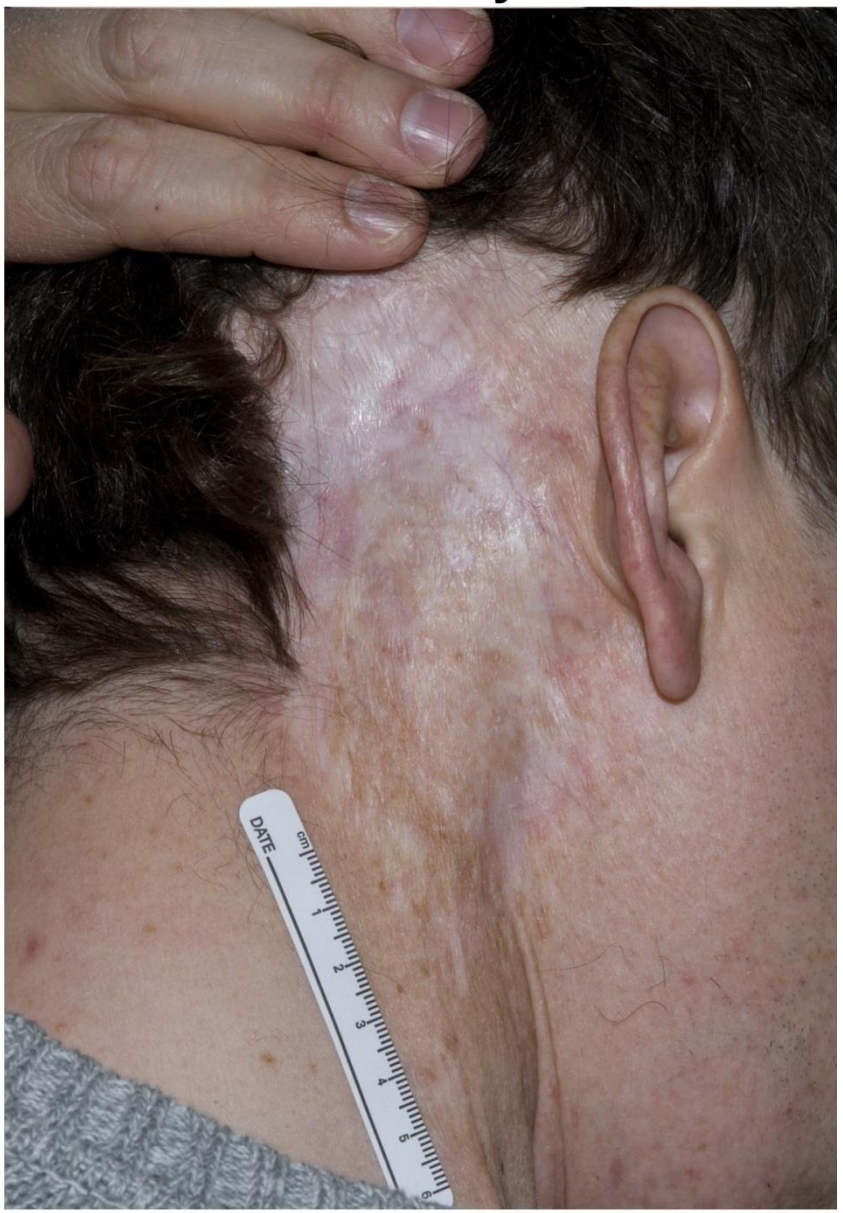
Nov 10, 2003

Feb 9, 2012

Other Sites: Lung

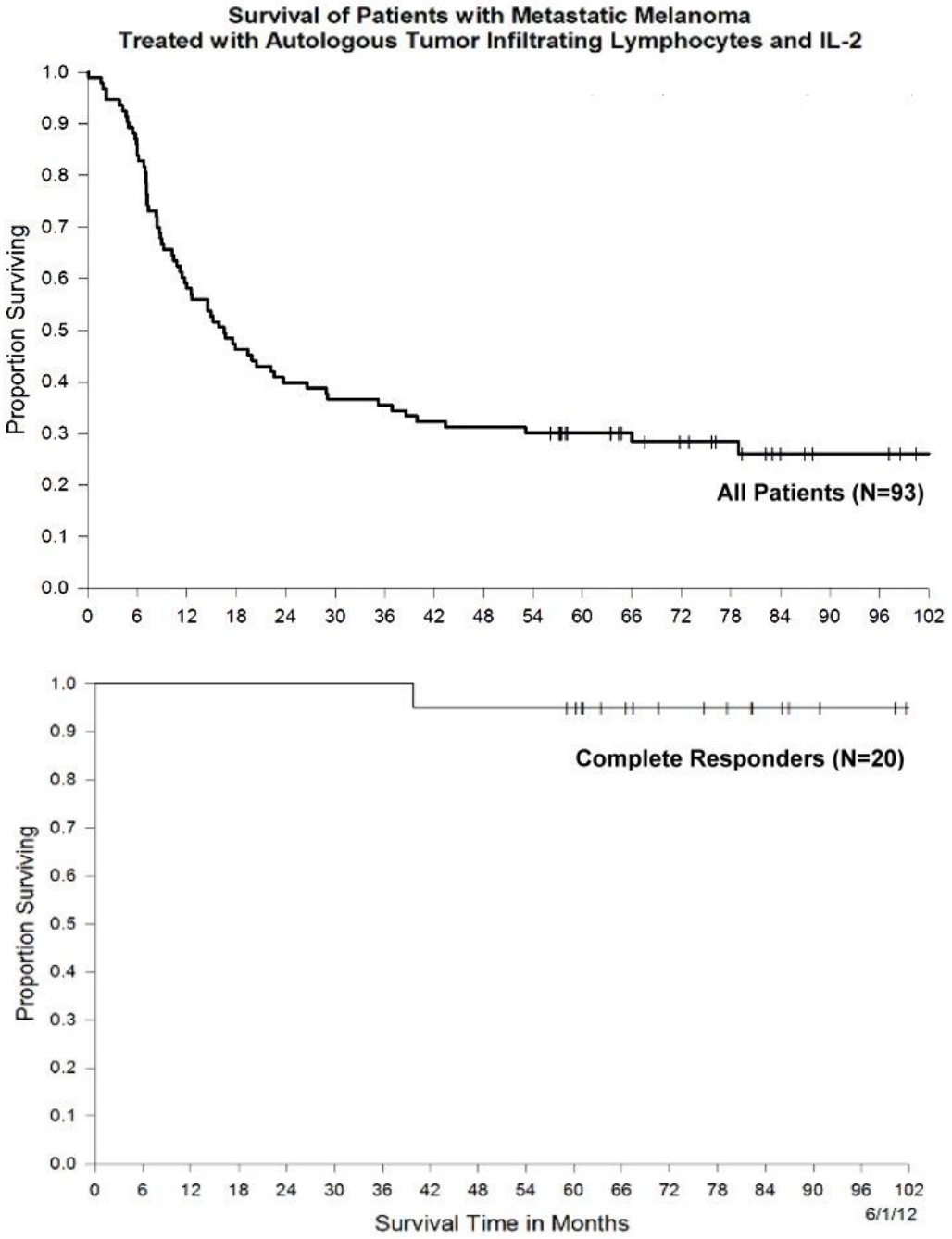
CR 6.7+ years





March 21, 2005

Dec 20, 2011

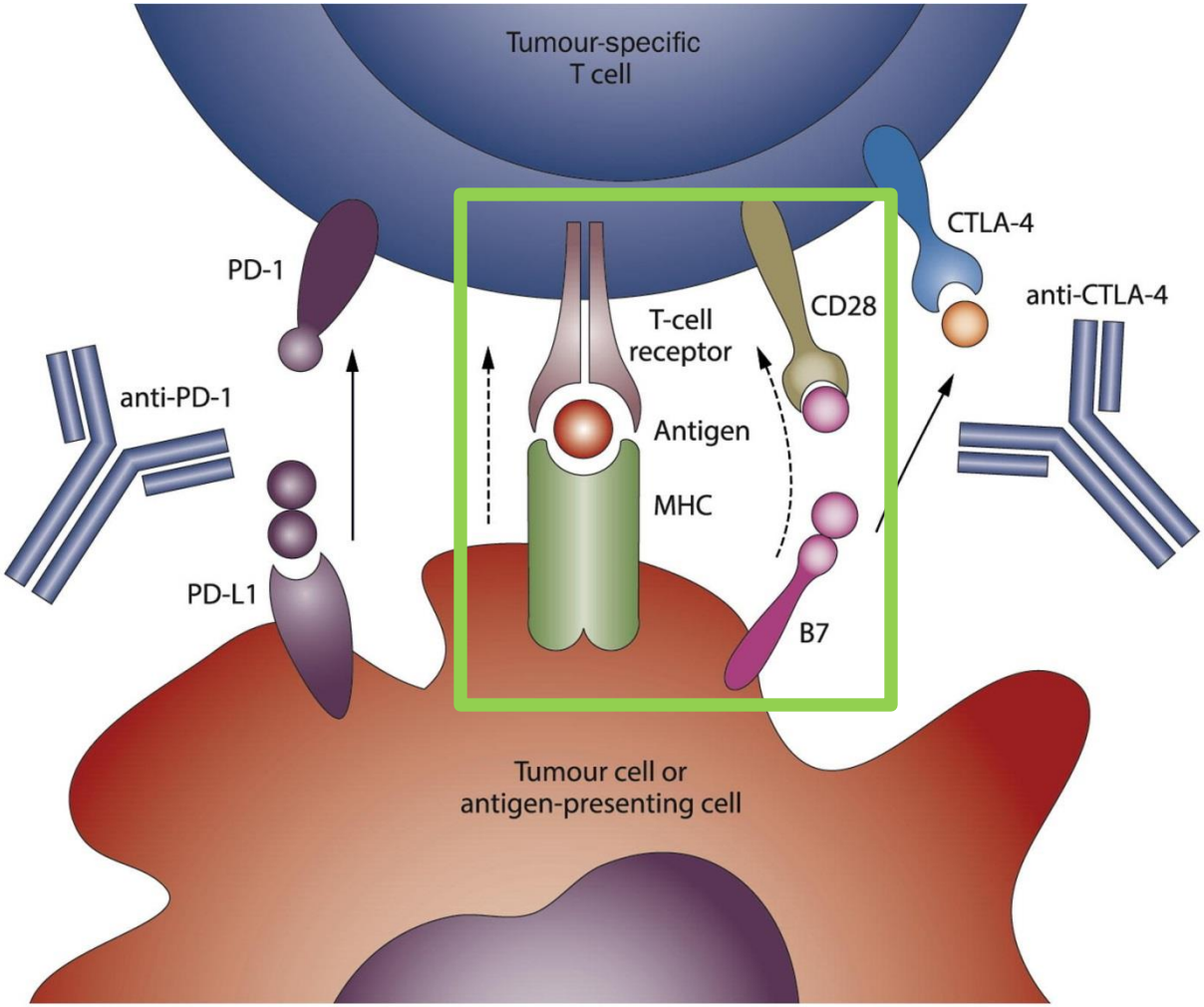

Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102
All Patients (N=93)
Complete Responders (N=20)
Survival Time in Months
6/1/12

What Other Factors Affect Tumor Rejection?

- T-cells are turned off by inhibitory receptors (activation "checkpoints")
  - CTLA4
  - PD1

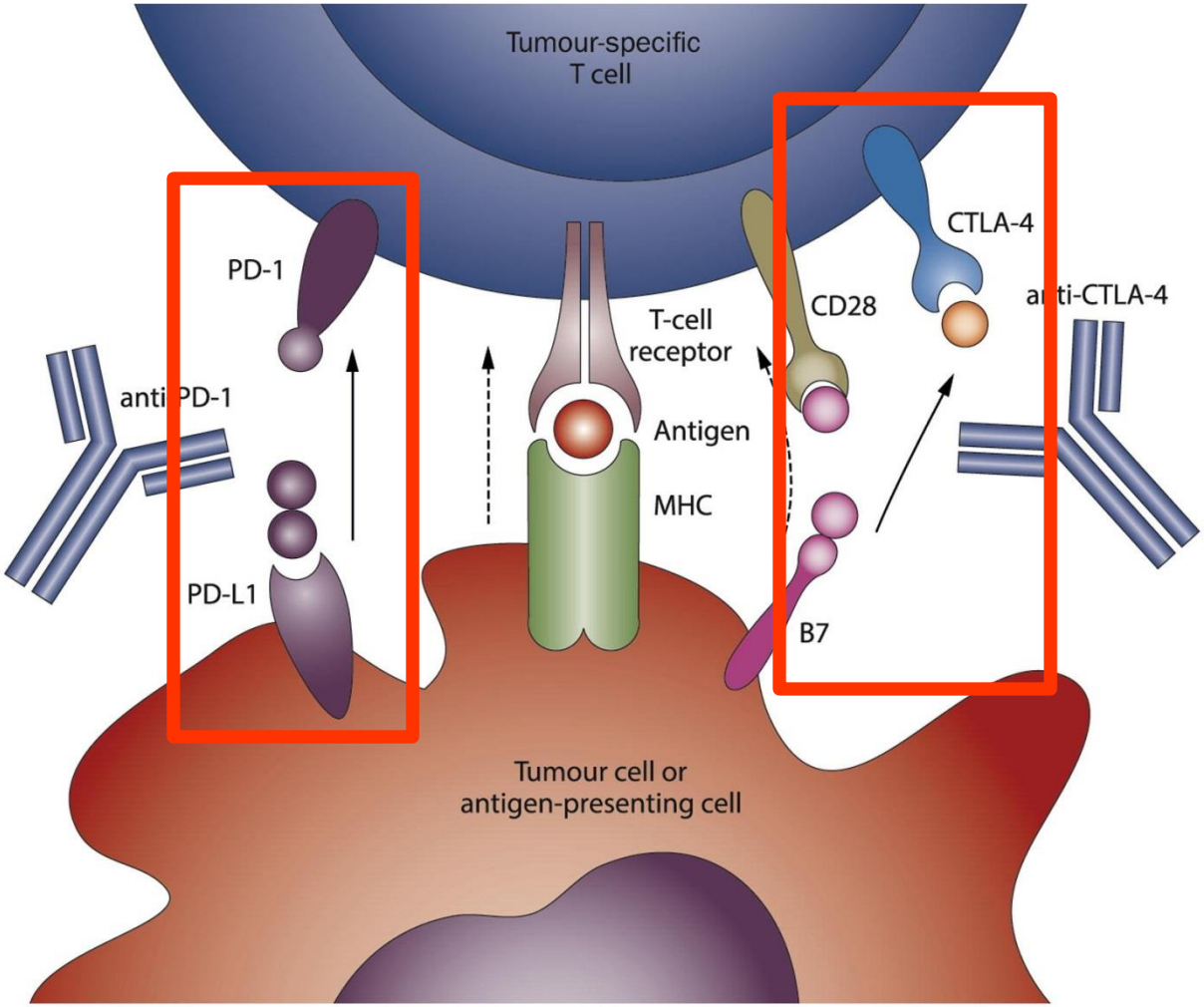
- Antibodies have been developed to block these "checkpoints" to preserve or sustain T-cell activation
  - Ipilimumab
  - Nivolumab

T-Cell Activation and Inhibition



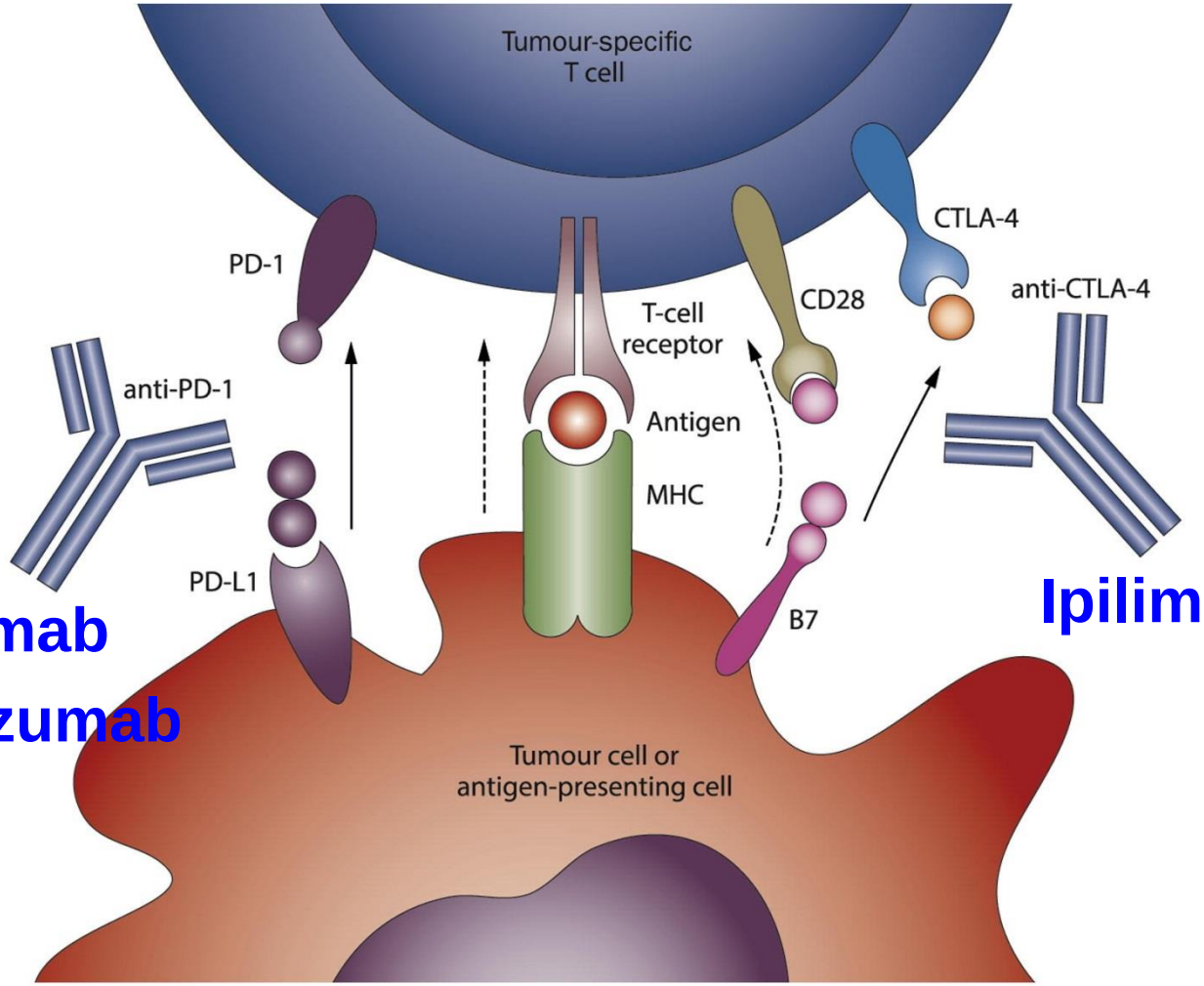
McDermott et al, Ca Treat Rev

T-Cell Activation and Inhibition



McDermott et al, Ca Treat Rev

T-Cell Activation and Inhibition



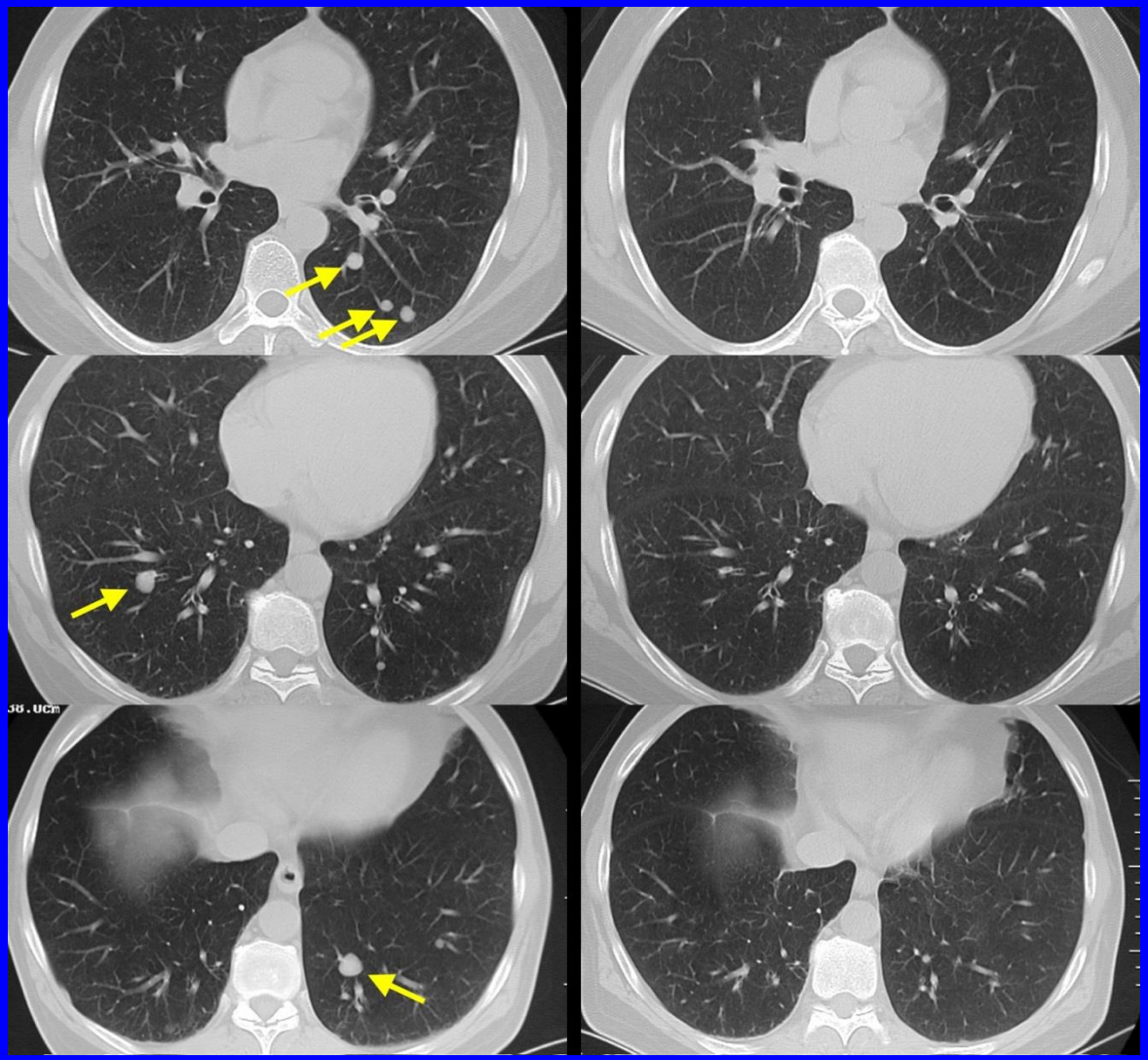
Nivolumab

Pembrolizumab

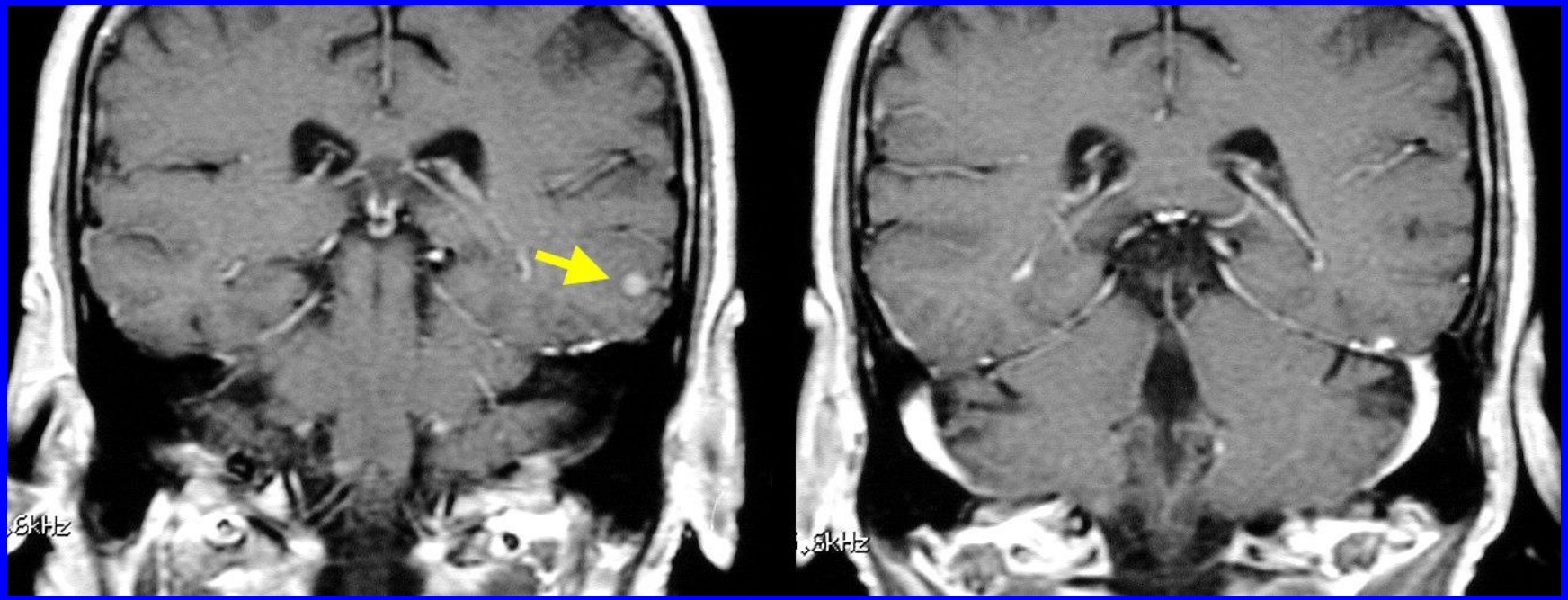
Ipilimumab

McDermott et al, Ca Treat Rev

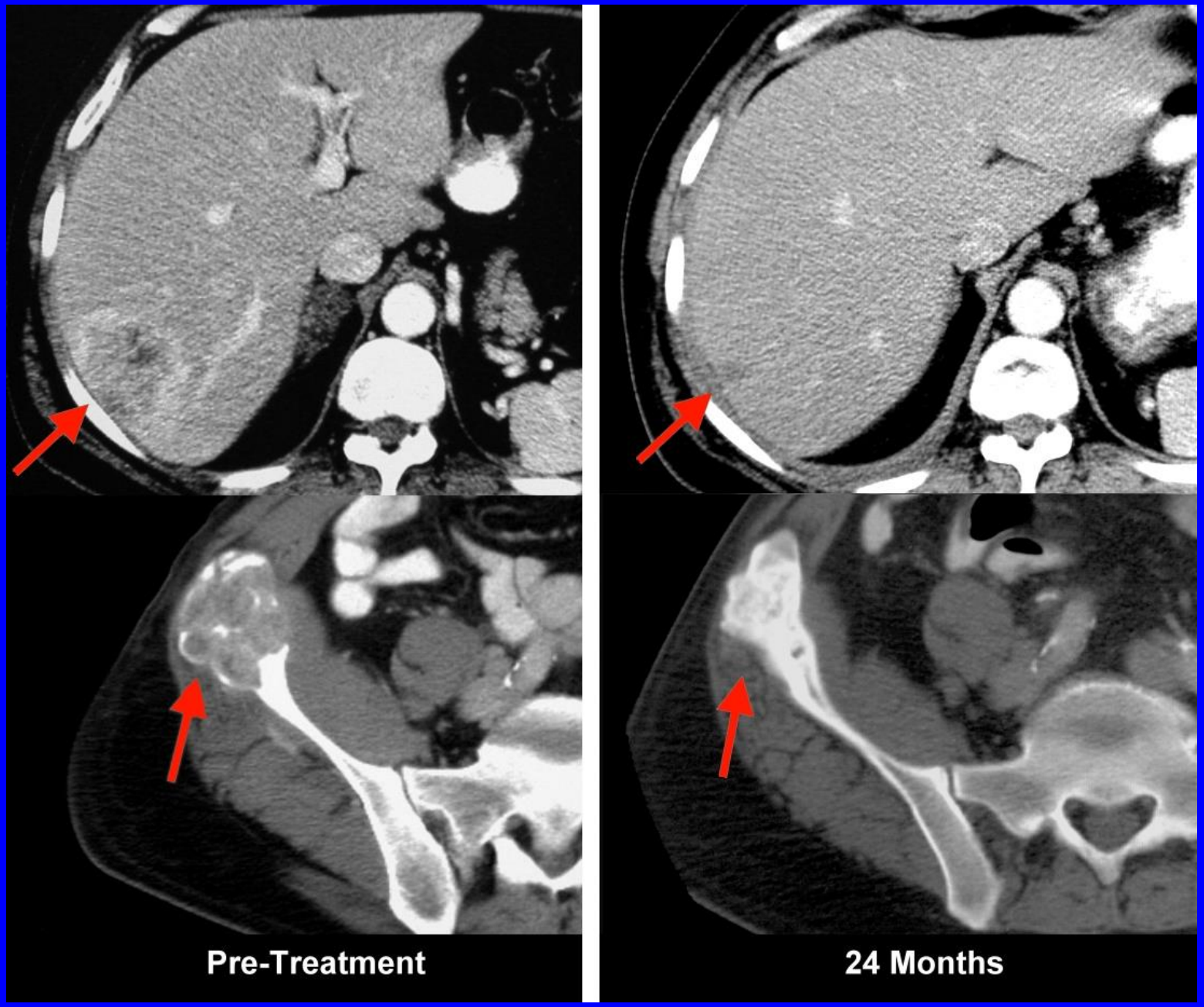
Metastatic Melanoma Treated with Ipilimumab (Anti-CTLA4)



Metastatic Melanoma Treated with Ipilimumab (Anti-CTLA4)



•Ipilimumab for RCC



Randomized Trial with Ipilimumab

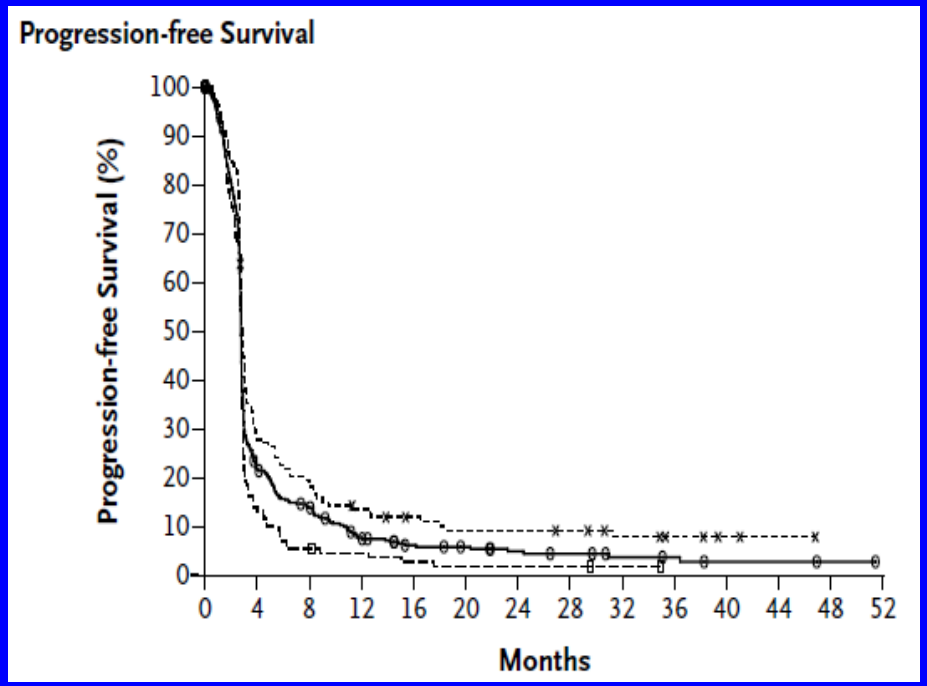
PR= 6.5%
CR= 0.5%

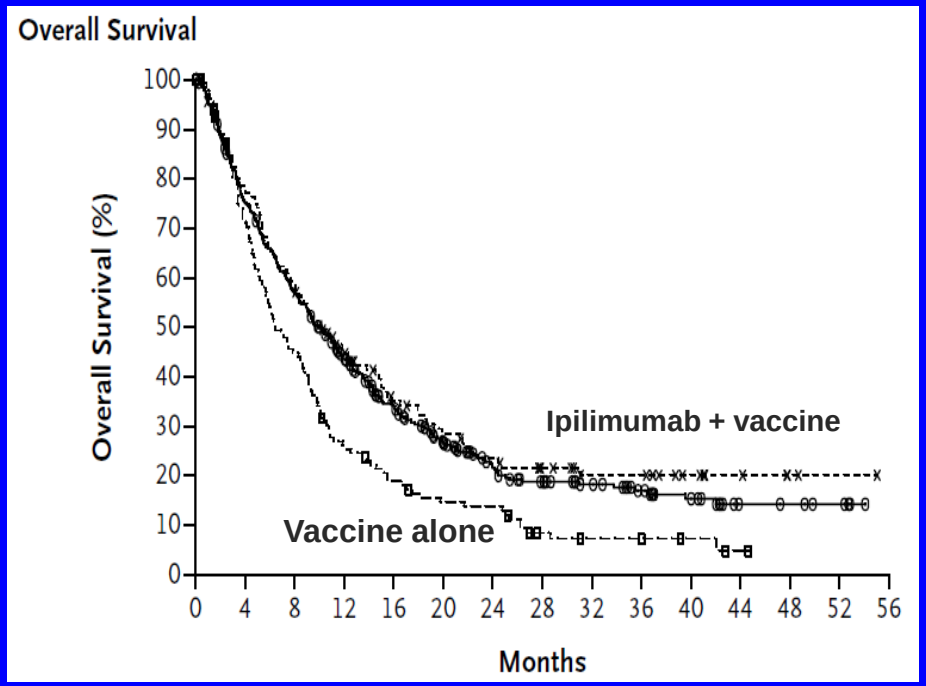
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 19, 2010 VOL. 363 NO. 8

Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

F. Stephen Hodi, M.D., Steven J. O'Day, M.D., David F. McDermott, M.D., Robert W. Weber, M.D., Jeffrey A. Sosman, M.D., John B. Haanen, M.D., Rene Gonzalez, M.D., Caroline Robert, M.D., Ph.D., Dirk Schadendorf, M.D., Jessica C. Hassel, M.D., Wallace Akerley, M.D., Alfons J.M. van den Eertwegh, M.D., Ph.D., Jose Lutzky, M.D., Paul Lorigan, M.D., Julia M. Vaubel, M.D., Gerald P. Linette, M.D., Ph.D., David Hogg, M.D., Christian H. Ottensmeier, M.D., Ph.D., Celeste Lebbé, M.D., Christian Peschel, M.D., Ian Quirt, M.D., Joseph I. Clark, M.D., Jedd D. Wolchok, M.D., Ph.D., Jeffrey S. Weber, M.D., Ph.D., Jason Tian, Ph.D., Michael J. Yellin, M.D., Geoffrey M. Nichol, M.B., Ch.B., Axel Hoos, M.D., Ph.D., and Walter J. Urba, M.D., Ph.D.



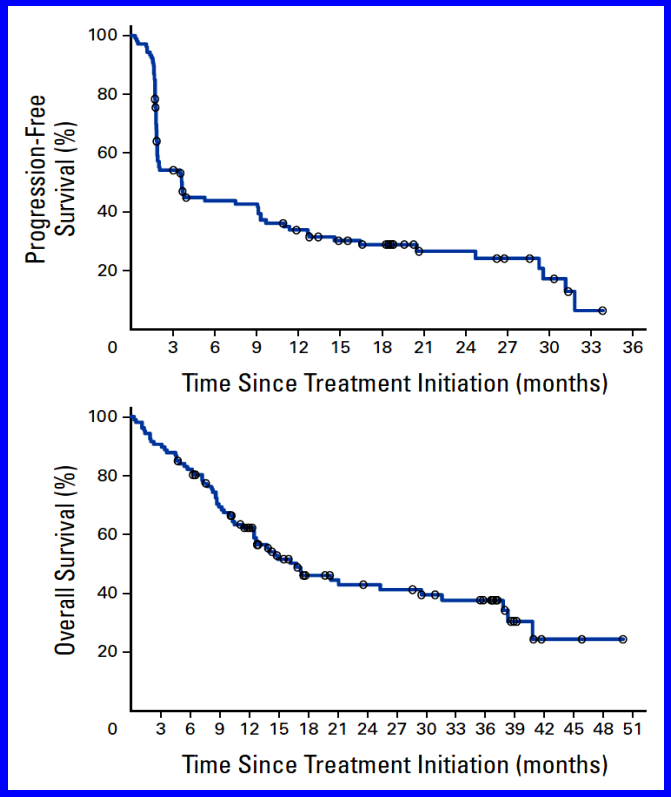


Anti-PD1 Antibodies for Melanoma

Nivolumab

ORR= 31%

CR= 3%?

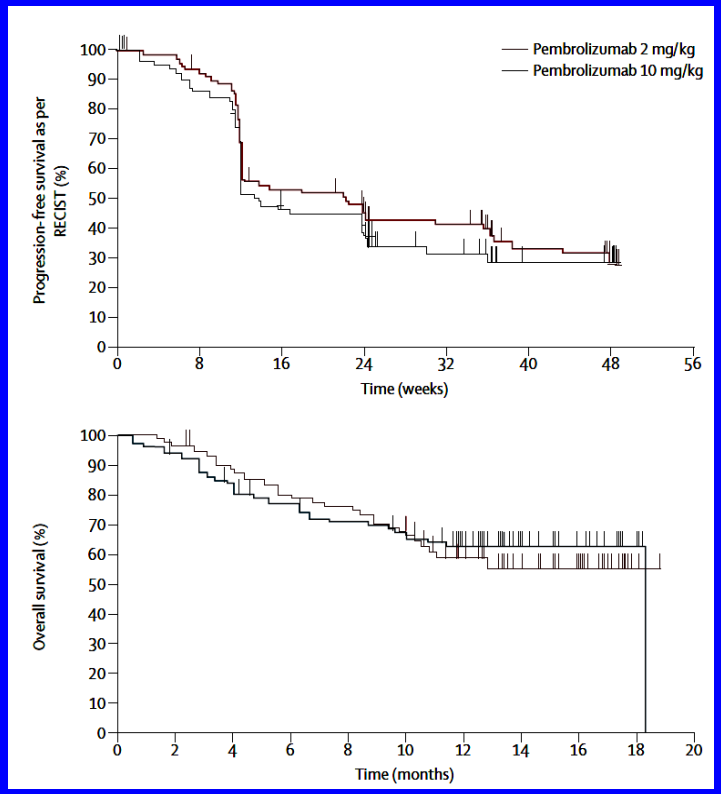


Topalian, JCO

Pembrolizumab

ORR= 26%

CR= 1%?



Robert, Lancet

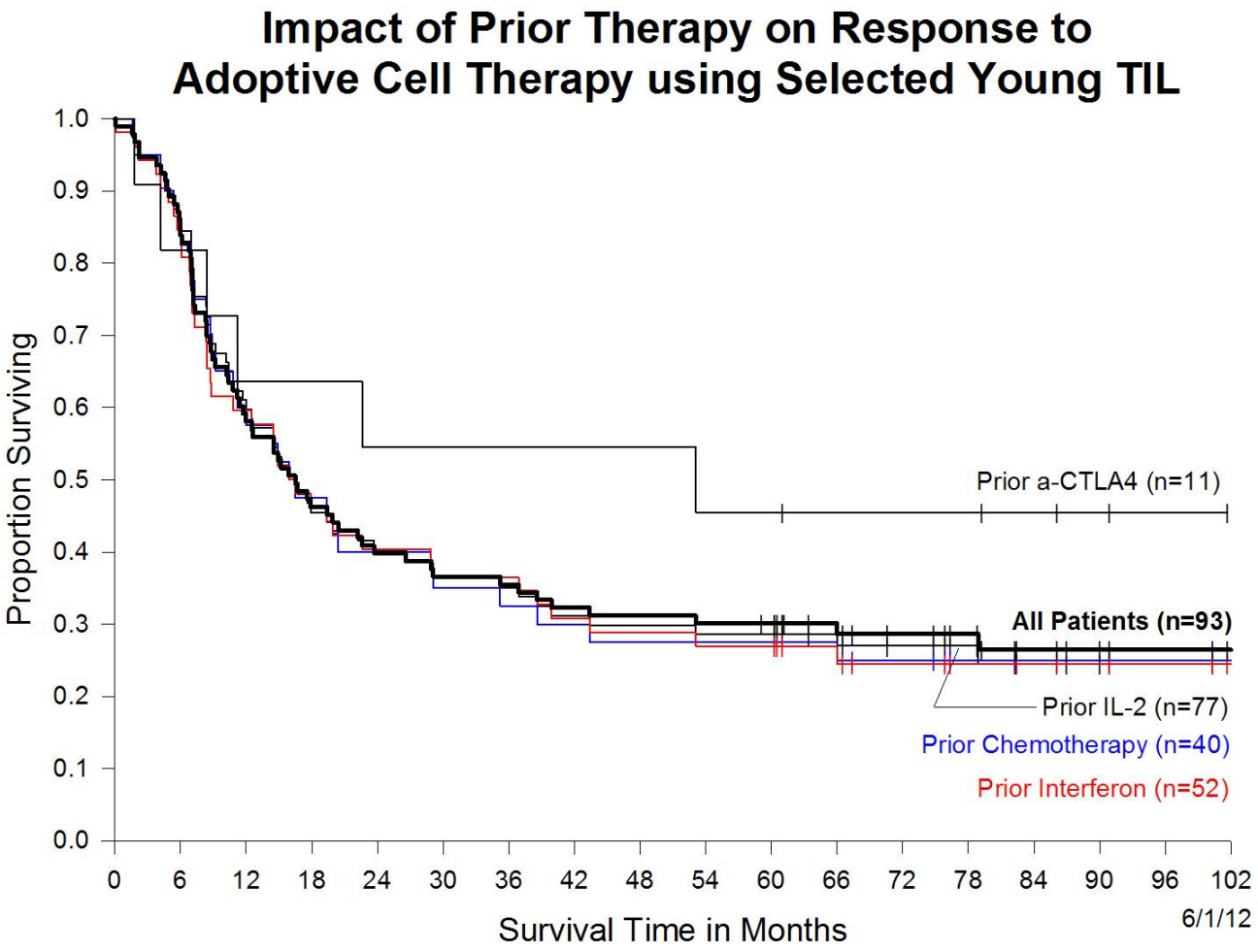
PD1/PDL1 Blockade for Other Cancers

Nivolumab Phase I Long-Term Results

Tumor Type	ORR (%; no patients)	Response Duration (median; mo)	OS (median; mo)	Survival (%)	
				1 yr	2 yr
Melanoma	31 (33/107)	24.0	16.8	62	43
NSCLC	17 (22/129)	17.0	9.6	42	14
RCC	29 (10/34)	12.9	>22	70	50

Topalian, Sznol, Brahmer et al. ASCO 2013

Are the Same Patients Responding to All Immunotherapies?



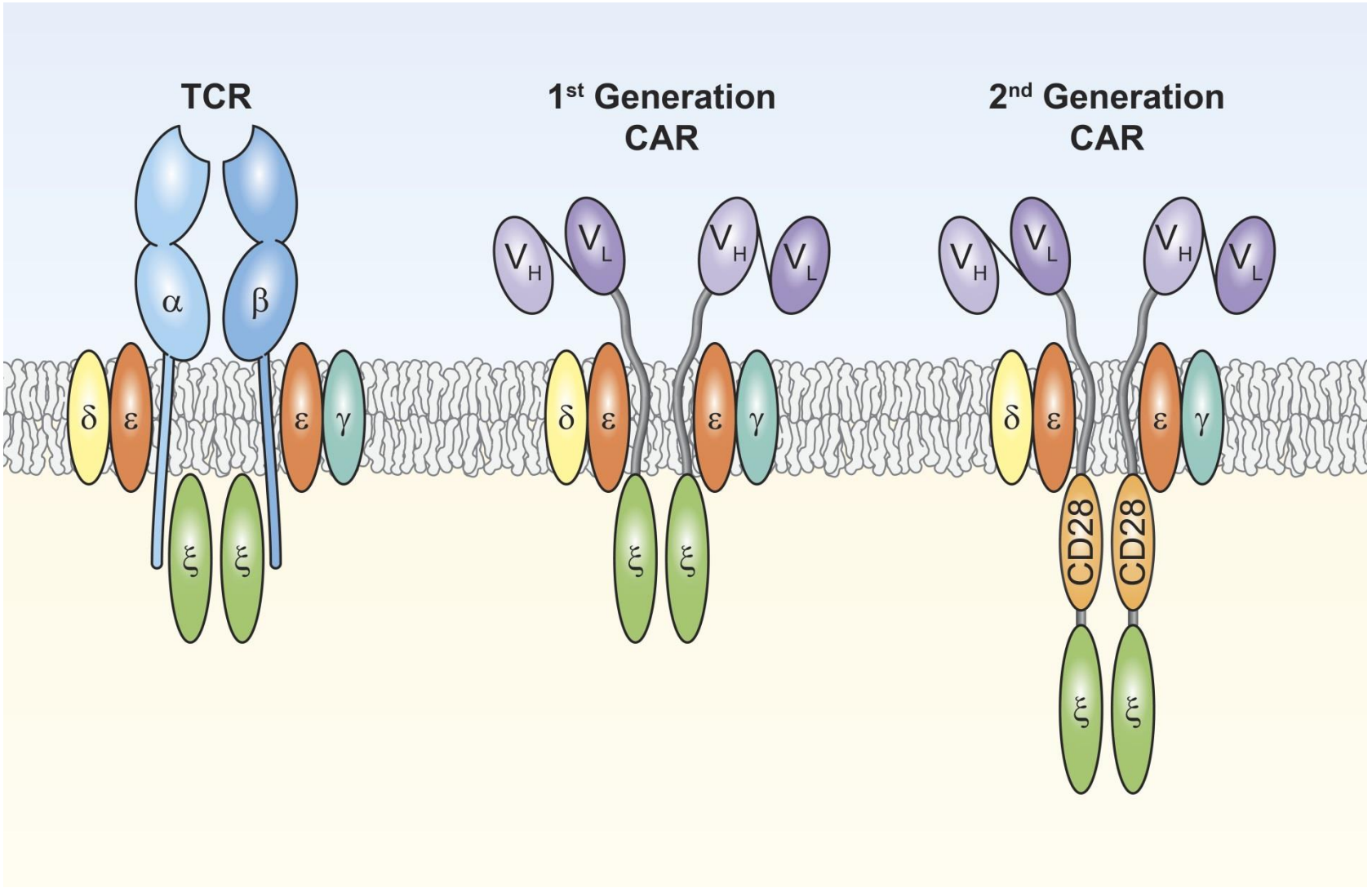
The Main Obstacle: Getting Tumor-Specific T-Cells

- Not all melanomas have reactive TIL, and some patients still do not respond
- The TIL from other cancers are rarely tumor-reactive
- Most cancer cells cannot even be grown in the lab for testing against T-cells

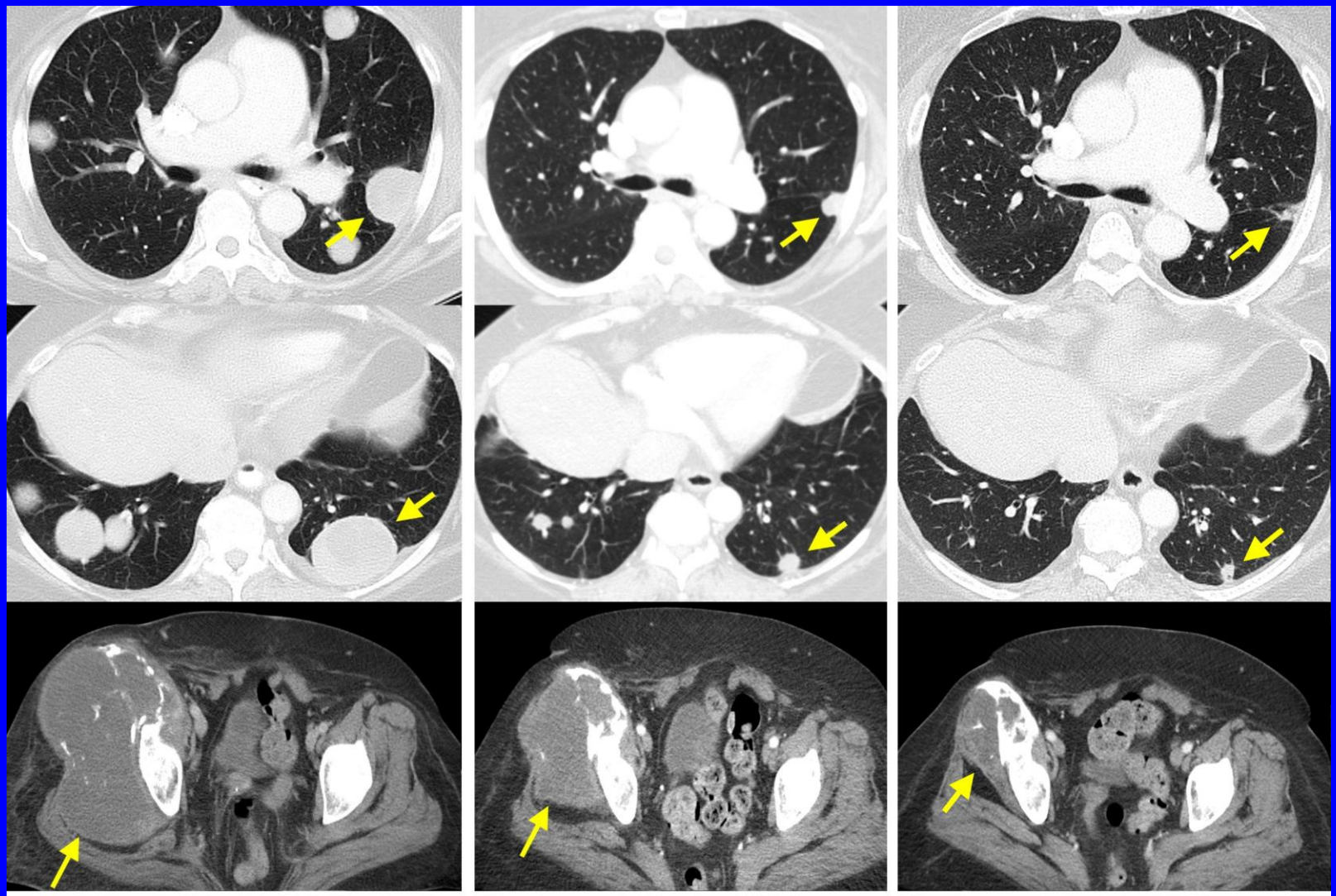
One Approach: Genetically Engineer Anti-Tumor Receptors into Peripheral Blood Lymphocytes

- If a tumor-reactive T-cell is found, its T-cell receptor can be retrovirally introduced into another patient's PBL
- Other "unnatural" receptors such as CAR (chimeric antigen receptors) can also be used
- These cells are then given exactly as native T-cells are administered

Gene-Engineered Anti-Tumor Receptors



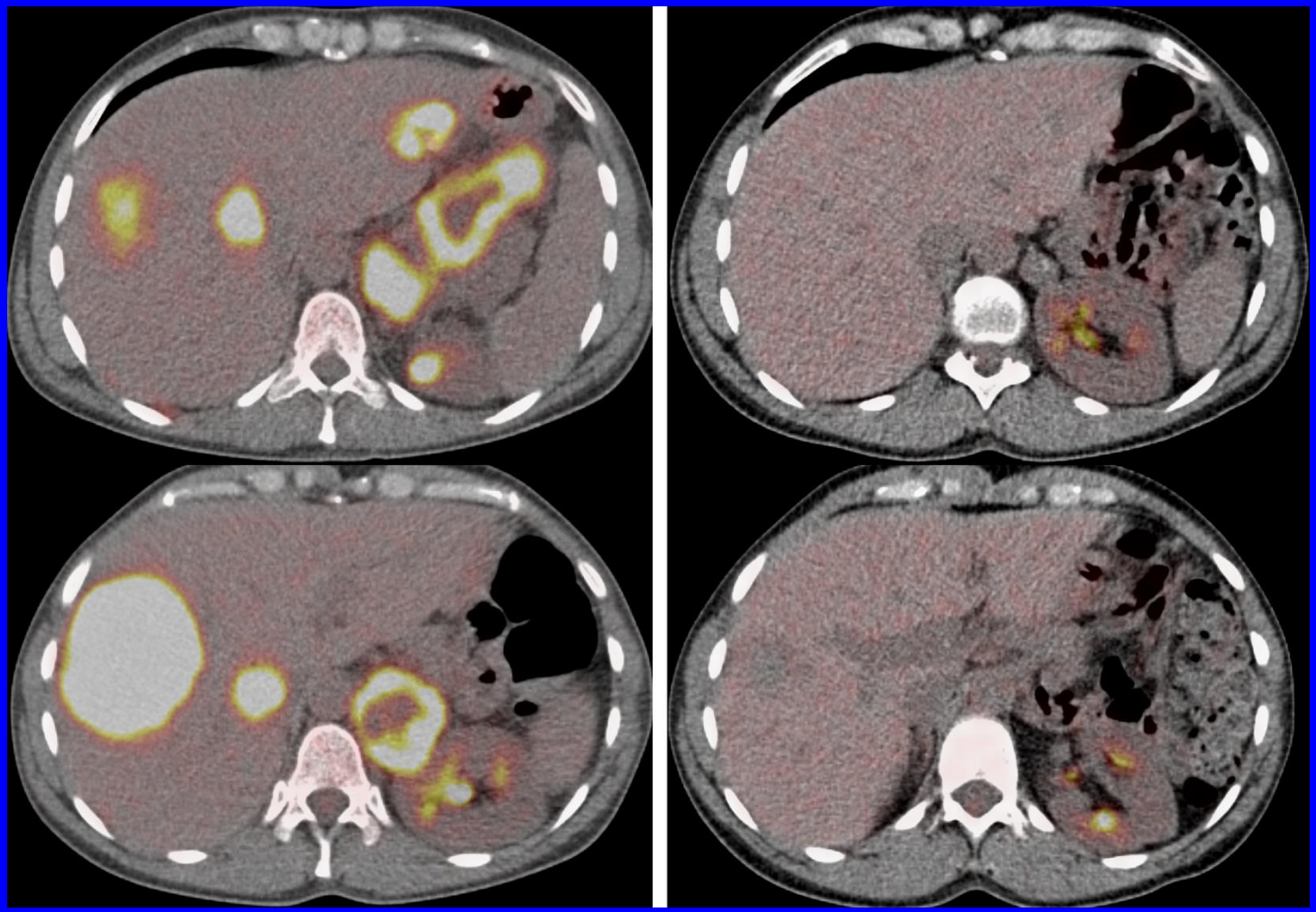
Anti-NY-ESO1 TCR (Synovial Sarcoma)



Anti-CD19 CAR
(Large B-Cell Lymphoma)

Prior Therapy:

R-CHOP
R-ICE
Brentuximab
R-HiDAC
Panobinostat
Lenalidomide
R-GDP
Anti-CD22
MAE



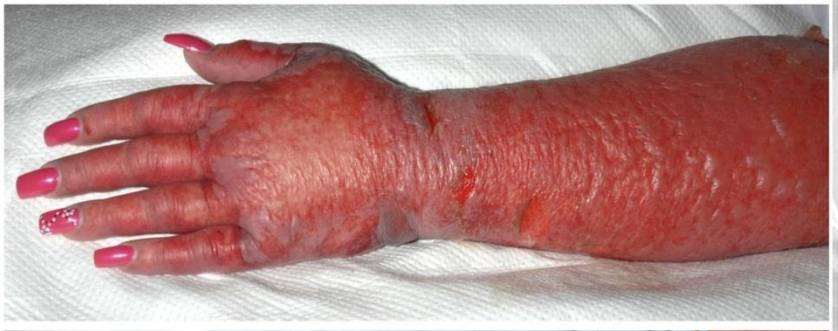
The Problem with Receptors Targeting Normal Tissue Antigens

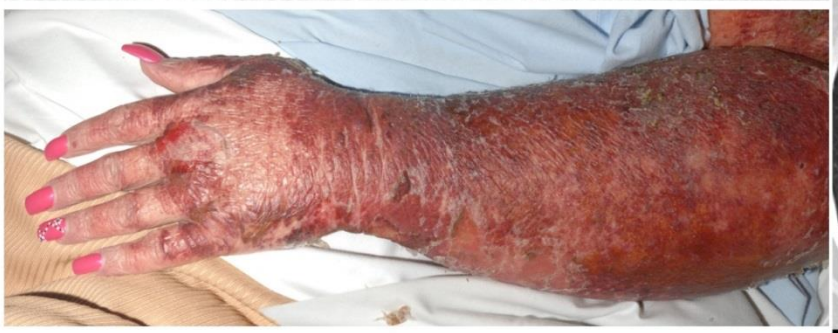
Some antigens are highly expressed on tumors but are also expressed by some normal tissues

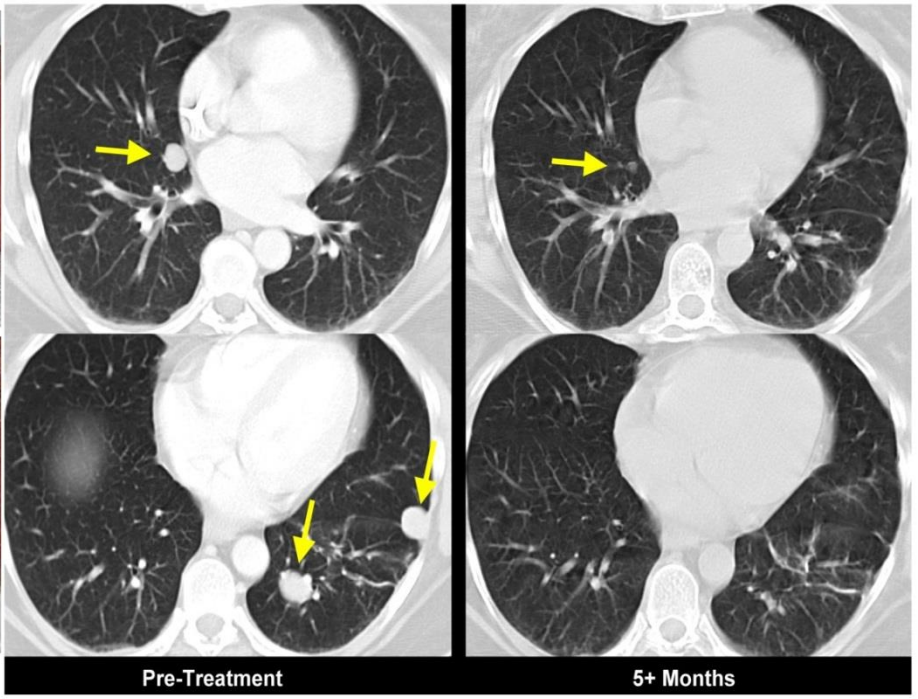
Gene-engineered T-cells can be used to specifically attack these targets hoping to impact the tumor but not the normal tissue

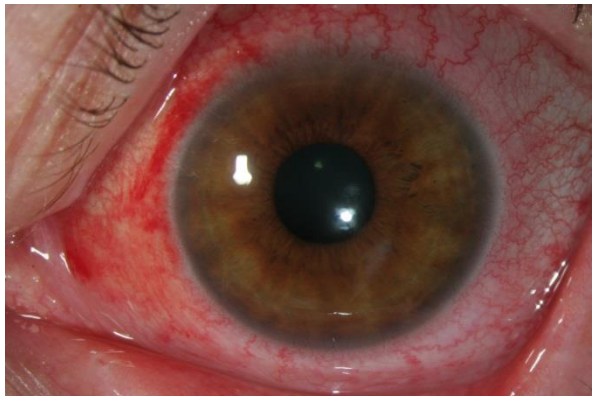
Accidental attack on important normal tissues can cause limiting toxicities

Targeting Melanocytic Proteins: MART-1

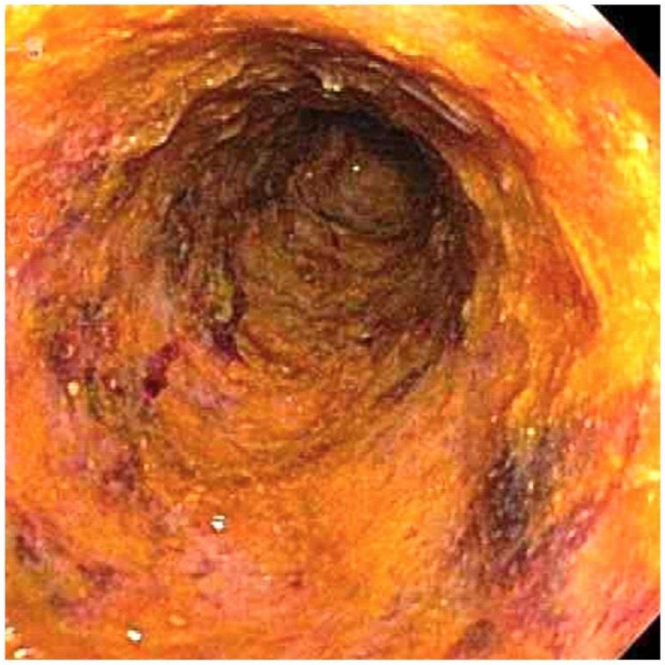


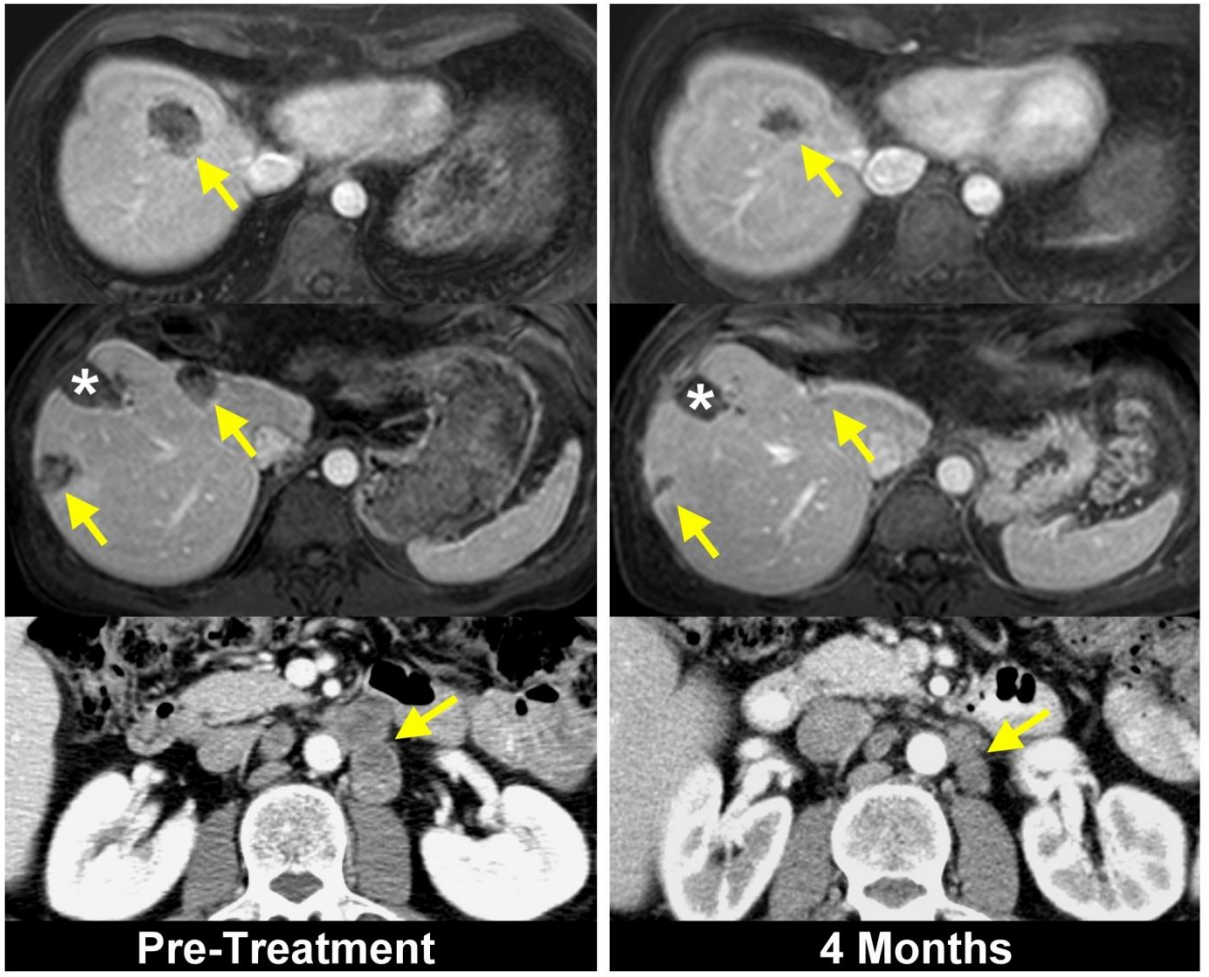






Targeting CEA



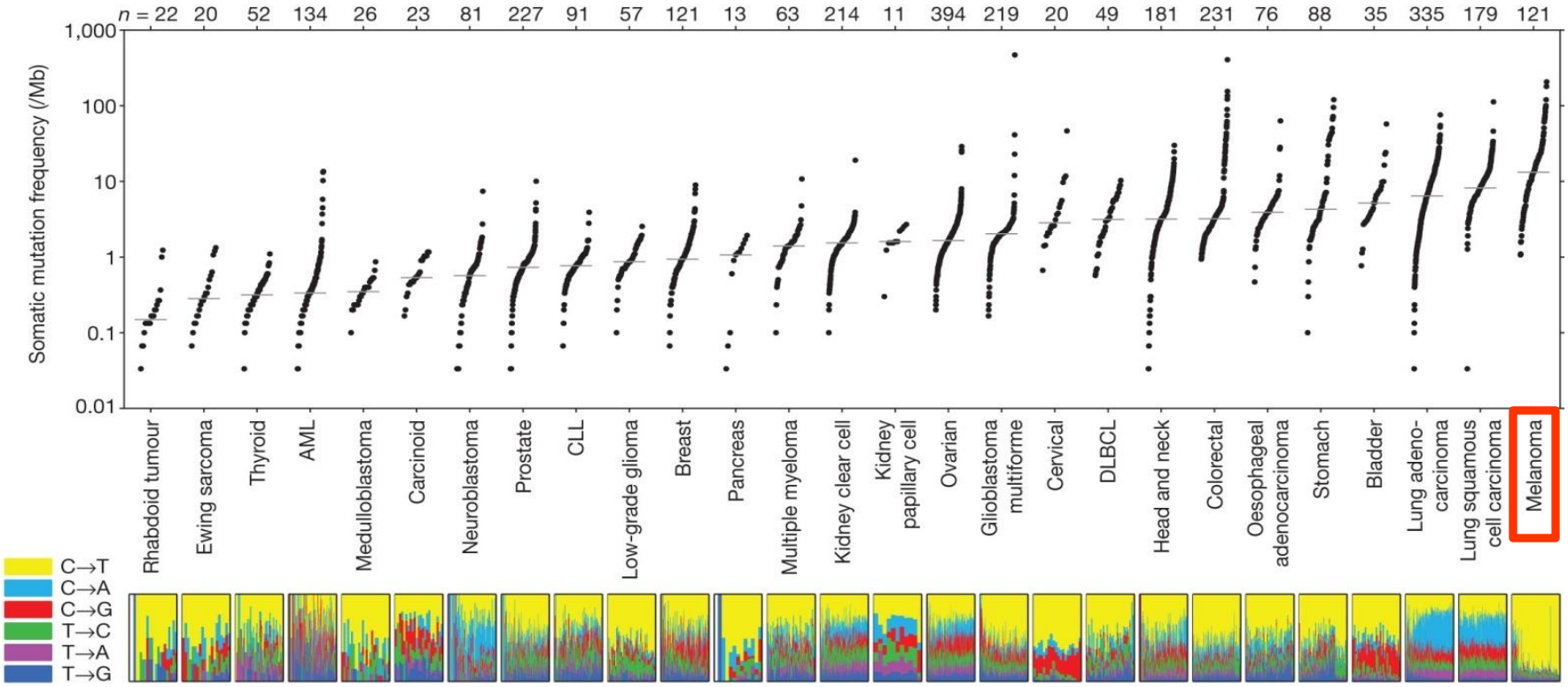


- Better targets are still needed

The Future of T-Cell Therapy for Melanoma and Other Cancers

- Some melanoma TIL were found which recognized mutated proteins in the patient's tumor
- All human cancers accumulate genetic mutations as the cause of their transformation
- The mutated proteins that result are completely tumor-specific and are "foreign" proteins to the host

Somatic mutation frequencies observed in exomes from 3,083 tumour–normal pairs.

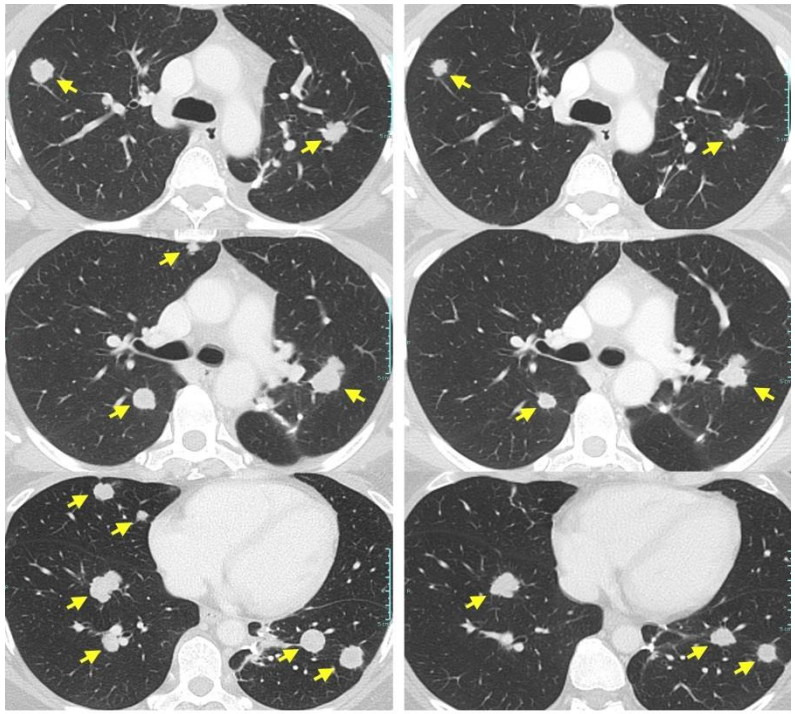


MS Lawrence *et al. Nature* 1-5 (2013) doi:10.1038/nature12213

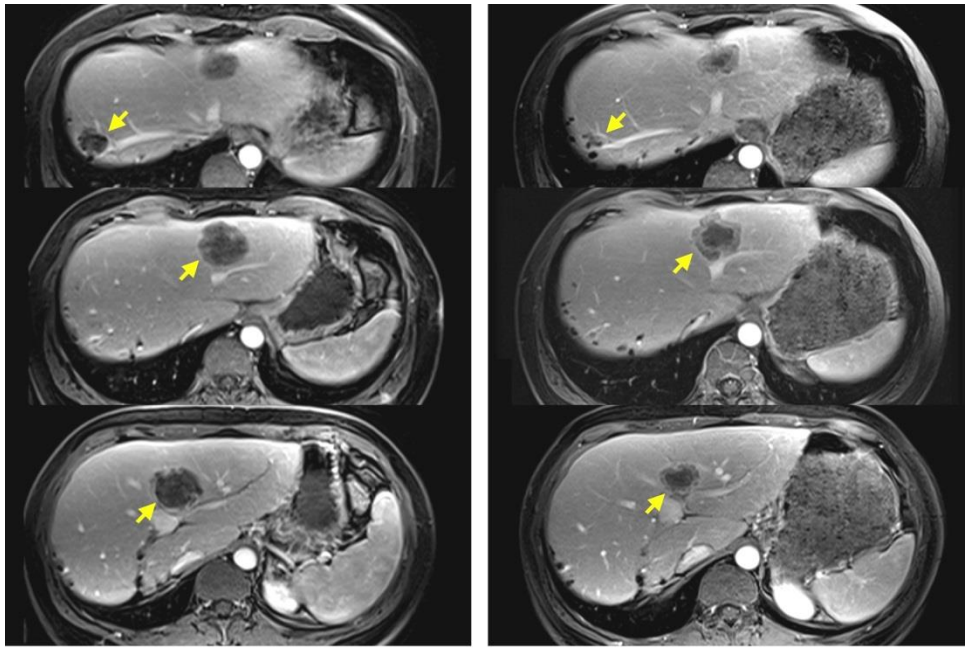
A Patient with Cholangiocarcinoma

- 43 yo F with metastatic cholangioCA who had progressed after hepatic and lung resections, cisplatin, gemcitabine and taxotere
- Had TIL grown from a lung metastasis
- Given Cy-Flu, $4x10^{10}$ TIL and 4 doses IL-2
- Had minimal response followed by tumor progression within a year

Best Response to Treatment #1



Pre-Treatment 7 Months



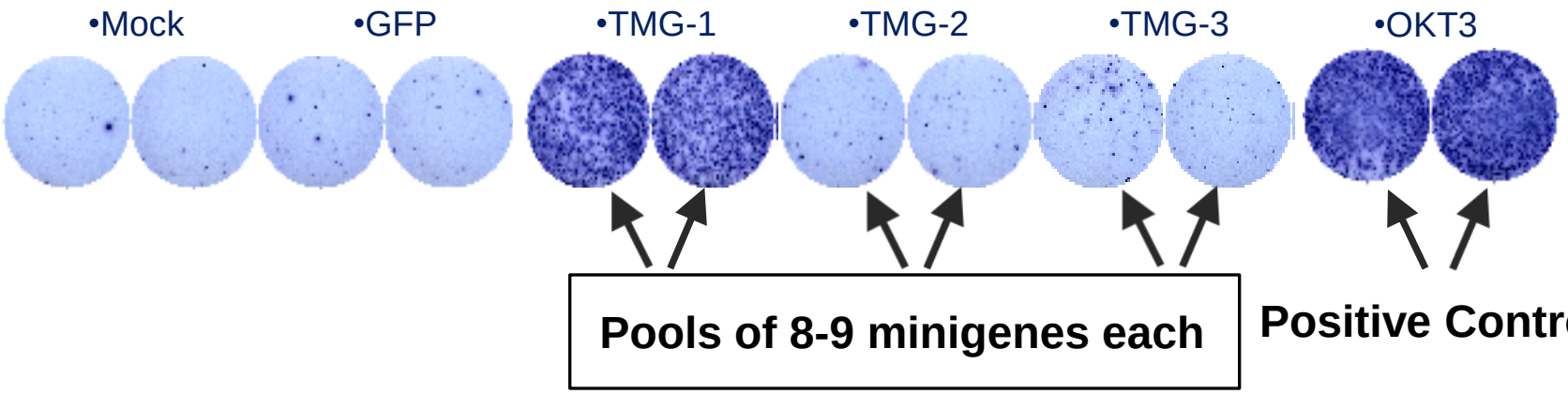
Pre-Treatment 7 Months

Continued…

- During that year, her tumor DNA was sequenced
- 26 mutations were found
- "Mini-genes" encoding just these mutated sequences were made and introduced into her own dendritic cells
- These were then tested for the ability to stimulate her TIL

ELISPOT ASSAY FOR TIL RECOGNITION OF 'MINIGENES'

Co-culture TIL + three pools of Minigenes--
Stain purple for TIL secreting Interferon-gamma
(each done in duplicate)



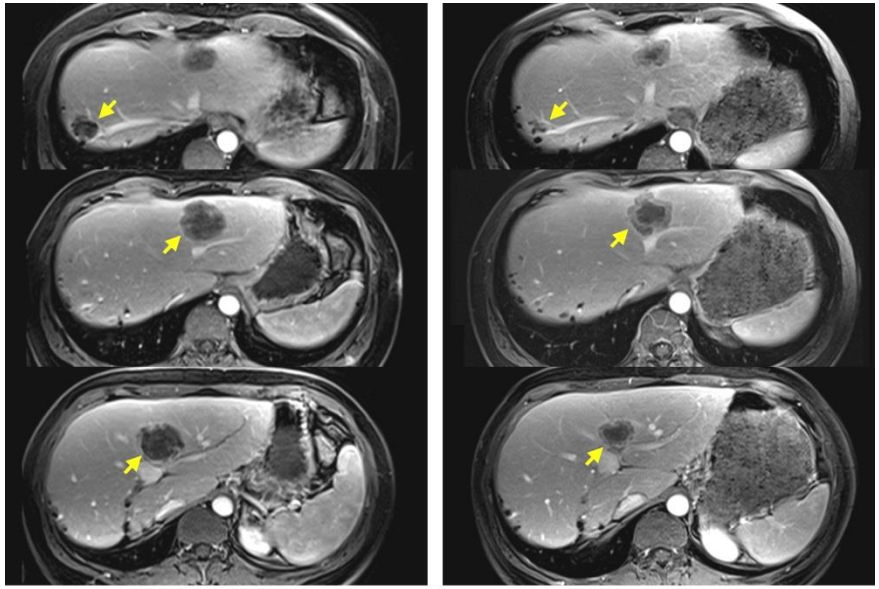
The specific mutated gene in pool TMG-1 encoded ERB-B2 interacting protein (ERBB2IP)

Continued…

- Her TIL cultures were examined for T-cells with this reactivity and one culture was found that was 95% pure
- Only these cells were grown in vitro and given in a second treatment
- This second infusion contained 12 times as many of these cells as the first treatment and she received the same chemo and 4 doses of IL-2

Liver

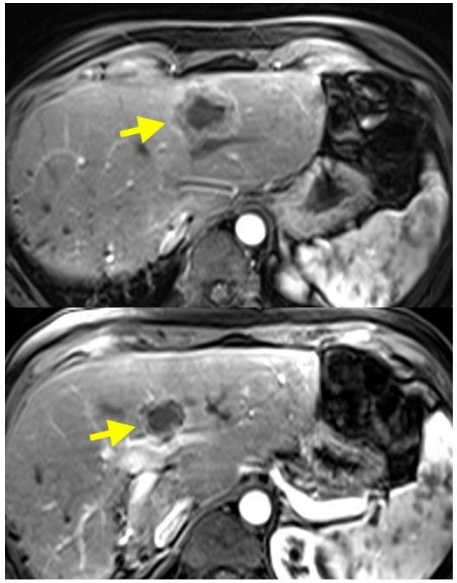
Treatment #1



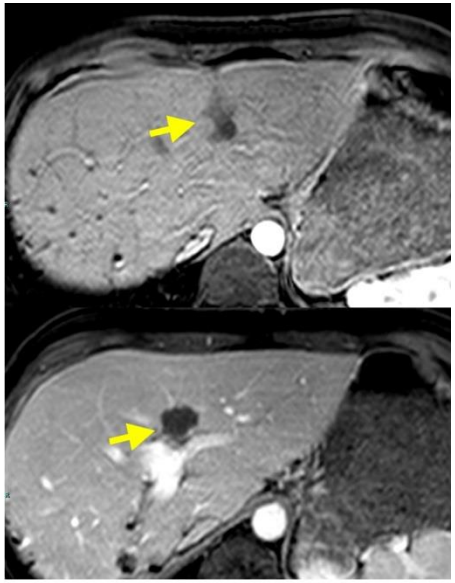
Pre-Treatment

7 Months

Treatment #2



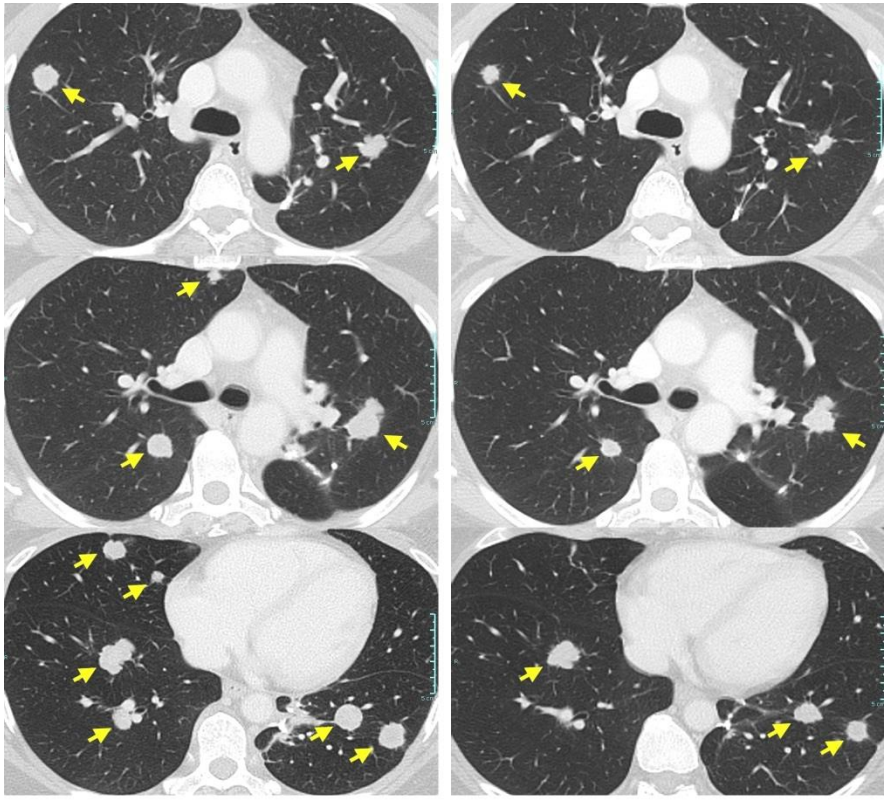
Pre-Treatment



8 months

Lungs

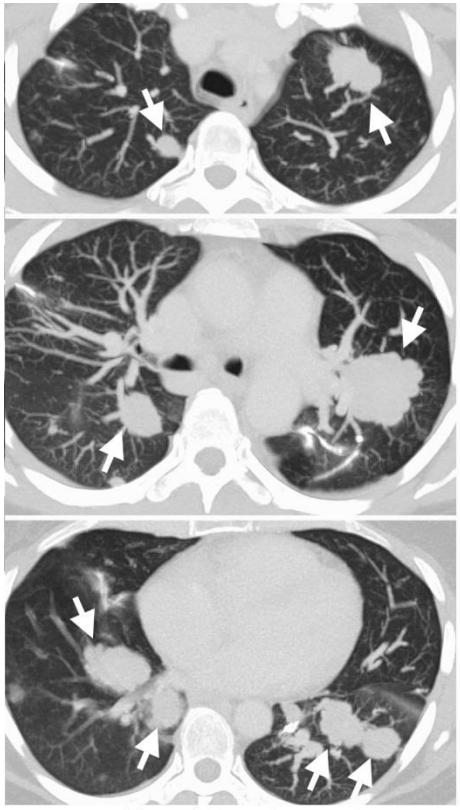
Treatment #1



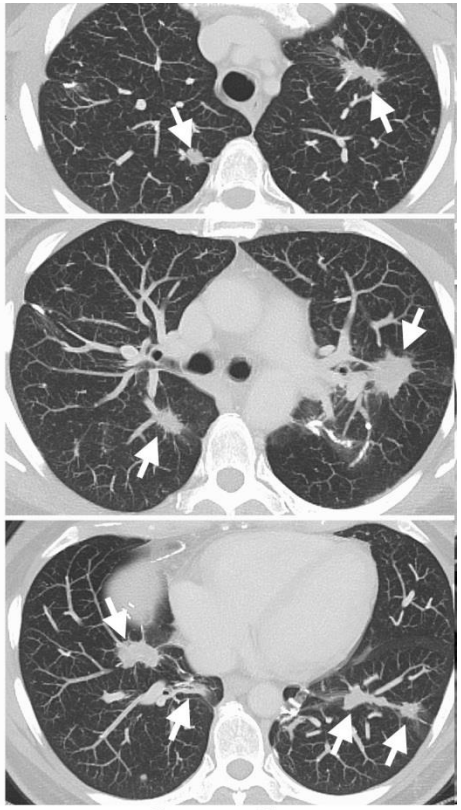
Pre-Treatment

7 Months

Treatment #2



Pre-Treatment

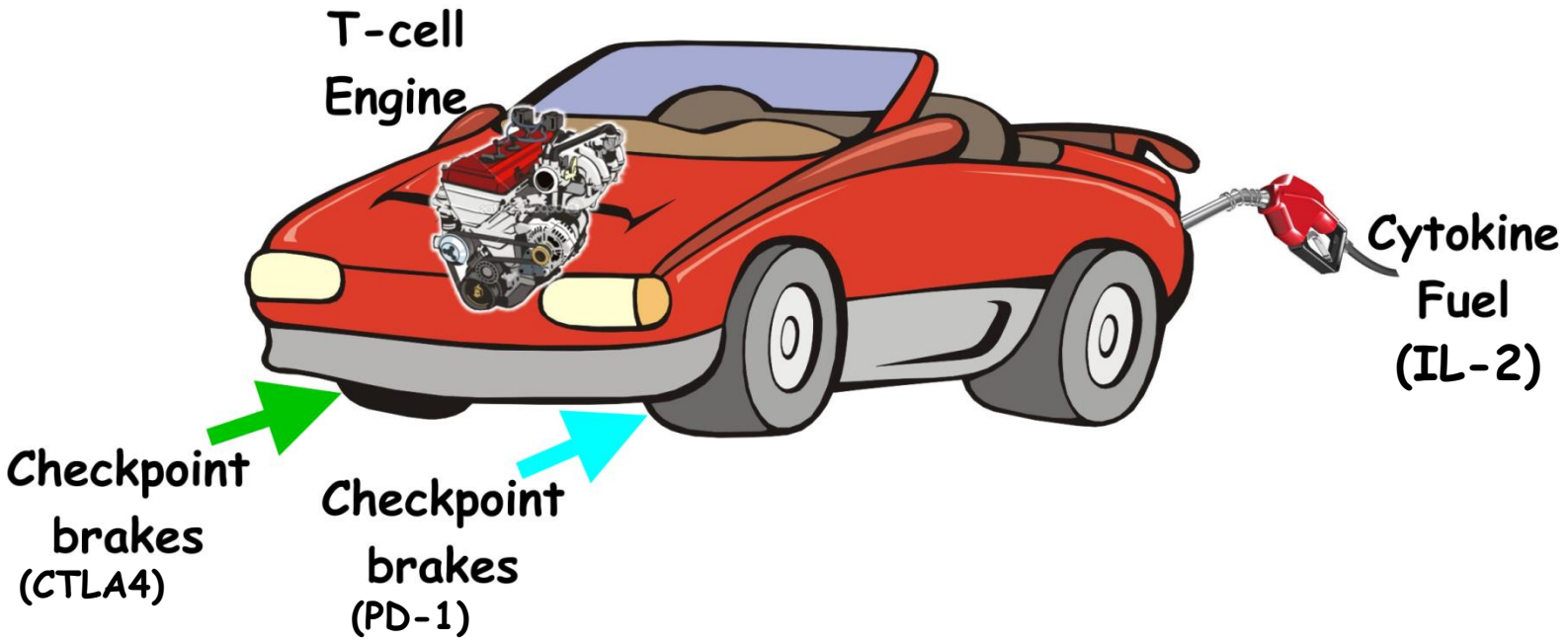


8 months

Hypotheses and Implications

- Mutated 'neo-antigens' drive the native immune response to cancer
- These responses are often weak but can be augmented by T-cell transfer
- Generic immunotherapies such as IL-2 and checkpoint inhibitors will work best in the most mutated tumors
- But any tumor could respond to the right T-cell

Driving Towards Tumor Rejection



Surgery Branch Protocols: Adoptive T-Cell Transfer for a Wide Variety of Human Cancers

Target Antigen	Type	Cancers
(Native)	TIL	Melanoma, bladder and GI cancers
(Native)	TIL	HPV+ cervical and head/neck CA
(Native)	TIL	Non-small cell lung cancer
NY-ESO-1	TCR	Melanoma & synovial sarcoma
CD19	CAR	Large B-cell lymphoma
EGFRvIII	CAR	Glioblastoma
Mesothelin	CAR	Pancreas, ovary & mesothelioma
MAGE-A3	TCR	Melanoma and adeno CA
Thyroglobulin	TCR	Differentiated thyroid CA

Immunotherapy for Human Cancers

("The Golden Age")

"It would be as difficult to reject the right ear and leave the left ear intact as it is to immunize against cancer."

W. H. Woglom