Adoptive T-Cell Transfer for Metastatic Cancer

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August 7, 2015

Disclosure

- Dr. Yang has no relevant financial relationships to disclose

T-Cell Adoptive Therapy: Concept and Principles

- The main obstacles to immune rejection of a patient's cancer are an inadequate anti-tumor T-cell repertoire and an immunosuppressive tumor microenvironment
- Vaccination has been largely inadequate to address the former and is performed in the ongoing presence of the latter

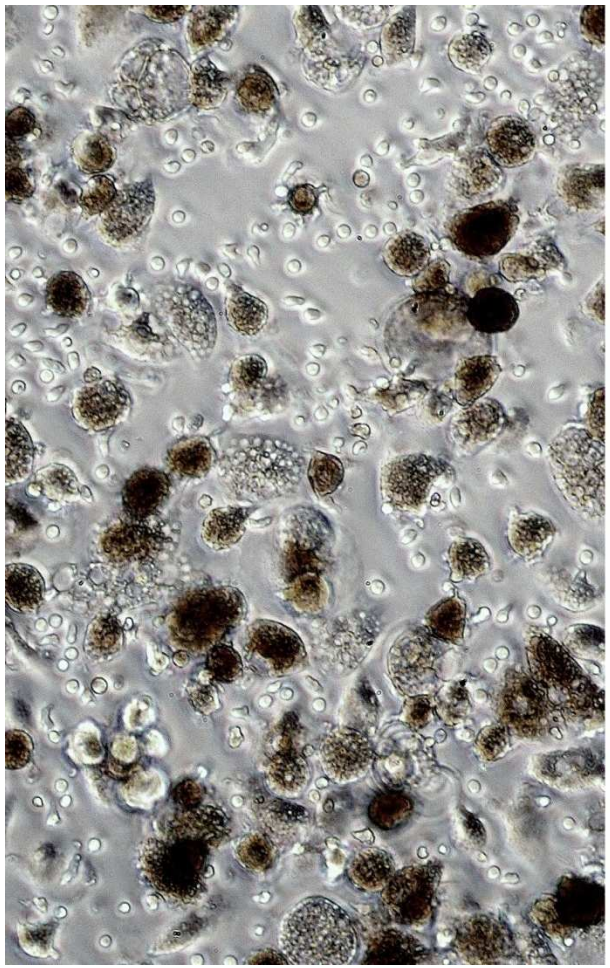
Adoptive Cell Therapy (ACT): Concept and Principles

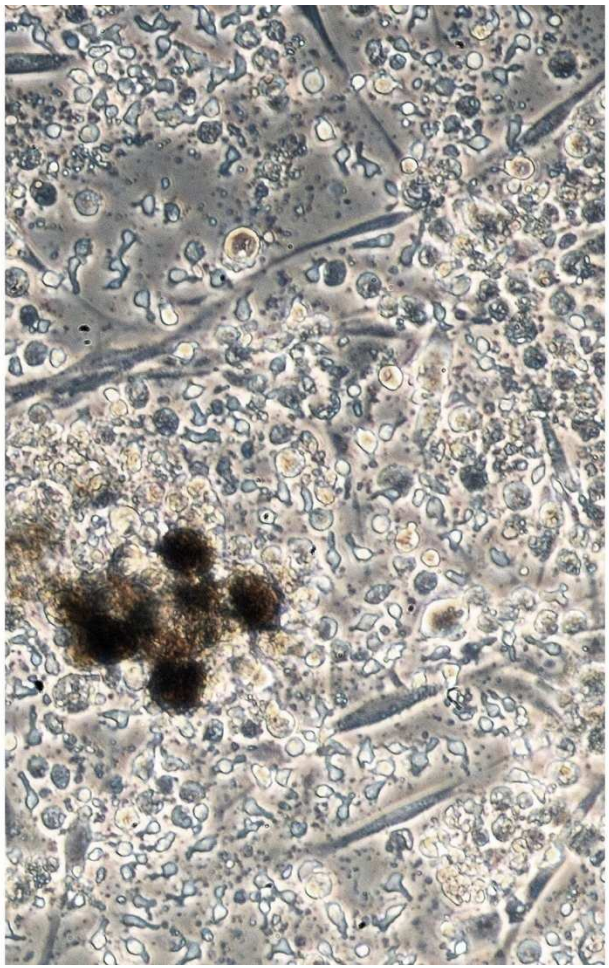
- Transferring tumor-reactive T-cells activated and expanded in vitro rapidly establishes an anti-tumor repertoire
- In vitro expansion permits the use of reagents and methods not tolerated in vivo
- This also allows independent manipulation of lymphocytes and the tumor microenvironment to optimize efficacy

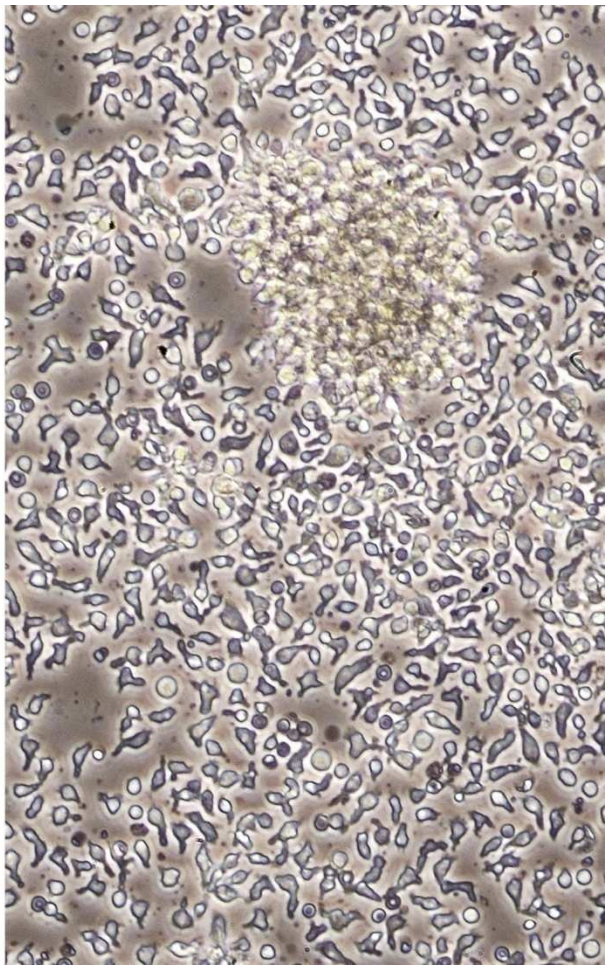
Sources of Tumor-Reactive T-Cells for Transfer

- Tumor infiltrating lymphocytes (TIL): Most human melanomas contain resident T-cells that can recognize the autochthonous tumor
- Cloning the T-cell receptor from a tumor-reactive T-cell allows it to be gene-engineered into other PBL and confer that tumor recognition
- Other novel receptors have also been devised to target tumor antigens

Melanoma TIL
(Tumor Infiltrating Lymphocytes)





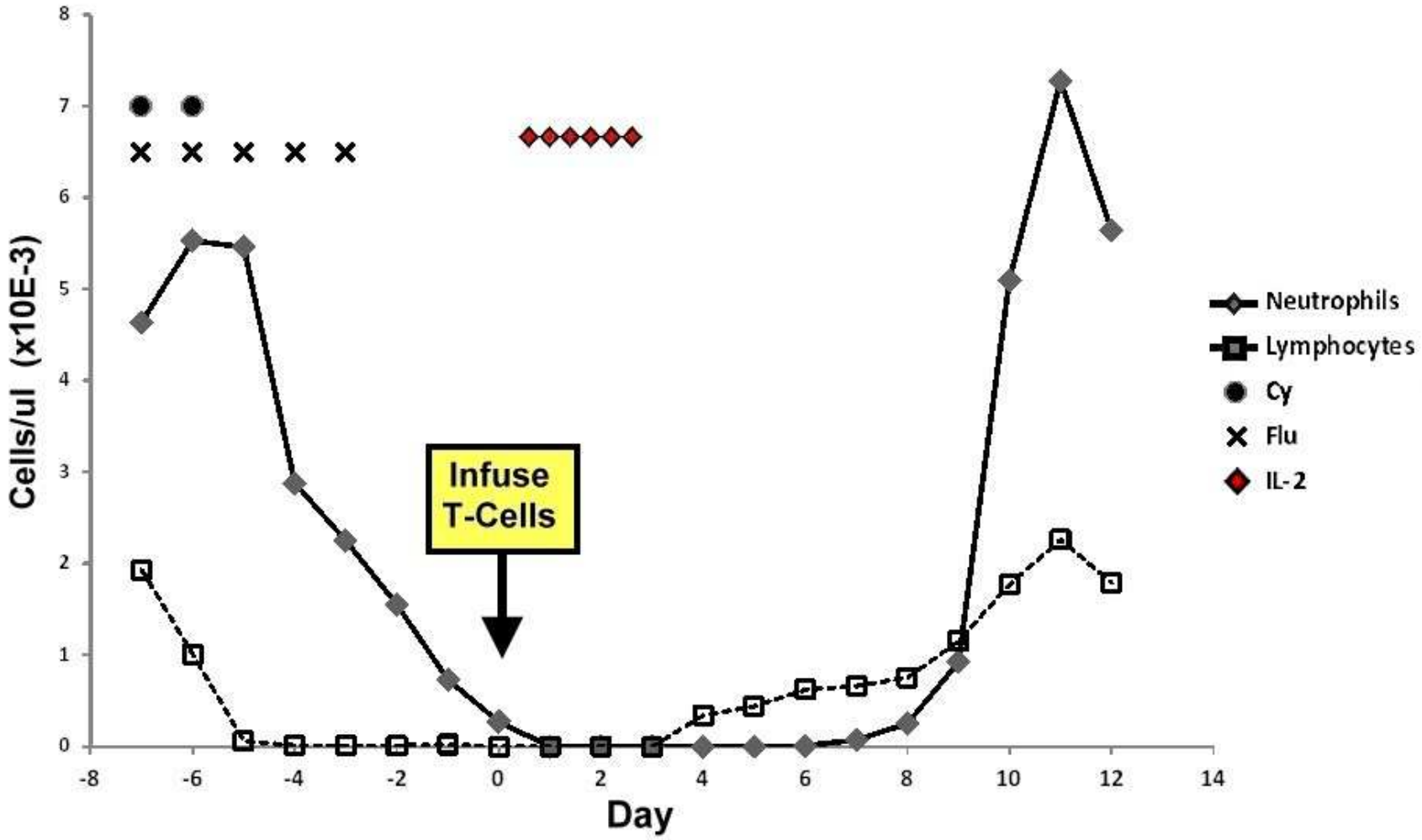


Fresh digest **One week** **Two weeks**

Preparative Host Immunosuppression Enhances ACT

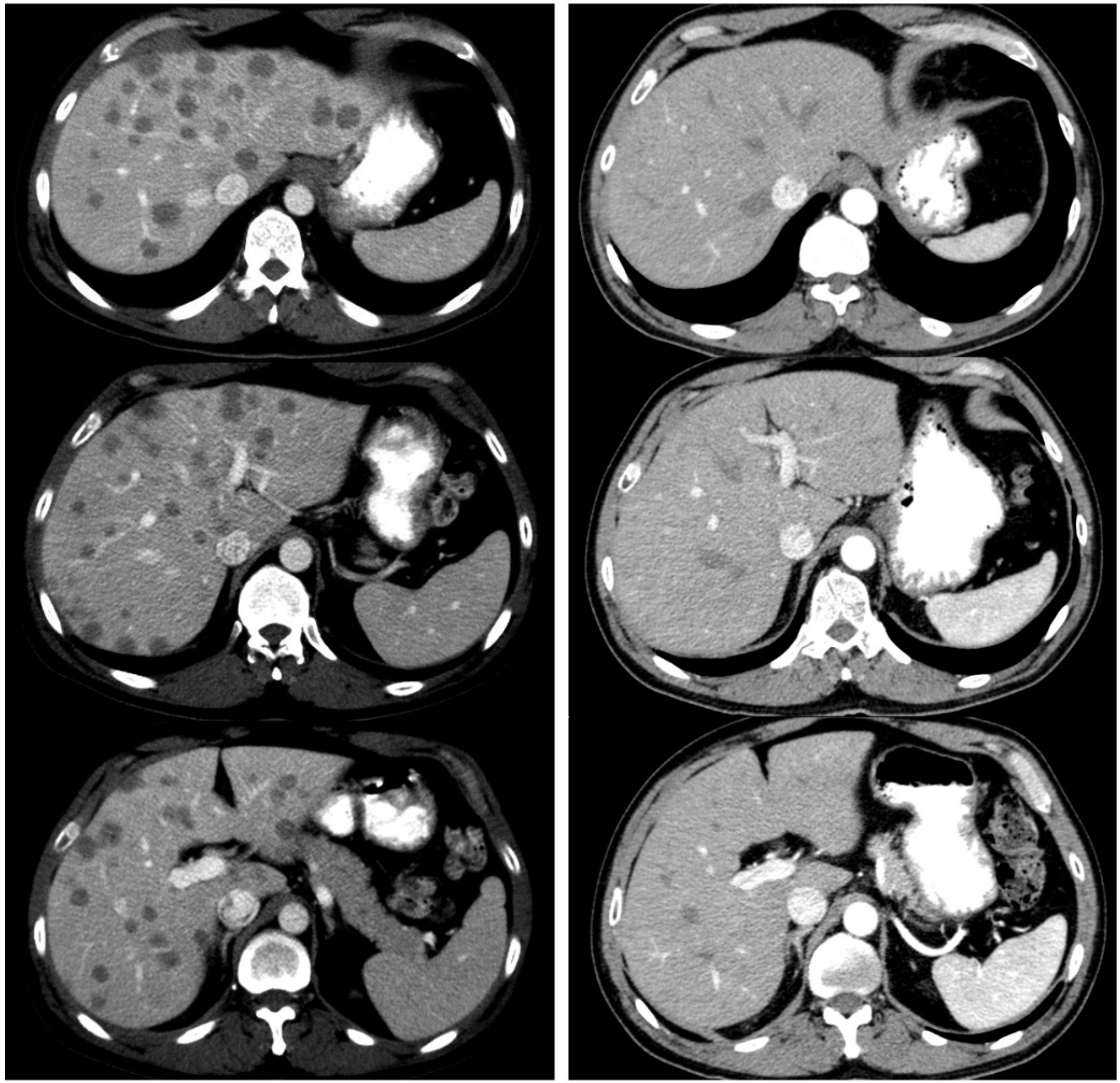
- Host immunosuppression prior to T-cell transfer increases T-cell survival and efficacy by:
  - Removing resident Tregs
  - Inducing homeostatic cytokines
  - Reducing competition for cytokines ('sinks')
  - Non-specifically increasing TLR ligands (LPS)

Cyclophosphamide + Fludarabine
Non-Myeloablative Chemotherapy



Other Sites: Lung

CR 99+ months



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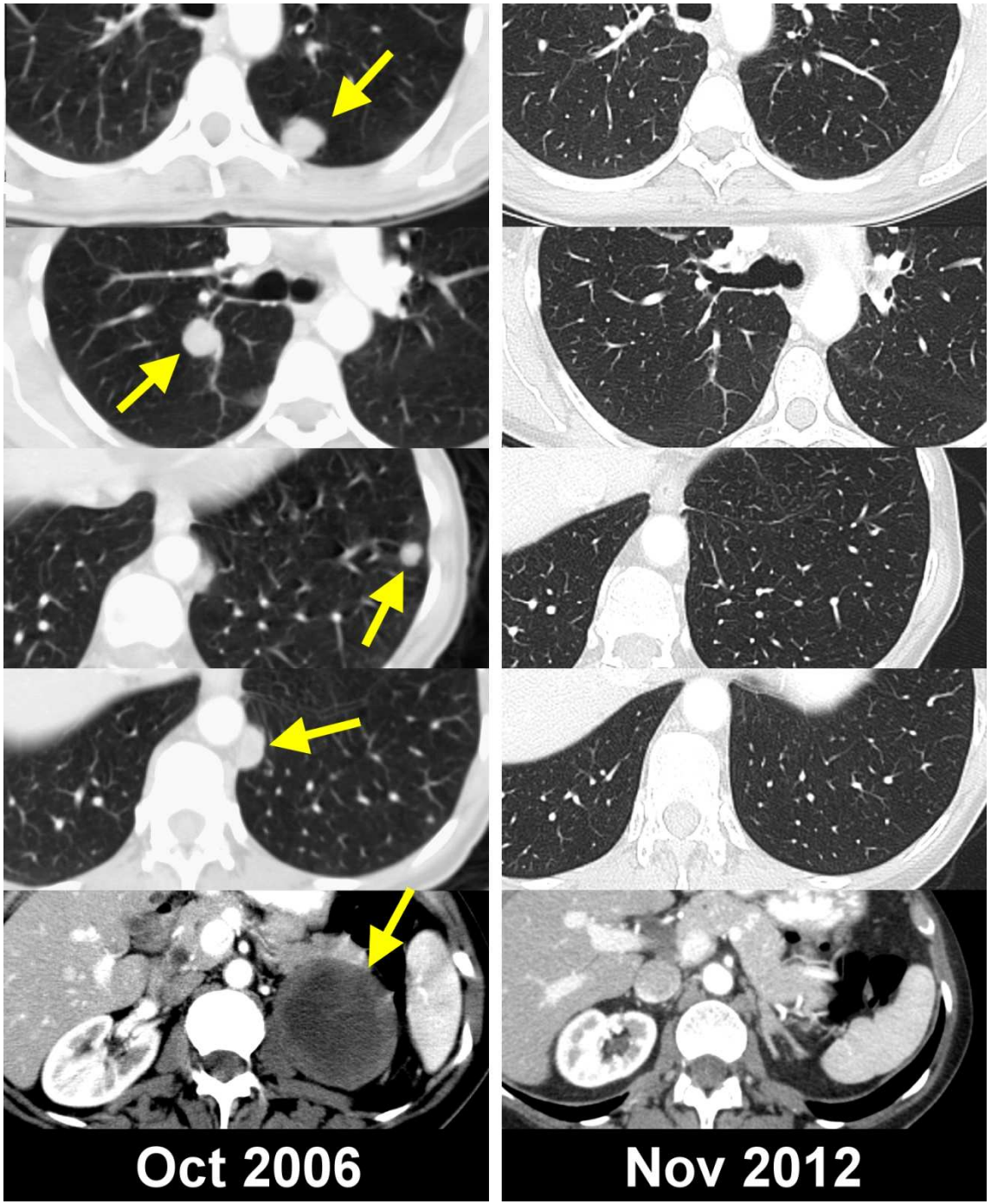
Feb 9, 2012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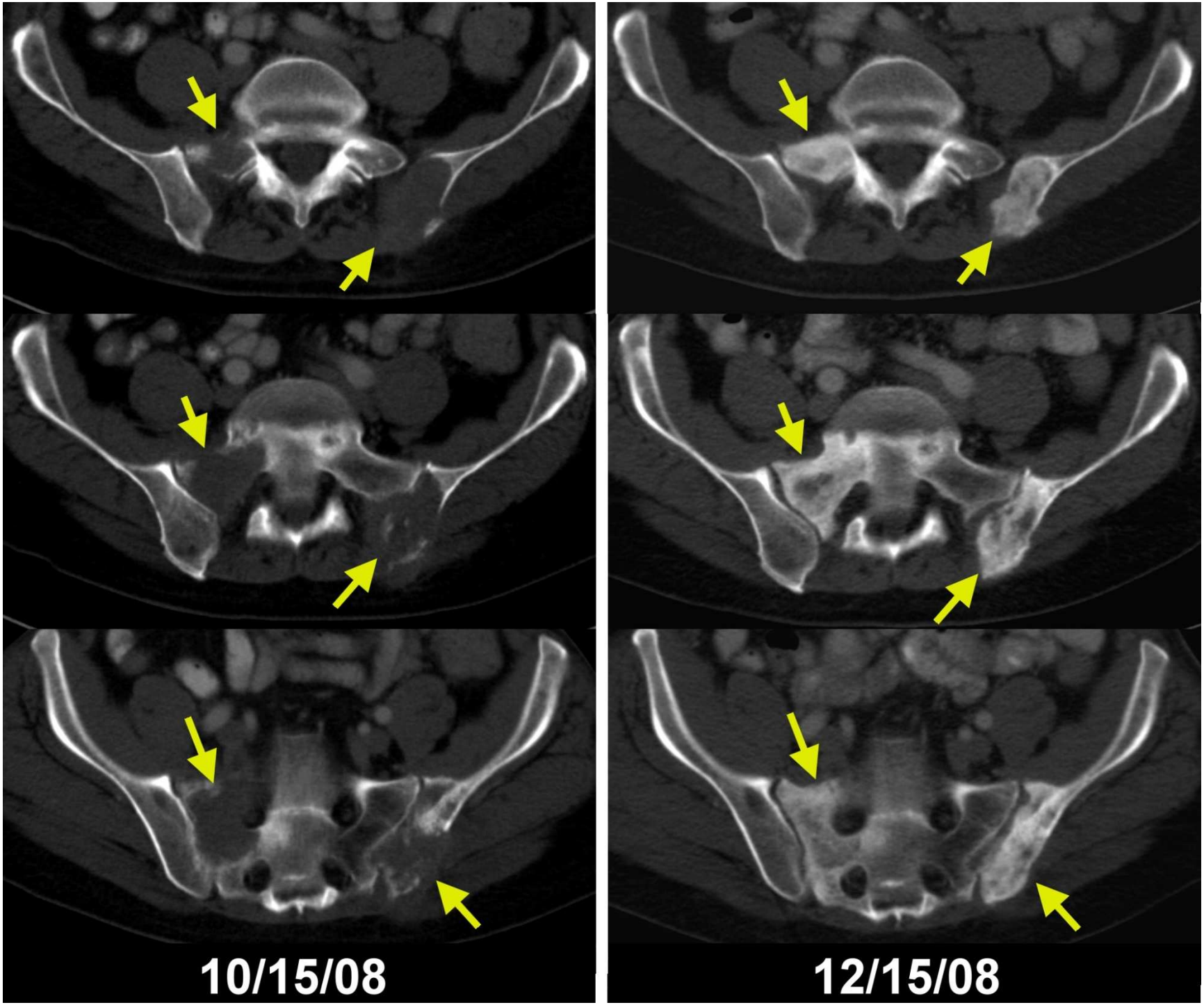
Nov 2012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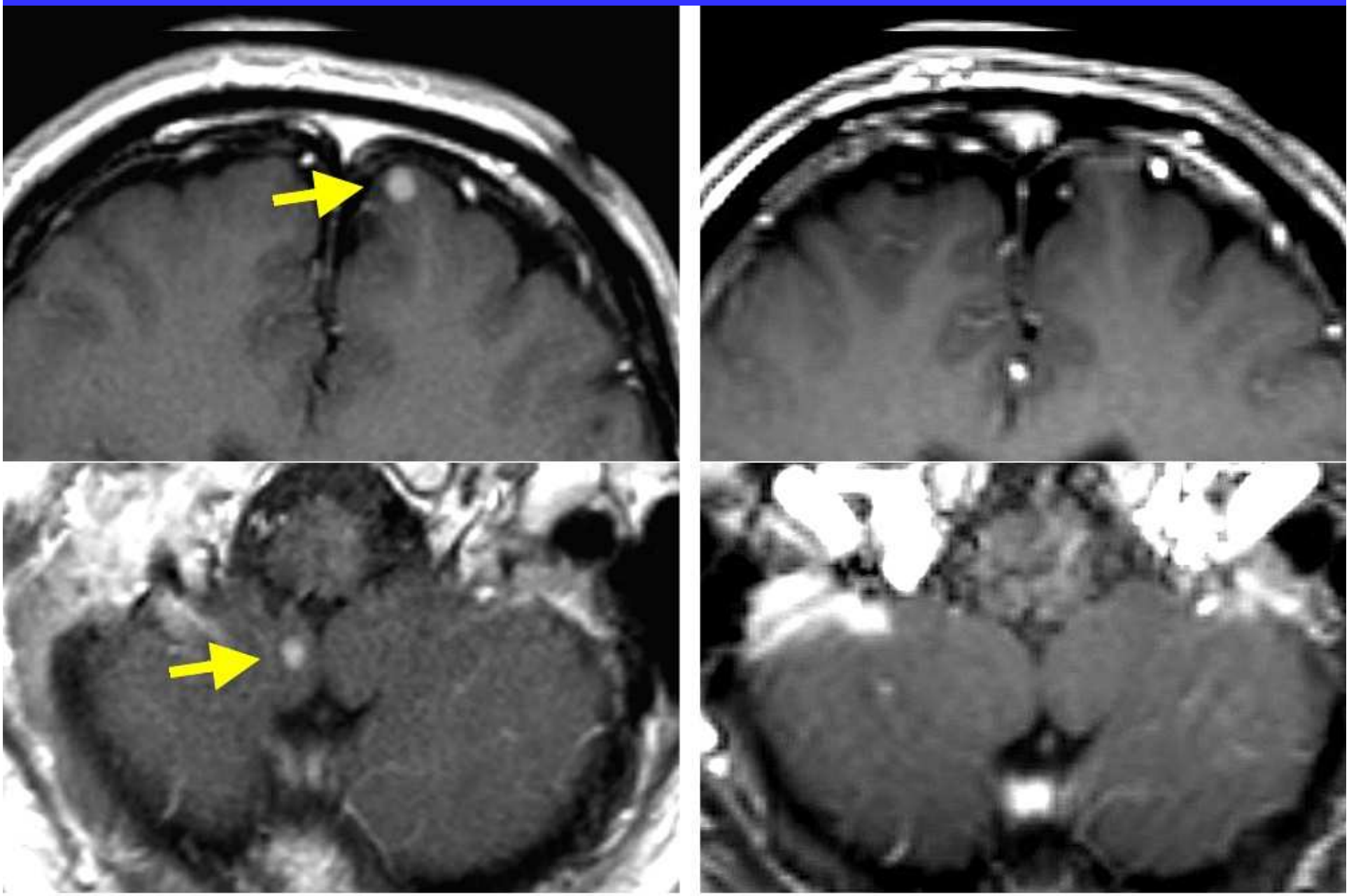


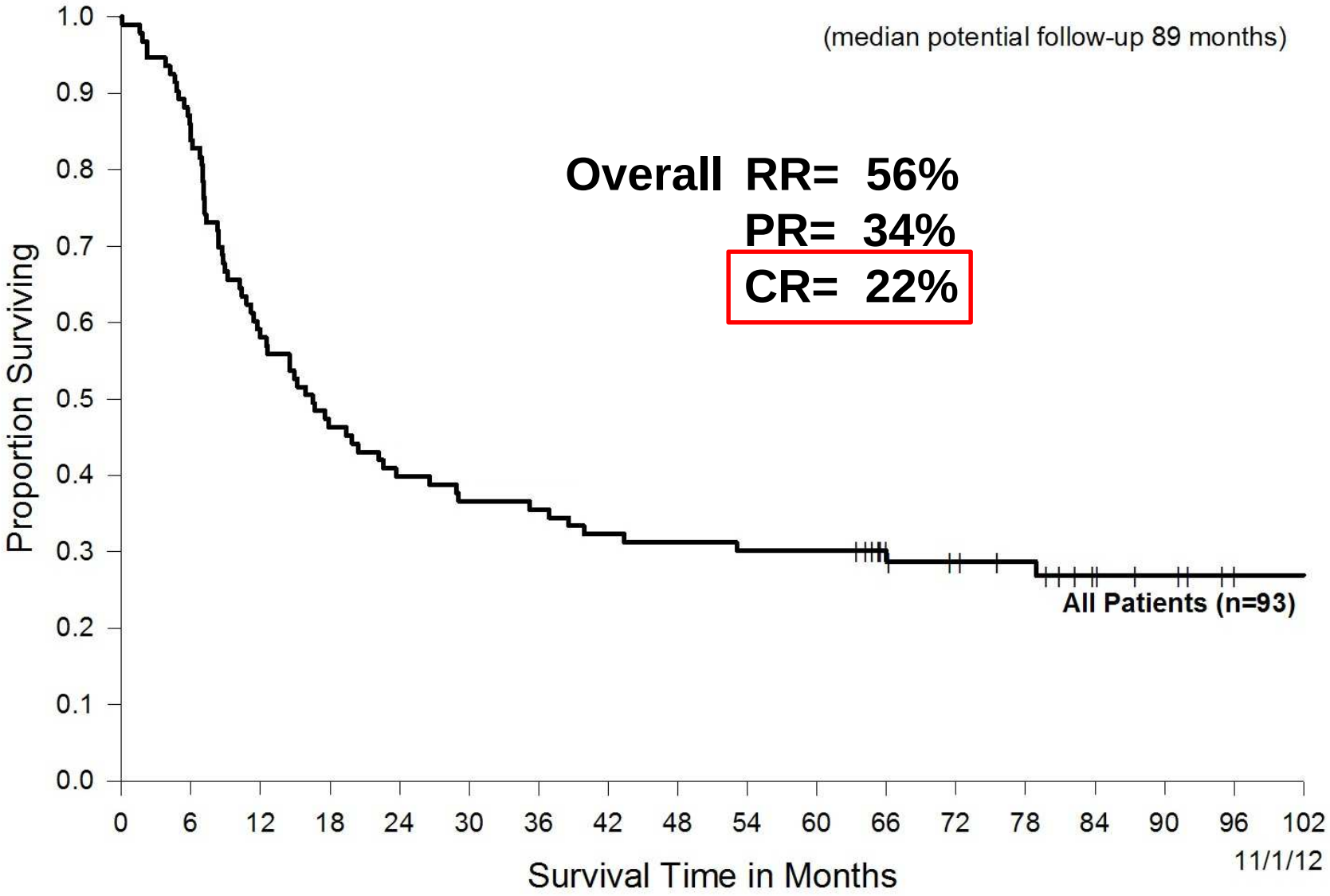
Pre-Treatment

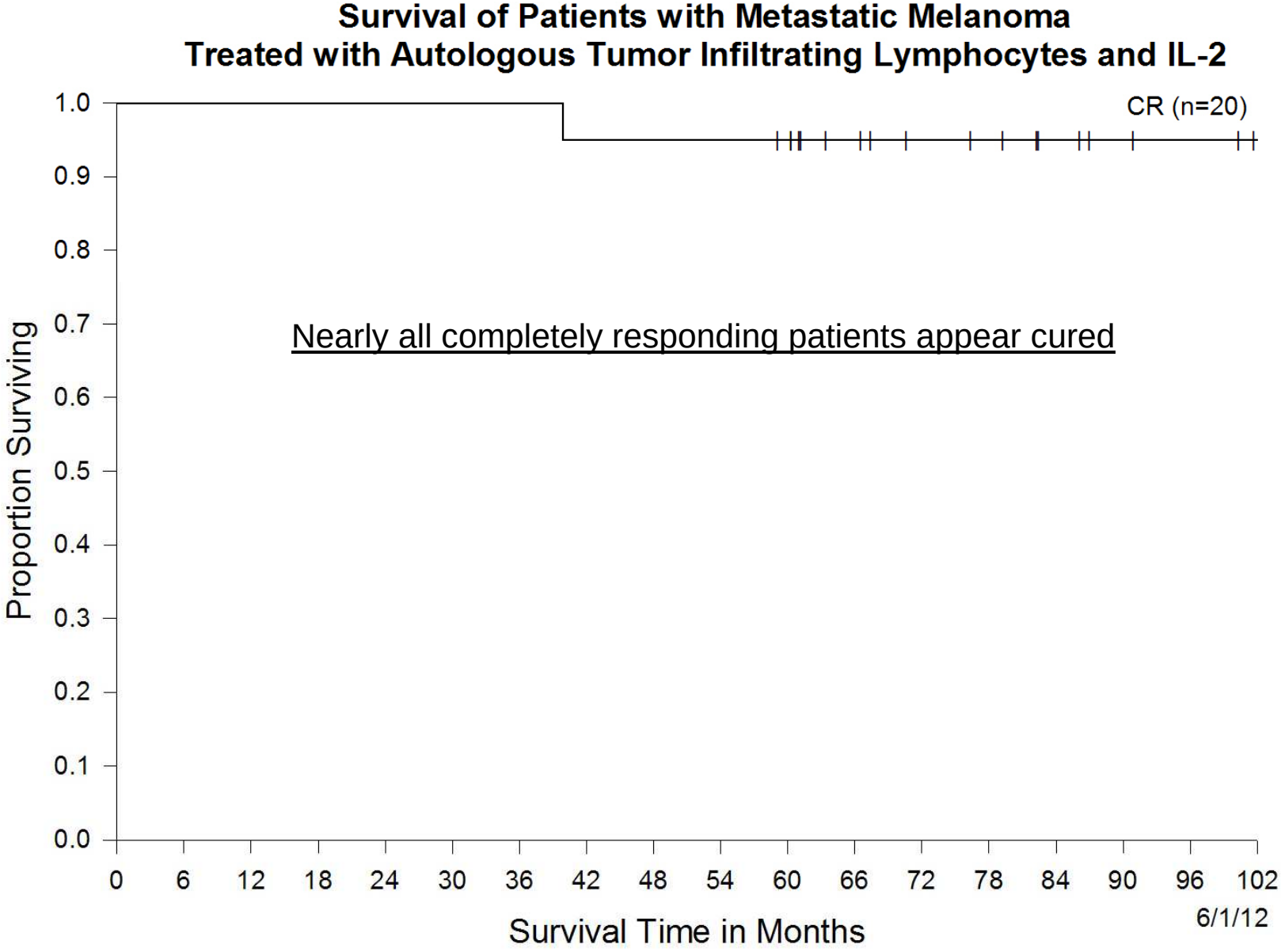
11 Months

TIL for Metastatic Melanoma

- Between 2000 and 2007, 93 patients with measurable metastatic melanoma were treated with a preparative lymphodepleting regimen followed by TIL and IL-2
- 86% had visceral metastases
- 83% had tried prior IL-2
- Only 2 patients were treated twice and there was one patient death during treatment due to sepsis

Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2




Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
CR (n=20)
Nearly all completely responding patients appear cured
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
Survival Time in Months
6/1/12

What Can Be Targeted on Cancers?
Categories of Tumor Associated Antigens

- Tissue differentiation antigens (MART1, gp100, CEA, CD19)
- Tumor germline antigens (NY-ESO1, MAGE)
- Overexpressed proteins supporting malignant phenotype (hTERT, EGFR)
- Proteins containing tumor specific mutations (MUM-1, CDK4, B-catenin, erbB2IP)
- Viral proteins (HPV, EBV, MCC)

'Self' and 'Non-Self' Antigens

- Tissue differentiation antigens
- Tumor testis antigens
- Overexpressed proteins supporting malignant phenotype
- Proteins containing tumor specific mutations
- Viral antigens (virally induced CA)

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Unmutated Self Antigens

- Constant between patients (off-the-shelf reagents)
- Potential for autoimmune toxicity
- T-cell repertoire may be limited by thymic deletion

Mutated Non-Self Antigens

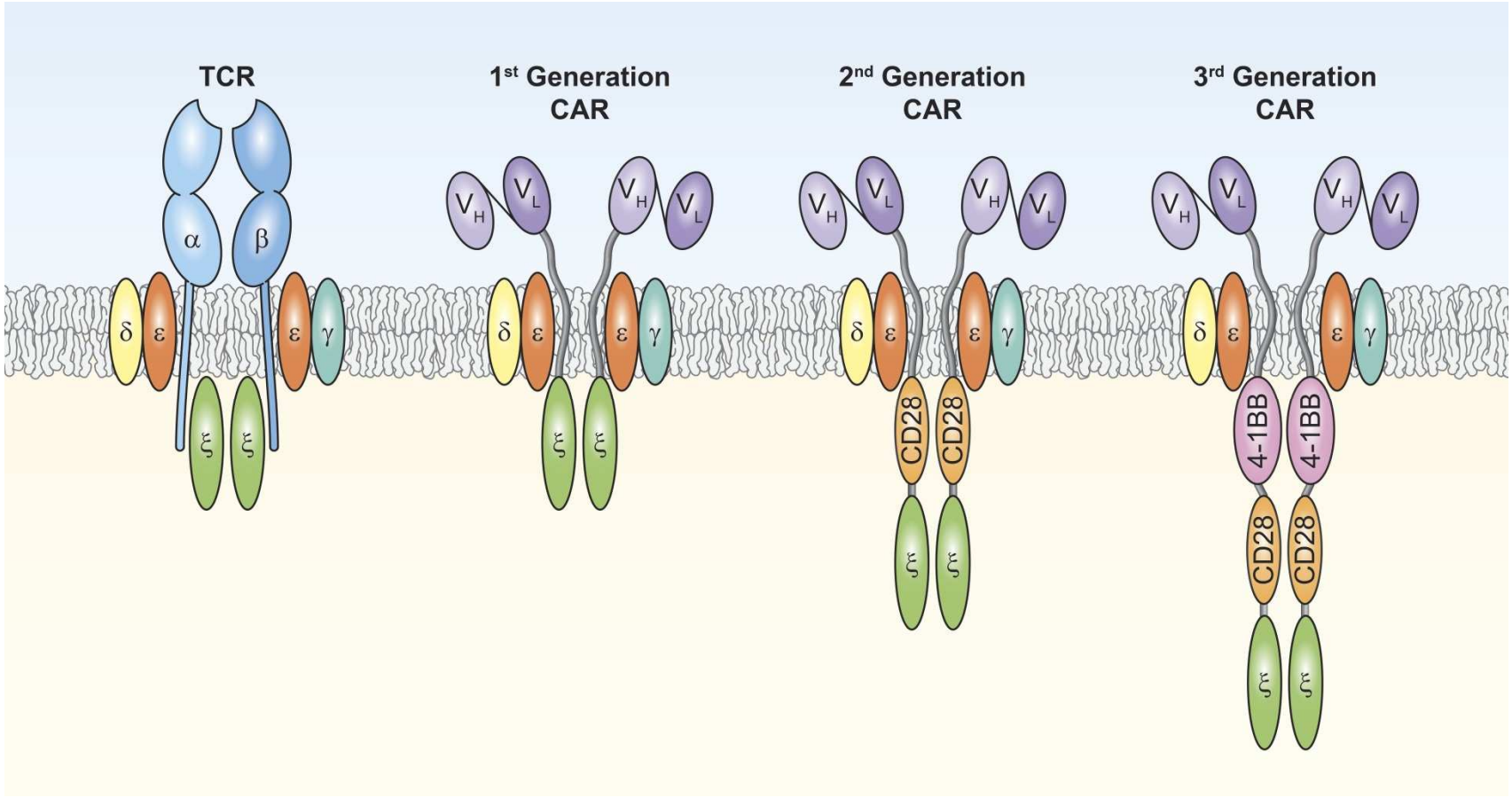
- Totally patient specific
- Very low potential for autoimmunity
- No central thymic tolerance ('neoantigens')

Targeting Shared Antigens with Receptor-Engineered T-Cells

- High-efficiency, stable gene insertion into mature human T-cells is possible using viral gene-therapy vectors
- Safe "one-shot" replication-incompetent retroviruses have been safely used to modify T-cells in hundreds of patients
- Native alpha-beta T-cell receptors (TCR) as well as chimeric antigen receptors (CAR) have been used

TCRs & Chimeric Antigen Receptors (CAR)

- Single chain MoAb antigen-binding domains can be covalently linked in tandem with intracellular costimulatory and T-cell activation moieties



TCRs	VS	CARs
• Target antigen is processed and presented by MHC • Any protein made in the cytoplasm could be recognized • Requires pt have correct HLA allele		• Target antigen is recognized intact • Only proteins on the external cell surface can be recognized • No HLA restriction

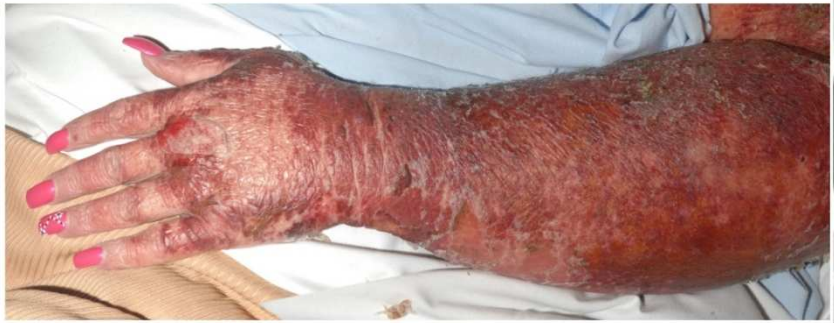
Retargeting PBL Against CD19 With a Chimeric Antigen Receptor (CAR)

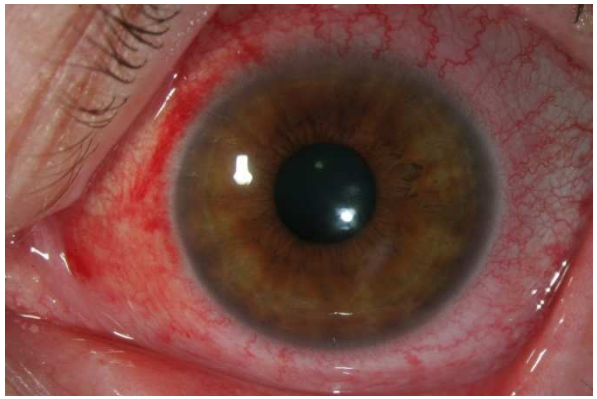
Effector cells	CD19-expressing targets			CD19-negative targets		Effectors alone
	Toledo	Nalm6	CD19-K562	NGFR-K562	CCRL-CEM	
	(IFN-g pg/mL)					
anti-CD19 CAR-transduced	**2180**	**4765**	**48050**	581	193	110
Not transduced	63	70	59	66	66	31

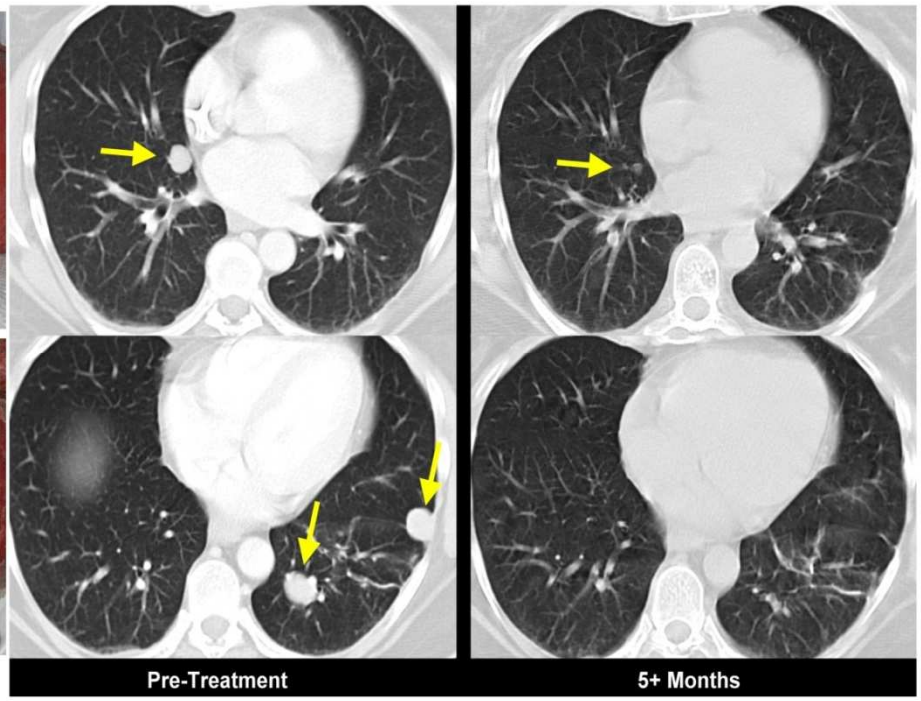
Dangers of Targeting Normal Self-Antigens

- TCRs recognizing tumor antigens were cloned and retrovirally introduced into the PBL of HLA-appropriate patients
- These were expanded in vitro and administered exactly as with TIL
- Initially, the melanoma/melanocyte antigens MART-1 and gp100 and the colon antigen CEA were targeted

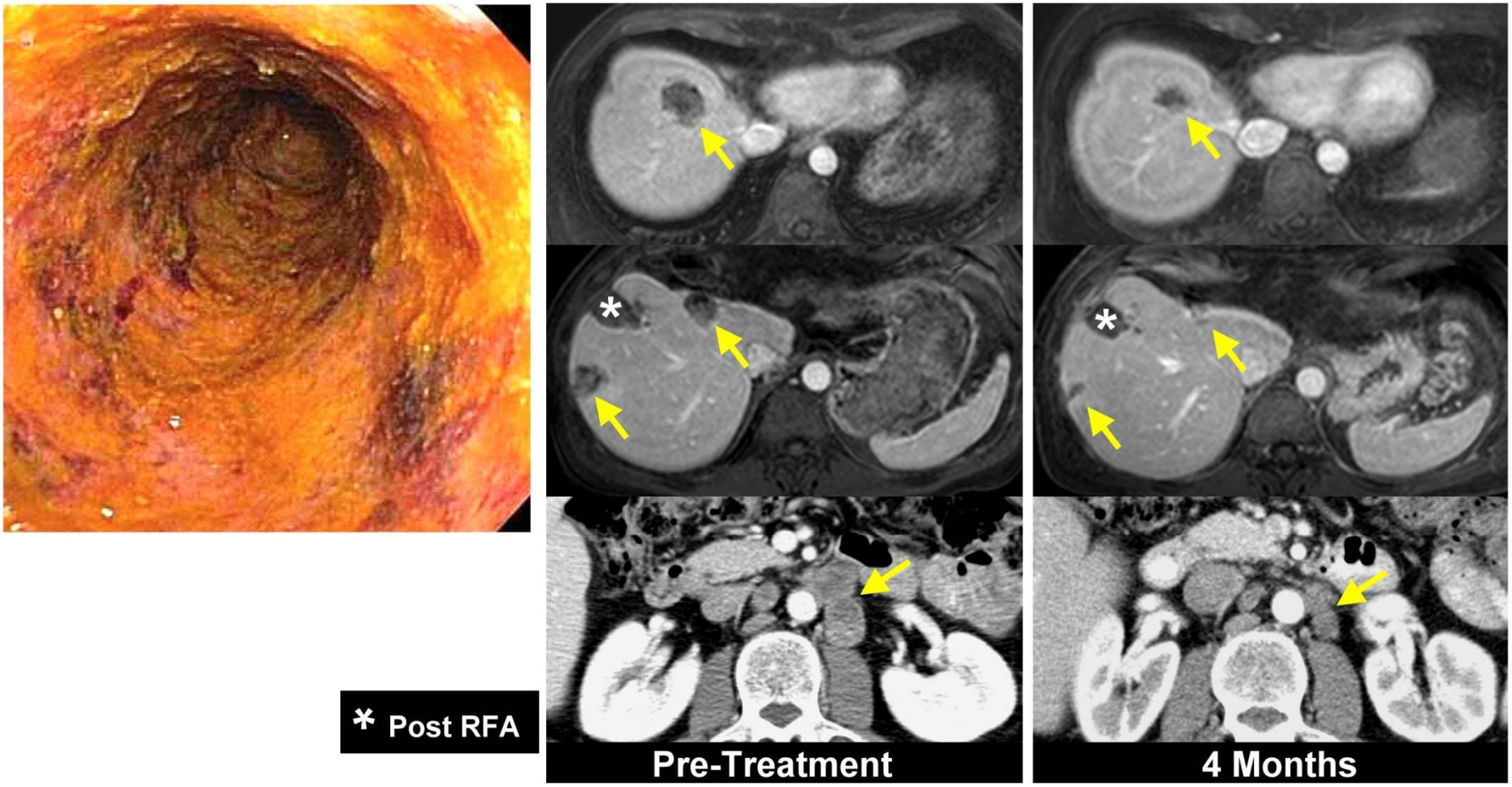
Targeting Melanocytic Proteins:
Anti-MART1 TCR-Engineered PBL







PBL with TCR Targeting CEA



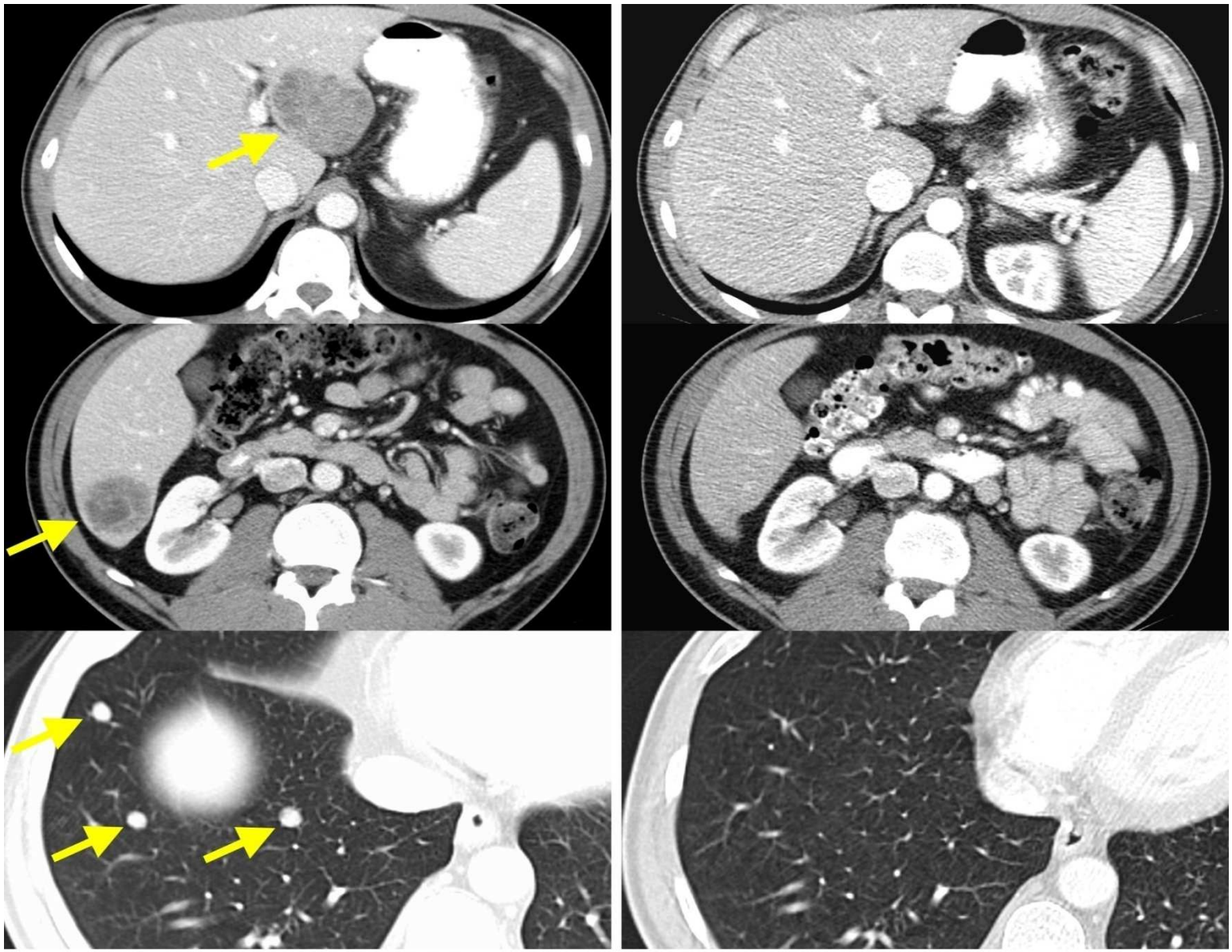
Effective attack on normal self-antigens may cause unacceptable autoimmunity

Are There Safe "Self" Antigens on Tumors?

- 38 patients with metastatic melanoma or synovial sarcoma expressing NY-ESO-1 were given their PBL transduced with a TCR recognizing NY-ESO-1
- The overall RRs were 55% for patients with melanoma and 61% for patients with synovial sarcoma
- Five patients achieved CR with 4 ongoing at 1-5 years
- No autoimmune toxicities were seen

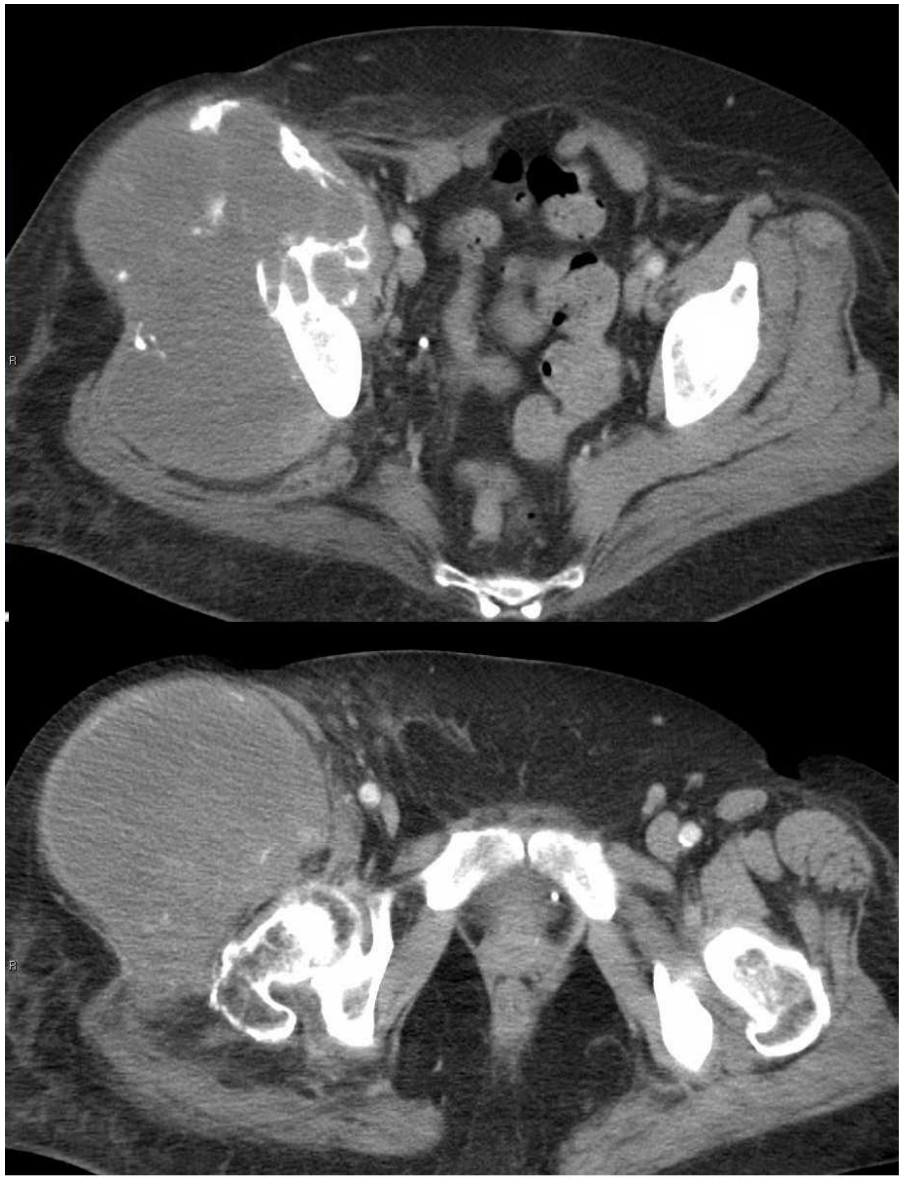
Robbins et al, Ca Res 2015

Gene Therapy with Anti-NY ESO-1 TCR (Melanoma)

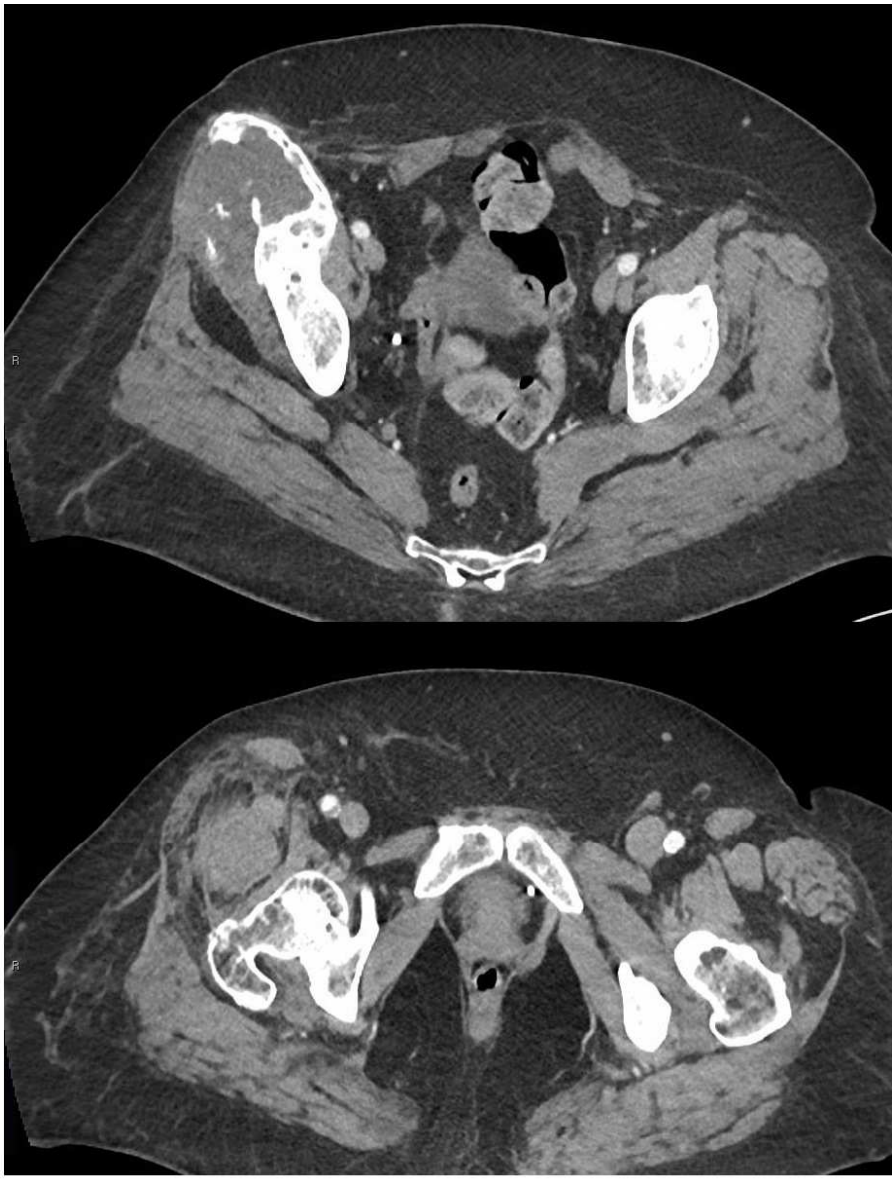


December 2009 March 2012

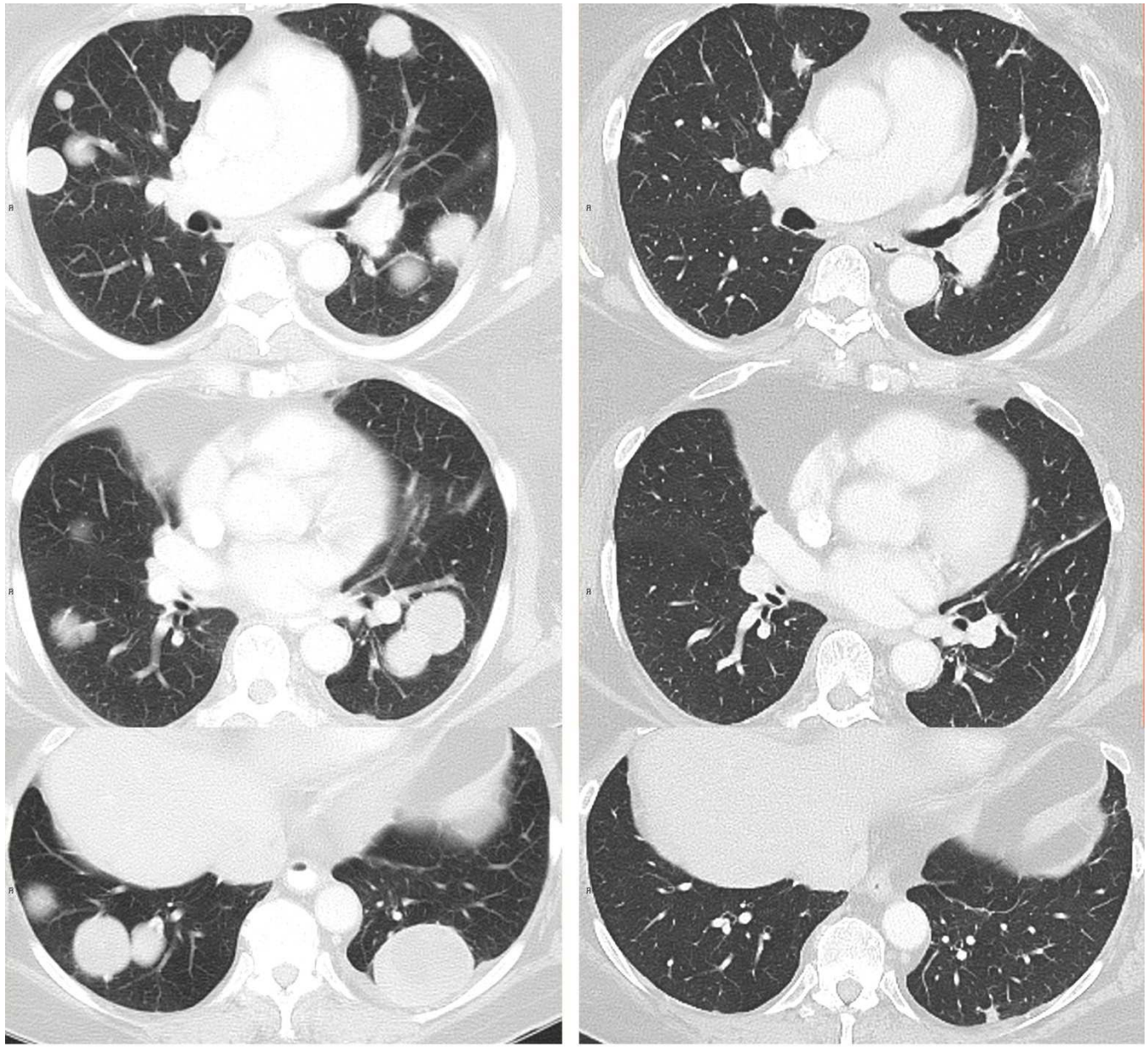
Synovial Sarcoma



August 2010



Feb 2015



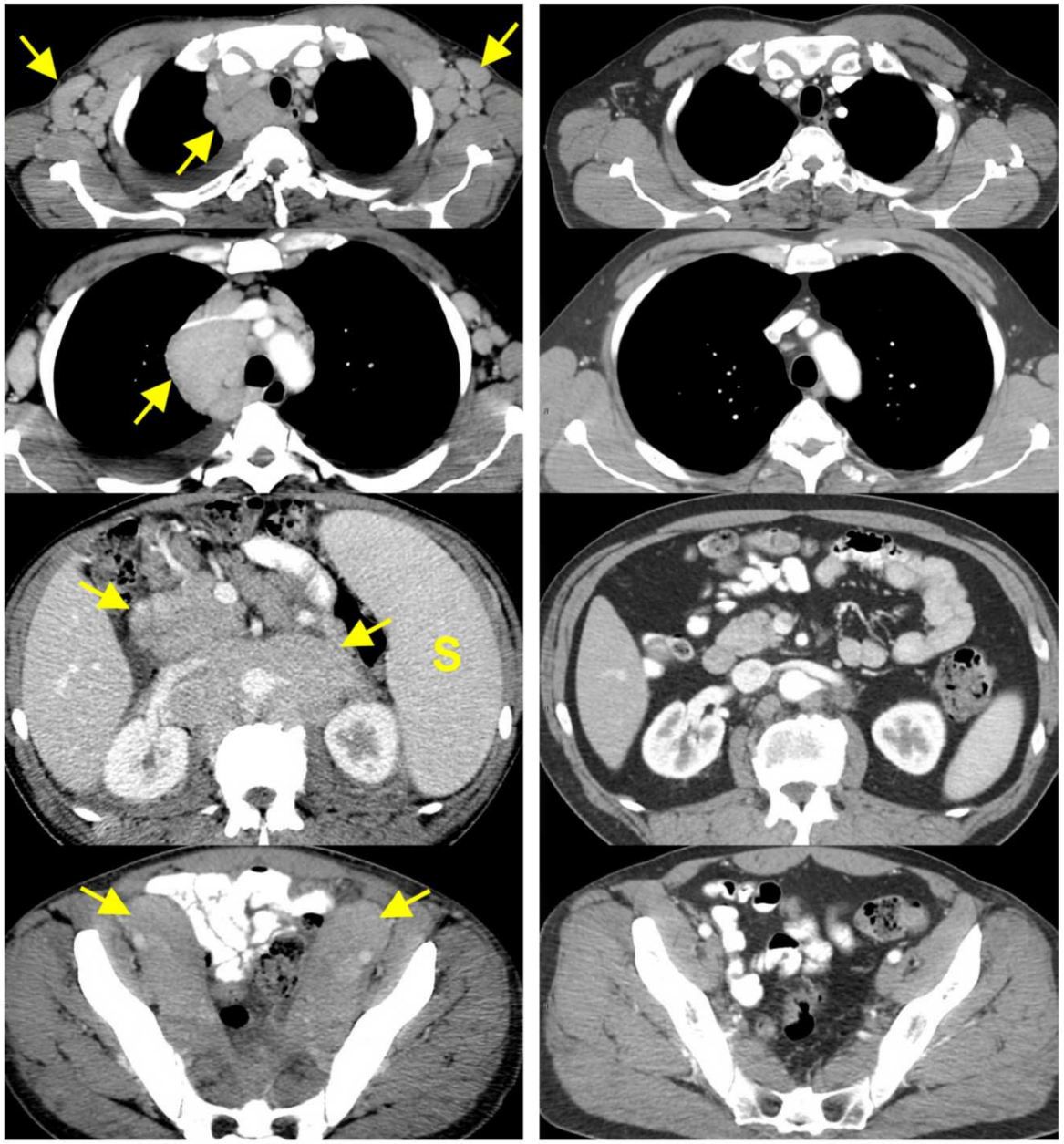
August 2010 Feb 2015

Targeting CD19

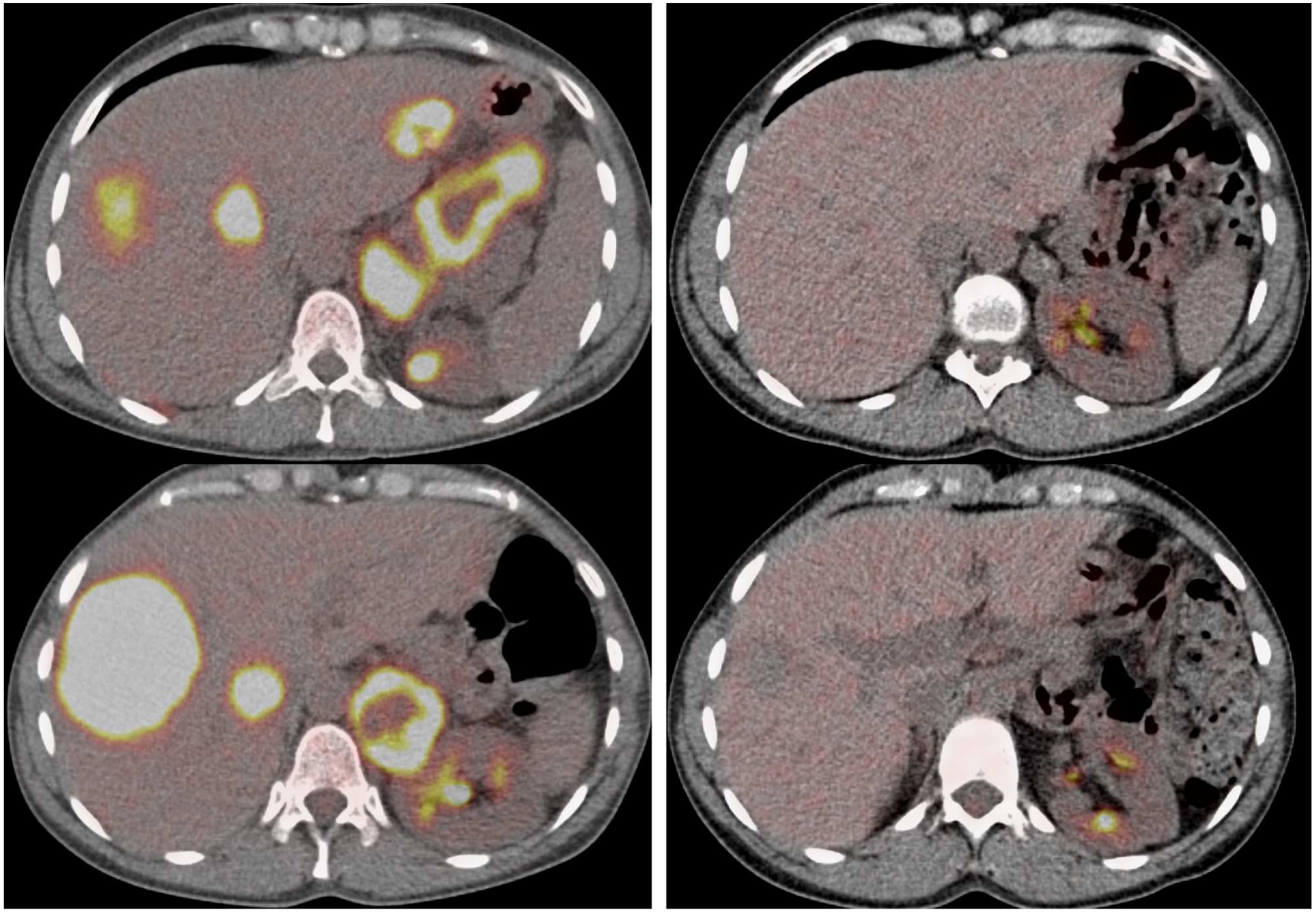
- Multiple investigators have reported dramatic responses in chemotherapy refractory lymphoma, CLL and ALL
- Concomitant IL-2 is not needed
- This therapy induces B-cell aplasia, but autoimmune B-cell destruction may be an acceptable toxicity when treating B-cell malignancies

Kochenderfer et al, Blood 2010
Porter et al, NEJM 2011
Maude et al, NEJM 2014
Lee et al, Lancet 2015

Anti-CD19 CAR: Follicular Lymphoma



Primary Mediastinal B-Cell Lymphoma



Pre-Treatment

22 Months

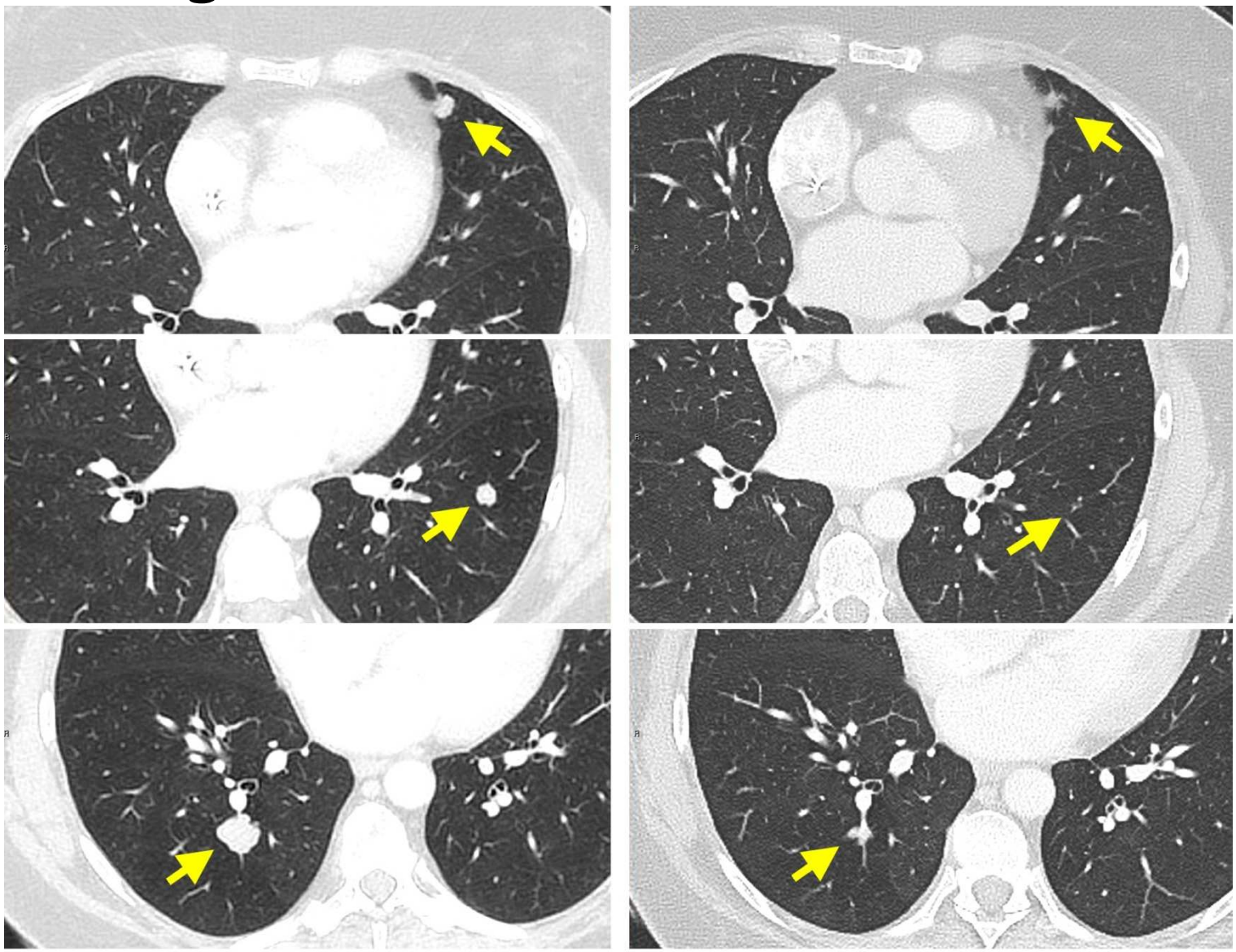
Gene-Engineered PBL Targeting Unmutated 'Self' Antigens

- Unacceptable toxicity:
  - Melanocyte proteins
  - CEA
  - MAGE-A3 (HLA-A2)
- Acceptable toxicity:
  - CD19
- No apparent autoimmunity:
  - NY-ESO-1

Targeting Viral Antigens in Virally-Induced Cancers

- Tumors have to retain expression of viral oncoproteins
- Cannot have essential normal tissues still expressing viral antigens
- Best current candidates are HPV, EBV and MCPyV

PBL Engineered with Anti-HPV E6 TCR



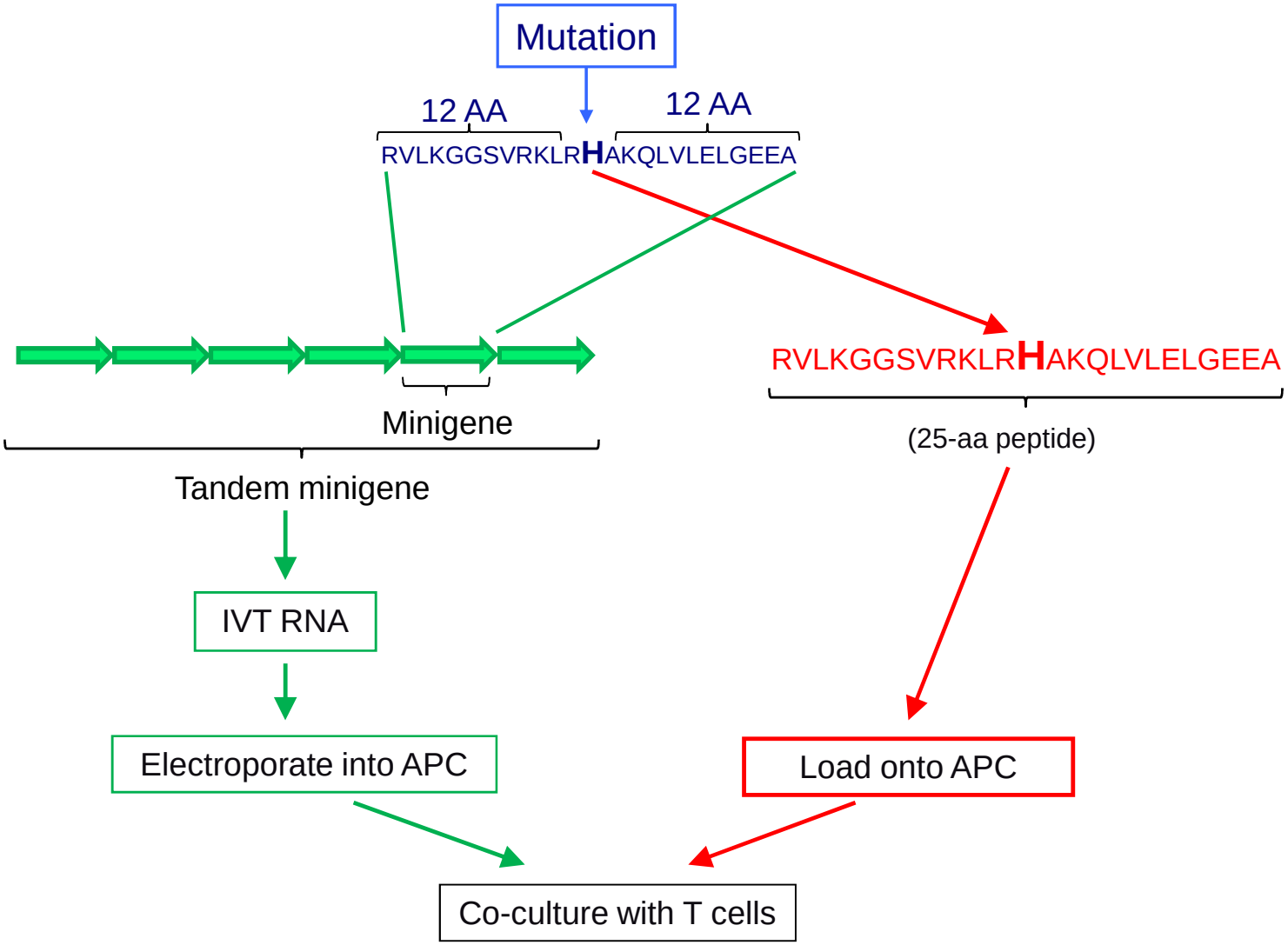
Pre-Treatment **4 Months**

Christian Hinrichs

T-Cells Targeting Tumor-Specific Mutations: The Ultimate Antigens

- Tumor-specific mutations are, by definition, confined to the tumor
- They are seen as 'foreign' by the immune system and are therefore more likely to be immunogenic
- High avidity T-cell responses against them have not been edited out by central thymic tolerance (neg selection)
- Melanoma TIL often contain such T-cells

Comprehensive Screening Techniques: Tandem Minigenes or Long Peptides to Display mutAgs



Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Eric Tran,[1] Simon Turcotte,[1]* Alena Gros,[1] Paul F. Robbins,[1] Yong-Chen Lu,[1] Mark E. Dudley,[1]† John R. Wunderlich,[1] Robert P. Somerville,[1] Katherine Hogan,[1] Christian S. Hinrichs,[1] Maria R. Parkhurst,[1] James C. Yang,[1] Steven A. Rosenberg[1]‡

Science, May 2014

43 yo F with cholangiocarcinoma

Treated with bulk TIL - minimal response

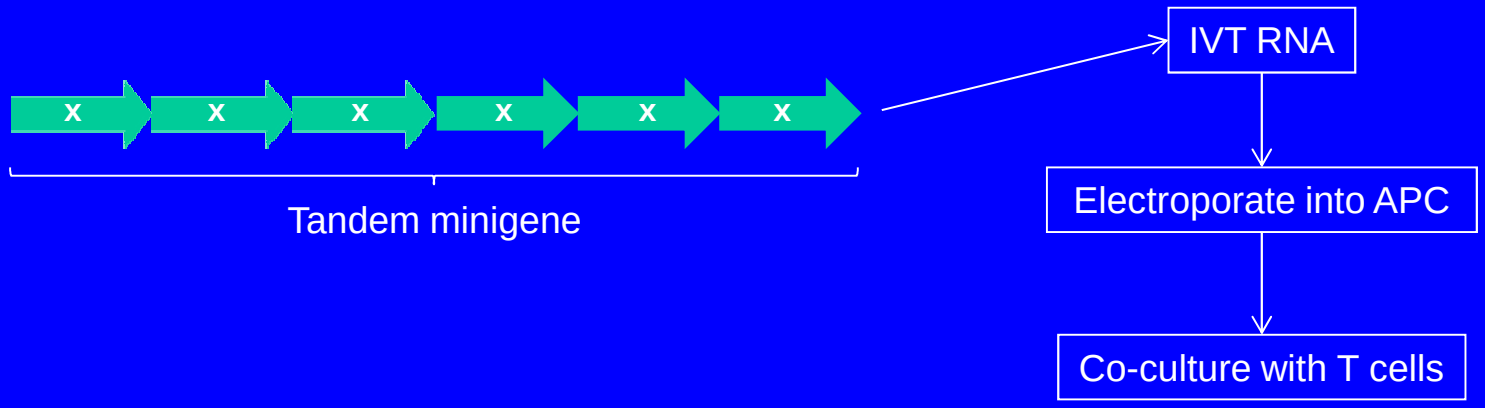
Tumor WES - 26 non-synonymous mutations

TIL screening with tandem minigenes - *mut*-ERBB2IP reactivity (CD4+)

First bulk TIL culture - 10^{10} of these T-cells (retrospectively)

New TIL selected and expanded - 12 x 10^{10} of these cells (95% reactive)

Tandem minigenes (TMG):

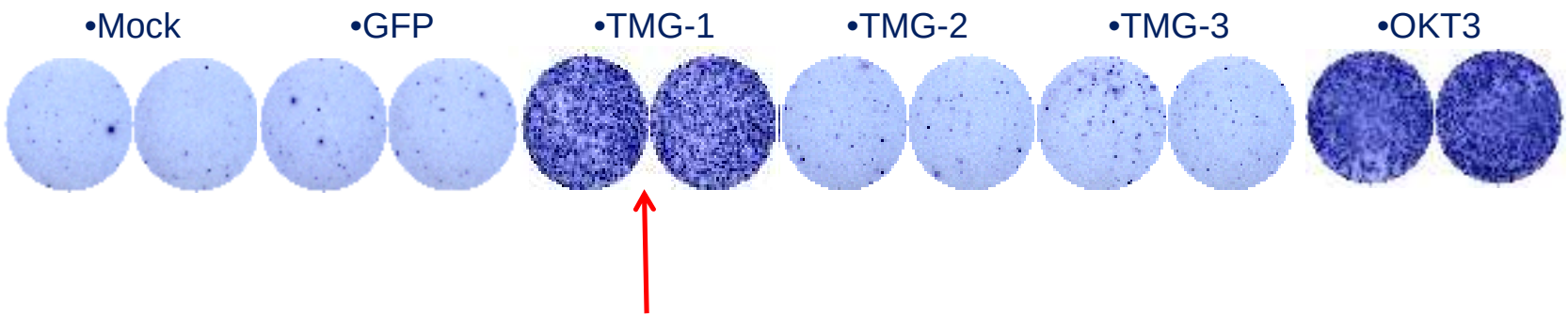


- Three TMGs generated for Pt. MB (26 mutations)

TMG-1	TMG-2	TMG-3
ALK	RAP1GDS1	SENP3
CD93	RASA1	LHX9
ERBB2IP	RETSAT	KLHL6
FCER1A	SEC24D	AR
GRXCR1	SLIT1	PDZD2
KIF9	TARBP1	HLA-DOA
NAGS	TGM6	LONRF3
NLRP2	TTC39C	
RAC3	POU5F2	

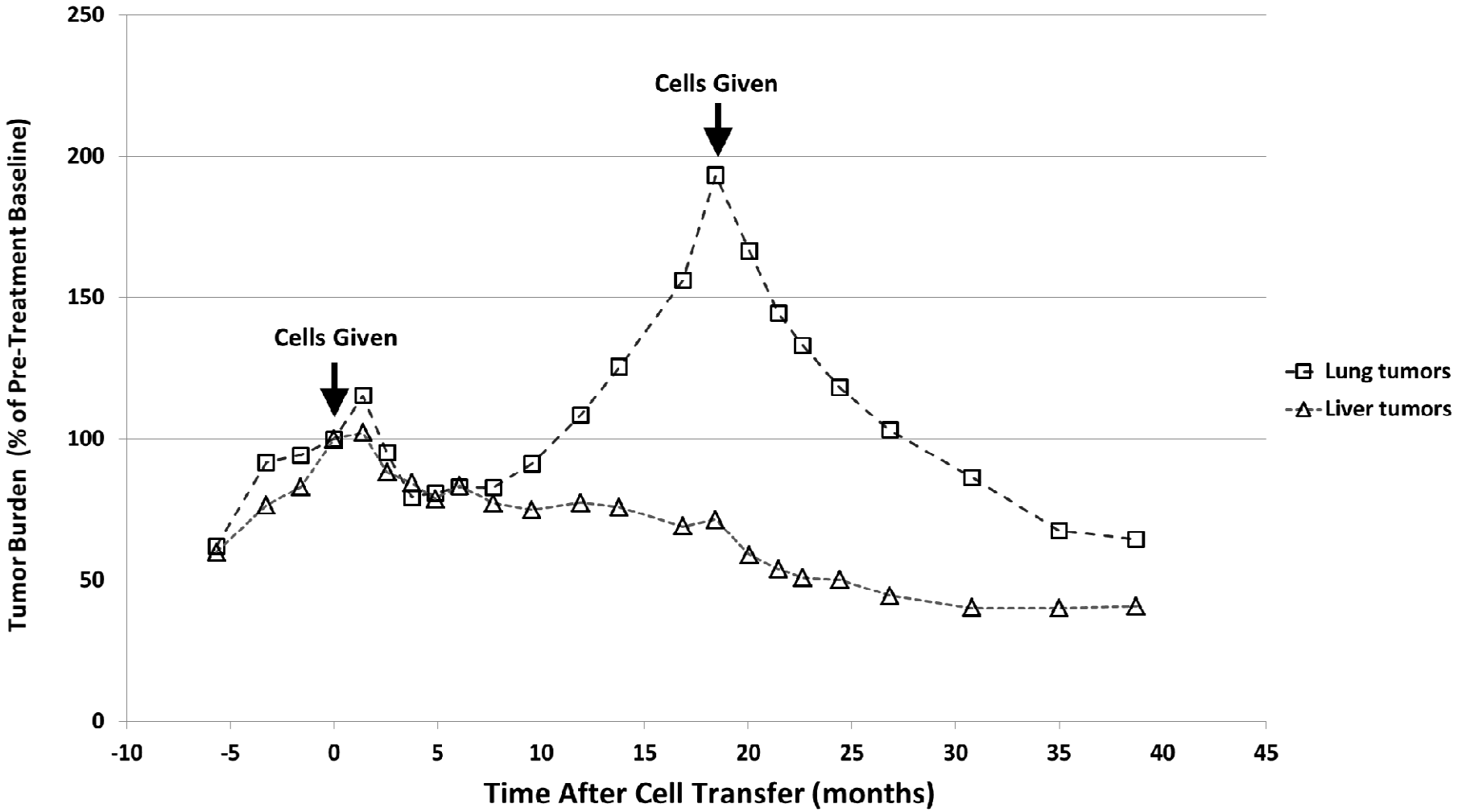
•Only TMG-1 induces IFN-g secretion and upregulation of the CD4+ T-cell activation marker OX40

•**Co-culture TIL + TMG-APC: IFN-g ELISPOT assay**

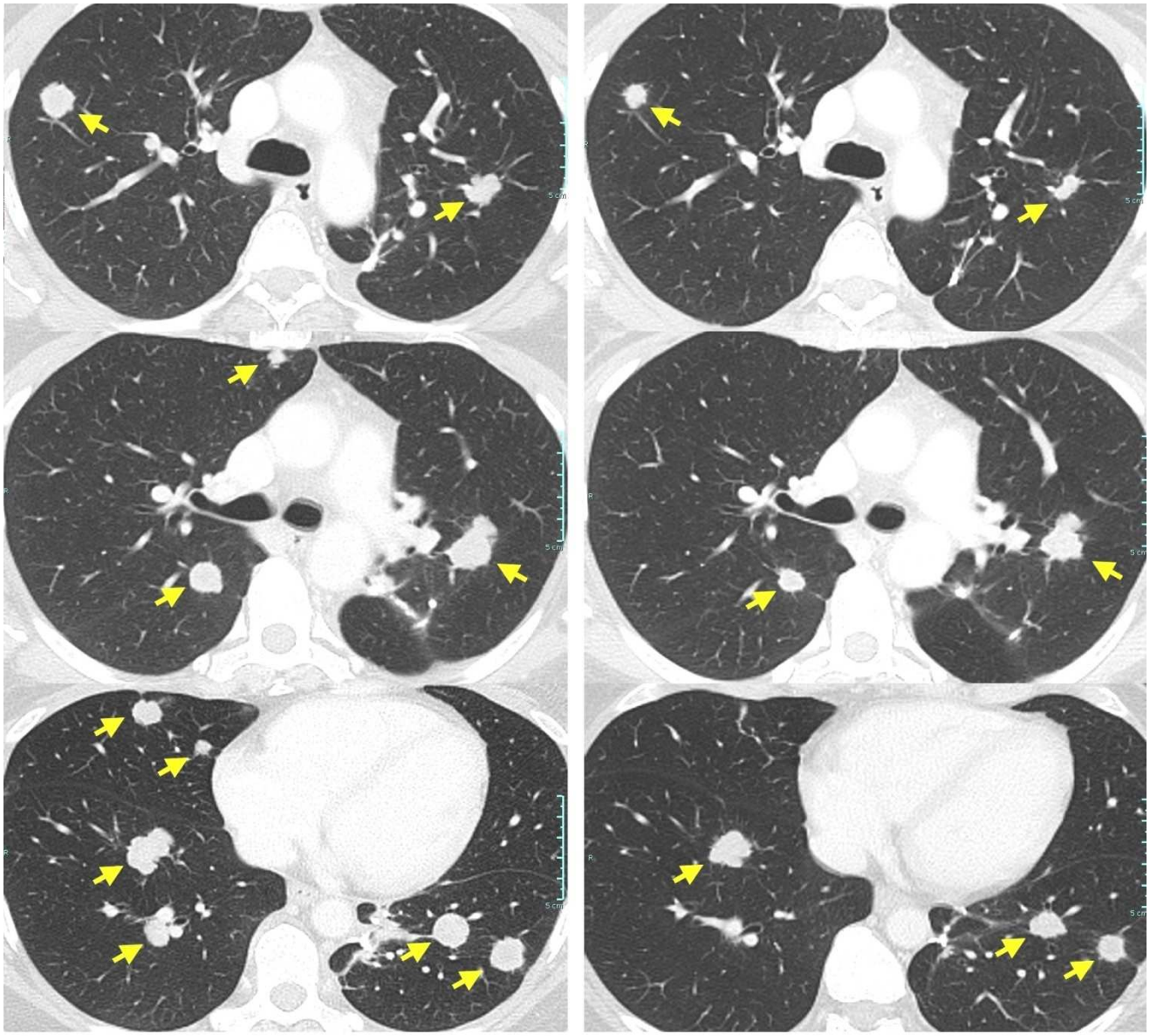


The recognized Ag in TMG-1 was ERBB2 Interacting Protein

Tumor Burden (RECIST)



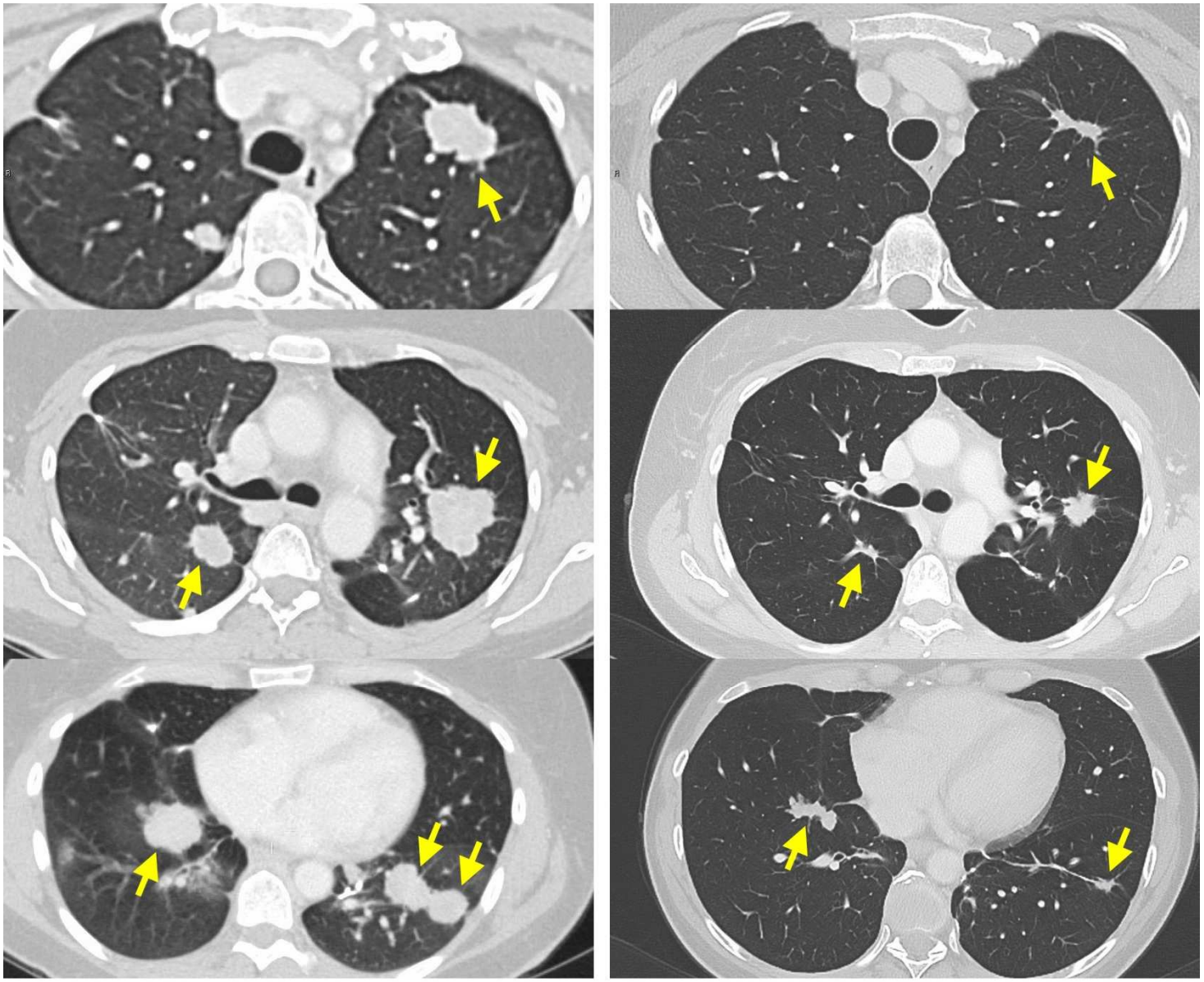
Treatment #1: Bulk TIL



Pre-Treatment

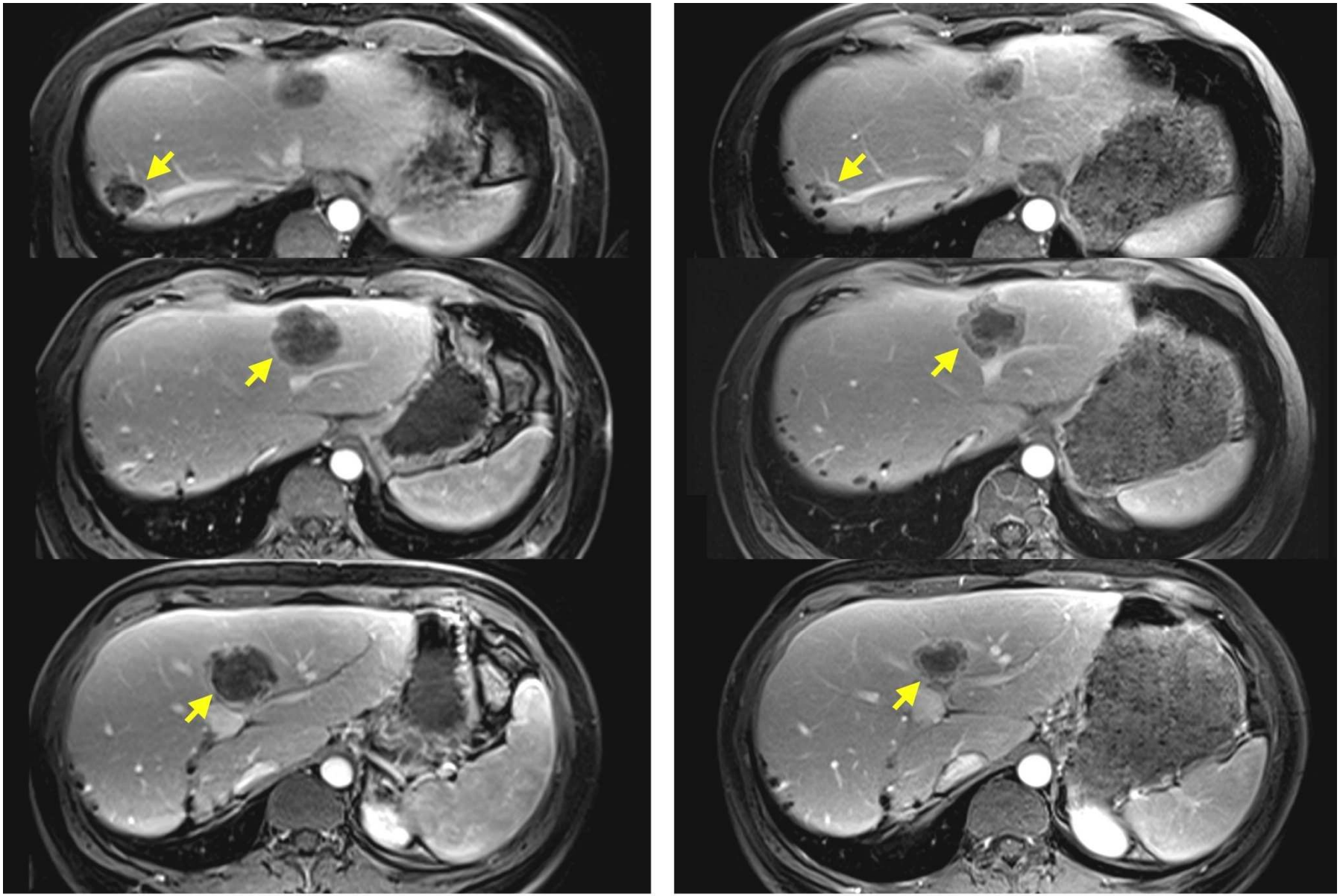
7 Months

Treatment #2: Selected TIL



October 2013 Feb 2015

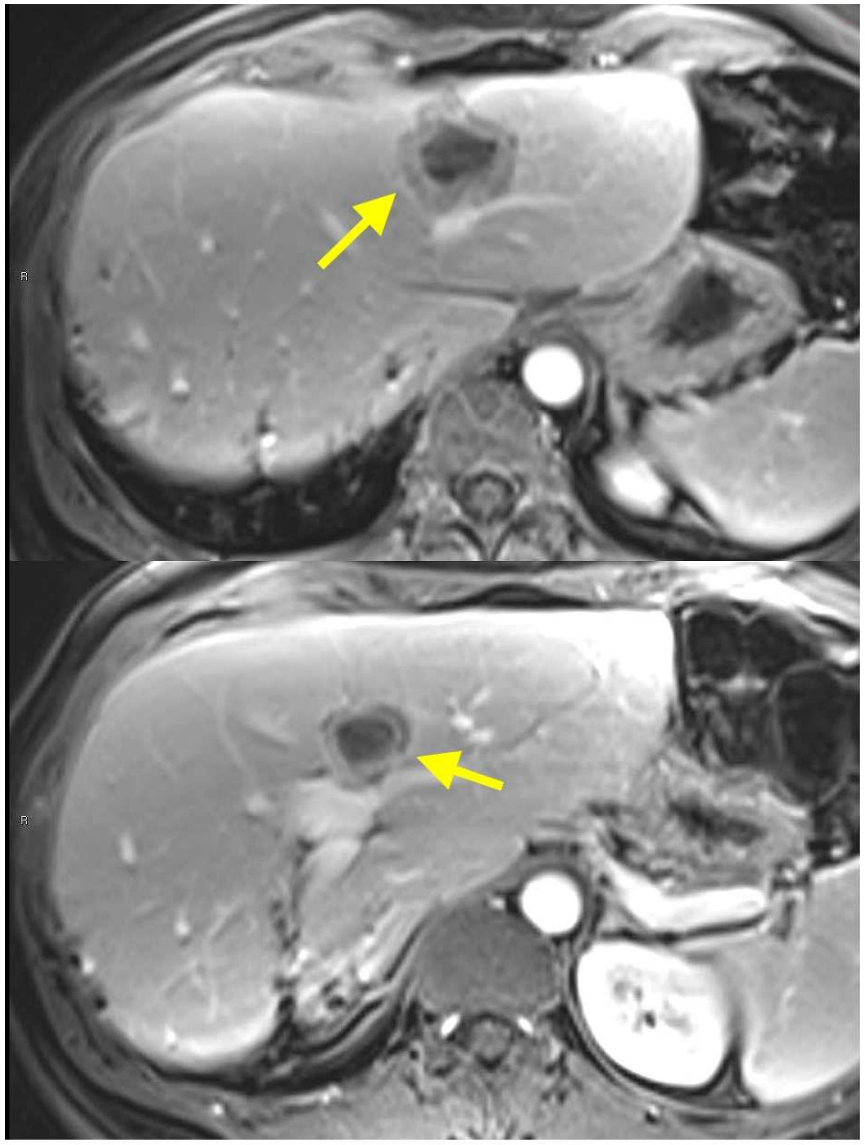
First Treatment- Bulk TIL



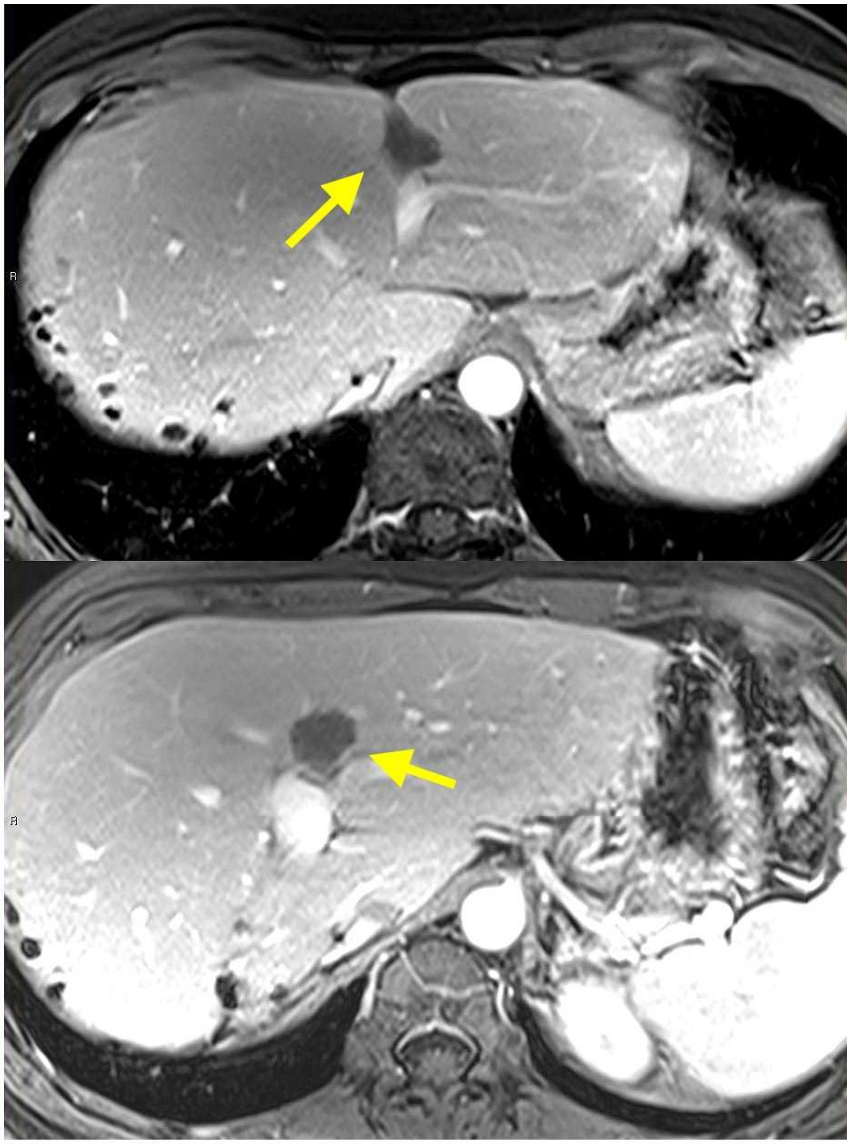
Pre-Treatment

7 Months

Second Treatment-Selected TIL



October 2013

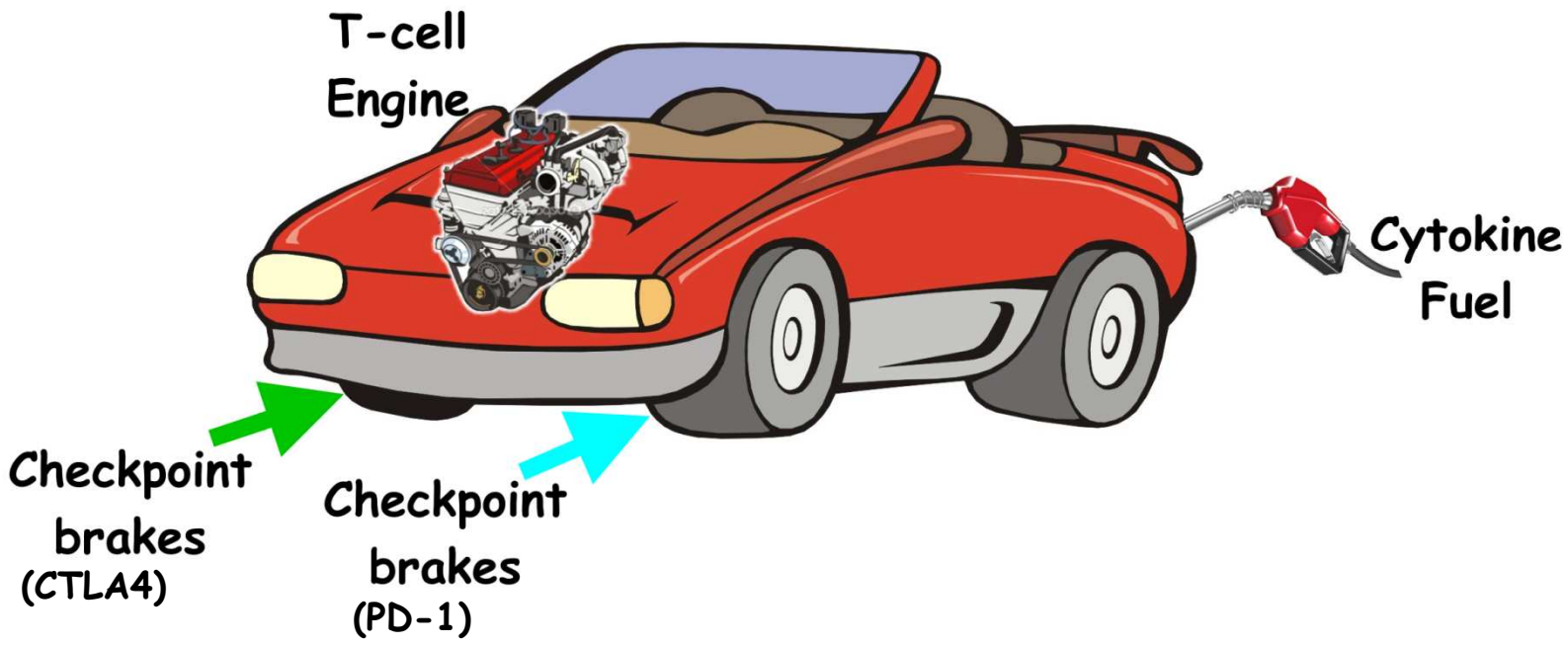


Feb 2015

Conclusions

- T-cell transfers can cure some patients of widespread metastatic melanoma
- Peptide and minigene techniques have shown that many tumor-reactive TIL frequently recognize tumor-specific mutations
- New hypotheses about the importance of T-cells targeting these 'neoantigens' need to be validated by evidence that they treat cancer successfully

Overview of Tumor Rejection



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