Adoptive T-Cell Transfer for Metastatic Melanoma:

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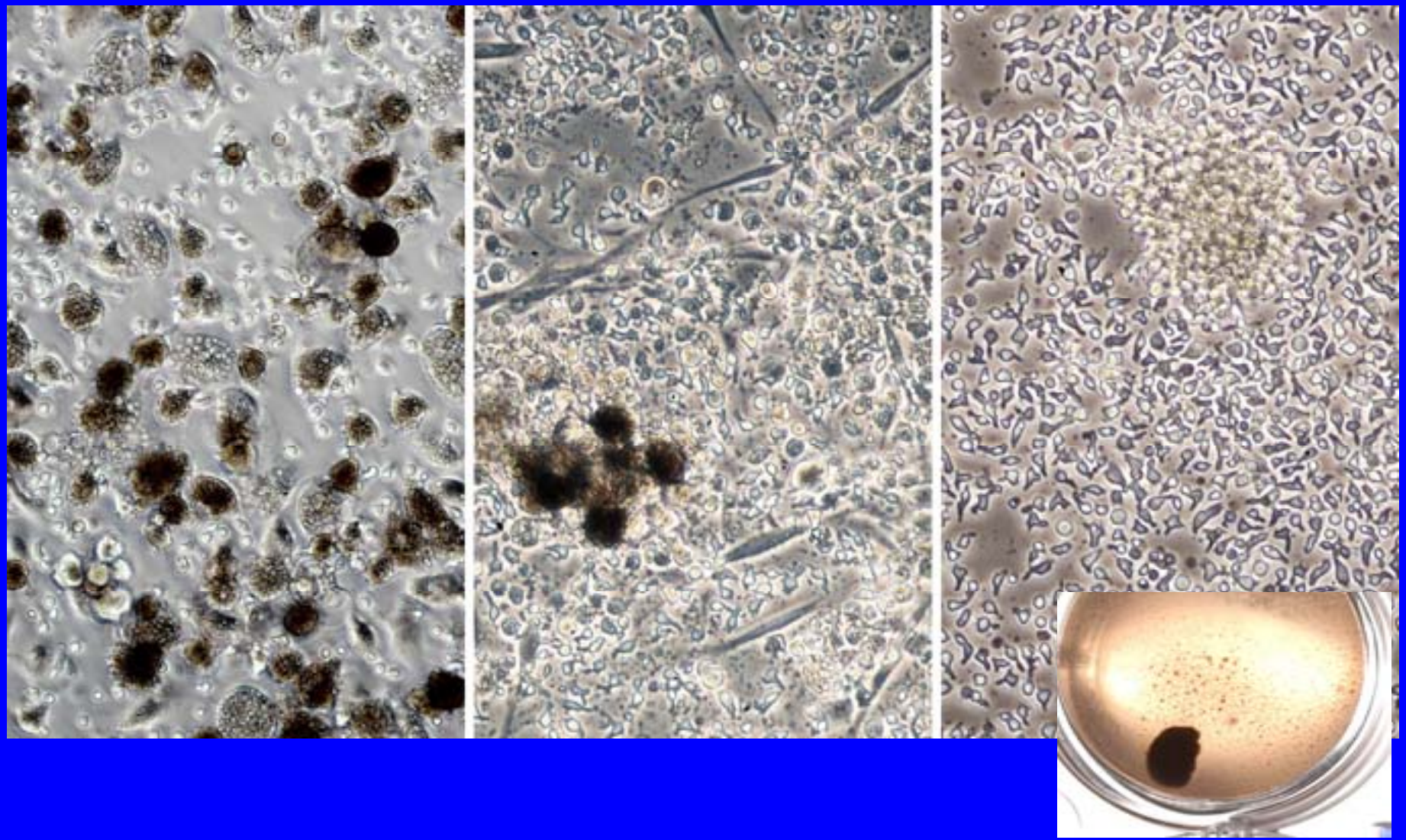
T-Cell-Mediated Tumor Rejection

- Dozens of human tumor-associated proteins which provoke T-cell responses have been discovered, many by studying the T-cells that infiltrate melanoma (TIL; tumor infiltrating lymphocytes)
- T-cell tolerance has been a major impediment to tumor rejection by these cells
- The tumor microenvironment and other host factors play active roles in regulating T-cell activity

Hypotheses

- Ex-vivo stimulation and expansion of tumor-reactive T-cells might overcome some of these impediments to T-cell function
- Manipulation of the recipient prior to the transfer of ex-vivo expanded T-cells could augment the efficacy of adoptive cellular therapy

Melanoma TIL
(Tumor Infiltrating Lymphocytes)



Giving TIL as Therapy

- From 1987-1994, eighty-six patients with metastatic melanoma were given their autologous, unselected, bulk-cultured TIL along with supportive systemic IL-2
- Some received one dose of cyclophosphamide (25 mg/kg) prior to cell transfer
- Twenty-eight had prior high-dose IL-2 therapy

Rosenberg et al. JNCI 1994

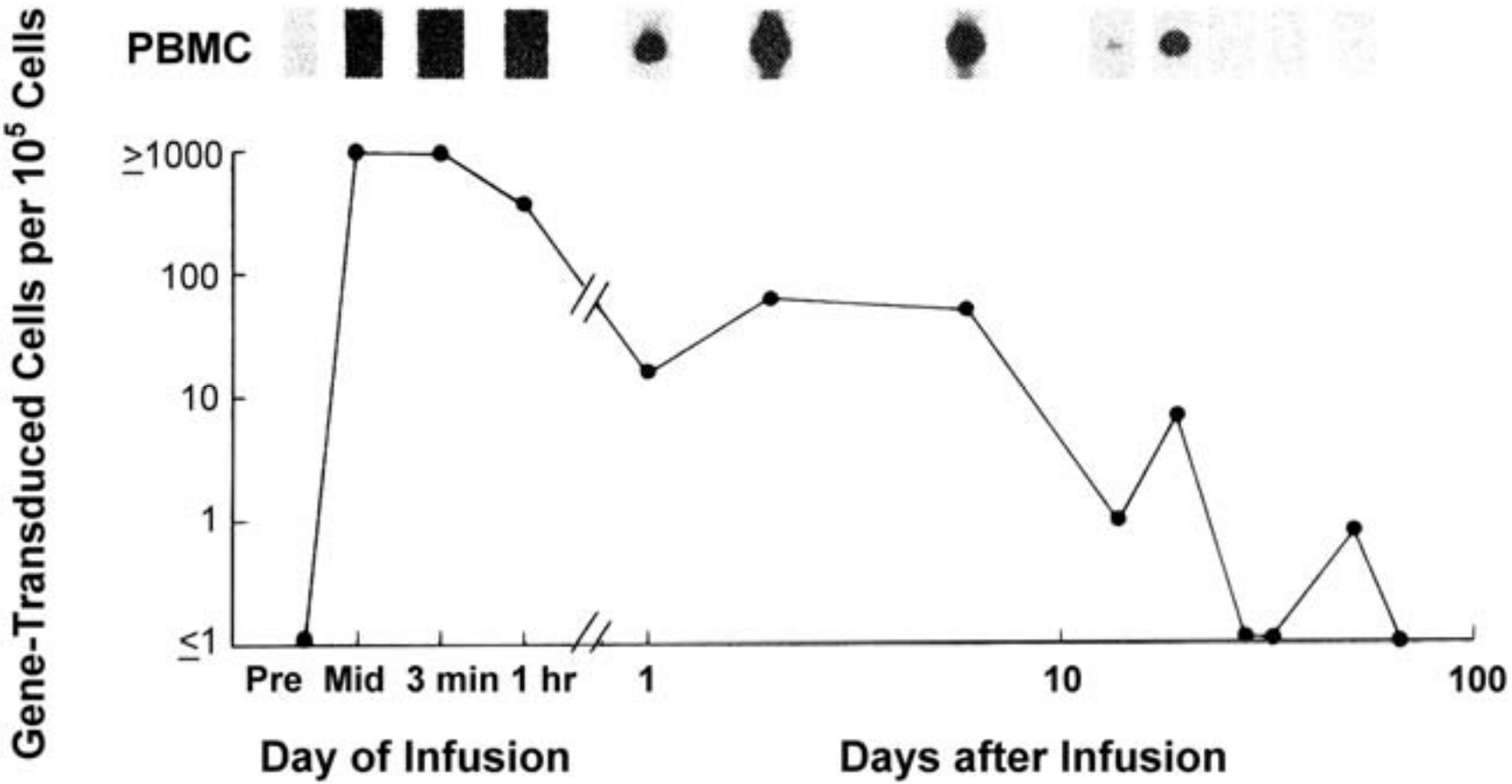
Objective Responses

- Objective RR= 32% prior IL-2
  34% no prior IL-2
-
- Response Durations (months):
  - PRs: 53+, 9, 8, 7, 7, 7, 7, 6, 5, 5, 4, 4, 4, 4, 2, 1, 1
  - CRs: 46+, 38, 21+, 23, 20

(5/6 durable or complete responses were in IL-2 naïve pts.)

Survival/Persistence of TIL in Vivo

- 2×10^{11} was the median number of TIL given
- When genetic marking was done with the neophosphotransferase gene and in vivo survival tracked after infusion, TIL could only be briefly detected by PCR


PBMC
Gene-Transduced Cells per 10^5 Cells
≥1000
100
10
1
≤1
Pre
Mid
3 min
1 hr
1
10
100
Day of Infusion
Days after Infusion

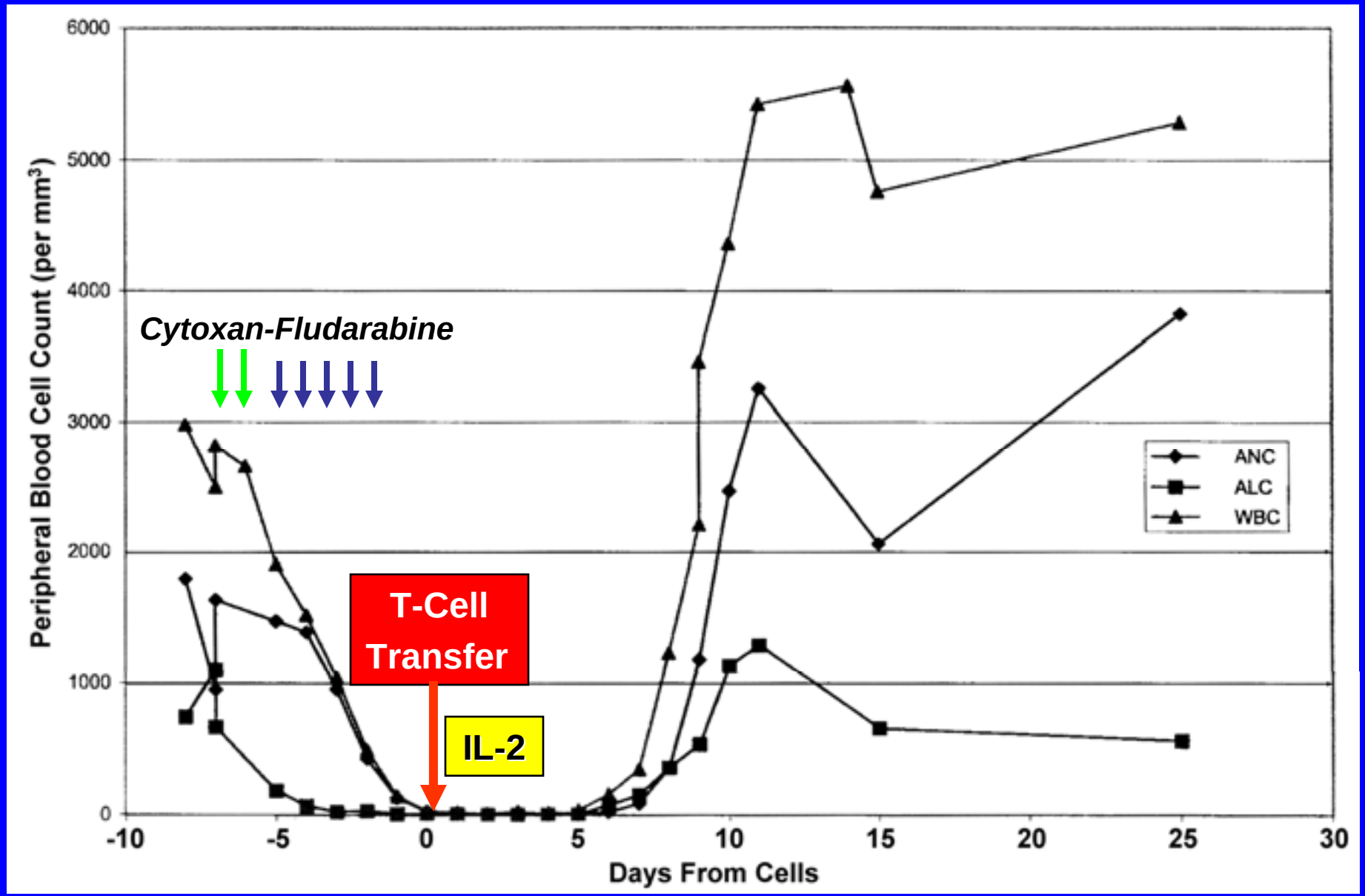
TIL Recognition of Tumor (IFN-gamma Release Assay)

T-Cells	Target Cells								
	Peptide on $A2^+$			Mel ($A2^-$)		Mel ($A2^+$)			
	None	MART	gp100	888 Mel	938 Mel	526 Mel	624 Mel	Autol Mel	Specificity
Patient TIL									
Patient 18	2,922	1,985	**37,895**	1,381	857	**16,130**	**18,665**	**20,365**	gp100
Patient 28	0	**9.725**	0	0	0	**6,895**	**528**	**16,435**	MART-1
Patient 6	ND	ND	ND	144	136	241	260	**5,980**	Autol
Controls									
gp 100 CTL	0	0	**2,503**	20	0	**23,695**	**14,900**	**16,875**	gp100
MART-1 CTL	0	**16,160**	407	0	0	**11,975**	**3,505**	**15,880**	MART-1
No T-Cells	0	0	0	0	0	0	0	0	None

Host Lymphodepletion?

- Animal models demonstrate that transferred lymphocytes proliferate better in an immunodepleted recipient (homeostatic proliferation)
- T-regulatory cells can impede tumor rejection and are reduced by host lymphodepletion prior to adoptive transfer (Antony et al, JI 2005)

Cyclophosphamide + Fludarabine Preparative Regimen

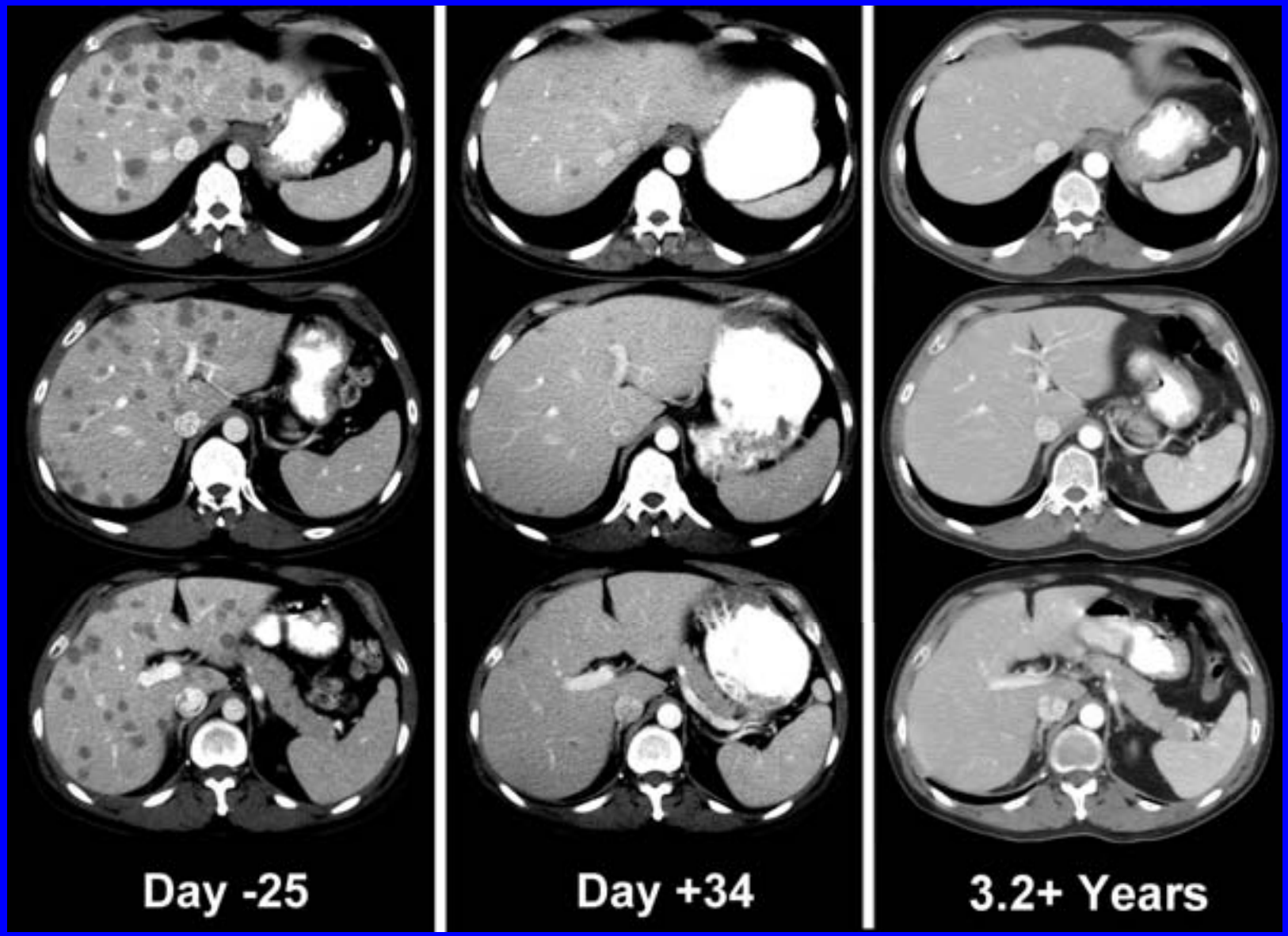


Clinical Trial: Cy-Flu, TIL and IL-2

Characteristics	No.	%
Total patients	35	100
Sex		
Male	21	60
Female	14	40
Age, years		
≤40	10	29
41-50	13	37
51-60	10	29
61-70	2	6
Performance status		
0	29	83
1	6	17
Prior treatments		
Surgery	35	100
Chemotherapy	18	51
Radiotherapy	11	31
Immunotherapy	35	100
Any, ≥ 2	35	100
Any, ≥ 3	22	63

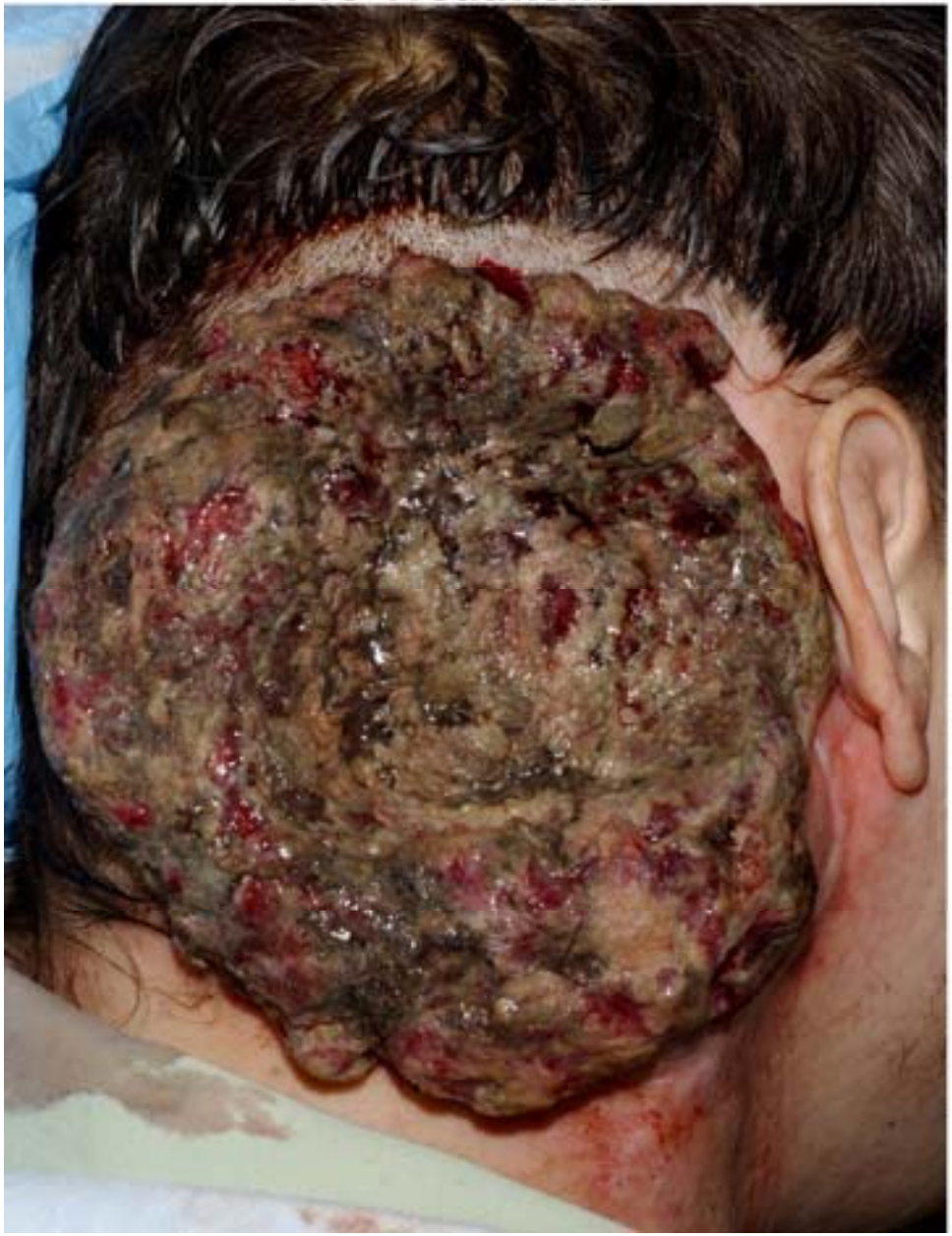
Dudley et al, JCO 2005

Chemotherapy, TIL Transfer and IL-2

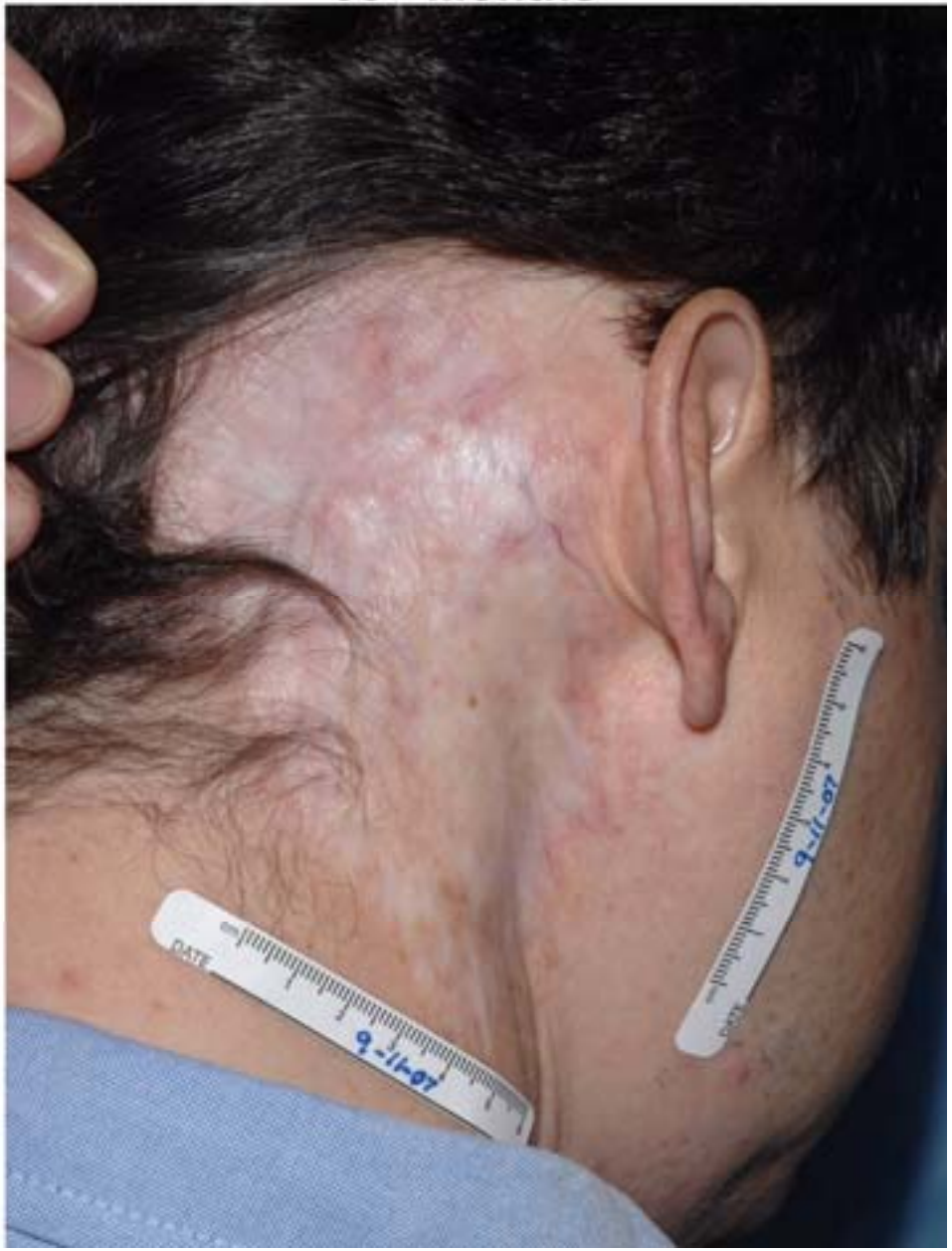


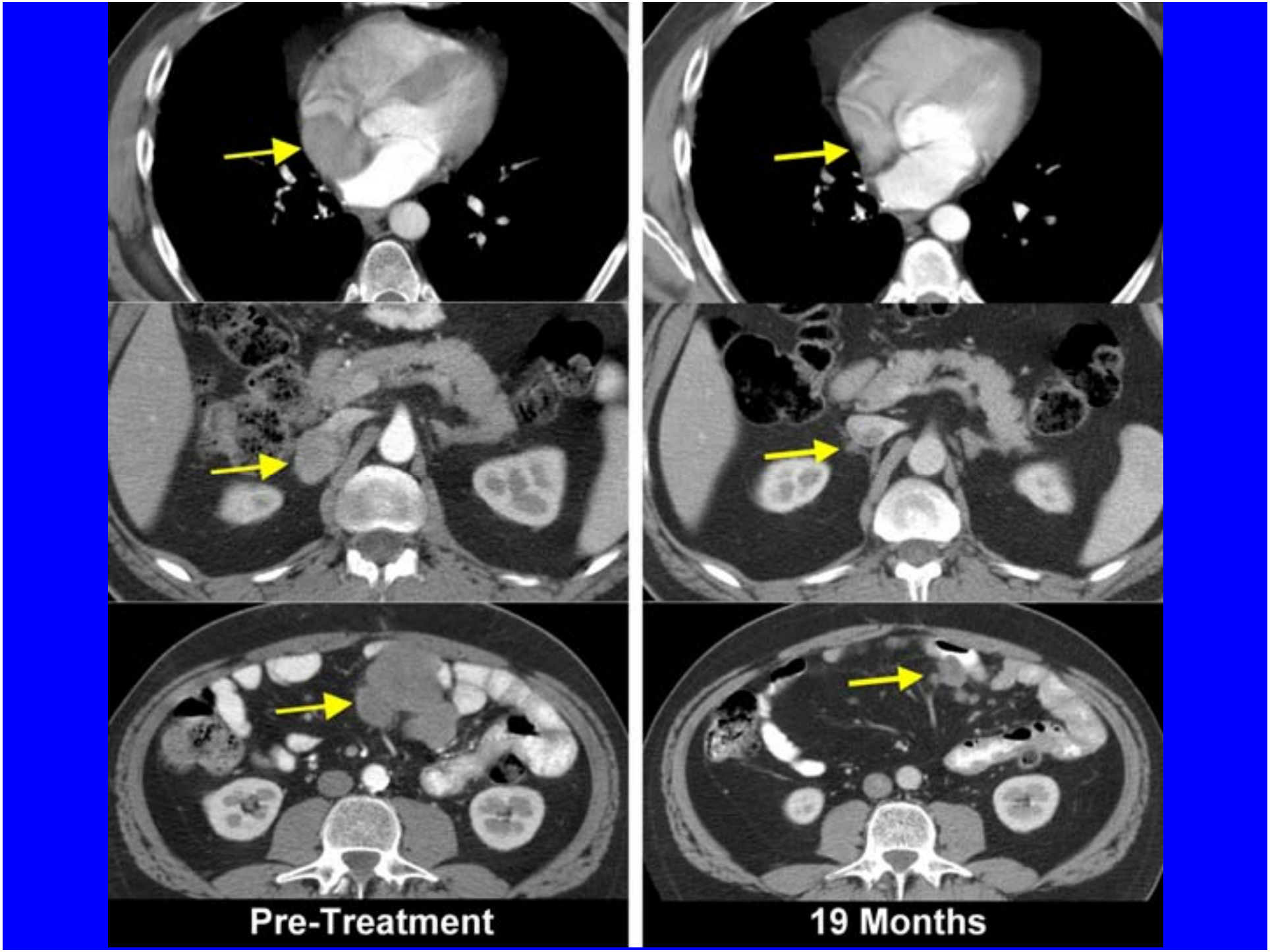
Metastatic Melanoma

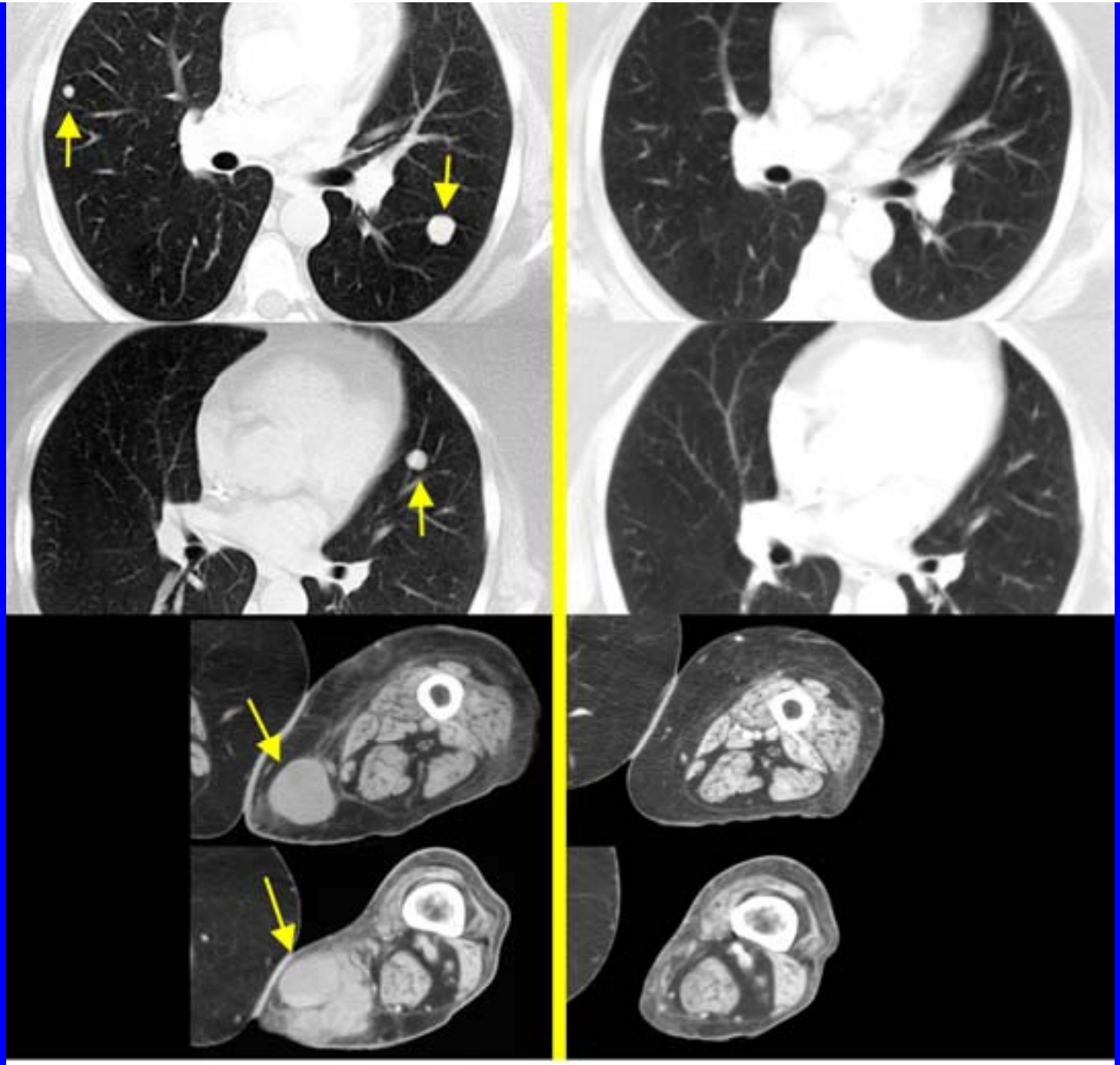
Pre-Treatment



30+ Months




Pre-Treatment
19 Months


Pre-Treatment
38+ Months

Results

- 43 patients treated currently (35 reported)
- 41 had previous IL-2
- Median number of TIL given 5×10^{10}
- Response Rate = 49% (17 PR, 4 CR)

Response Durations: Cy-Flu, TIL and IL-2 (Months)

- PR: 62+, 30+, 29, 28, 18+, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2
- CR: 61+, 56+, 46+, 45+
- Median duration= 13 mo

Oct 2007

Persistence of T-Cell Clones After TIL Transfer Into a Lymphodepleted Patient

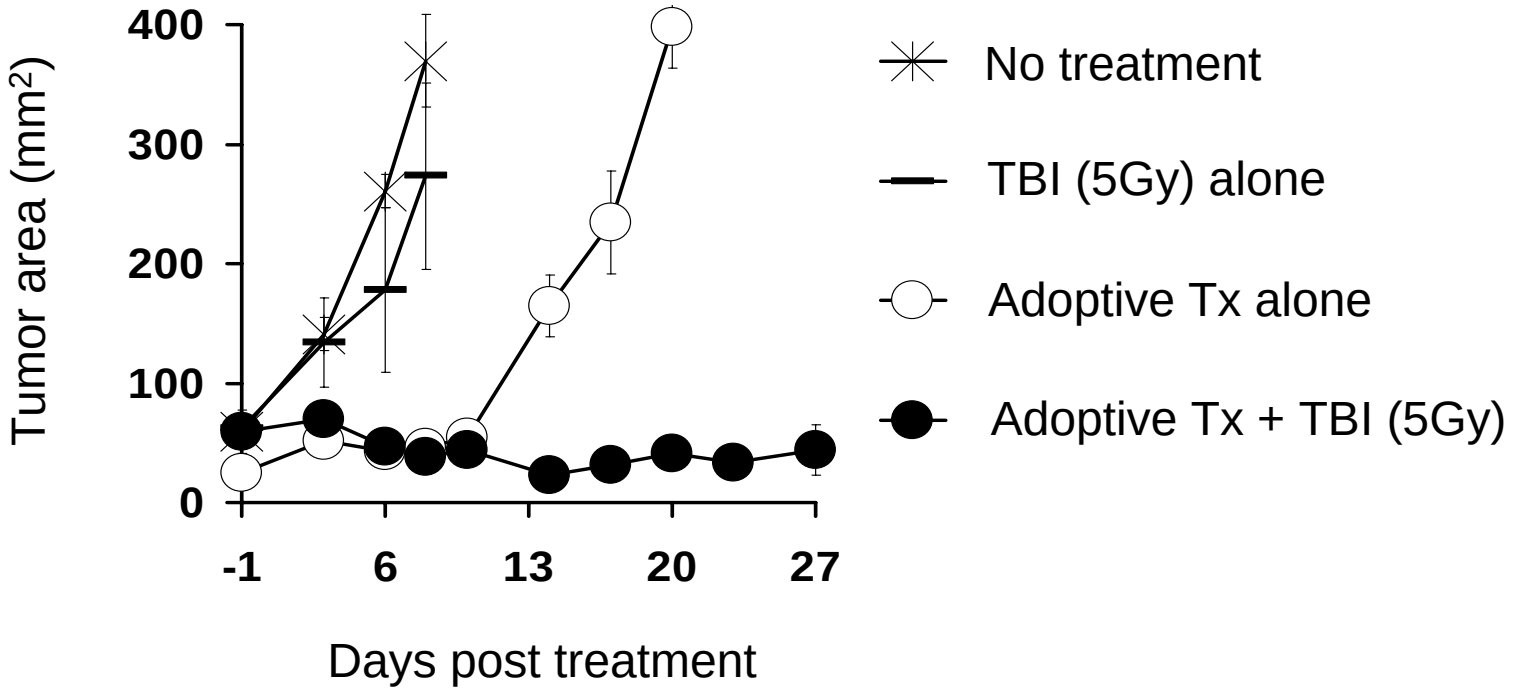
TCR BV Genes	TIL	Day 9	Day 19	Day 46
	(% of lymphocytes)			
BV3	--	--	--	6
BV4-1	--	--	3	--
BV6-4	23	5	--	--
BV6-6	--	--	6	--
BV7-6	--	--	--	3
BV7-9	--	--	--	3
BV10-3	34	69	88	88
BV20-1	21	8	--	--
BV27	5	12	--	--
BV29-1	2	--	--	--
BV30	15	3	--	--
CD8+tetramer+	89	n.d.	60	n.d.

Augmentation of Adoptive T-Cell Transfer by Host Immunosuppression

Hypotheses Based on Murine Modeling

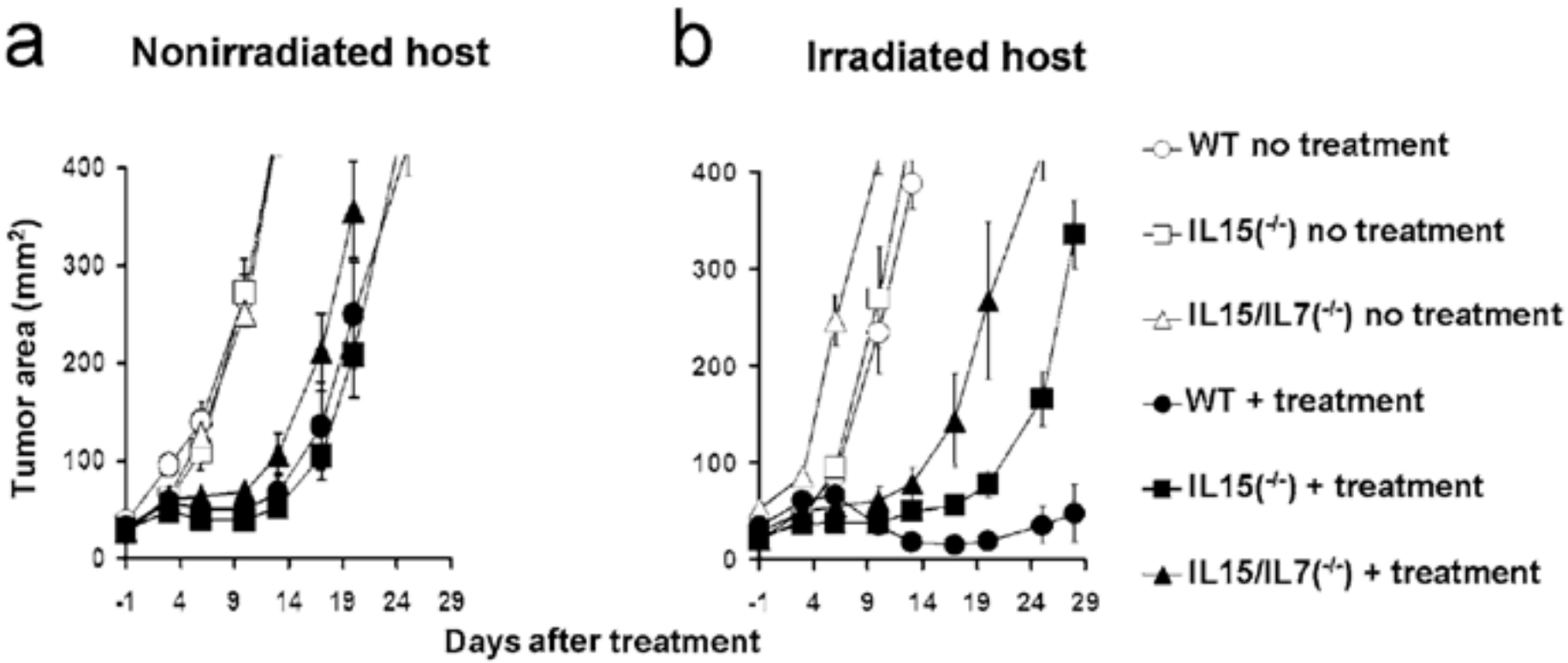
- Elimination of cytokine "sinks"
- Elimination of T reg Cells
- TLR stimulation via enteric organisms

Enhanced Function of Adoptively Transferred pmel-1 T-cells after Total Body Irradiation (TBI)



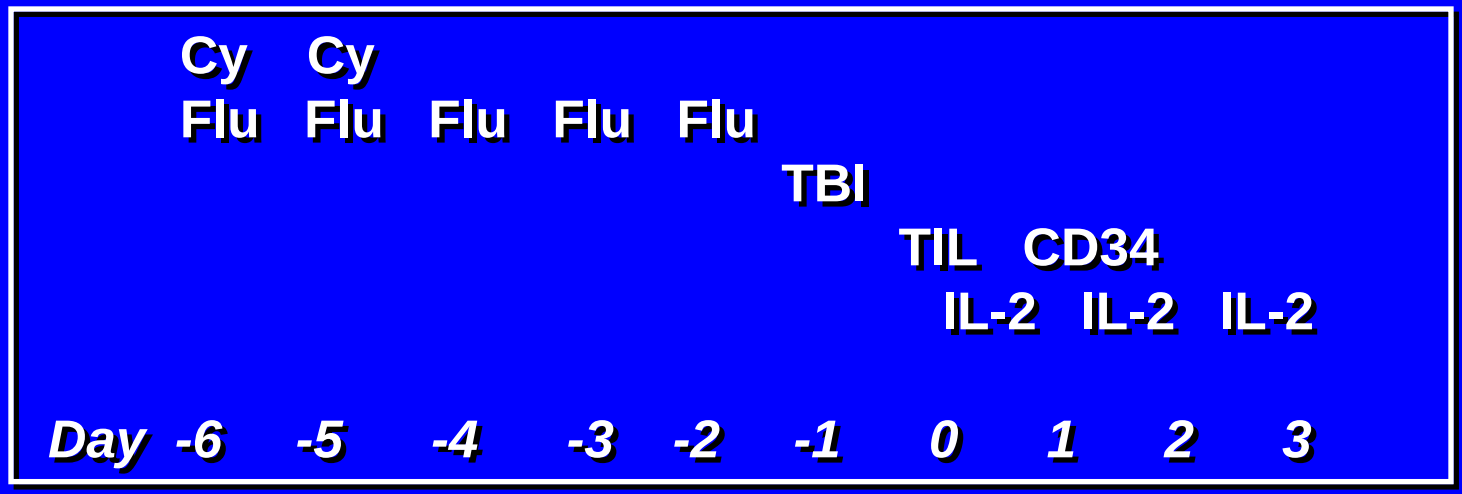
Gattinoni et al, JEM, 2005 202:907

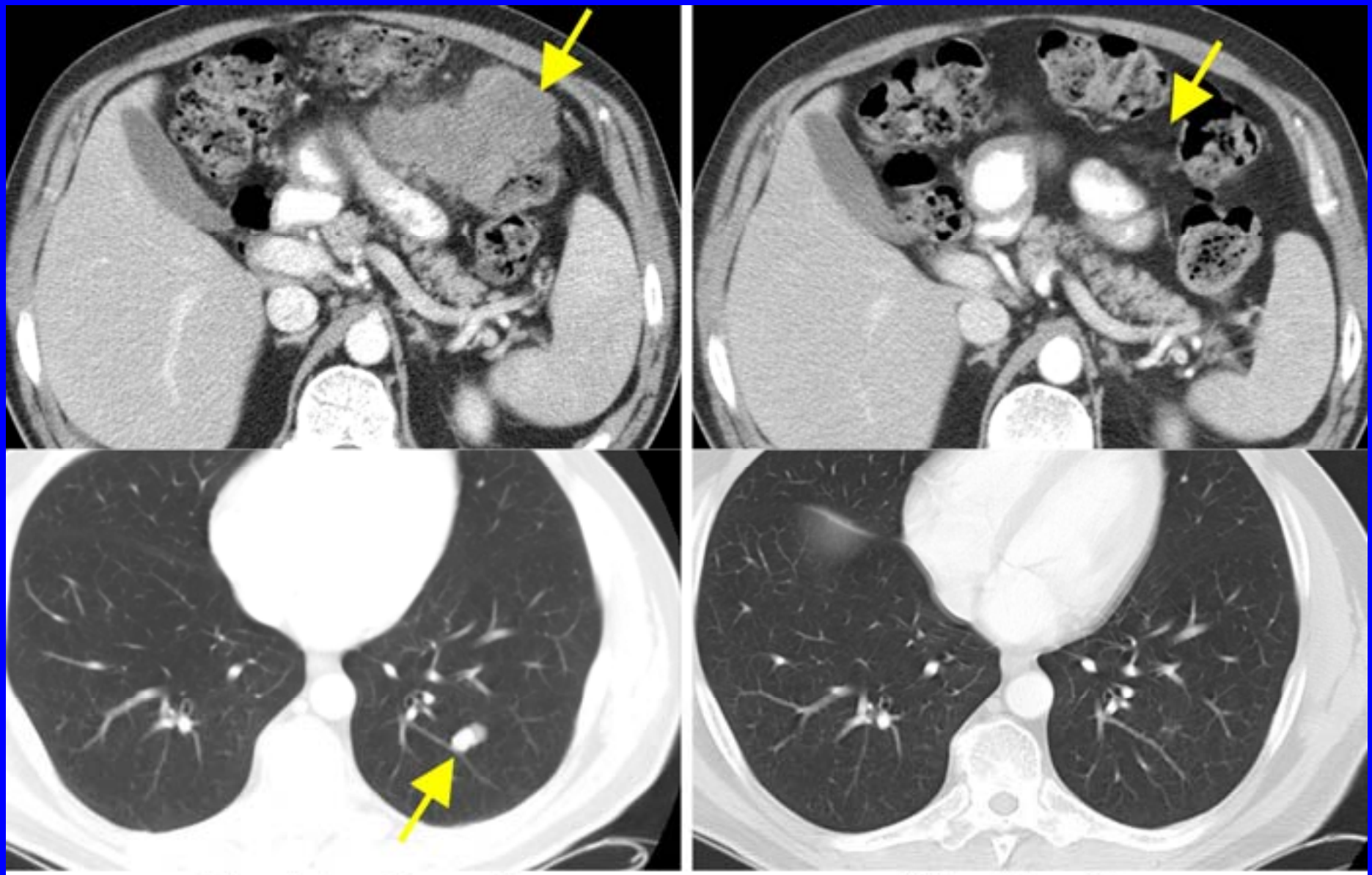
IL-7 and IL-15 Contribute to the Benefit of Host Irradiation

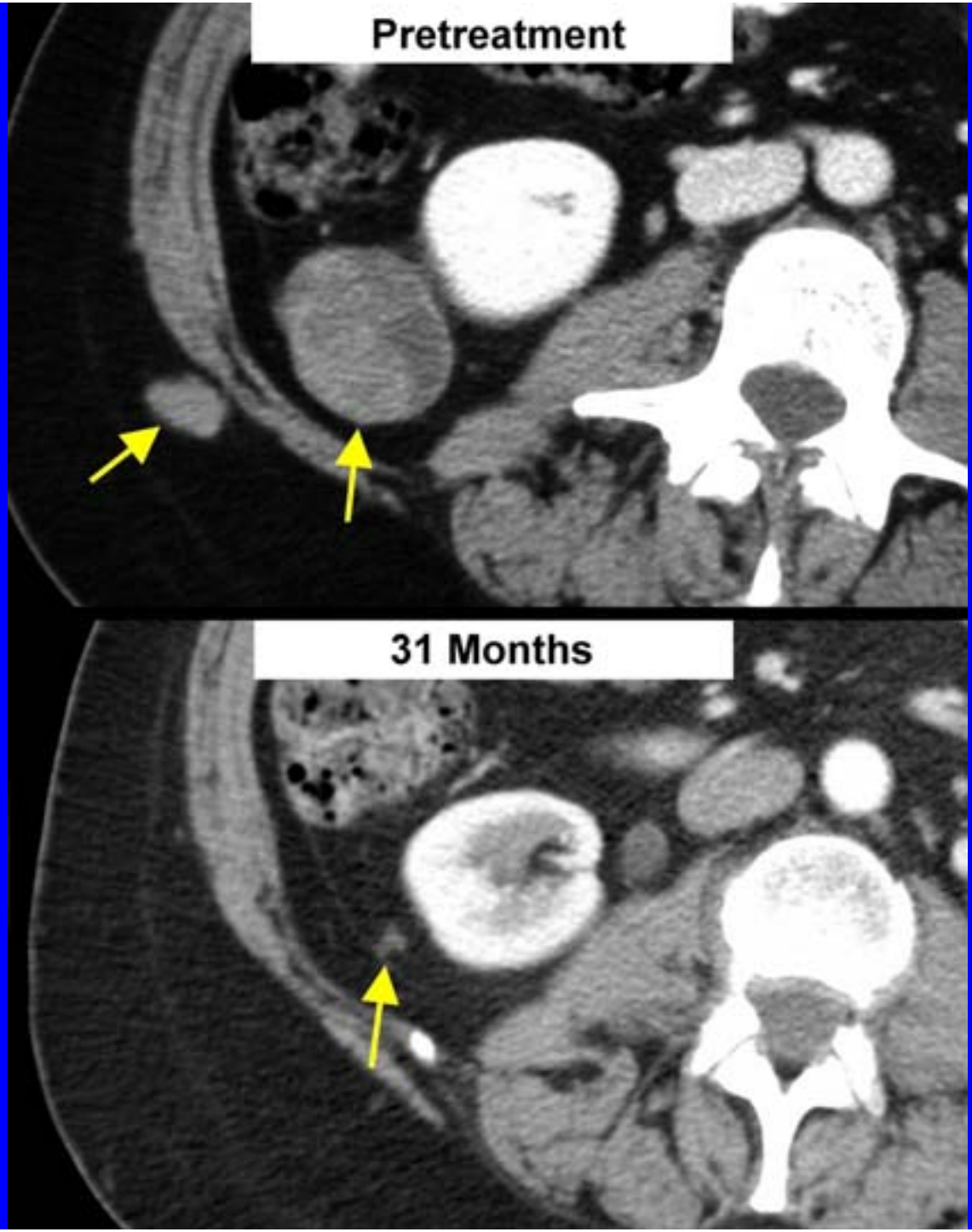


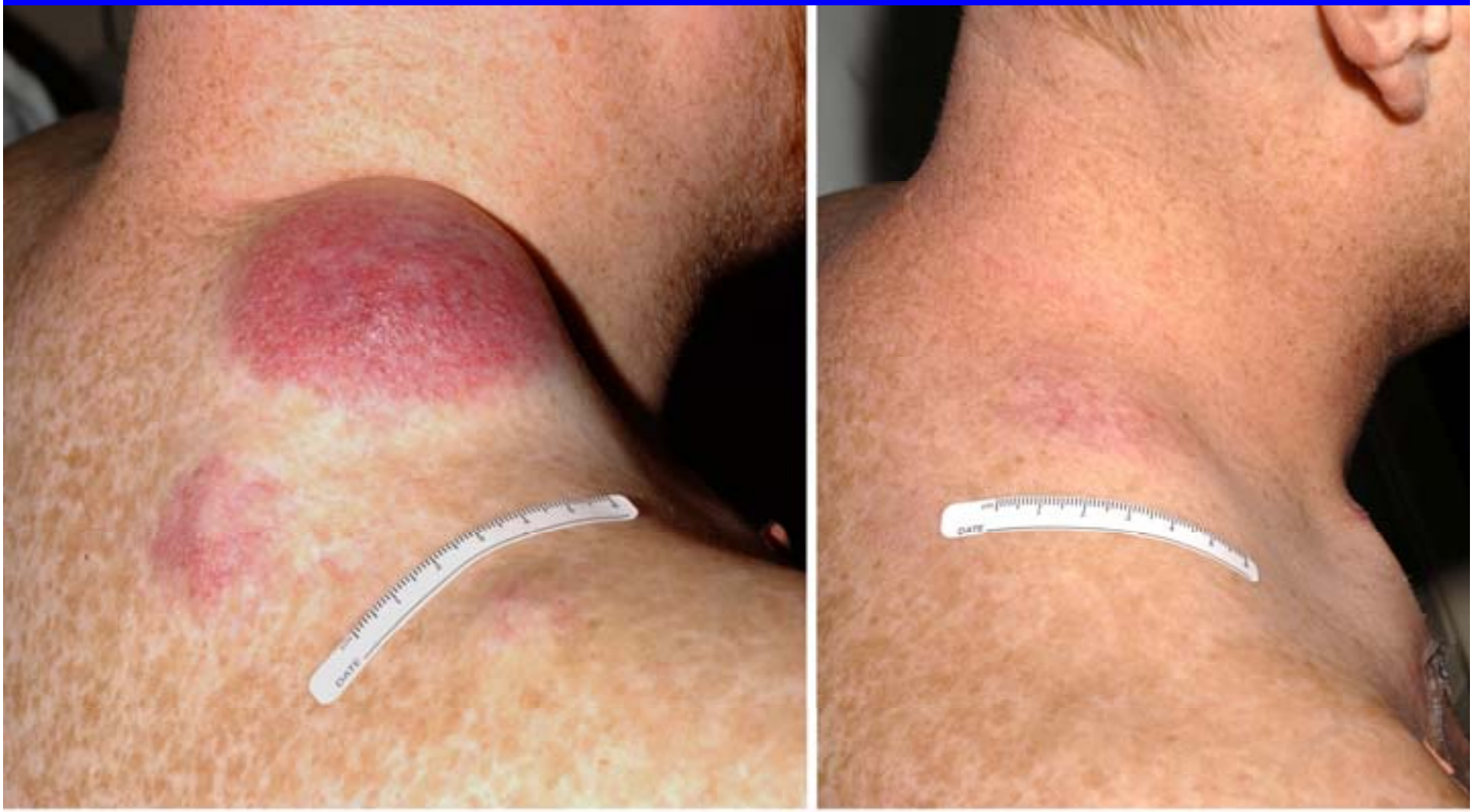
Revised TIL Protocol Adding 200cGy TBI

- Same dose of cyclophosphamide and fludarabine with 200cGy TBI added just prior to cell transfer
- Autologous g-CSF-mobilized, purified CD34+ stem cell support given one day after T-cell transfer



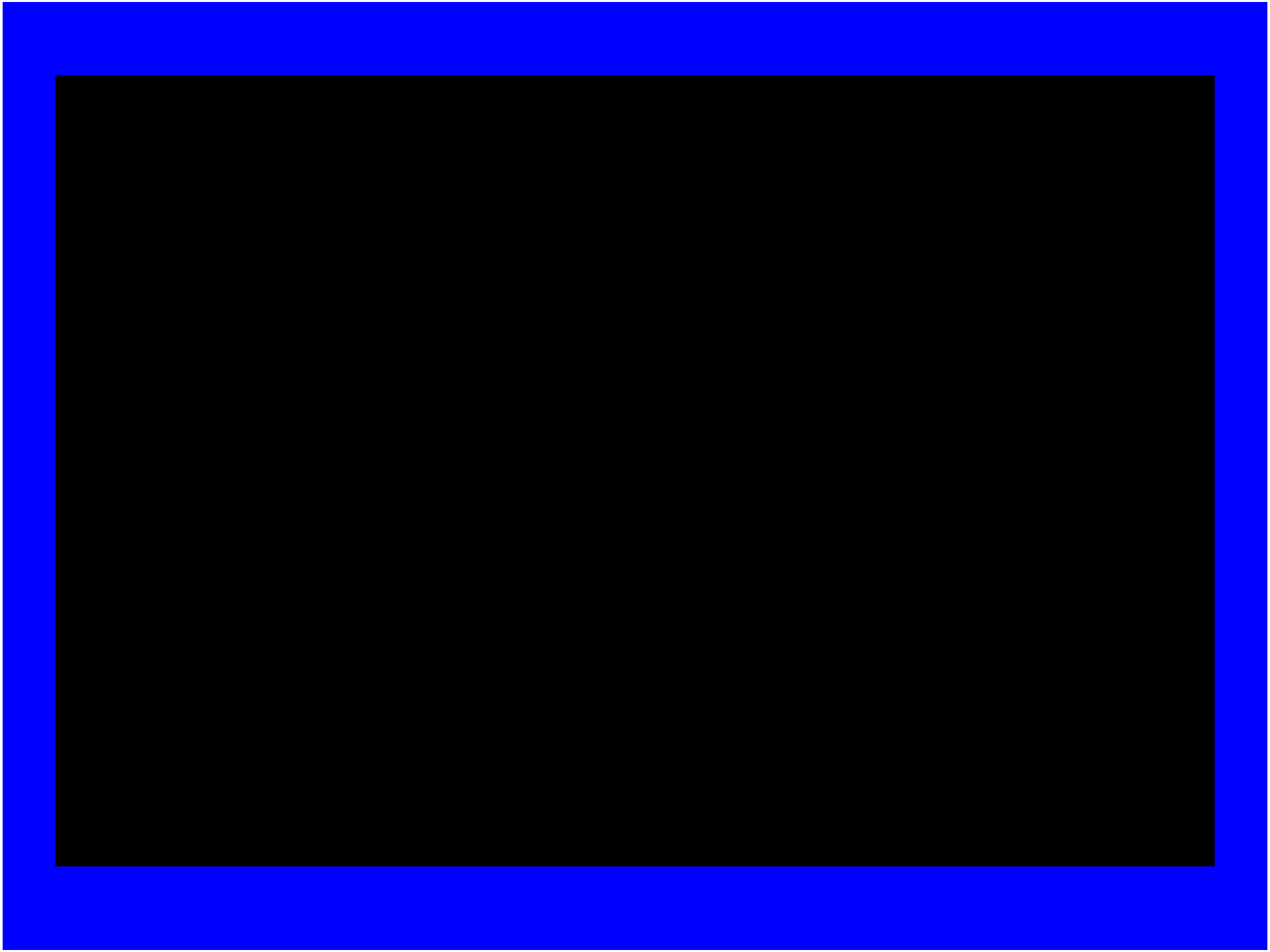

Pre-Treatment
21+ Months


Pretreatment
31 Months



Pre-Treatment

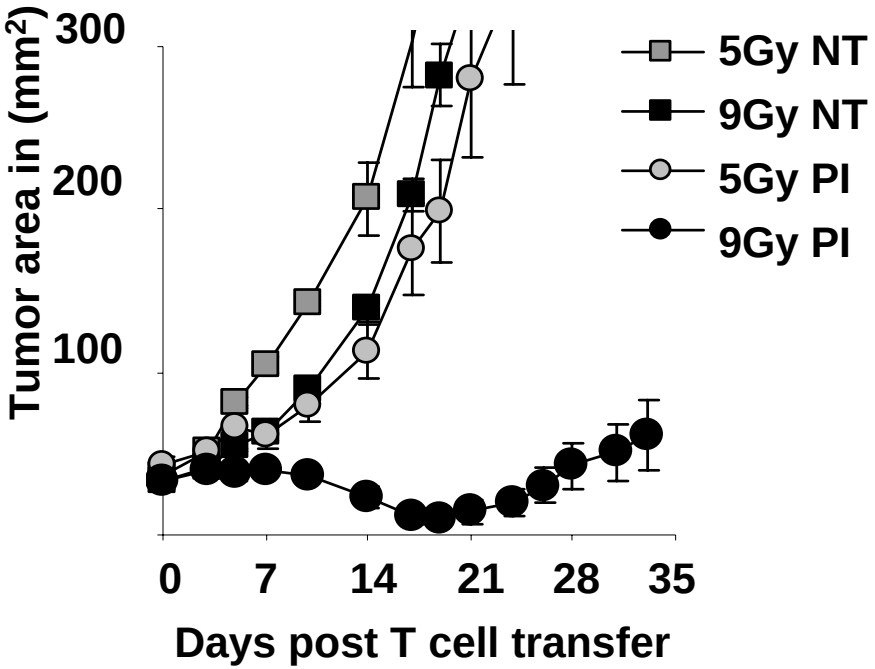
12 Days after Cell Transfer



TIL After Cy+Flu+200cGy TBI

- 25 patients treated; median number of TIL given= $5x10^{10}$
- RR= 52% (13/25)
- Response Durations (months):
  - CR: 35+, 23+
  - PR: 31+, 27+, 21+, 14, 10, 6, 5, 5, 4, 3, 3

900 cGy TBI + Hematopoietic stem cells (HSC) significantly improves tumor response in the absence of a vaccination



P: 1e6 1 week cultured pmel-1 transgenic T cells;
I: IL-2 (100K CU/bid x 3d).
9Gy irradiated animal received a HSC transplant

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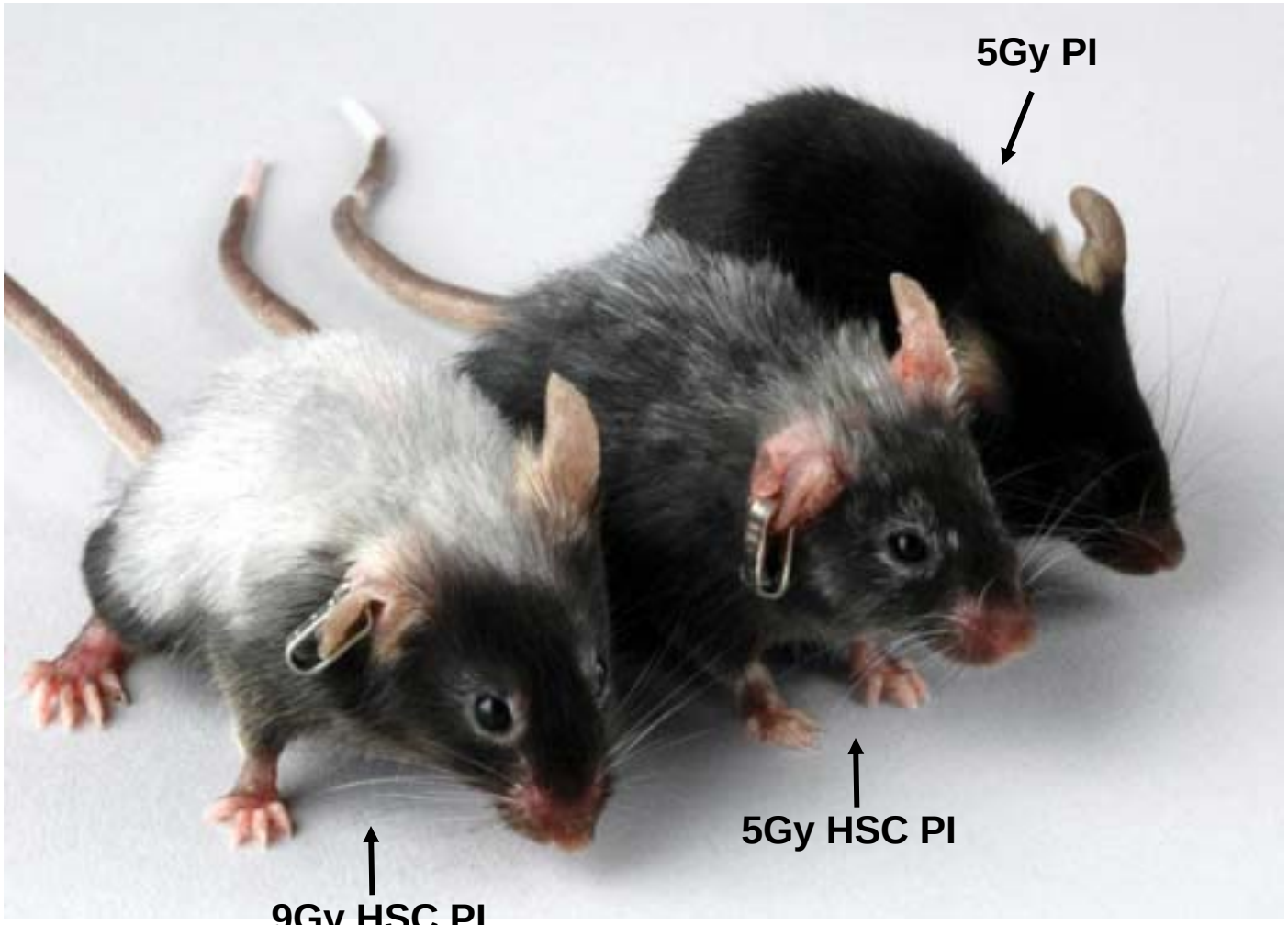
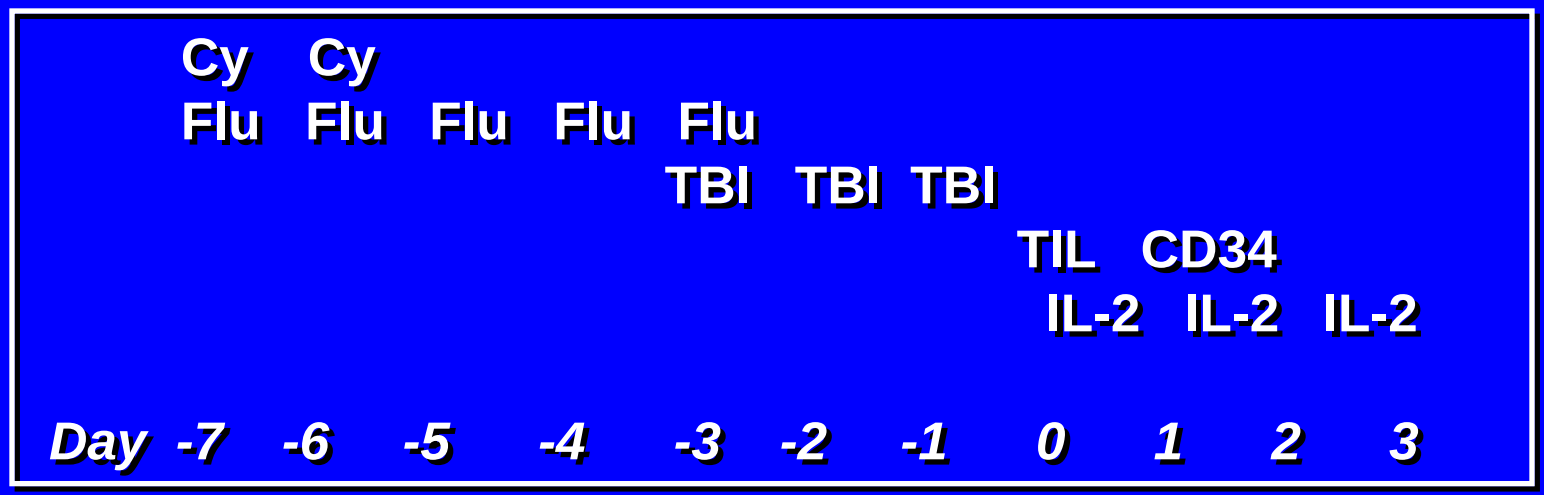
Induction of profound vitiligo after 9Gy TBI and HSC transplantation in the absence of vaccination



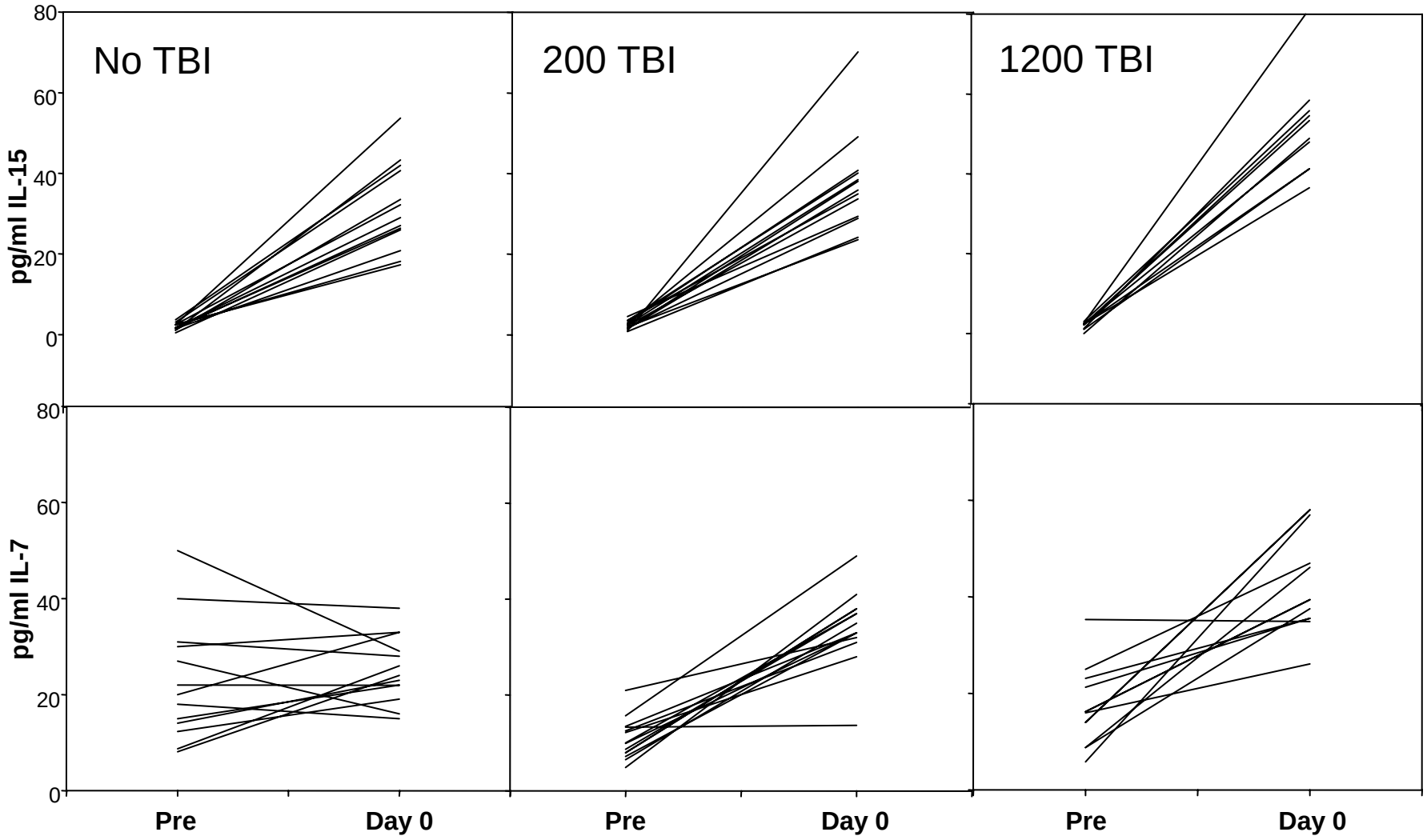
TIL with Cy+Flu+1200cGy TBI



Grade 3/4 Toxicities in TIL/TBI Protocols

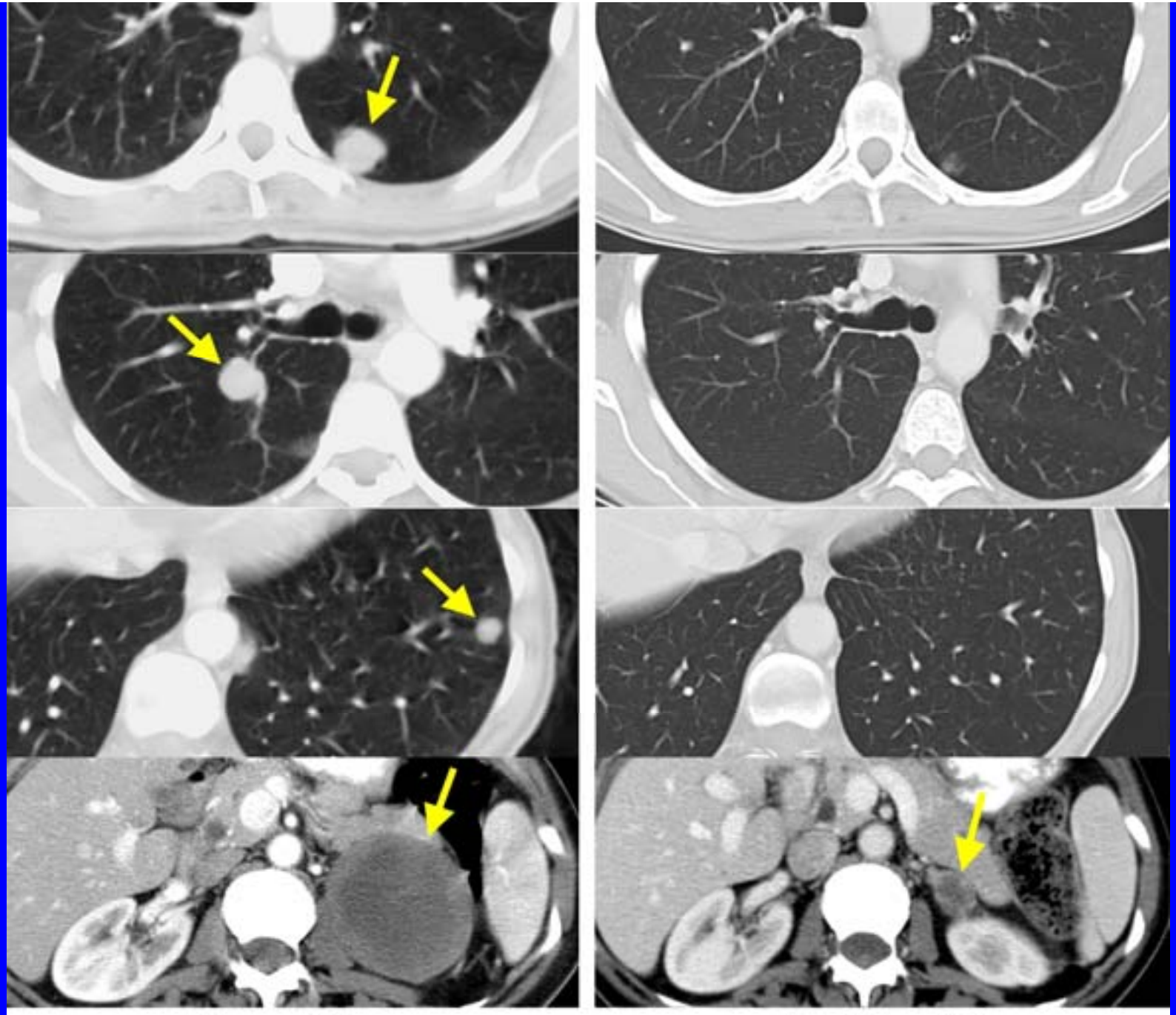
	200 TBI (n = 25)		1200 TBI (n = 18)	
	Grade 3	Grade 4	Grade 3	Grade 4
	(number of patients)			
Febrile neutropenia	12	0	11	0
Sepsis	2	0	3	0
Pneumonia	3	0	0	0
PTT elevation	6	0	4	0
Hypoalbuminemia	7	0	1	0
ALT/AST increase	2	0	2	0
Alkaline p'tase increase	0	0	11	0
Hyperbilirubinemia	1	0	1	0
Hypocalcemia	3	1	4	1
Hypomagnesemia	2	0	0	0
Hypophosphatemia	4	0	7	0
Hyponatremia	3	0	4	0
Hyperuricemia	0	1	0	2
Somnolence	2	0	4	0
Dypsnea	7	1	3	0
Renal failure	0	1	2	0
Autoimmunity	0	0	1	0
Thrombotic microangiopathy	0	0	1	0
Desquamating rash	0	0	2	0
Death		1		

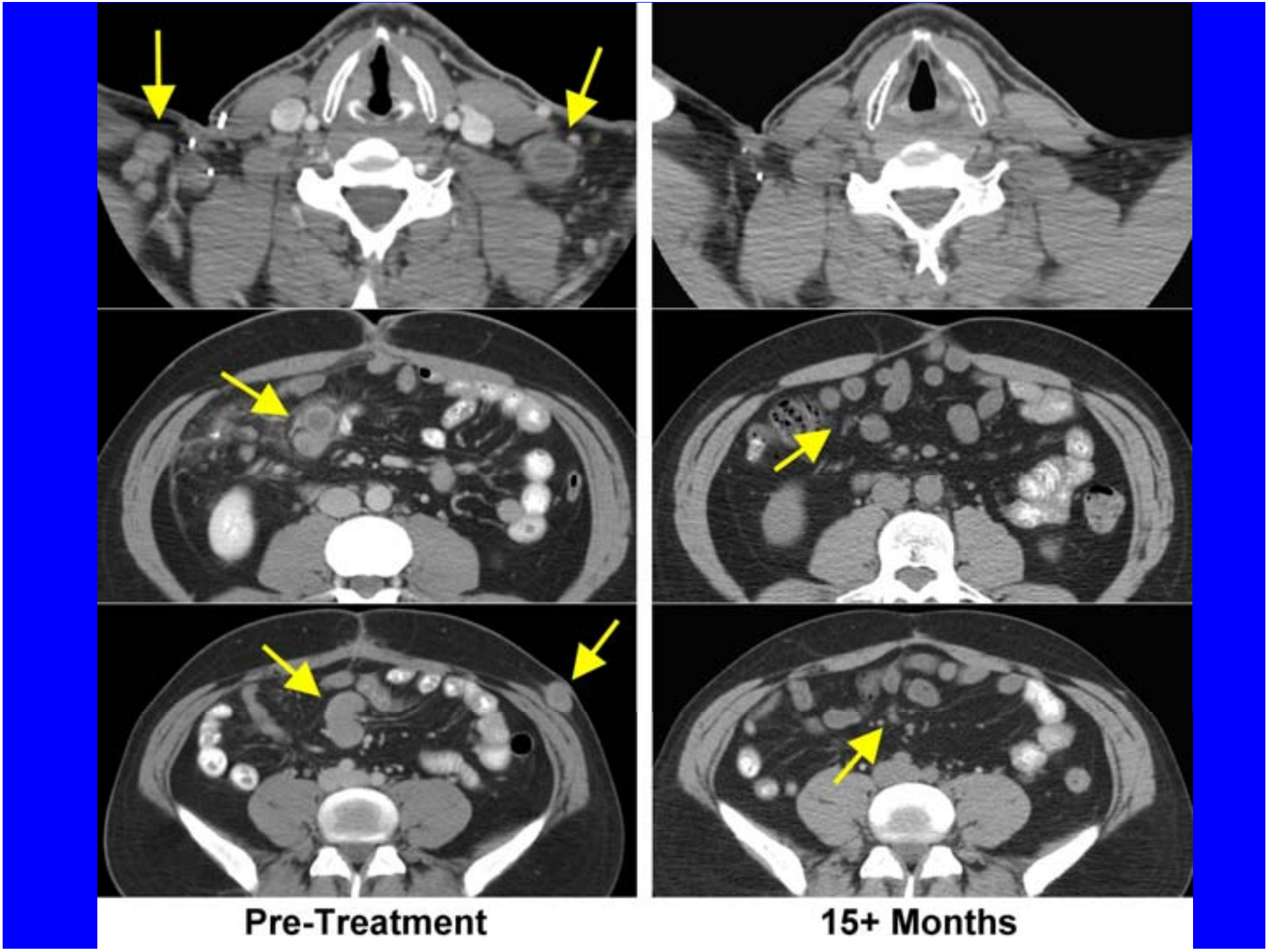
Impact of Lymphodepletion on Serum Levels Of IL-15 and IL-7



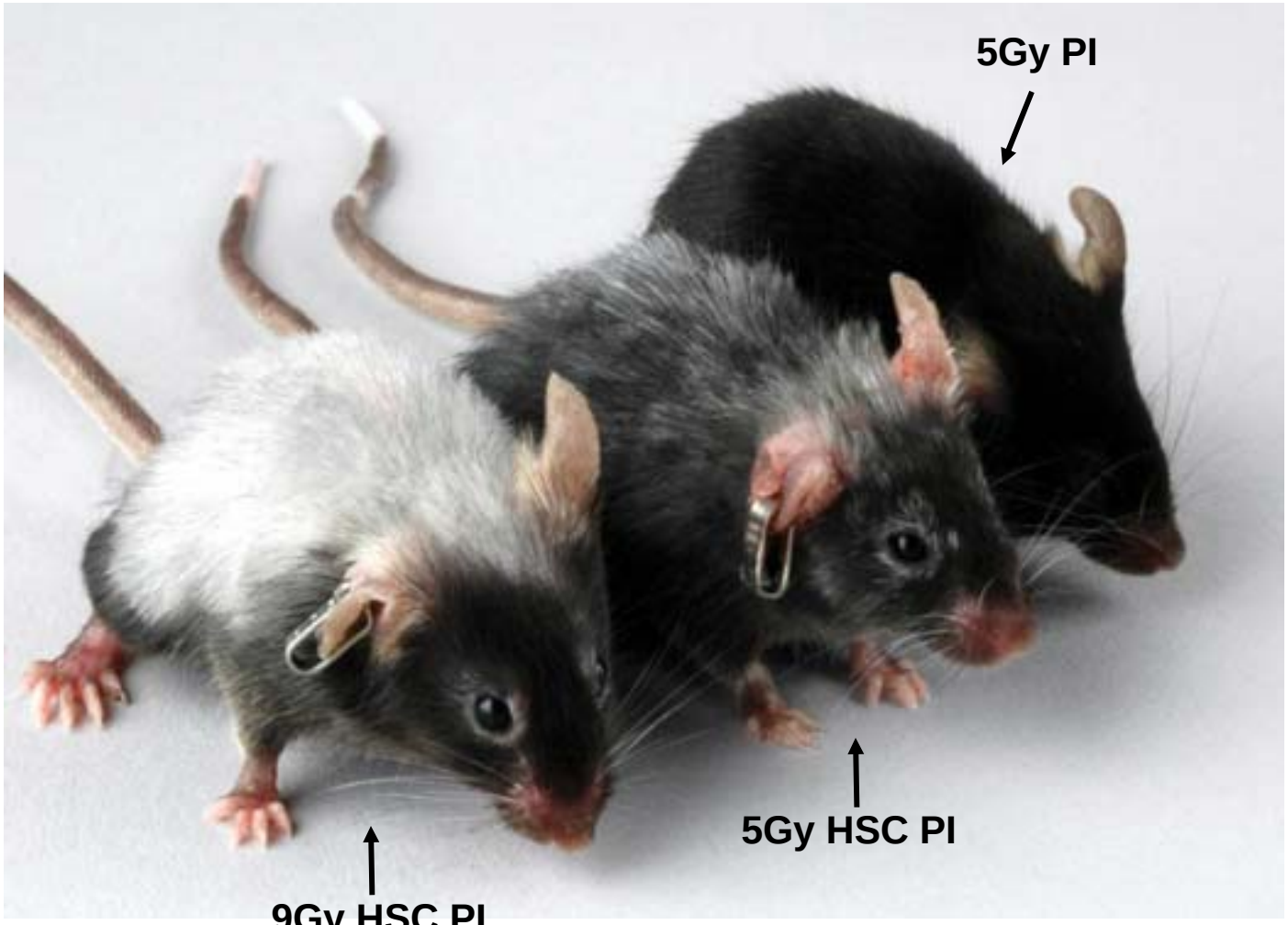
TIL with Cy+Flu+1200cGy TBI : (Preliminary Results)

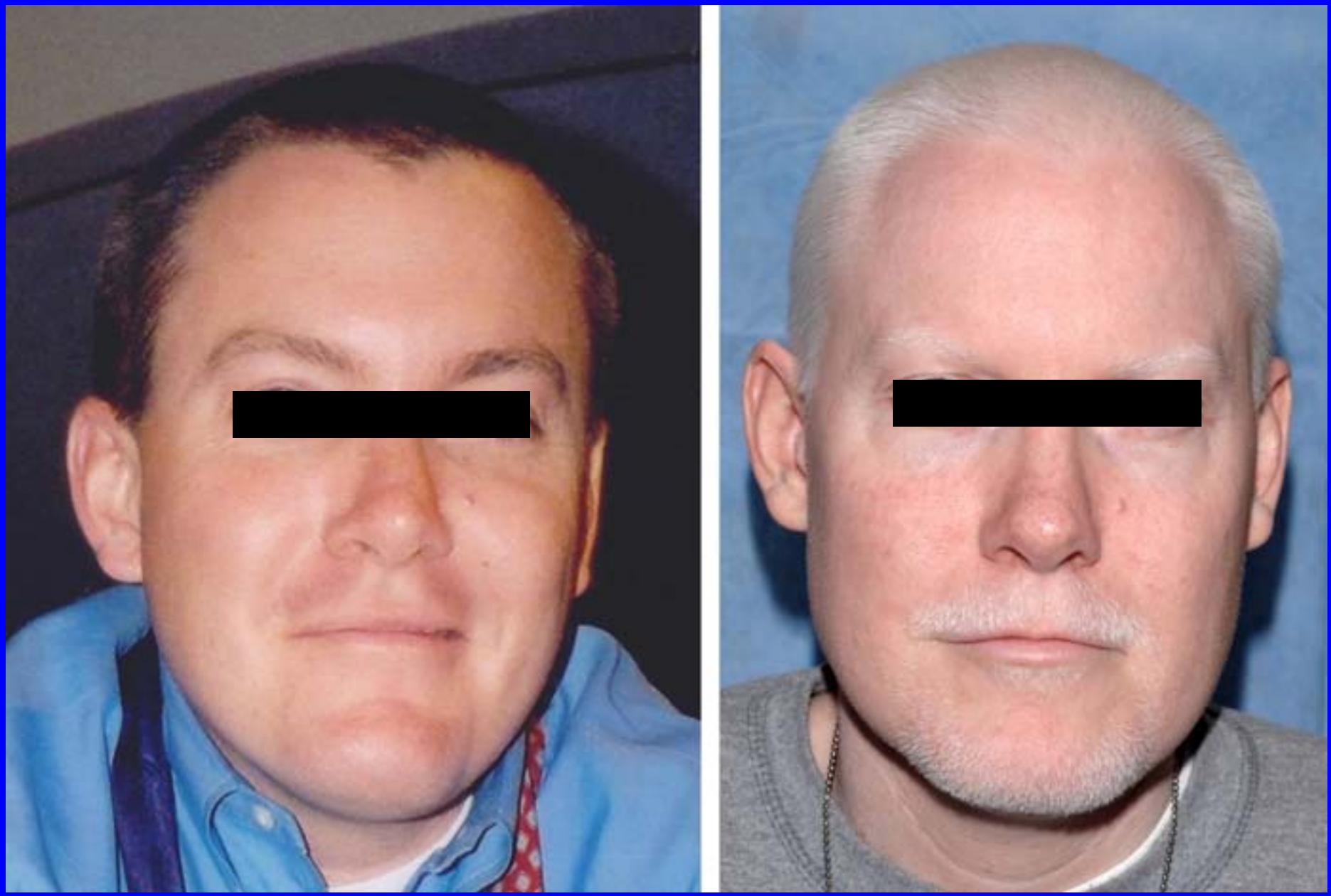
- 25 patients treated
- RR= 72% (18/25)
- Response Durations (months):
  - CR: 15+, 11+
  - PR: 13+, 12+, 11+, 8+, 7, 6, 6, 5+, 5+, 5+, 4+, 4+, 4, 3+, 3, 2+

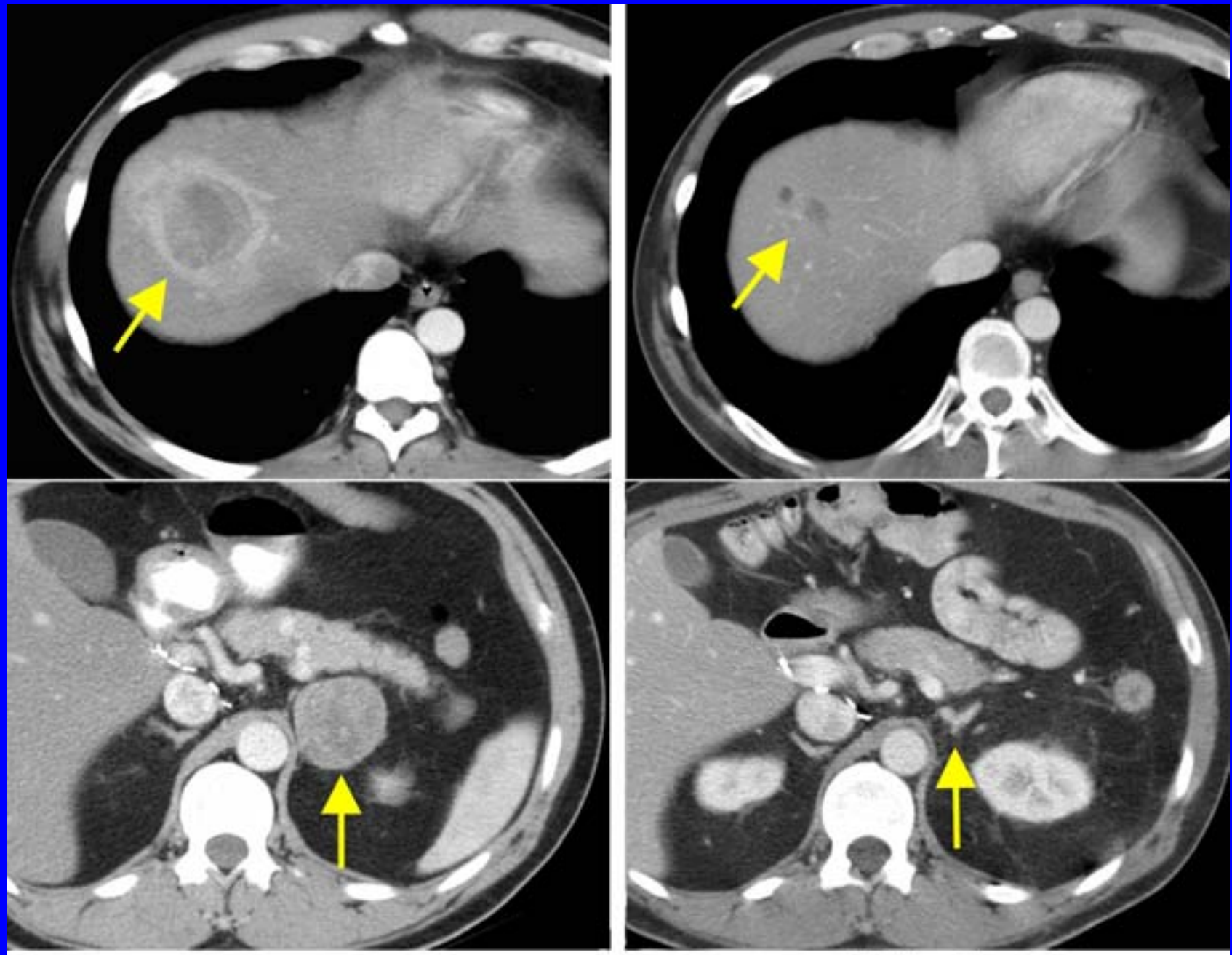

Baseline
11 Months

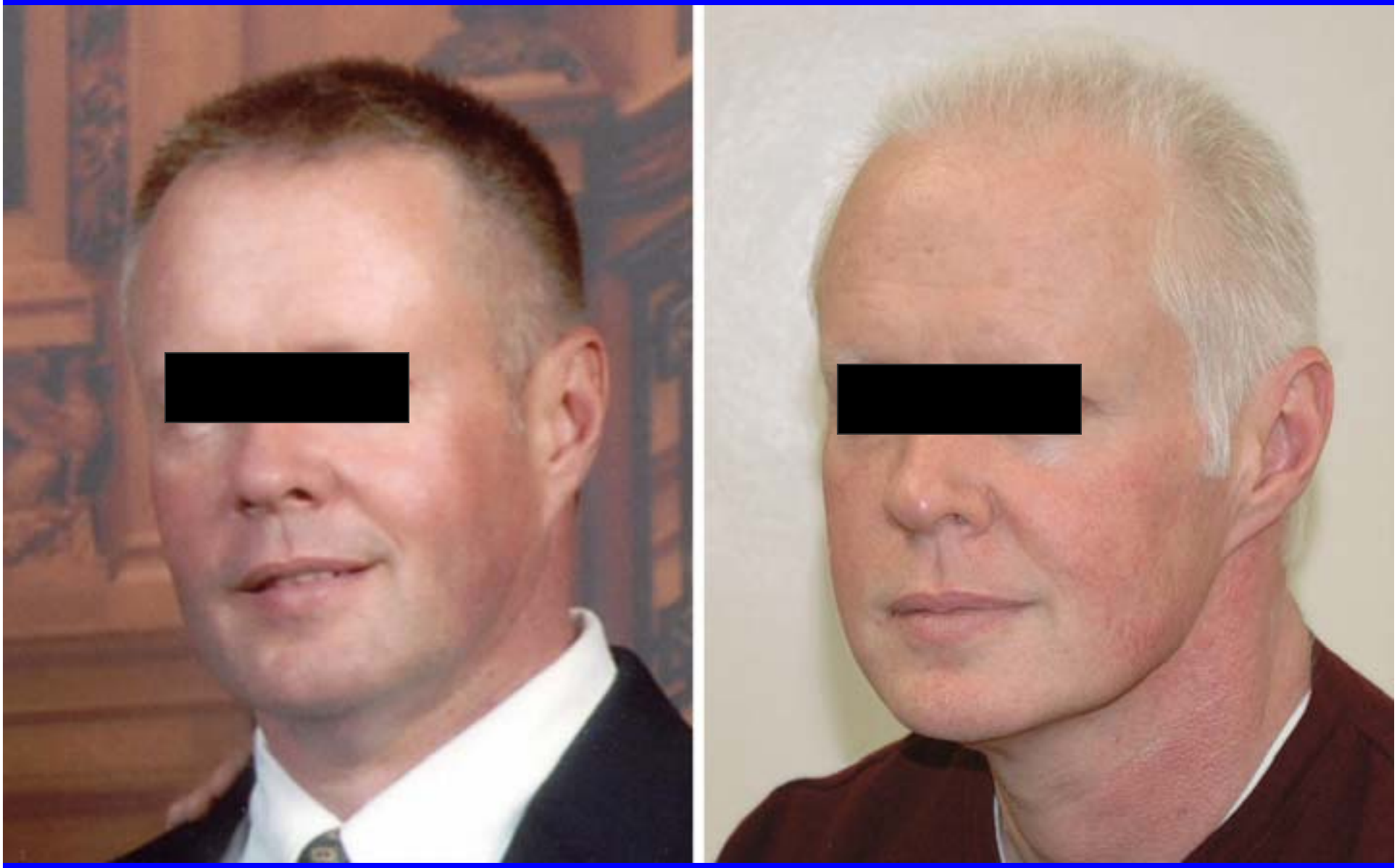

Pre-Treatment
15+ Months

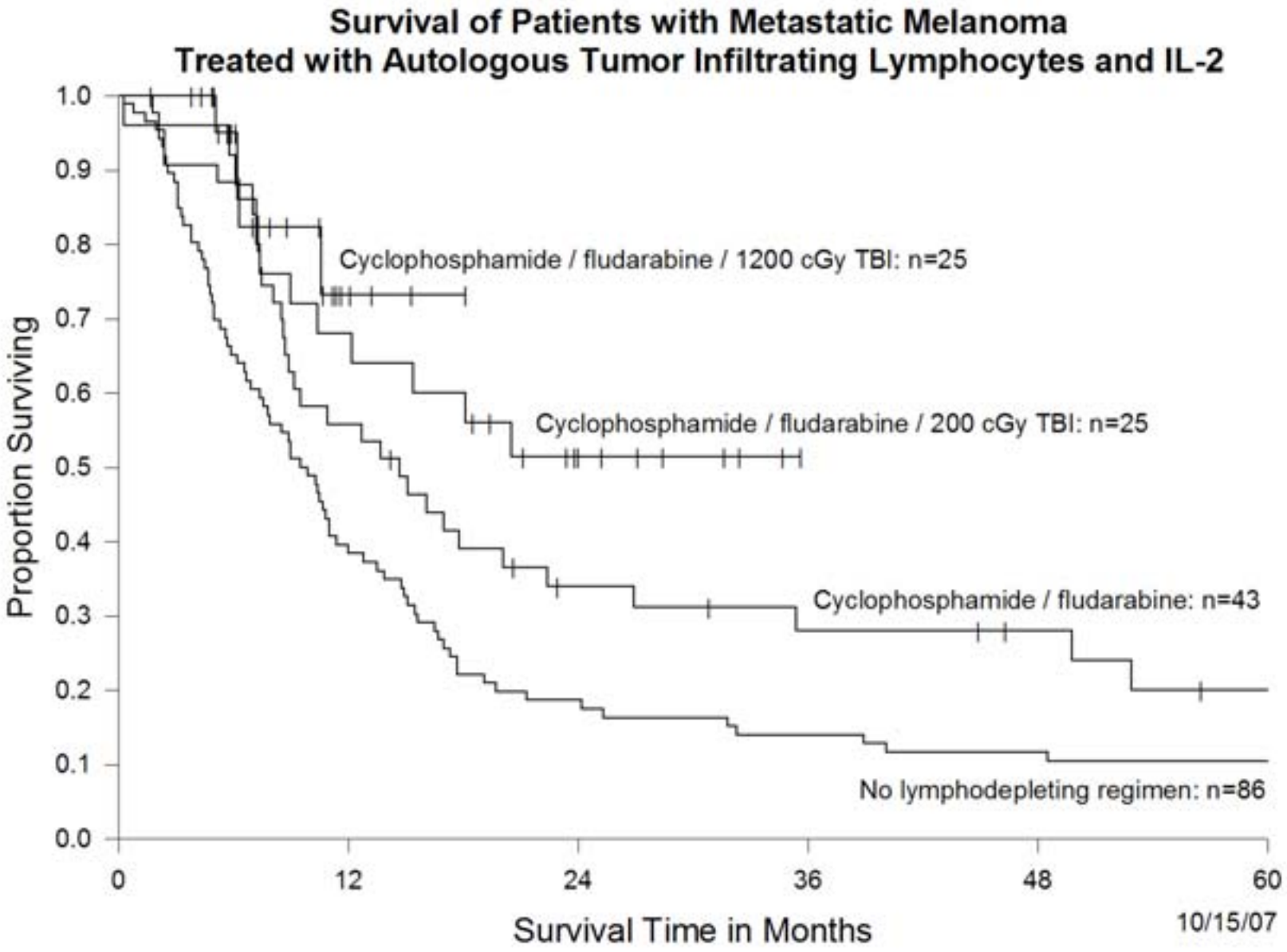
Induction of profound vitiligo after 9Gy TBI and HSC transplantation in the absence of vaccination






Pre-Treatment
11 Months




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
Cyclophosphamide / fludarabine / 1200 cGy TBI: n=25
Cyclophosphamide / fludarabine / 200 cGy TBI: n=25
Cyclophosphamide / fludarabine: n=43
No lymphodepleting regimen: n=86
0
12
24
36
48
60
Survival Time in Months
10/15/07

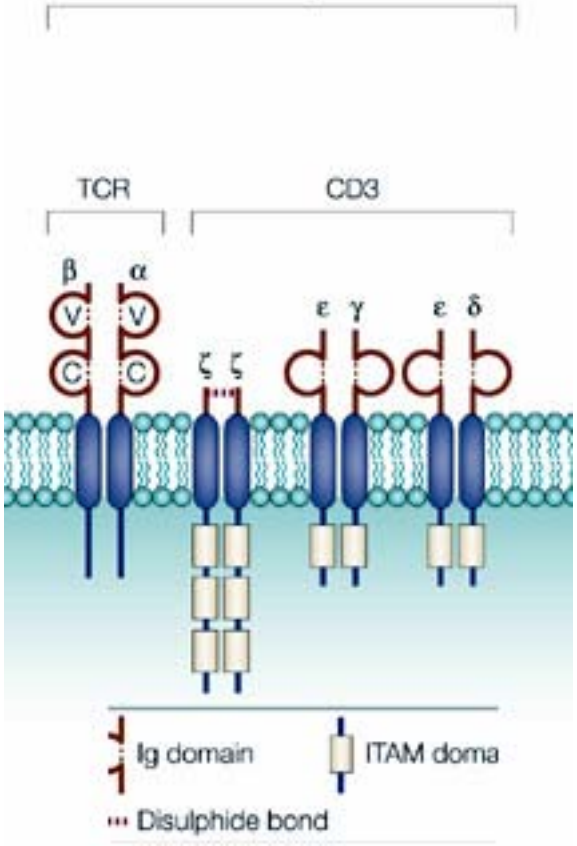
Problems with TIL Therapy

- Some patients cannot grow reactive TIL
- Effective TIL can only be found consistently in melanoma
- Every treatment must be individualized

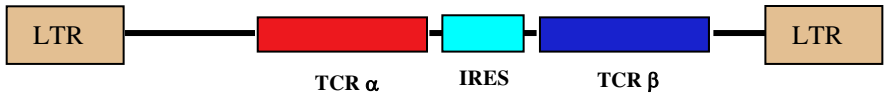
Q: Can effective tumor-reactive T-cells be genetically constructed?

TCR Gene Therapy Trial

The T-Cell Receptor Complex

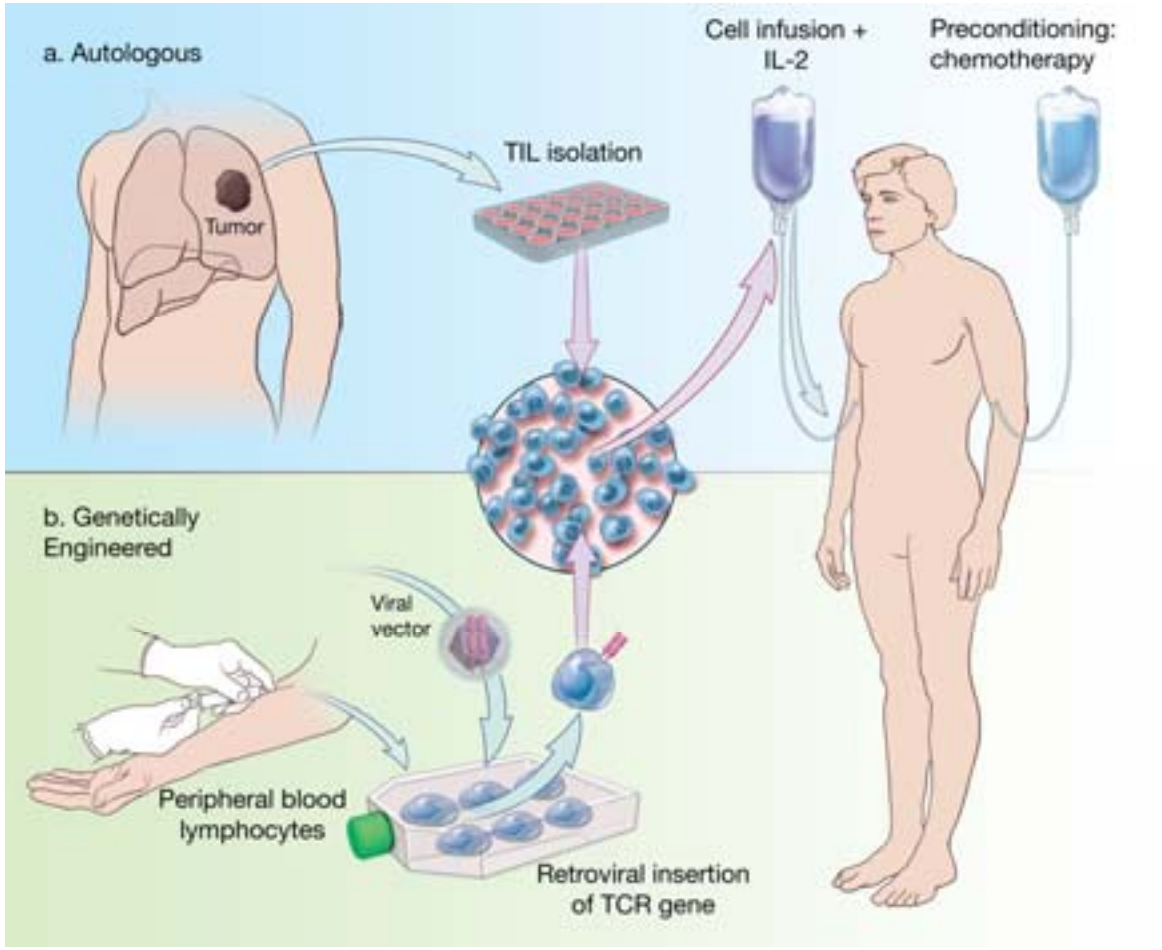


Isolate and clone TCRa/b chains into retroviral vector



MSGV1martAIB

TCR Gene Therapy Protocol Schema



Isolate PBL and stimulate with OKT-3

Day 2: transduce PBL with retroviral vector encoding TCR and culture in IL-2

Infuse transduced cells after pt. lymphodepletion instead of TIL

REPORTS

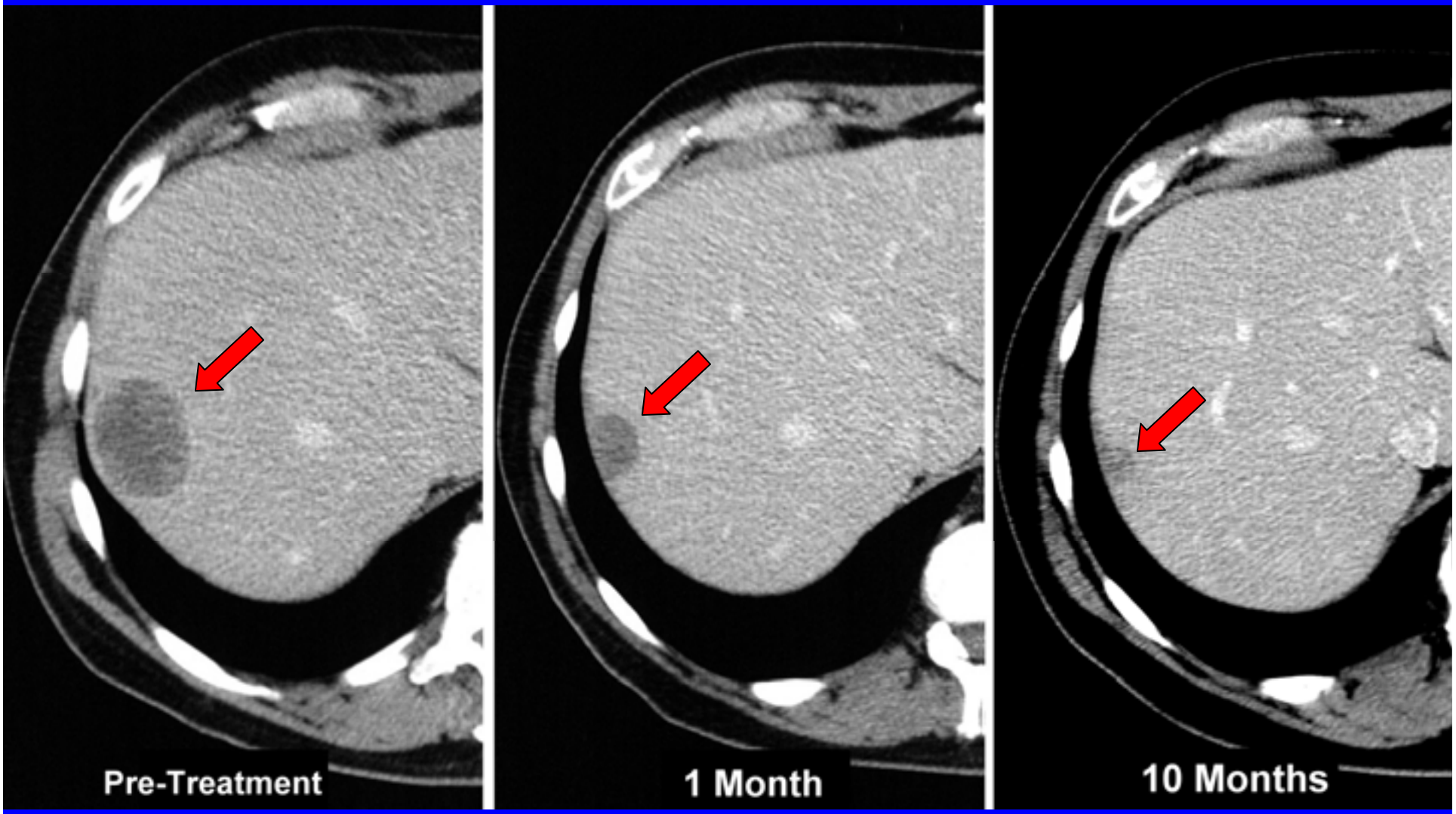
Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

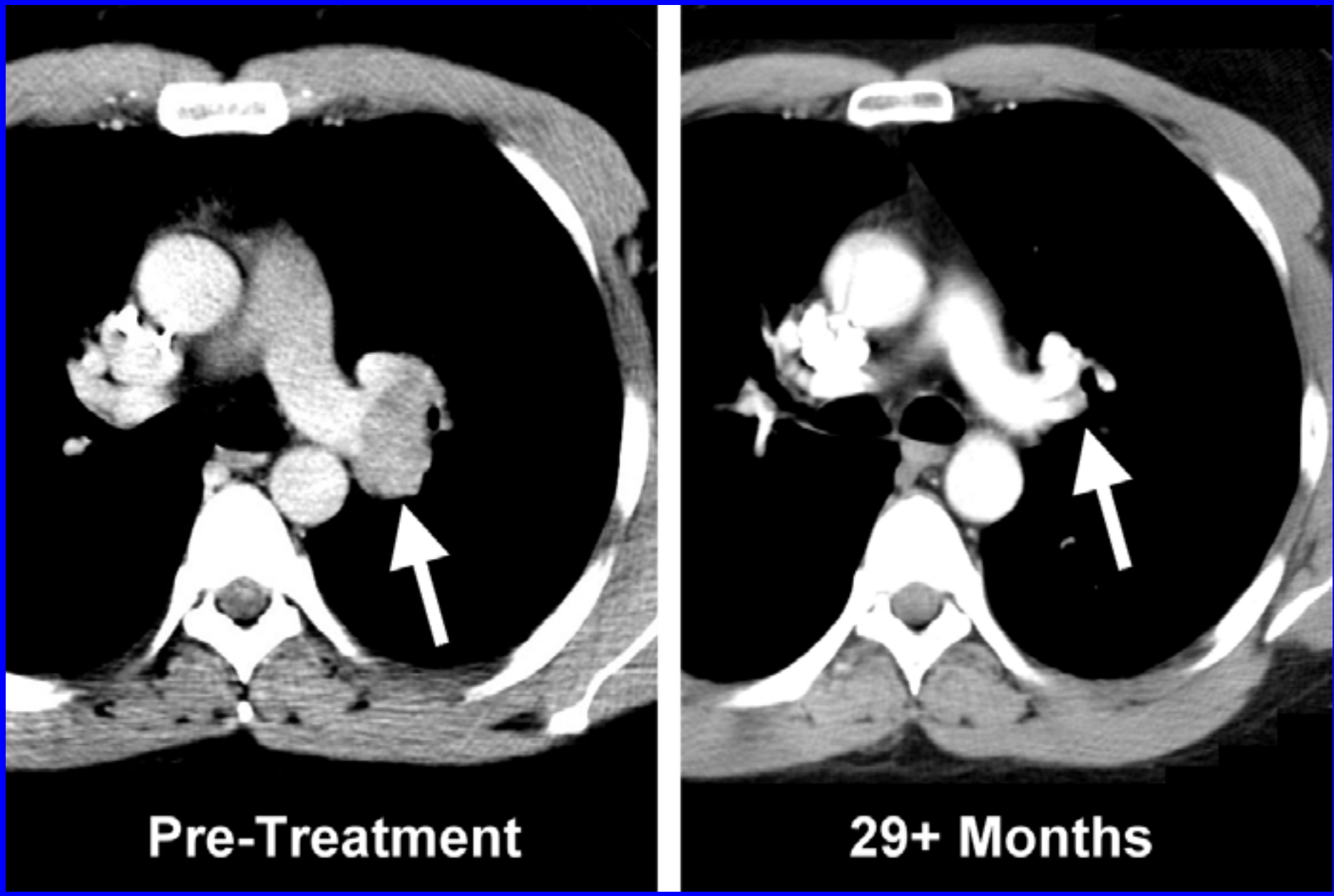
Richard A. Morgan, Mark E. Dudley, John R. Wunderlich, Marybeth S. Hughes, James C. Yang, Richard M. Sherry, Richard E. Royal, Suzanne L. Topalian, Udai S. Kammula, Nicholas P. Restifo, Zhili Zheng, Azam Nahvi, Christiaan R. de Vries, Linda J. Rogers-Freezer, Sharon A. Mavroukakis, Steven A. Rosenberg*

Through the adoptive transfer of lymphocytes after host immunodepletion, it is possible to mediate objective cancer regression in human patients with metastatic melanoma. However, the generation of tumor-specific T cells in this mode of immunotherapy is often limiting. Here we report the ability to specifically confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a T cell receptor. Adoptive transfer of these transduced cells in 15 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after the infusion. We observed high sustained levels of circulating, engineered cells at 1 year after infusion in two patients who both demonstrated objective regression of metastatic melanoma lesions. This study suggests the therapeutic potential of genetically engineered cells for the biologic therapy of cancer.

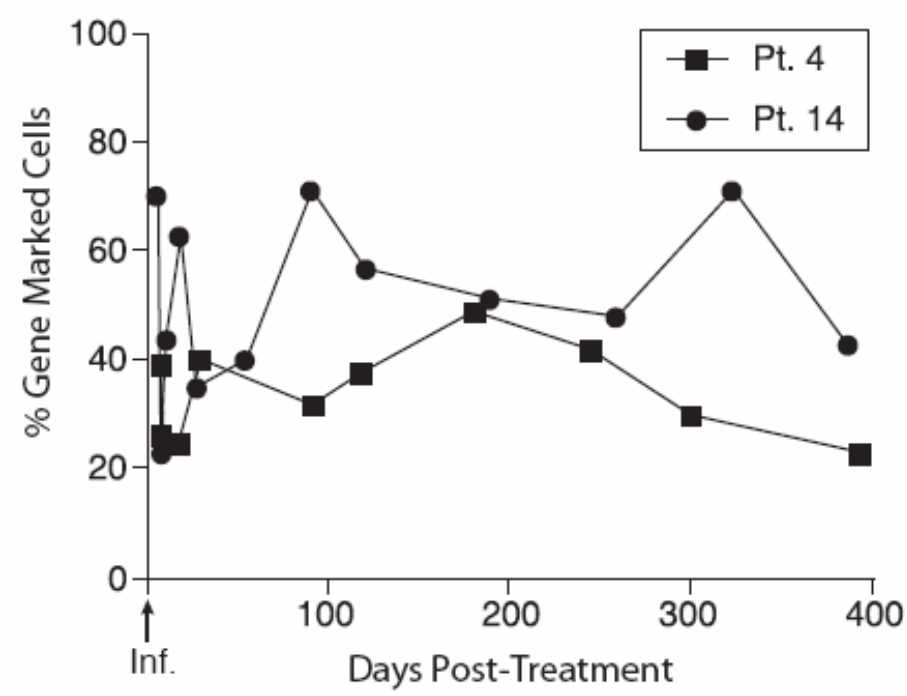
Science, Oct 2006

Tumor Regression After Receiving TCR-Transduced T Cells

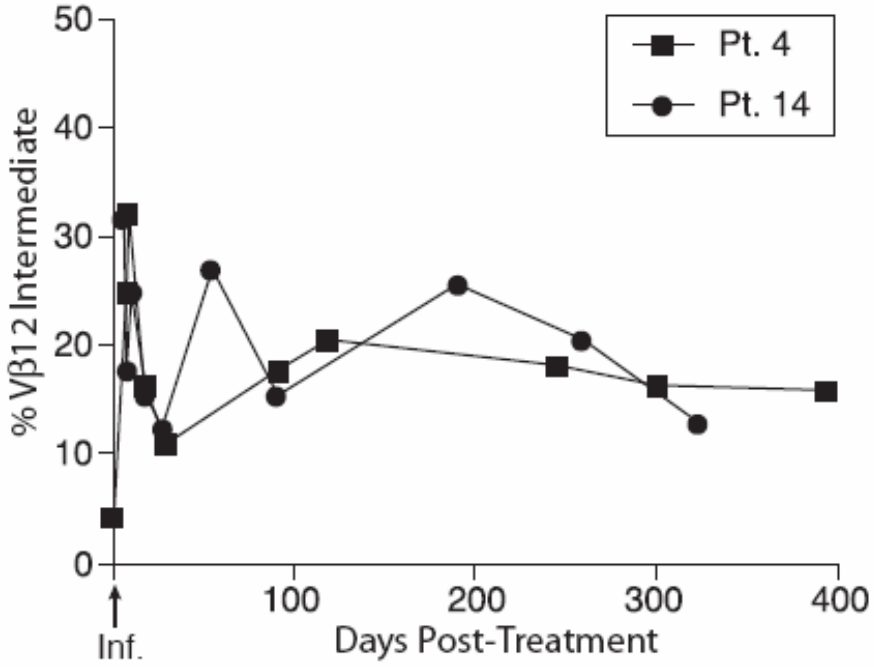



Pre-Treatment
29+ Months

Long-Term Persistence of TCR Transduced Cells in Responding Patients



Quantitative PCR (TaqMan)

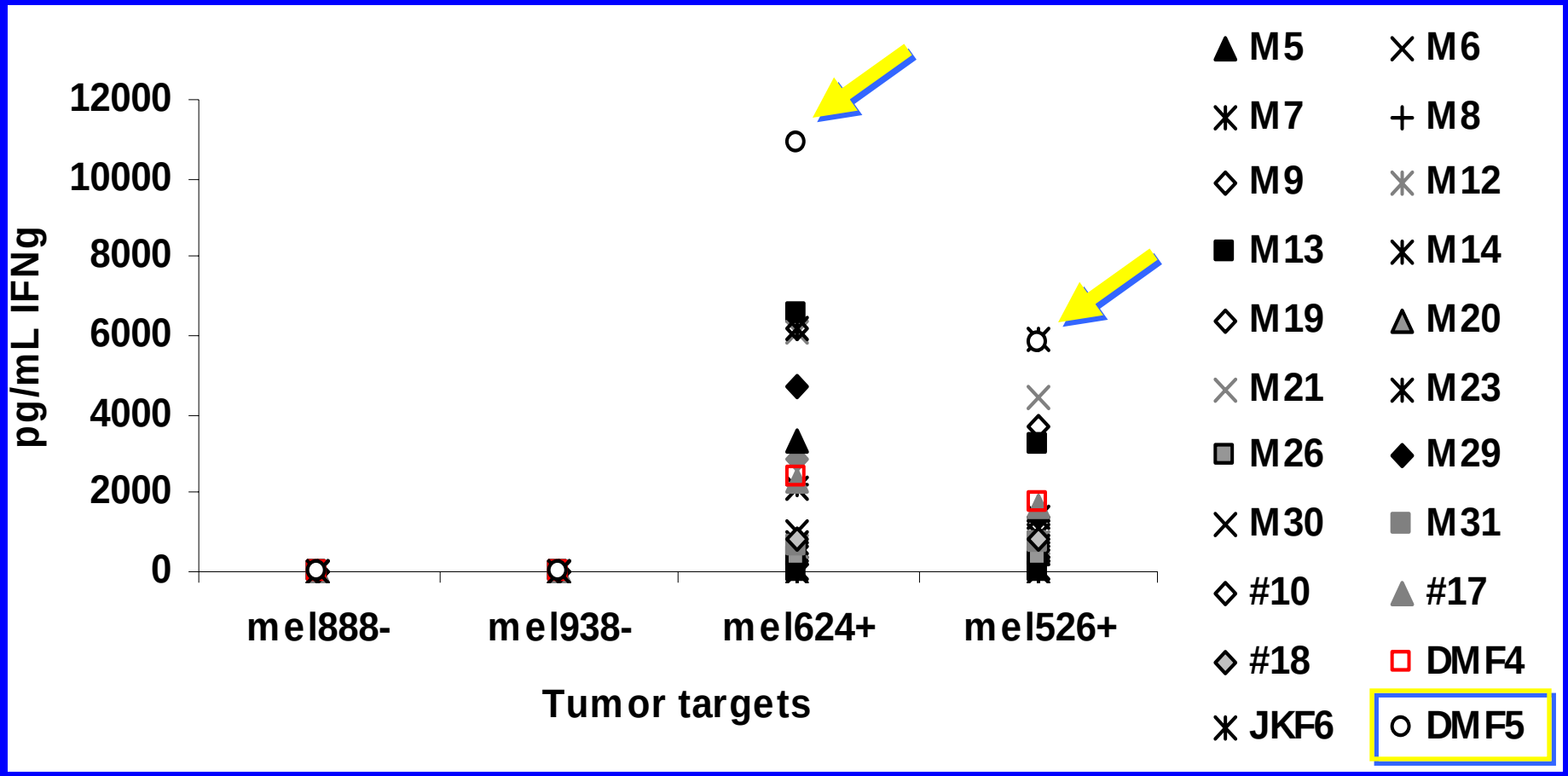


FACS for Vb12

Second Generation TCRs: Higher Avidity

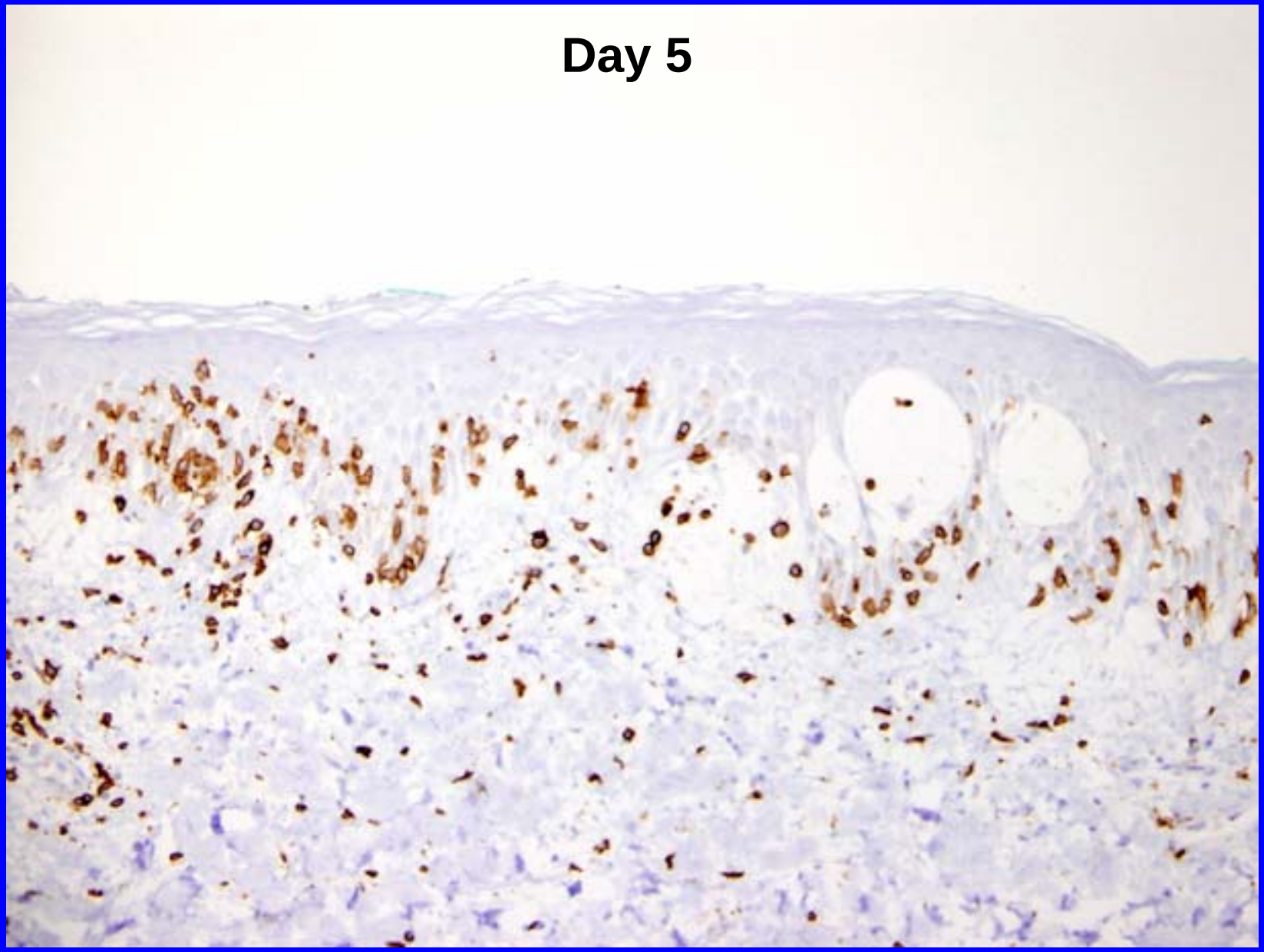
- The MART-1-reactive TCR selected for this study was from a patient who responded to TIL transfer after Cy-Flu and was a clone that persisted in vivo
- Extensive cloning of other MART-1-reactive TIL showed a wide range of TCR avidities

Tumor Recognition of MART-1-Reactive TIL clones: IFN Release



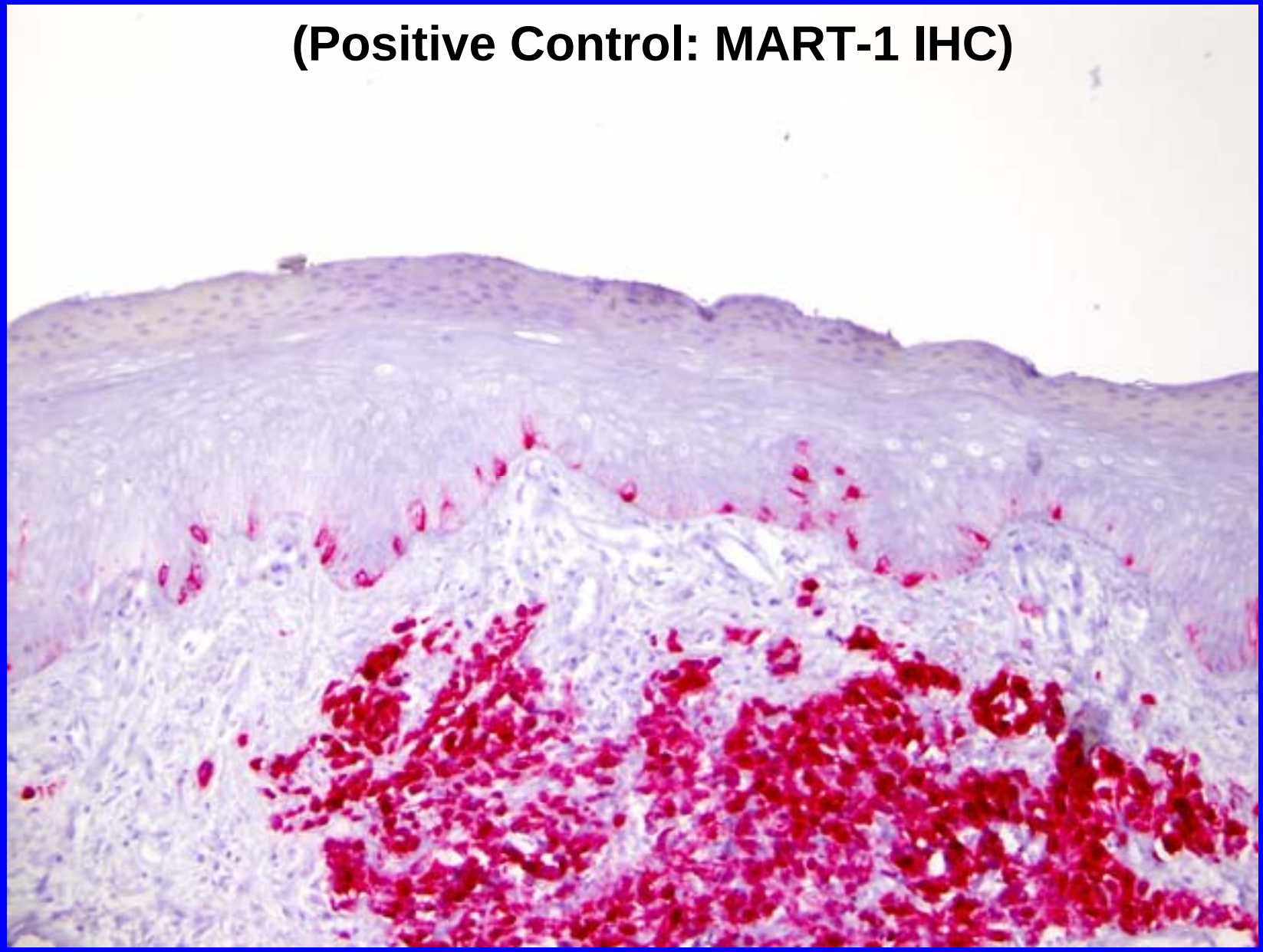
RNA Electroporation of PBL

Day 5

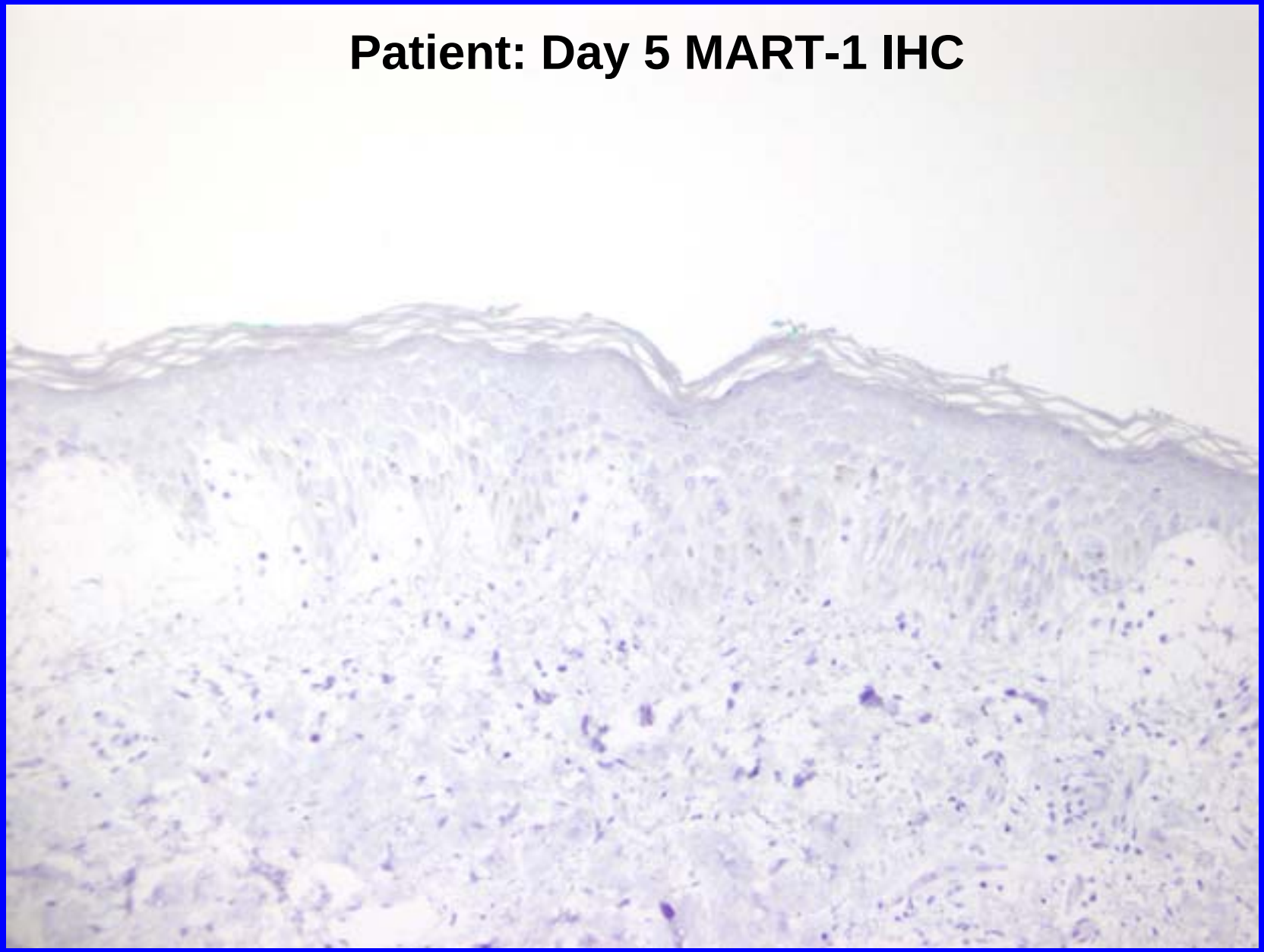


CD8 positive cells

(Positive Control: MART-1 IHC)

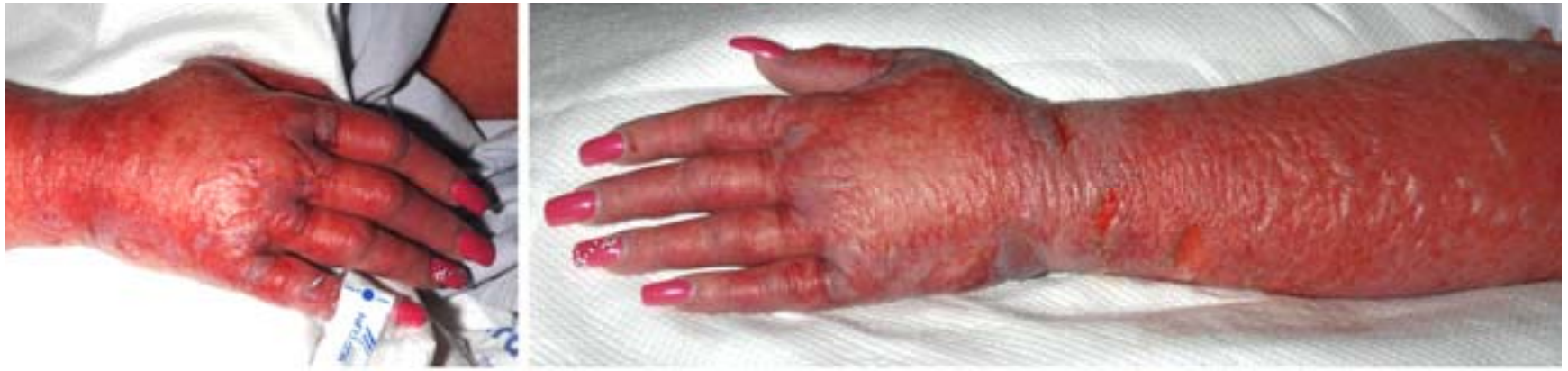


Patient: Day 5 MART-1 IHC

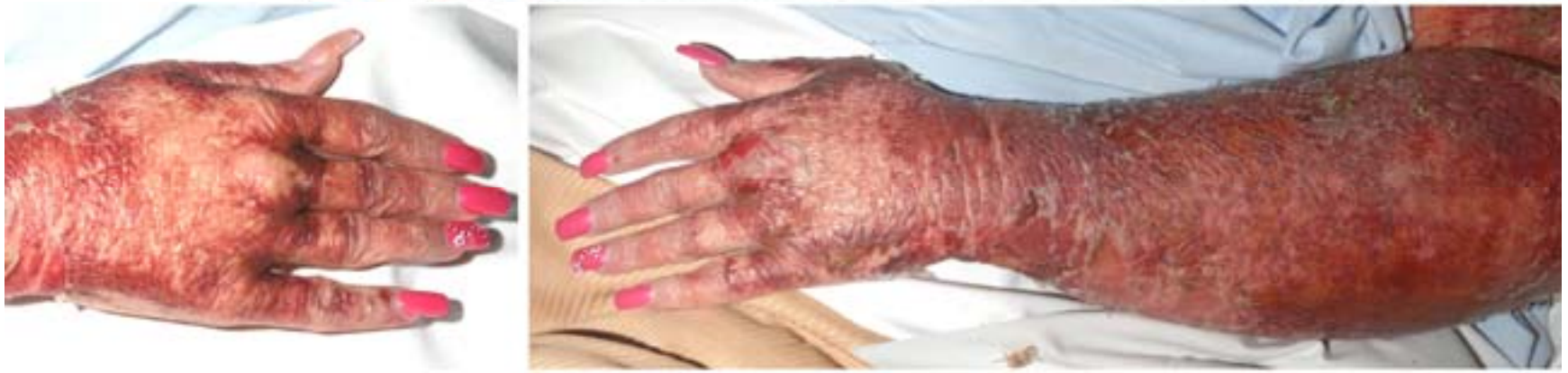


F5 High-Avidity Anti-MART1 TCR

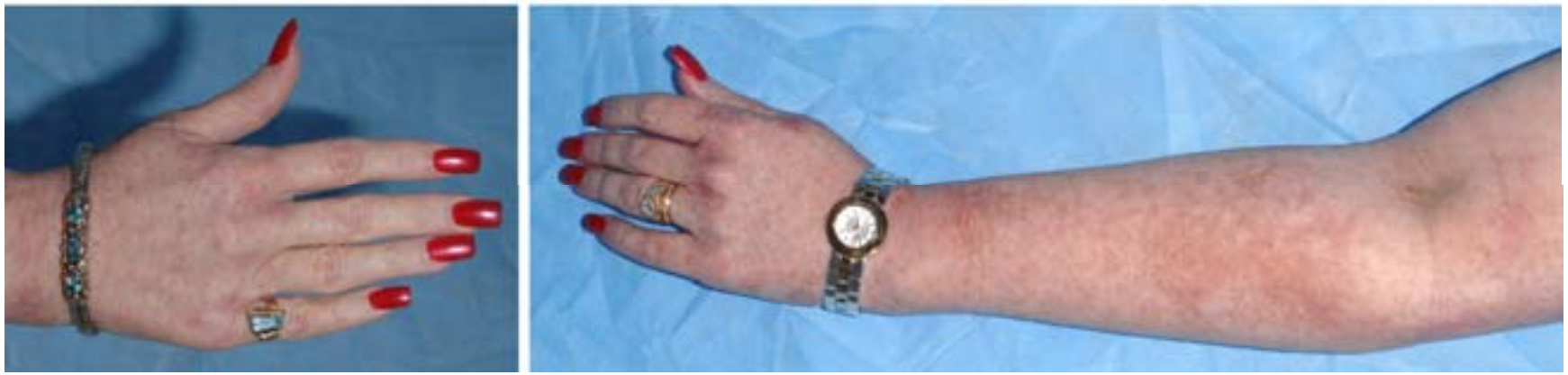
D8



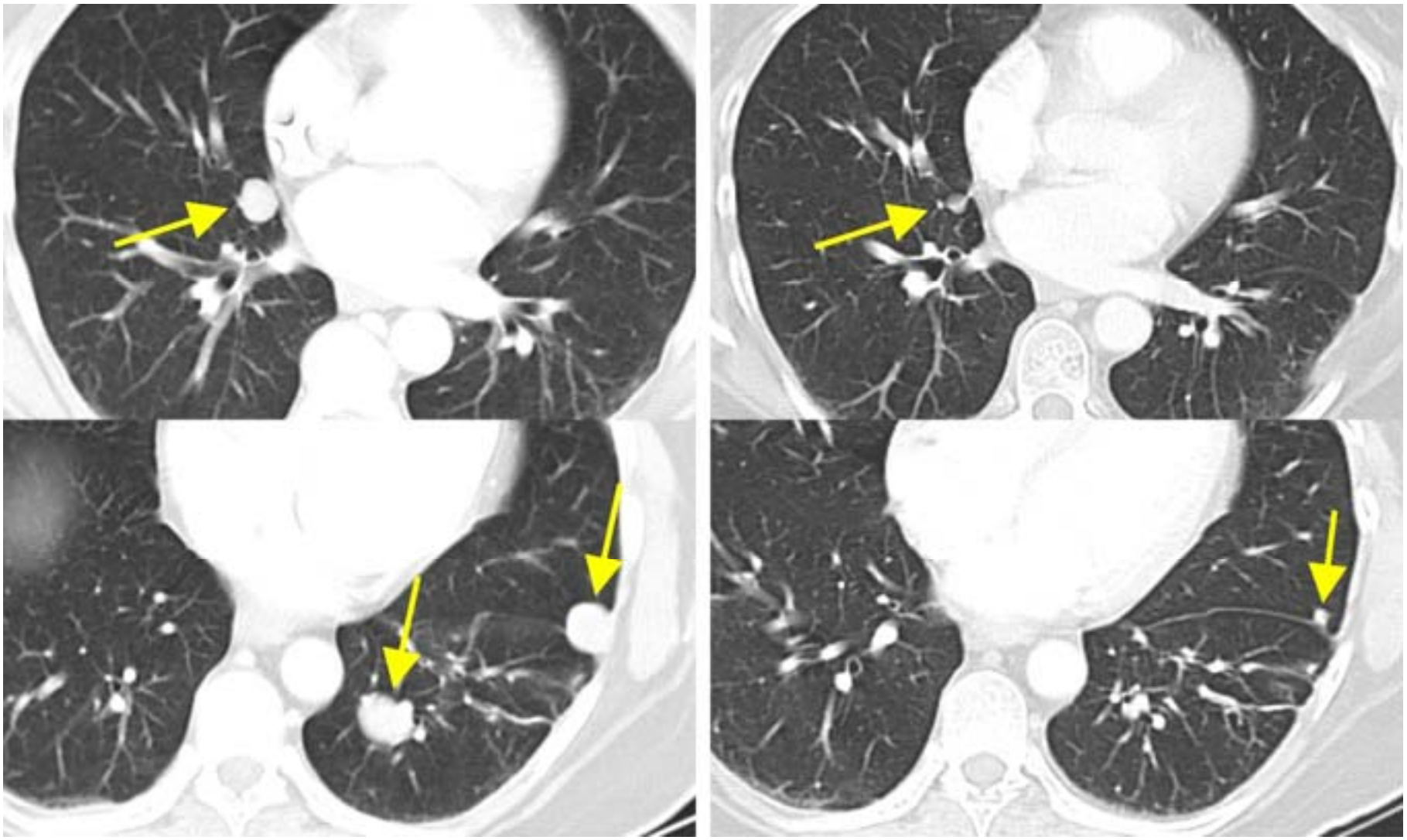
D12



D57



F5 High-Avidity Anti-MART1 TCR



2 Month Follow-Up

Conclusions

- Transfer of tumor-reactive T-cells after recipient lymphodepletion can cause dramatic regressions of metastatic melanoma in $\geq$50% of patients, with some complete and durable responses
- The principle that tumor-reactive T-cells generated by introducing the genes encoding tumor-reactive TCRs can cause tumor regression has been shown
- Genetic construction of tumor-reactive T-cells has potential for significant improvement and can allow novel immunomodulatory strategies that may greatly enhance the efficacy of T-cell transfer

Future Directions

- New gene therapy vectors and methods are being developed to improve TCR expression in PBL
  - Lente viruses
  - Transposons
  - 2A peptides
  - CDR2/CDR3 modified receptors
- Receptors that recognize antigens on non-melanoma tumors have been developed to treat more common cancers with T-cell transfer
- Adding vaccines and alternative in vivo cytokine support (eg. IL-7 or IL-15)
- Manipulating T-cell function with gene therapy (eg. Mir 181a, zeta chain, CD8)

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