DEVELOPMENT OF CELLULAR IMMUNOLOGY

1880's: Antibodies described
(dominated studies of immunology until 1960's)

1958: Journal of Immunology (137 papers)
"lymphocyte" not listed in index
Two papers on transfer of lymph node cells were the only papers dealing with lymphocytes

1960's: Importance of cellular immunology recognized as mediator of: allograft rejection
protection against transfer of mouse tumors

1970's: No convincing evidence for human lymphocytes reactive with cancer or the existence of human cancer antigens

No successful immunotherapies for cancer in humans

“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. Woglum
Cancer Research

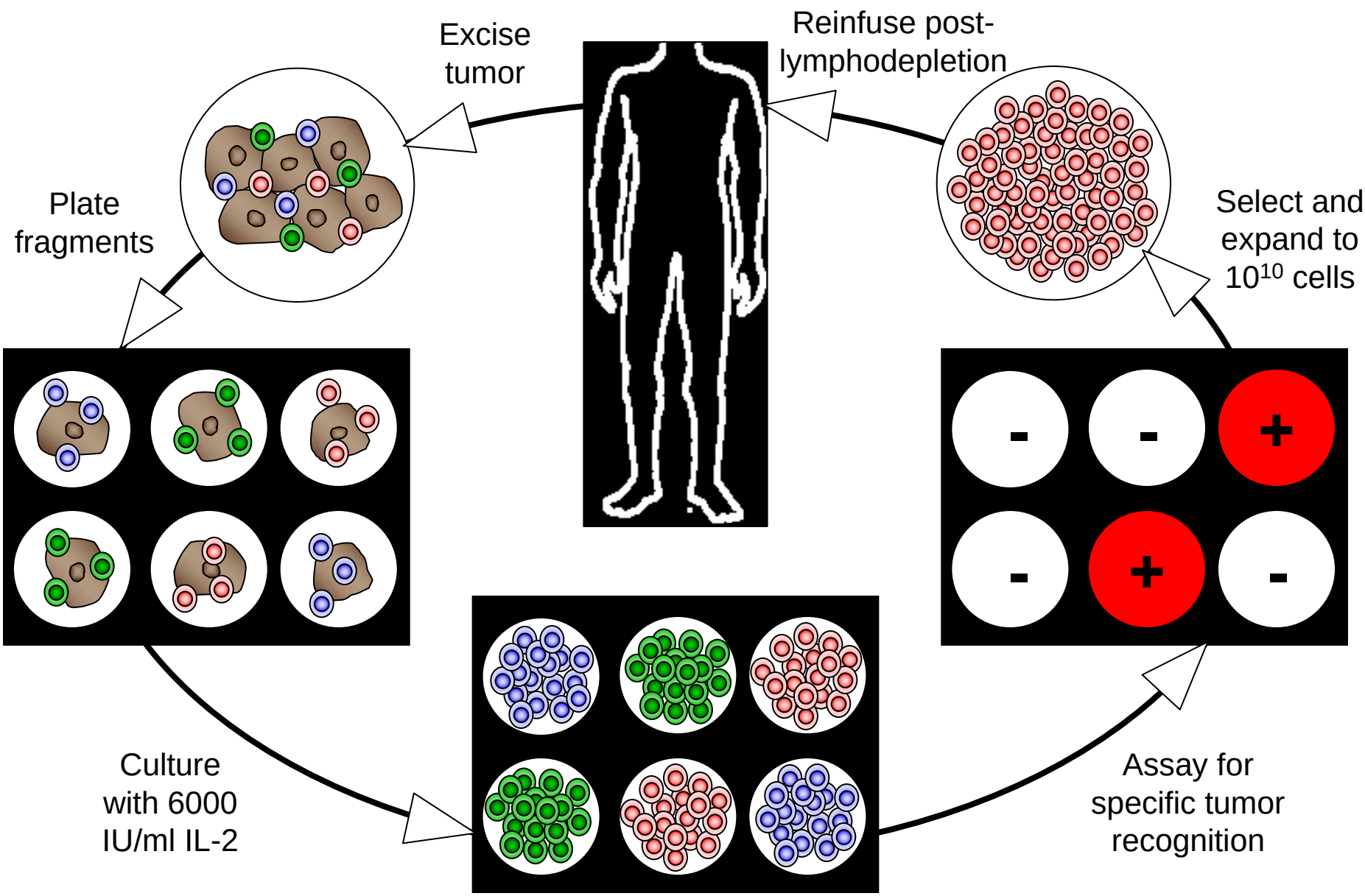
ADVANTAGES OF CELL TRANSFER THERAPY

1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

2. Administer cells activated ex-vivo to exhibit anti-tumor effector function.

3. Manipulate host prior to cell transfer to provide altered environment for transferred cells.

Adoptive transfer of tumor infiltrating lymphocytes (TIL)



INITIAL RESULTS WITH CELL TRANSFER THERAPY FOLLOWING LYMPHODEPLETING CHEMOTHERAPY

Six of 13 (46%) patients with metastatic melanoma experienced objective cancer regression.

Four patients had mixed or minor responses.

All had previously been refractory to IL-2 administration and eight had prior chemotherapy.

(Science 298:850-854, 2002)

Cell Transfer Therapy

(10/1/09)

Treatment	Total	PR	CR	OR (%)
No TBI	43	16 (84, 36, 29, 28 14, 13, 11, 8 8, 7, 4, 3, 3, 2, 2, 2)	5 (85+, 79+, 69+, 68+, 54+)	21 (49%)

Preparative Regimens for Cell Transfer

	Days										
	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Non-myeloablative	Cy	Cy	Flu	Flu	Flu	Flu	Flu				
								Cells			
									IL-2	IL-2	IL-2
Ablative		Cy	Cy								
		Flu	Flu	Flu	Flu	Flu					
							TBI				
								Cells			
									IL-2	IL-2	IL-2
									CD34+		

Cy: Cyclophosphamide 60 µg/kg
Flu: Fludarabine 25 mg/m^2
IL-2: 720,000 IU/kg q8h
Cells: Autologous TIL (1-5 x 10^{10})
$CD34^+$: $\geq$2 x 10^6/kg
TBI: 200 cGy total body irradiation

Cell Transfer Therapy

(10/1/09)

Treatment	Total	PR	CR	OR (%)
No TBI	43	16 (84, 36, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2)	5 (85+, 79+, 69+, 68+, 54+)	21 (49%)
200 TBI	25	11 (54+, 50, 44+, 14, 10, 6, 5, 5, 4, 3, 3)	2 (58+, 47+)	13 (52%)

Preparative Regimens for Cell Transfer

	Days										
	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Non-myeloablative	Cy	Cy	Flu	Flu	Flu	Flu	Flu				
								Cells			
								IL-2	IL-2	IL-2	
Ablative		Cy	Cy								
(200cGy)		Flu	Flu	Flu	Flu	Flu					
							TBI				
								Cells			
								IL-2	IL-2	IL-2	
									CD34+		
Ablative	Cy	Cy									
(1200cGy)	Flu	Flu	Flu	Flu	Flu						
					TBI	TBI	TBI				
								Cells			
								IL-2	IL-2	IL-2	IL-2
									CD34+		

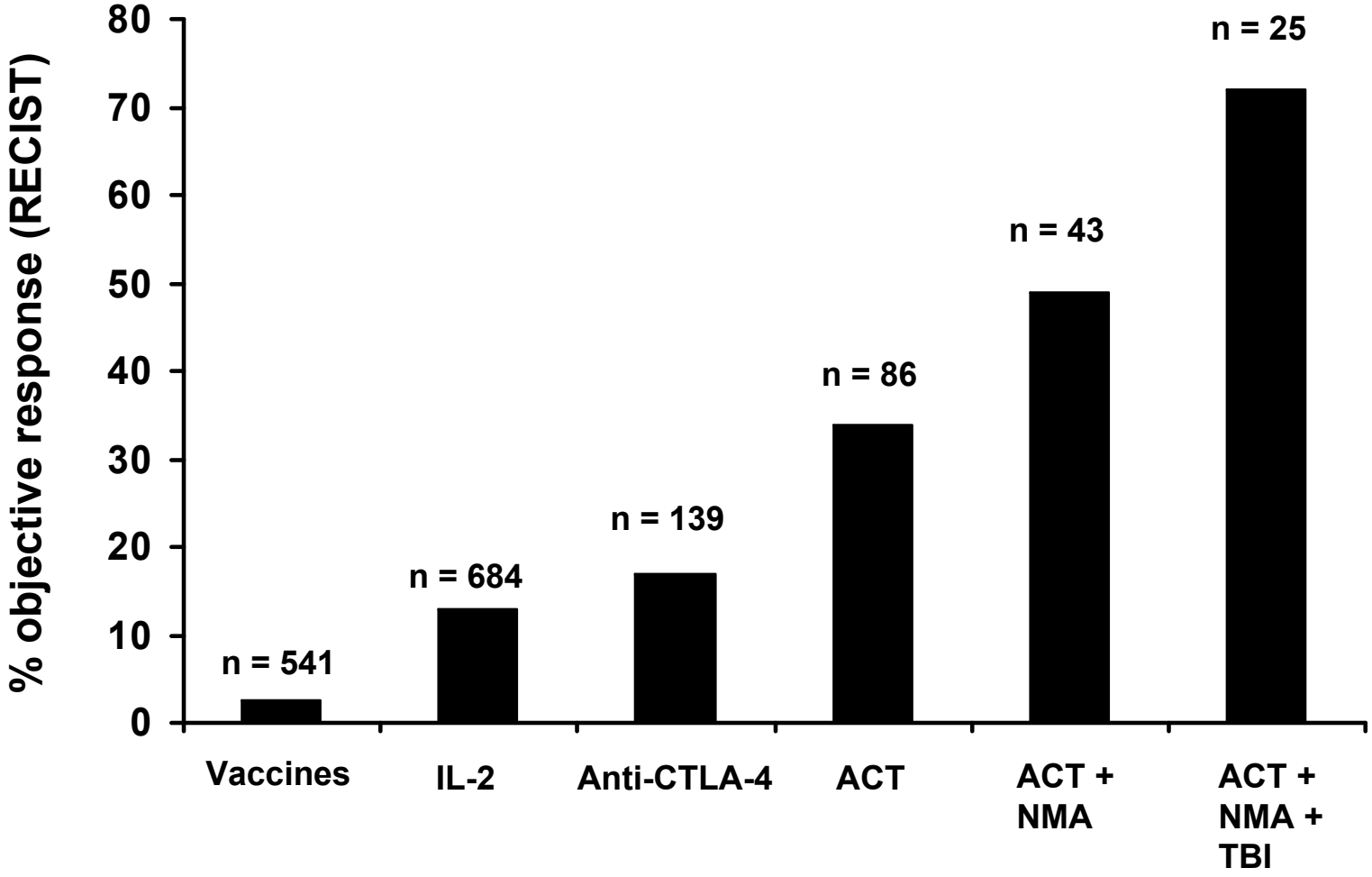
Cell Transfer Therapy

(10/1/09)

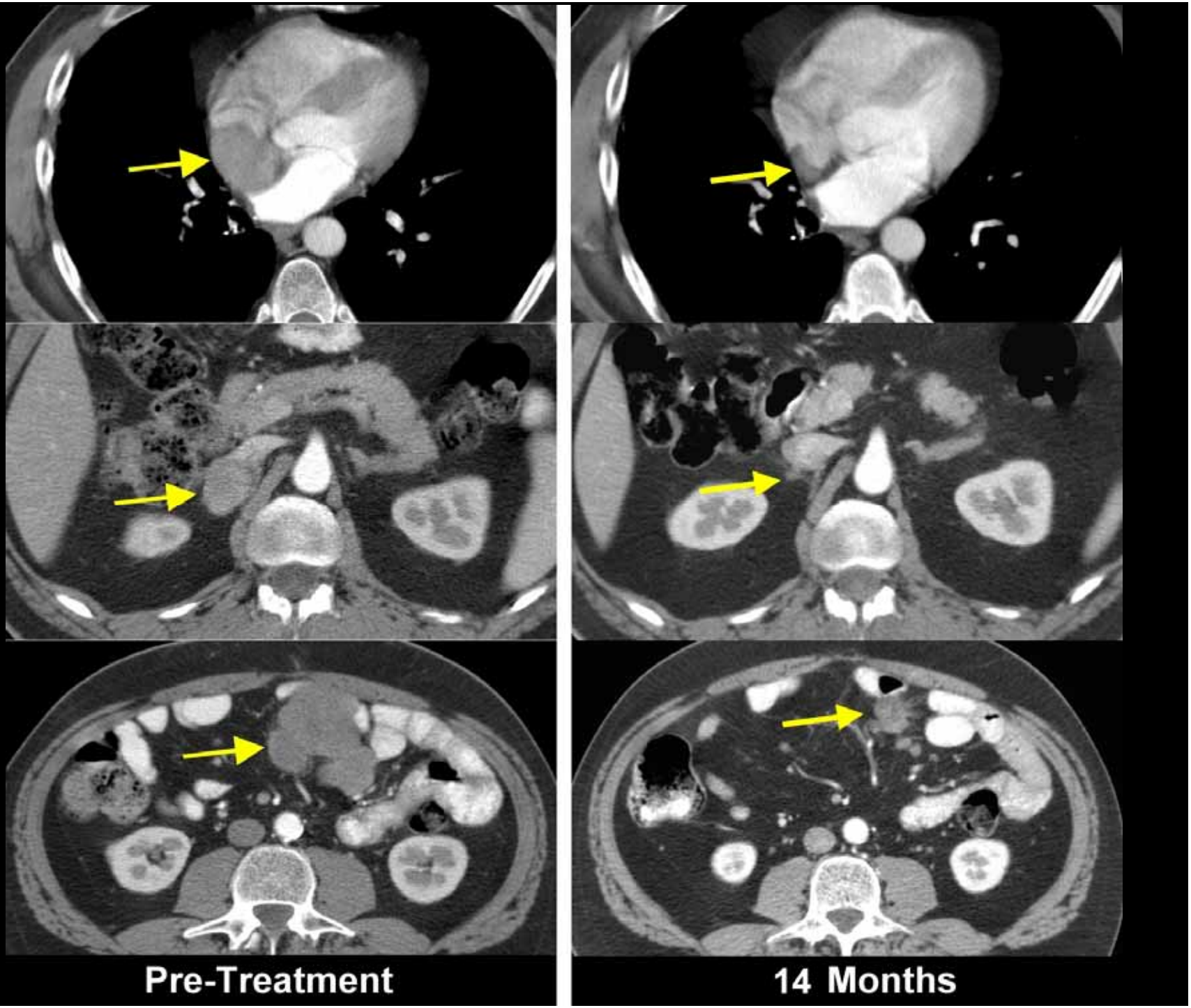
Treatment	Total	PR	CR	OR (%)
No TBI	43	16 (84, 36, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2)	5 (85+, 79+, 69+, 68+, 54+)	21 (49%)
200 TBI	25	11 (54+, 50, 44+, 14, 10, 6, 5, 5, 4, 3, 3)	2 (58+, 47+)	13 (52%)
1200TBI	25	10 (35+, 28+, 21, 13, 7, 6, 6, 5, 4 , 3)	8 (38+, 19, 34+, 34+, 29+, 28+, 28+, 27+)	18(72%)

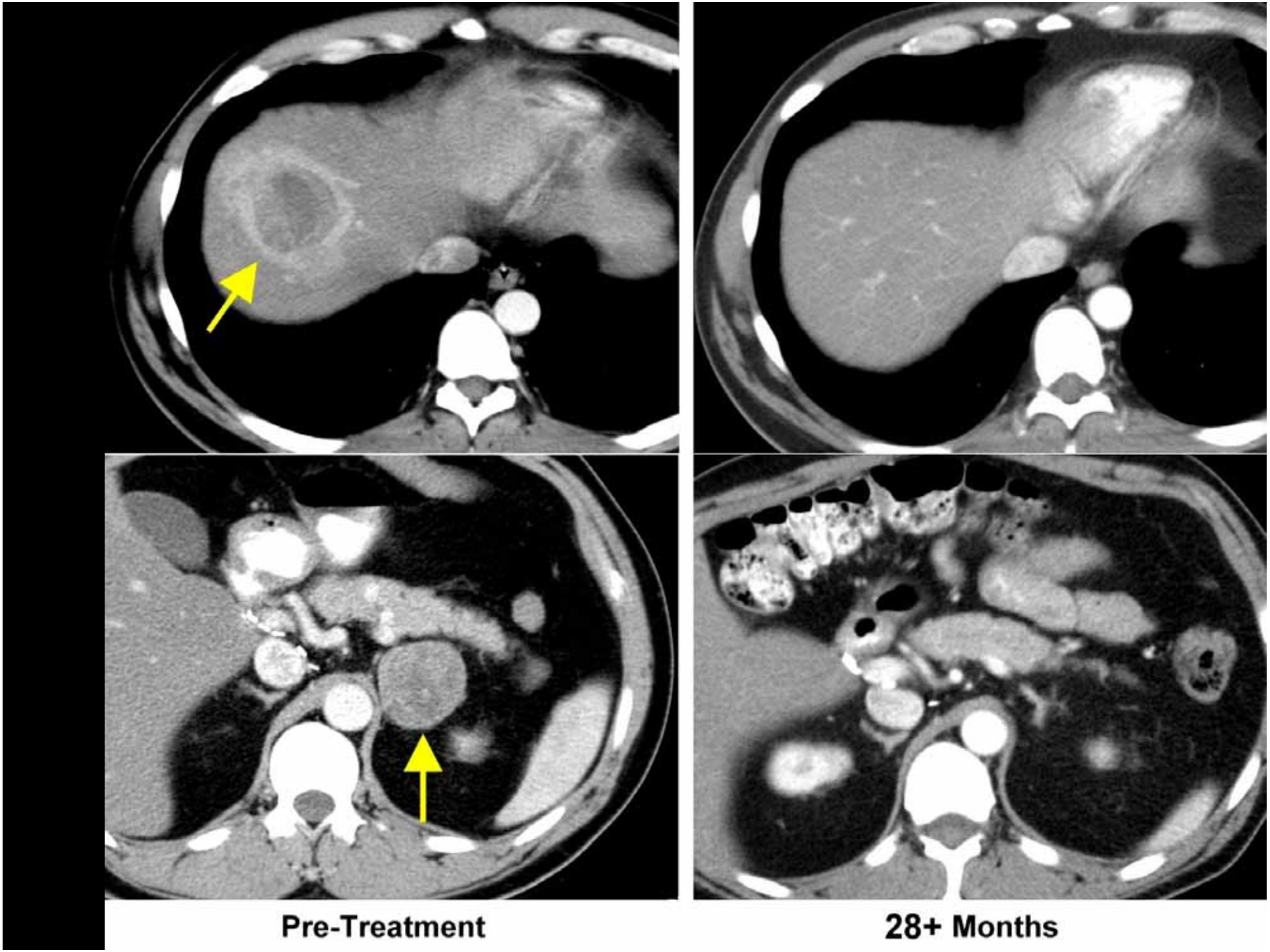
(52 responding patients: 42 had prior IL-2, 21 had prior IL-2 + chemotherapy)

Objective response rates (RECIST) in metastatic melanoma patients treated in the Surgery Branch, NCI

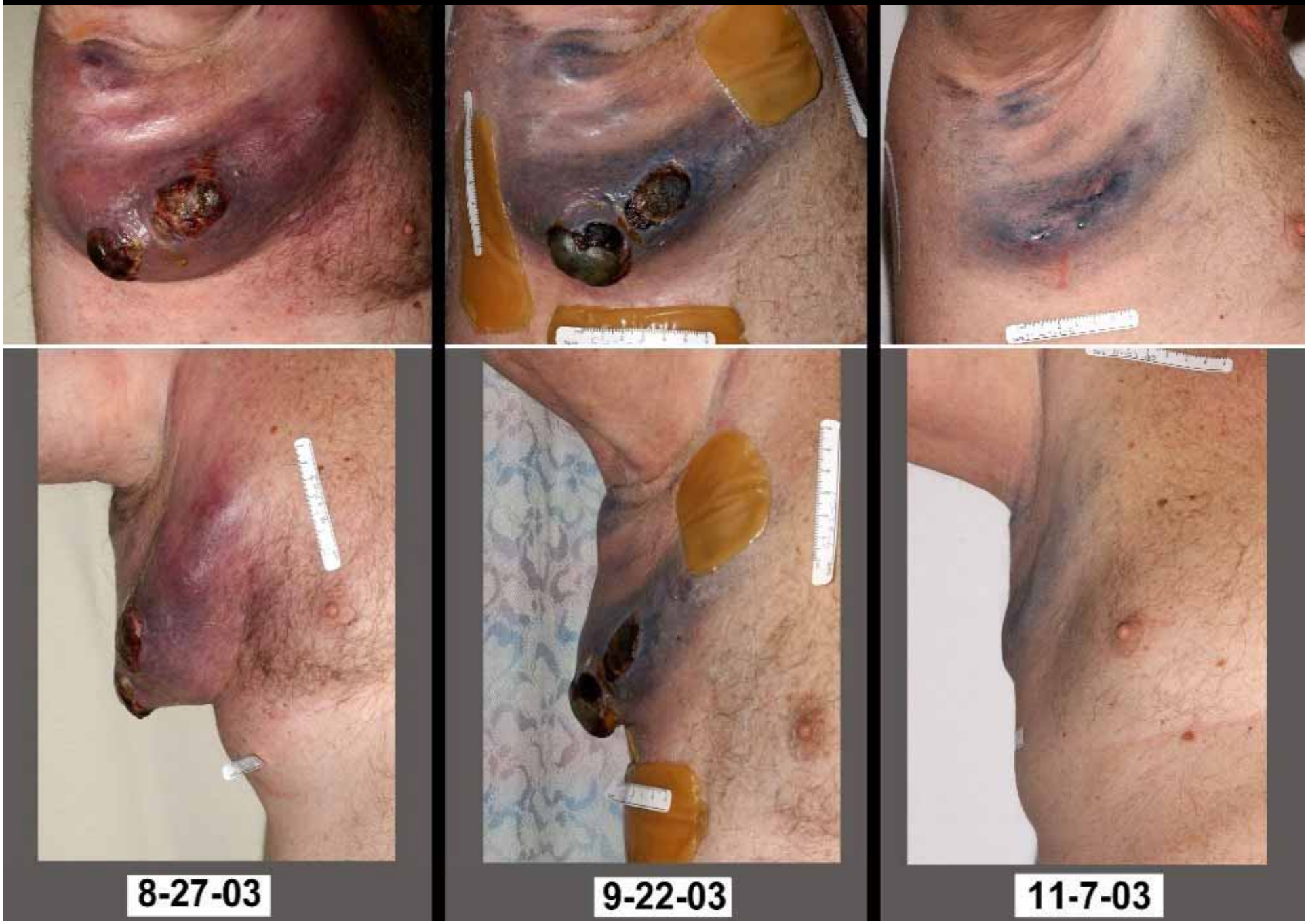


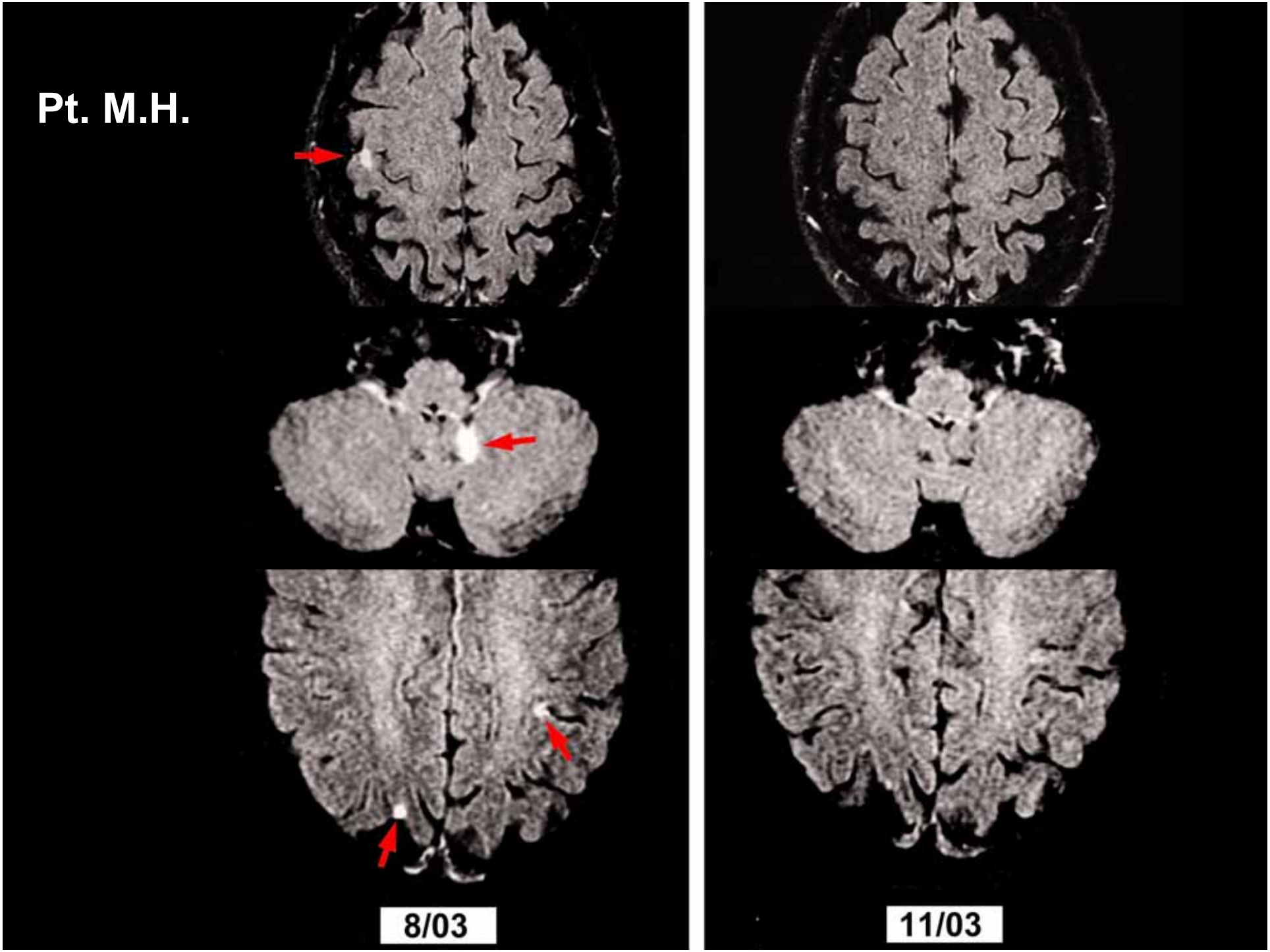
R.K.
(NMA)

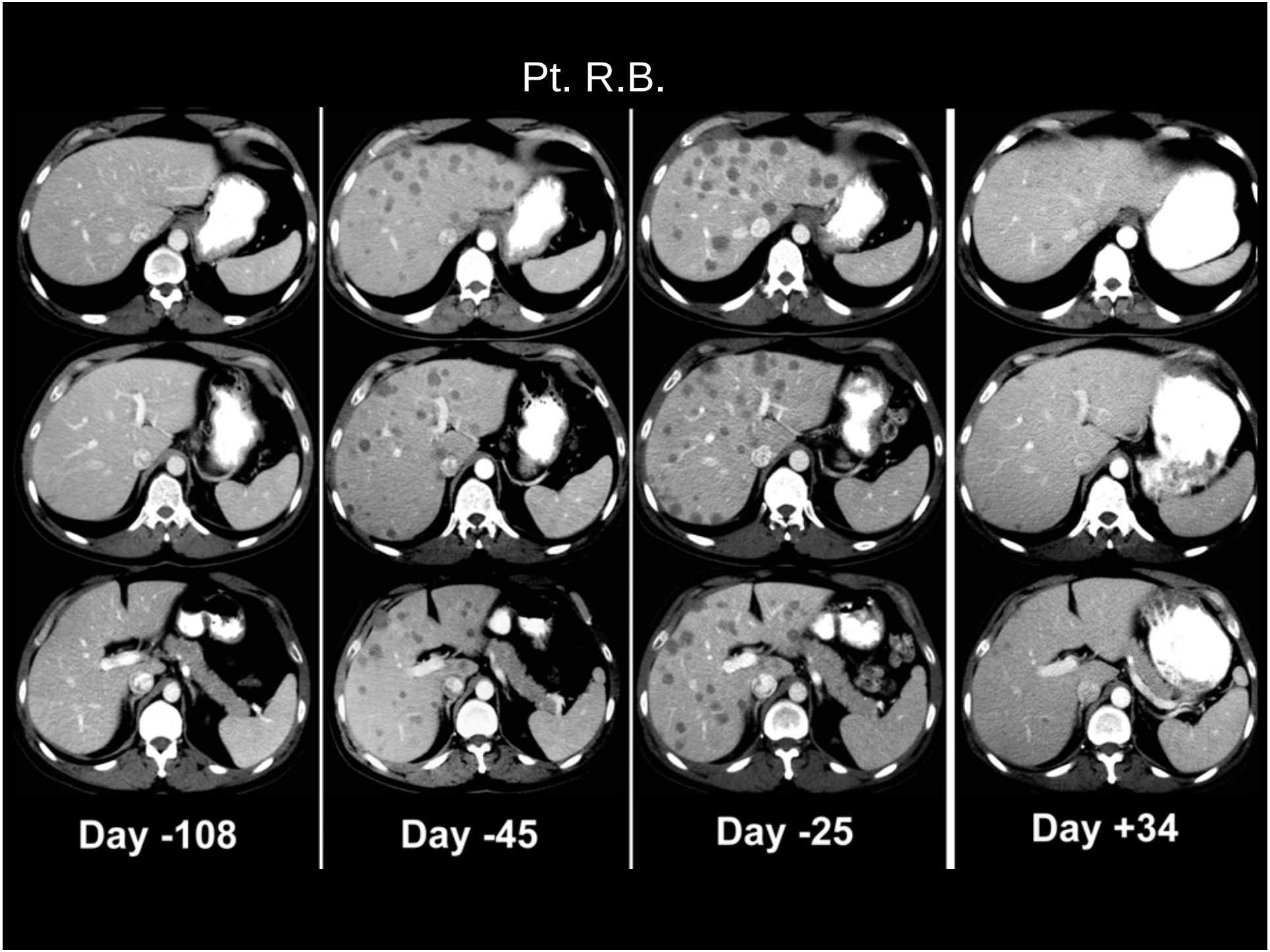


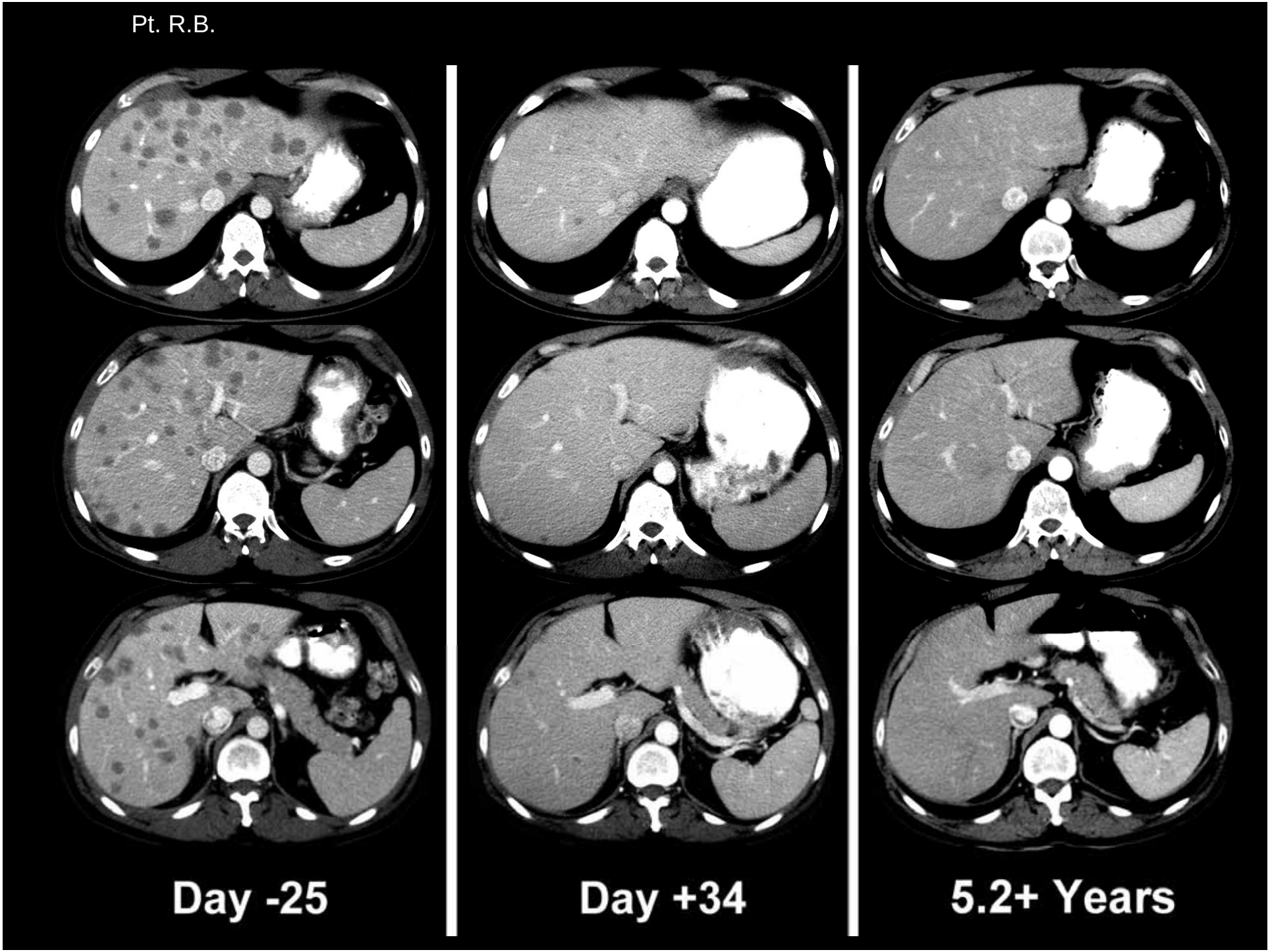

Pre-Treatment
28+ Months

Pt. M.H.

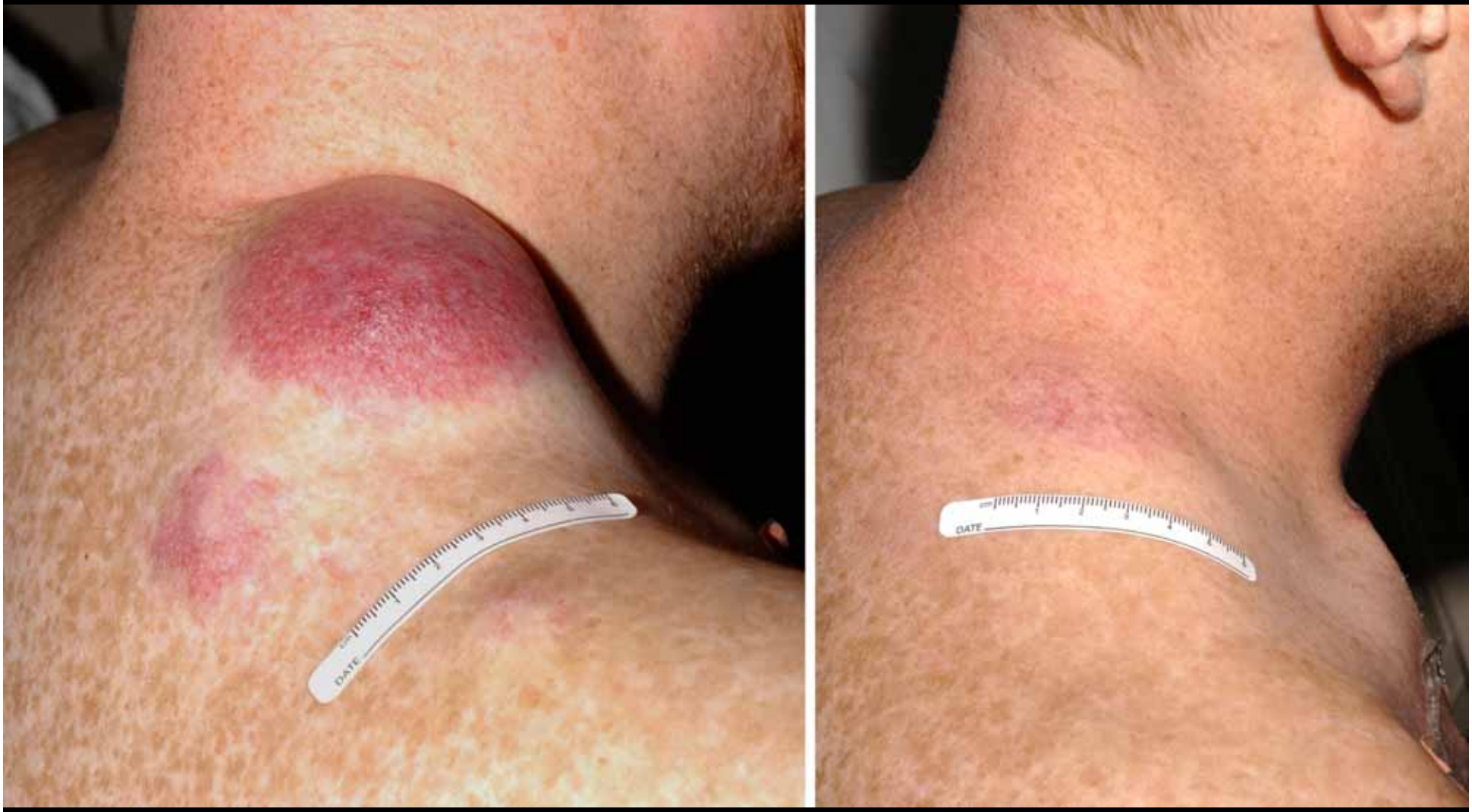



Pt. M.H.
8/03
11/03


Pt. R.B.
Day -108
Day -45
Day -25
Day +34

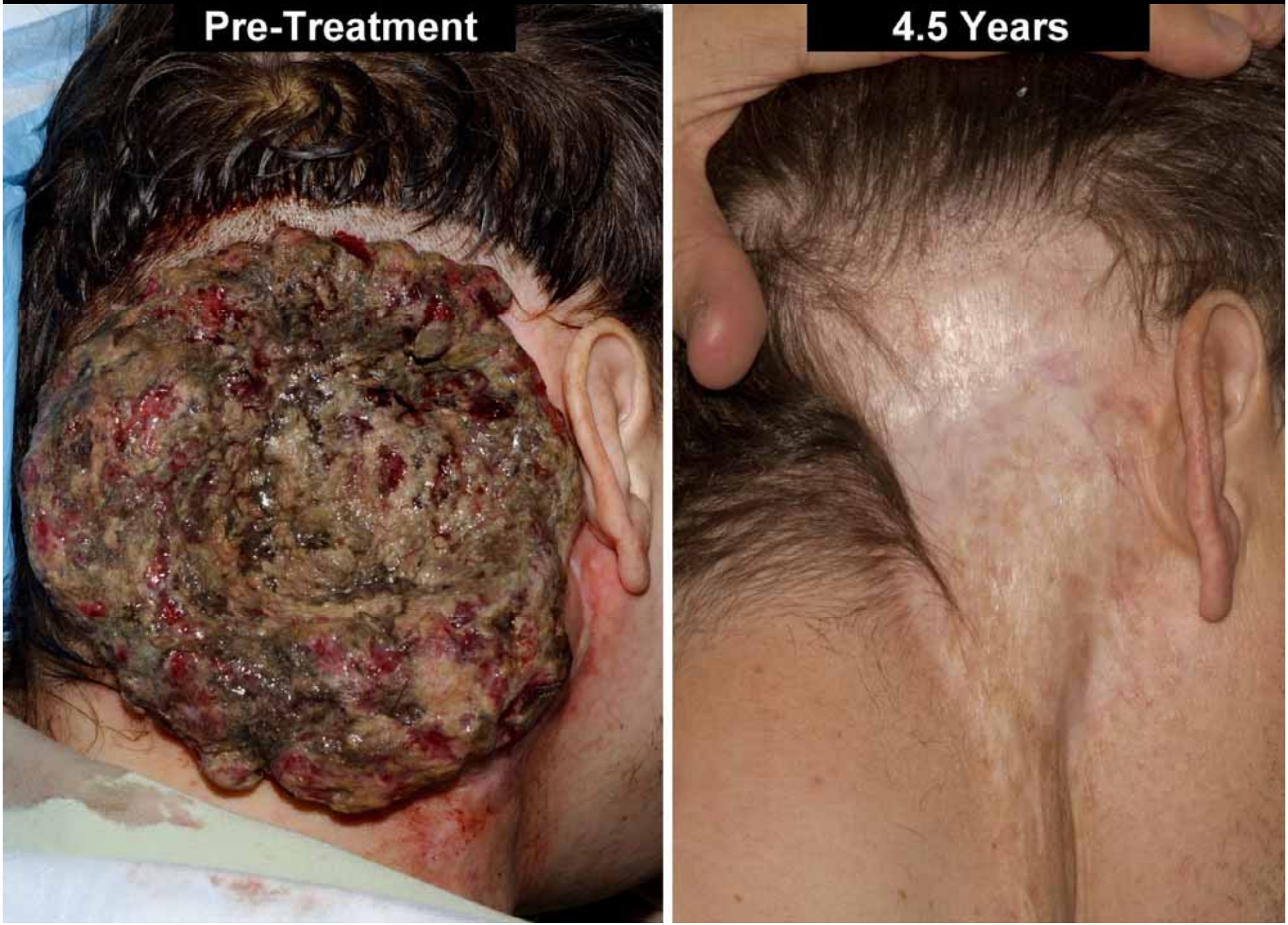

Pt. R.B.
Day -25
Day +34
5.2+ Years

C.K. (200cGy) Pre 12 days



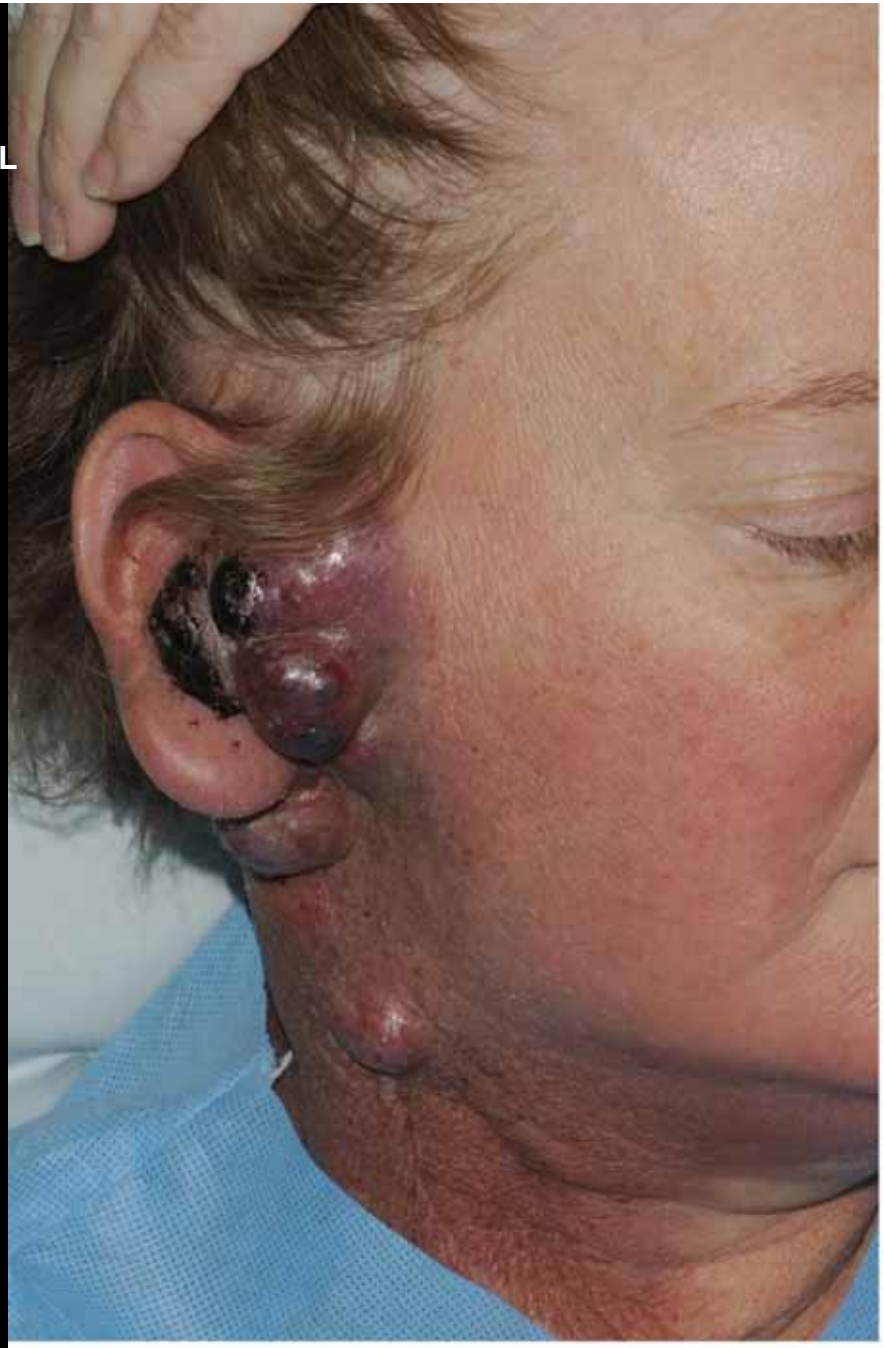
A.H.: N-M cell transfer

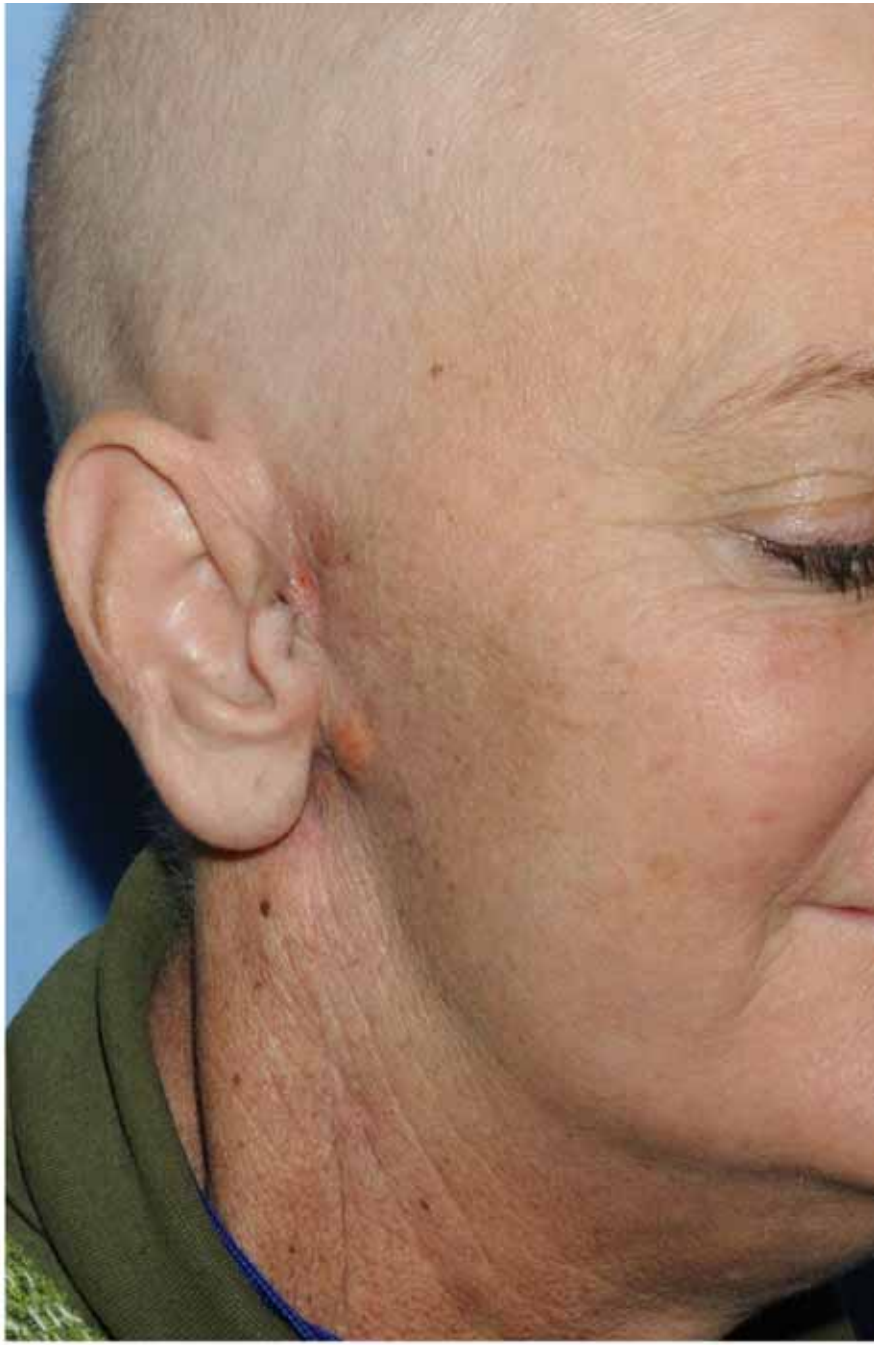
Pt. A.H. NMA



P.T.

Young TIL
CD8

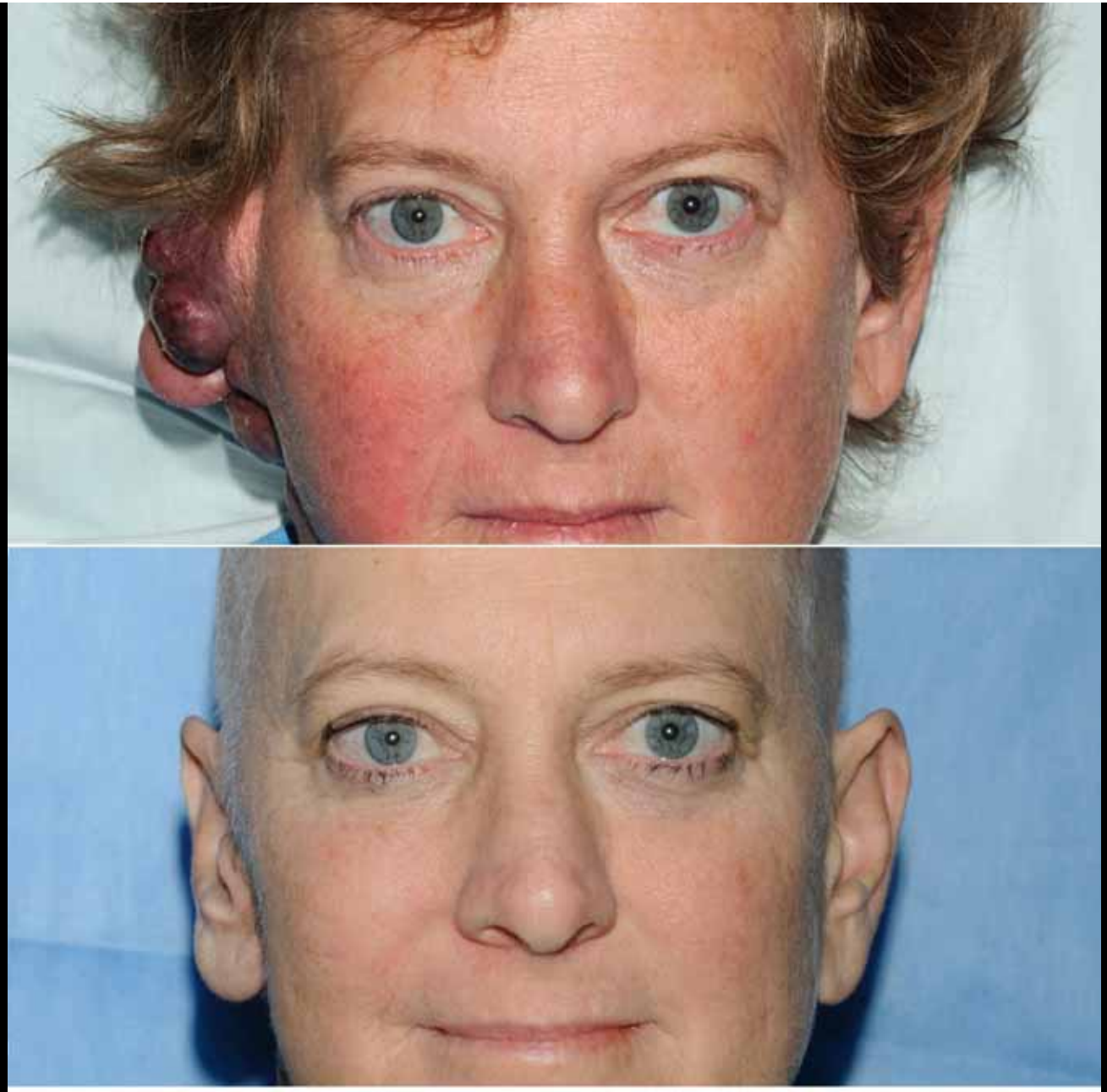




Pre-Treatment

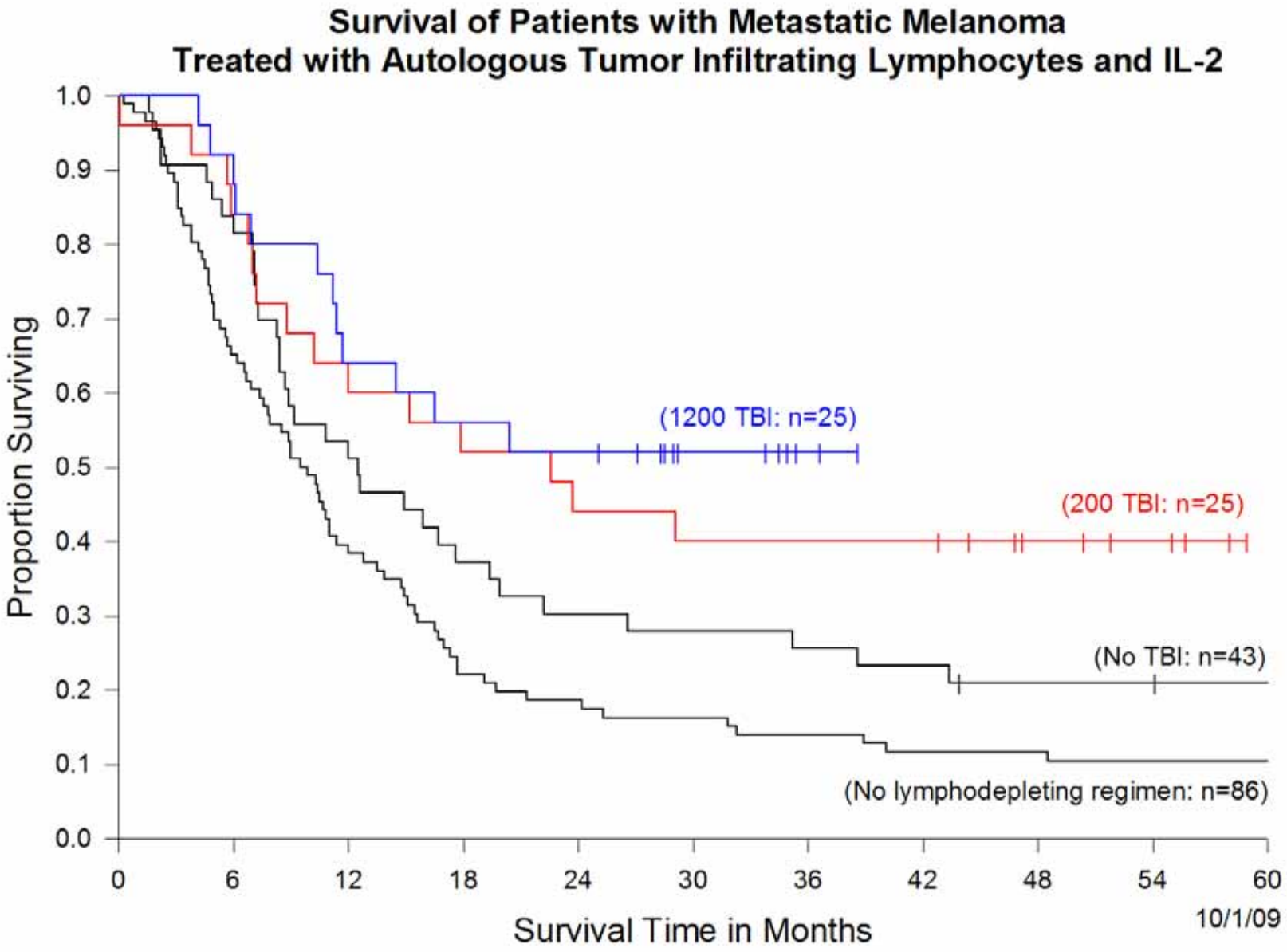
3 Months

P.T.
Young
TIL
CD8



"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 Cancer Research


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
(1200 TBI: n=25)
(200 TBI: n=25)
(No TBI: n=43)
(No lymphodepleting regimen: n=86)
0
6
12
18
24
30
36
42
48
54
60
Survival Time in Months
10/1/09

Hypothesis of Mechanism of Cancer Regression Following Cell Transfer

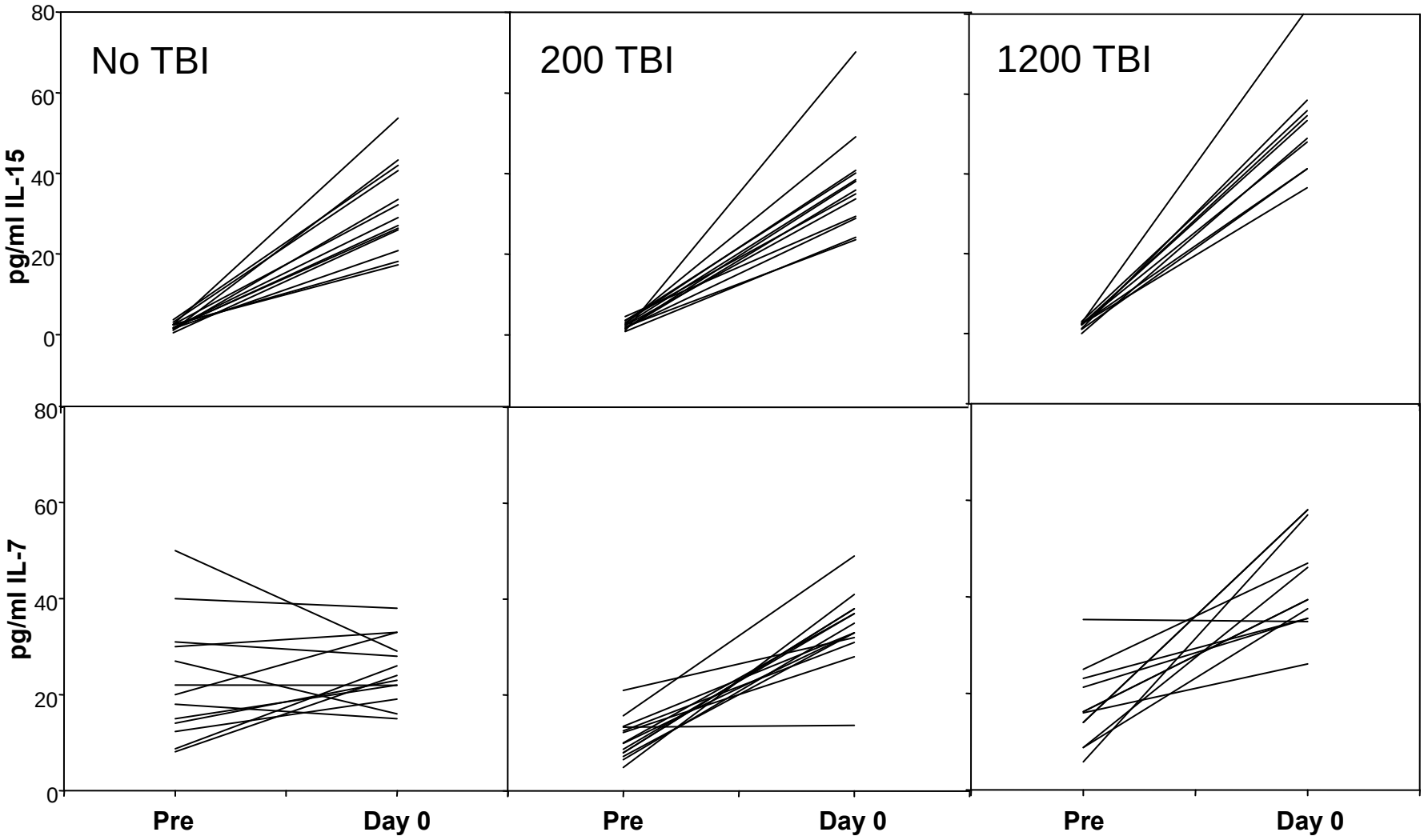
The lymphophenic environment

1) eliminates T regulatory (suppressor) cells

2) eliminates competition for homeostatic cytokines (IL-7, IL-15) vital for T cell survival

In the lymphopenic host, anti-tumor T cells proliferate, persist, infiltrate organs, recognize cancer antigens and destroy cancer cells.

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



Three Factors that Correlate with Cancer Regression Following Cell Transfer Therapy

	Responders	Non-Responders	
	(mean)		
Persistence in PBMC at 1-2 months (% CD3)	18.5	1.0	p<0.001
Telomere length in infusion TIL (kb)	6.5	5.4	p<0.01
CD27+CD8+ cells infused ($x10^{-10}$)	1.5	0.46	p<0.0001

Simplification of adoptive immunotherapy to make it more widely applicable

---

Administer "Young TIL" (new method of cell preparation)

single cell suspension of the entire tumor,
grow for 2 weeks
REP for 2 weeks and administer
no in vitro testing

Advantages:

short time in culture
cells are less differentiated with shorter telomeres
heterogeneous tumor antigen recognition
less labor intensive (no multiple cultures and no functional tests)
more patients qualify for treatment

Cell Transfer Therapy

(10/1/09)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
Young TIL	24	4 (25+,10,5,2)	1 (16+)	5 (21%)

Cell Transfer Therapy

(10/1/09)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
Young TIL	24	4 (25+,10,5,2)	1 (16+)	5 (21%)
CD8 Young TIL	33	15 (12+,11+,10+,9+, 9+,8+,6+,6+, 4+,9,8,6,5,3,2)	3 (10+,8+,6+)	18 (55%)

CONCLUSION

T cell based immunotherapy is capable of mediating the regression of large vascularized, invasive metastatic melanoma in humans

(The widely-held belief that immunotherapy can only affect minimal disease in the adjuvant setting is not the case.)

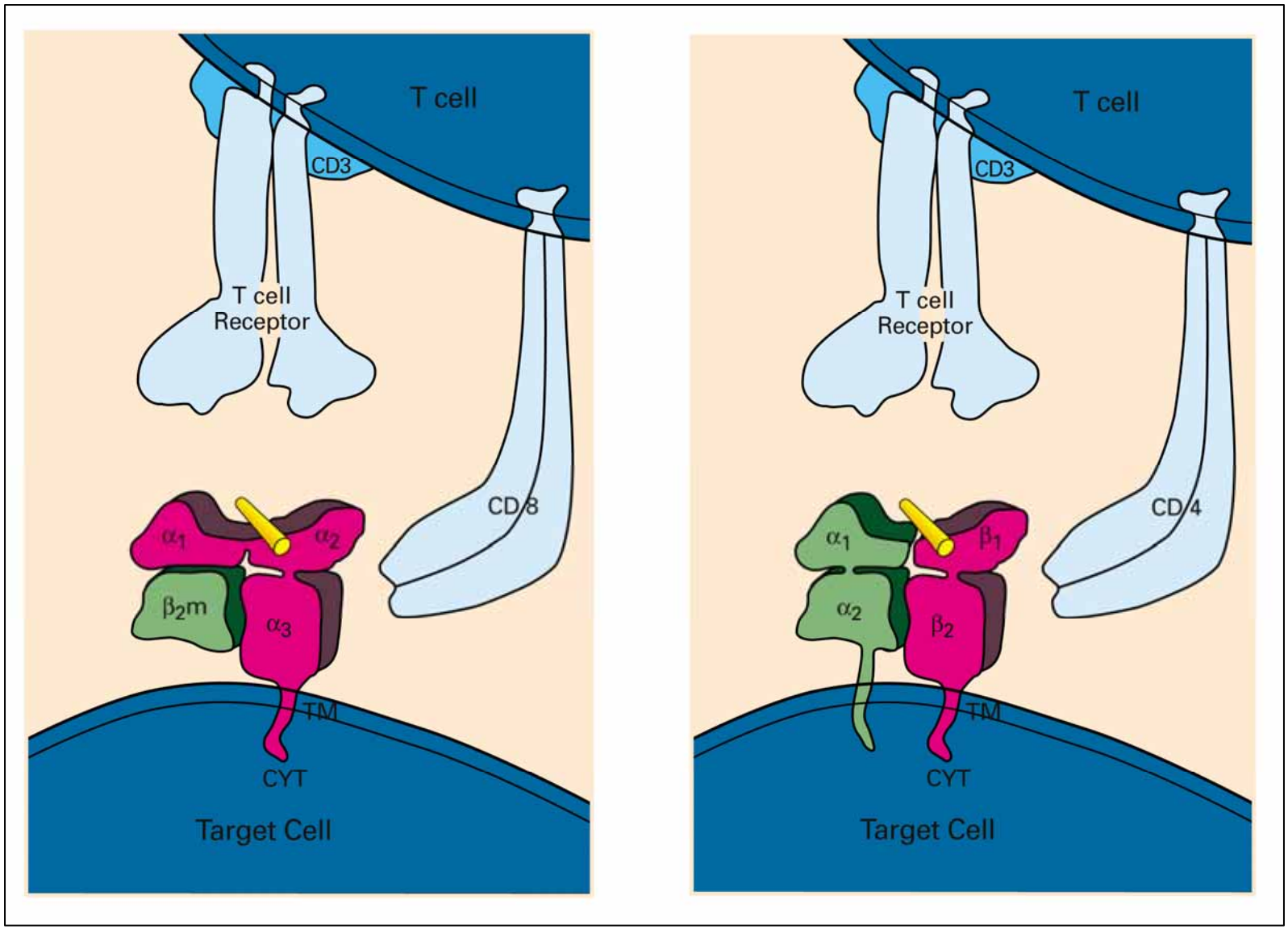
CHALLENGE

Determine ways to extend this approach to:

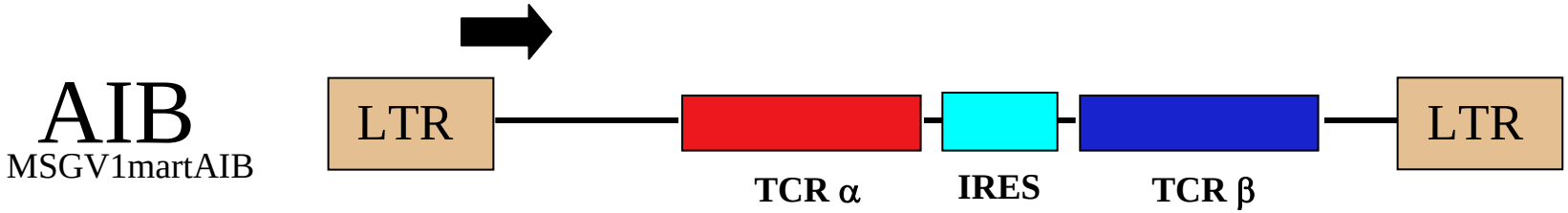
1) additional melanoma patients

2) patients with common epithelial cancers

Antigen recognition by $CD4^+$ and $CD8^+$ T lymphocytes



anti-Mart-1 retroviral vector



(Science 314:126,2006)

Treatment with MART-1 TCR transduced autologous lymphocytes

- **Stimulate circulating PBL with OKT-3**
- **On day 2 and 3 transduce PBL with MART-1 TCR retroviral vector and culture in IL-2**
- **Infuse transduced cells following lymphodepletion of the host and administer IL-2**

(Science 314:126, 2006)

First Trial of Cell Transfer Therapy using TCR Gene-Modified Cells

---

17 patients with metastatic melanoma (Science, 314:126, 2006)

2 (12%) with objective regressions

(both disease free over three years later)

14 additional patients treated

2 further objective regressions

<u>Overall</u>: 4/31 (13%) objective regressions

(Science 314:126, 2006)

DMF4 and DMF5 MART1 and gp100(154) TCR retroviral constructs

DMF4 (previous MART1 clinical trial)

DMF5

gp100(154) (high-affinity murine TCR)

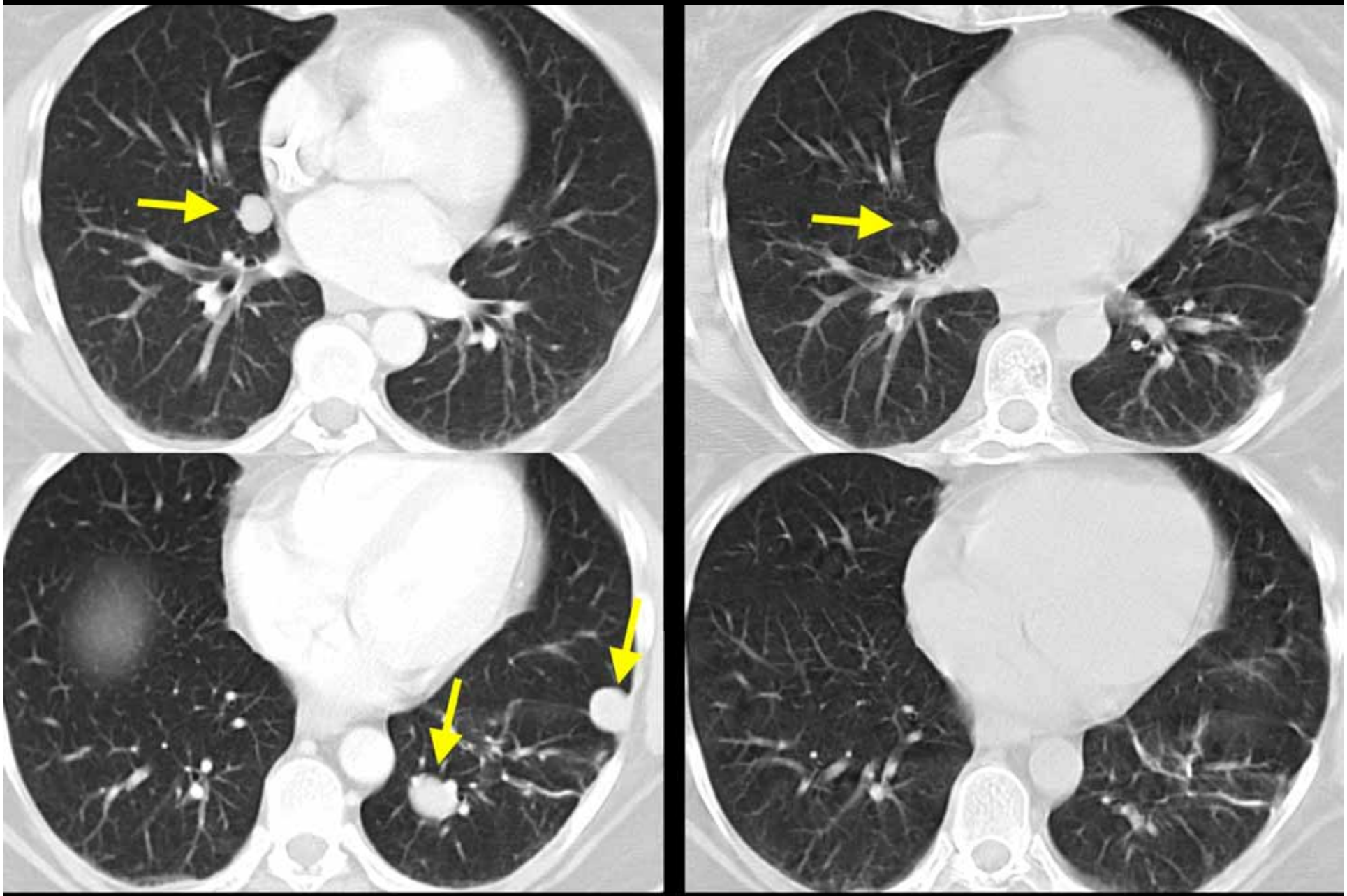
Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

Cohort	Cell#	IL-2	Response	
			Total	OR
1	$1\text{-}3x10^{10}$	limited	6	2
2	$\sim 3x10^{9}$	to tolerance	6	2
3	$1\text{-}8x10^{10}$	to tolerance	8	2
		Total	20	6(30%)

(All patients were refractory to prior treatment with IL-2.)

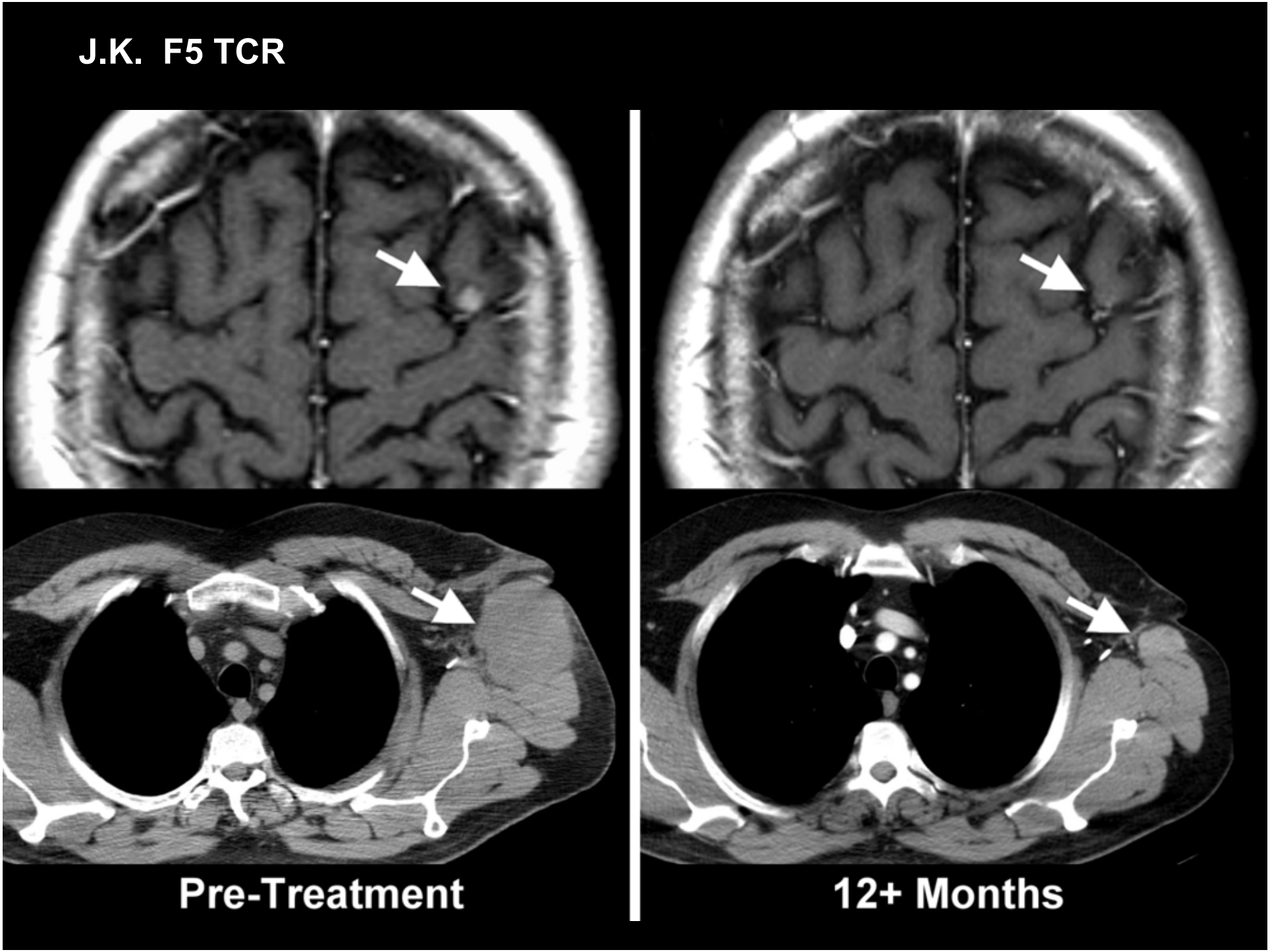
(Blood 114:535-546, 2009)

D.T. DMF5 TCR

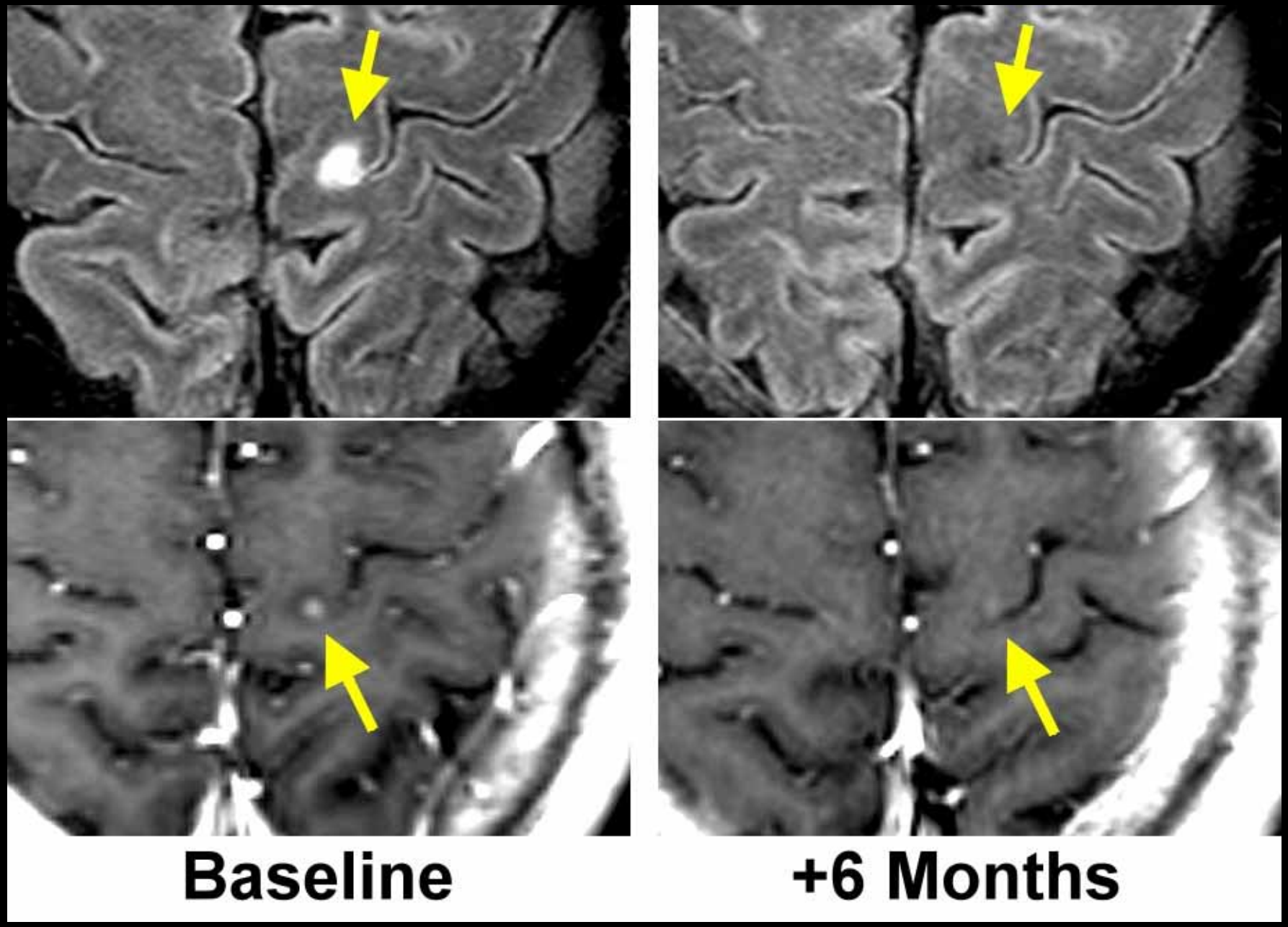


Pre-Treatment

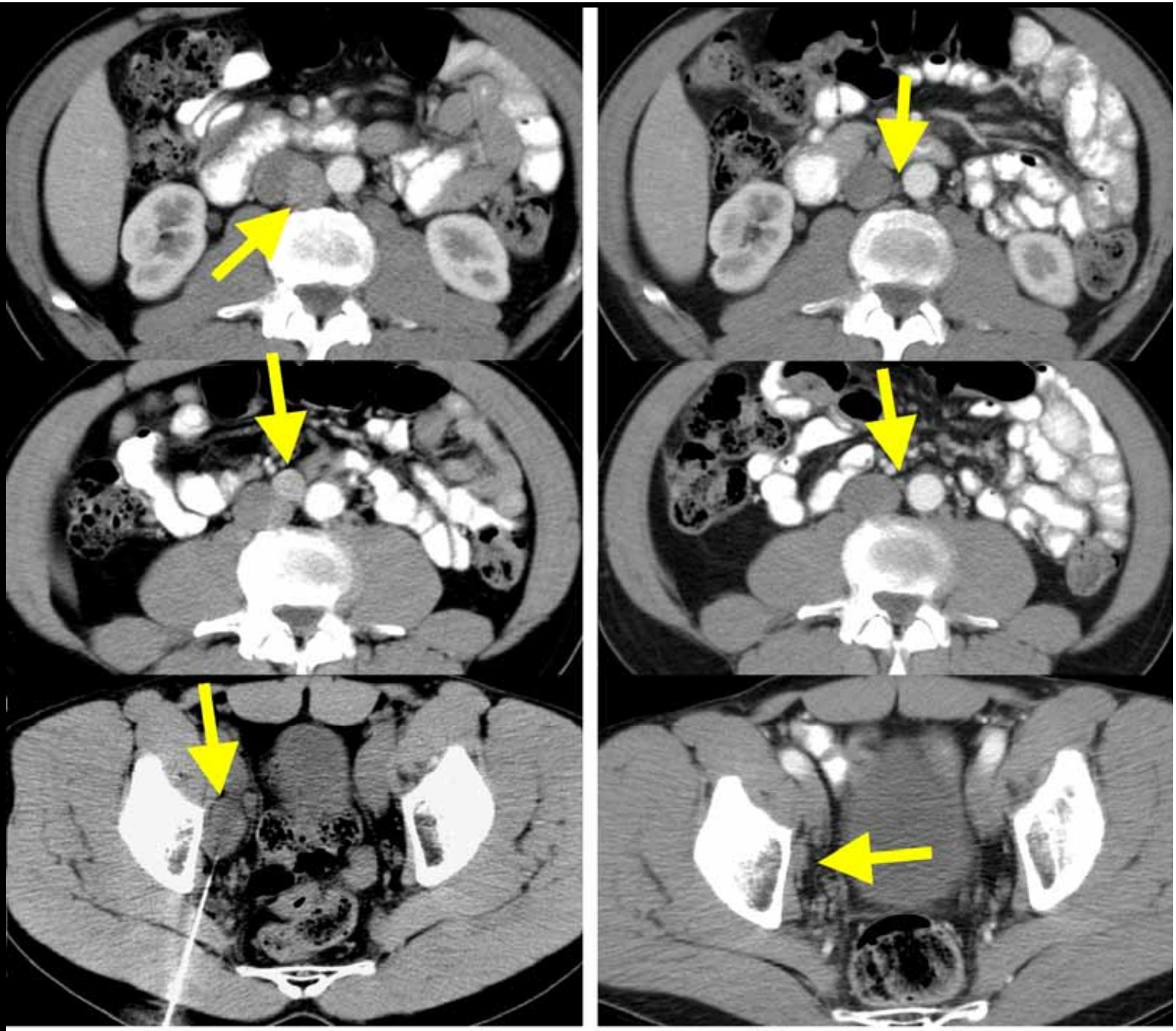
5+ Months


J.K. F5 TCR
Pre-Treatment
12+ Months

T.D. F5 TCR



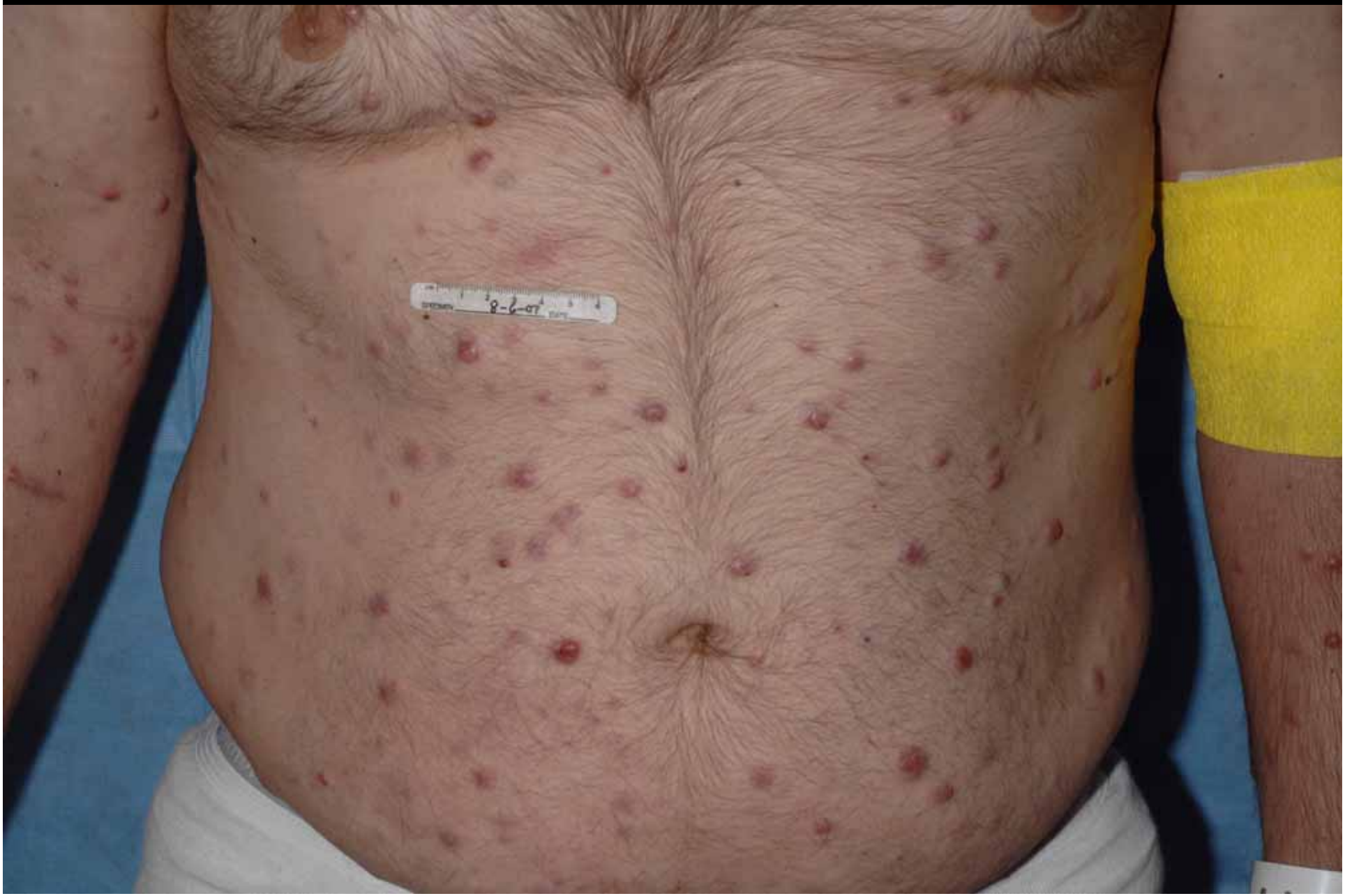
S.S. 154 TCR



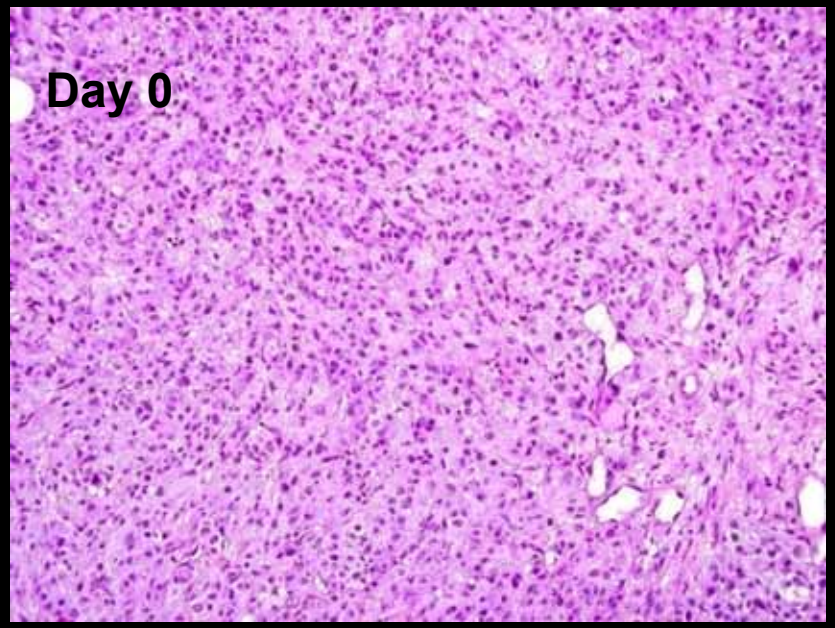
Pre-Treatment

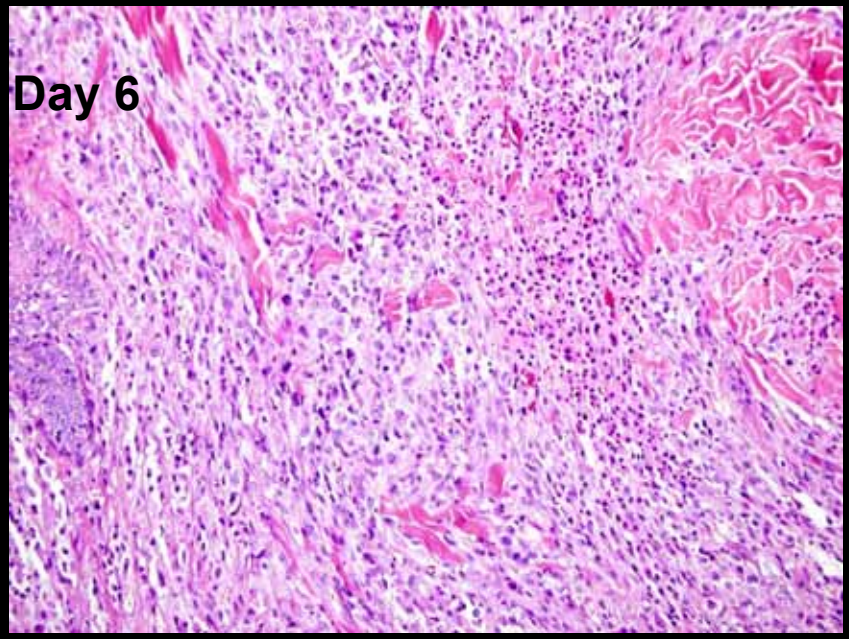
10+ Months

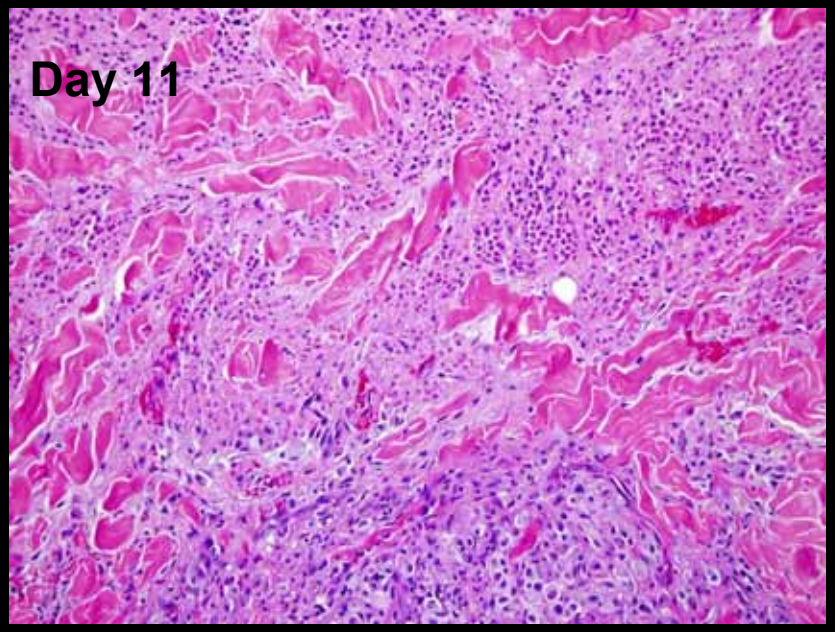
D.Th. F5 TCR Pretreatment

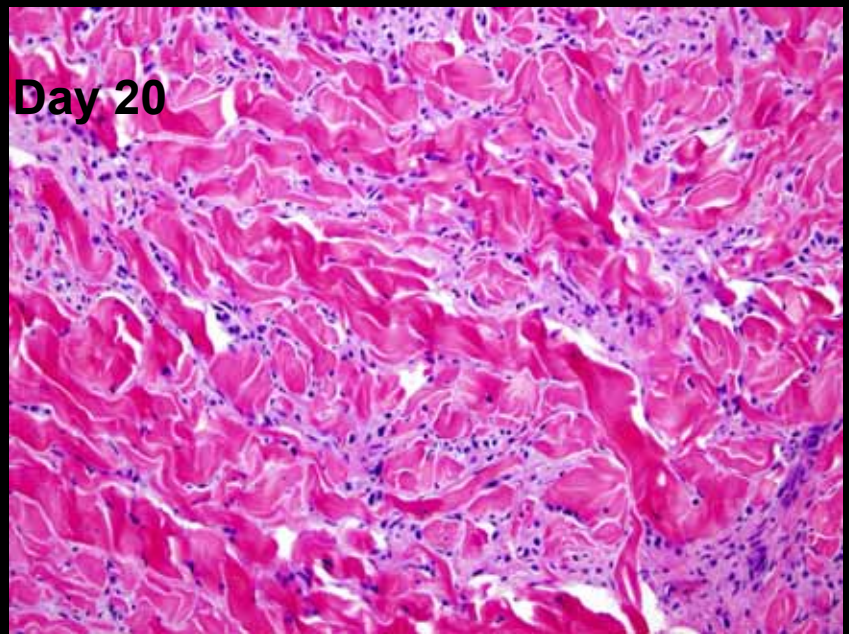


Sequential tumor biopsies (D.Th.): MART TCR



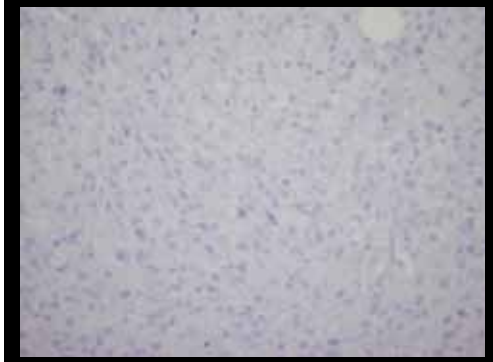




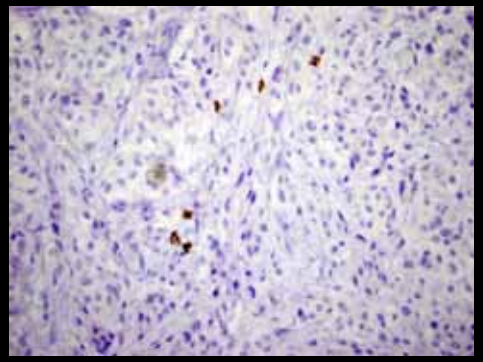


Sequential tumor biopsies (D.Th.): MART TCR (CD8, 40x)

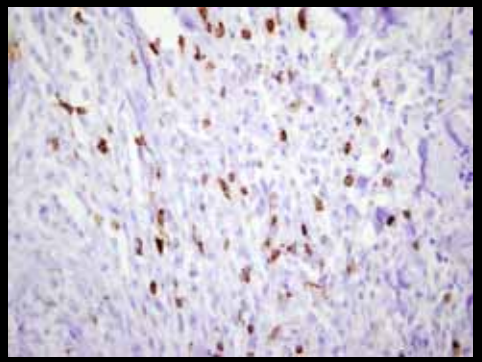
day 0



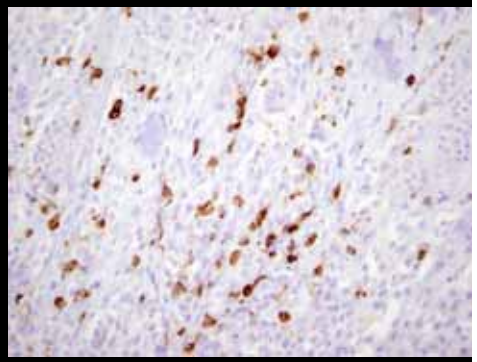
day 5



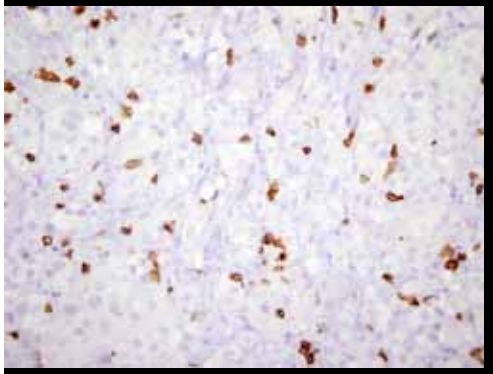
day 6



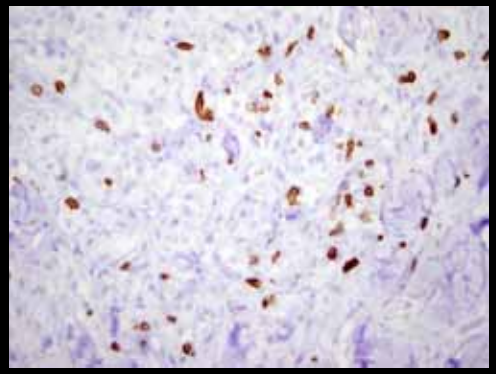
day 9



12 day



day 16



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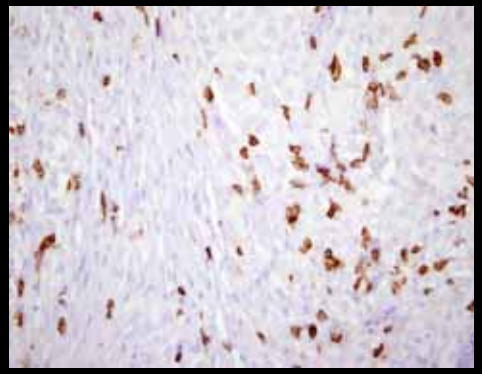
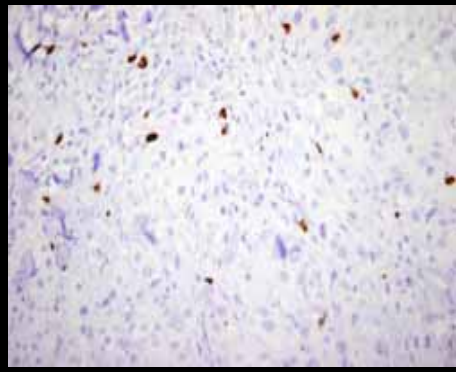


day 26



Two methods to improve the effectiveness of transduced T cell receptors

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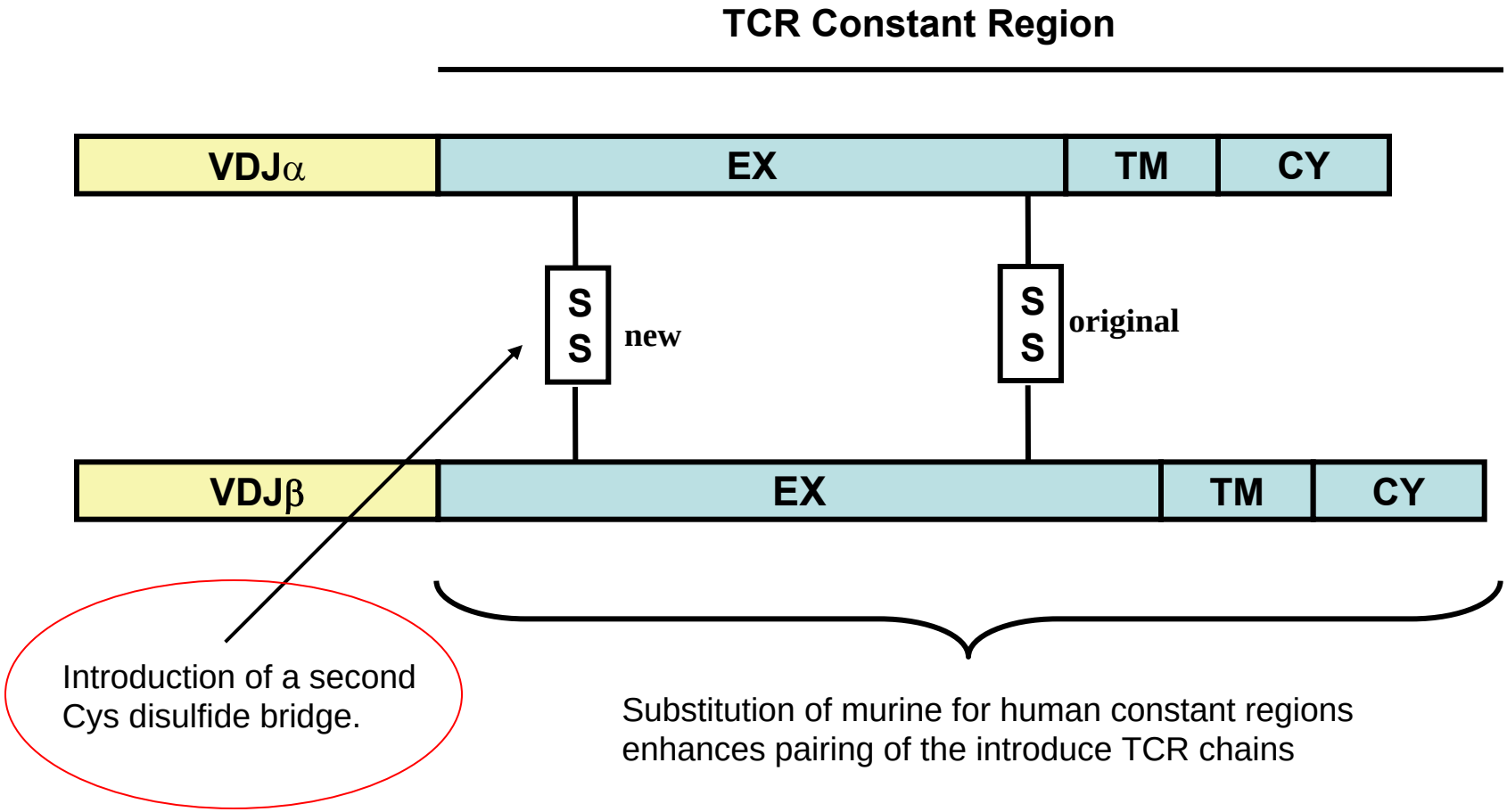
Reduce mispairing of the transduced alpha and beta chains

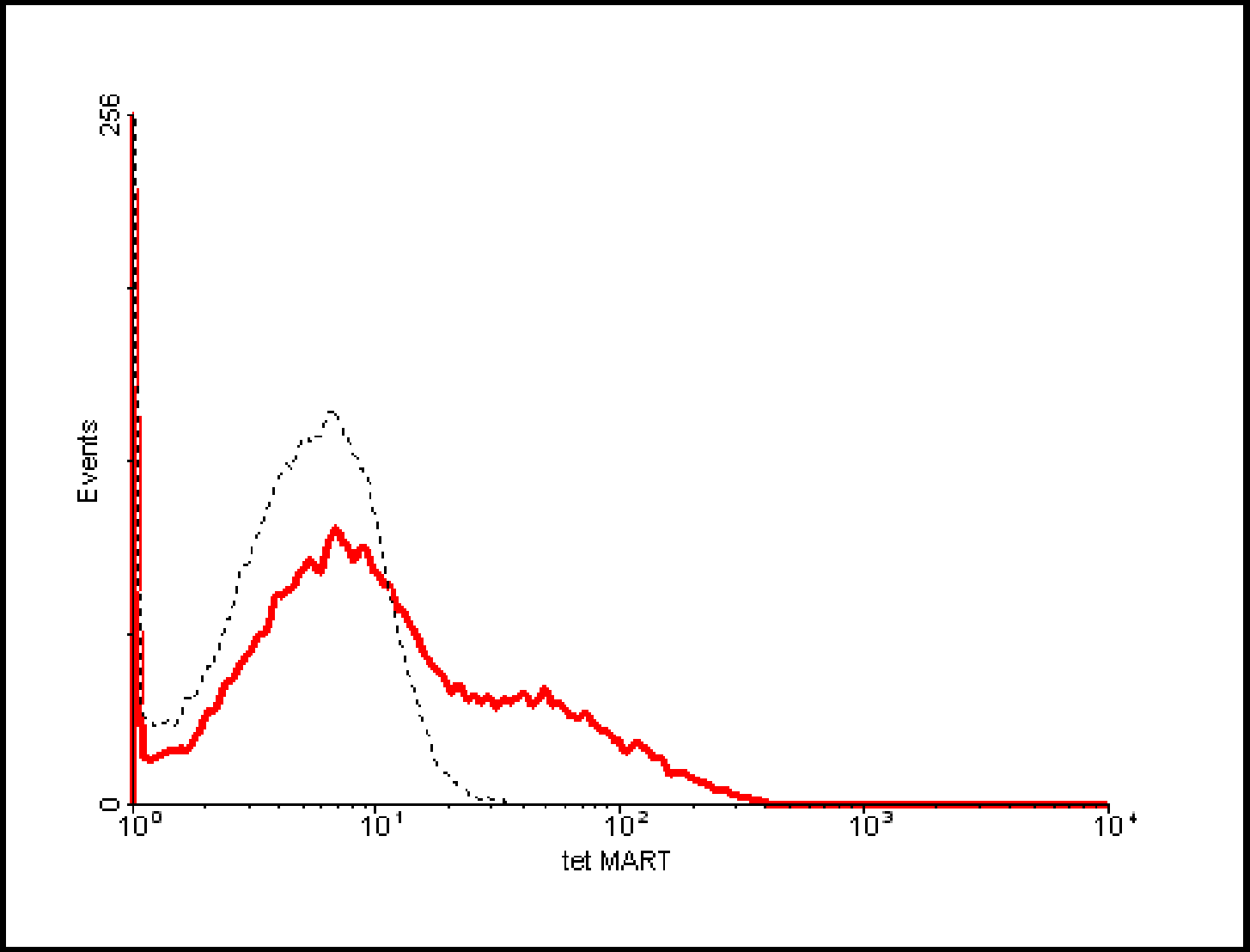
Modify the CDR2 and CDR3 regions of the TCR to improve antigen recognition

Optimizing Expression of the TCR Vector Constructs

Protein engineering of TCR chains

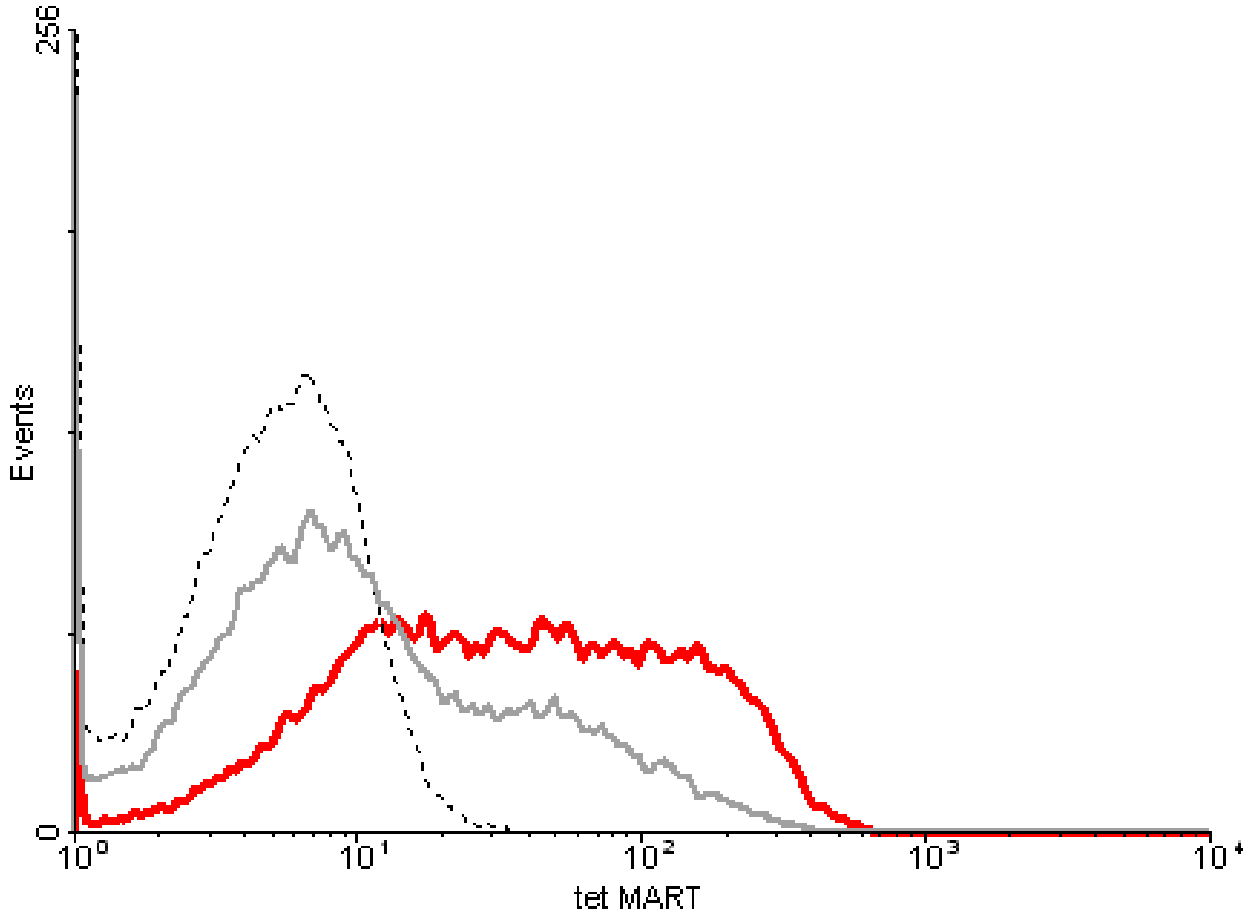


Human Constant Regions



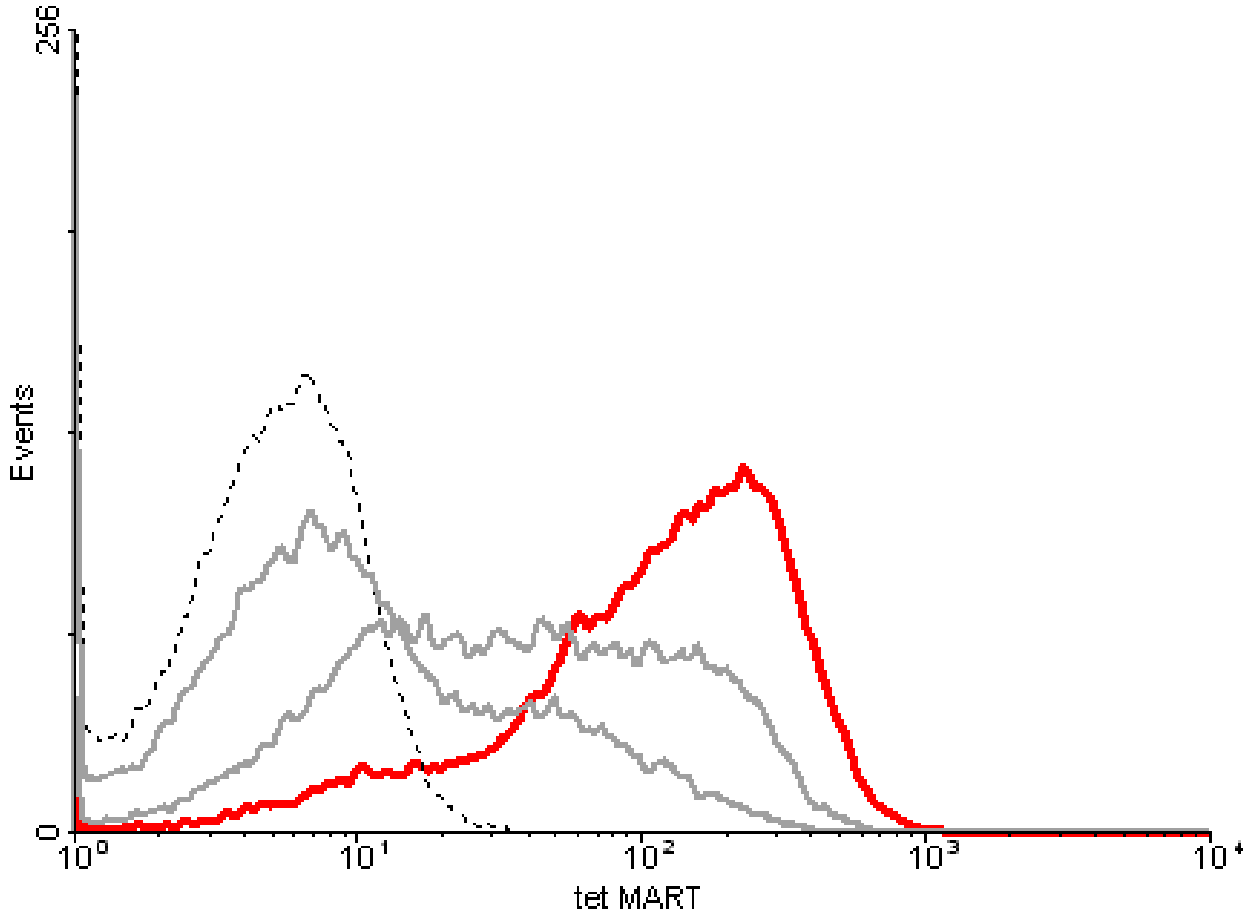
Constant Regions: H

Human CR+ Cysteines



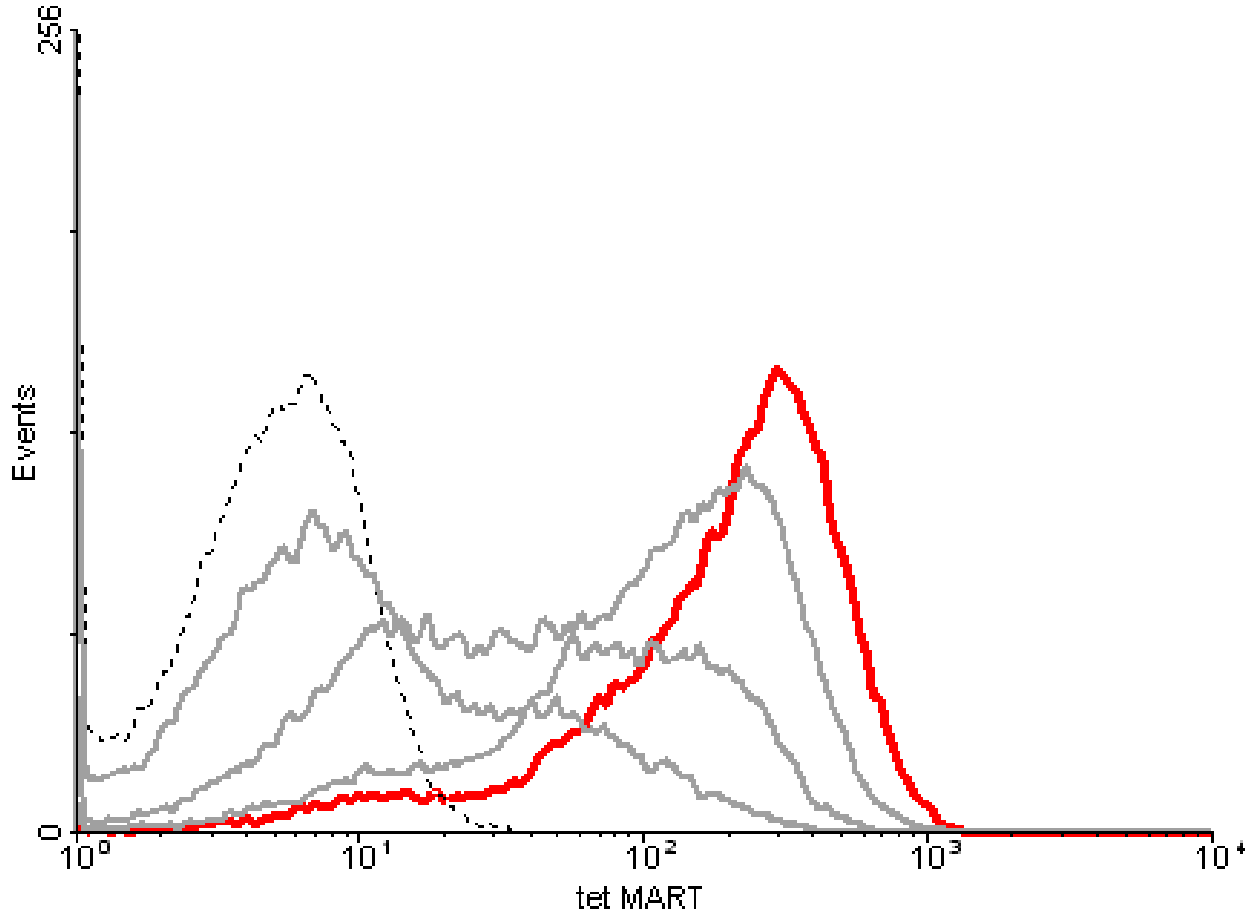
Constant Regions: H<H-Cys

Mouse Constant Regions



Constant Regions: H<H-Cys<M

Mouse CR + Cysteines



Constant Regions: H<H-Cys<M<M-Cys

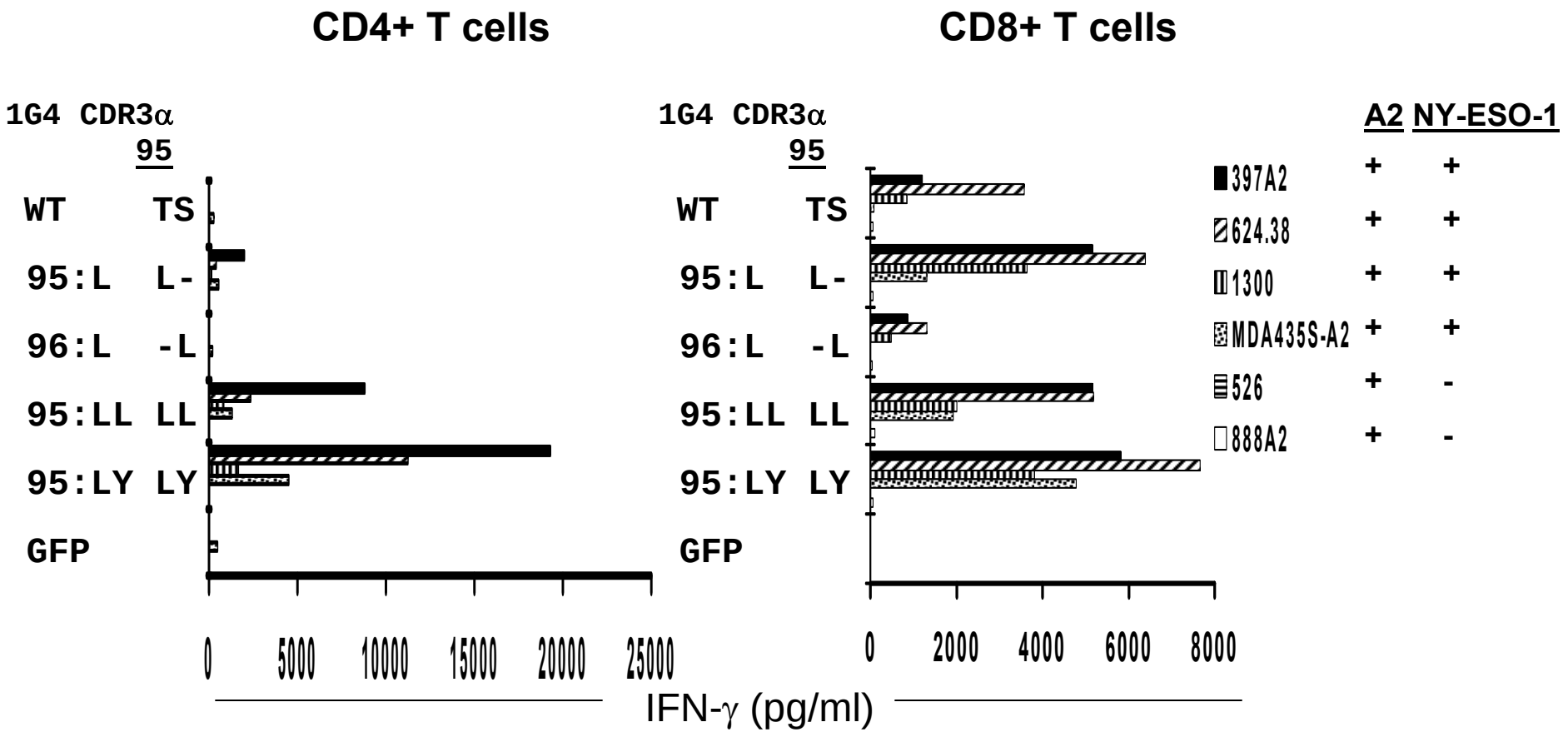
Method for increasing the affinity of T cell receptors

CDR2 and CDR3 regions of the T cell receptor are responsible for binding to the peptide/MHC complex.

Selective substitution of individual amino acids in the CDR2 and CDR3 regions can increase the affinity of the TCR.

(J. Immunol. 180:6116-6131, 2008.)

Reactivity of Wild-type and Substituted Anti-ESO T Cell Receptor



CONCLUSION

T cell based immunotherapy is capable of mediating the regression of large vascularized, invasive metastatic melanoma in humans

(The widely-held belief that immunotherapy can only affect minimal disease in the adjuvant setting is not the case.)

CHALLENGE

Determine ways to extend this approach to:

1) additional melanoma patients

2) patients with common epithelial cancers

T-Cell Receptor Cell Transfer Gene Therapy for Cancers Other than Melanoma

NY-ESO-1	**Cancer-testes antigen expressed on about one-third of common cancers**
CEA	**Overexpressed on selected colorectal and other G-I cancers**
CD19	**Expressed on B-cell lymphomas and leukemias and normal B cells**

NY-ESO-1 CANCER ANTIGEN

No expression on adult human tissues except for testis

Expressed on about 25% of common epithelial cancers such as lung, breast, prostate