IMMUNOLOGIC BACKGROUND

Humoral immunity: Antibodies recognize three-dimensional regions on intact protein molecules.

Cellular immunity: T cells recognize peptides from intracellular proteins presented on cell surface MHC molecules.

T cells are responsible for the rejection of allografts and transplanted tumors in mice and the rejection of organ grafts in humans

THREE MAIN APPROACHES TO CANCER IMMUNOTHERAPY

---

1. **Nonspecific stimulation of immune reactions**
   a) **Stimulate effector cells (IL-2)**
   b) **Inhibit suppressive factors (anti CTLA4)**

2. **Active immunization to enhance anti-tumor reactions (cancer vaccines)**

3. **Passively transfer activated immune cells with anti-tumor activity (adoptive immunotherapy)**

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.

PROTOCOL 99-C-95

Treatment of Patients with Metastatic Melanoma Using Cloned Lymphocytes Plus IL-2

Infuse up to 10^{11} cloned antitumor lymphocytes selected for high avidity for tumor recognition

Simultaneously begin IL-2 administration given either subcutaneously or intravenously

T-CELL CLONES USED FOR THE FIRST INFUSION CYCLE FOR EACH PATIENT SECRETE IFN-γ SPECIFICALLY TO g209 PEPTIDE AND HLA-A2$^+$ MELANOMAS

	Stimulator cell							
	T2 pulsed with peptide (μM)				Melanoma cell line			
Patient	g280 (1.0)	g209 (1.0)	g209 (0.01)	g209 (0.0001)	526-mel (A2$^+$)	624-mel (A2$^+$)	888-mel (A2$^-$)	938-mel (A2$^-$)
	(pg γ-IFN/ml)							
1	2	7633	1180	25	1000	1678	4	2
2	3	33025	3065	4	3263	6087	4	4
3	6	6190	4134	126	2040	4604	4	3
4	4	14103	5411	1096	5461	4078	12	0
5	6	>53402	18618	>5209	>49780	22835	0	0
6	0	5998	4609	164	973	940	0	0
7	10	17260	11229	816	3776	2760	7	4
8	72	7892	6321	988	2365	1624	0	0
9	1	15585	6624	840	92	401	0	0
10	9	33375	29720	>1259	699	2446	1	2
11	15	17435	20580	>1263	500	2848	1	0
12	1	9280	8051	>986	614	1596	ND	ND
13	35	2844	2175	148	2637	2155	16	24

(J. Immunother. 24:363-373, 2001)

PROTOCOL 99-C-0158

Treatment of patients with Metastatic Melanoma Using Cloned Lymphocytes Following Administration of a Non-Myeloablative but Lymphocyte Depleting Regimen

---

a) Cyclophosphamide (30-60 mg/kg x2 days
Fludarabine (25 mg/m^2 x5 days)

b) After lymphocytes completely depleted (day 7):

Infuse up to 10^{11} cloned antitumor lymphocytes selected for high avidity for tumor recognition

Simultaneously (day 7) begin IL-2 administration

Phase I Study of Non-Myeloablative Chemotherapy Plus Transfer of Cloned T-Cells Plus IL-2

Patient	Cyclo-phosphamide (mg/M^2)	Fludarabine (IU/kg)	IL-2 ($x10^{-9}$)	Cloned Cells
LR	30	25	--	22.4
SC	30	25	--	21.5
FB	30	25	--	15.0
CK	60	25	--	9.3
JB	60	25	--	4.1
JH	60	25	--	5.5
BL	60	25	72,000	11.0
MD	60	25	72,000	6.8
CC	60	25	72,000	3.2
KS	60	25	720,000	2.8
AK	60	25	720,000	11.3
AT	60	25	720,000	0.9
CS	60	25	720,000	4.9
DK	60	25	720,000	12.6
ML	60	25	720,000	24.2

(J. Immunother. 25:243-251, 2002)

PROTOCOL 99-C-0158 (amended)

Treatment of patients with Metastatic Melanoma Using Cloned Lymphocytes Following Administration of a Non-Myeloablative but Lymphocyte Depleting Regimen

---

a) **Cyclophosphamide (30-60 mg/kg x2 days)**
Fludarabine (25 mg/m^2 x5 days)

b) **After lymphocytes completely depleted (day 7):**

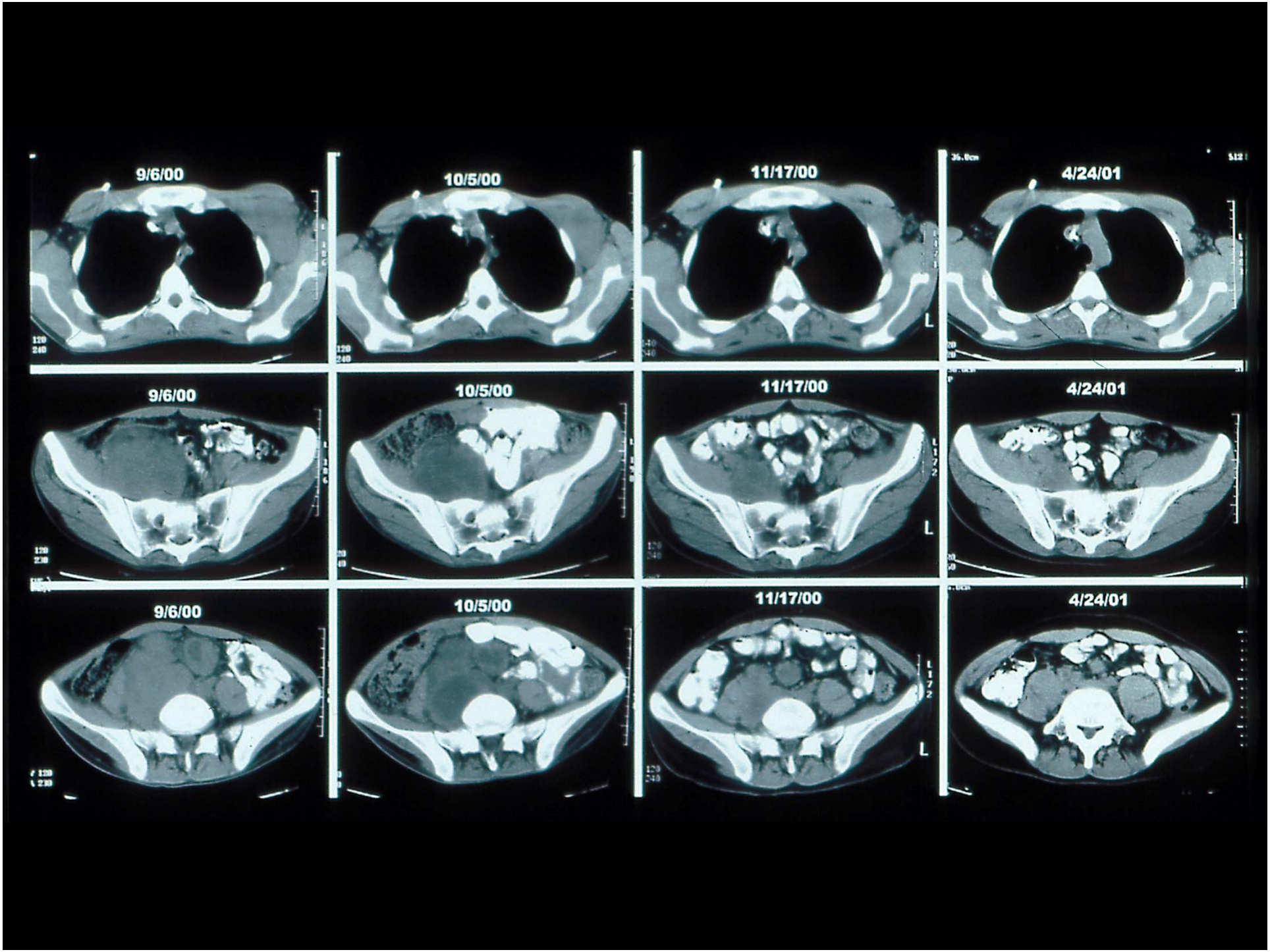
Infuse up to 10^{11} heterogeneous TIL
(containing both CD4 and CD8 cells)
selected for high avidity for tumor recognition.

Simultaneously (day 7) begin IL-2 administration

PATIENT A.K.

16 year old male referred to NCI with metastatic melanoma to multiple subcutaneous sites

Oct. 1996	**Excision of a 3.4 mm deep melanoma of left knee**
March 1997	**Lymph node dissection, 1/9 positive; treated with alpha-interferon**
Oct. 1997	**Developed multiple subcutaneous metastases To NCI; treated with experimental 4 peptide vaccine; progressive disease**
Jan. 1998	**Multiple cycles of high-dose IL-2; progressive disease**
May 1998	**New subcutaneous masses; resected for TIL**
Jan. 2000	**Increasing subcutaneous, pelvic, axillary metastases Treated with cisplatin & dacarbazine; progressive disease**
March 2000	**Non-myeloablative chemotherapy plus cloned lymphocytes plus high-dose IL-2; progressive disease**
May 2000	**Brain metastasis; resected**
July 2000	**Non-myeloablative chemotherapy plus cloned lymphocytes (4 days) plus high-dose IL-2**
Sept. 2000	**Progressive bulky disease in axilla, pelvis and intraperitoneum; bedridden; narcotics for pain**


9/6/00
10/5/00
11/17/00
4/24/01
9/6/00
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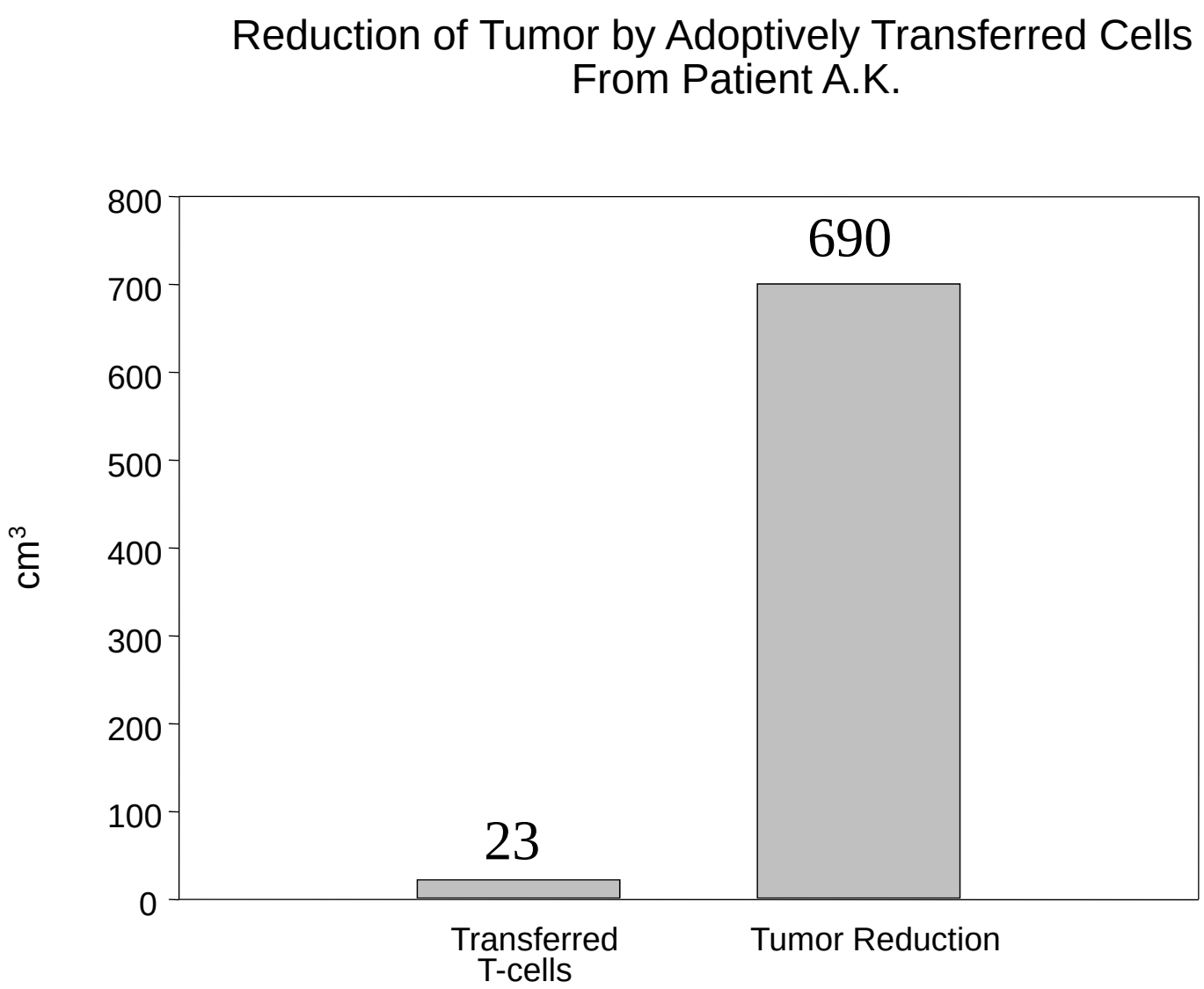
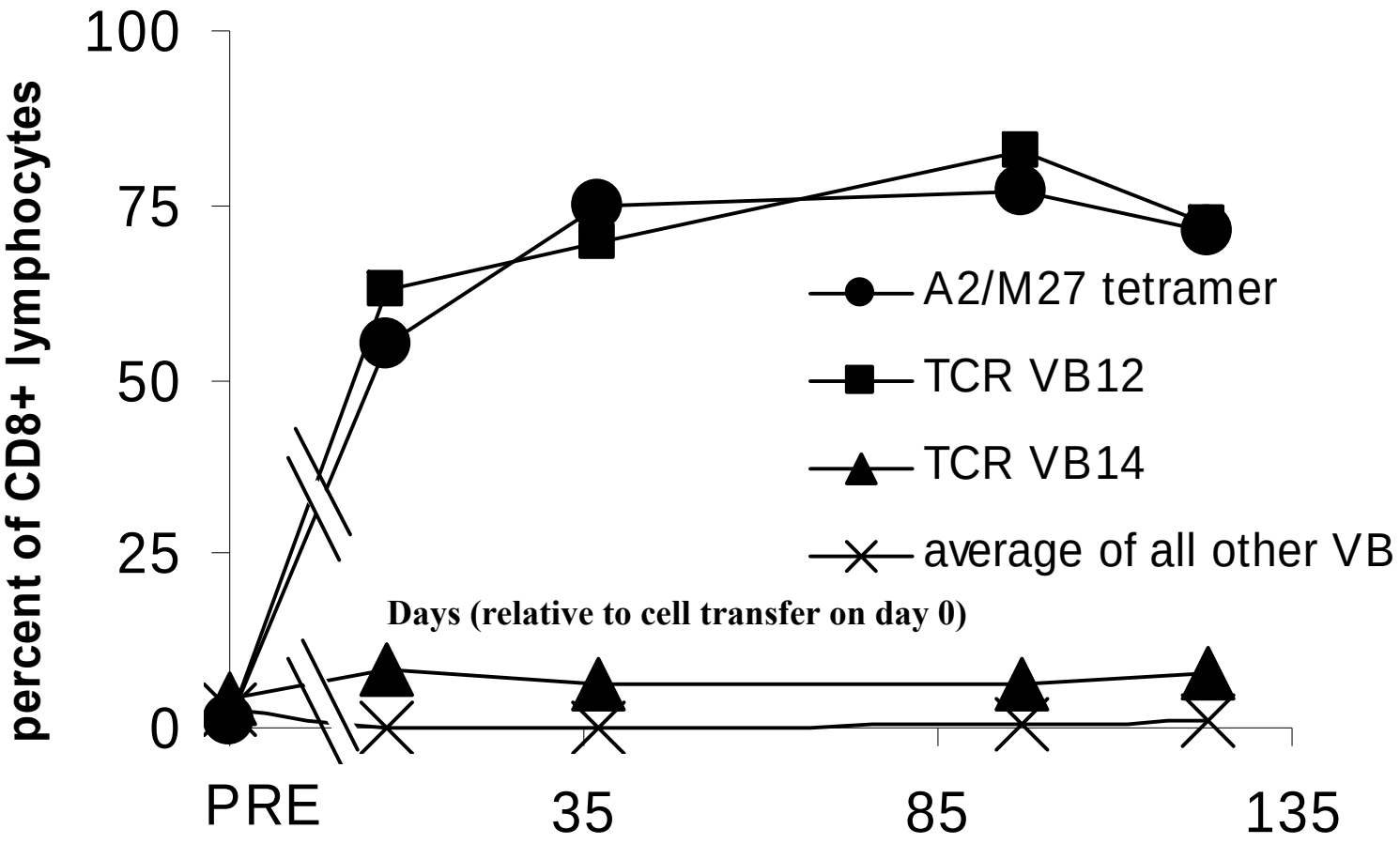
10/5/00
11/17/00
4/24/01

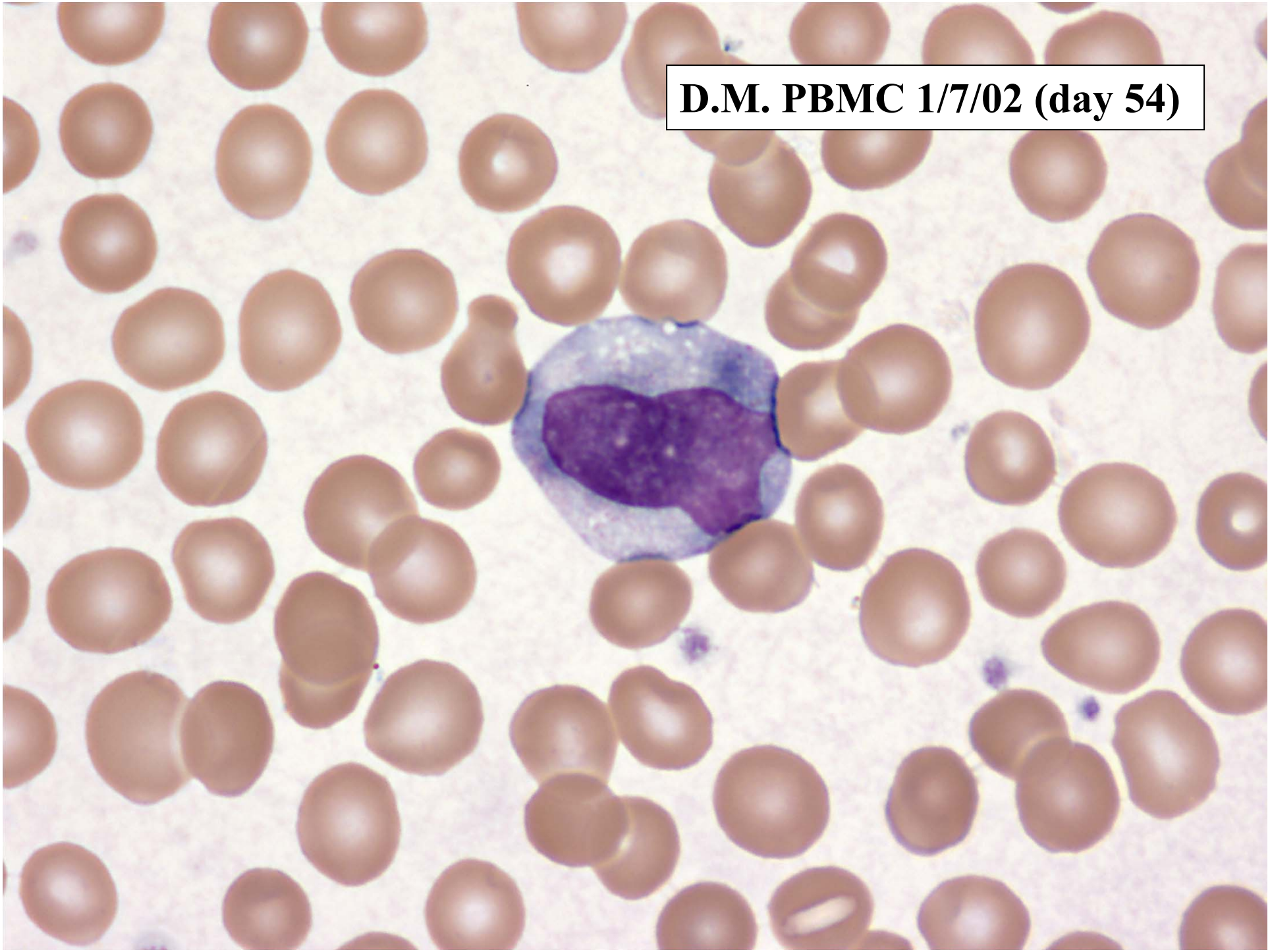

Reduction of Tumor by Adoptively Transferred Cells
From Patient A.K.
800
700
600
500
400
300
200
100
0
cm3
690
23
Transferred
T-cells
Tumor Reduction

Persistence of transferred cells – Patient DM

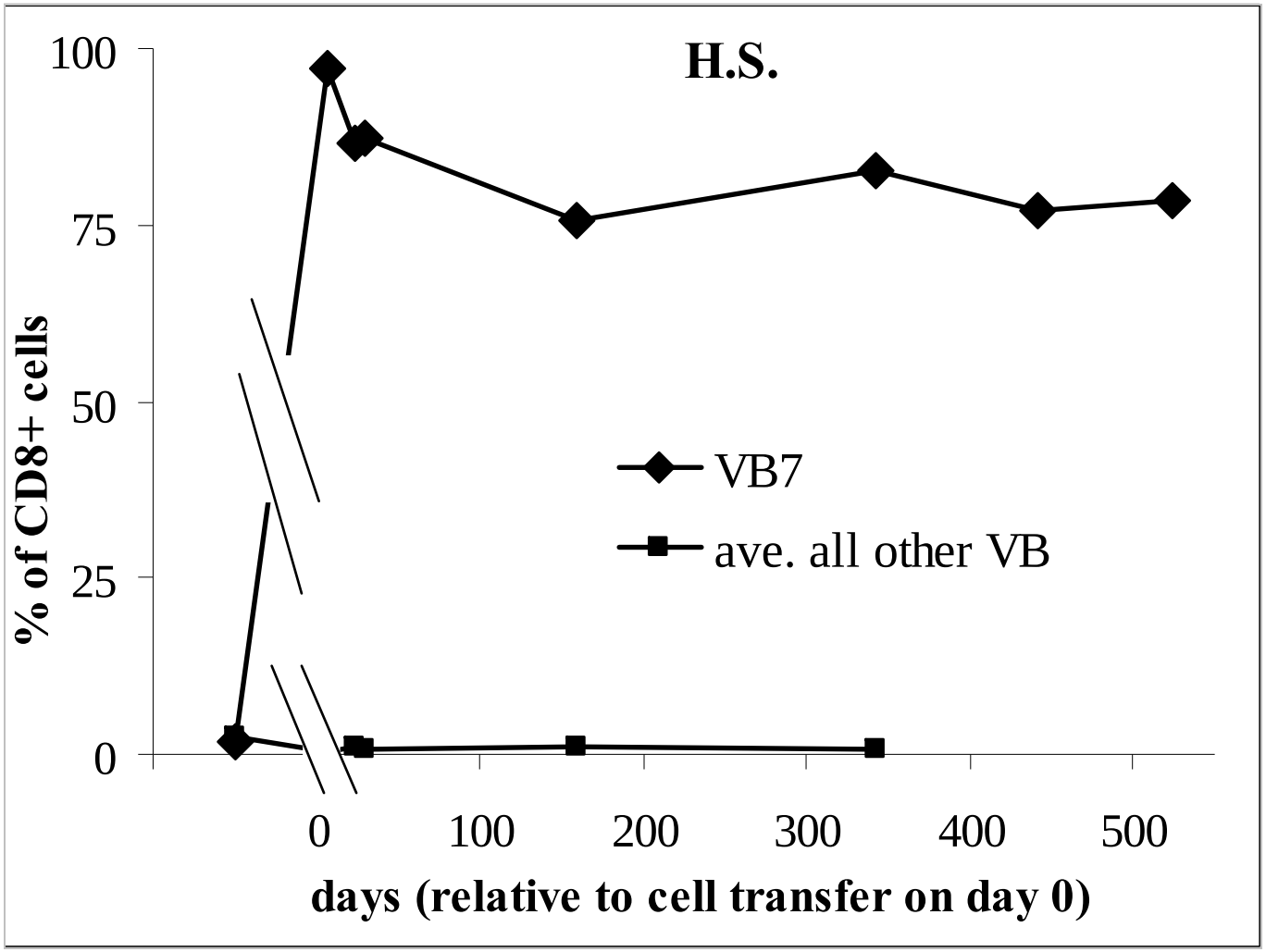


$CD8^+$ Vβ12 LYMPHOCYTES IN PATIENT D.M.

$CD8^+$ Vβ12 MART-1 reactive lymphocytes		
		% of $CD8^+$ that are $MART^+$
Administered in TIL:	1.3×10^{10}	90%
Circulating in Blood: day 7	6.4×10^{10}	71%
Circulating in Blood: day 19	3.8×10^{10}	78%
Circulating in Blood: day 55	2.6×10^{9}	83%


D.M. PBMC 1/7/02 (day 54)

HS: Persistence of VB7 clone



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

(4/1/08)

	Total	PR	CR	OR (%)
Number	43	17	4	21 (49%)
Duration (mos)		(67+, 35+, 23+, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2)	(66+, 61+, 51+, 50+)	

All patients received cyclophosphamide 60mg/kg x 2d + fludarabine 25mg/m^2x5d.

(Science, 2002; J Clin Oncol, 2005.)

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

(4/1/08)

Treatment*	Total	PR	CR	OR (%)
No TBI**	43	17 (67+, 35+, 23+, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2)	4 (66+, 61+, 51+, 50+)	21 (49%)
200 TBI	25	11 (36+, 32+, 26+, 14, 10, 6, 5, 5, 4 3, 3)	2 (40+, 28+)	13 (52%)

*All patients received cyclophosphamide 60mg/kg x 2d + fludarabine 25mg/m^2x5d.

**Patients who received REPed TIL plus the full preparative regimen as first REPed TIL treatment.

Preparative Regimens for Cell Transfer

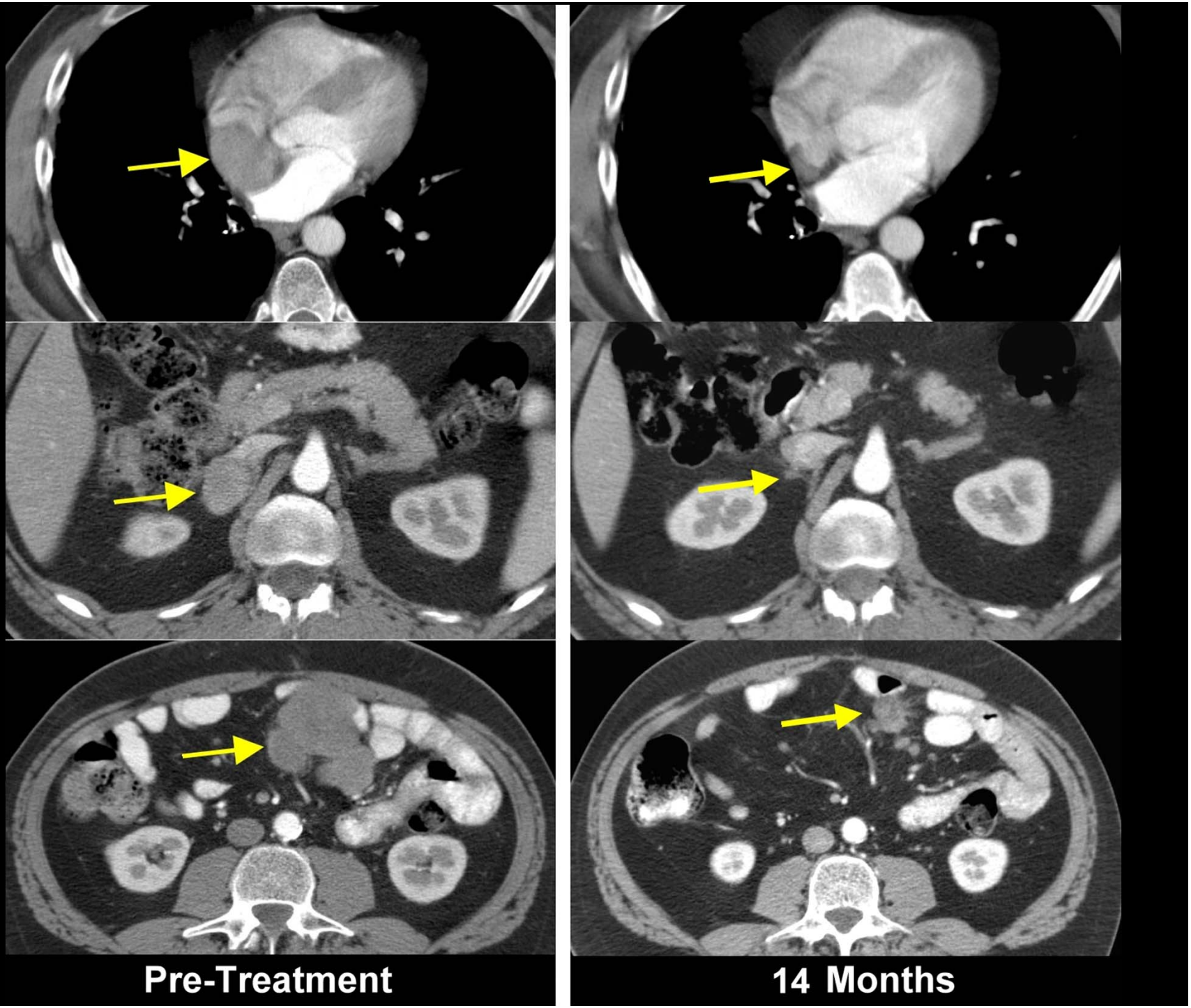
	Days										
	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Non-myeloablative	Cy	Cy	Flu	Flu	Flu	Flu	Flu				
								Cells			
								IL-2	IL-2	IL-2	
Ablative		Cy	Cy								
(200cGy)		Flu	Flu	Flu	Flu	Flu					
							TBI				
								Cells			
								IL-2	IL-2	IL-2	
									CD34+		
Ablative	Cy	Cy									
(1200cGy)	Flu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

Cell Transfer Therapy

(4/1/08)

Treatment	Total	PR	CR	OR (%)
No TBI	43	17 (67+, 35+, 23+, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2)	4 (66+, 61+, 51+, 50+)	21 (49%)
200 TBI	25	10 (36+, 32+, 26+, 14, 10, 6, 5, 5, 4 3, 3)	2 (40+, 28+)	13 (52%)
1200TBI	25	14 (17+, 16+, 13+, 10+, 10+, 10+, 9+, 9+, 7+, 7, 6, 6, 4, 3)	4 (20+, 18+, 16+, 11+)	18(72%)

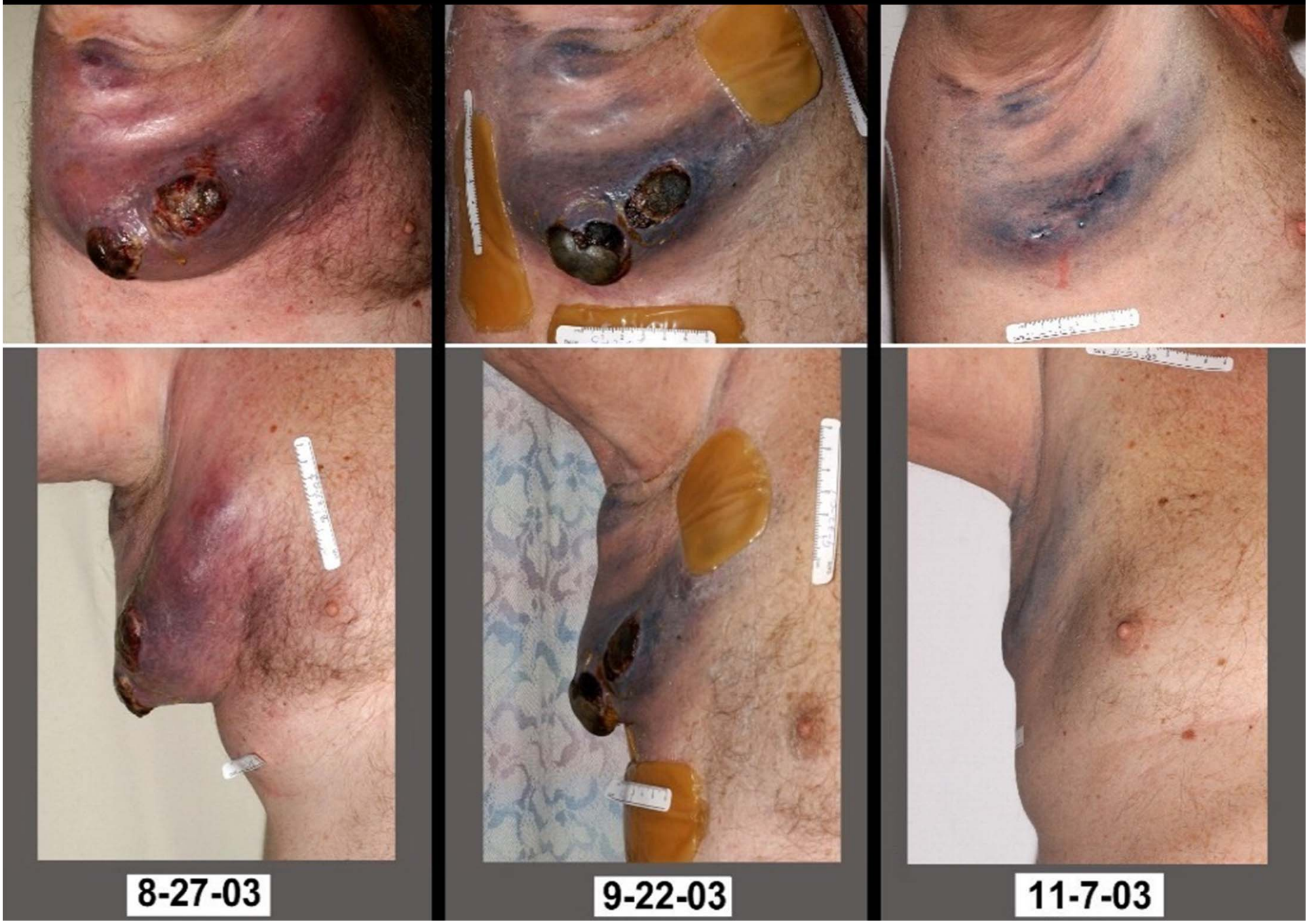
R.K.
(NMA)

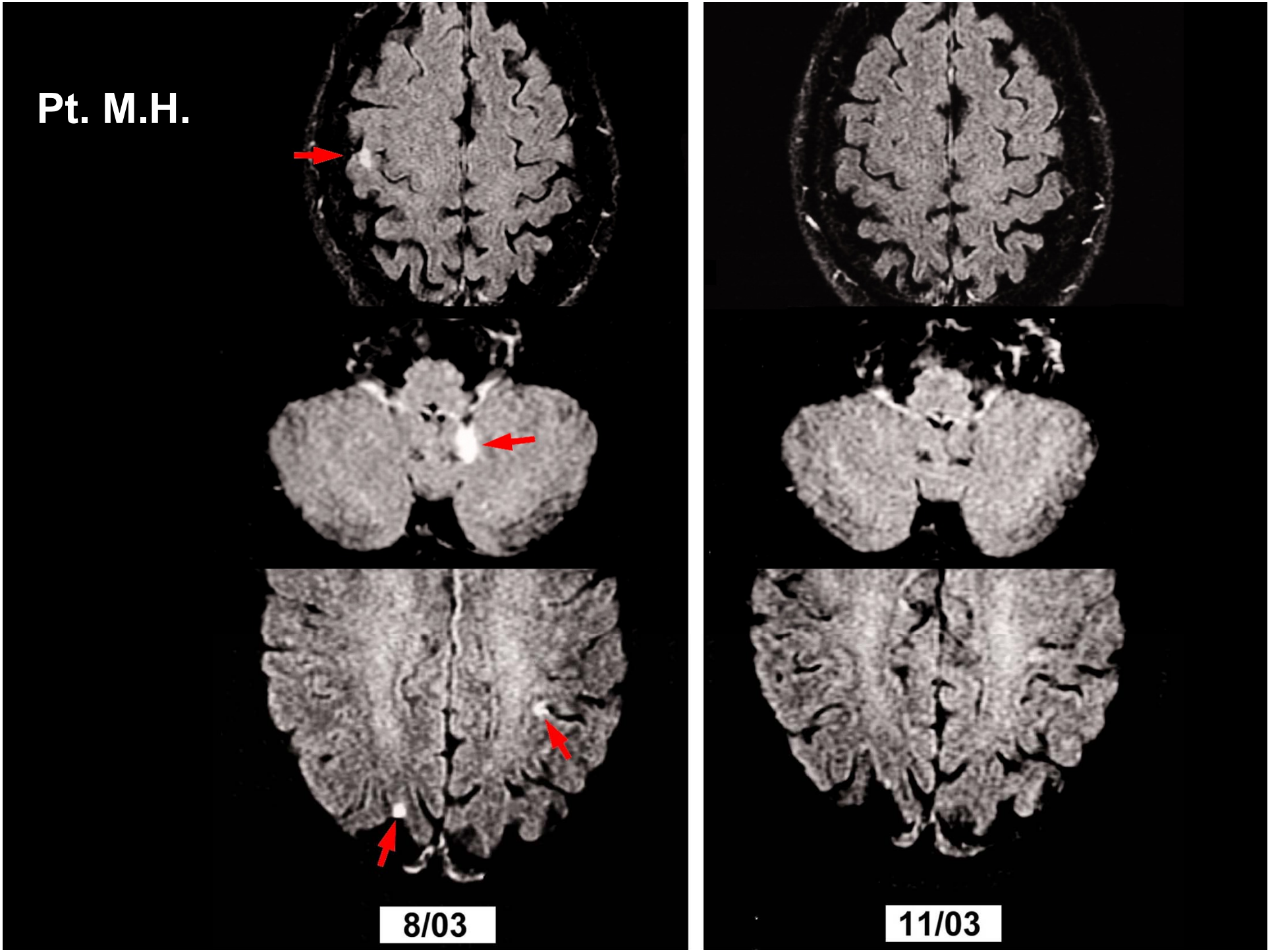


Pre-Treatment

14 Months

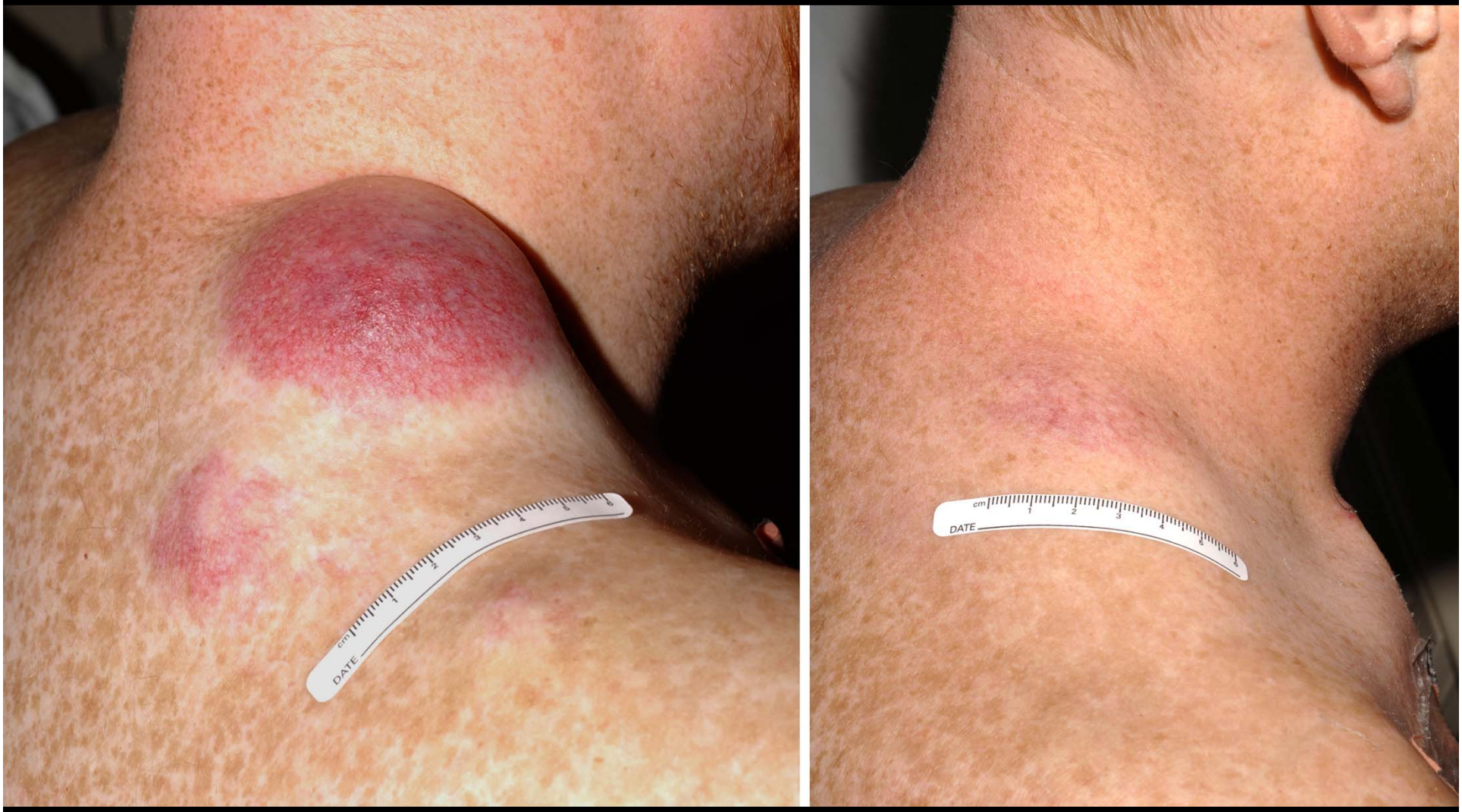
Pt. M.H.

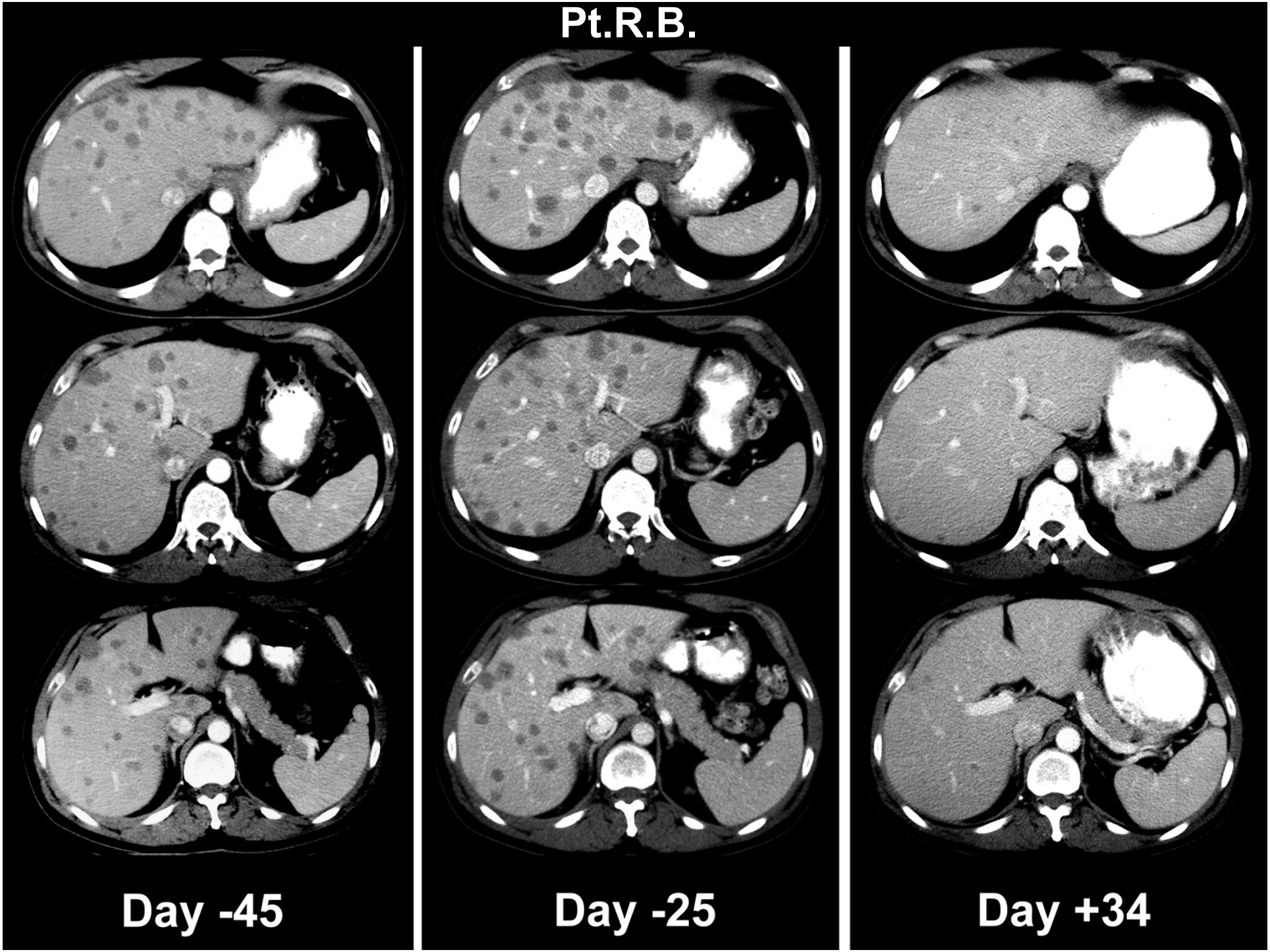


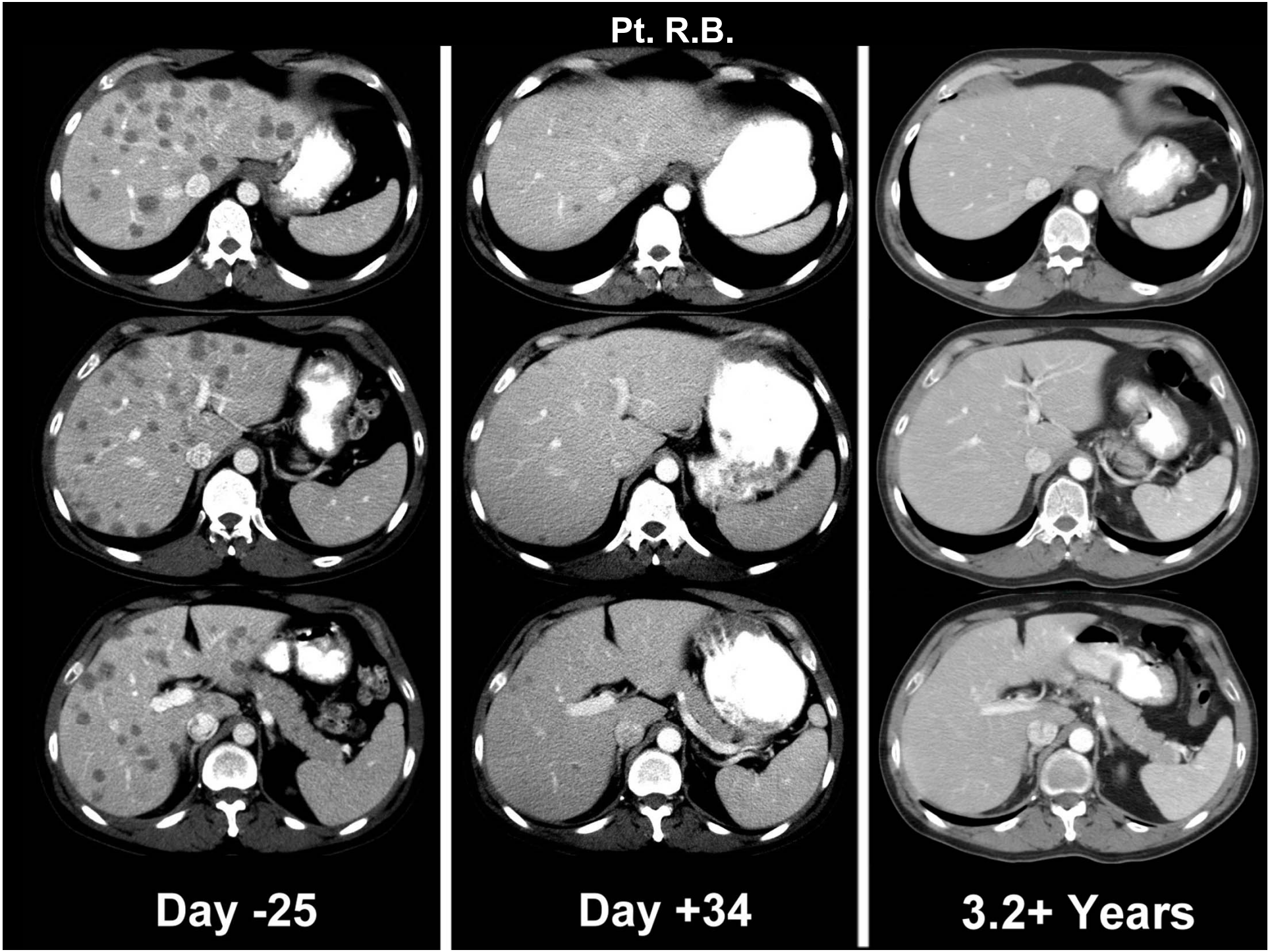

Pt. M.H.
8/03
11/03

C.K. (200cGy) Pre

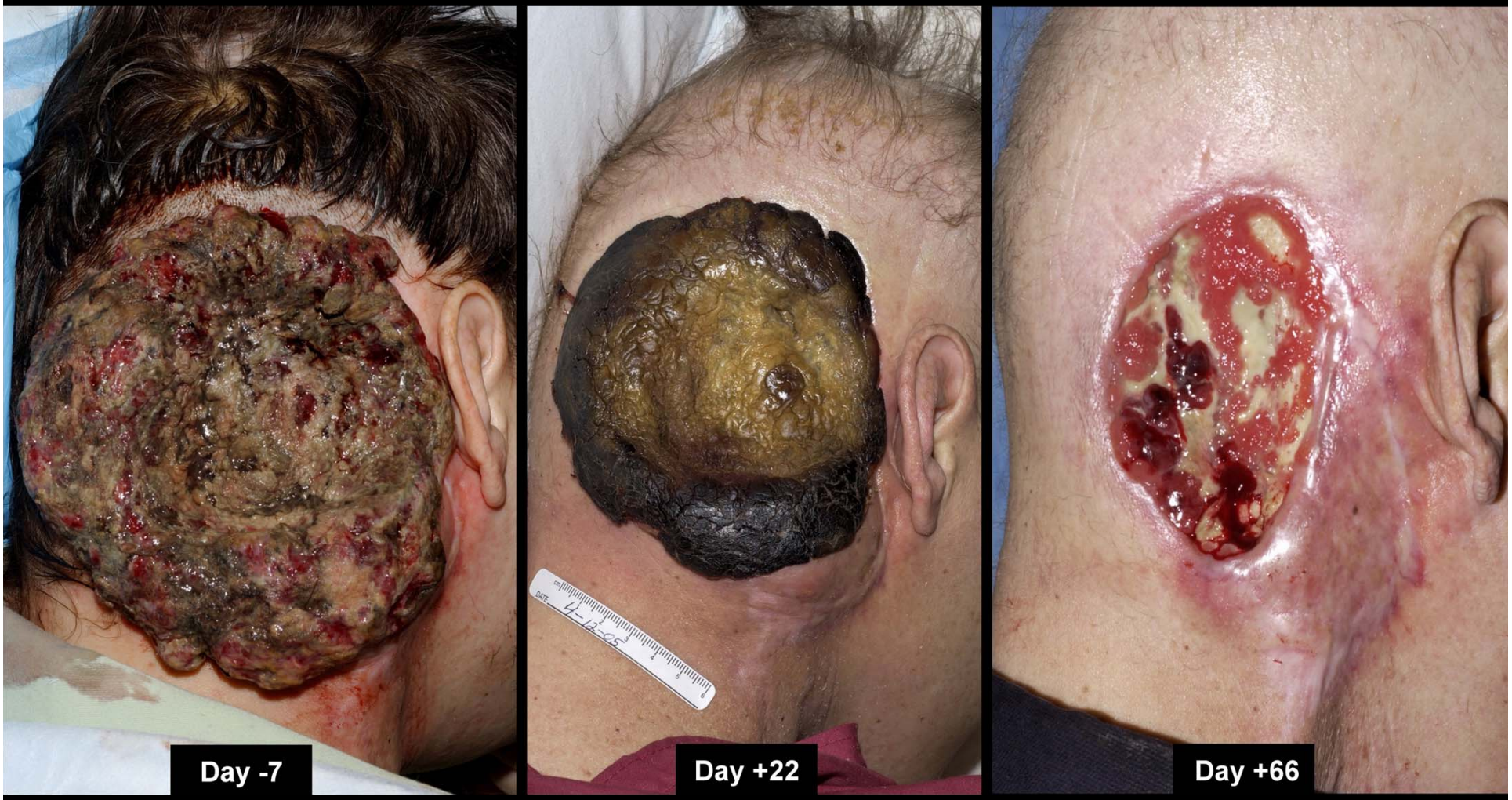
12 days

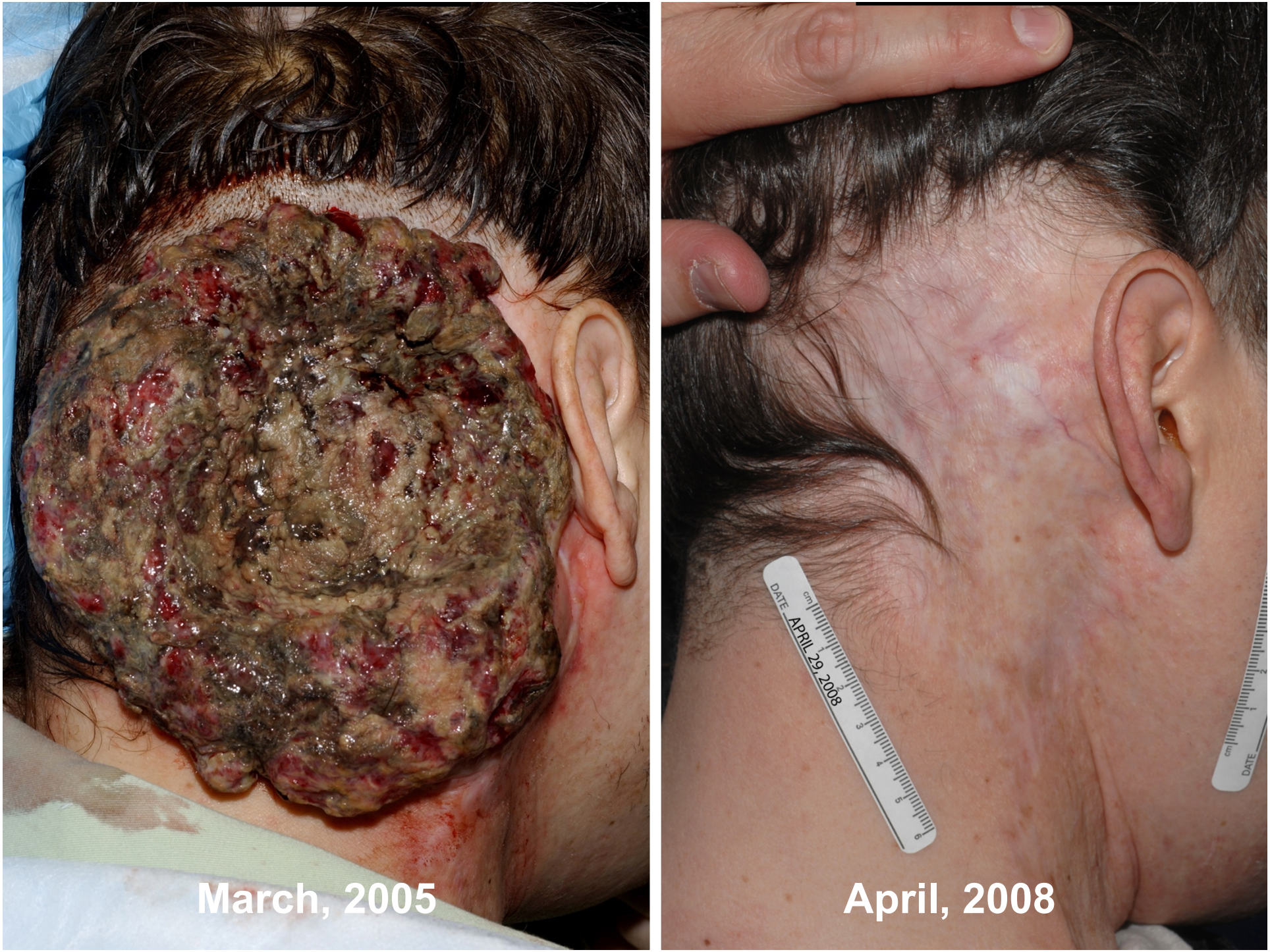



Pt.R.B.
Day -45
Day -25
Day +34


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

A.H.: N-M cell transfer




March, 2005
April, 2008
DATE APRIL 29, 2008

Hypothesis of Mechanism of Cancer Regression Following Cell Transfer

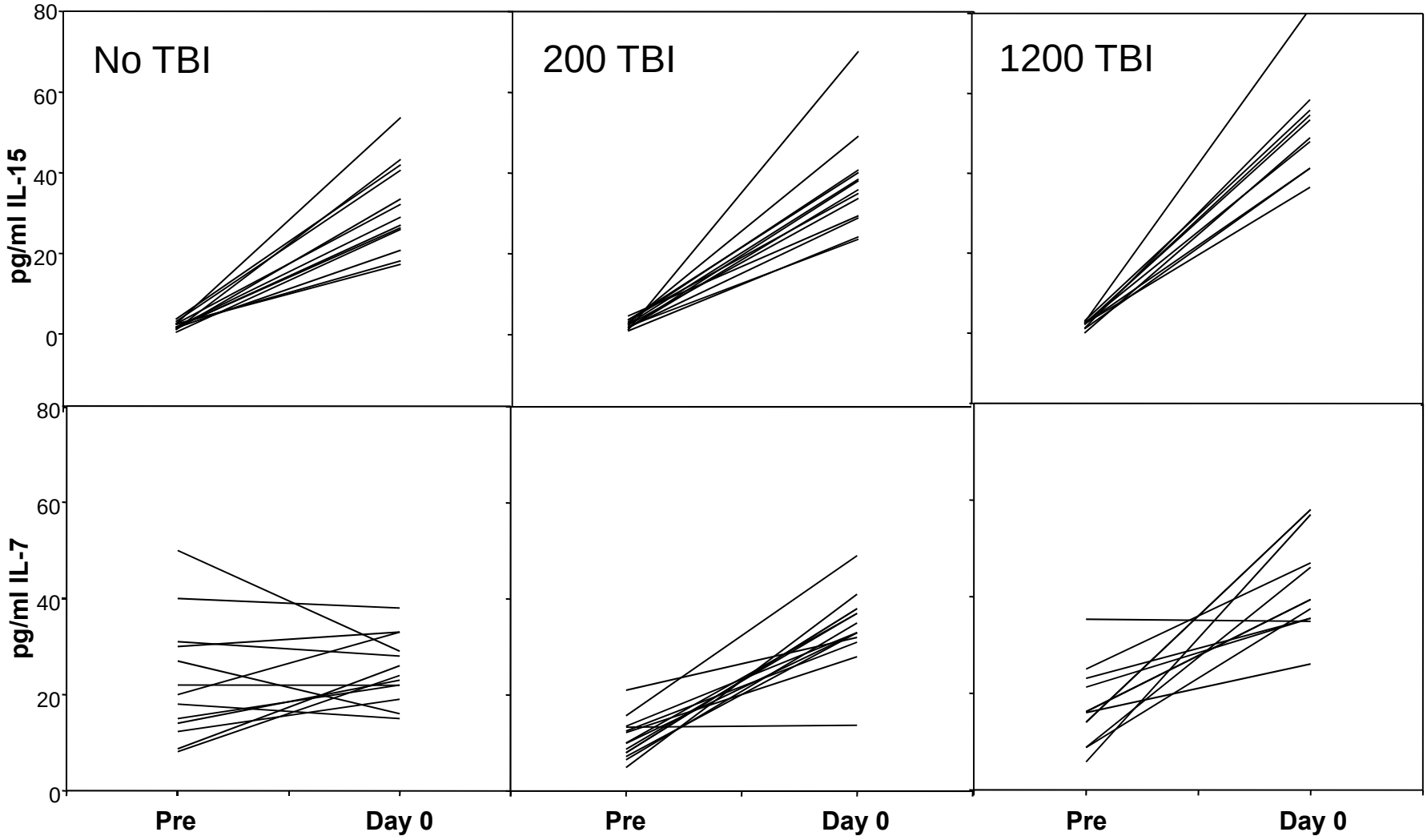
The lymphophenic environment

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

---

Persistence of the transferred cells

Telomere length

Expression of CD27 (following IL-2 withdrawal)

(Many other functional, phenotypic and gene array studies of the transferred cells and tumor showed no correlation with cancer regression.)

CONCLUSION

A highly avid T cell that recognizes a tumor antigen is capable of mediating the regression of large, vascularized, invasive metastatic melanoma in humans

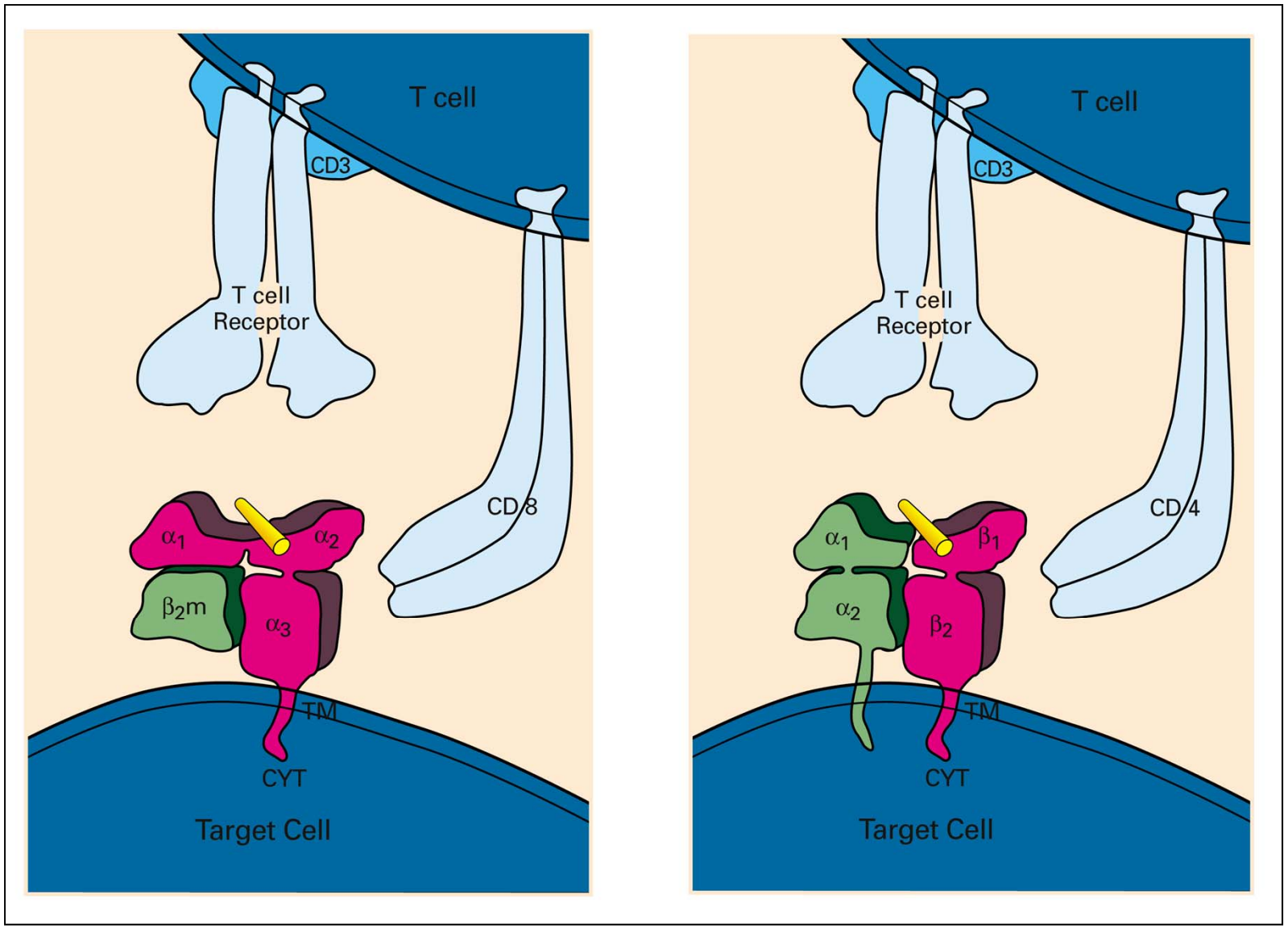
CHALLENGE

Determine ways to extend this approach to:

additional melanoma patients

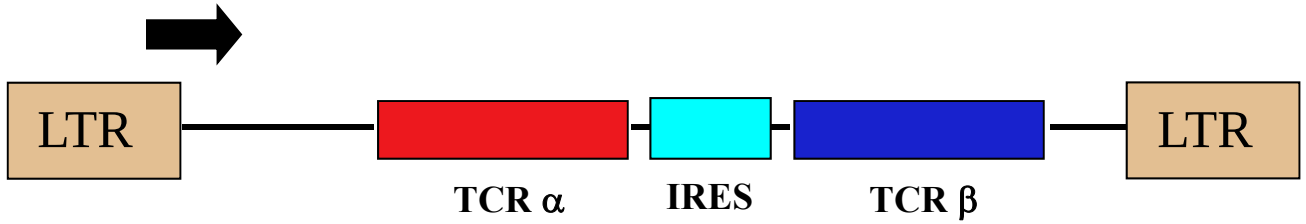
patients with common epithelial cancers

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



anti-Mart-1 retroviral vector

AIB
MSGV1martAIB



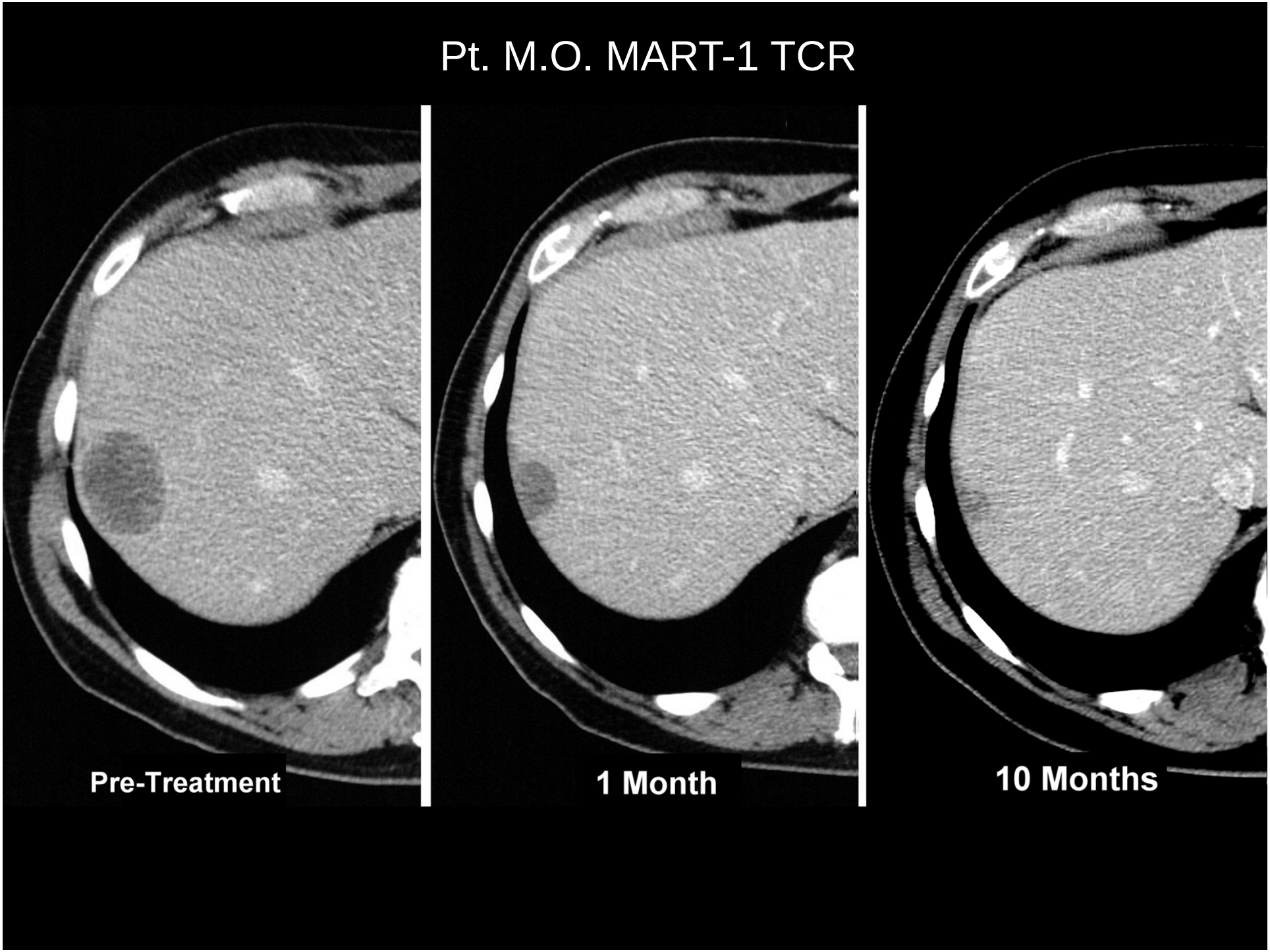
(Science 314:126,2006)

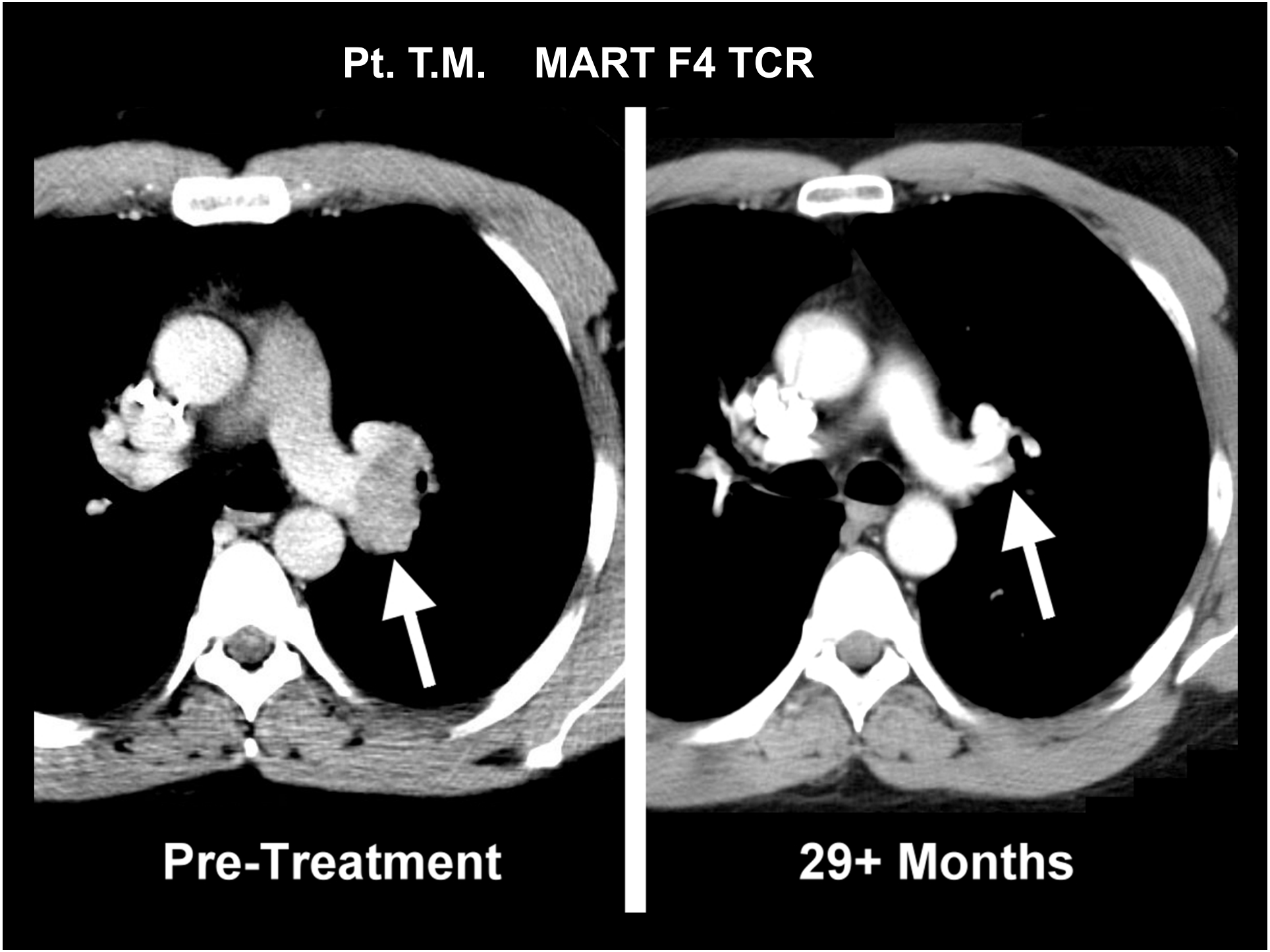
Treatment with MART-1 TCR transduced autologous lymphocytes

- **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)

Pt. M.O. MART-1 TCR




Pt. T.M. MART F4 TCR
Pre-Treatment
29+ Months

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

---

16 patients with metastatic melanoma (Science, 2006)

2 (13%) with objective regressions

(both disease free over two years later)

15 additional patients treated

2 further objective regressions

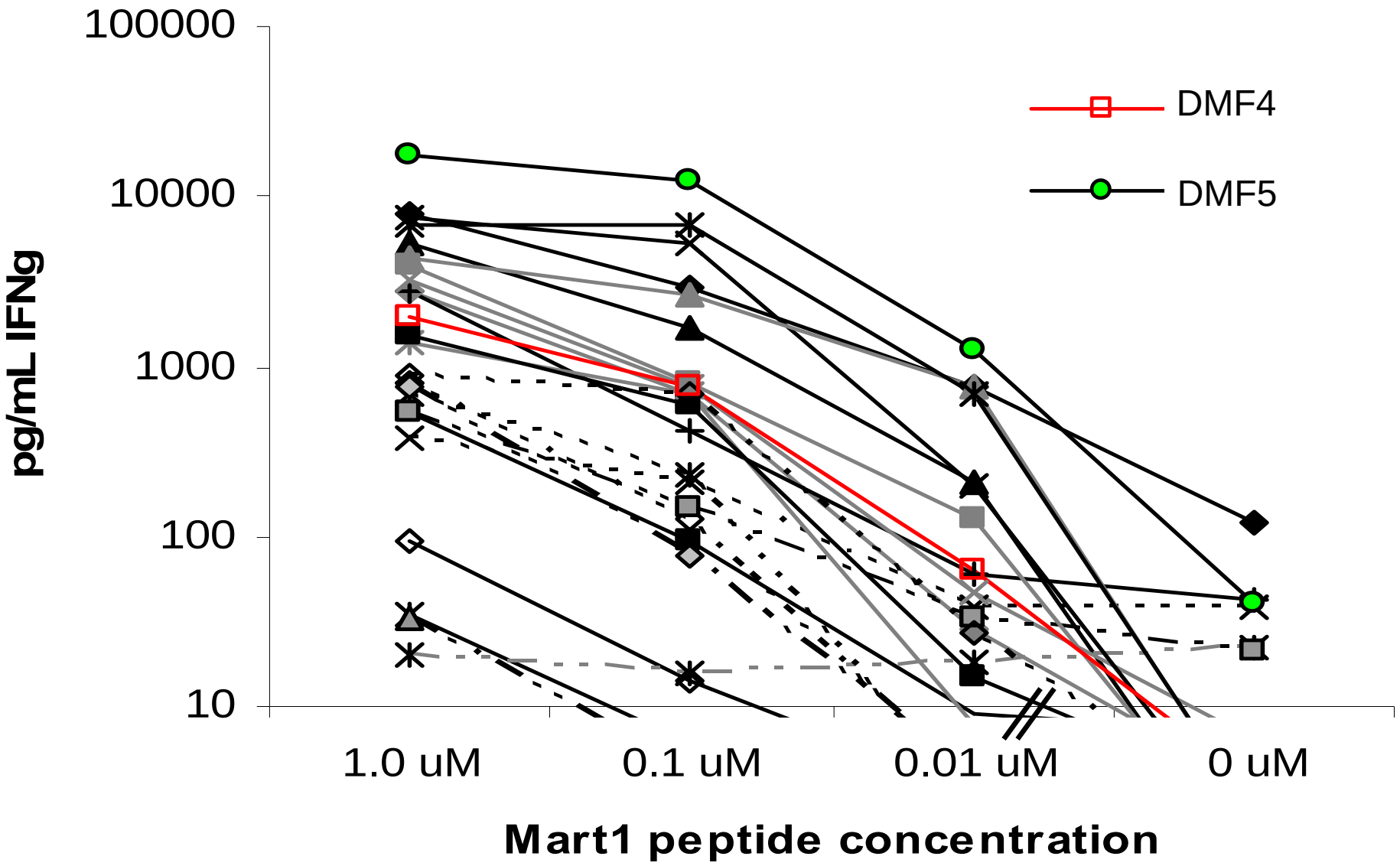
<u>Overall</u>: 4/31 (13%) objective regressions

Attempts to Improve Cell Transfer Therapy Using TCR Gene-Modified Cells

1. **Identify higher affinity TCRs**
    1. screen multiple anti-tumor TIL and PBL clones
    2. immunize HLA transgenic mice (bypass tolerance to human self peptides)
    3. mutagenesis of CDR2 and CDR3 regions

2. **Avoid mispairing of inserted and endogeneous alpha and beta chains**
    1. mouse constant region chimeric TCR
    2. substitute additional cystines to form disulfide bond

MART-1 TIL clones show diverse avidities to MART-1 peptide on target cells



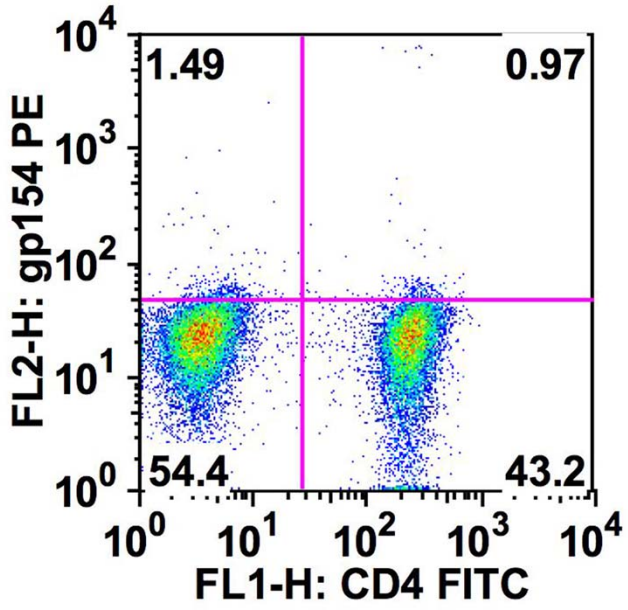
DMF4 and DMF5 MART1 and gp100(154) TCR retroviral constructs

DMF4 (previous MART1 clinical trial)

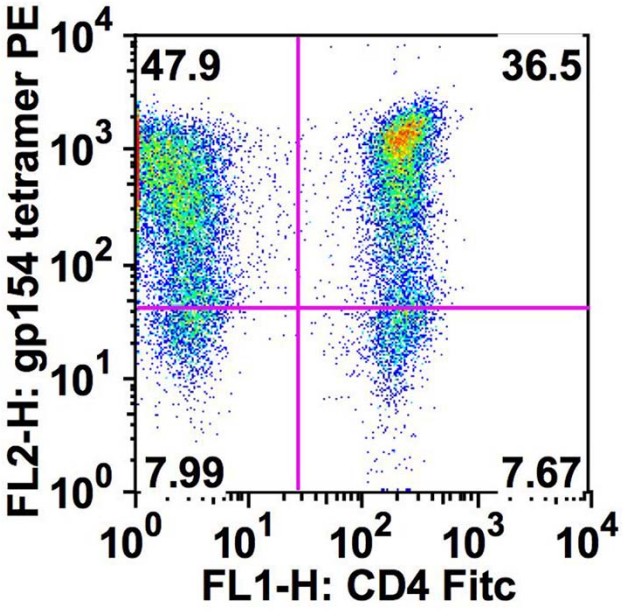
DMF5

gp100(154) (high-affinity murine TCR)

Efficient TCR gene transfer into PBL patient A.C.

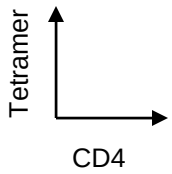


UnTd



gp100(154) TCR

84% Tet+



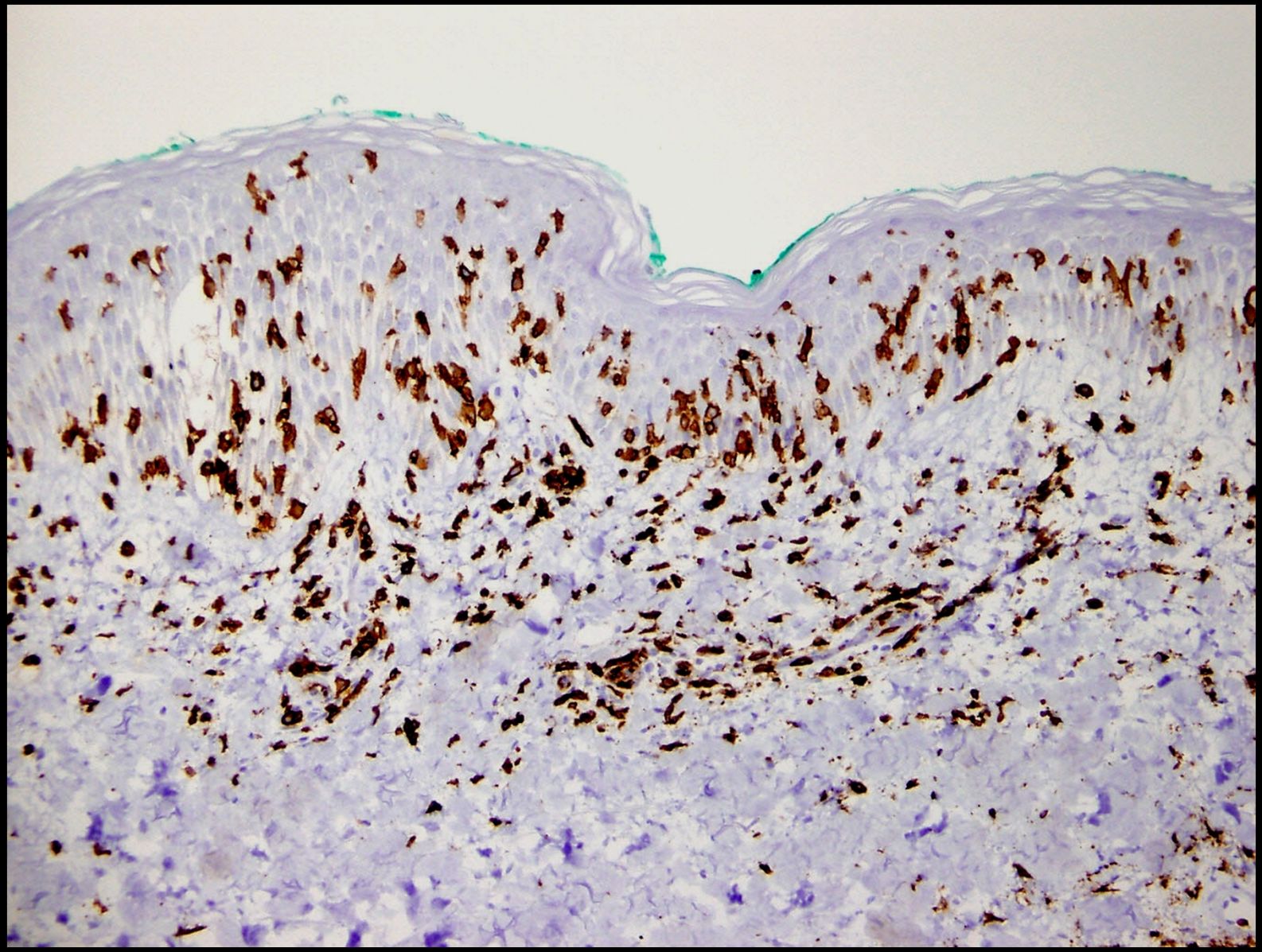
Analyzed 4 days post-transduction, (stim1)

Preliminary Evaluation of Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

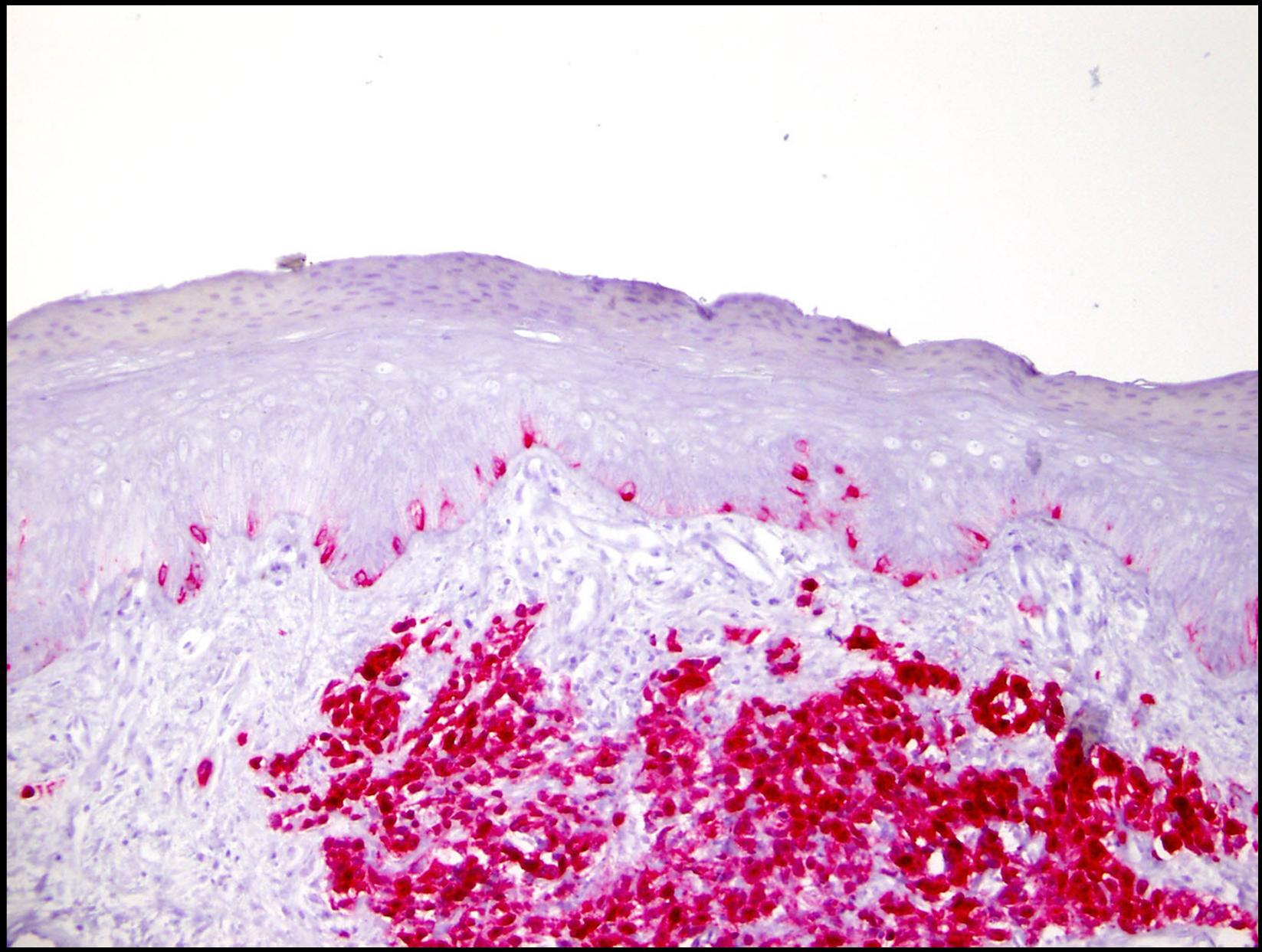
(first patient treated 7/13/07; followup as of 5/1/08)

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

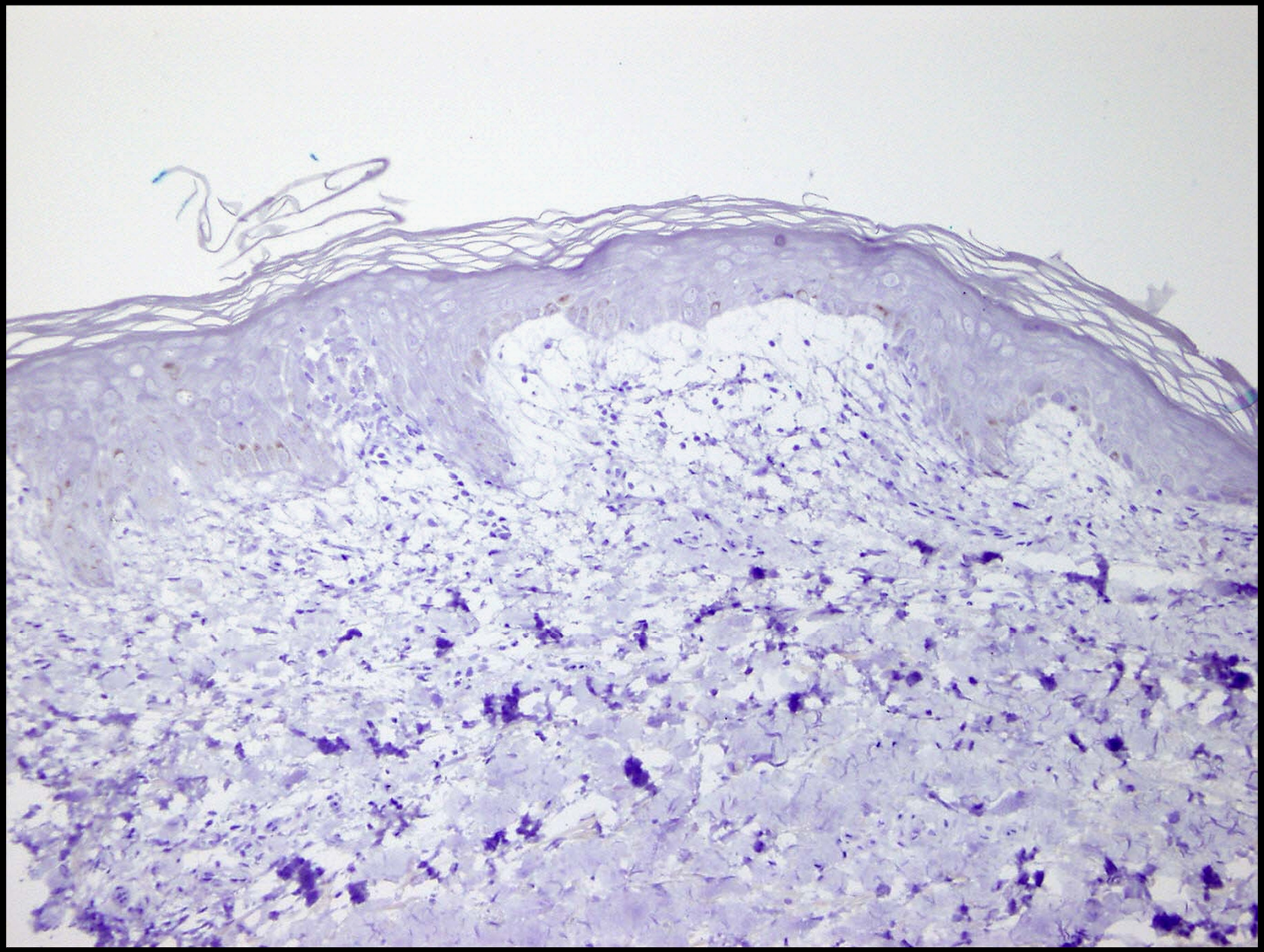
(All patients were refractory to prior treatment with IL-2.)



Day 5 post F5 TCR cell infusion (D. Tu)
Skin: CD8 positive cells

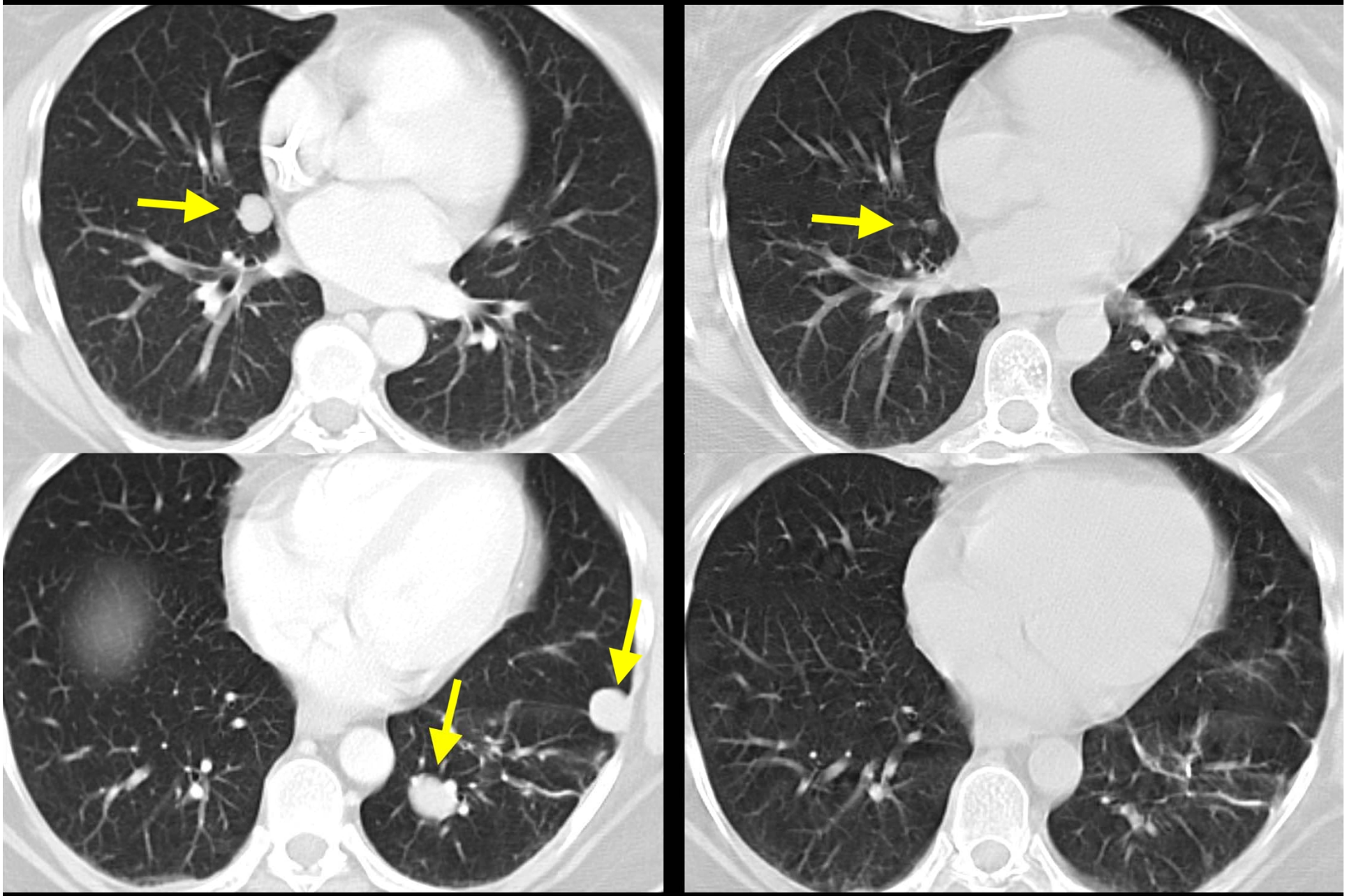


Skin / melanoma: Melan-A positive control slide



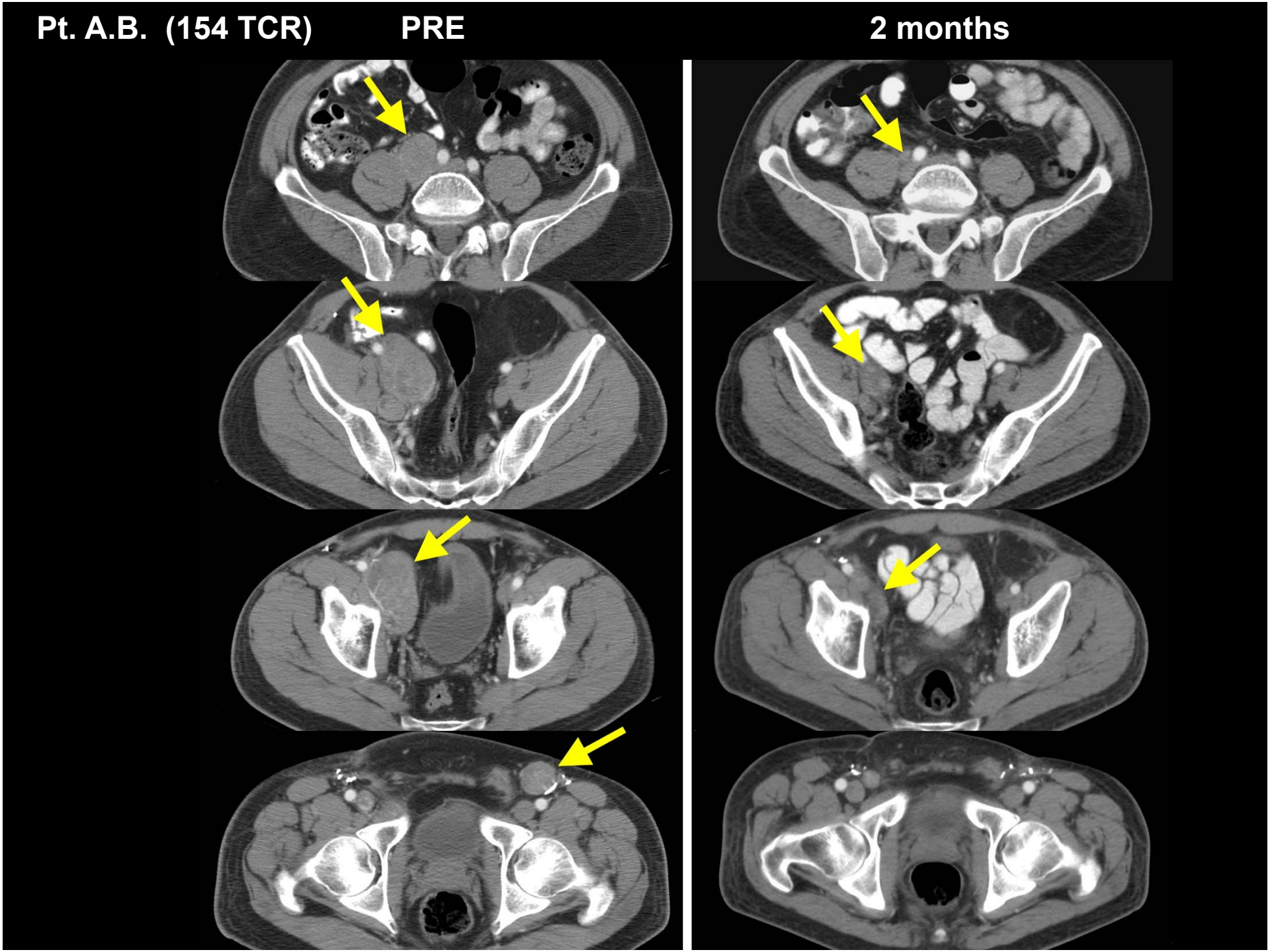
Day 5 post F5 TCR cell infusion (D. Tu)
Skin: Melan-A staining

D.T. DMF5 TCR



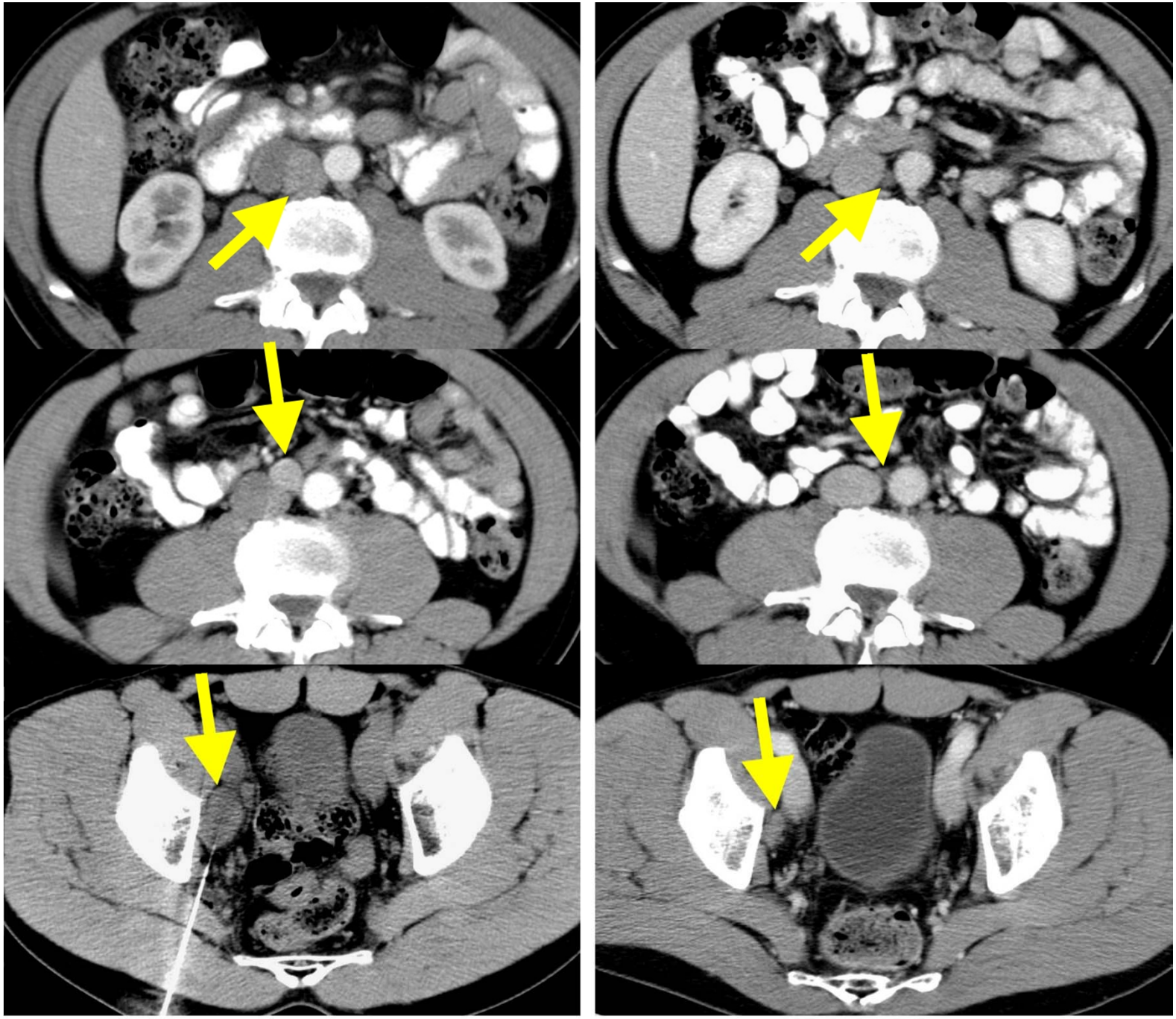
Pre-Treatment

5+ Months


Pt. A.B. (154 TCR)
PRE
2 months

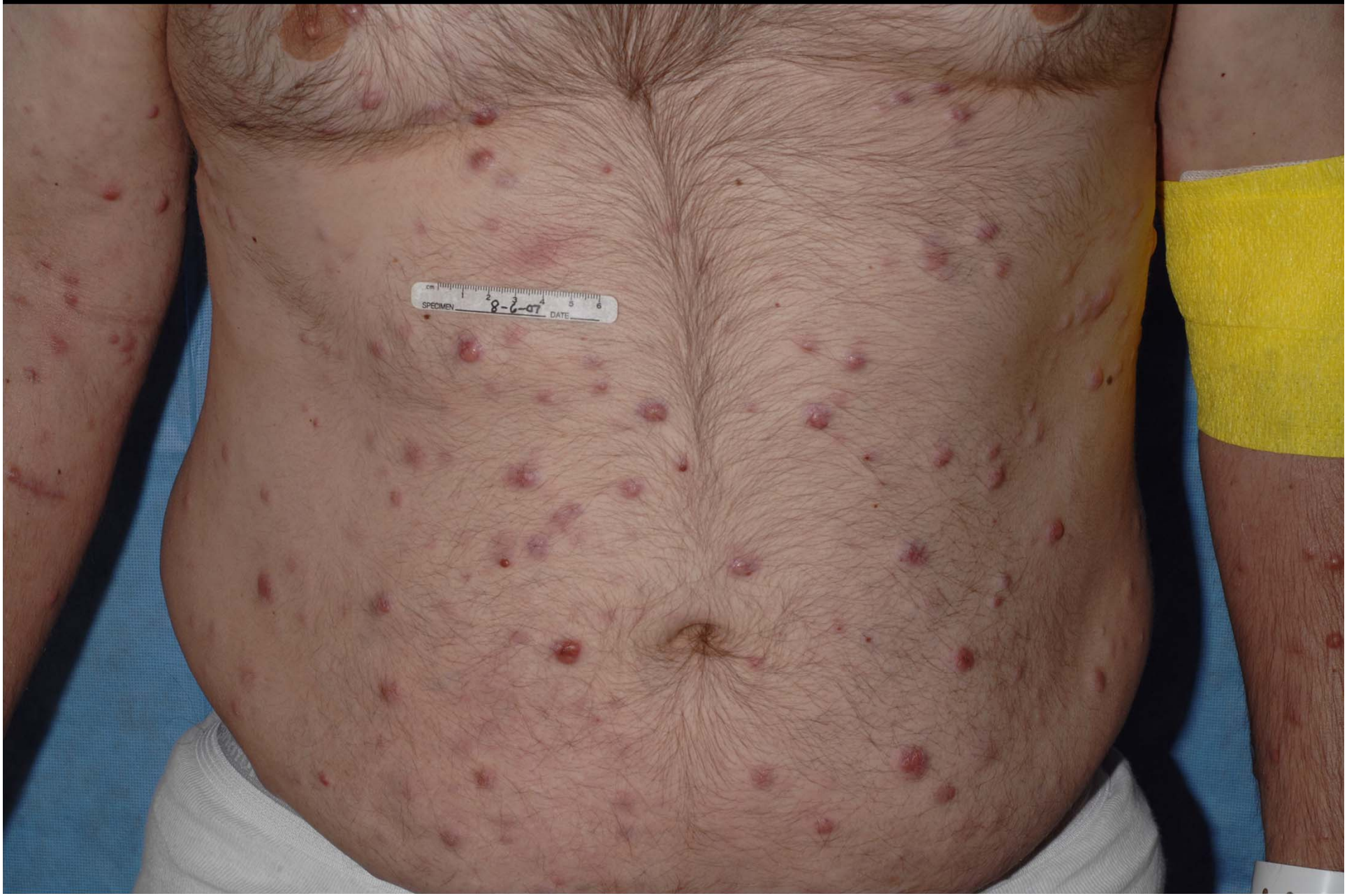
S.S.

154 TCR

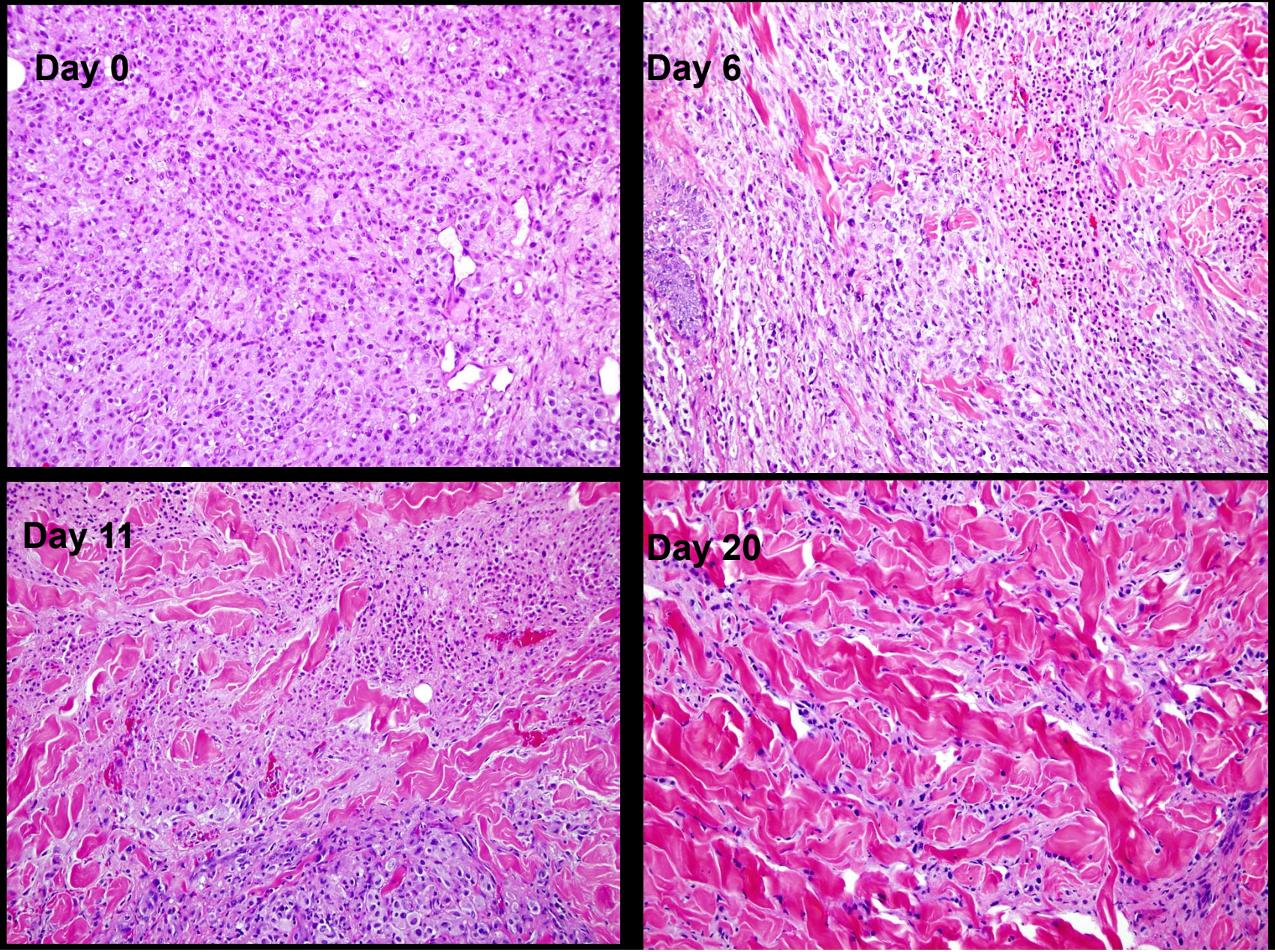


Pre-Treatment **4+ Months**

D.Th. F5 TCR Pretreatment

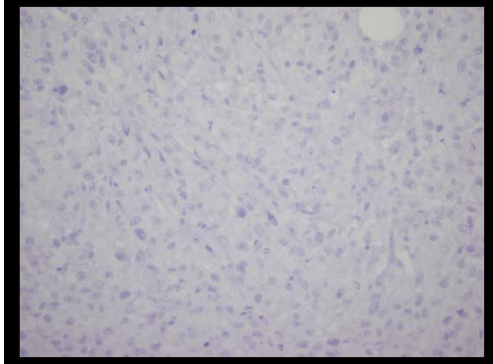


Sequential tumor biopsies (D.Th.): MART TCR

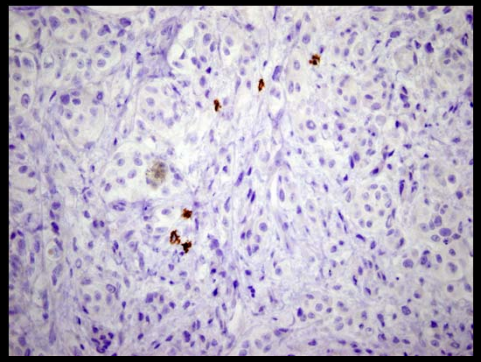


Sequential tumor biopsies (D.Th.): MART TCR (CD8, 40x)

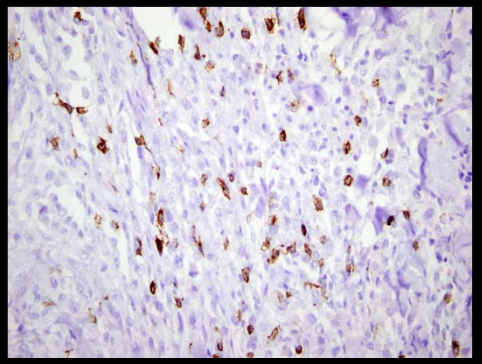
day 0



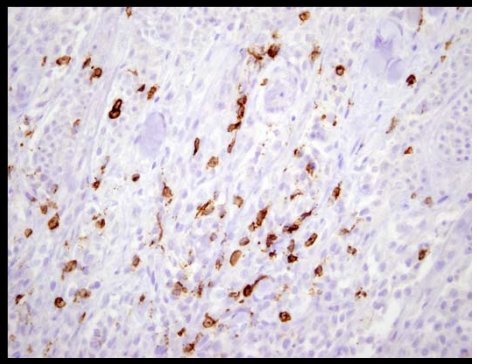
day 5



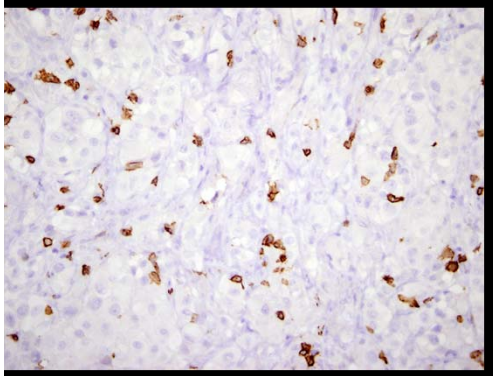
day 6



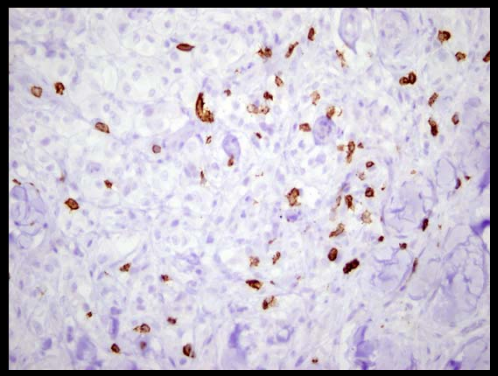
day 9



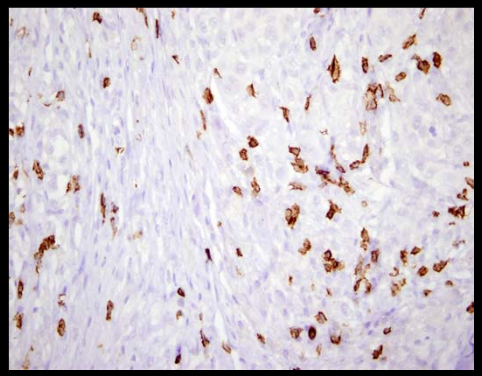
12 day



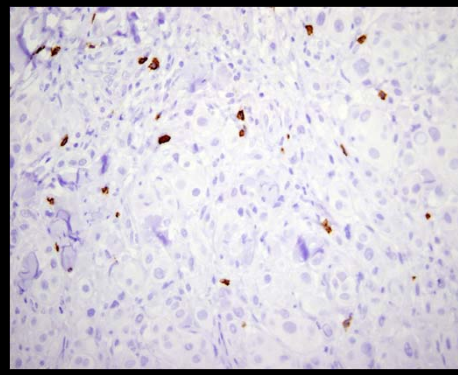
day 16



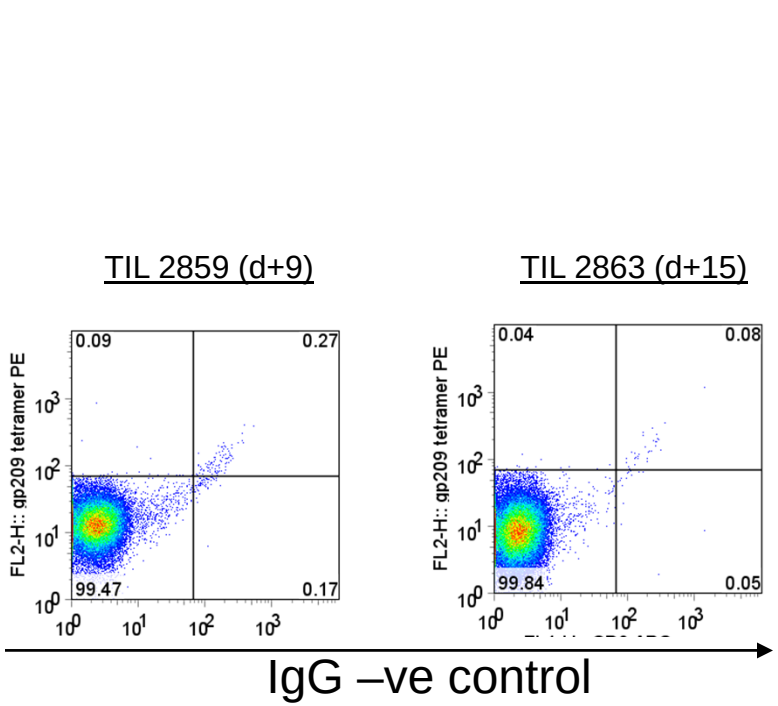
day 19

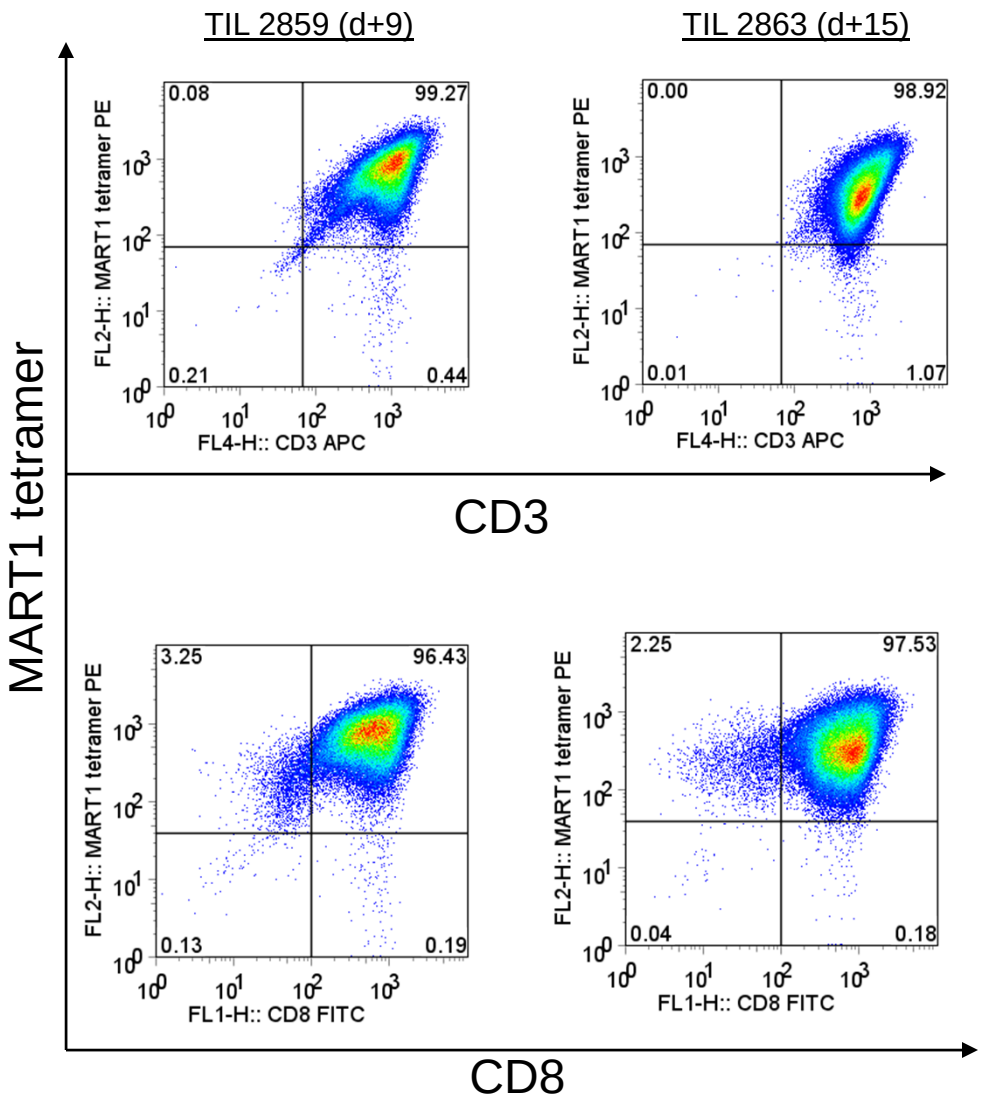


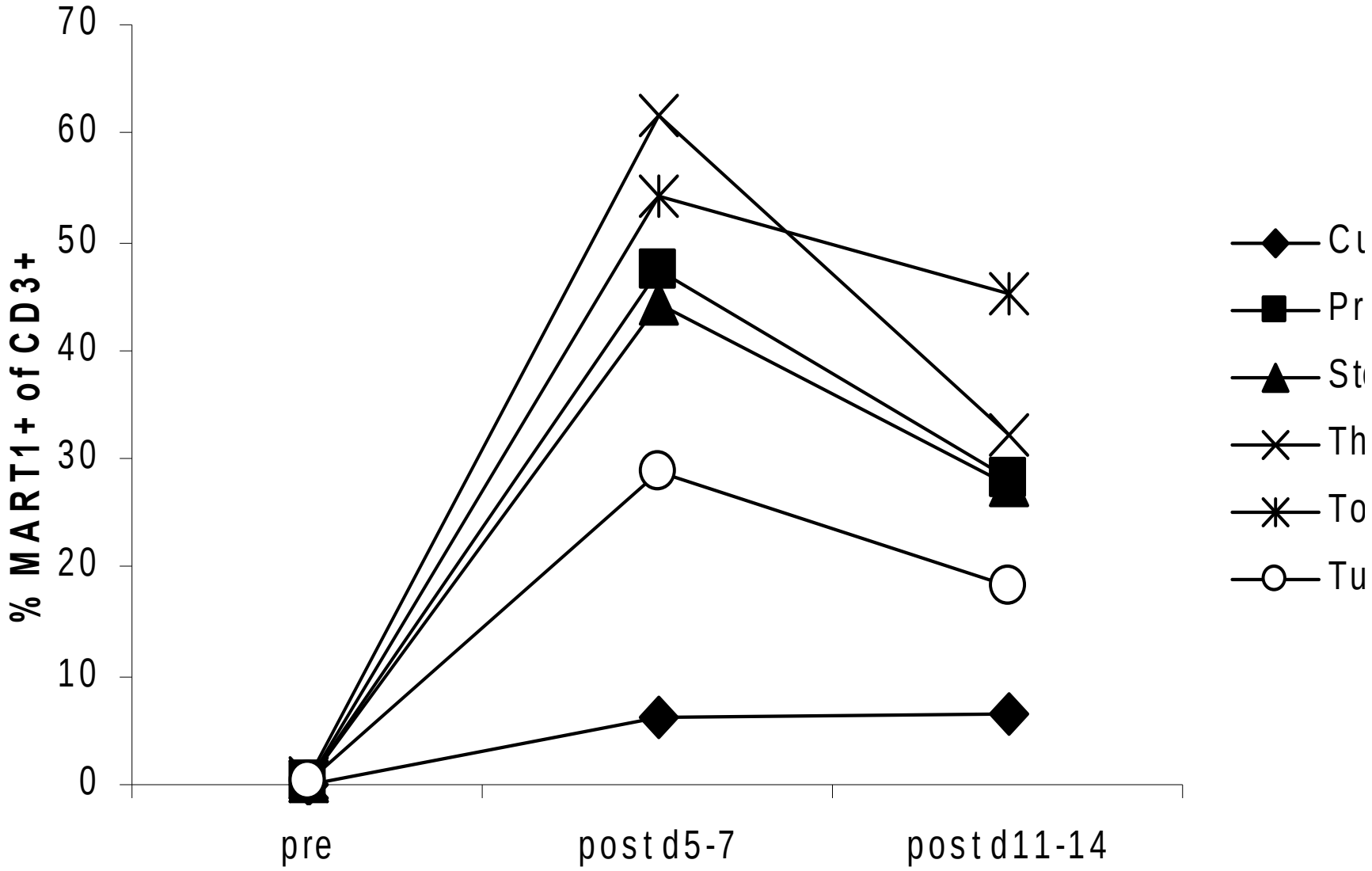
day 26



D.Th. TIL (d40+d46 in-vitro) from 2 tumor biopsies taken d+9, +15 post F5 TCR treatment.



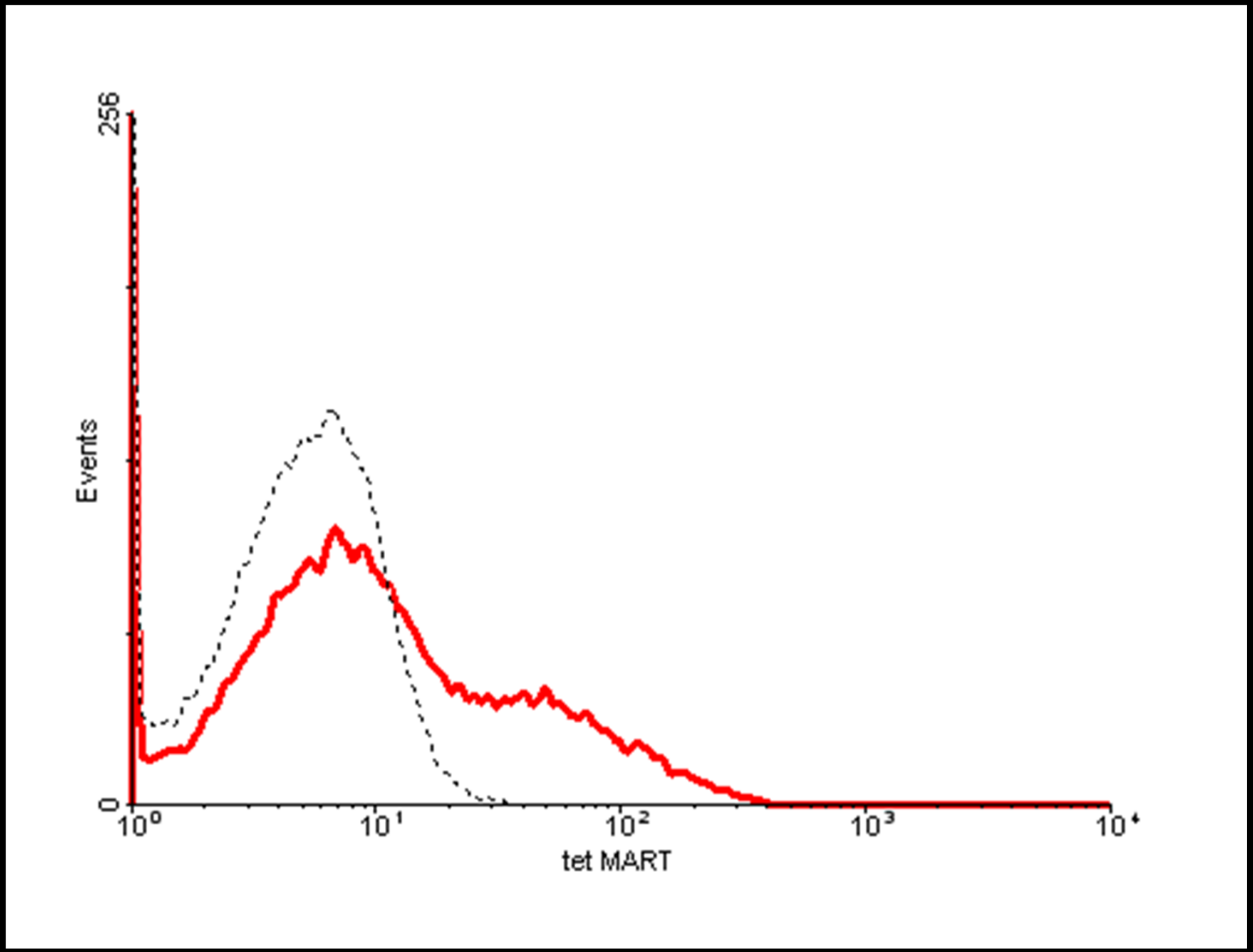



Persistence MART1+ T-cells in patient PBL
% MART1+ of CD3+
70
60
50
40
30
20
10
0
pre
post d5-7
post d11-14
Cu
Pr
St
Th
To
Tu

Attempts to Improve Cell Transfer Therapy Using TCR Gene-Modified Cells

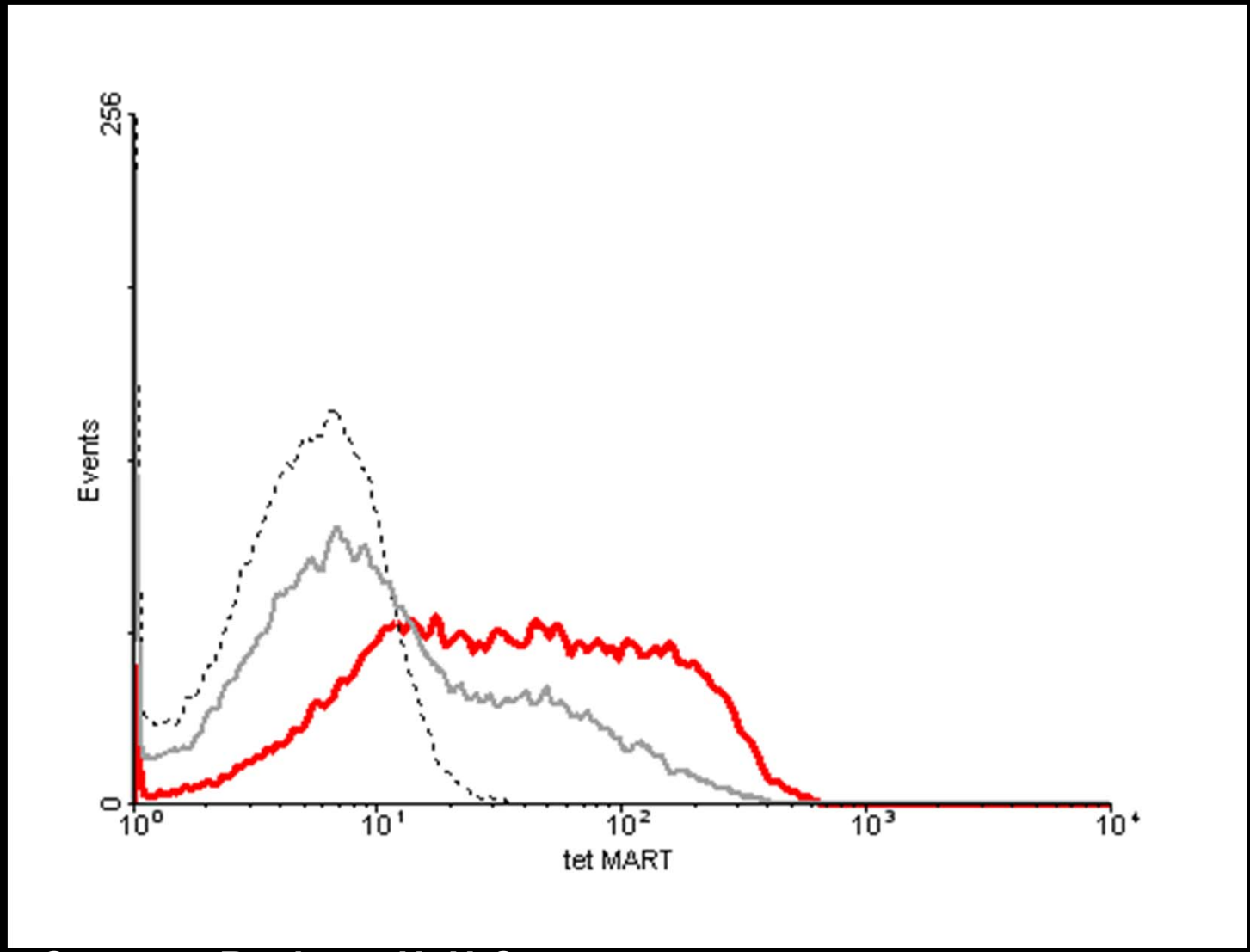
1. Identify higher affinity TCRs
    1. screen multiple anti-tumor TIL and PBL clones
    2. immunize HLA transgenic mice (bypass tolerance to human self peptides)
    3. mutagenesis of CDR2 and CDR3 regions
2. Avoid mispairing of inserted endogenous alpha and beta chains
    1. substitute mouse constant regions for human constant regions
    2. substitute additional cystines in the alpha and beta chains to form a second disulfide bond

Human Constant Regions



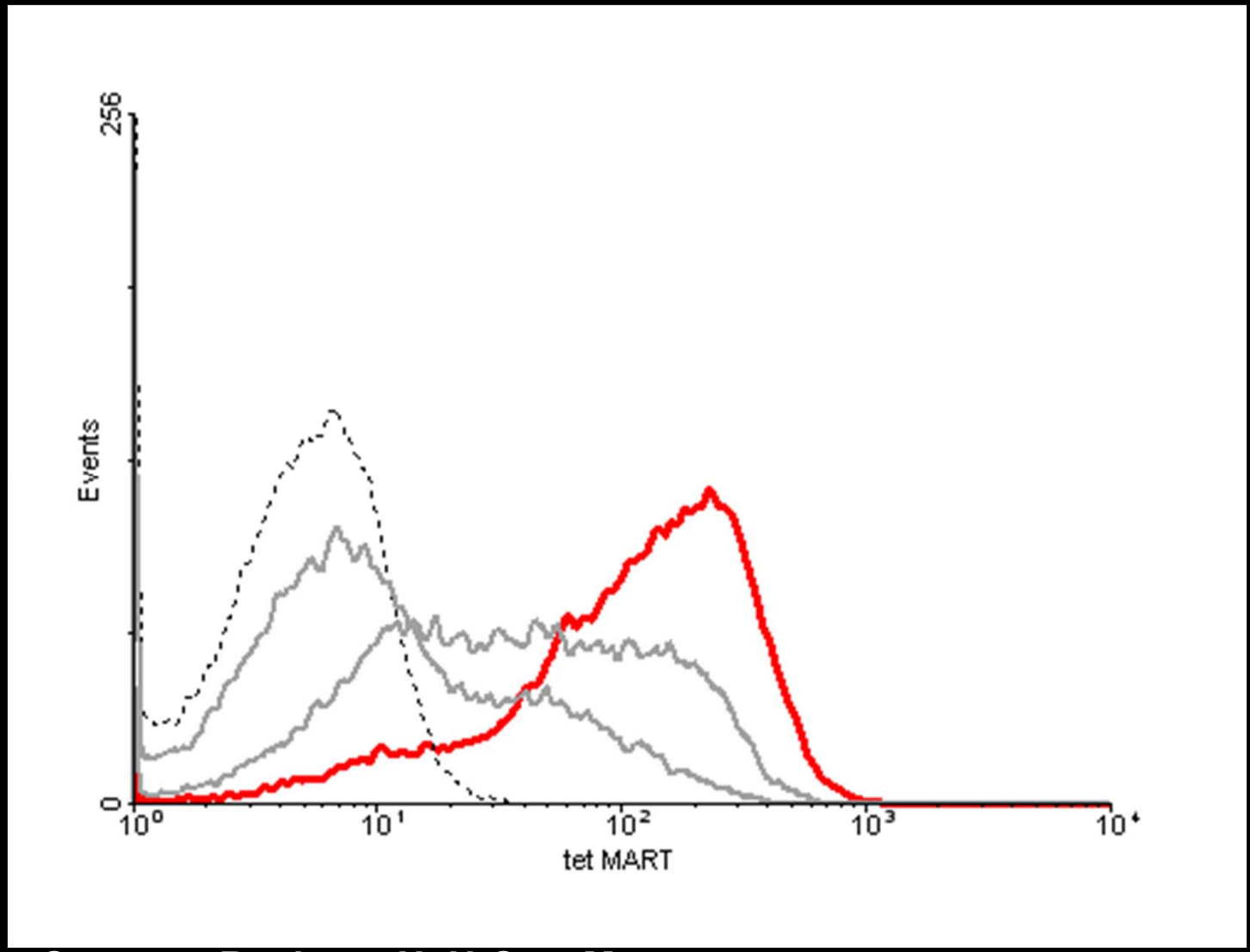
Constant Regions: H

Human CR+ Cysteines



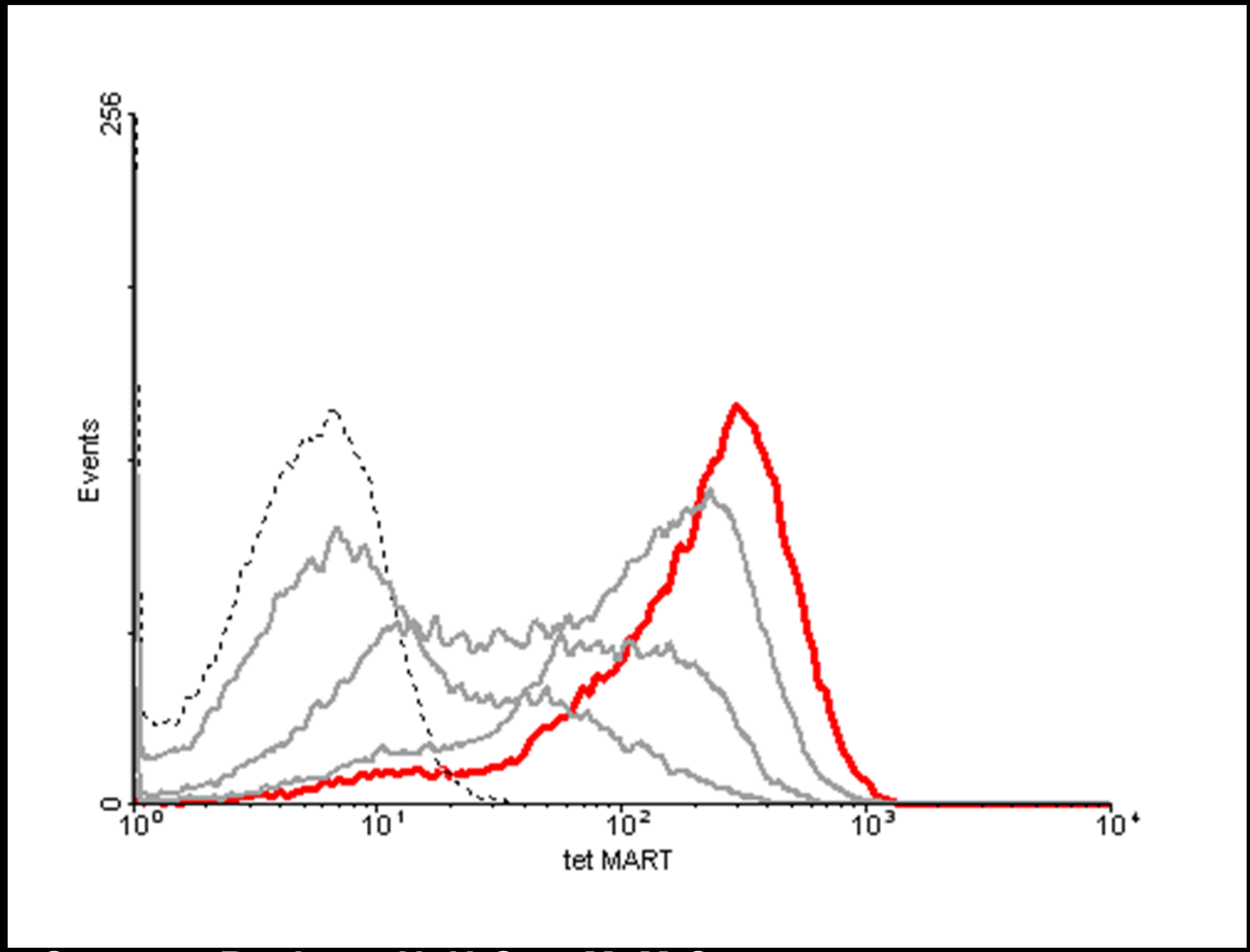
Constant Regions: H<H-Cys

Mouse Constant Regions



Constant Regions: H<H-Cys<M

Mouse CR + Cysteines



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

CONCLUSION

A highly avid T cell that recognizes a tumor antigen is capable of mediating the regression of large, vascularized, invasive metastatic melanoma in humans

CHALLENGE

Determine ways to extend this approach to:

additional melanoma patients

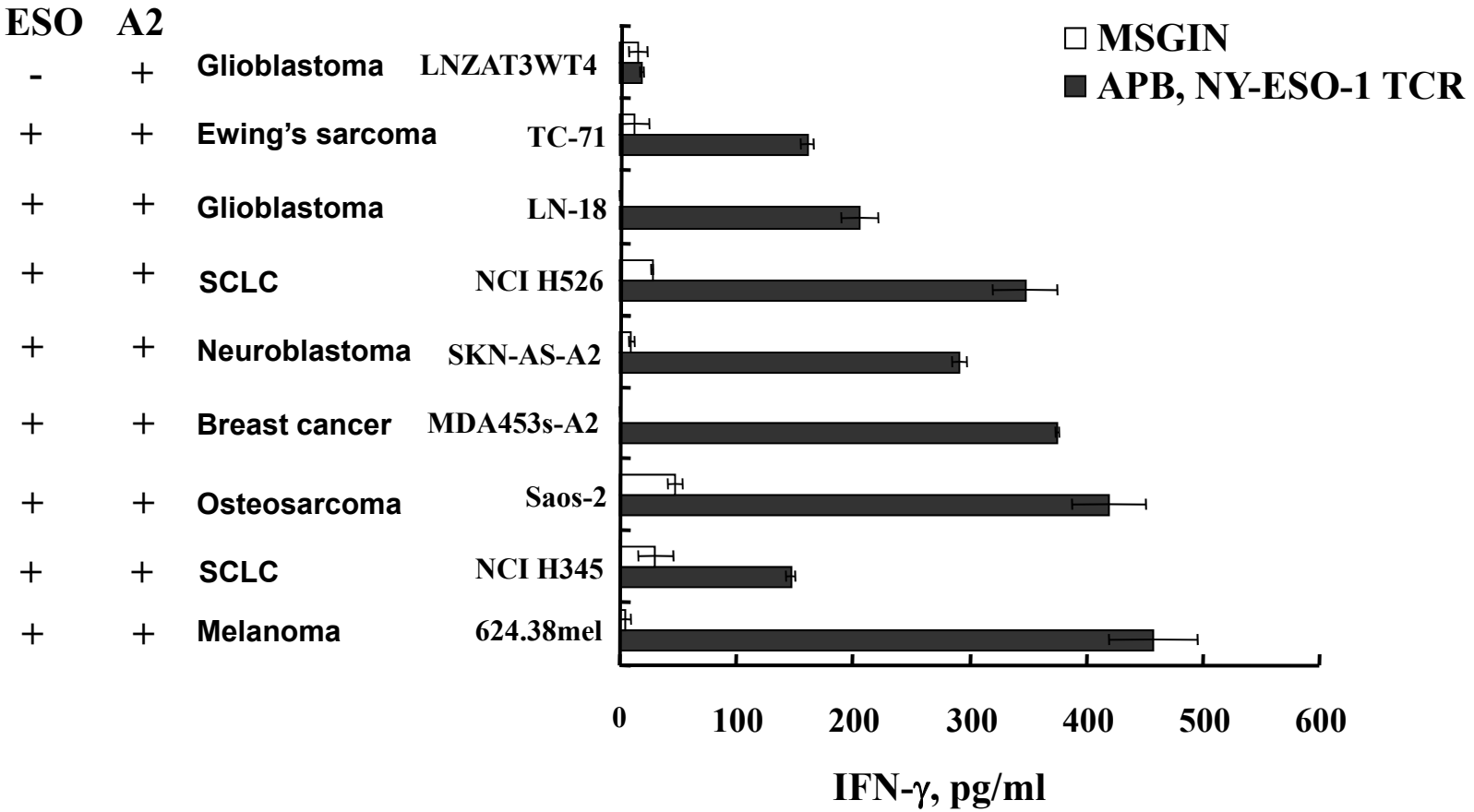
patients with common epithelial cancers

NY-ESO-1 CANCER ANTIGEN

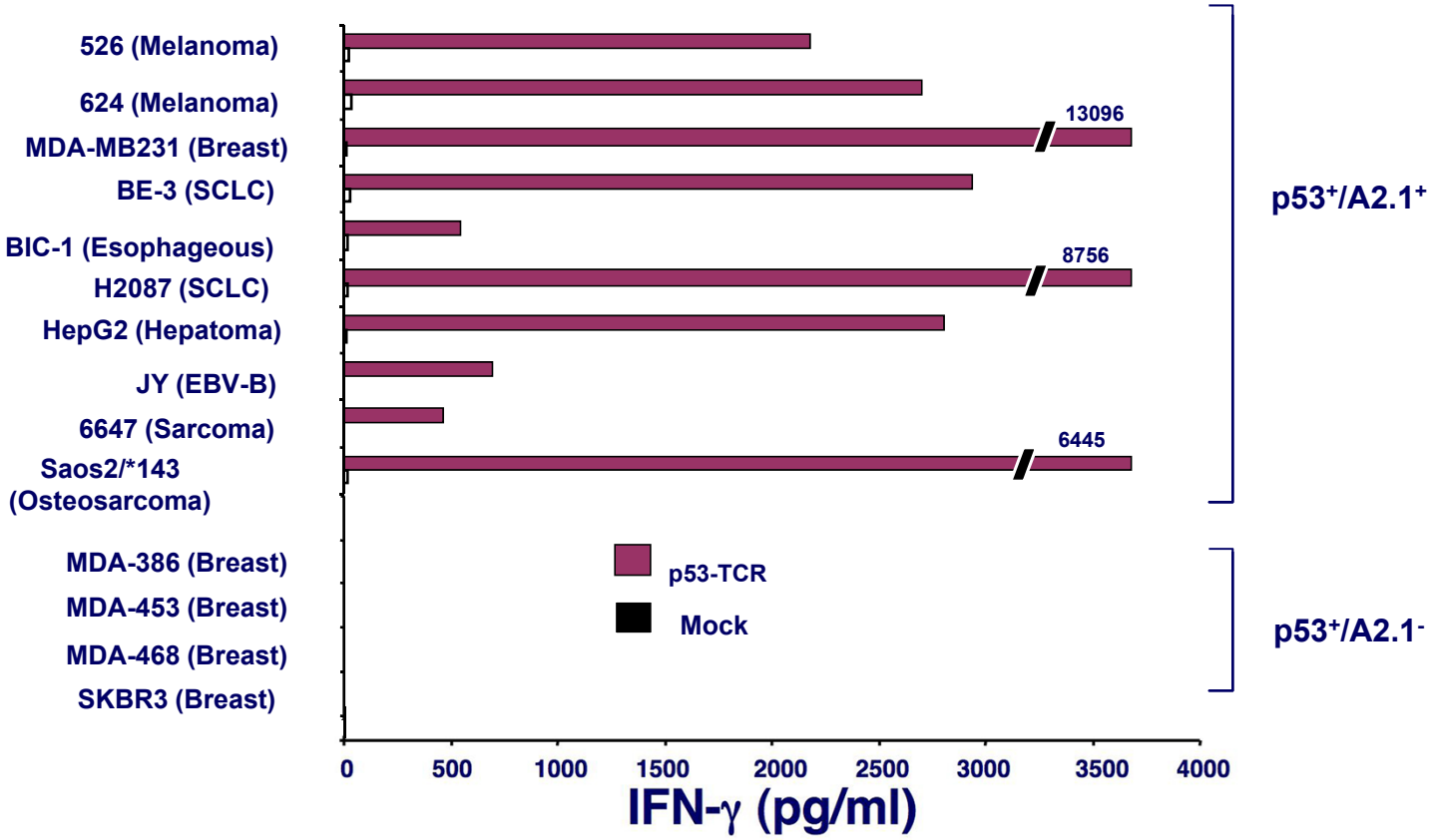
No expression on adult human tissues except for testis

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

Recognition of Non-melanoma Tumors by NY-ESO-1 TCR Transduced PBL

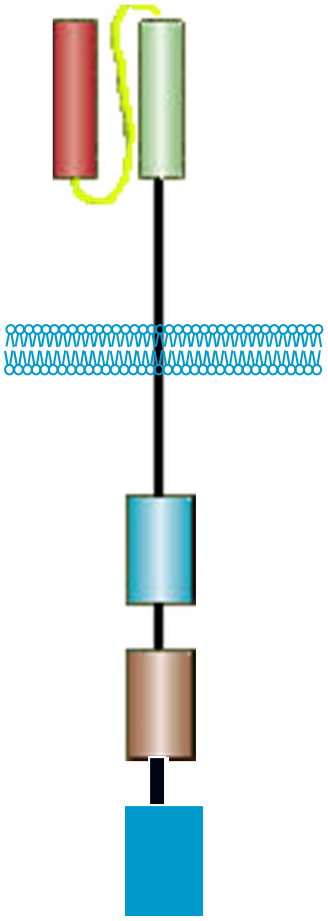


Extended TCR gene transfer to common cancers: p53 TCR transduced PBL



Cohen, et.al., J. Immunol., 2006

Construction of a chimeric TCR: the combining site of the Her2/neu antibody fused to intracellular signalling chains



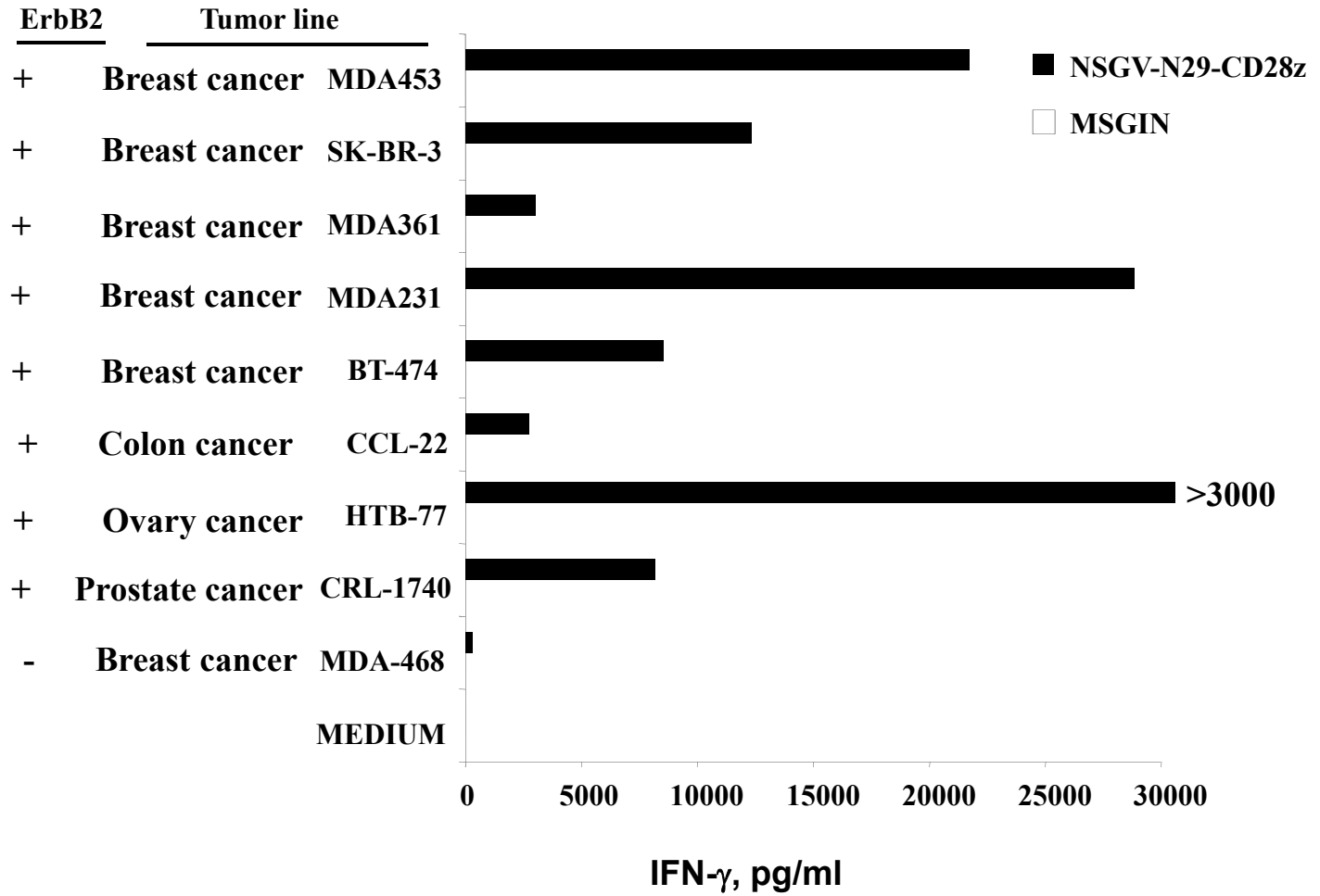
Single chain construct of the antigen binding sites of the Her2/neu antibody

CD8 hinge and transmembrane region

CD28, 41BB, zeta cytoplasmic signalling domain

This construct provides the T cell with the recognition of the Her2/neu antibody.

Recognition of Non-melanoma Tumors by ErbB2 Chimeric Receptor Transduced PBL



Studies to Improve the Genetic Modification of T Cells to Improve Cancer Immunotherapy

Bcl-2	reduce apoptosis
telomerase	prolong in vivo proliferation
IL-2, IL-15	cells provide their own cytokine
41BBL, CD80	cells provide their own co-stimulation
combine antigen receptors	avoid antigen escape
dominant negative TGF-β	eliminate lymphocyte inhibitors

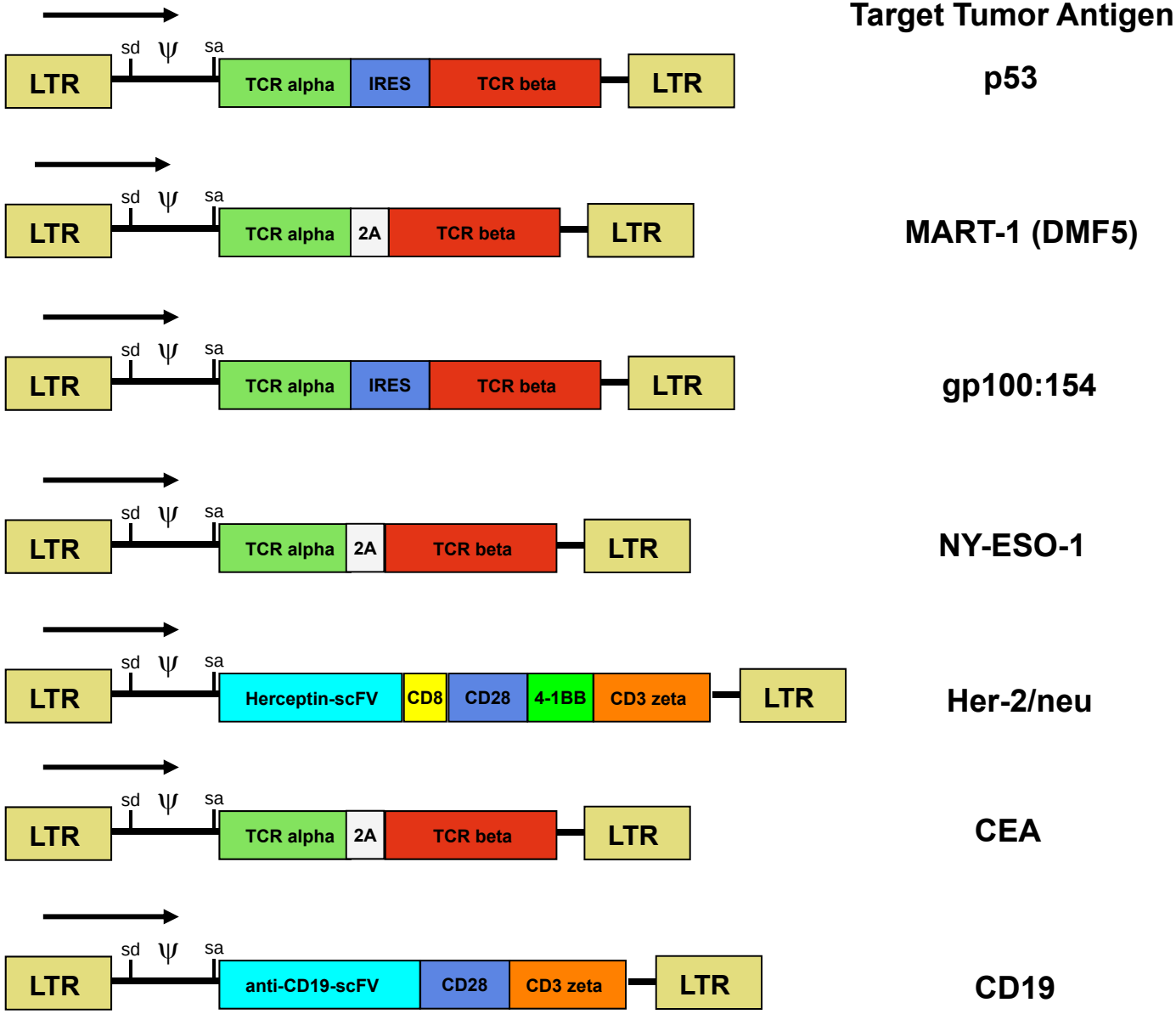
Conclusion

Cell transfer immunotherapy can mediate the regression of metastatic cancer in humans.

Autologous peripheral lymphocytes genetically modified to express anti-tumor T cell receptors can mediate cancer regression in vivo.

The ability to genetically modify human T cells opens possibilities to improve the effectiveness of cell transfer immunotherapy and extend it to patients with common epithelial cancers.

Anti-tumor antigen receptor containing retroviral vectors



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

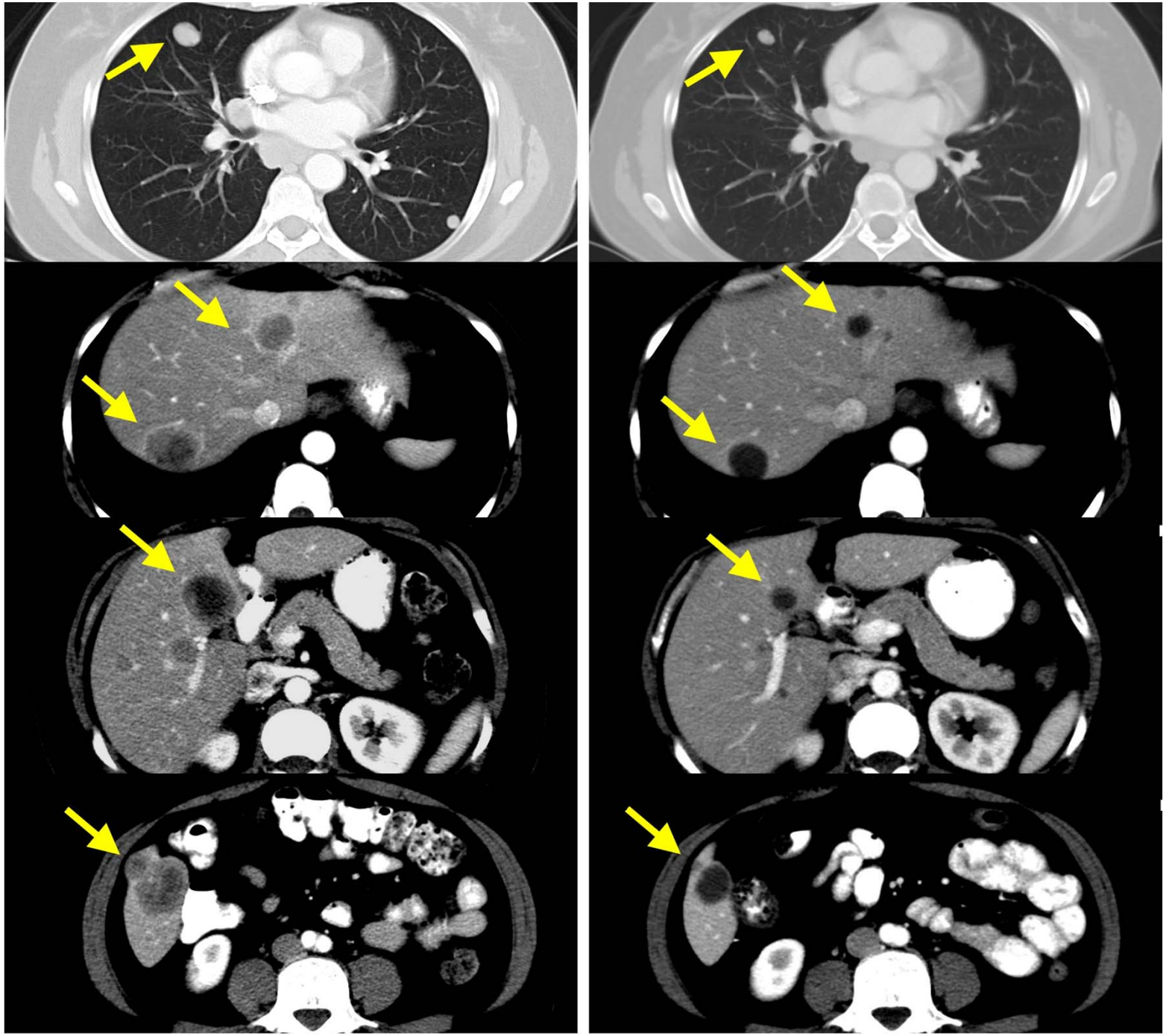
Determine ways to extend this approach to:

additional melanoma patients

patients with common epithelial cancers

J.W.

154 TCR



Pre-Treatment **2 Months**

CONCLUSION

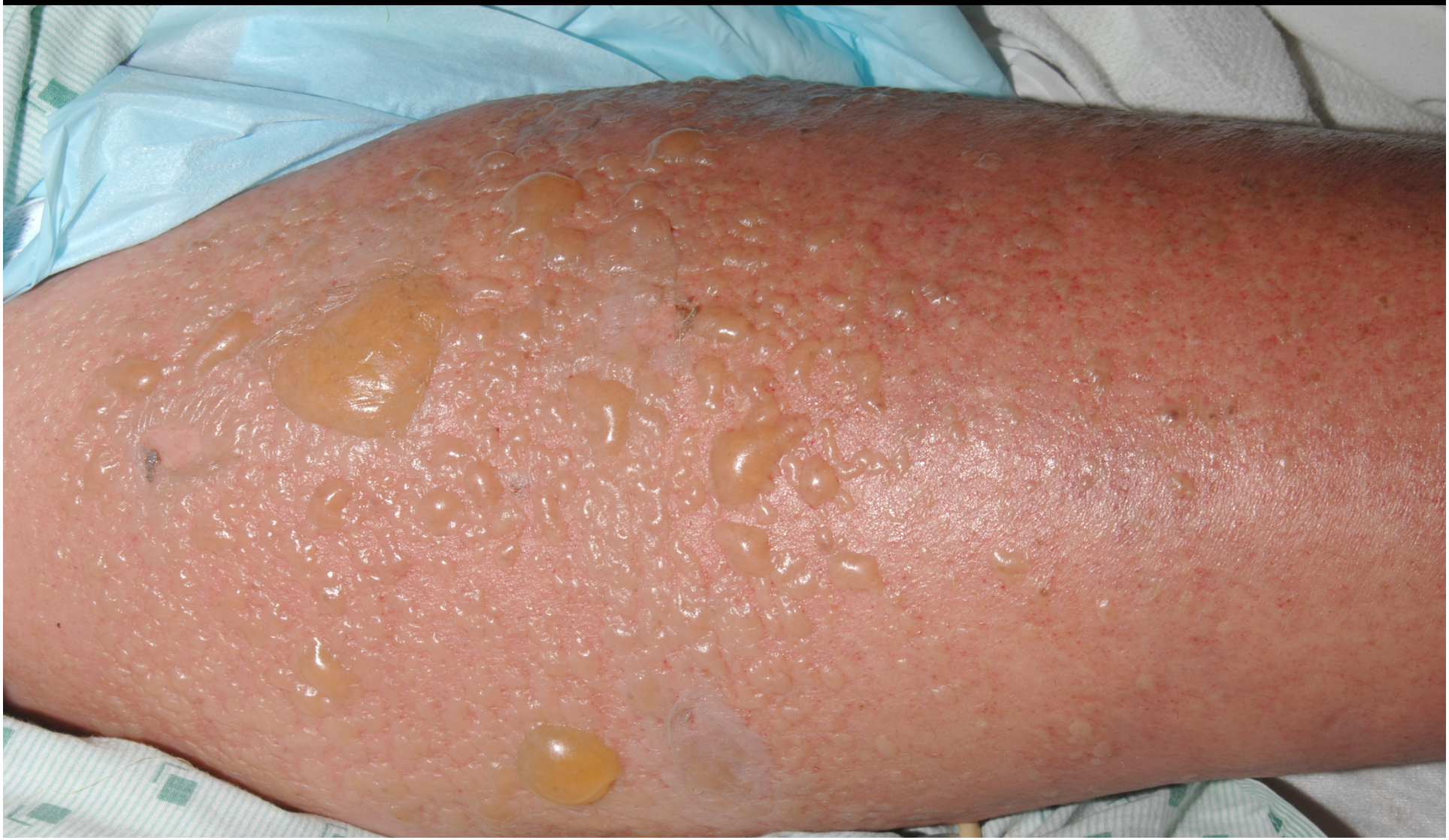
T cell based immunotherapy is capable of mediating the regression of large vascularized, invasive metastatic melanoma in humans

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

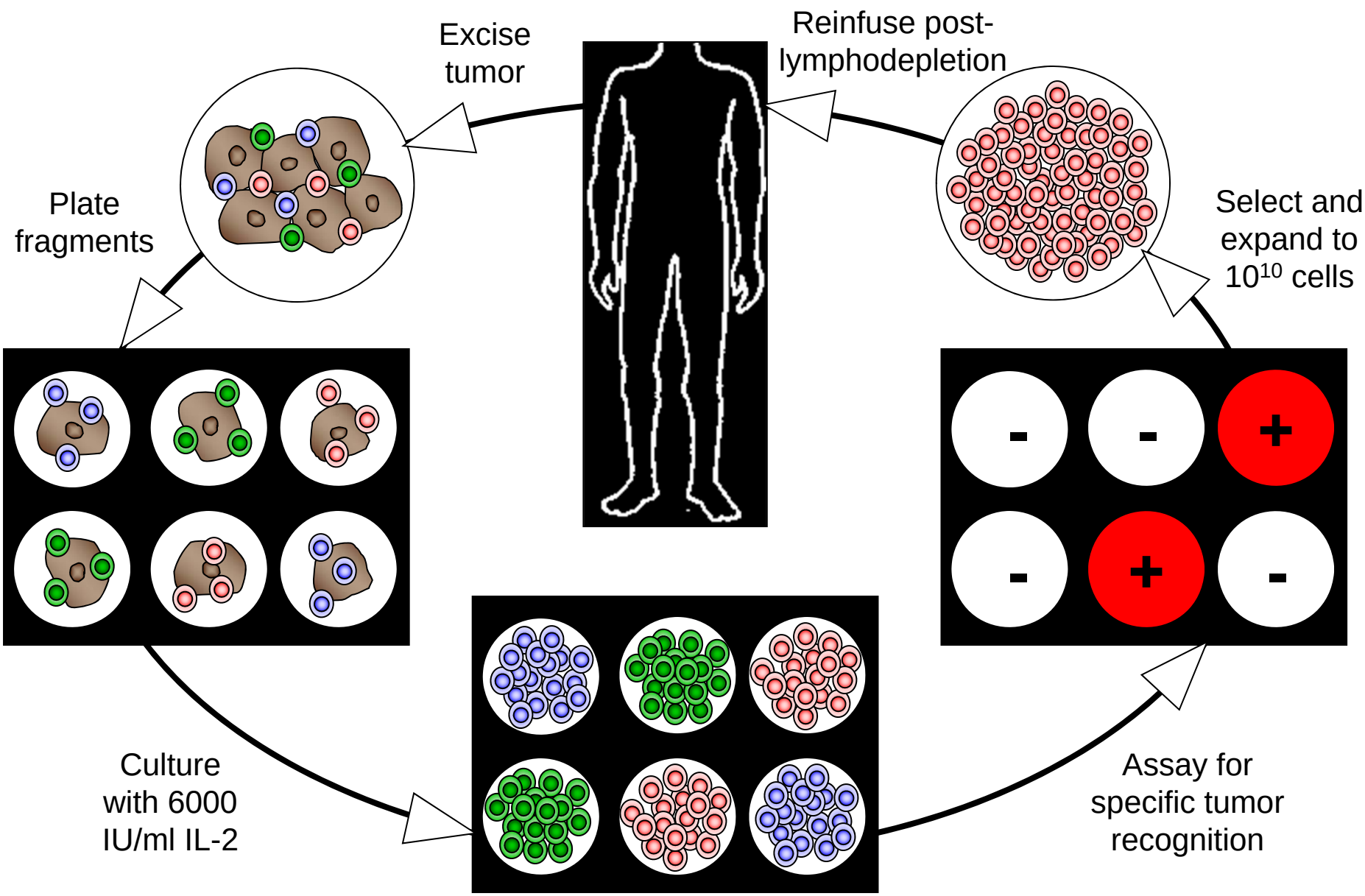
CHALLENGE

Determine ways to extend this approach to additional melanoma patients and patients with common epithelial cancers

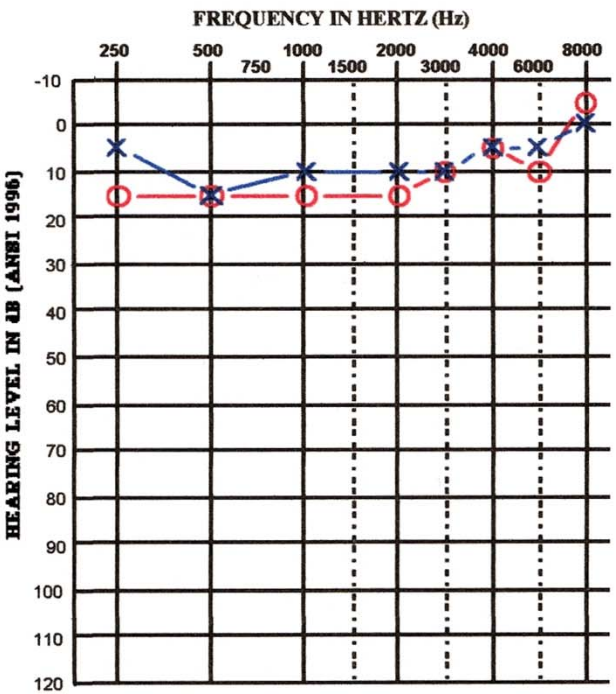
D.D. gp100:154 TCR day 7



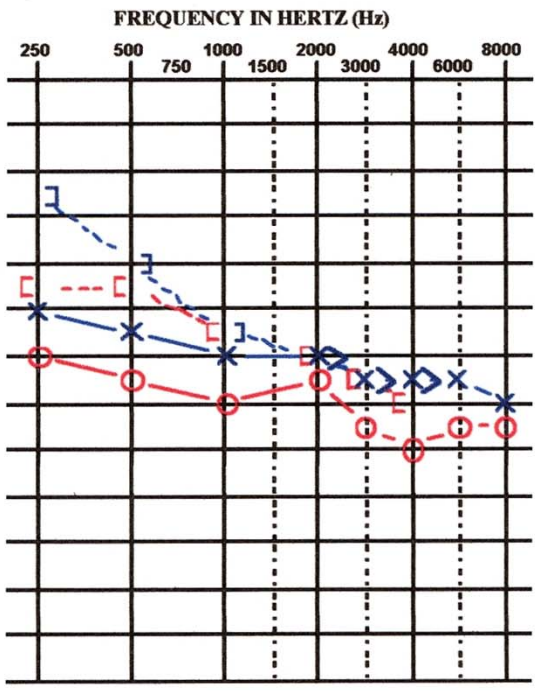
Adoptive transfer of tumor infiltrating lymphocytes (TIL)



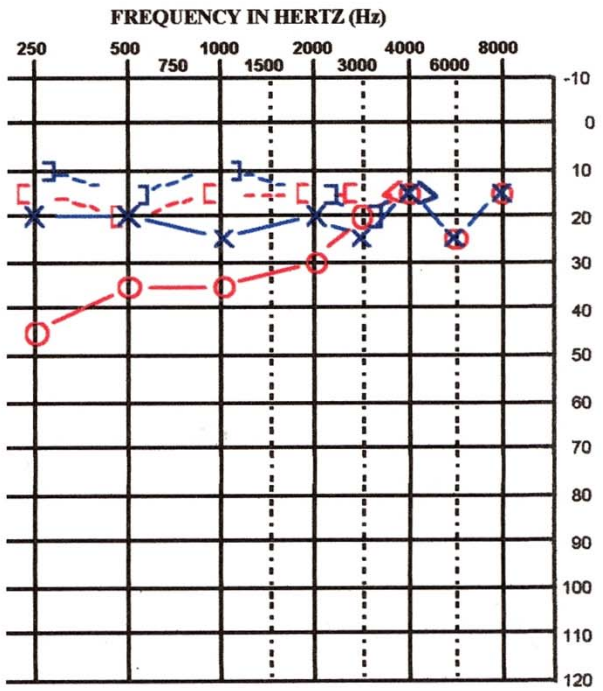
Pt. D.T. DMF5 TCR



Day -2

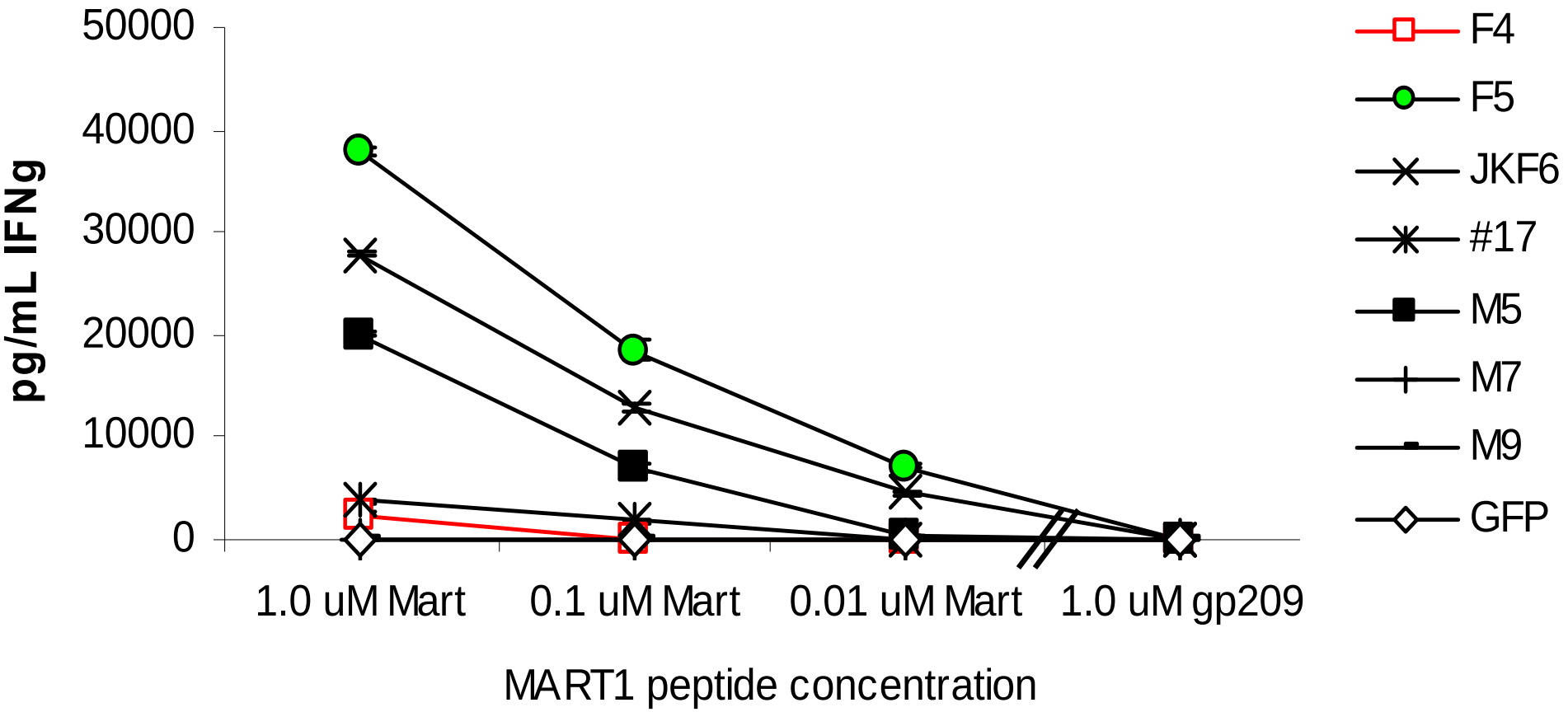


Day +13



Day +60

Electroporation of different MART-1 TCR RNA into the same normal PBL



Results: RNA electroporated PBL showed the same relative avidities as the TIL clones

DMF5 > JKF6 > M5 > #17 = DMF4 > M7 = M9

CEA Reactivity of PBL Transduced with anti-CEA TCR

Target	HLA-A2	CEA	Patient 1		Patient 2	
			No	Tx	No	Tx
			(pg/ml IFN-g)			
T2 +HBV	+	-	219	266	299	202
+ 10-12 M CEA	+	+	218	416	237	267
+ 10-11 M CEA	+	+	209	**789**	313	**762**
+ 10-10 M CEA	+	+	180	**2653**	229	**2695**
+ 10 -9 M CEA	+	+	203	**9839**	240	**7534**
+ 10 -8 M CEA	+	+	161	**16214**	151	**18043**
COS-A2-ESO	+	+	34	176	56	88
COS A2-CEA	+	-	44	**1436**	64	**1058**
LS 174T	-	+	27	22	43	25
LS 180	-	+	26	24	44	23
SW 620	+	-	84	344	42	88
SW 480	+	-	69	147	52	43
624 ml	+	-	76	217	56	67
526 ml	+	-	56	202	46	67
SW 403	+	+	15	**2719**	20	**1111**
SW 1463	+	+	22	**1710**	41	**895**
T84	+	+	30	**4330**	68	**3009**
H 508	+	+	14	**8535**	16	**8501**

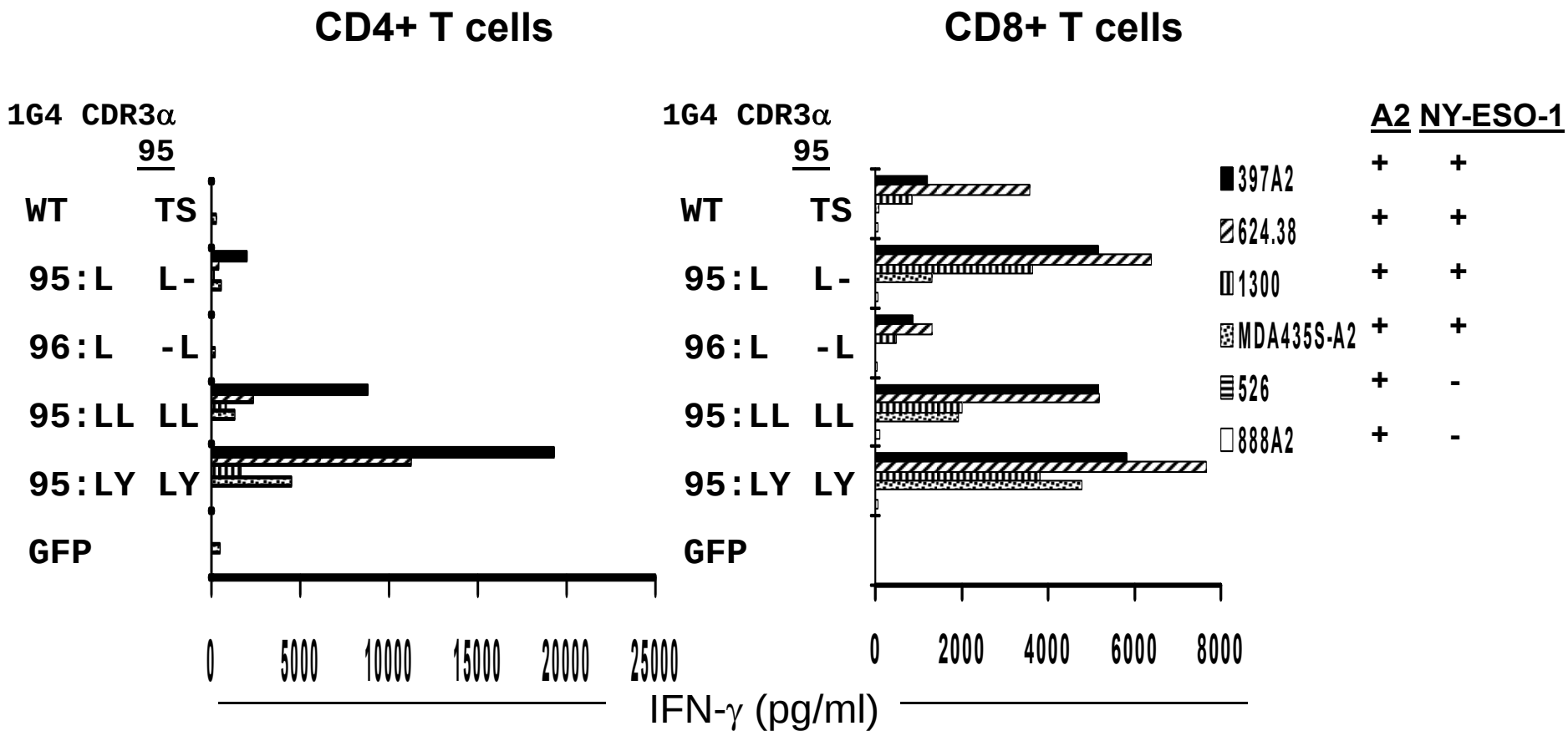
Method for increasing the affinity of T cell receptors

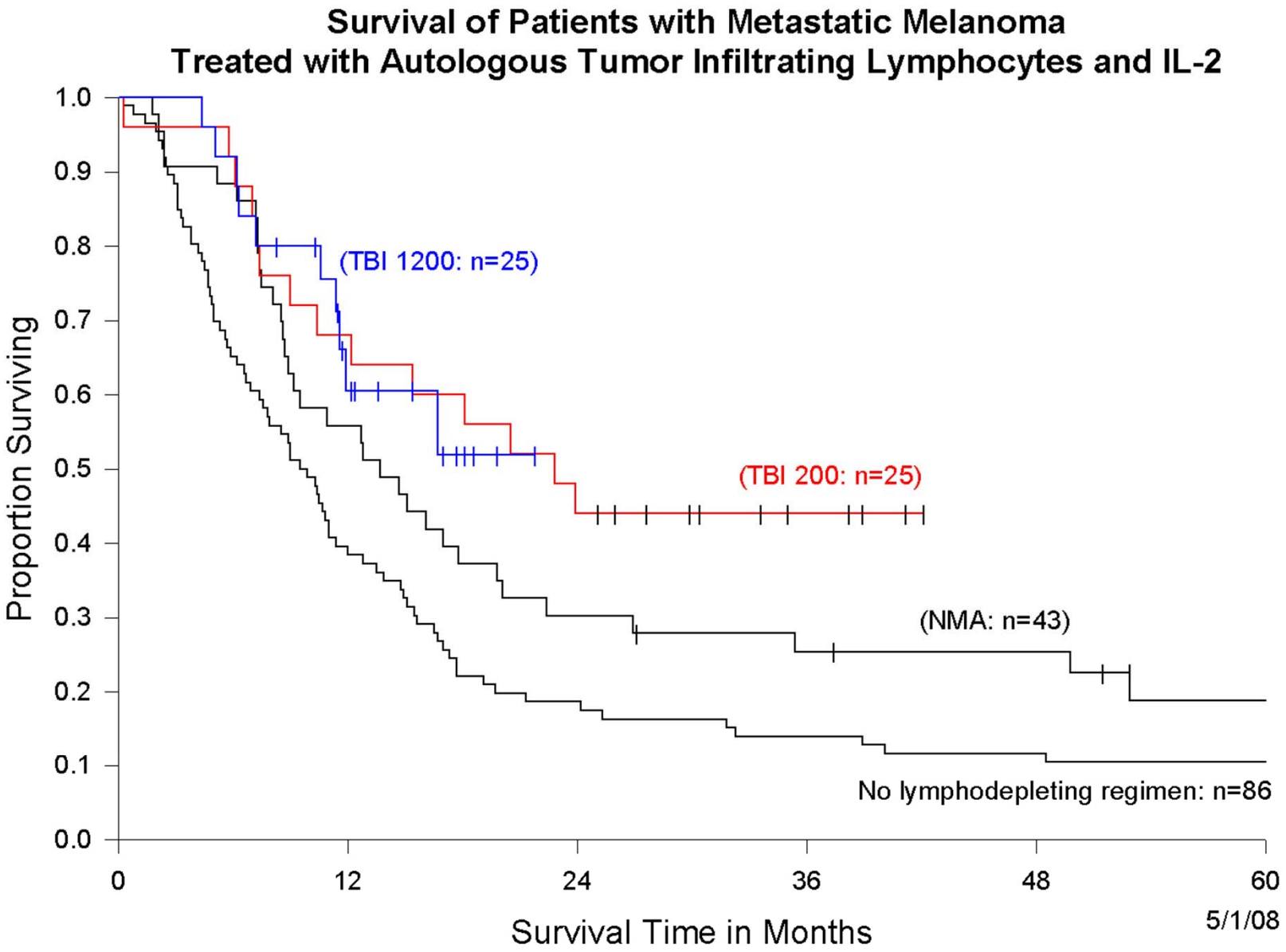
CDR2 and CDR3 regions of the T cell receptor are responsible for binding to the peptide/MHC complex.

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




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
(TBI 1200: n=25)
(TBI 200: n=25)
(NMA: n=43)
No lymphodepleting regimen: n=86
0
12
24
36
48
60
Survival Time in Months
5/1/08

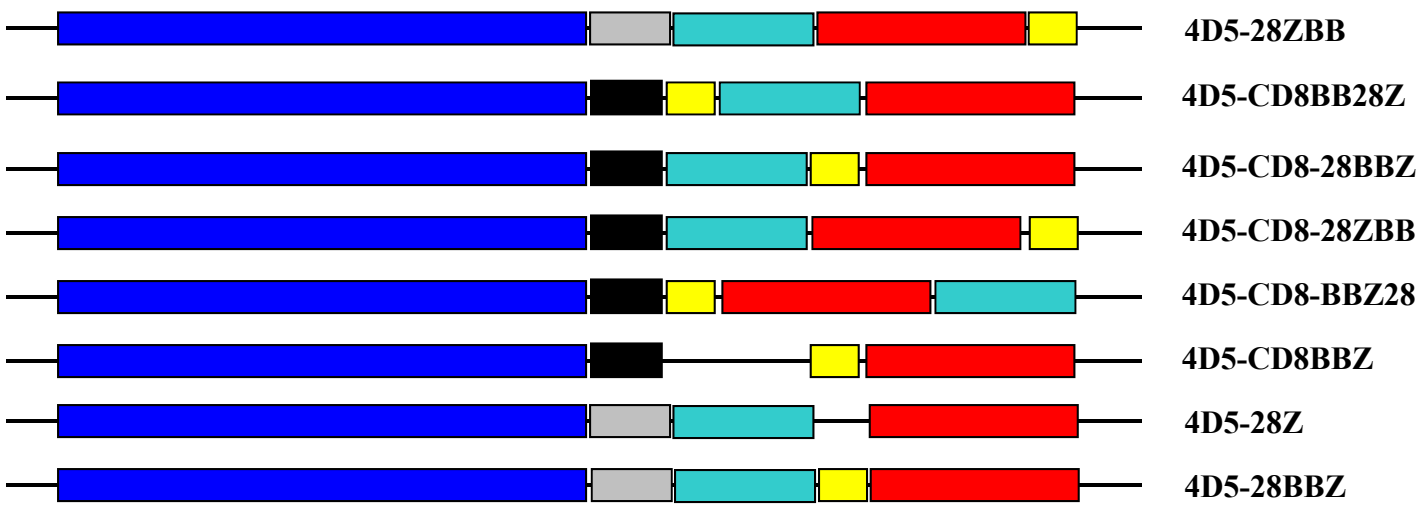
Preliminary Evaluation of TCR Gene Therapy in Patients with Metastatic Melanoma

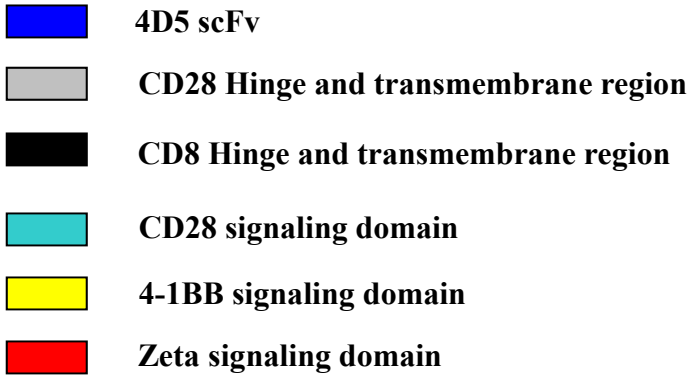
(first patient treated 7/13/07; follow up as of 5/30/08)

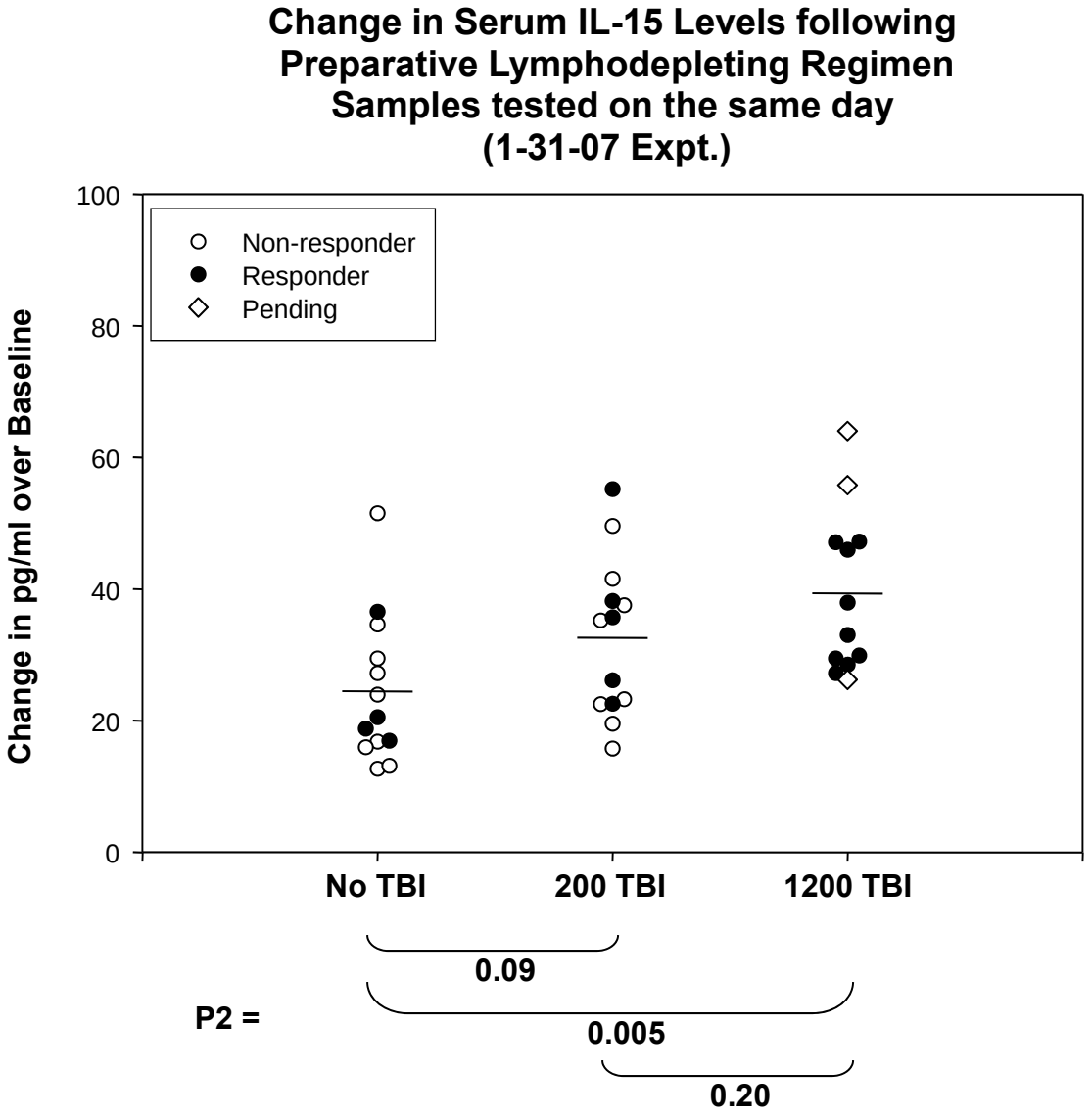
Cohort	Cell#	IL-2	MART (DMF5) Response Total	MART (DMF5) Response OR	MART (DMF5) Toxicity Uveitis	MART (DMF5) Toxicity Auditory	gp100 (m154) Response Total	gp100 (m154) Response OR	gp100 (m154) Toxicity Uveitis	gp100 (m154) Toxicity Auditory
			number of patients (duration in months)							
1	1-3x10^{10}	limited	6	2 (8+,8+)	4	3(0)	6	0	0	0(0)
2	~3x10^{9}	to tolerance	5	2 (7+,6+)	0	0(1)	4	0	1	0
3	1-8x10^{10}	to tolerance	9	2 (3,3)	5	5(1)	6	3 (5+,2+,2+)	3	1(2)
		Total	20	6 (30%)	9	8(2)	16 (19%)	3	4	1(2)

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

Protein-protein interaction may account for the low transgene expression






Change in Serum IL-15 Levels following
Preparative Lymphodepleting Regimen
Samples tested on the same day
(1-31-07 Expt.)
Change in pg/ml over Baseline
100
80
60
40
20
0
Non-responder
Responder
Pending
No TBI
200 TBI
1200 TBI
P2 =
0.09
0.005
0.20

PHENOTYPIC ANALYSIS OF PERSISTENT AND NON-PERSISTENT T CELL CLONOTYPES

---

p>0.1

4-1BB	CD30	CD70	CXCR4
CCR5	CD40L	CD71	DR3
CCR6	CD45RA	CD80	HVEM
CCR7	CD45RO	CD86	ICOS
CD2	CD48	CD94	KLRG1
CD5	CD50	CD95	NKB1
CD8αα	CD54	CD102	NKG2D
CD8β	CD56	CD122	Notch1
CD11a	CD57	CD127	PD-1
CD16	CD58	CD161	TRAIL
CD25	CD62L	CD178	
CD27	CD69	CD231	

p<0.05

CD28 (higher in cells that persist)

Very Preliminary Evaluation of Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

Cohort	Cell#	IL-2	Response		Autoimmune toxicity		
			Total	OR	Skin	Ear	Eye
1	1-3x10^{10}	limited	6	2	2	0	2
2	~3x10^{9}	to tolerance	5	1	0	0	0
3	1-8x10^{10}	to tolerance	8	2	3	4	5
		Total	19	5(26%)			

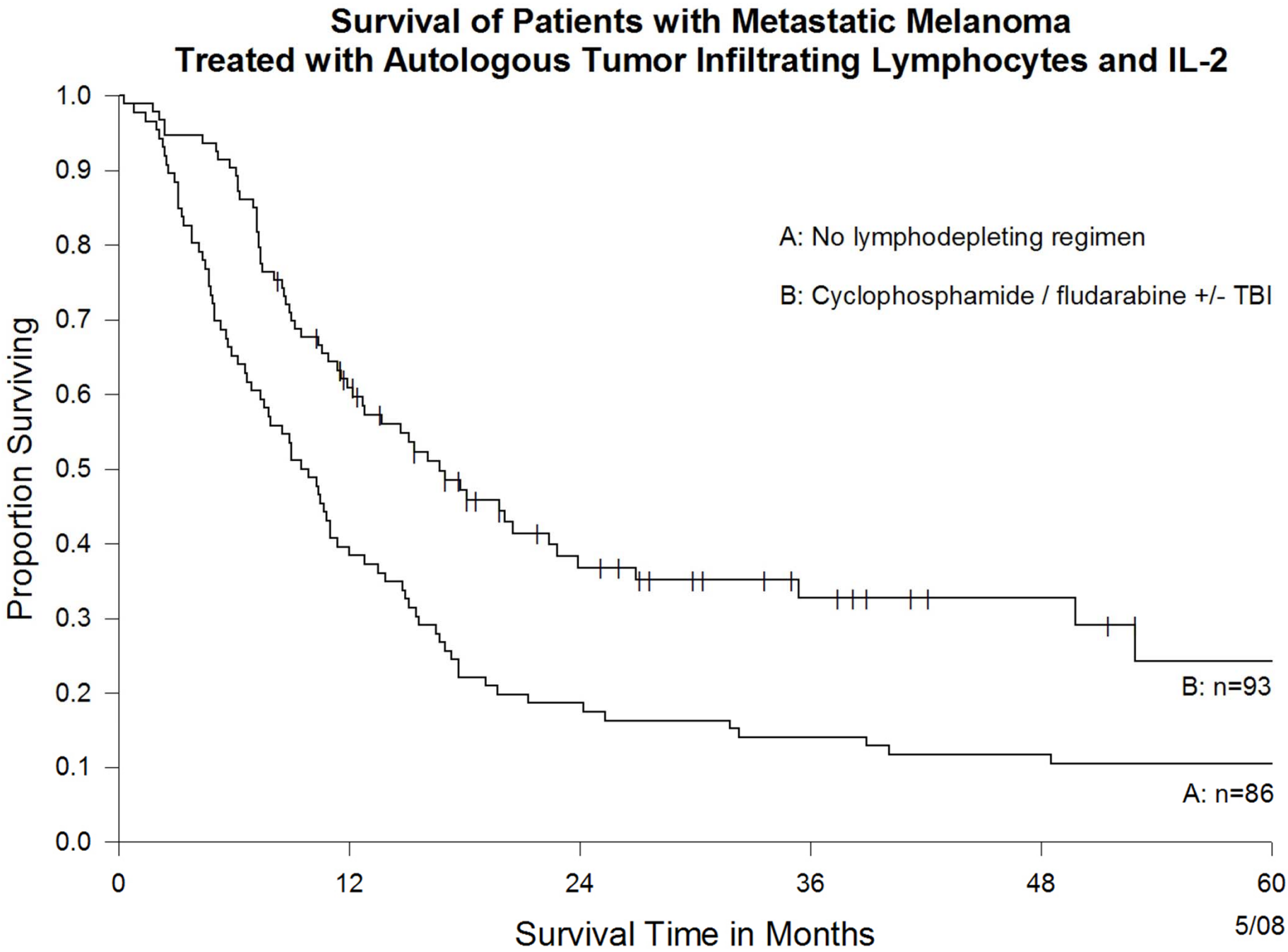
(All patients were refractory to prior treatment with IL-2.)

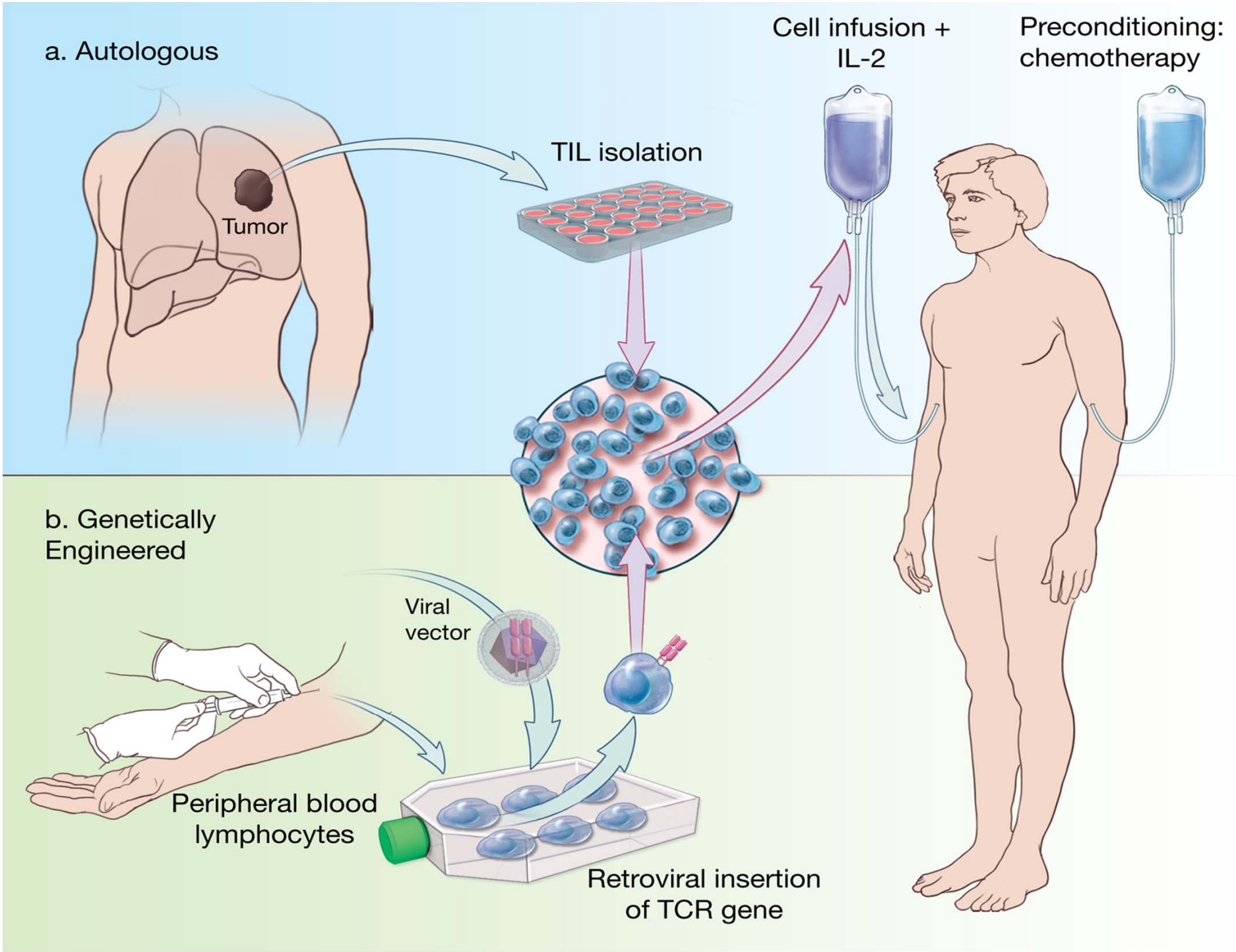
Preliminary Evaluation of Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

(first patient treated 7/13/07; follow up as of 5/1/08)

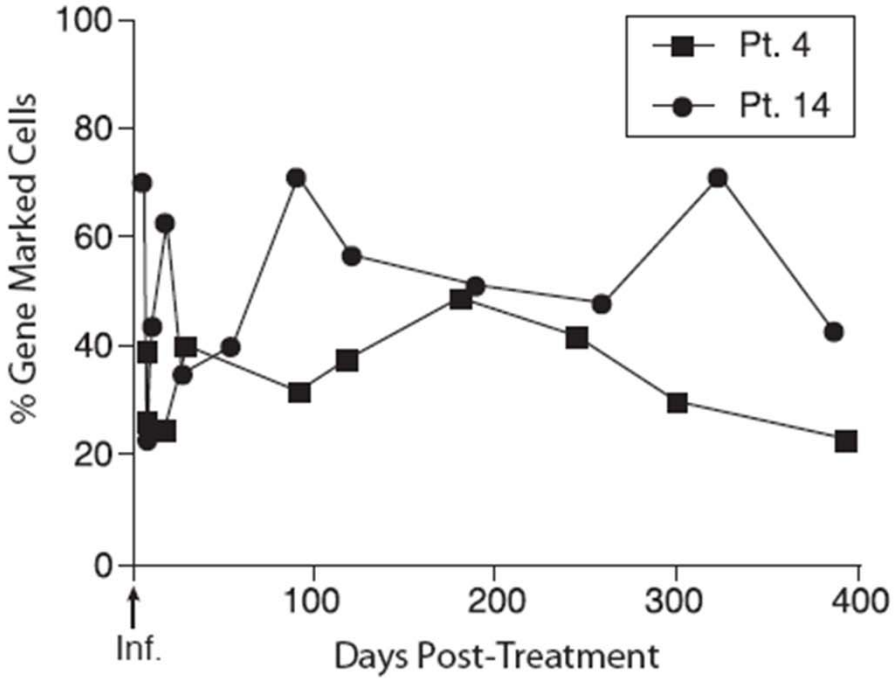
Cohort	Cell#	IL-2	Response		Toxicity	
			Total	OR (duration mos)	Uveitis	Auditory
				(number of patients)		
1	1-3x10^{10}	limited	6	2 (8+,8+)	5	3
2	~3x10^{9}	to tolerance	5	1 (6+)	0	0
3	1-8x10^{10}	to tolerance	8	2 (3,3)	5	4
		Total	19	5 (26%)	10	7

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

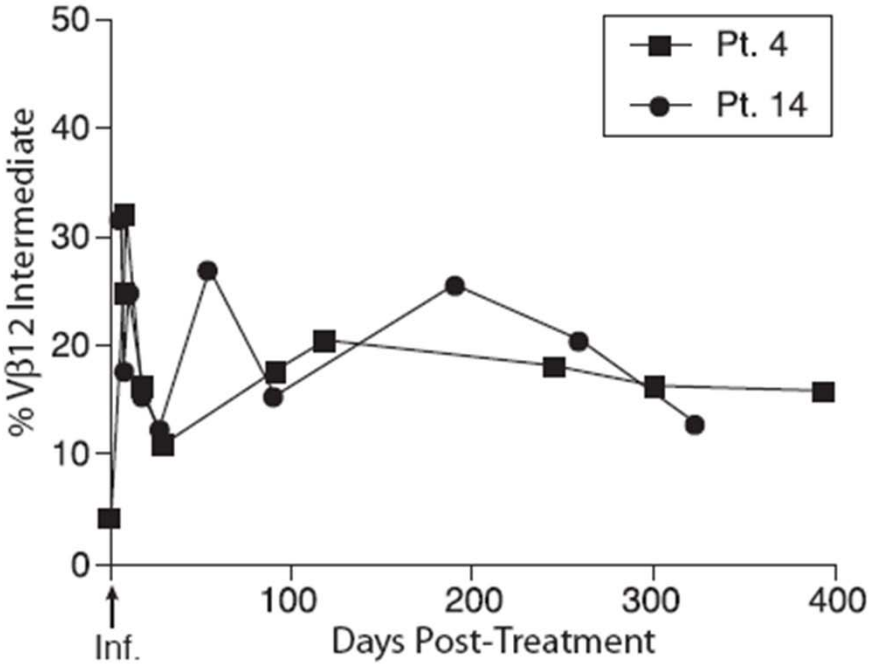

Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A: No lymphodepleting regimen
B: Cyclophosphamide / fludarabine +/- TBI
B: n=93
A: n=86
0
12
24
36
48
60
Survival Time in Months
5/08


a. Autologous
Tumor
TIL isolation
Cell infusion +
IL-2
Preconditioning:
chemotherapy
b. Genetically
Engineered
Viral
vector
Peripheral blood
lymphocytes
Retroviral insertion
of TCR gene

Persistence of TCR transduced cells in responding patients

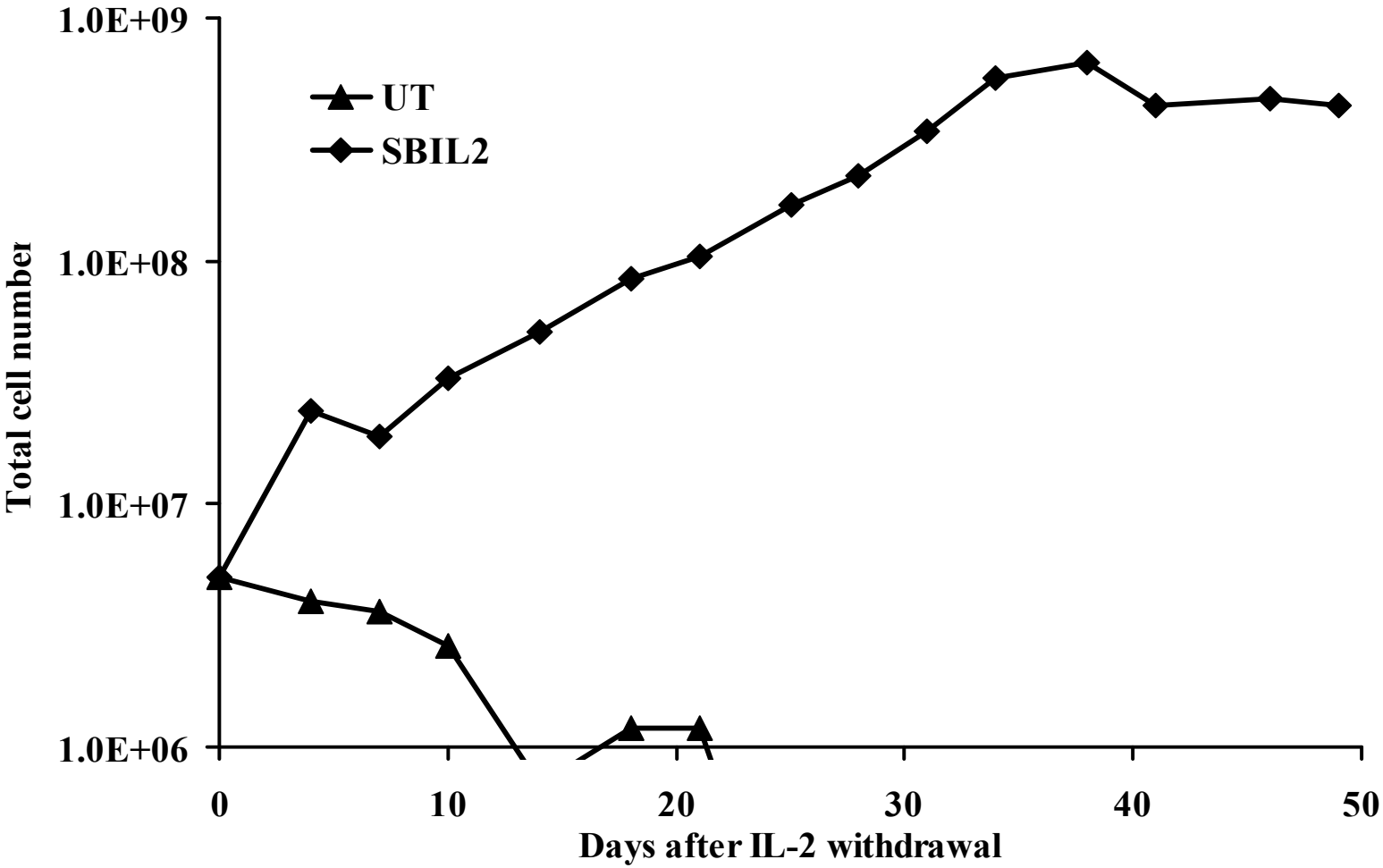


Quantitative PCR (TaqMan)



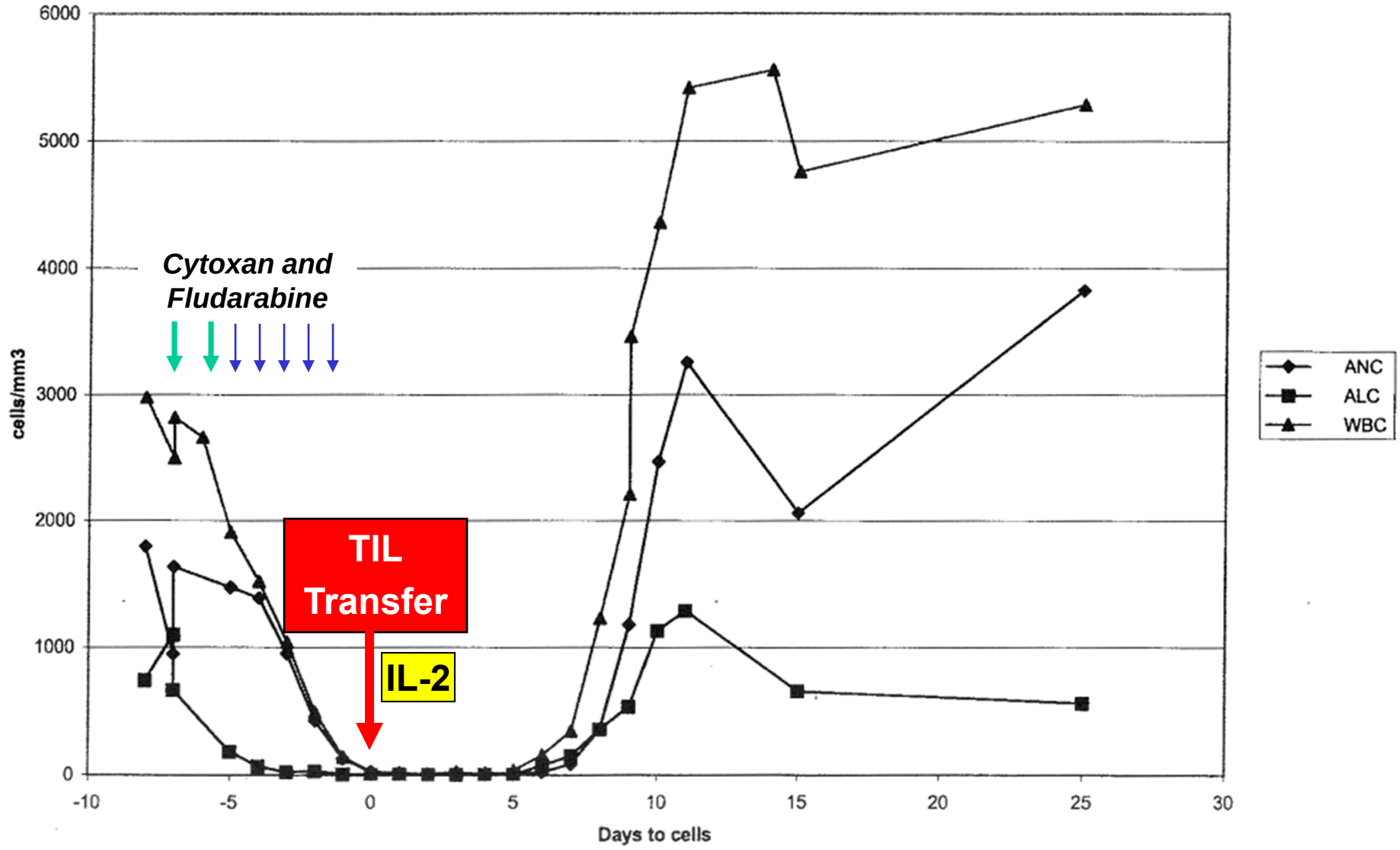
FACS for Vβ12

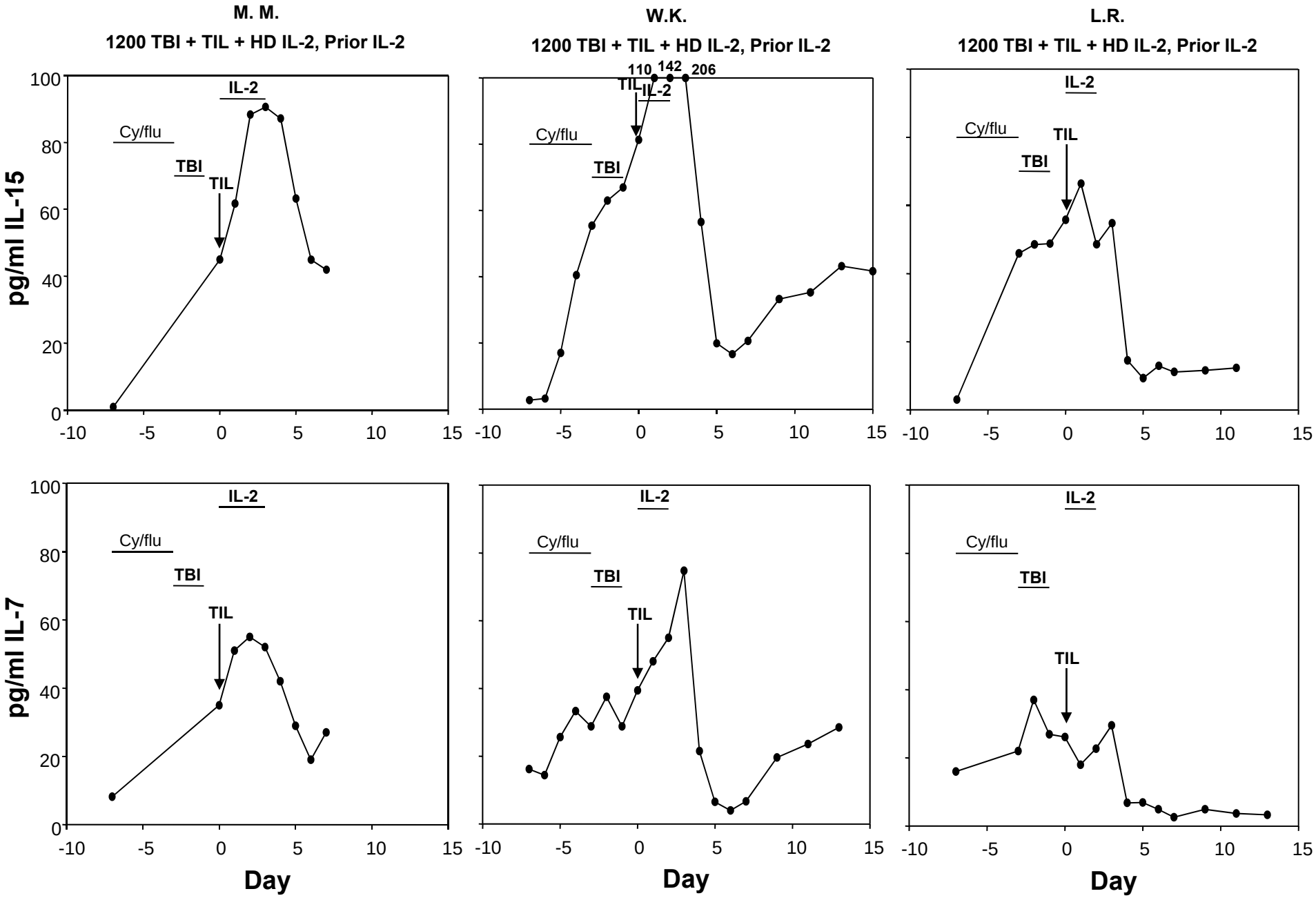
IL-2 withdrawal on day 9 of rapid expansion (patient K.S.)



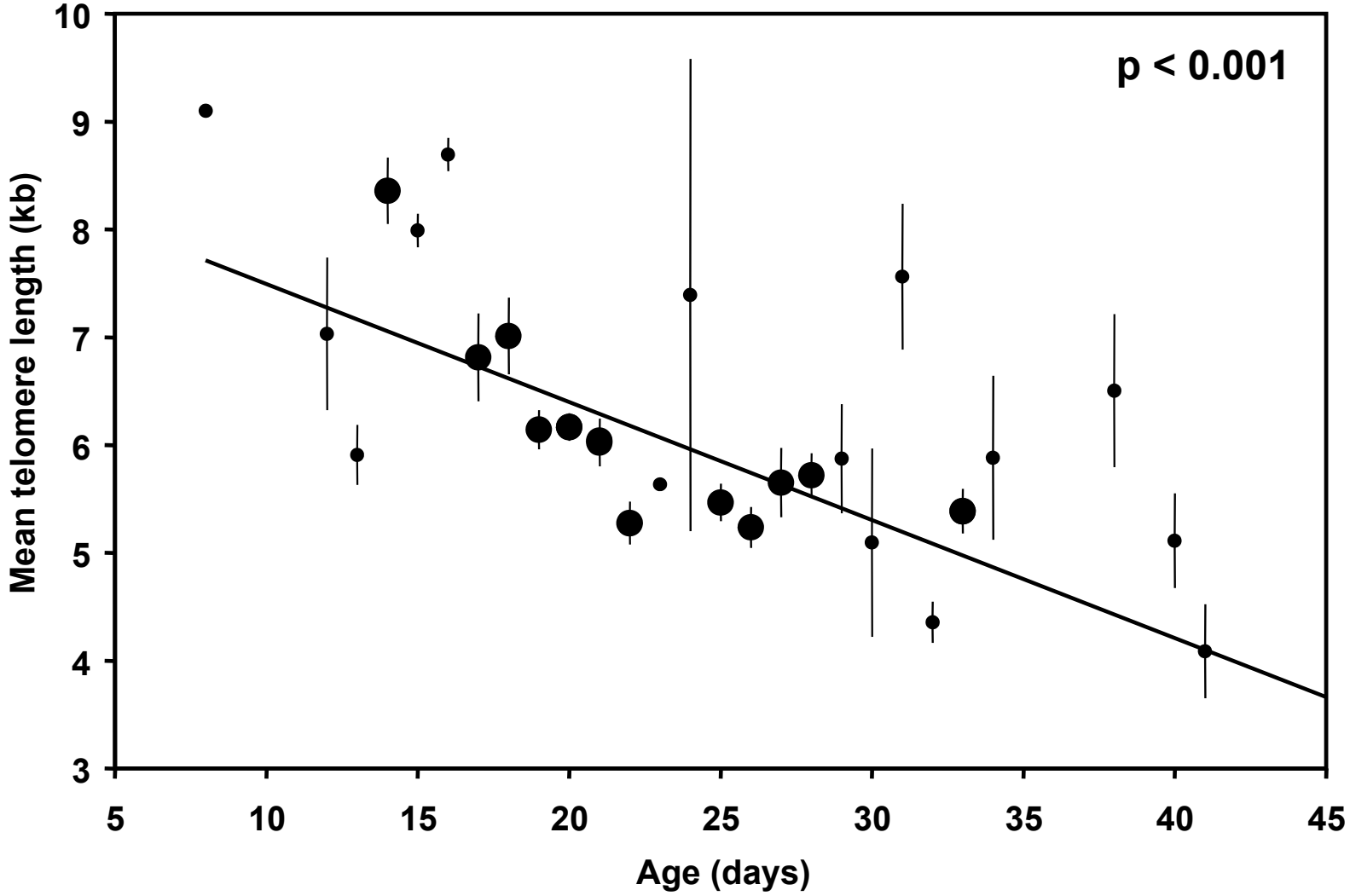
(J. Immunol. 167:6356-6365, 2001)

Integrating Preparative Nonmyeloablative Chemotherapy With Adoptive T-Cell Transfer

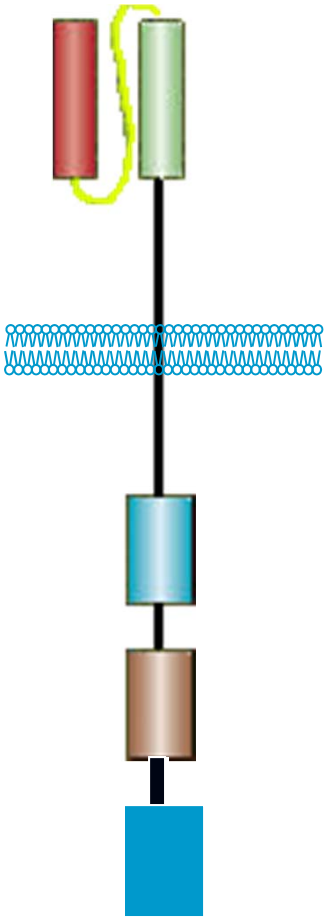



M. M.
1200 TBI + TIL + HD IL-2, Prior IL-2
W.K.
1200 TBI + TIL + HD IL-2, Prior IL-2
L.R.
1200 TBI + TIL + HD IL-2, Prior IL-2
pg/ml IL-15
pg/ml IL-7
Cy/flu
TBI
TIL
IL-2
110
142
206
Day
0
20
40
60
80
100
-10
-5
0
5
10
15

Telomere length decreases over time as bulk TIL are maintained in culture



Schematic representation of the Her2/neu chimeric receptor



Single chain construct of the antigen binding sites of the Her2/neu antibody

CD8 hinge and transmembrane region

CD28, 41BB, zeta cytoplasmic signalling domain

ADVANTAGES OF CELL TRANSFER THERAPY

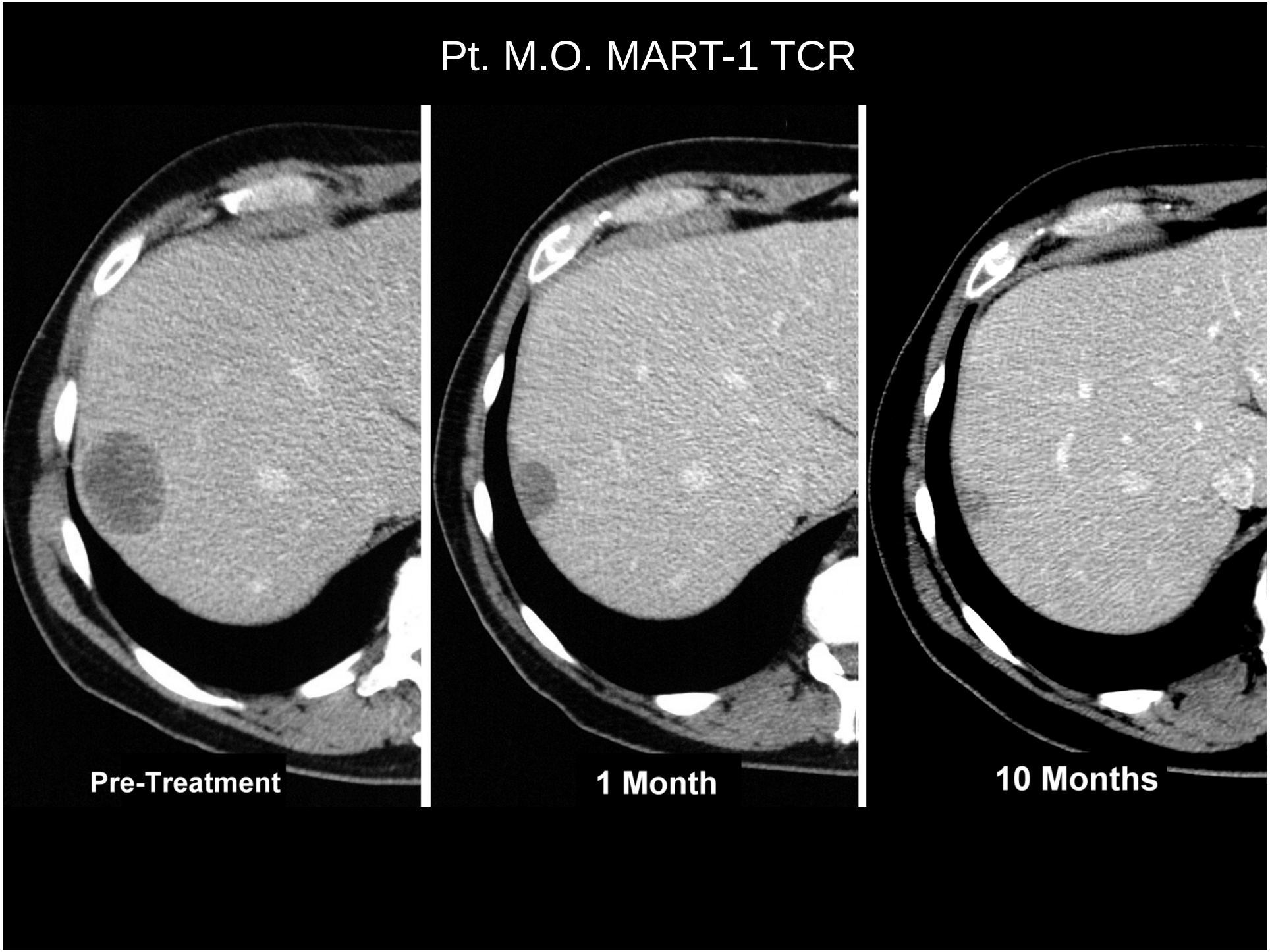
1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

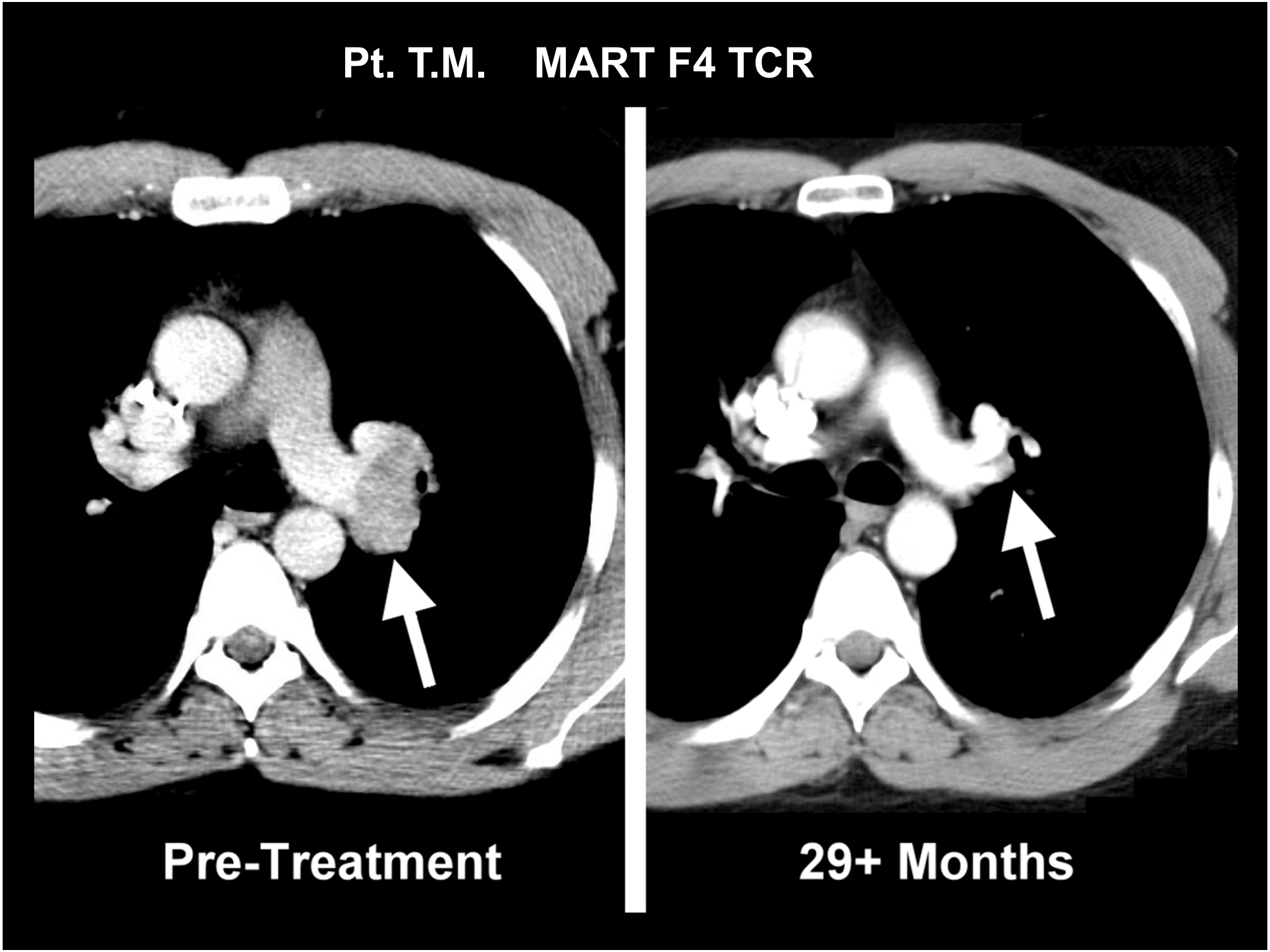
2. Administer cells activated ex-vivo to exhibit anti-tumor effector function.

3. Potentially identify exact cell subpopulations and effector functions required for cancer regression in vivo.

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

Pt. M.O. MART-1 TCR



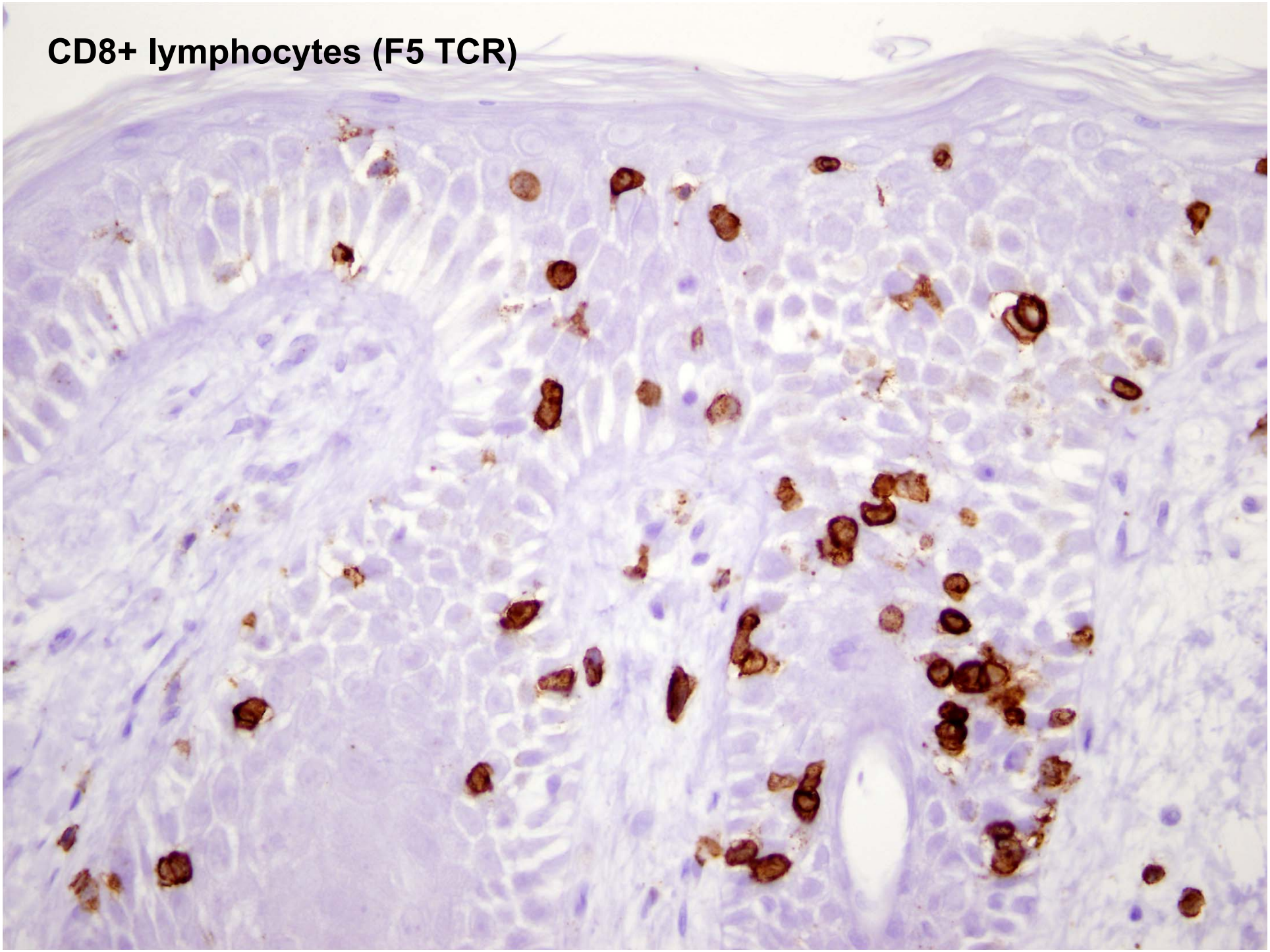

Pt. T.M. MART F4 TCR
Pre-Treatment
29+ Months

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

Multiple antigenic epitopes on NY-ESO-1 recognized by human T lymphocytes

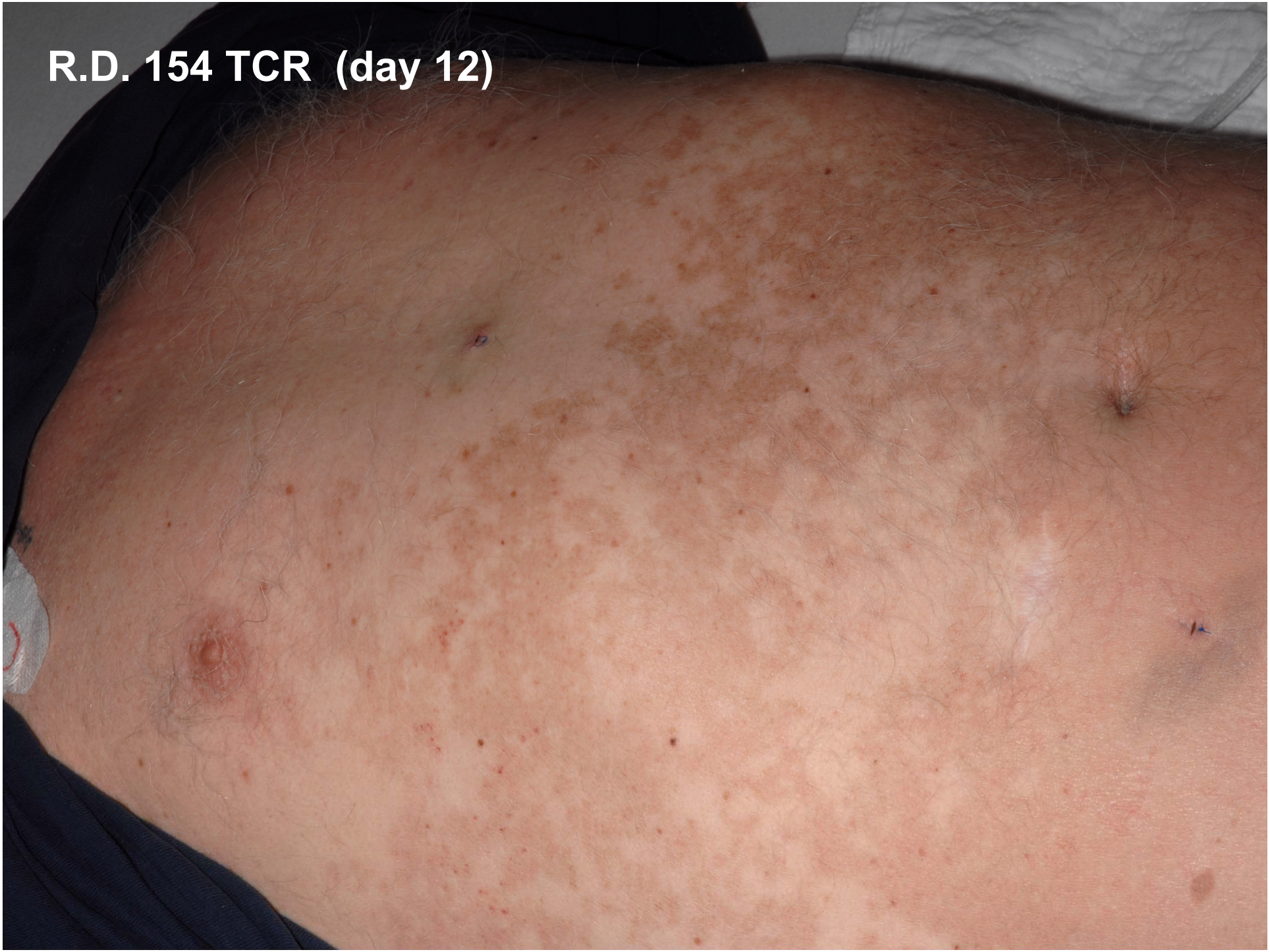

CD8+ lymphocytes (F5 TCR)

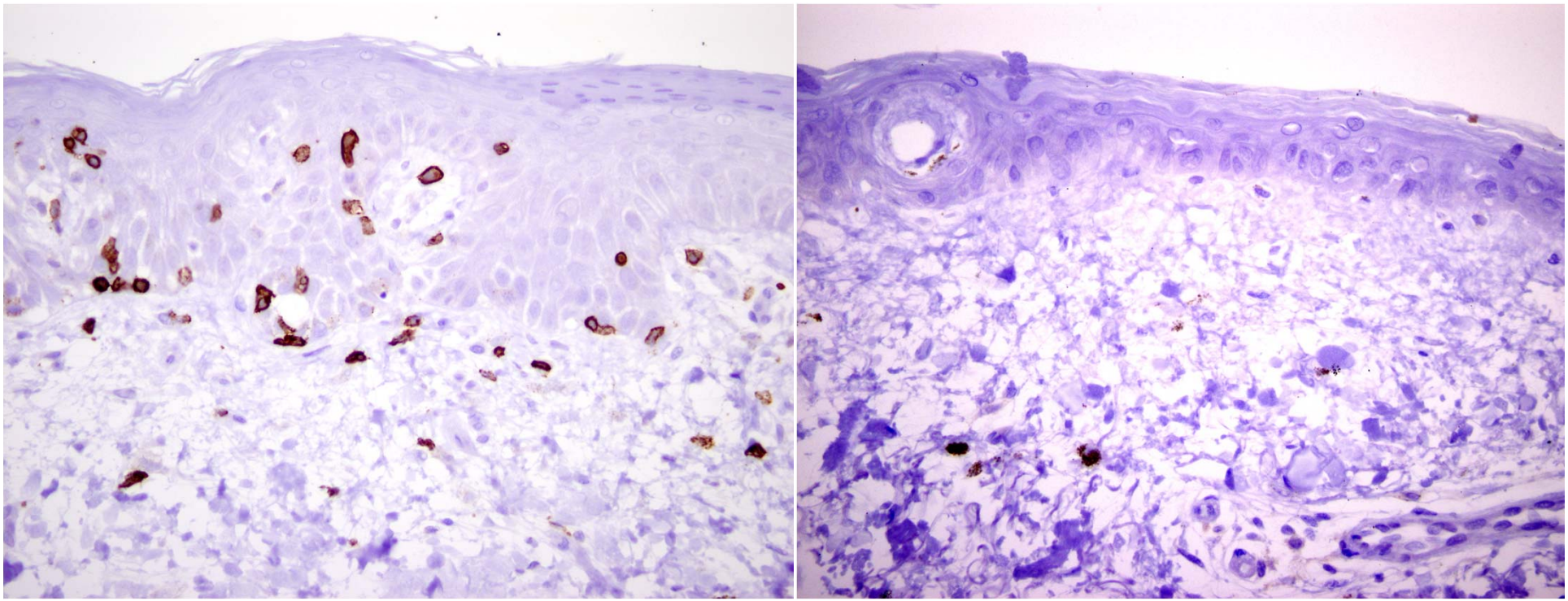
Preliminary Evaluation of Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

(first patient treated 7/13/07; follow up as of 5/1/08)

Cohort	Cell#	IL-2	Response		Toxicity	
			Total	OR (duration mos)	Uveitis	Auditory
				(number of patients)		
1	1-3x10^{10}	limited	6	2 (8+,8+)	5	3
2	~3x10^{9}	to tolerance	5	1 (6+)	0	0
3	1-8x10^{10}	to tolerance	8	2 (3,3)	5	4
		Total	19	5 (26%)	10	7

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


R.D. 154 TCR (day 12)




D.Tu. F5 TCR (day 8)


D.Tu. F5 TCR (day 63)

Approach

Identify high-affinity T cell receptors (TCR) that recognize cancer antigens

Transduce the genes encoding these TCRs into the autologous PBL of patients and use these cells for treatment

Utilize the principles learned from studies of TIL to improve the effectiveness of this gene therapy approach

CELL TRANSFER PROTOCOL

Treatment of patients with Metastatic Melanoma Using Cloned Lymphocytes Following Administration of a Non-Myeloablative but Lymphocyte Depleting Regimen

---

a) **Cyclophosphamide (60 mg/kg x2 days)**
Fludarabine (25 mg/m^2 x5 days)

b) **After lymphocytes completely depleted (day 7):**

Infuse up to 10^{11} heterogeneous TIL
(containing both CD4 and CD8 cells)
selected for high avidity for tumor recognition.

Simultaneously (day 7) begin IL-2 administration

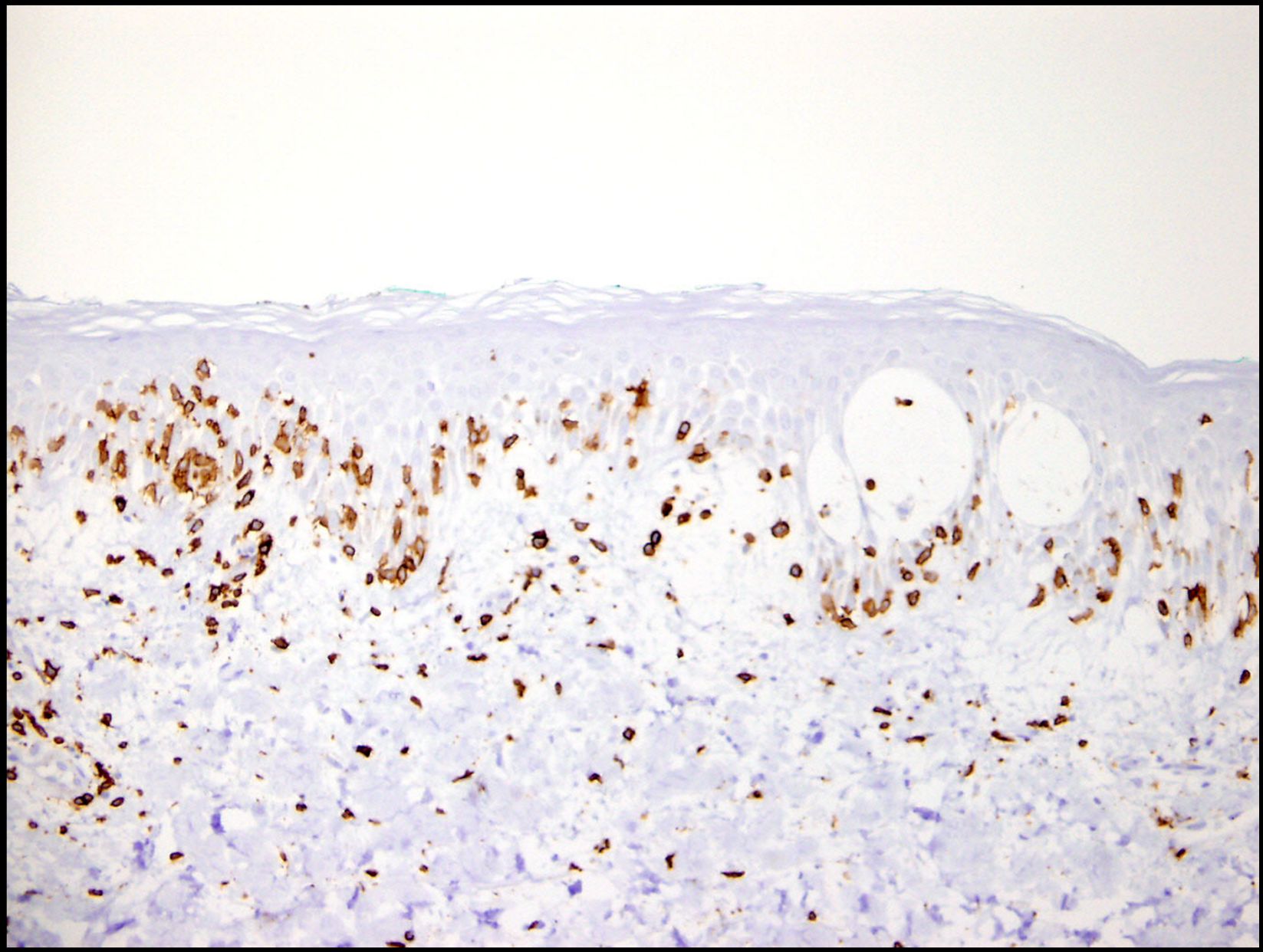
Goal (9/04 - 5/08)
To develop effective cell transfer immunotherapies for patients with cancer

---

1. **Development and analysis of cell transfer therapies using tumor infiltrating lymphocytes for patients with cancer.**

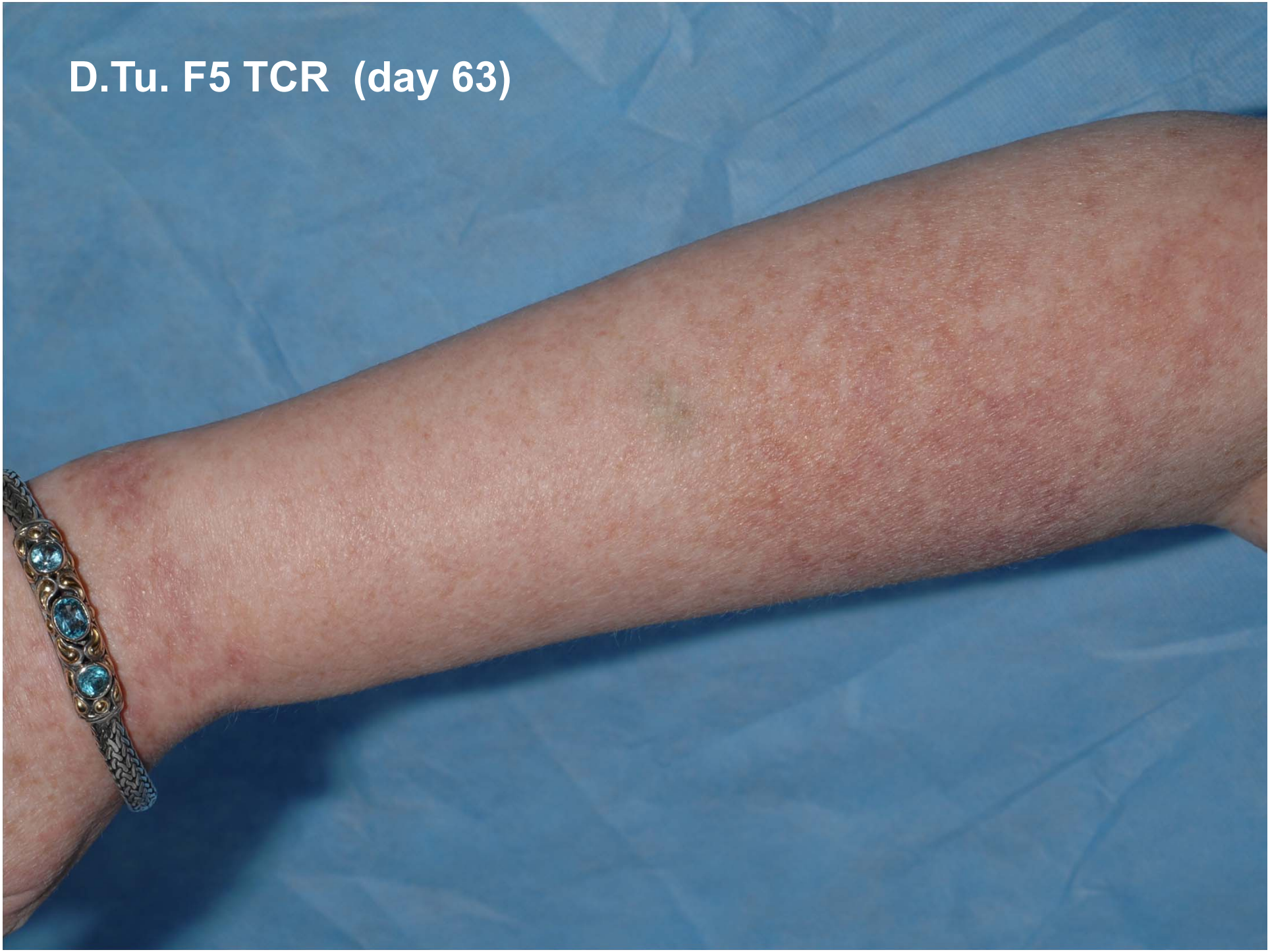
   By the site visit in Sept, 2004, 13 patients had received cell transfer with TIL following lymphodepletion, published in 2002 (Science); 35 patients published in April 2005 (J Clin Oncol)

2. **Development of cell transfer therapies based on the genetic modification of autologous lymphocytes.**



Day 5 post F5 TCR cell infusion (D. Tu)
Skin: CD8 positive cells


D.Tu. F5 TCR (day 8)

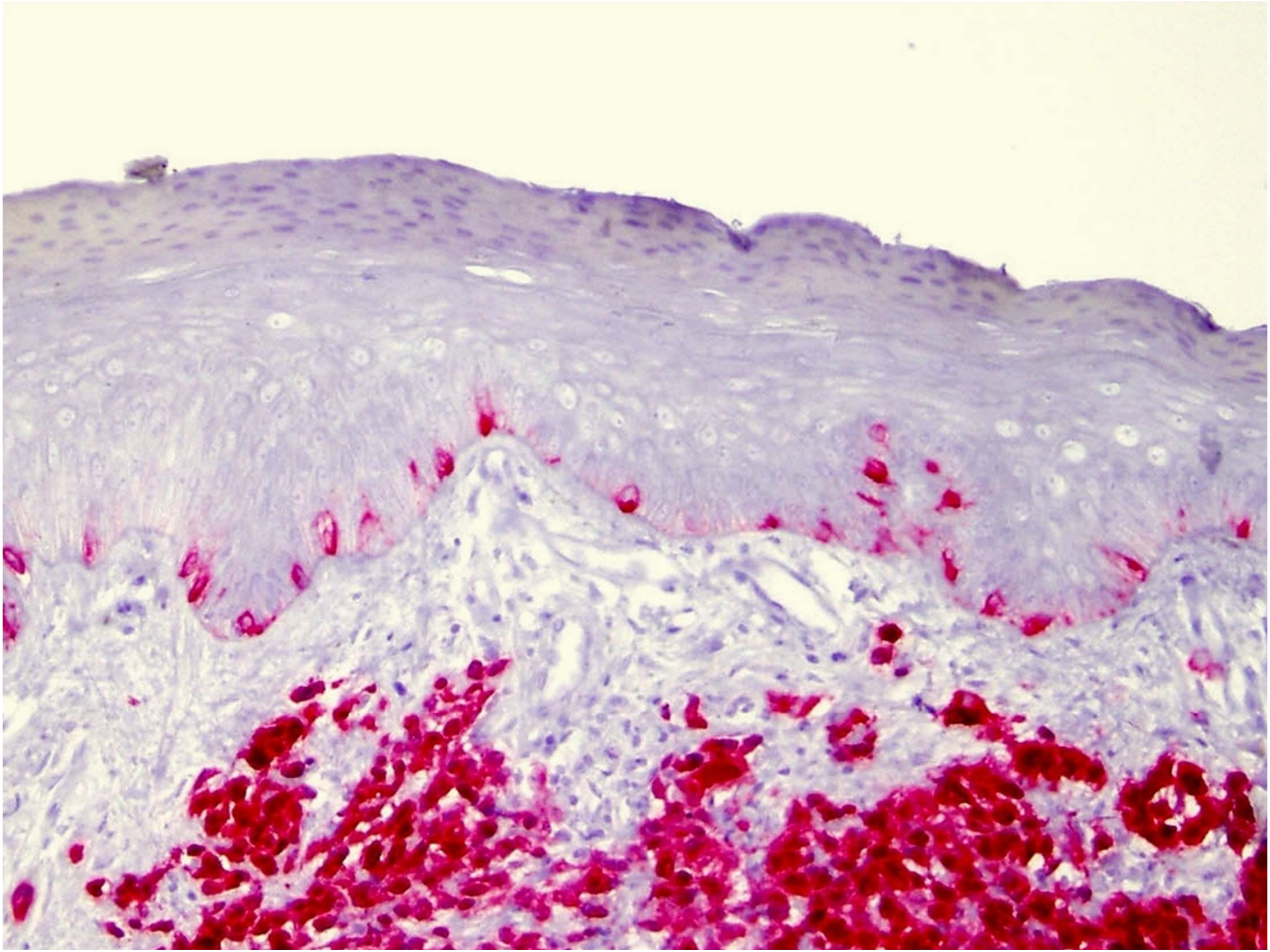

D.Tu. F5 TCR (day 63)

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

---

Using a higher affinity TCR (DMF5 vs DMF4) it appears that:

- **higher response rate (but still only 26%)**
- **targeting of relatively rare cells in the uvea and inner ear (corrected by local steroid administration)**



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

---

16 patients with metastatic melanoma

2 (13%) with objective regressions

(both disease free over two years later)

Subsequently 15 additional patients treated

2 further objective regressions

4/31 (13%) objective regressions

Approach

Identify high-affinity T cell receptors (TCR) that recognize cancer antigens

Transduce the genes encoding these TCRs into the autologous PBL of patients and use these cells for treatment

Utilize the principles learned from studies of TIL to improve the effectiveness of this gene therapy approach

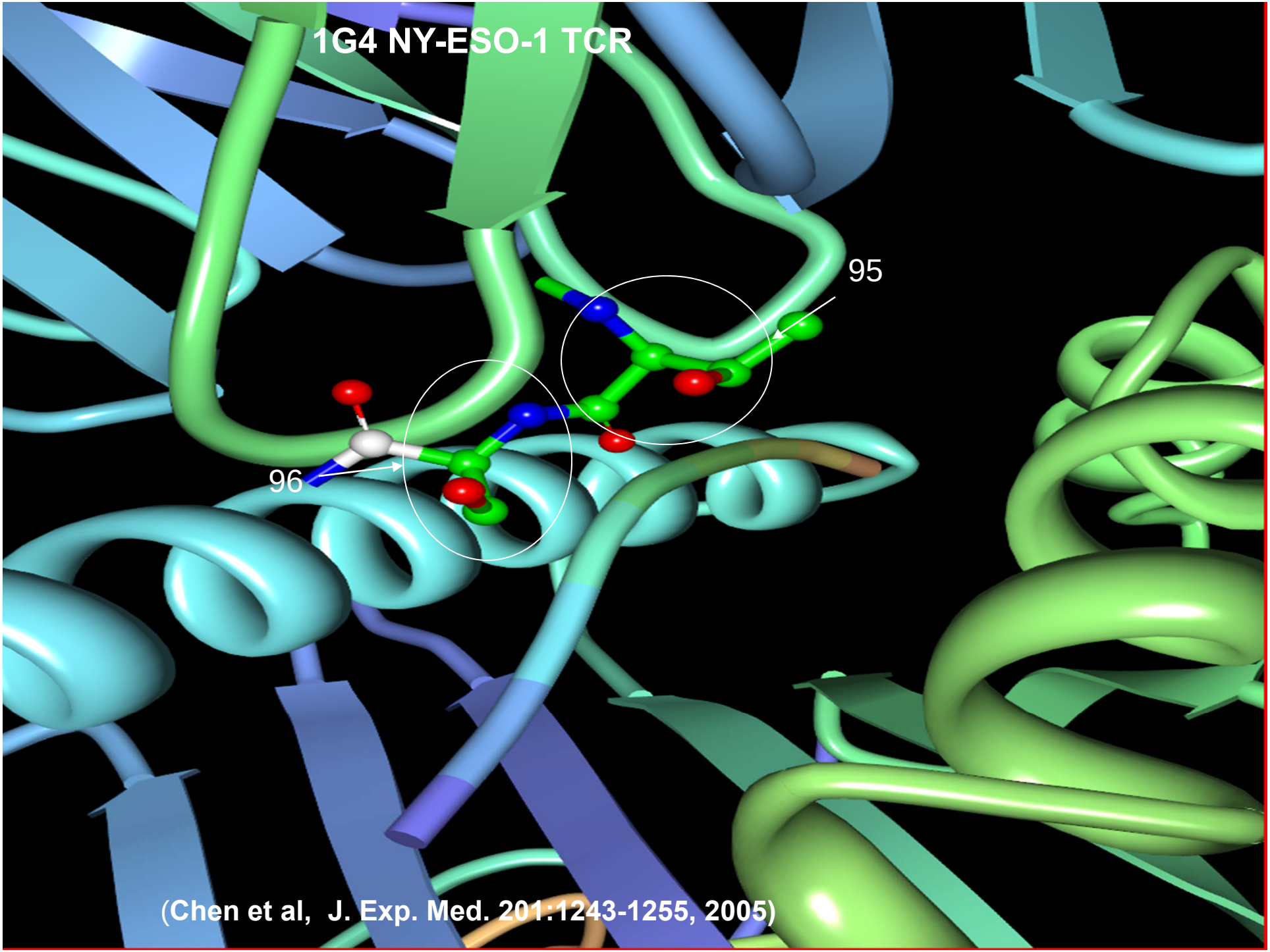
Three Factors that Correlate with Cancer Regression Following Cell Transfer Therapy

---

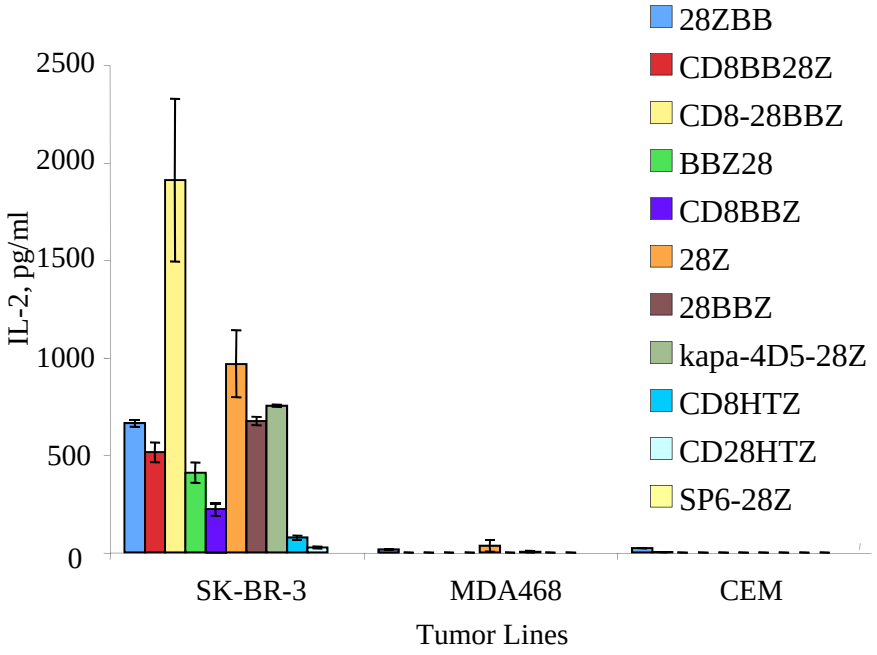
Persistence of the transferred cells

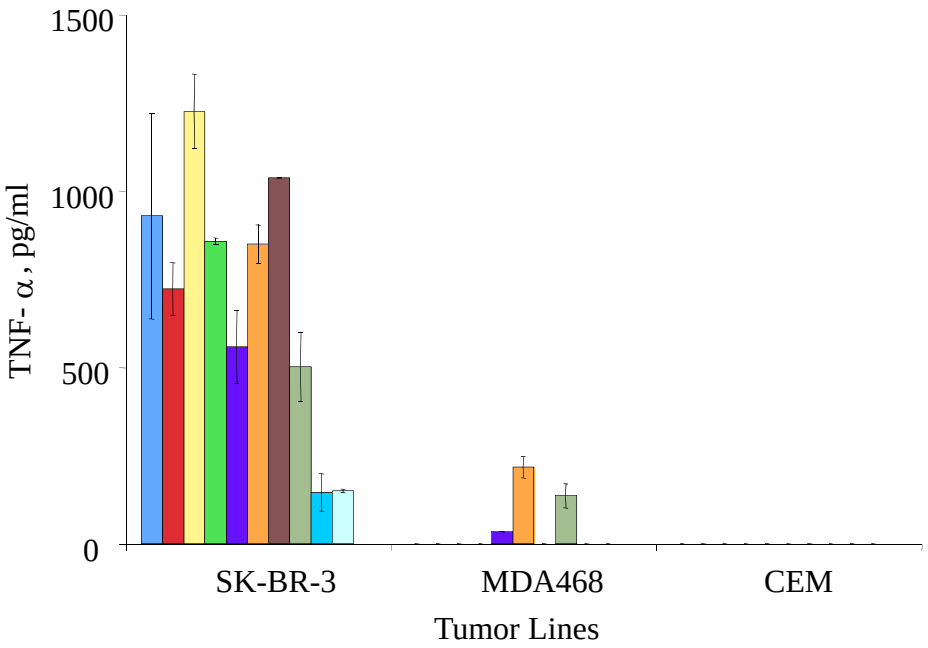
Telomere length

Expression of CD27 (following IL-2 withdrawal)


1G4 NY-ESO-1 TCR
95
96
(Chen et al, J. Exp. Med. 201:1243-1255, 2005)

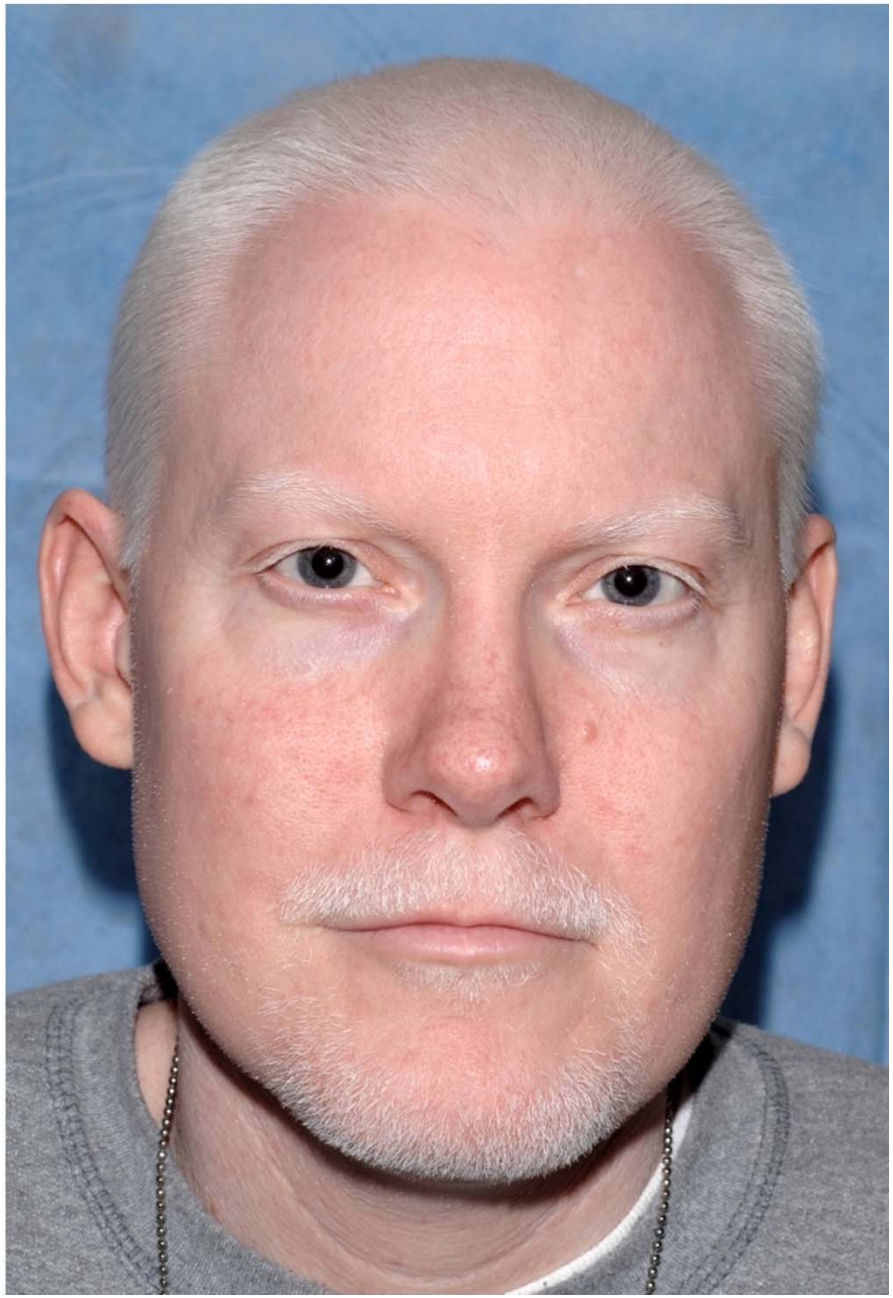
Analysis of the number and order of signalling chains in anti-Her/2 CAR

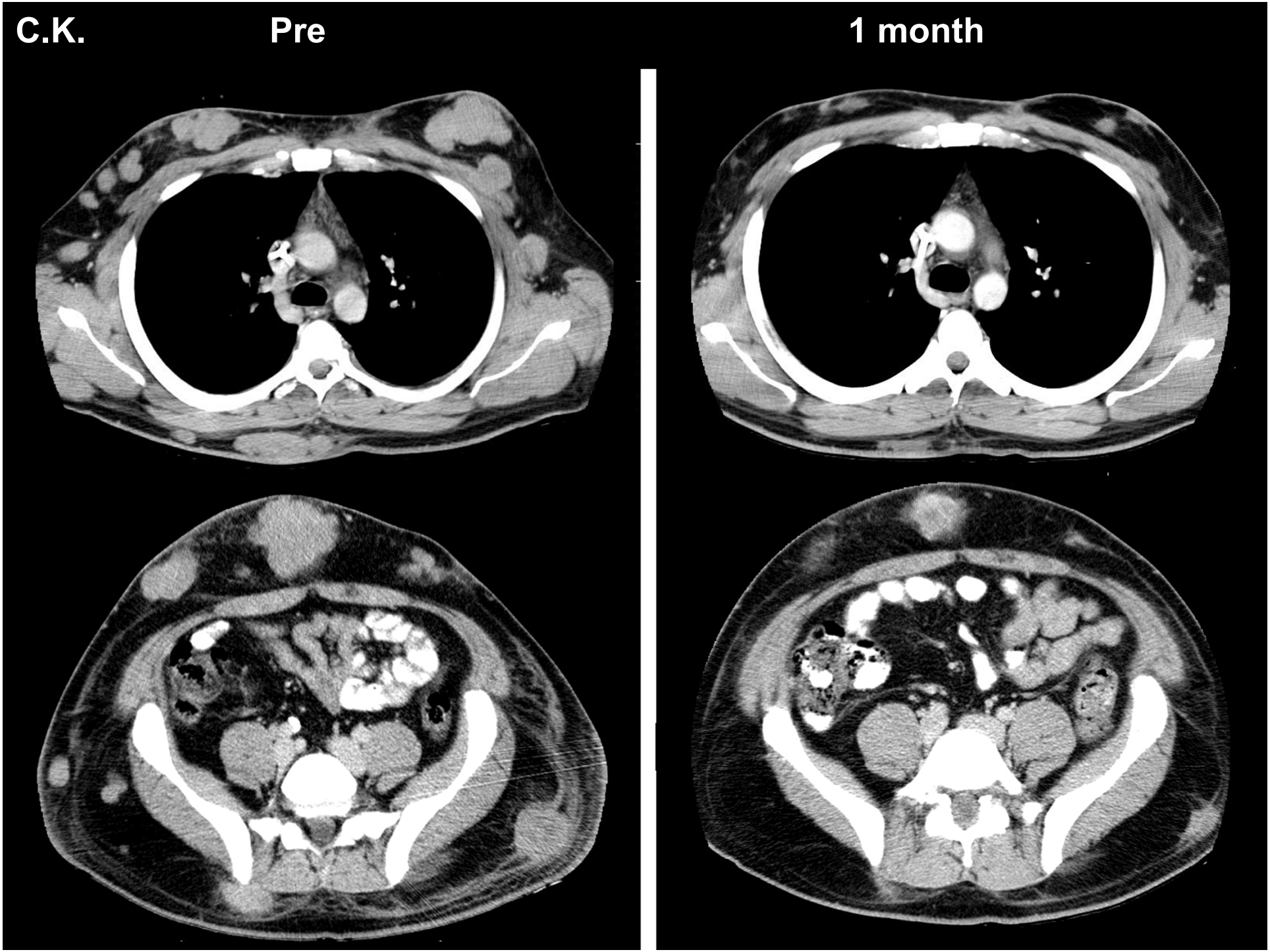




D.W. Pre

5 months

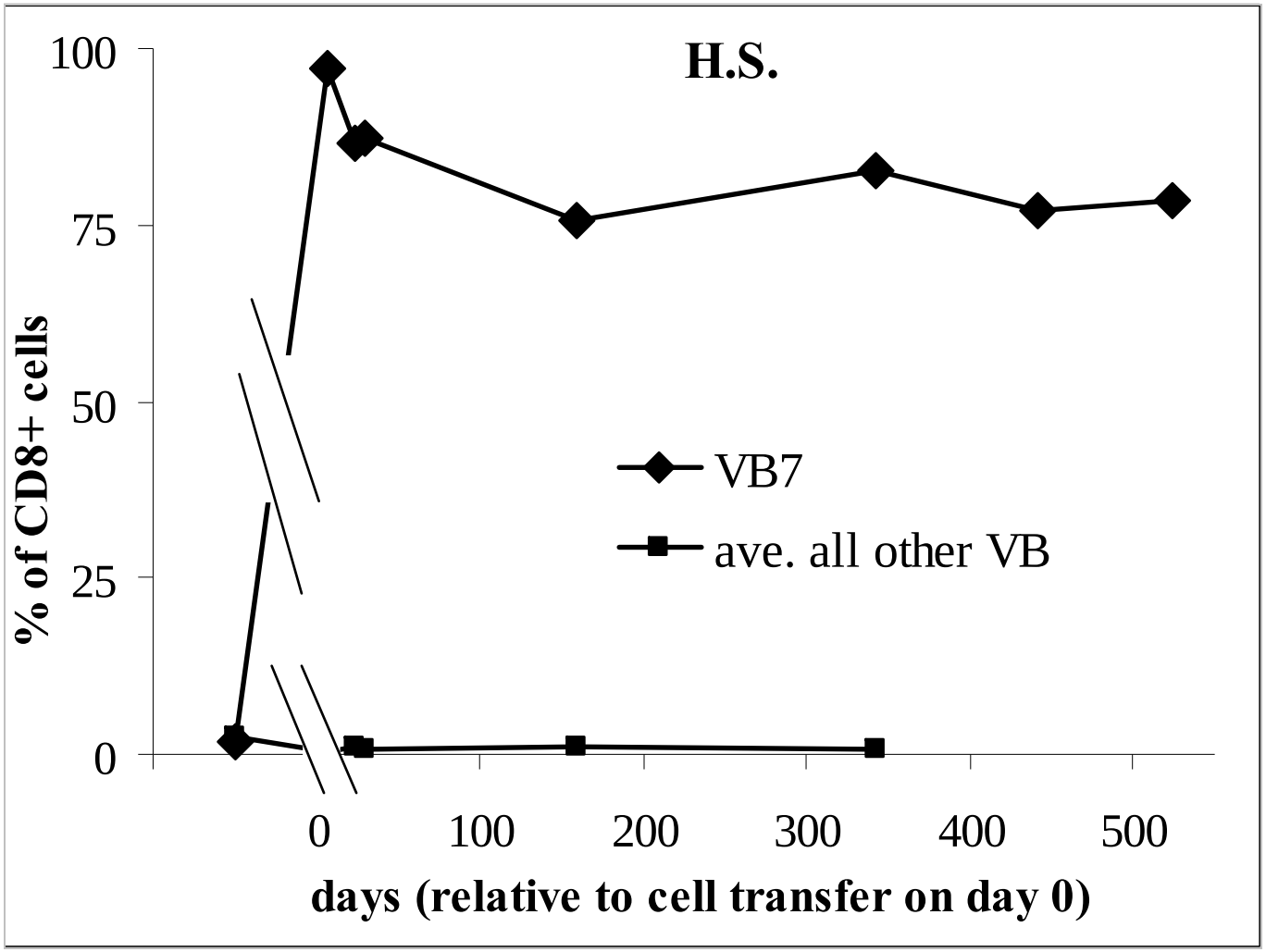



C.K.
Pre
1 month

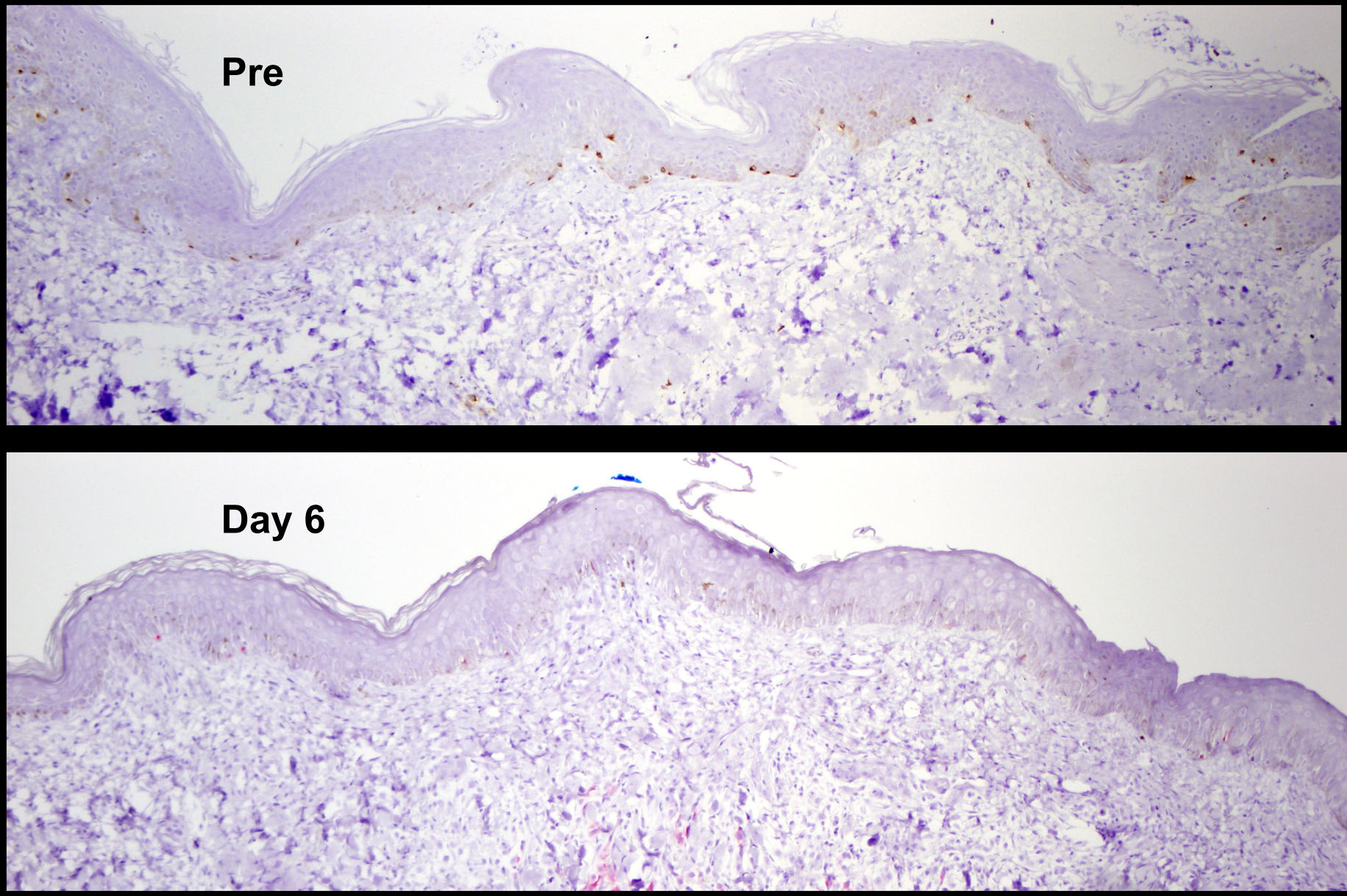
$CD8^+$ Vβ12 LYMPHOCYTES IN PATIENT D.M.

$CD8^+$ Vβ12 MART-1 reactive lymphocytes		
		% of $CD8^+$ that are $MART^+$
Administered in TIL:	1.3×10^{10}	90%
Circulating in Blood: day 7	6.4×10^{10}	71%
Circulating in Blood: day 19	3.8×10^{10}	78%
Circulating in Blood: day 55	2.6×10^{9}	83%

HS: Persistence of VB7 clone

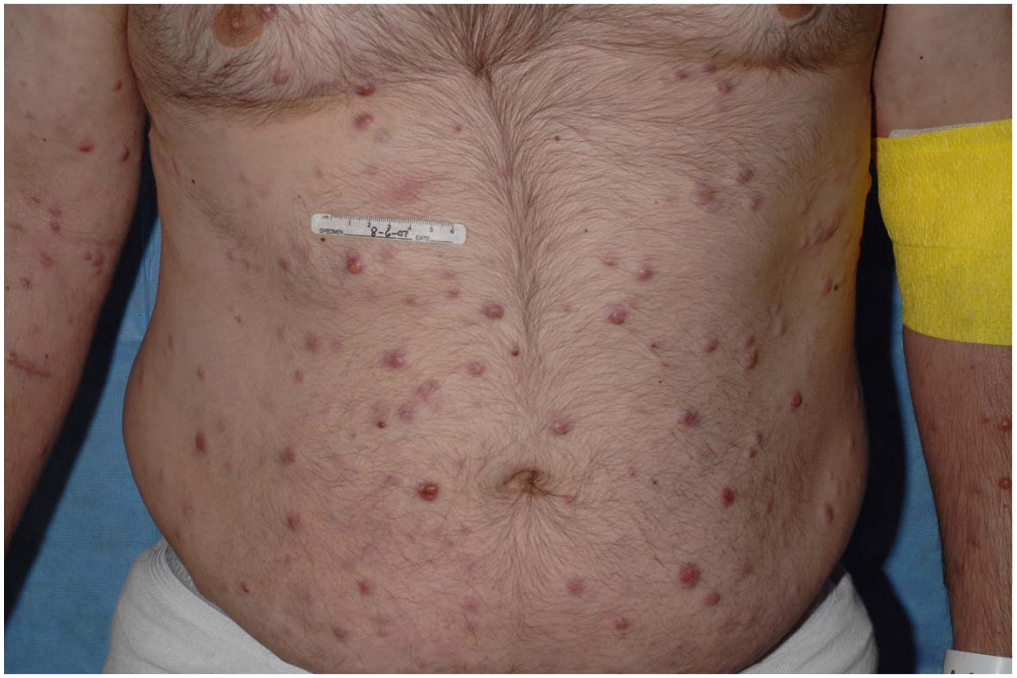


Elimination of melanocytes: MART F5 TCR cell transfer

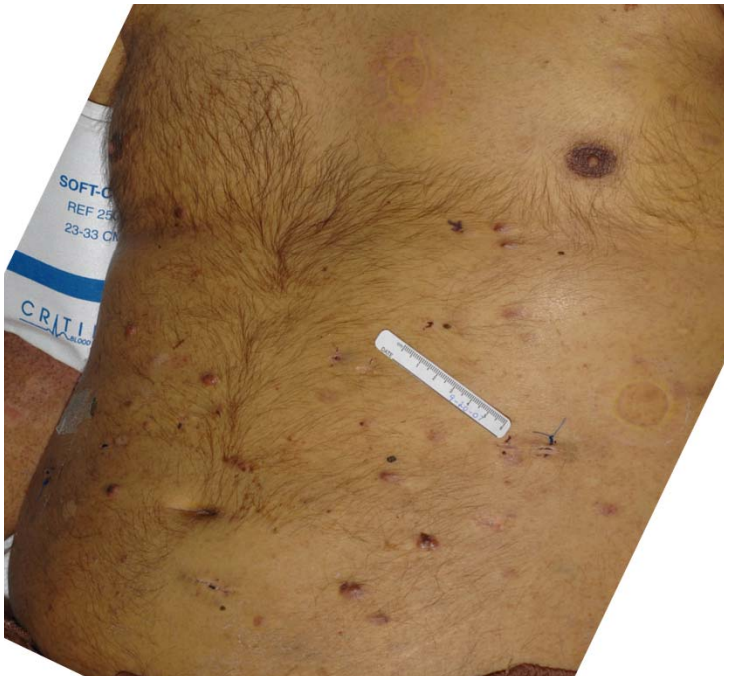


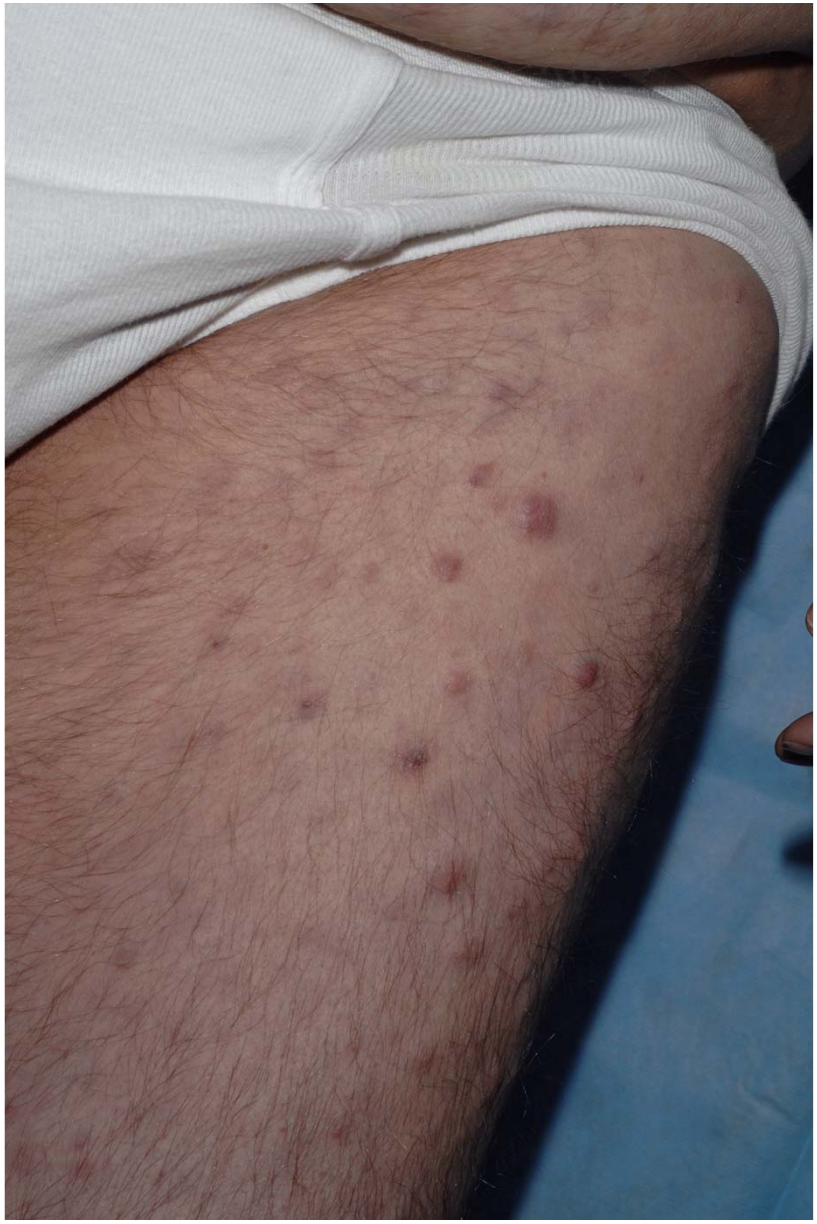
D.Th. F5 TCR

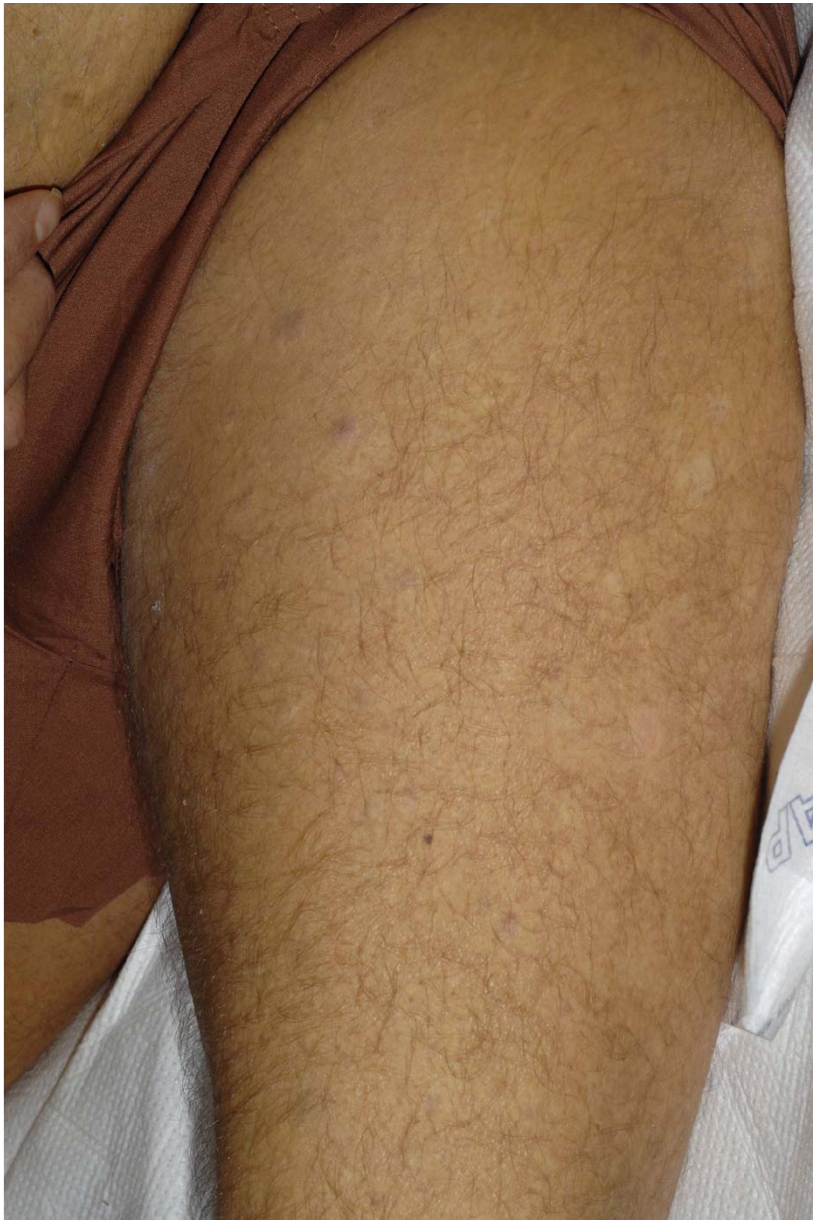
Pre



Day 36







8-6-2007 Pre-treatment Left thigh 9-20-2007 Day +36

UPDATED RESULTS OF CELL TRANSFER PROTOCOL IN PATIENTS WITH METASTATIC MELANOMA

	(Number of patients)
Total	35
Objective response	18 (51%)
Minor/mixed response	8 (23%)
No response	9 (26%)

(J. Clin. Oncol., 23:2346-2357,2005)

Modifications of adoptive immunotherapy to make it more widely applicable

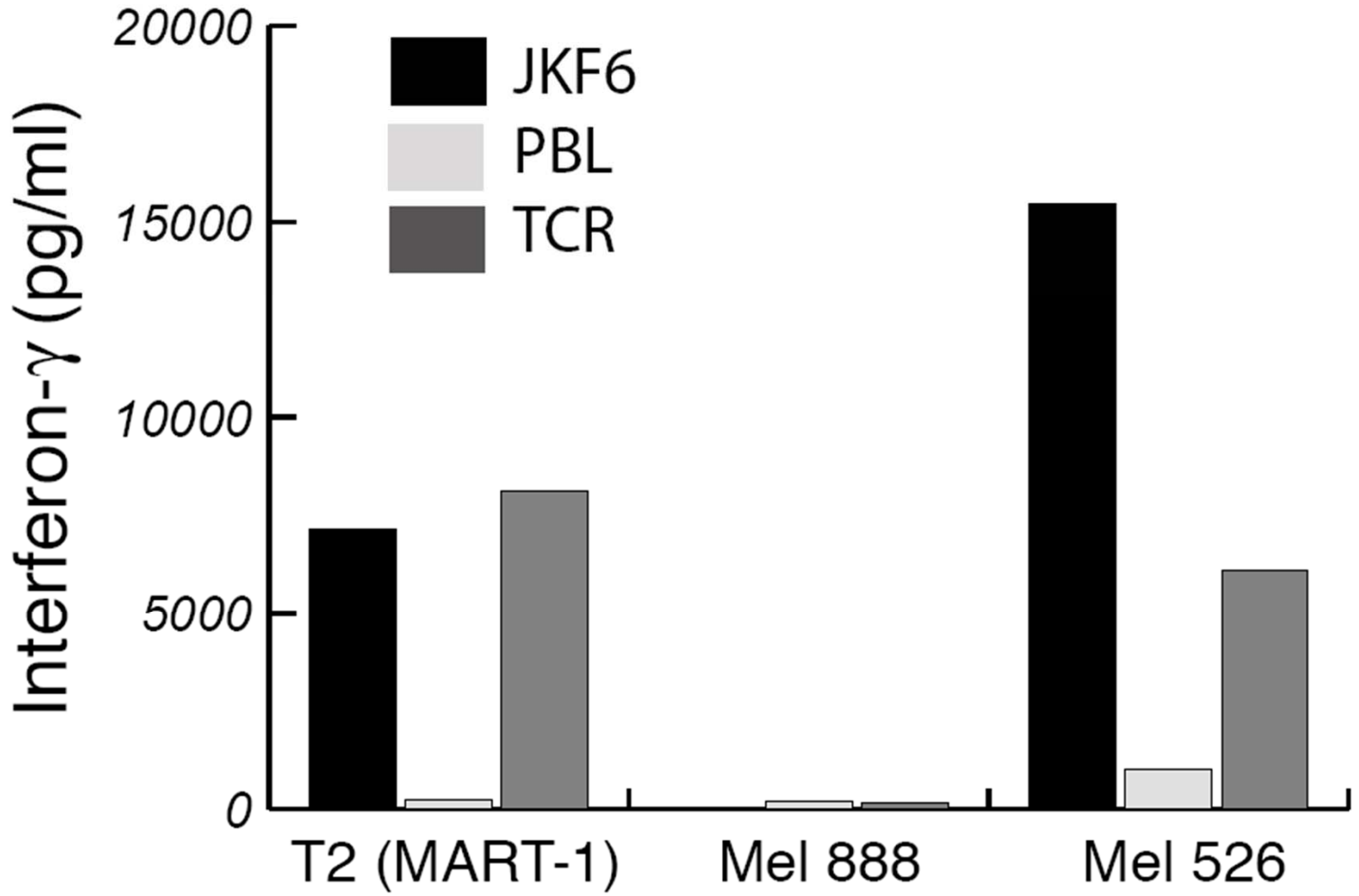
---

1. Simplify the generation of the transferred cells ("young TIL")
    1. single cell suspension of the entire resected tumor
    2. grow in IL-2 for 10-14 days (obtain 5e7 lymphocytes)
    3. rapid expansion in anti-CD3, autologous feeders and IL-2 for 2 weeks (1,000 fold expansion)
    4. administer cells following host lymphodepletion (no cell testing)

2. Administer low-dose subcutaneous IL-2 (instead of high-dose i.v.)
    1. 125,000 IU/kg s.c. qdx5, 2 day rest, 6 cycles (6 weeks)
    2. can be administered as an outpatient

(Use of "young TIL" and low-dose IL-2 could result in a faster, cheaper, outpatient treatment)

Transduced PBL specifically recognize tumor cells



SUMMARY

1. High affinity anti-tumor TCR can be identified by screening clones from anti-tumor TIL or PBL.
2. Transduction of anti-melanoma/melanocyte TCR provides normal T cells with a high level of in vitro activity.
3. Administration of these transduced cells to patients can result in destruction of melanocytes and melanoma.
4. These transduced T cells can survive for prolonged periods in vivo.

Status at the time of the last site visit report (September 10, 2004)

---

1. **Identification of Cancer Associated Antigens and Their Use in the Active Immunization of Patients with Cancer**
    1. 24 new cancer antigens
    2. active immunization protocols with fowlpox, DNA, peptides
    3. first studies in humans of anti-CTLA-4

2. **Development and Evaluation of Cell Transfer Immunotherapies for Patients with Cancer**
    1. 13 patients received cell transfer with TIL following lymphodepletion (published in 2002; 35 patients published in April 2005)
    2. in vitro studies of anti-tumor T cell receptors

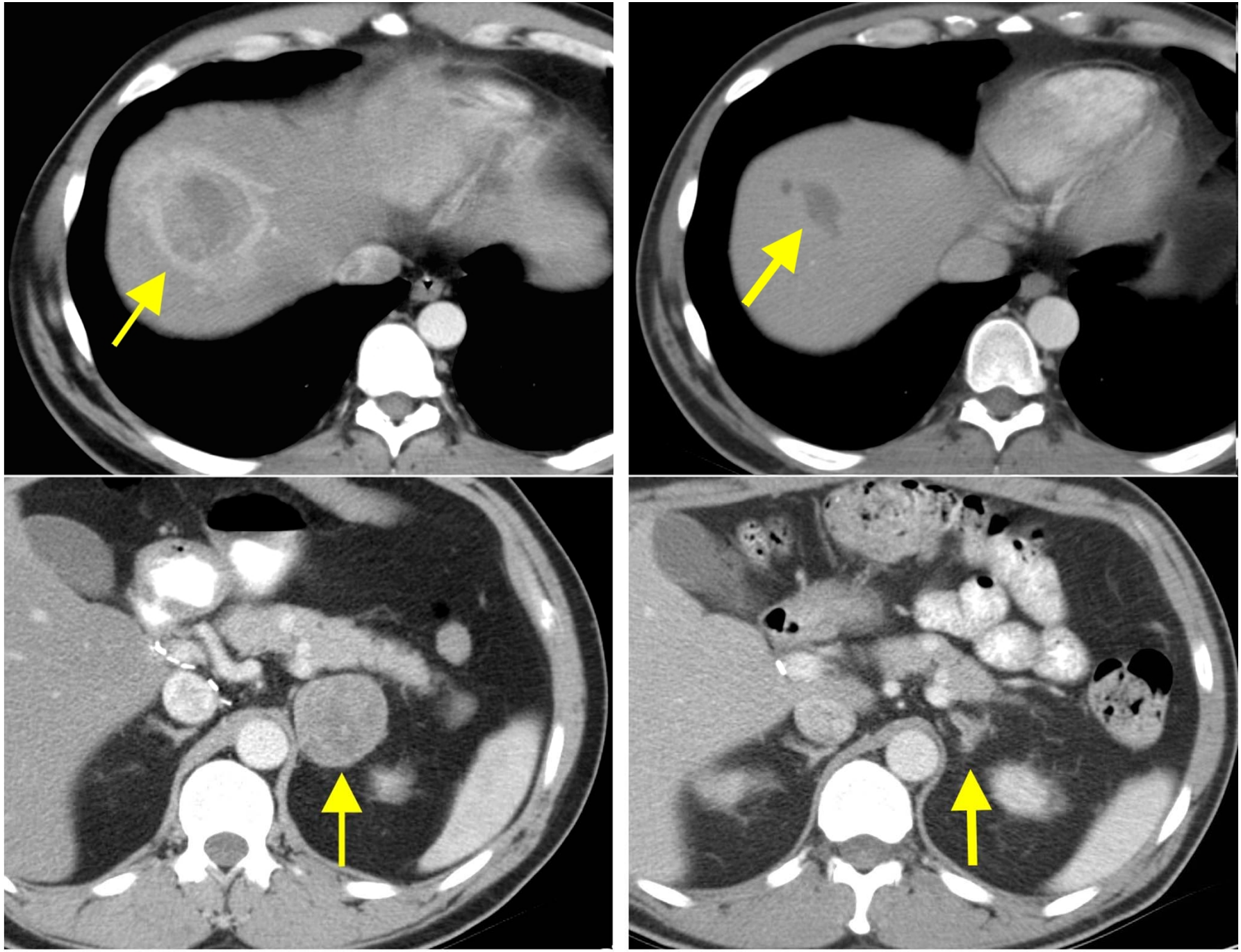
Goal (9/04 - 5/08)
To develop effective cell transfer immunotherapies for patients with cancer

---

1. **Development and analysis of cell transfer therapies using tumor infiltrating lymphocytes for patients with cancer.**

2. **Development of cell transfer therapies based on the genetic modification of autologous lymphocytes.**

J.B.
(1200
TBI)



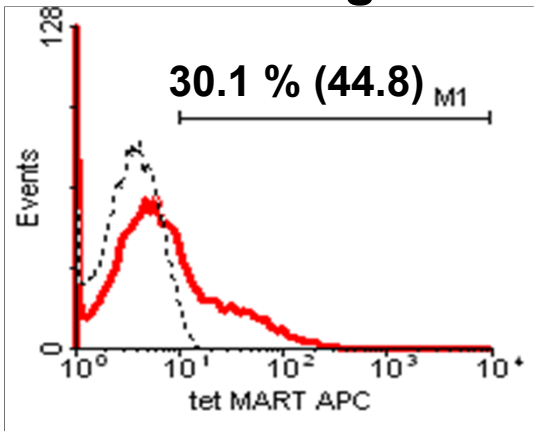
Pre-Treatment

3 Months

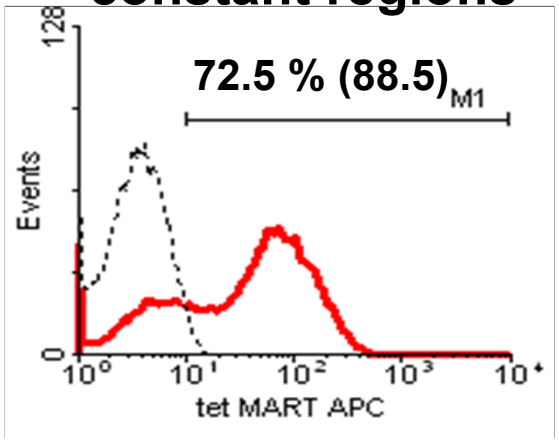
Expression of original and mouse-hybrid TCRs

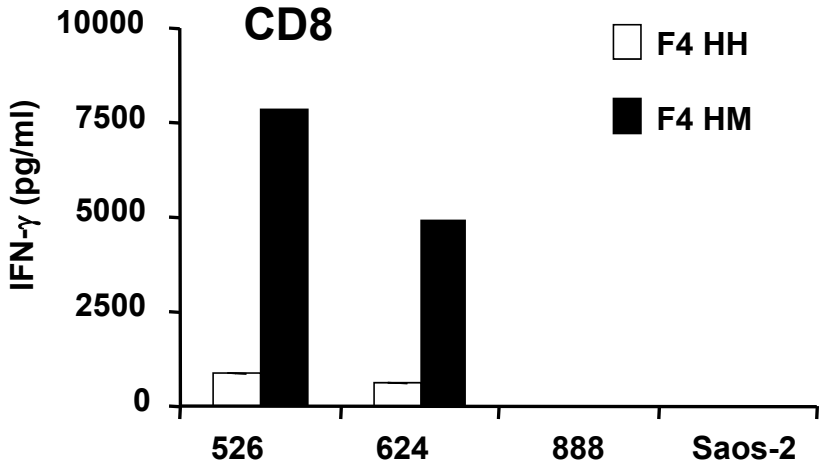
F4

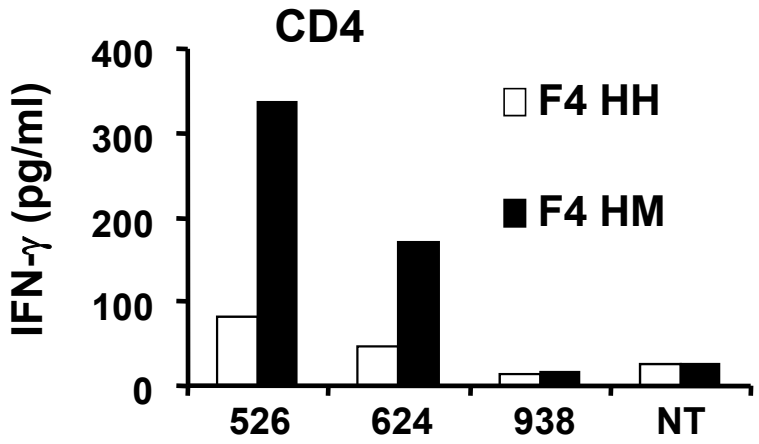
Human constant regions



Murine constant regions



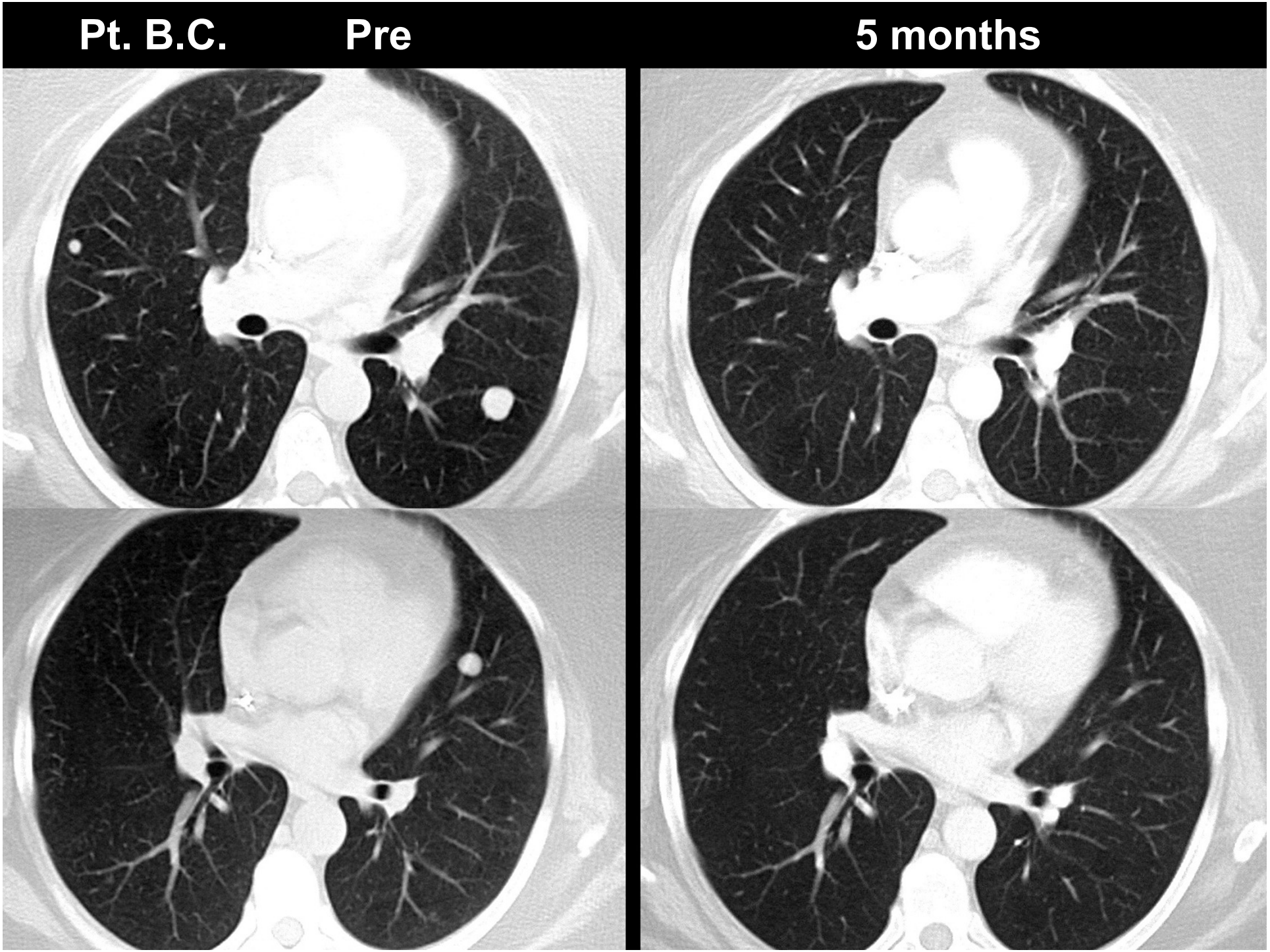


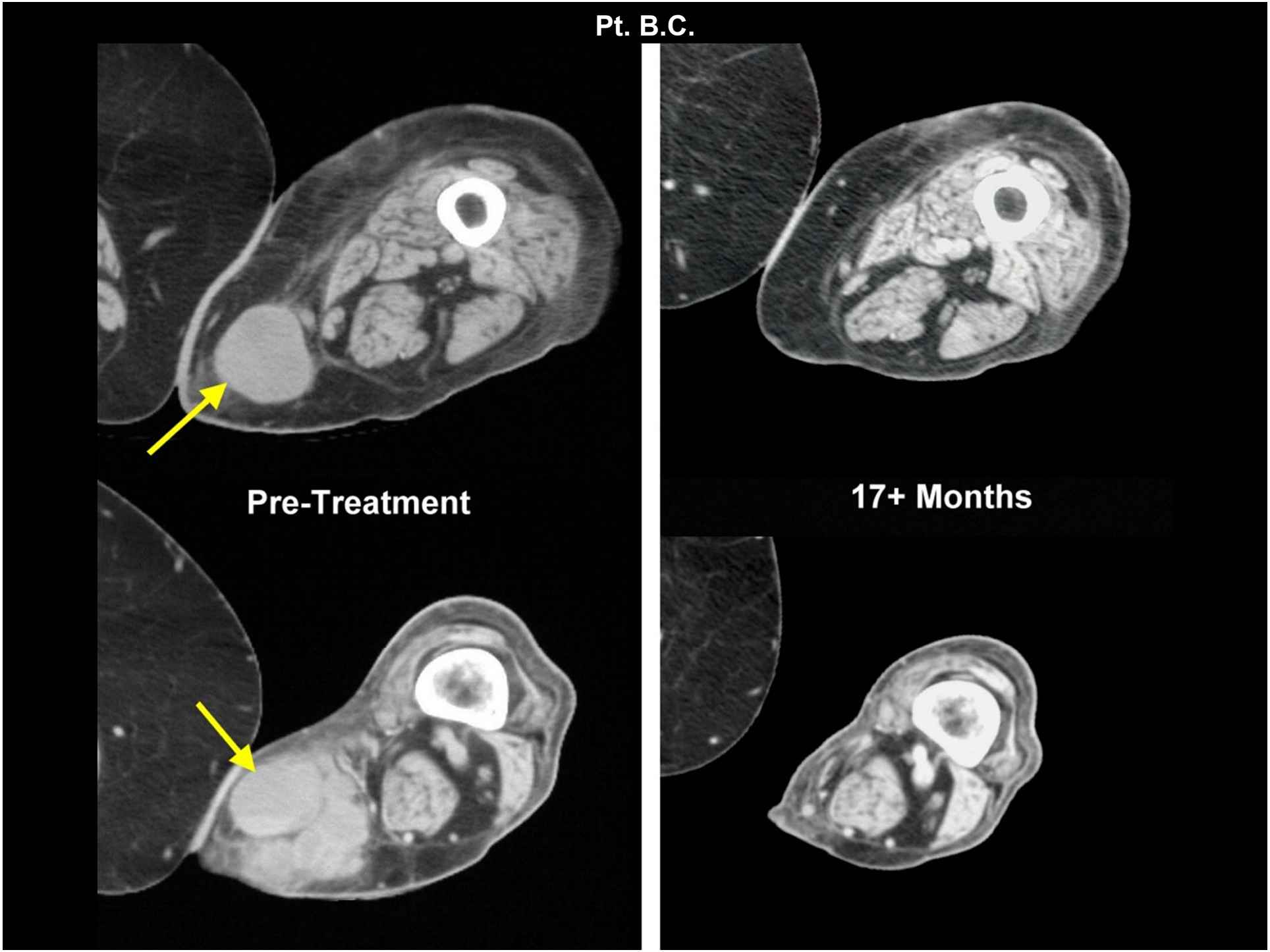


(Cohen et al, Cancer Res., 2006)

Approaches to Improve the Effectiveness of TCR-based Cell Transfer Therapy

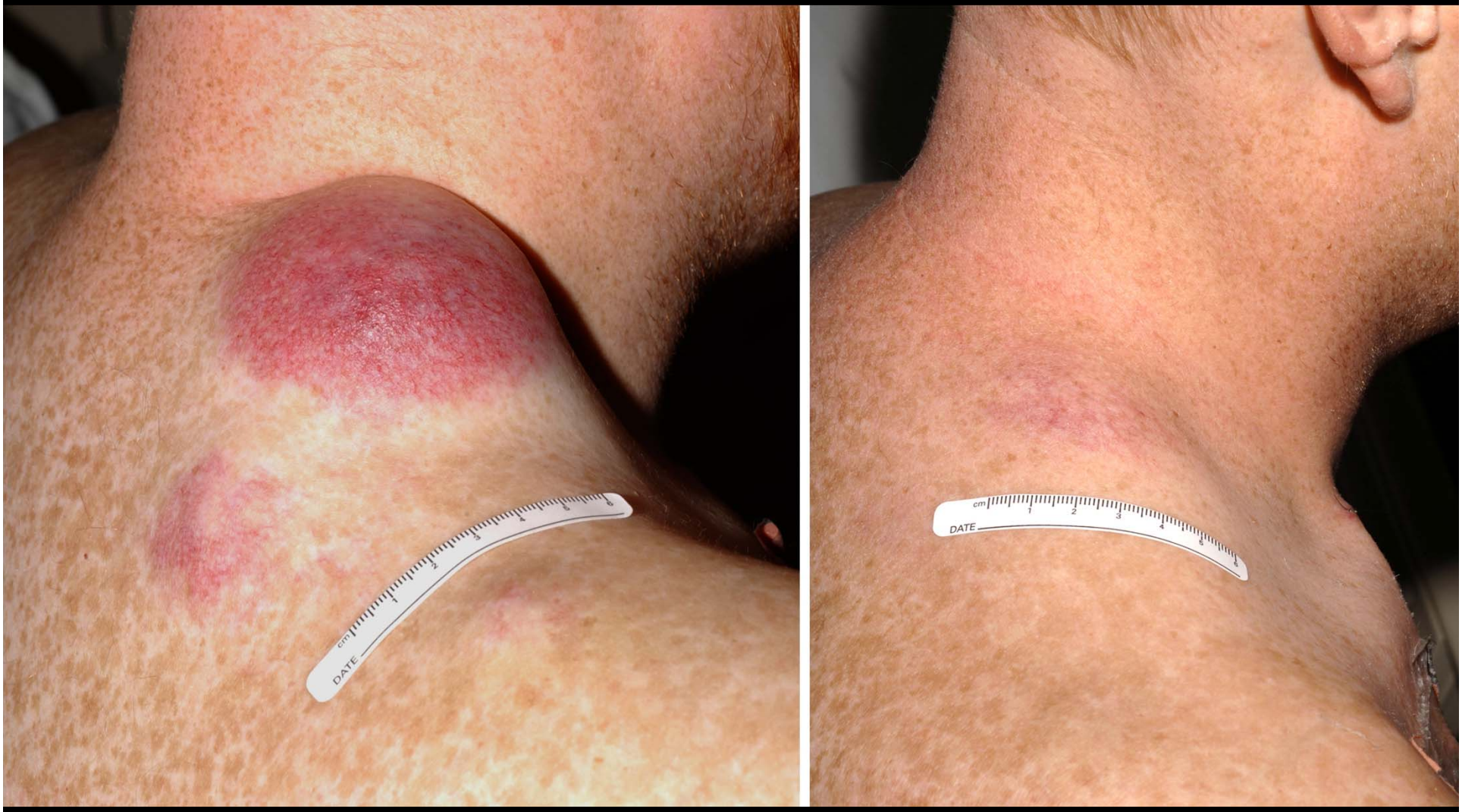
1. Add vaccines
2. Stimulate antigen present cells (TLR agonists)
3. IL-15 (instead of IL-2)
4. IL-12 (plus IL-2)
5. Block immune inhibitory factors
    1. Selective removal of T regulatory cells
    2. anti-CTLA-4
    3. anti-PD1/PDL1
6. Combine with anti-angiogenic approaches to increase lymphocyte infiltration into tumors
7. Additional gene-modification of TCR transduced PBL
    1. cytokines (IL-2, IL-15)
    2. costimulatory molecules (41BB, CD28)
    3. homing molecules (CD62L, CCR7)
    4. anti-apoptosis molecules (Bcl-2)

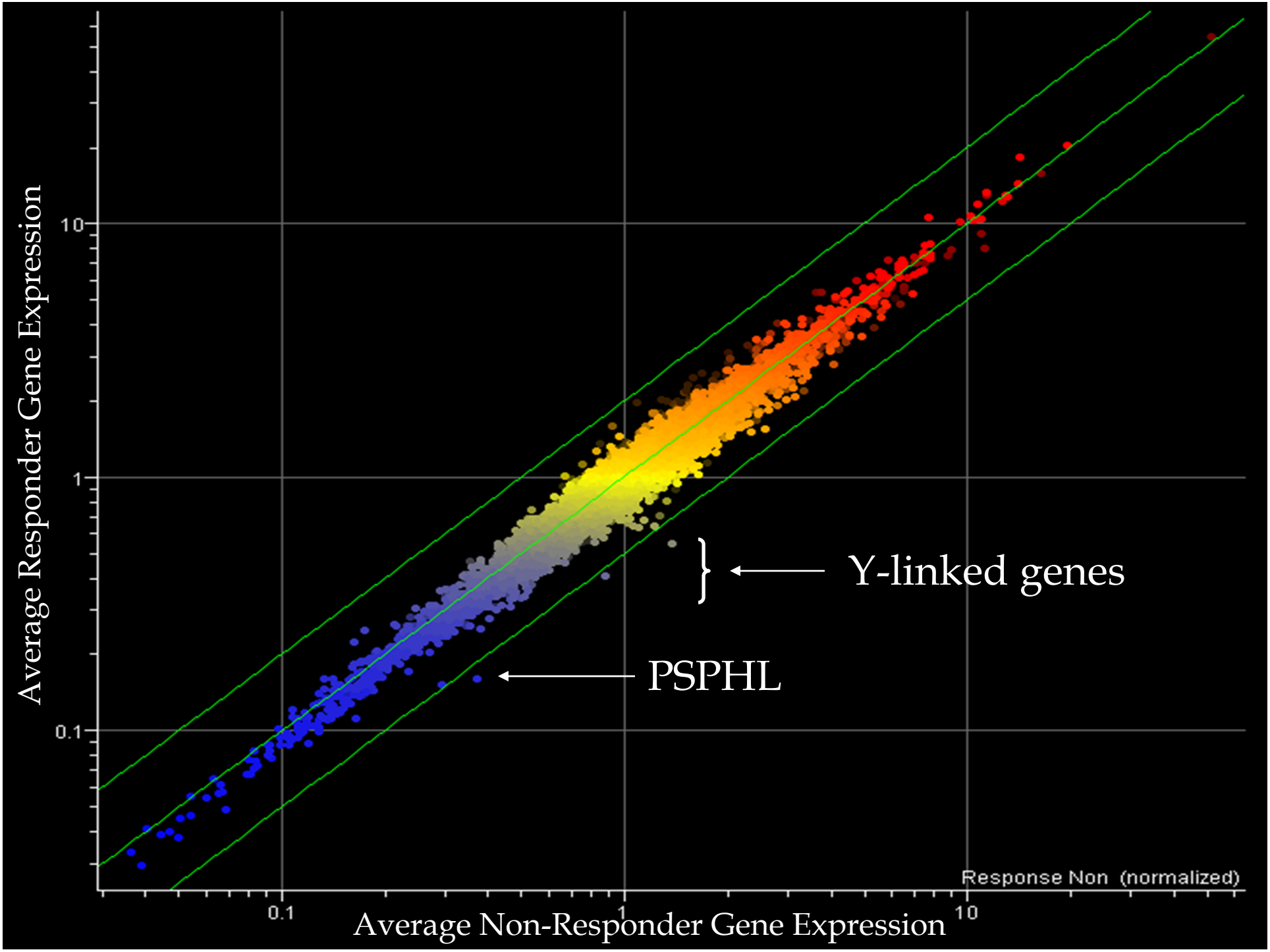

Pt. B.C.
Pre
5 months

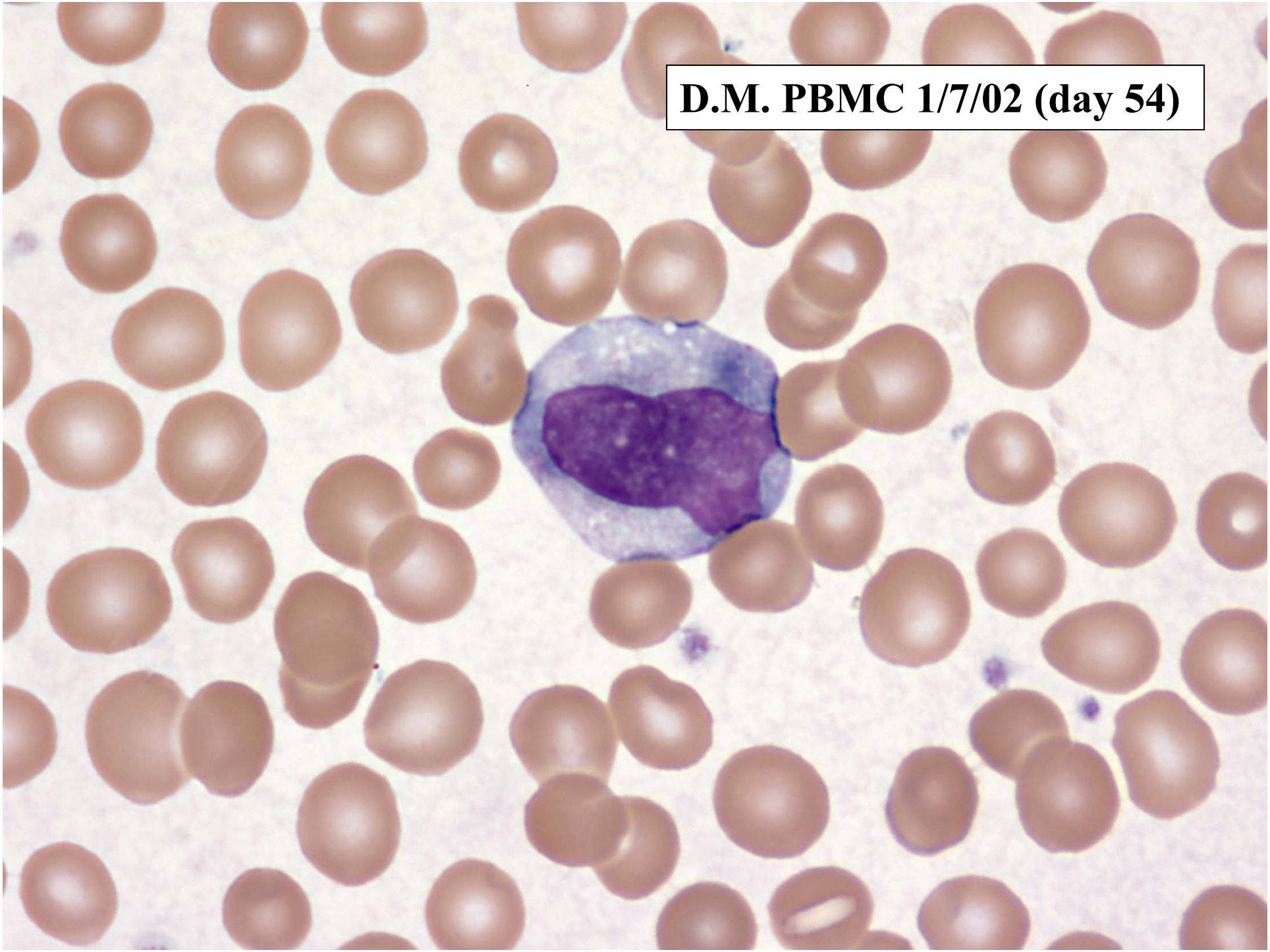

Pt. B.C.
Pre-Treatment
17+ Months

C.K. (200cGy) Pre

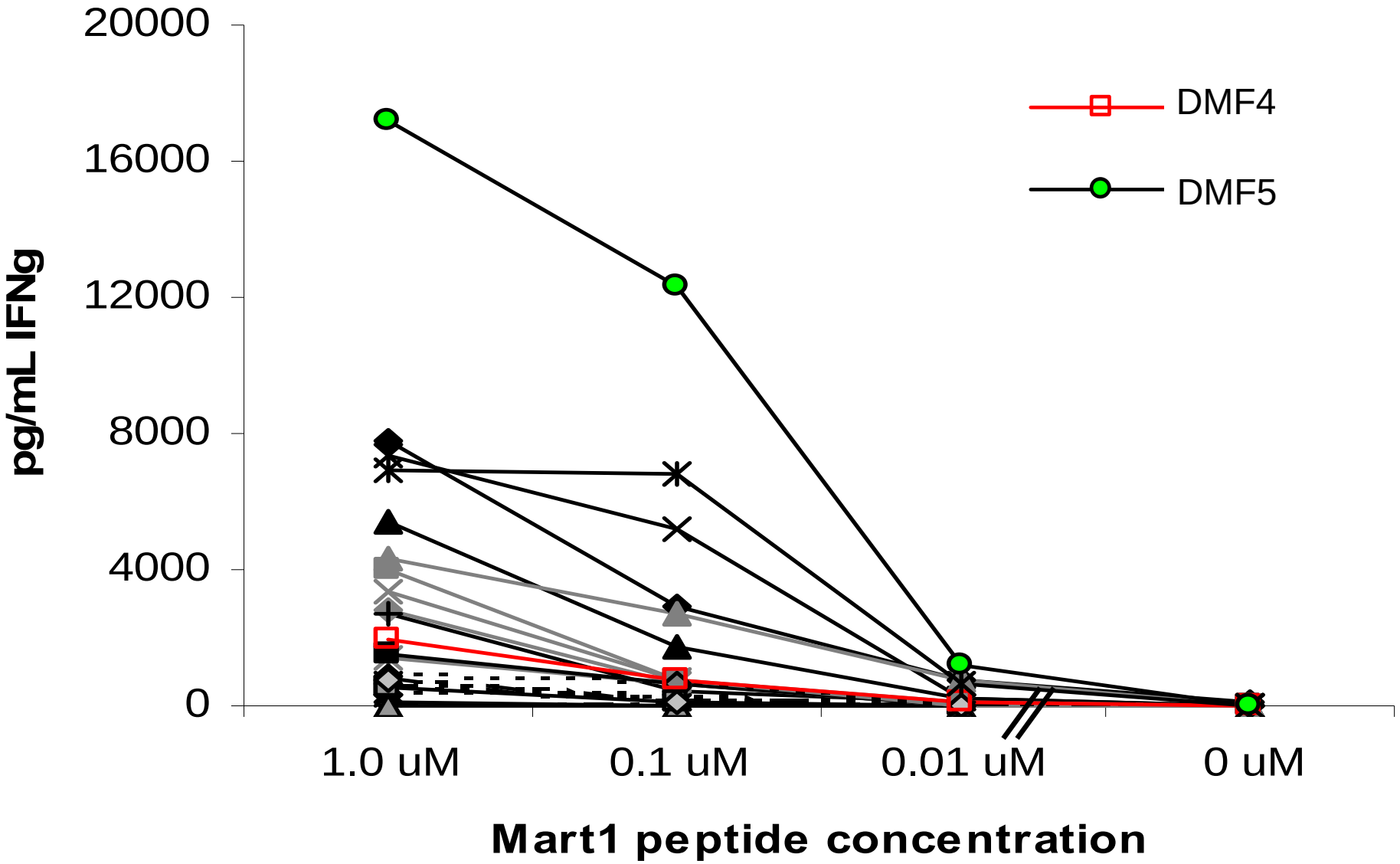
12 days




Average Responder Gene Expression
10
1
0.1
Y-linked genes
PSPHL
Response Non (normalized)
0.1
1
10
Average Non-Responder Gene Expression


D.M. PBMC 1/7/02 (day 54)

MART-1 TIL clones show diverse avidities to MART-1 peptide on target cells



What are some potential reasons for the low response rate?

Suboptimal TCR

Mispairing of the inserted alpha and beta chains with the endogenous chains

Low function of the transduced TCR

Poor traffic of the transduced cells to lymph nodes or tumor

Identification of High-Affinity T Cell Receptors for Use in Cell Transfer Gene Therapy

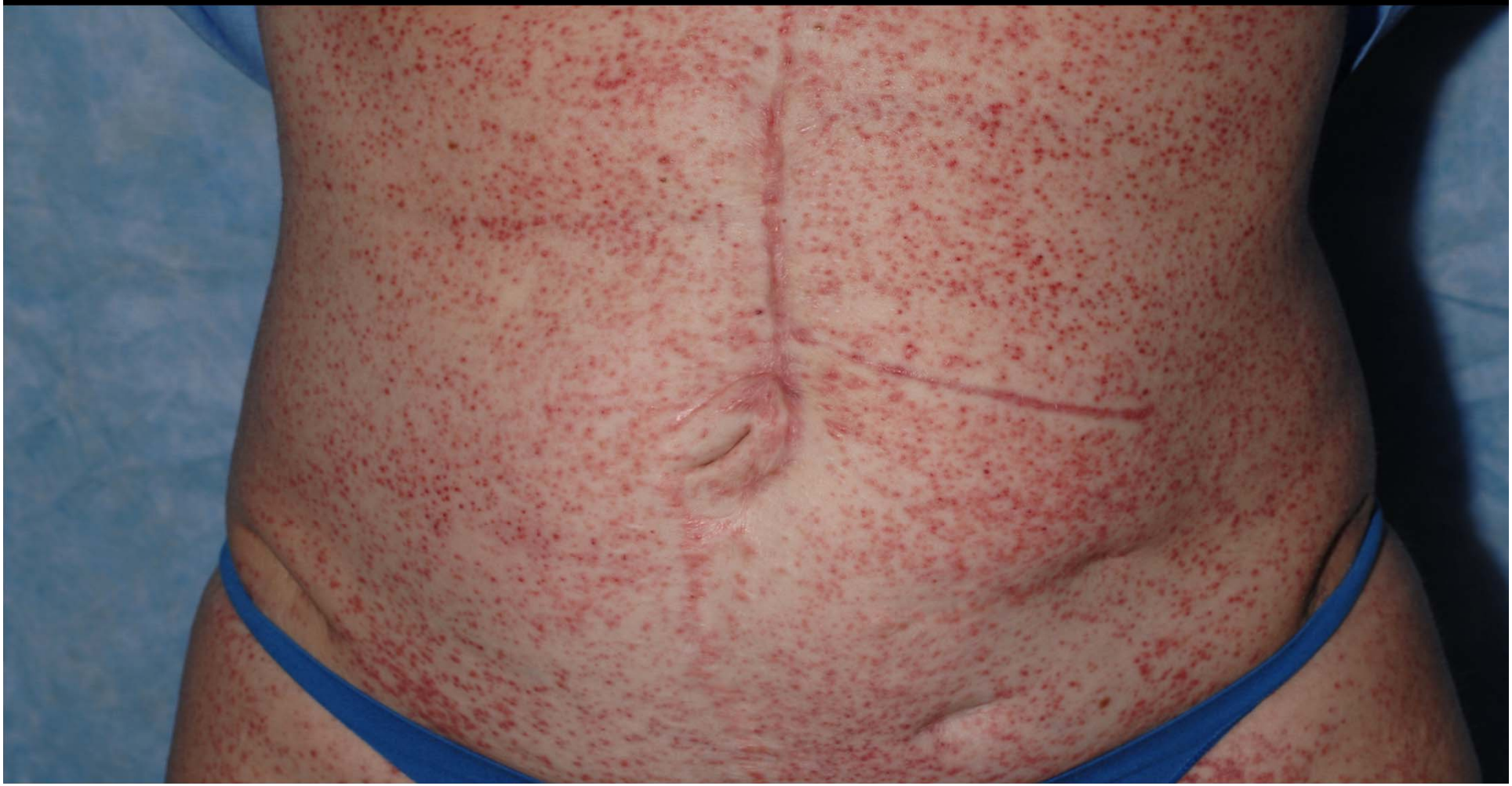
---

1. Screen large numbers of anti-tumor TIL and PBL clones

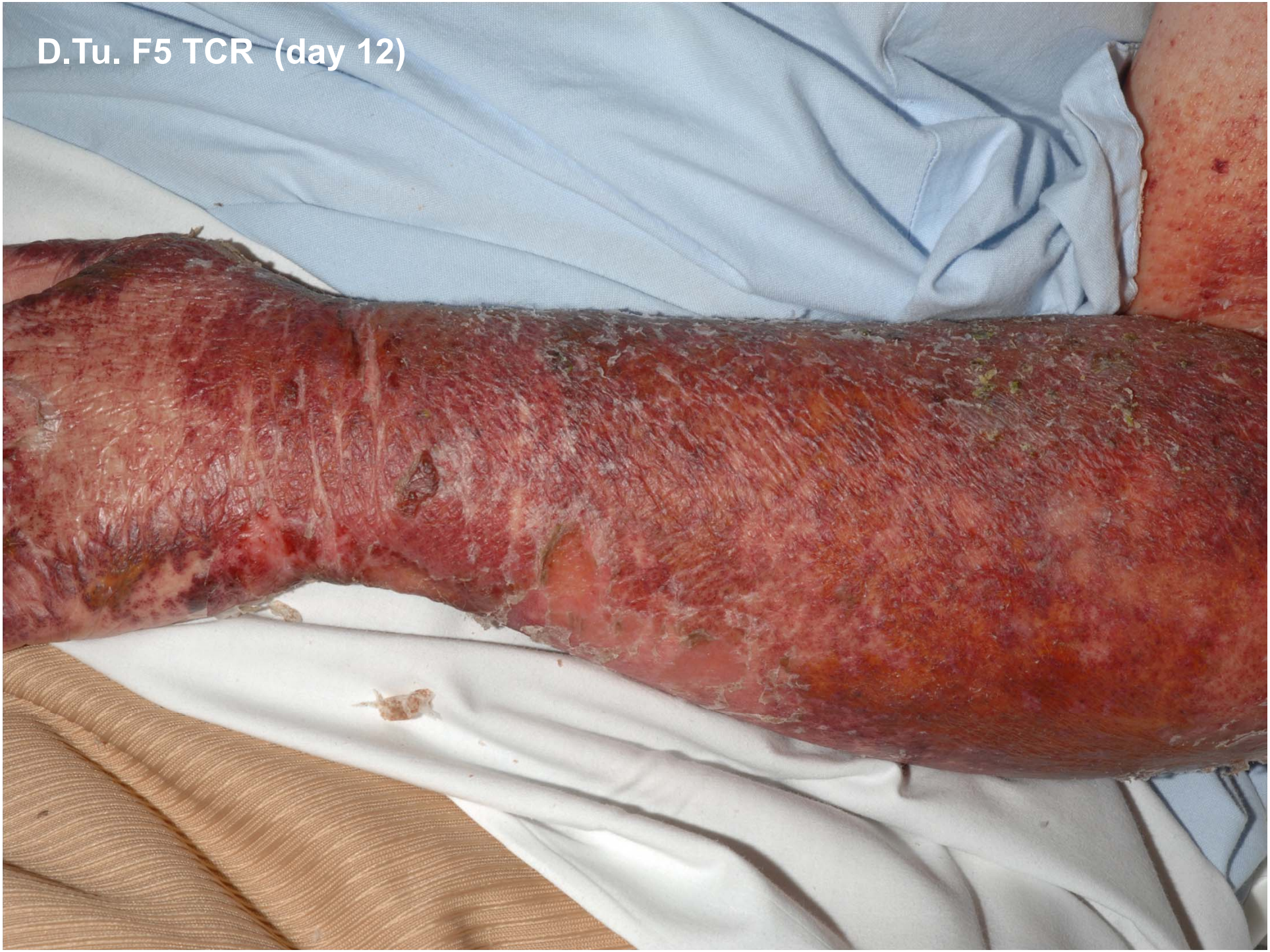
2. Immunize HLA transgenic mice (bypass tolerance to human self peptides)

3. Mutagenesis of CDR2 and CDR3 regions of anti-tumor TCRs.

J.W gp100:154 TCR day 6



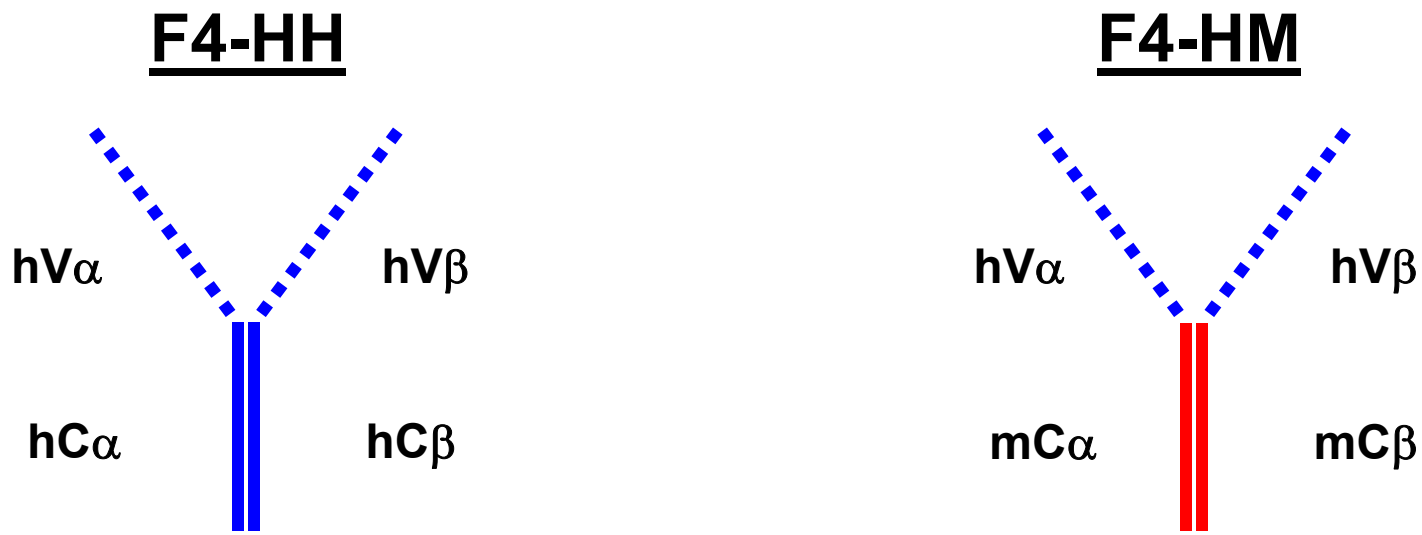
What is the target of the transferred cells in the skin?


D.Tu. F5 TCR (day 12)

Anti-Mart-1 F5 TCR Patients			Anti-gp100:154 TCR Patients		
Patient	Peak IFN-γ pg/ml	Day	Patient	Peak IFN-γ pg/ml	Day
DTu*	202	5	JW*	65	6
JS*	25	4	DG*	438	7
DTh*	3,062	5	JS*	20	3
SC*	0	6	DH*	0	5
CP*	19	7	DD*	92	13
JT*	4	6	RM	64	6
VB	33	4	RD	270	2
JK	90	6	MP	283	2
GG	38	2	AC	80	5
CF	136	4	SS	142	3
AP	73	5	LM	66	4
NG	305	3			
SS	23	7			
GK	256	5			

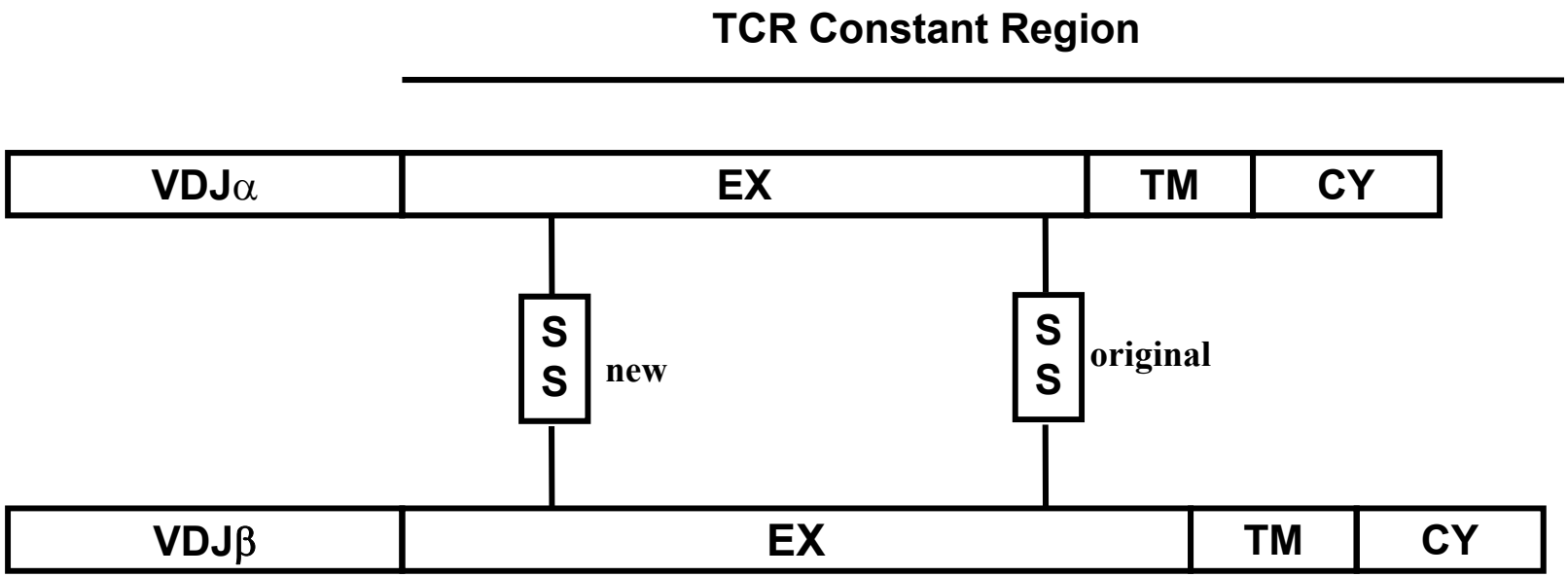
(Normal levels < 5 pg/ml)

Building human/mouse hybrid TCRs



* H: Human, M: Mouse; HM = Human VR+ Mouse CR

Engineering of an additional disulfide bond between the TCR chains



What are some potential reasons for the low response rate?

Suboptimal TCR

Mispairing of the inserted alpha and beta chains with the endogenous chains

Low function of the transduced TCR

Poor traffic of the transduced cells to lymph nodes or tumor

DEVELOPMENT OF HUMAN CANCER IMMUNOTHERAPY

1. Stimulation of T cells with IL-2 can mediate regression of metastatic cancer in patients with melanoma and renal cancer.

2. Blockade of lymphocyte inhibitory signals can mediate cancer regression (but also induces autoimmunity).

3. Transfer of anti-tumor T cells following lymphodepletion mediates objective regressions in 50-70% of patients with metastatic melanoma

4. Normal human peripheral lymphocytes can be genetically modified and mediate effective cancer immunotherapy.

5. Improvements in this gene therapy approach such as the use of high affinity TCR and prevention of mispairing are being developed.

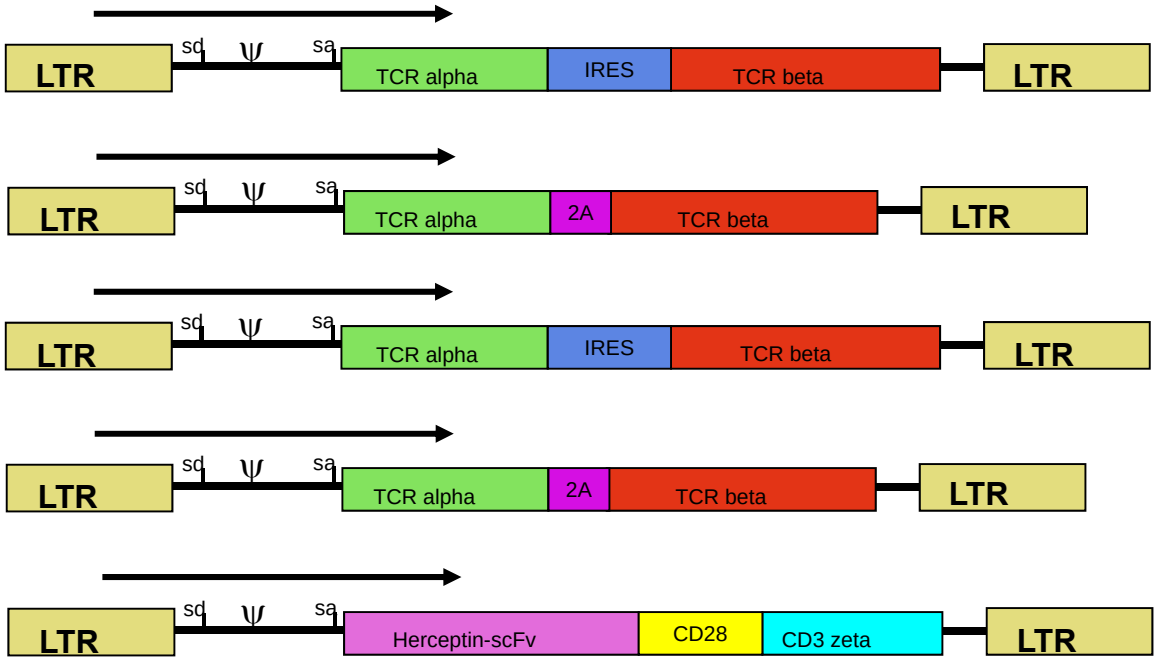
6. This approach is currently being explored for the immunotherapy of patients with melanoma and common epithelial cancers.

Current efforts to enhance cell transfer therapy by genetic modification of the transferred cells

---

- **Provide high affinity T cell receptors**
- **Improve function of transferred cells**
- **Extend cell transfer therapy to patients with common epithelial cancers**

Anti-tumor antigen receptor containing retroviral vectors



Target
Tumor Antigen

p53

MART-1

gp100

NY-ESO-1

Her2/neu

Current efforts to enhance cell transfer therapy by genetic modification of the transferred cells

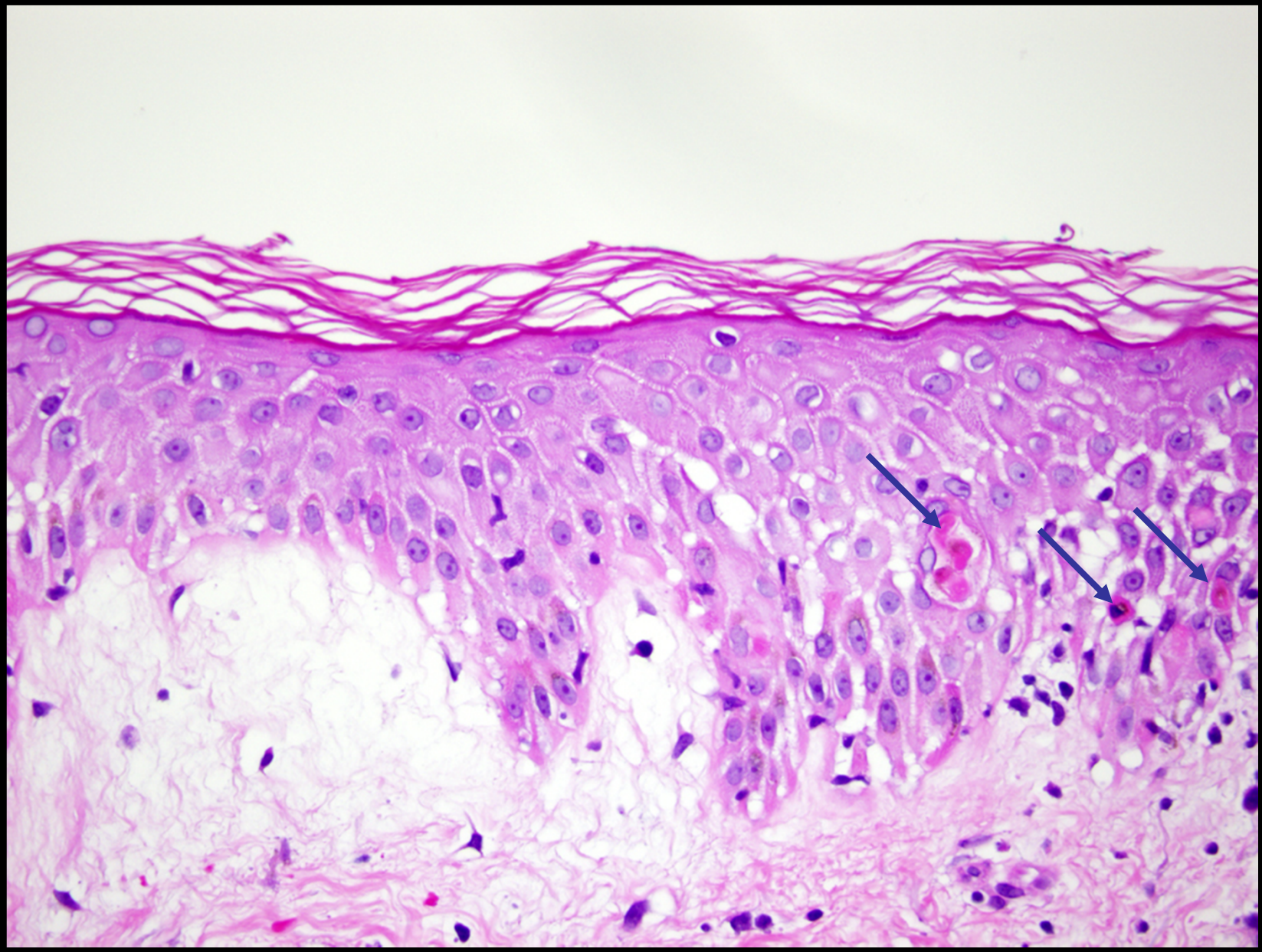
---

- **Provide high affinity T cell receptors**
- **Improve function of transferred cells**
- **Extend cell transfer therapy to patients with common epithelial cancers**

Very Preliminary Evaluation of Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

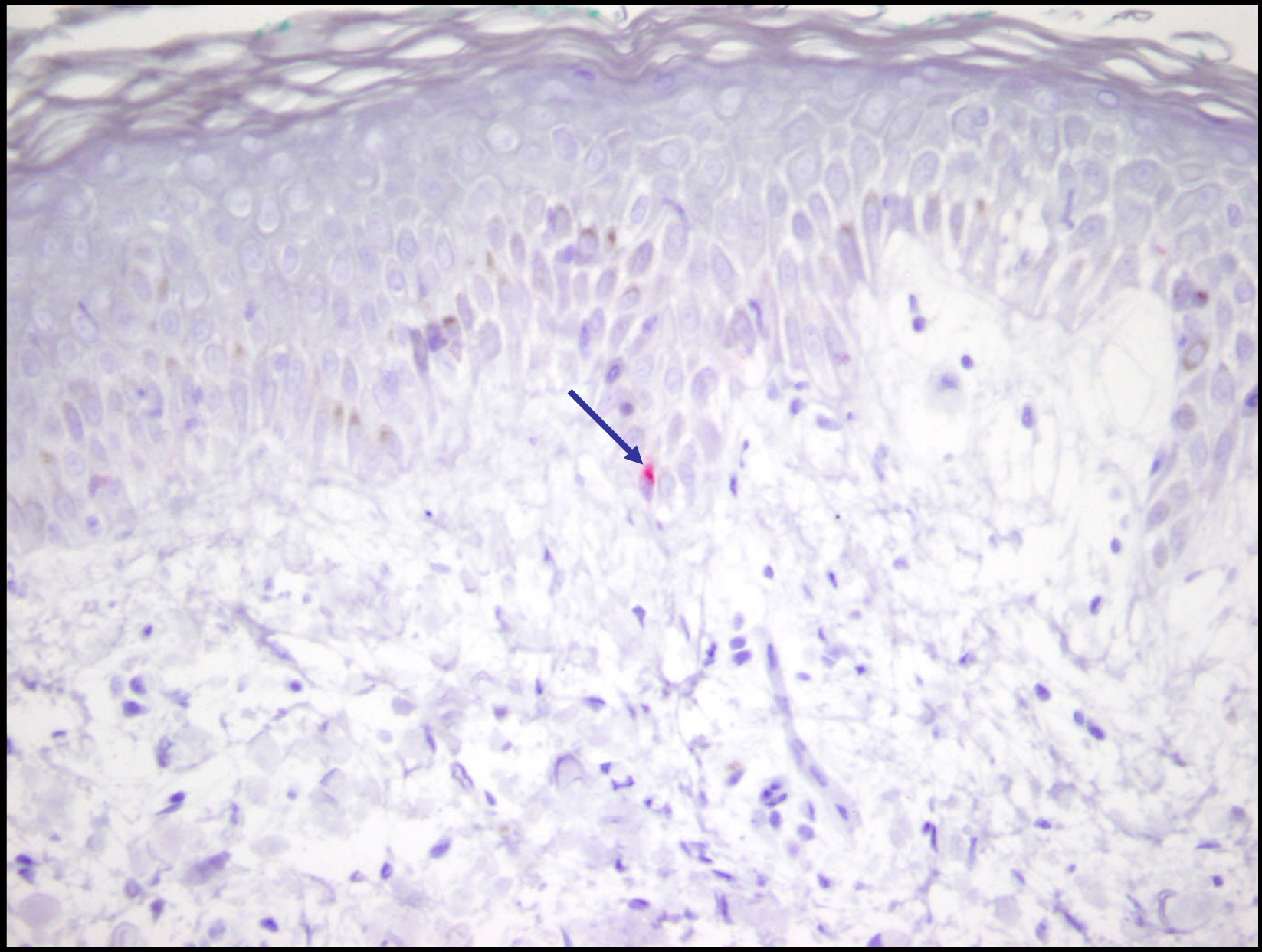
Cohort	Cell#	IL-2	Response	
			Total	OR
1	1-3x10^{10}	limited	6	2
2	~3x10^{9}	to tolerance	5	1
3	1-8x10^{10}	to tolerance	8	2
		Total	19	5 (26%)

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

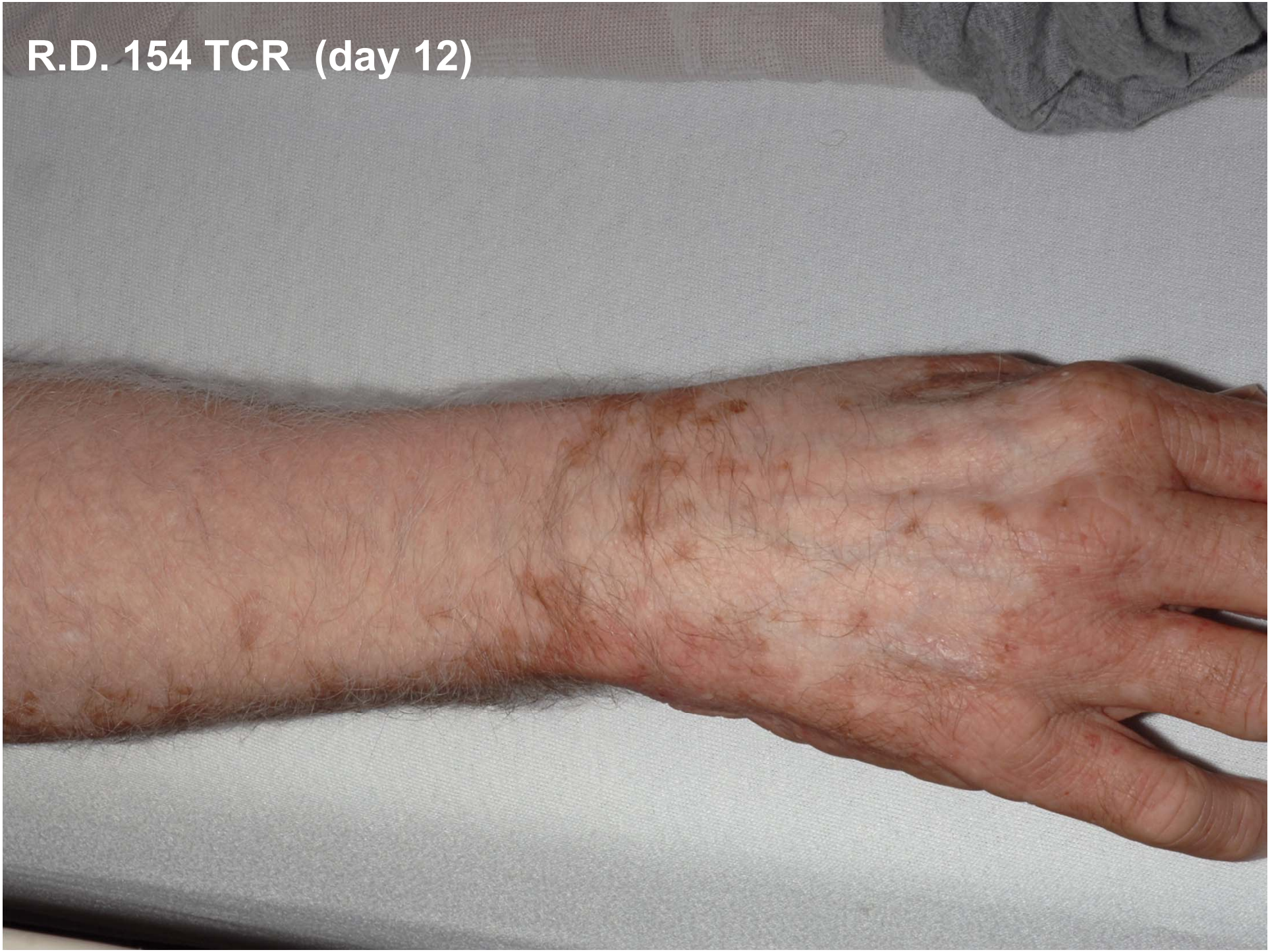


Day 5 post F5 TCR cell infusion (D. Tu)

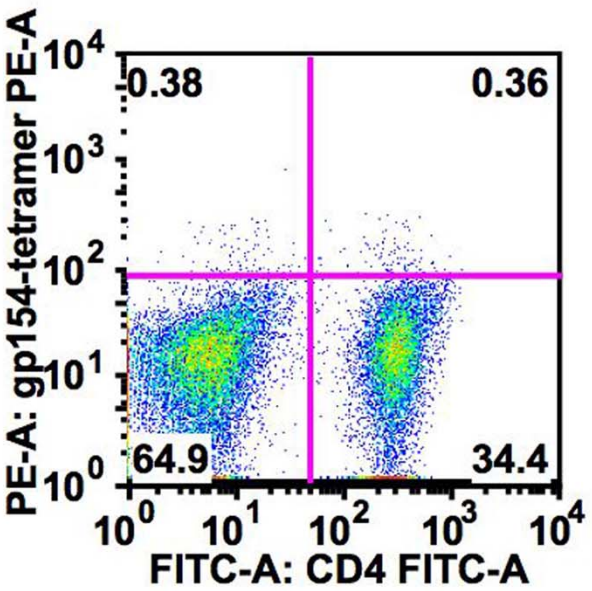
Skin: Spongiotic vesicles + necrotic/dyskeratotic keratinocytes



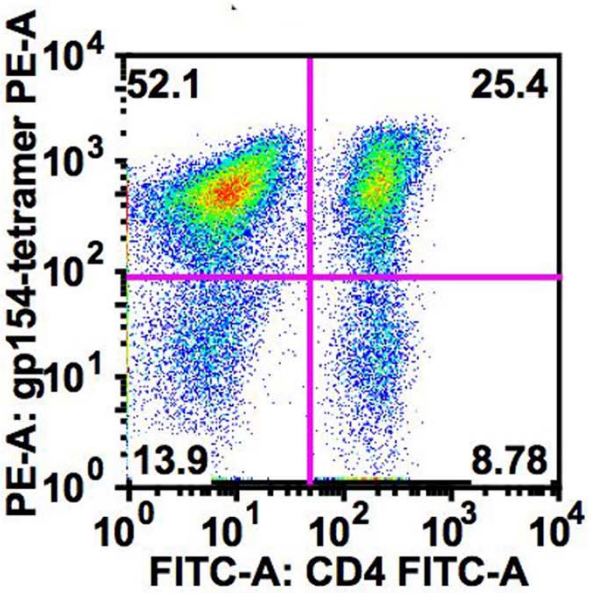
Melan-A (rare specific staining)


R.D. 154 TCR (day 12)

Efficient TCR gene transfer into PBL, patient J.S.



UnTd



gp100(154) TCR

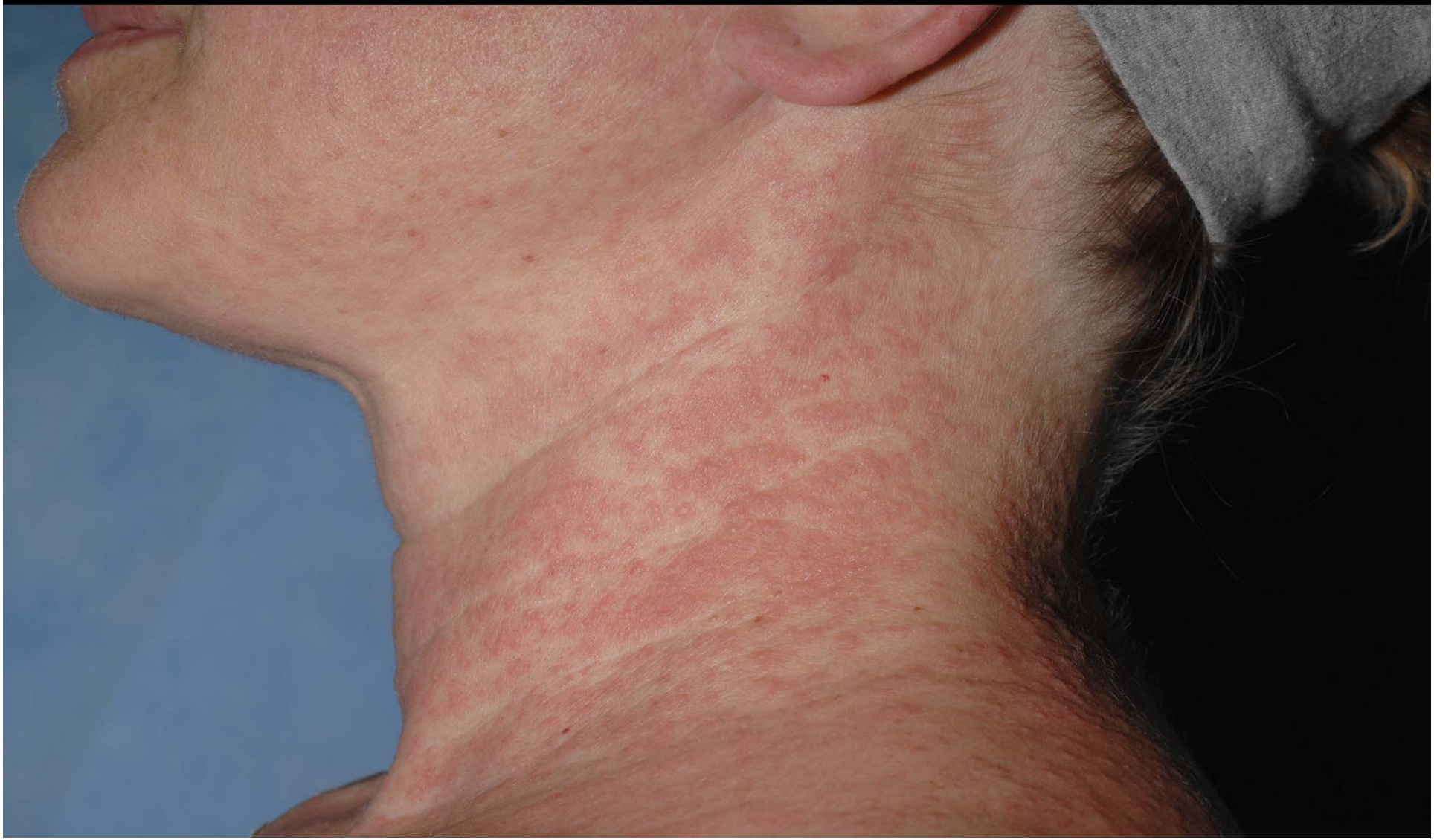
78% Tet+

Tetramer

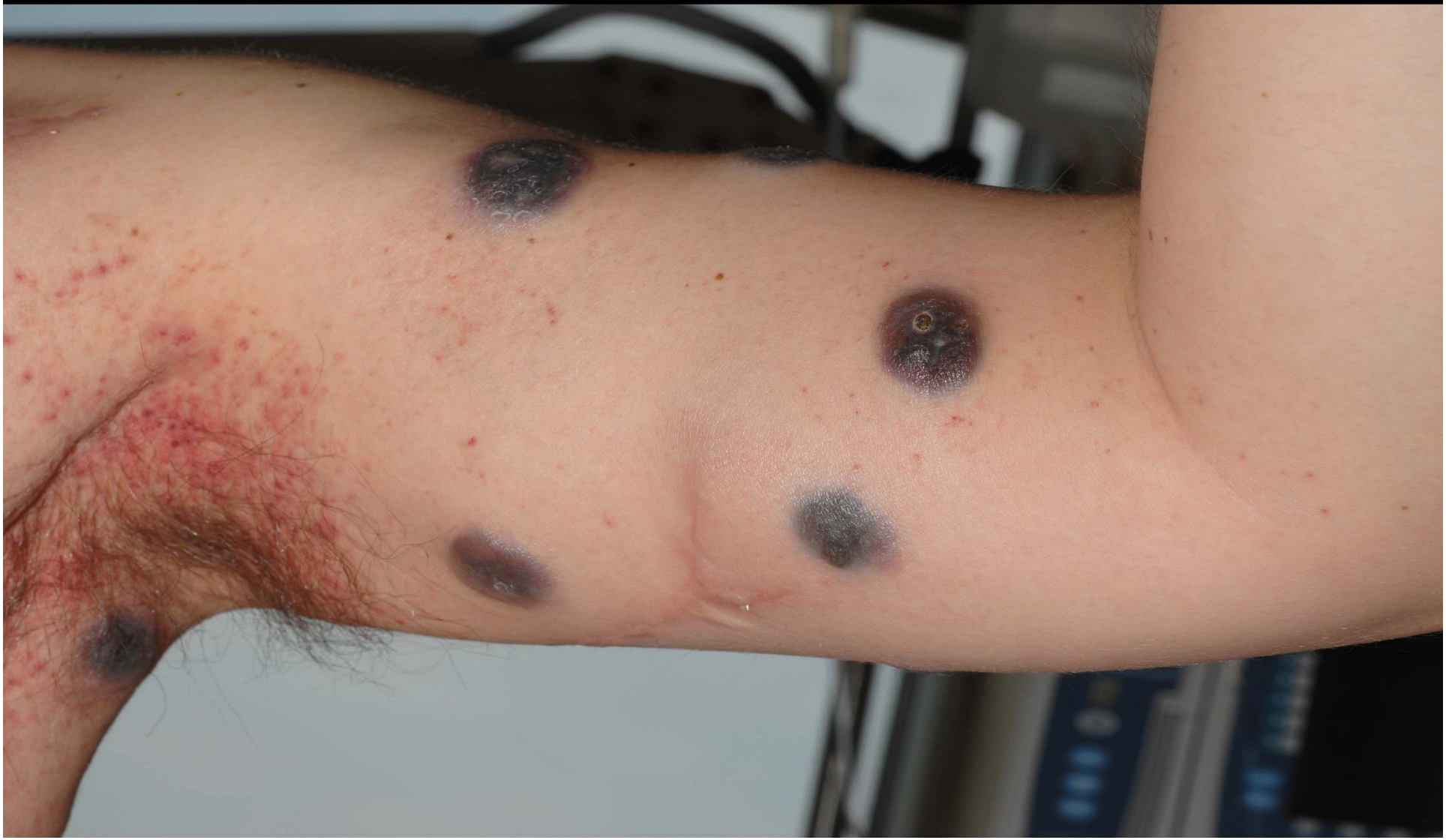
CD4

Analyzed 6 days post-transduction, (stim1)

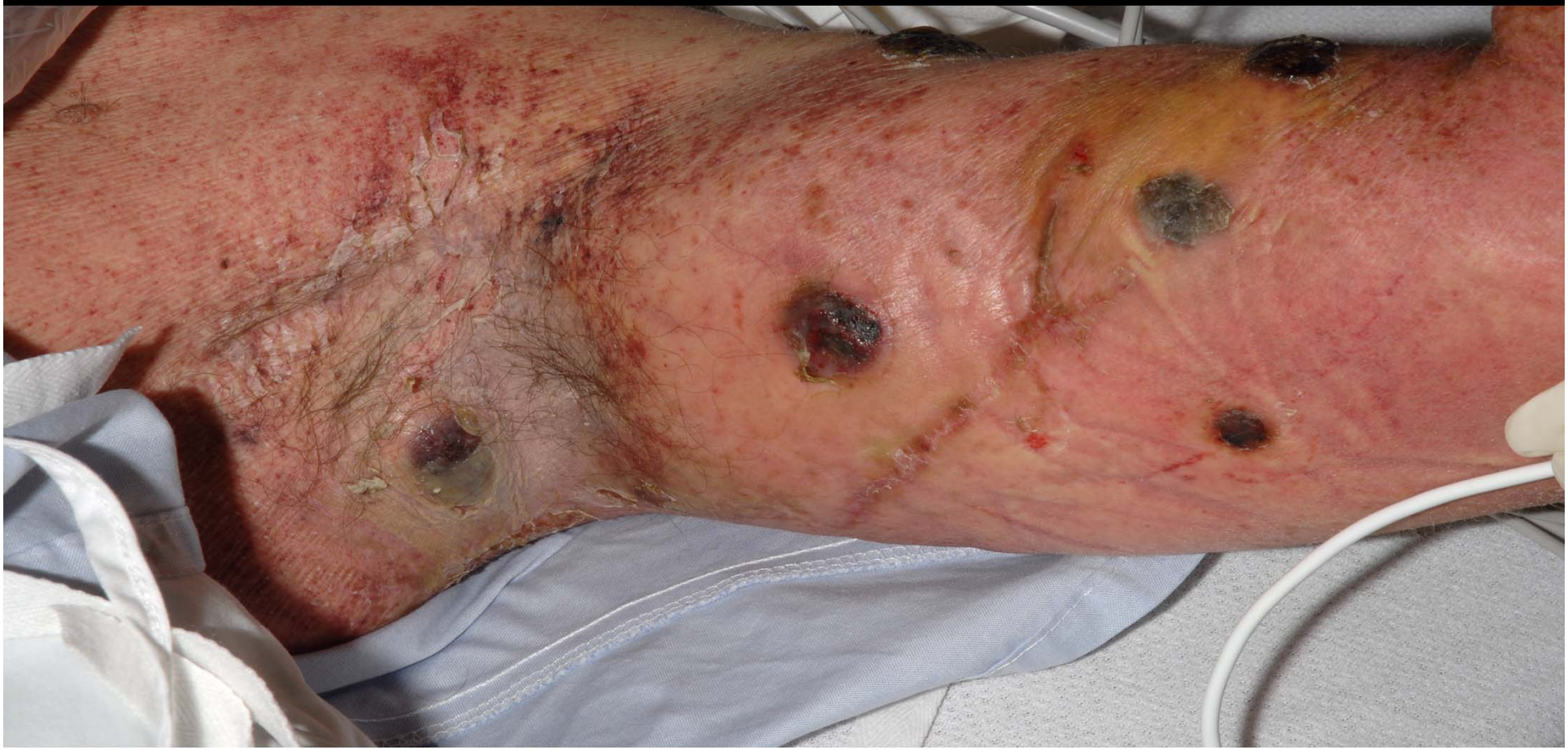
R.M. Gp100:154 TCR day 5



D.G. gp100:154 TCR day 4



D.G. Gp100:154 TCR day 12

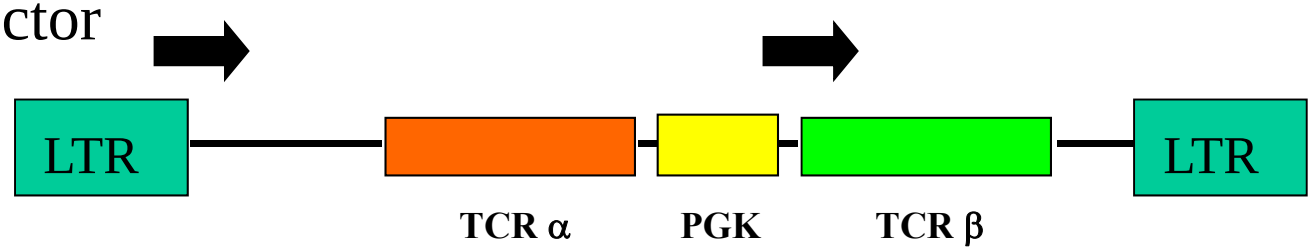


TCR VECTORS:

Anti-gp100 vector

APB

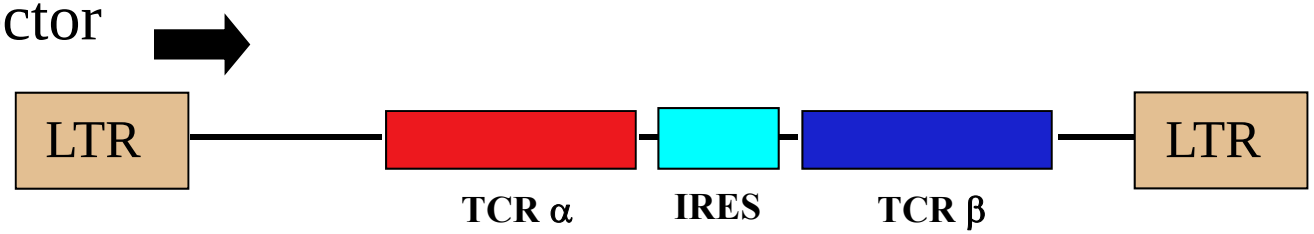
GCsamgp100APB



Anti-Mart-1 vector

AIB

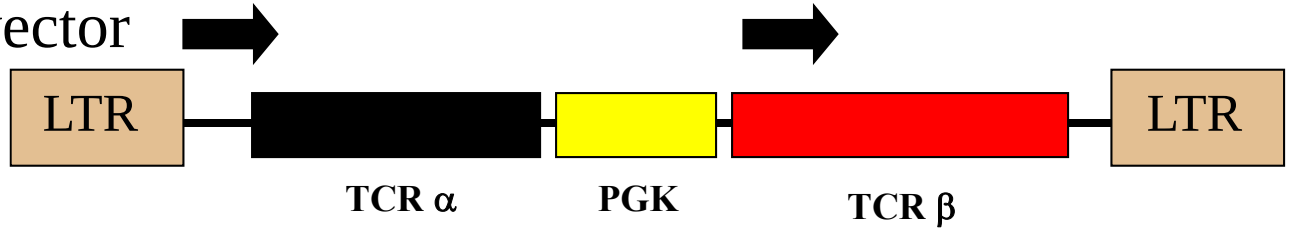
MSGV1martAIB



Anti-NY-Eso-1 vector

E1APB

MSGV1NYEso-1APB

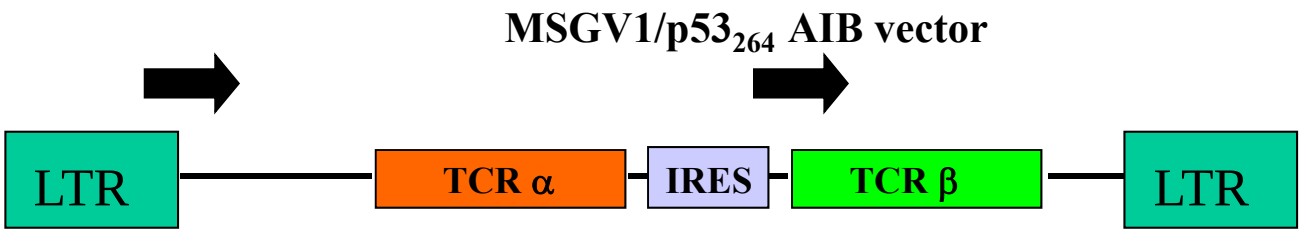


Summary

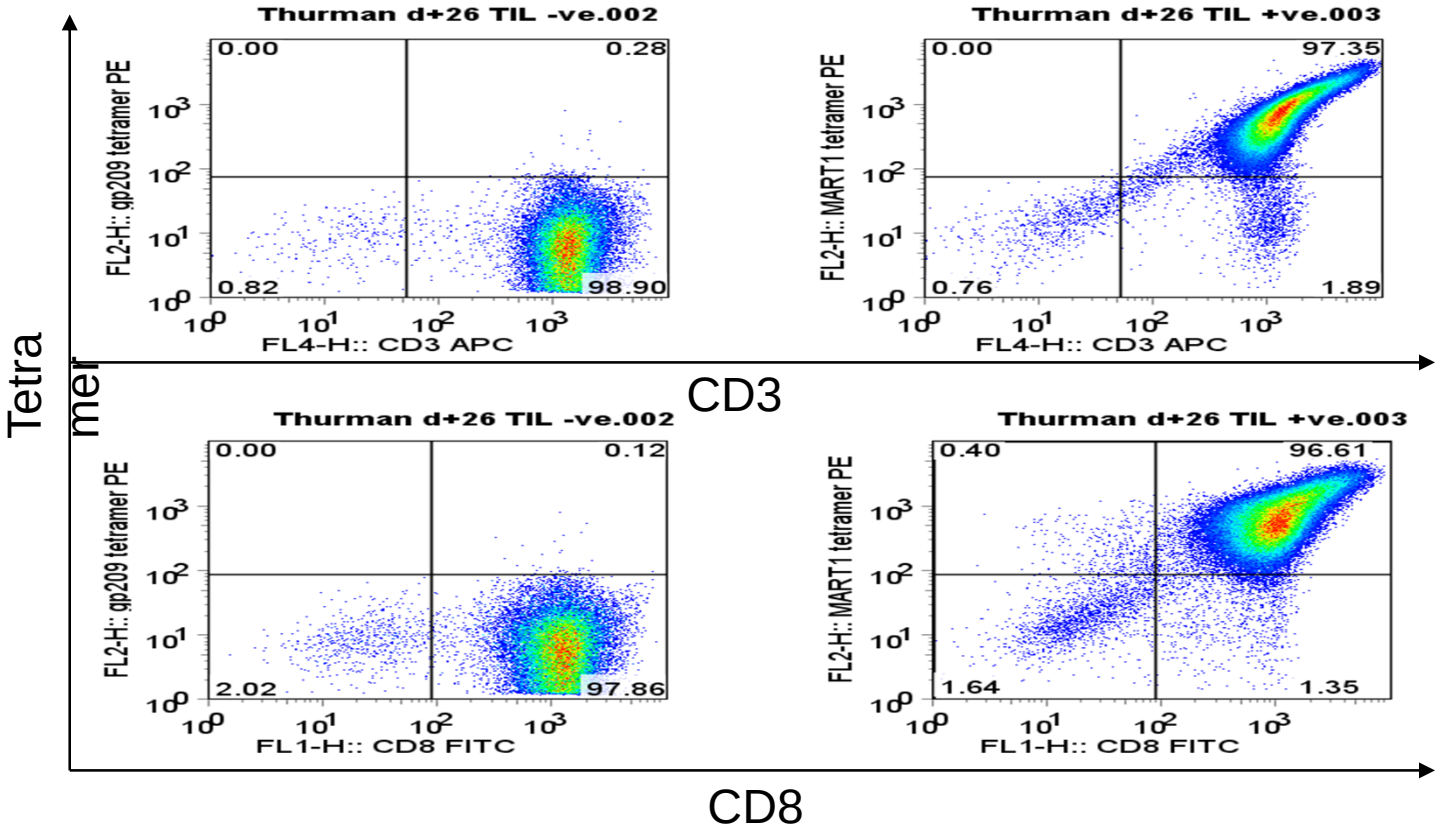
- **The adoptive transfer of activated antitumor T cells can mediate the objective regression of cancer in 50 to 70% of patients with metastatic melanoma.**

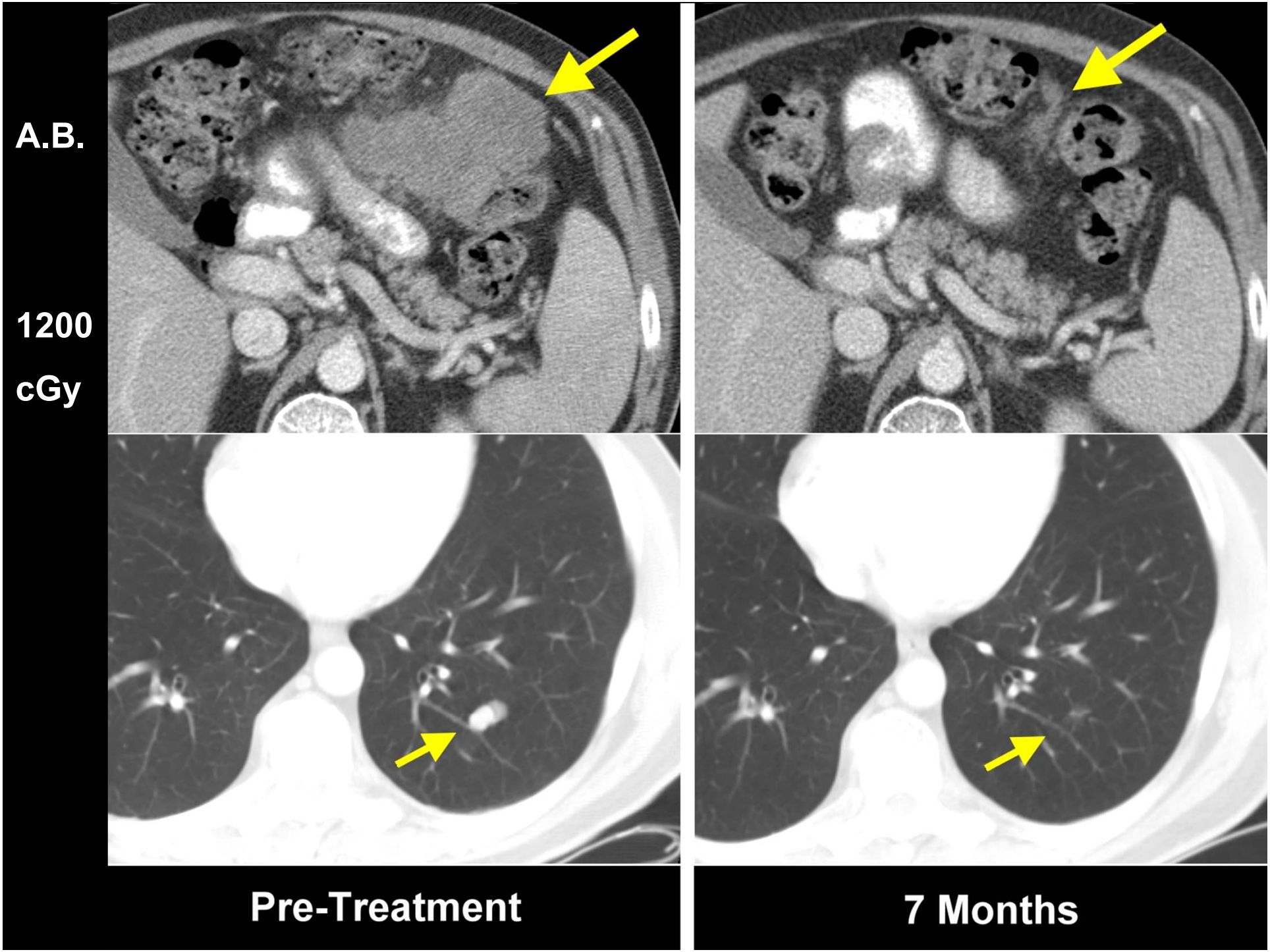
- **The persistence of the transferred cells, the telomere length of the cells and the numbers of CD27+CD8+ cells in TIL correlate with the effectiveness of treatment.**

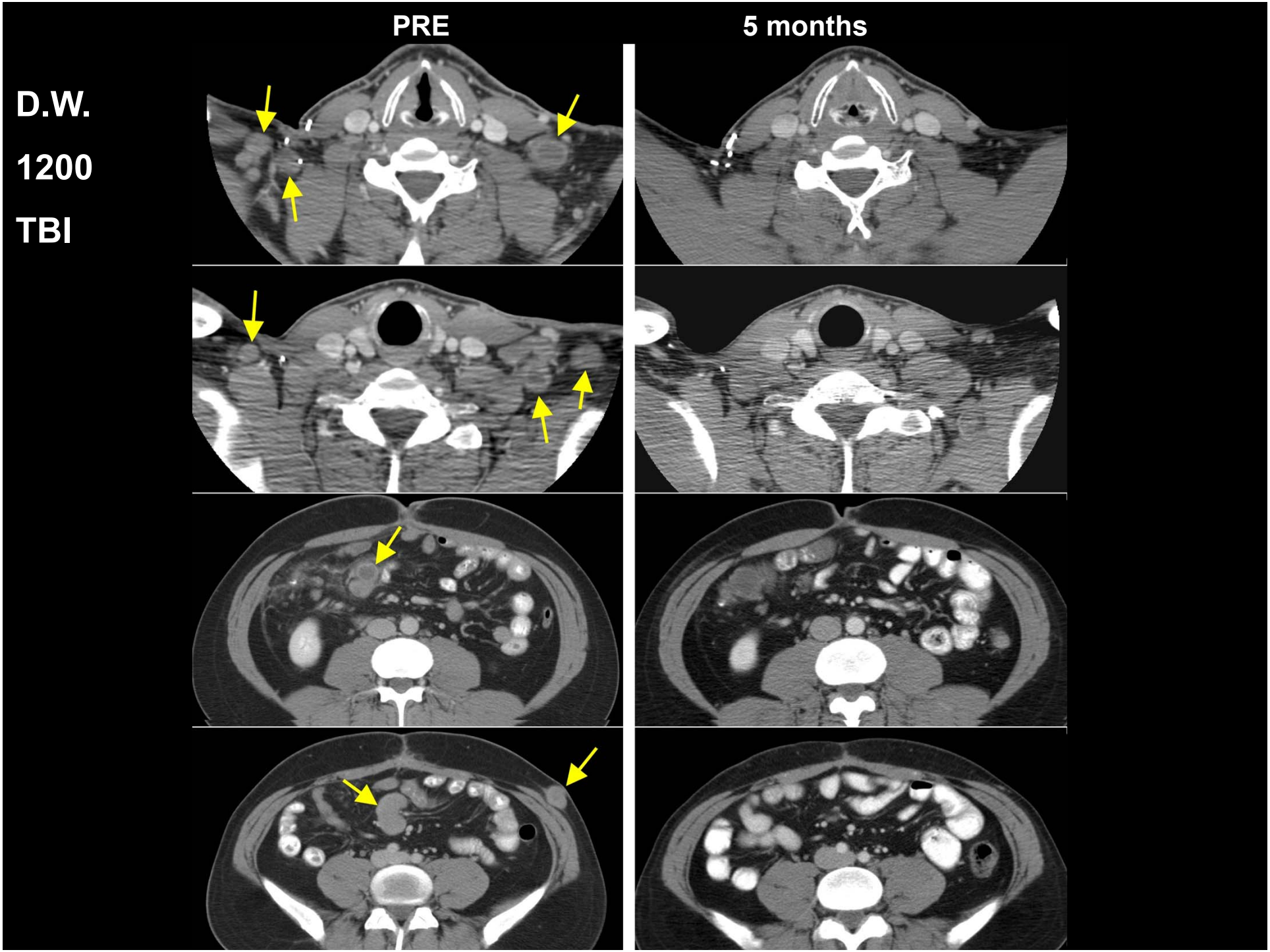
Schematics of anti-p53/264 TCR encoding retroviral vector



D.Th. cells grown in vitro from tumor excised d+26 post F5 TCR treatment.




A.B.
1200
cGy
Pre-Treatment
7 Months


PRE
5 months
D.W.
1200
TBI

What are some potential reasons for the low response rate?

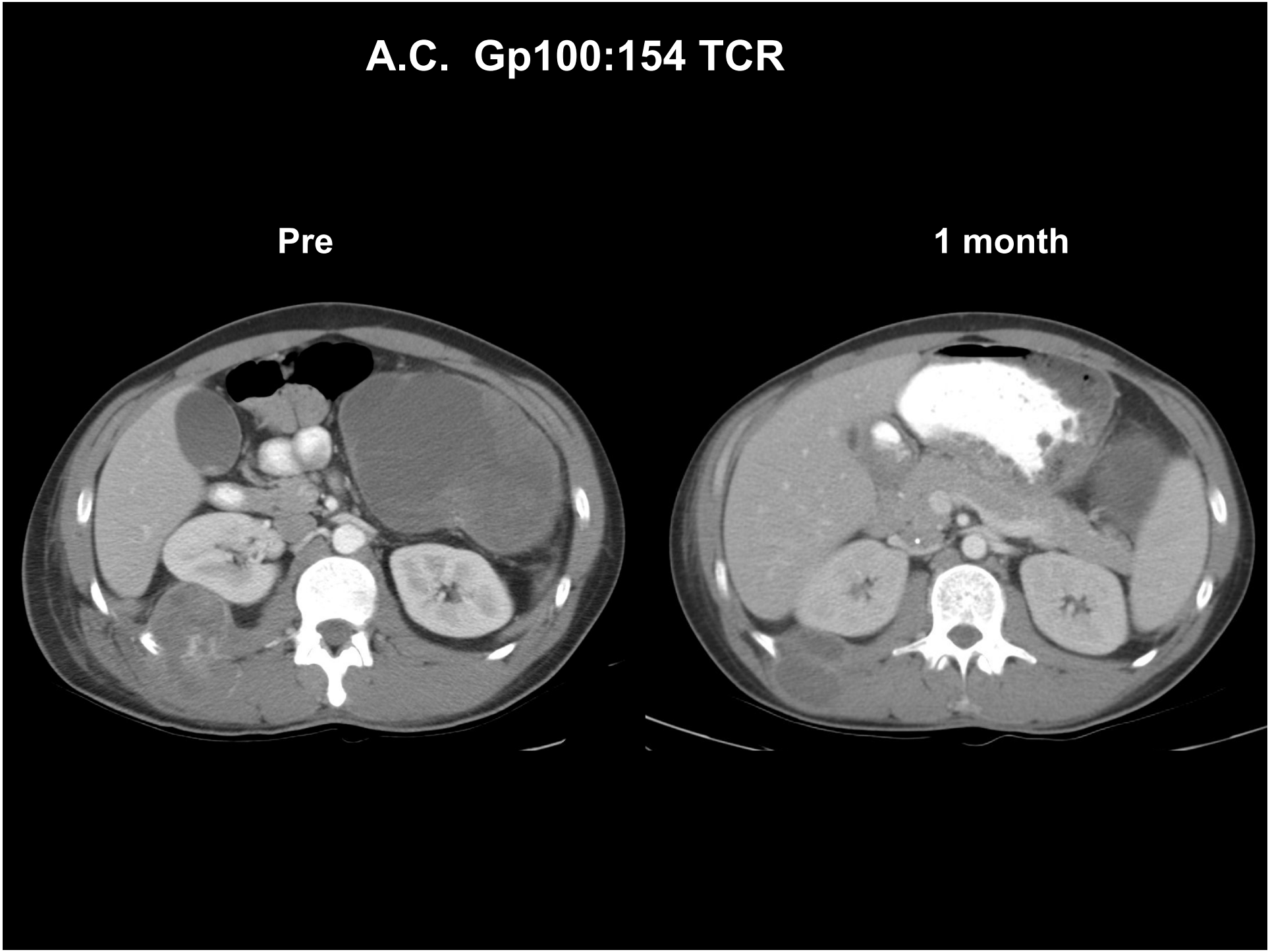
Suboptimal TCR

Mispairing of the inserted alpha and beta chains with the endogenous chains

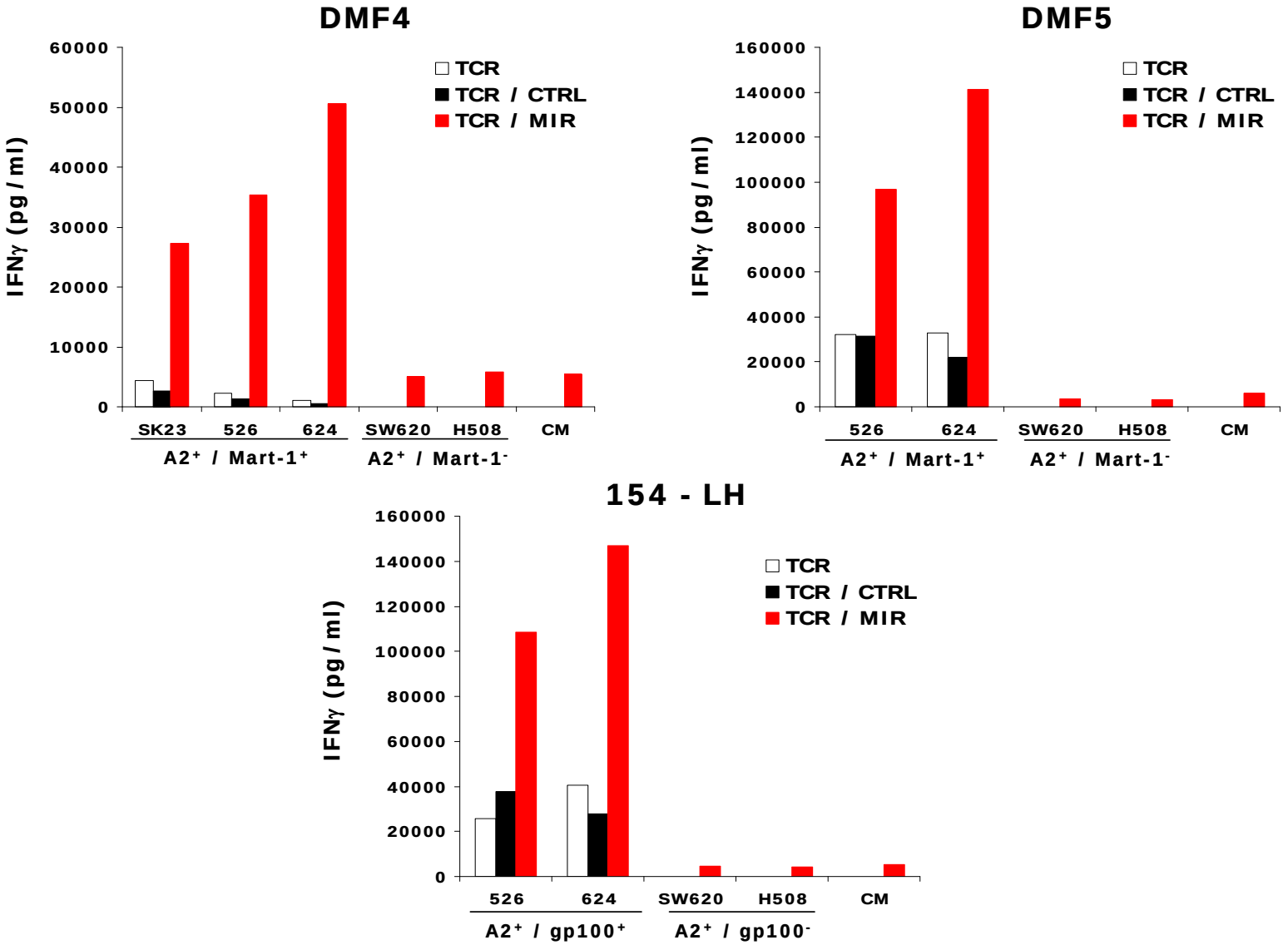
Low function of the transduced TCR

Poor traffic of the transduced cells to lymph nodes or tumor

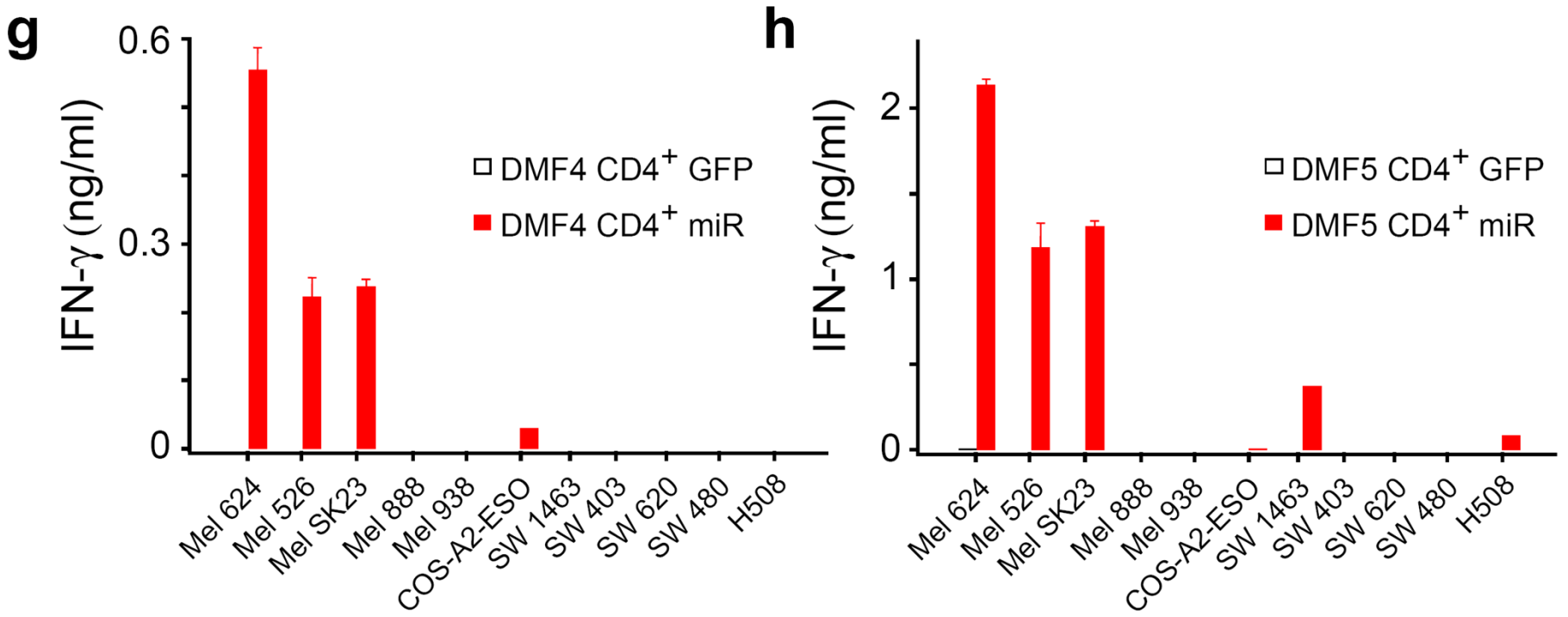
A.C. Gp100:154 TCR



Co-transduction of human PBL with TCR and miRNA-181a increased their functional avidity against tumors

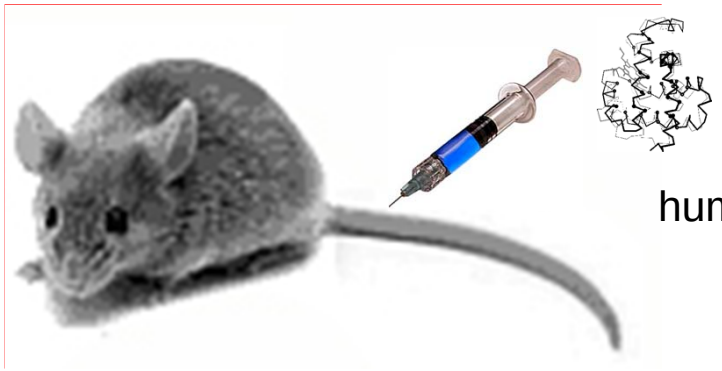


Overexpression of miR181a converts non-reactive TCR-modified human CD4+ cells into tumor-reactive ones



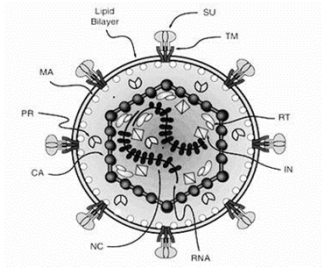
Using transgenic mice to generate T cells specific for human tumor antigens

Clone human MHC class I

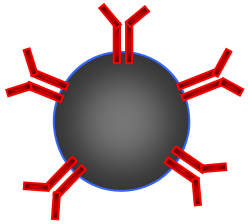


Immunize with human cancer antigen

Mouse transgenic for Human MHC class I



Clone mouse TCR into retroviral vector



Human T cell expressing **mouse TCR**

Treat humans with genetically engineered T cells

Sequence of the gp100:154-162 melanoma antigen epitope

---

human sequence: **KTWGQYWQV**

corresponding murine sequence: **KTWGKYWQV**

(glutamine to lysine switch at position 158)

Approaches to the generation of high avidity, highly active anti-tumor T cell receptors

---

Screen limiting dilution clones from tumor infiltrating lymphocytes or PBL from immunized patients

Immunize mice transgenic for human HLA molecules (avoid tolerance)

Modify individual residues in the CDR2 and CDR3 antigen binding regions of the TCR to increase TCR affinity

Method for increasing the affinity of human T cell receptors

- The CDR2 and CDR3 regions (each about 4 - 6 amino acids) of the T cell receptor bind to the peptide/MHC complex on the cell surface.

- Substitutions of individual amino acids in the CDR2 and CDR3 regions can profoundly alter (improve) the affinity of the T cell receptor.

(P. Robbins et al, in preparation)

DM F5 MART-1 TCR

CDR2β (47-52)

52

WT T

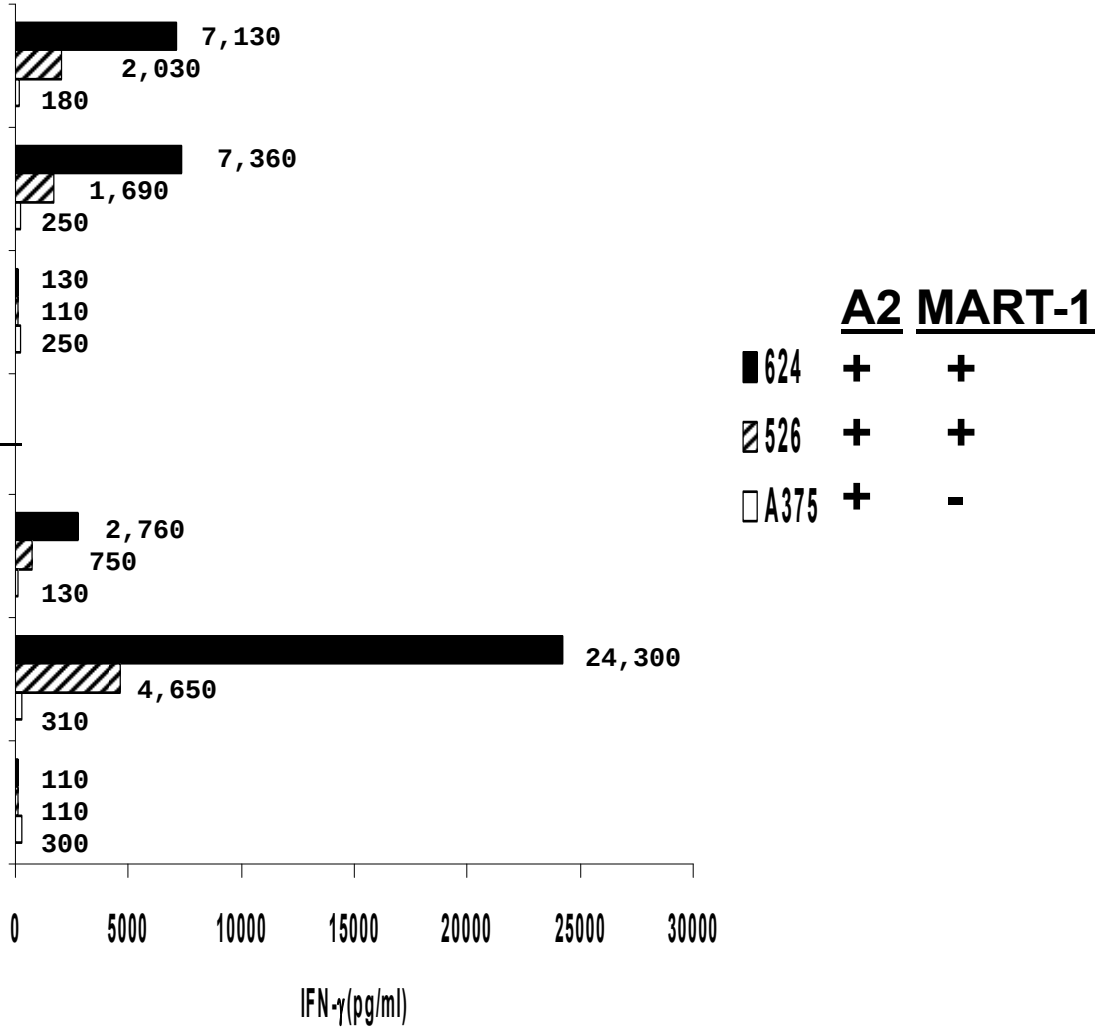
CD8+ A

None

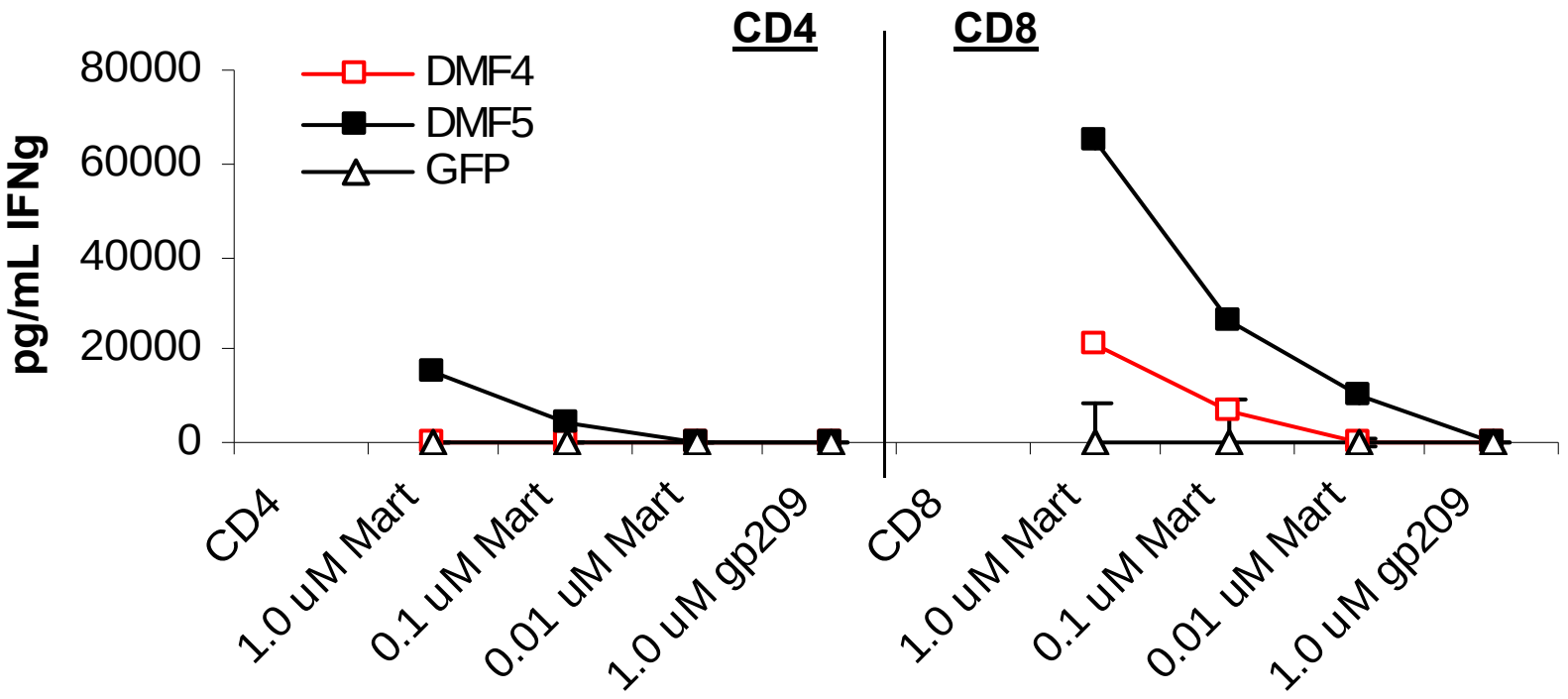
WT T

CD4+ A

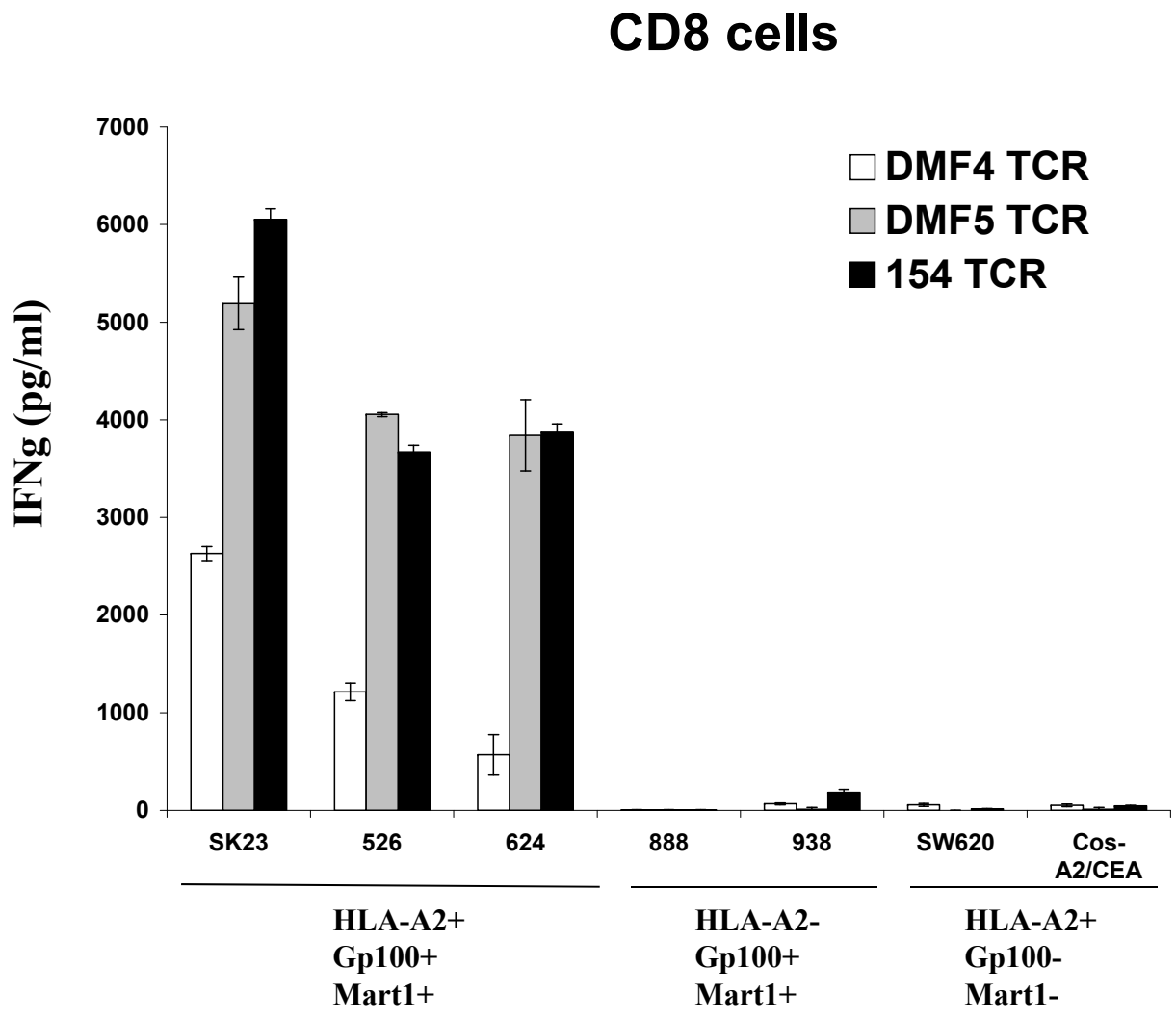
None



High-affinity TCRs confer functional recognition of MART-1 peptide to CD4+ as well as CD8+ PBMC



Secretion of IFNg by CD8 cells transduced with DMF4, DMF5 or 154 TCR



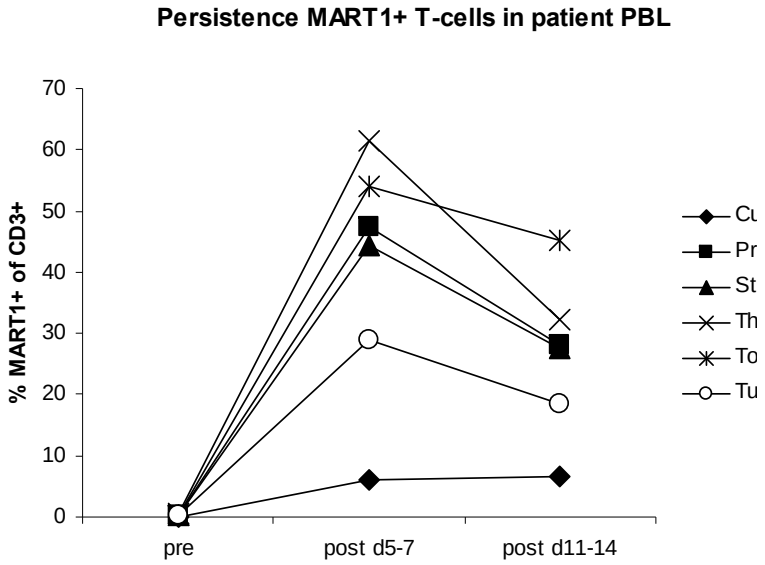
D.T.: MART-1 F5 TCR: Serum Values

day	IL-5	IL-7	IL-8	IL-10	IL-15	IL-18	GMCSF	TNFa	IFNg
	(pg/ml)								
-21	2	8	295	2	3	115	212	<5	1
-7	5	2	314	1	4	102	359	<5	2
0	4	32	76	1	33	135	88	<5	1
5	58	4	29,700	40	23	1181	1646	25	605
9	20	3	178	11	27	1246	294	18	13
12	2	4	89	5	24	1183	271	6	2
14	26	11	103	8	47	1469	602	9	2
20	16	2	85	8	36	1433	523	10	1

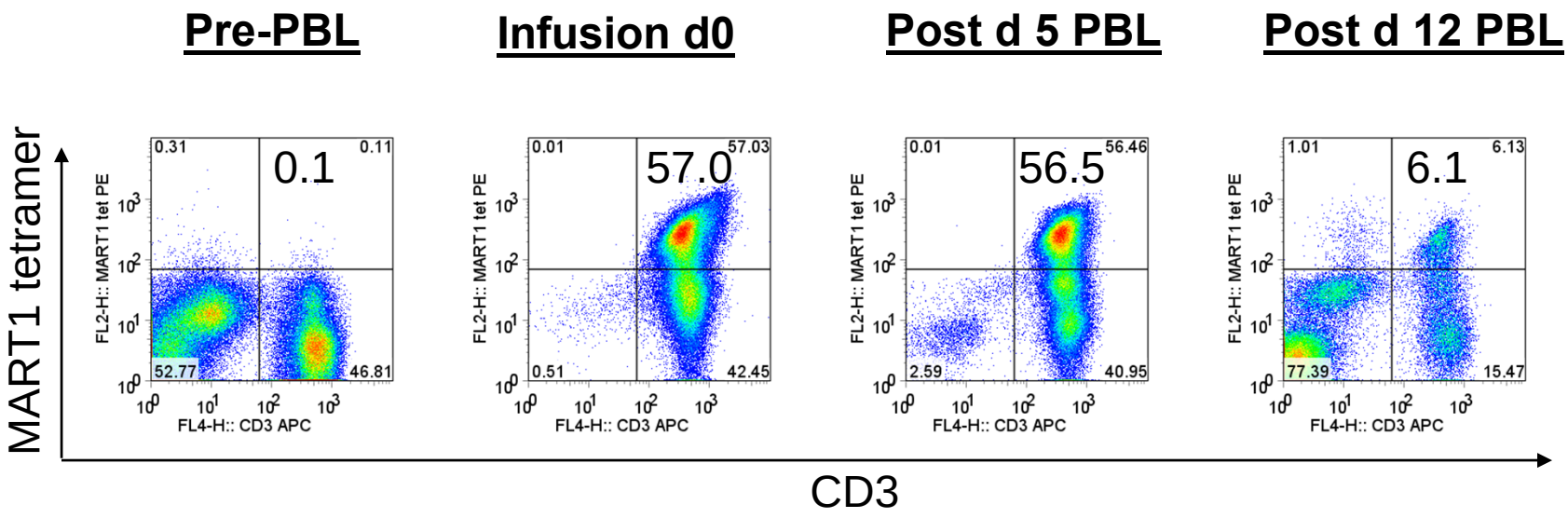
F5 TCR Transduced PBL Persistence in vivo

% of CD3+ PBL binding MART1+ tetramer

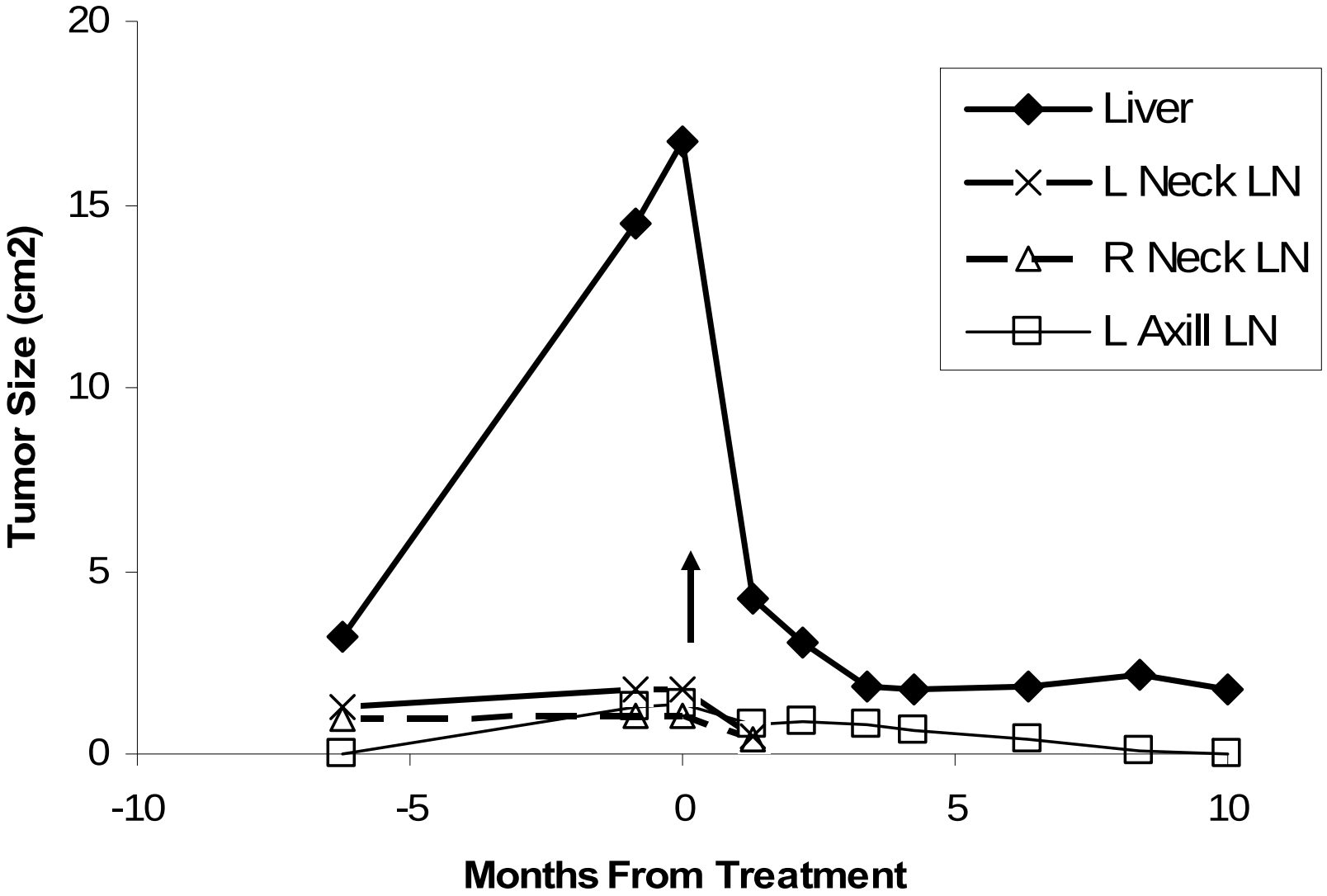
Patient	Cu	Pr	St	Th	To	Tu
pre	0.1	0.2	0.2	0.5	0.3	0.4
post d5-7	6.1	47.4	44.4	61.5	54.1	28.8
post d11-14	6.6	28.2	27.5	32.2	45.2	18.4



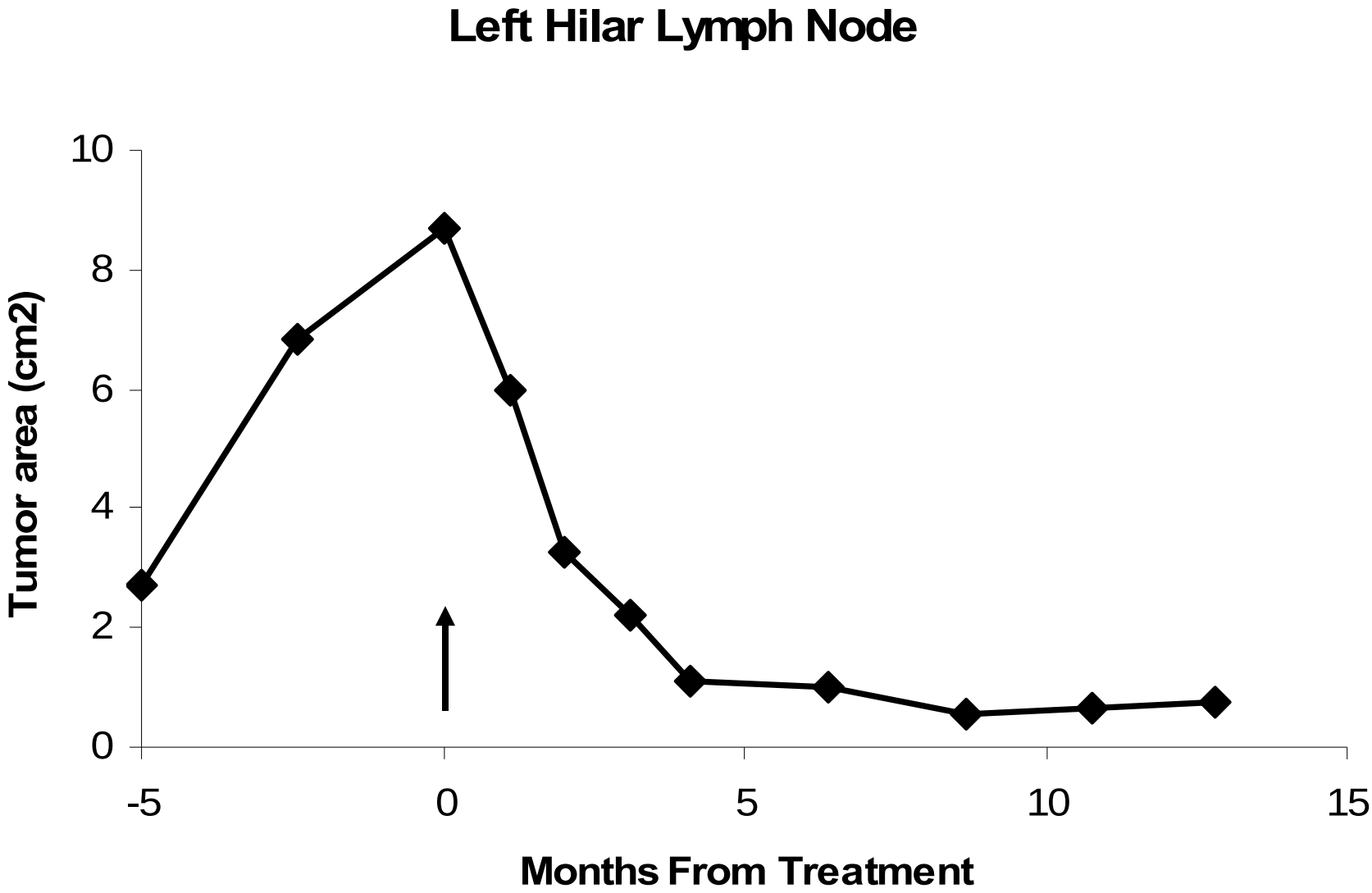
F5 TCR Transduced PBL Persistence in vivo



Tumor regression in patient M.O. – MART TCR



Tumor regression in Pt. T.M. – MART TCR



Opportunities for Improving ACT for the Treatment of Human Cancer

	Approach	Selected Examples
1.	Genetic modification of lymphocytes to introduce new recognition specificities	alpha-beta TCR chimeric TCR
2.	Genetic modification of lymphocytes to alter function of T cells	costimulatory molecules (CD8, 41BB) cytokines (IL-2, IL-15) homing molecules (CD62L, CCR7) prevention of apoptosis (Bcl-2) global alteration of signaling (miR-181a)
3.	Modify host lymphodepletion cells	selective depletion of CD4+ cells or T regulatory
4.	Block inhibitory signals on reactive lymphocytes	antibodies to CTLA-4 or PD-1
5.	Administer vaccines to stimulate transferred cells	recombinant virus encoding antigen
6.	Administer alternative cytokines to support cell growth	IL-15, IL-21
7.	Stimulate antigen presenting cells	toll-like receptor agonists
8.	Generate less differentiated lymphocytes	alternate culture conditions and growth promoting cytokines in vitro

Current Efforts to Improve Cell Transfer Therapy

Host modification

Administer a vaccine encoding the antigen recognized by the transferred T cells

Lymphocyte modification by gene transfer

Improve the in vivo survival of the transferred cells

Provide higher affinity T cell receptors

Extend cell transfer therapy to patients with common epithelial cancers

“What profits wisdom when there is nothing to be done?”

Sophocles
450 B.C.

CLASS I RESTRICTED MELANOMA ANTIGENS RECOGNIZED BY TIL

Name of antigen	TILs used for identification	HLA Restriction	Immunodominant epitopes	Characteristics
MART-1	1235 and others	A2	AAGIGILTV	
	Normal differentiation antigen			
gp100	1200 and others	A2	KTWGQYWQV	
	Normal differentiation antigen			
			ITDQVPFSV	
			YLEPGPVTA	
	1351	A3	LIYRRRLMK	
	888	A24	VYFFLPDHL	
	Intronic sequence			
Tyrosinase	888&1413	A24	AFLPWHRLF	
	Normal differentiation antigen			
	1388	A1	SSDYVIPIGTY	
	1138	A2	YMDGTMSQV	
p15	1290	A24	AYGLDFYIL	
	Post-transcriptional control			
β-catenin	1290	A24	SYLDSGIHF	
	Single base mutation			
TRP-1	586	A31	MSLQRQFLR	
	Translated from alternative open			
				reading frame
TRP-2	586	A31	LLGPGRPYR	
	Normal differentiation antigen			
	1790	A2	SVYDFFVWL	
MART-2	TIL 1362	A1	FLEGNEVGKTY	
	Mutation in phosphate binding			
				loop; loss of ability to bind GTP

CURRENT EFFORTS TO IMPROVE CELL TRANSFER THERAPY

1. Increase the preparative lymphodepletion by adding whole body irradiation.

2. Transduce the gene encoding IL-2 or IL-15 into antitumor lymphocytes used for therapy.

3. Transduce the genes encoding highly avid, anti-tumor T-cell receptors into PBMC used for therapy.

FREQUENCY OF $CD8^+$ T-CELLS REACTIVE WITH NATIVE gp100:209-218 PEPTIDE (TETRAMER ASSAY)

Patient	After one course	After four courses
	(% of $CD8^+$ cells $tetramer^+$)	
1	2.57% (1/39)	13.2% (1/8)
2	0 (0)	6.4% (1/16)
3	0.12% (1/833)	4.8% (1/21)
4	1.80% (1/56)	19.1% (1/5)
5	0.54% (1/185)	38.1% (1/3)

MAJOR PARADOX OF HUMAN CANCER IMMUNOTHERAPY

Human cancers express antigens naturally recognized by the immune system

Humans with cancer have T-lymphocytes that recognize these antigens

BUT

The cancer grows

GENE TRANSFER INTO HUMANS — IMMUNOTHERAPY OF PATIENTS WITH ADVANCED MELANOMA, USING TUMOR-INFILTRATING LYMPHOCYTES MODIFIED BY RETROVIRAL GENE TRANSDUCTION

STEVEN A. ROSENBERG, M.D., PH.D., PAUL AEBERSOLD, PH.D., KENNETH CORNETTA, M.D., ATTAN KASID, PH.D., RICHARD A. MORGAN, PH.D., ROBERT MOEN, M.D., EVELYN M. KARSON, PH.D., M.D., MICHAEL T. LOTZE, M.D., JAMES C. YANG, M.D., SUZANNE L. TOPALIAN, M.D., MARIA J. MERINO, M.D., KENNETH CULVER, M.D., A. DUSTY MILLER, PH.D., R. MICHAEL BLAESE, M.D., AND W. FRENCH ANDERSON, M.D.

Abstract *Background and Methods.* Treatment with tumor-infiltrating lymphocytes (TIL) plus interleukin-2 can mediate the regression of metastatic melanoma in approximately half of patients. To optimize this treatment approach and define the in vivo distribution and survival of TIL, we used retroviral-mediated gene transduction to introduce the gene coding for resistance to neomycin into human TIL before their infusion into patients — thus using the new gene as a marker for the infused cells.

Results. Five patients received the gene-modified TIL. All the patients tolerated the treatment well, and no side effects due to the gene transduction were noted. The presence and expression of the neomycin-resistance gene were demonstrated in TIL from all the patients with Southern blot analysis and enzymatic assay for the neomycin phosphotransferase coded by the bacterial gene. Cells from four of the five patients grew successfully in high concentrations of G418, a neomycin analogue otherwise toxic to eukaryotic cells.

With polymerase-chain-reaction analysis, gene-modified cells were consistently found in the circulation of all five patients for three weeks and for as long as two months in two patients. Cells were recovered from tumor deposits as much as 64 days after cell administration. The procedure was safe according to all criteria, including the absence of infectious virus in TIL and in the patients.

Conclusions. These studies demonstrate the feasibility and safety of using retroviral gene transduction for human gene therapy and have implications for the design of TIL with improved antitumor potency, as well as for the possible use of lymphocytes for the gene therapy of other diseases. (N Engl J Med 1990; 323:570-8.)

PRINCIPLES OF THE DEVELOPMENT OF HUMAN CANCER IMMUNOTHERAPY

1. Natural body defenses can lead to the regression of metastatic cancer in humans.

2. Stimulation of T cells with IL-2 can mediate regression of metastatic cancer in patients with melanoma and renal cancer.

3. Transfer of immune cells can mediate the regression of human cancer. These cells can be used to identify cancer antigens.

4. Prior depletion of the patient's natural immune system can improve the effectiveness of cell transfer immunotherapy.

5. Normal human peripheral lymphocytes can be genetically modified ex vivo for use in effective cancer immunotherapy.

RECOMBINANT INTERLEUKIN-2

1. T-cell Growth Factor (IL-2) described by Morgan et al (Science 193:1007,1976).

2. DNA sequence of the gene coding for IL-2 was determined by Taniguchi, et al. (*Nature* 302:305, 1983).

3. IL-2 gene was expressed in E. coli; the biologic characteristics of this recombinant IL-2 were determined; Lymphokine Activated Killer (LAK) cells described (Rosenberg, S.A., et al., *Science* 223:1412, 1984).

PRINCIPLES OF THE DEVELOPMENT OF HUMAN CANCER IMMUNOTHERAPY

---

1. **Stimulation of T cells with IL-2 can mediate regression of metastatic cancer in patients with melanoma and renal cancer.**

“What profits wisdom when there is nothing to be done?”

Sophocles
450 B.C.

WHY IS TUMOR REGRESSION MINIMAL DESPITE THE IN VIVO GENERATION OF ANTITUMOR PRECURSORS?

1. Tumor cells may lose or not express the relevant antigen

2. Number of anti-tumor cells too low

3. Avidity of T-cells too low

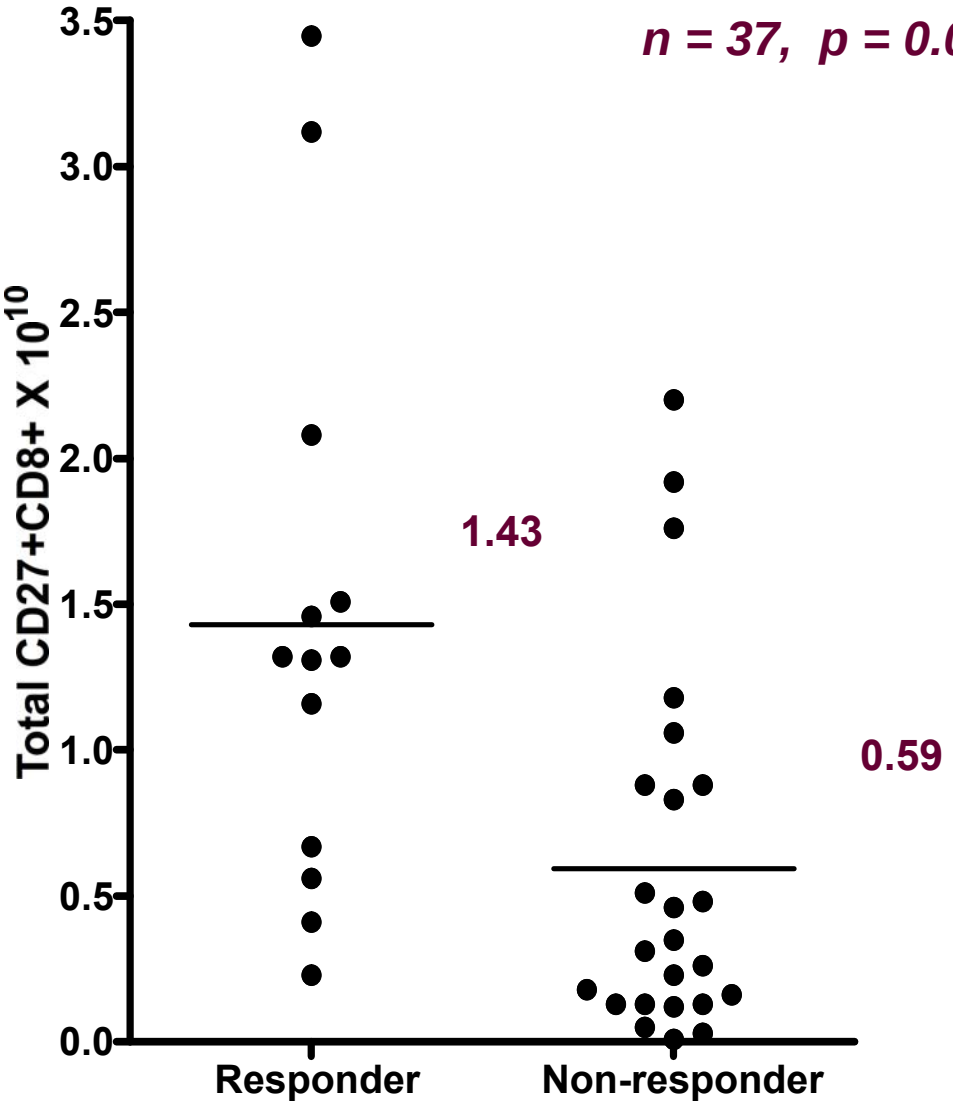
4. Regulatory lymphocytes may suppress the immune reaction

PATIENT M.G.

26 year old female referred to NCI with metastatic melanoma to lungs, oral mucosa, tonsils, and multiple subcutaneous sites

Dec. 1988	Excision of a 14 mm deep melanoma of right arm; 2/30 positive lymph nodes
June 1989	Developed metastases to both lungs, soft palate, tonsils, >20 soft tissue masses
July 1989	Soft tissue mass resected; treated with autologous TIL + IL-2 Complete regression
Sept. 1992	Developed 10 cm pelvic mass; resected and TIL grown
March 1993	**Developed new pelvic mass; treated with TIL (from Sept. 1992 mass) + IL-2 Complete regression**

Number of CD27+CD8+ cells in TIL (after IL-2 withdrawal) correlates with response



Current Efforts to Improve Cell Transfer Therapy

Host modification

Increase preparative lymphodepletion by adding whole body irradiation

Administer a vaccine encoding the antigen recognized by the transferred T cells

Lymphocyte modification by gene transfer

Improve the in vivo survival of the transferred cells

Provide higher affinity T cell receptors

Extend cell transfer therapy to patients with common epithelial cancers

CURRENT EFFORTS TO IMPROVE CELL TRANSFER THERAPY

1. Increase the preparative lymphodepletion by adding whole body irradiation.

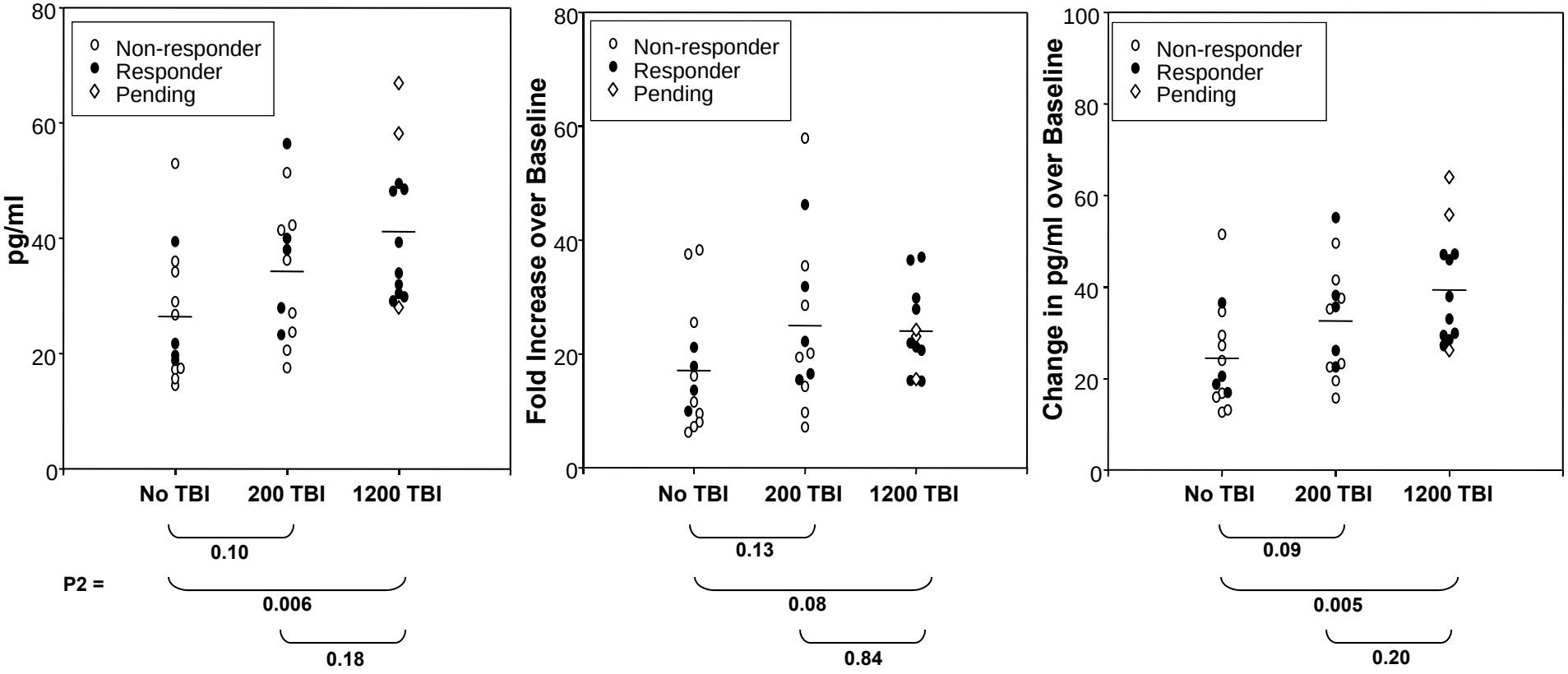
2. Transduce the gene encoding IL-2 or IL-15 into antitumor lymphocytes used for therapy.

3. Transduce the genes encoding highly avid, anti-tumor T-cell receptors into PBMC used for therapy of patients with melanoma and common epithelial cancers.

TREATMENT OF PATIENTS WITH METASTATIC MELANOMA USING TUMOR INFILTRATING LYMPHOCYTES

	Total	PR + CR	%PR + CR
	(Number of Patients)		
No previous interleukin-2 therapy	52	19	37%
Previous interleukin-2 therapy	21	7	33%
Total	73	26	36%

Serum IL-15 Levels on Day 0 of TIL Administration following Preparative Lyphodepleting Regimen
Samples tested on the same day
(1-31-07 Experiment)



PERSISTENCE OF SELECTED CLONES FROM PATIENT D.M. FOLLOWING LYMPHOCYTE TRANSFER INTO THE LYMPHODEPLETED HOST

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.

Current Efforts to Improve Cell Transfer Therapy

Host modification

Administer a vaccine encoding the antigen recognized by the transferred T cells

Lymphocyte modification by gene transfer

Improve the in vivo survival of the transferred cells

Provide higher affinity T cell receptors

Extend cell transfer therapy to patients with common epithelial cancers

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

Multiple antigenic epitopes on NY-ESO-1 recognized by human T lymphocytes

BIOLOGIC THERAPY

Cancer treatment that acts primarily through natural host defense mechanisms or by the administration of natural mammalian substances.

HISTORY OF CANCER - INCIDENCE

First Tumor	**Cretaceous Period Dinosaur (130,000,000 years ago)**	**<u>2 cases:</u> Benign osteoma of vertebra Hemangioma of vertebra**
First Cancer	**Pleistocene Epoch Cave Bear (1,750,000 years ago)**	**Osteosarcoma**
First Cancer in Humans	**3rd to 5th Dynasty in Egypt (5,000 years ago)**	**<u>6 cases:</u> 3 Osteosarcomas 3 Nasopharyngeal cancers**

FIRST CLINICAL TRIAL

BOOK OF DANIEL (1.11-15)

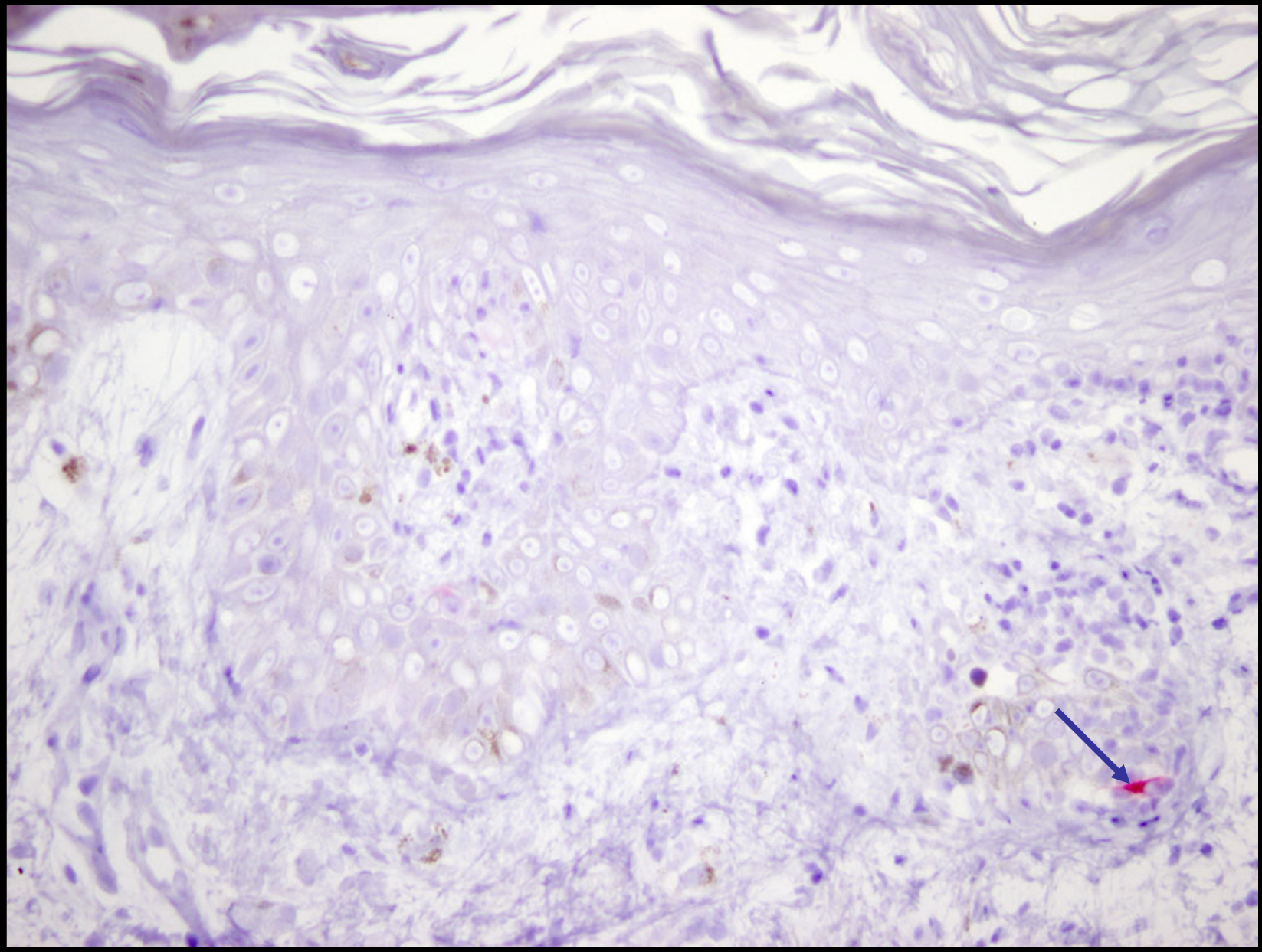
Rationale: (1.5) Nebuchadnezzar, king of Babylon, conquered Jerusalem and commanded Daniel and the children of Judah to eat the king's meat or die.

Method: (1.12) Daniel proposed that for 10 days:
one group eat king's meat
one group eat only kosher food

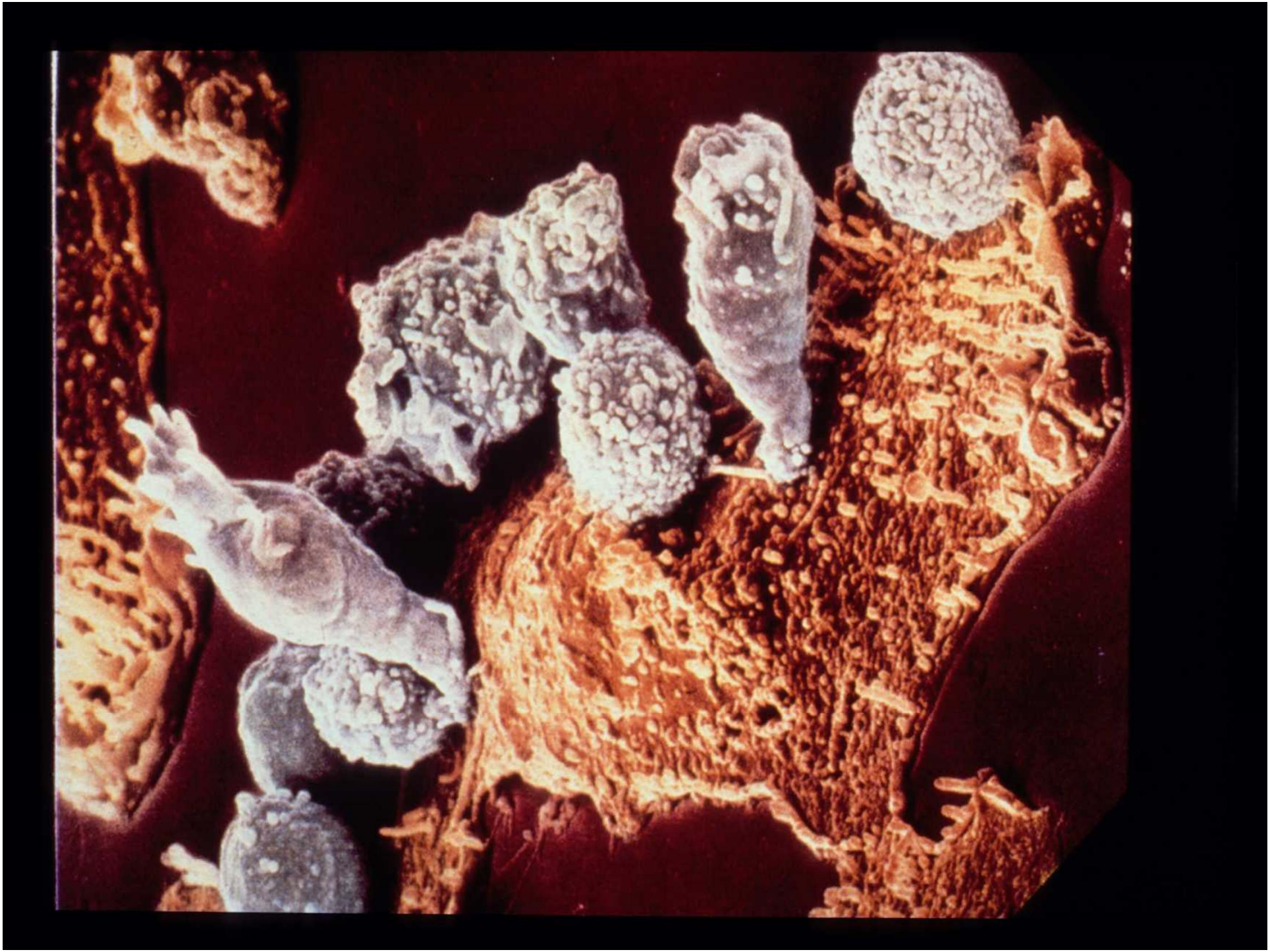
Result: (1.15) "And at the end of 10 days their countenances appeared fairer and fatter in flesh than all the children which did eat the portion of the king's meat".

HISTORY OF CANCER -TREATMENT

Surgery	**1600 B.C.**	**Edwin Smith Papyrus, Egypt: tumors removed with a knife or red-hot iron red-hot iron**
Radiation therapy	**1896**	**One year after discovery of x-rays; E.H. Grubbe treated a patient with advanced breast cancer (Chicago).**
Chemotherapy	**1942**	**Nitrogen mustard (bis -chlorethylamin used by G.D. Lindskog to treat a patient with x-ray resistant lymphosarcoma.**

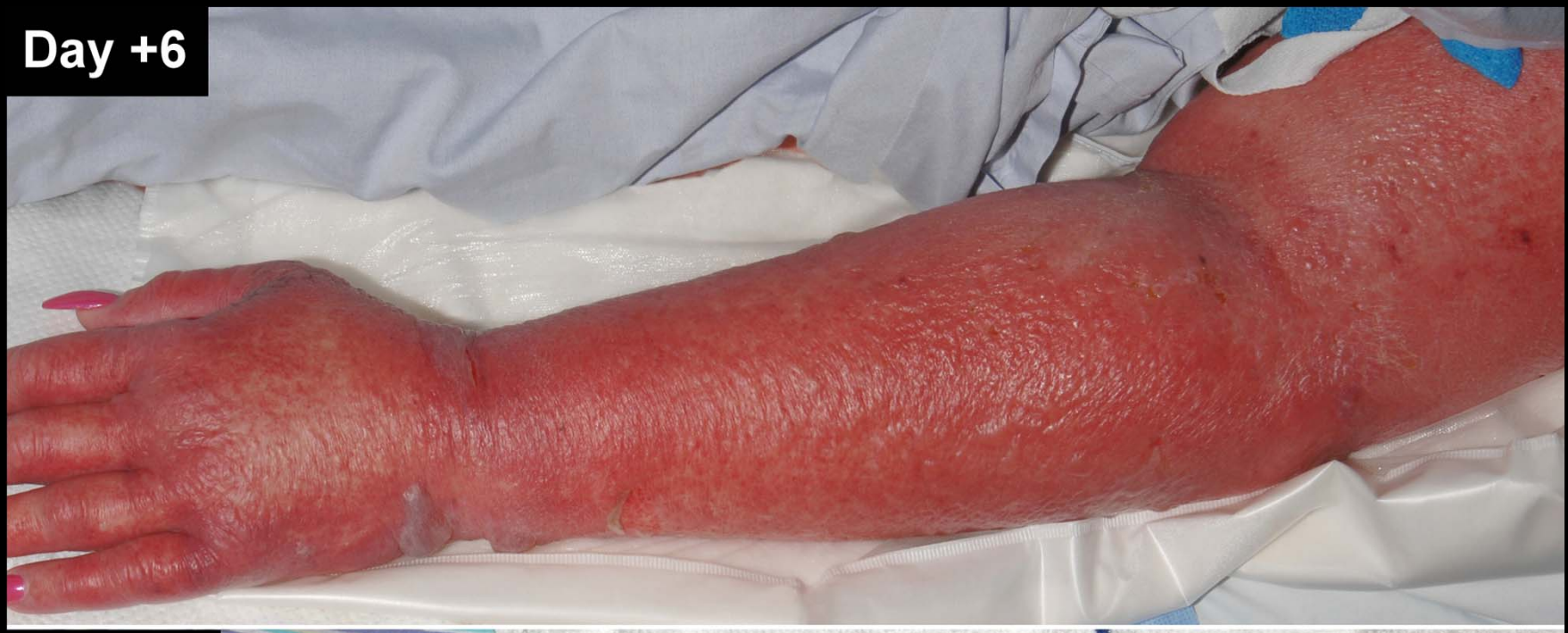


Mart-1 (rare staining in melanocytes)

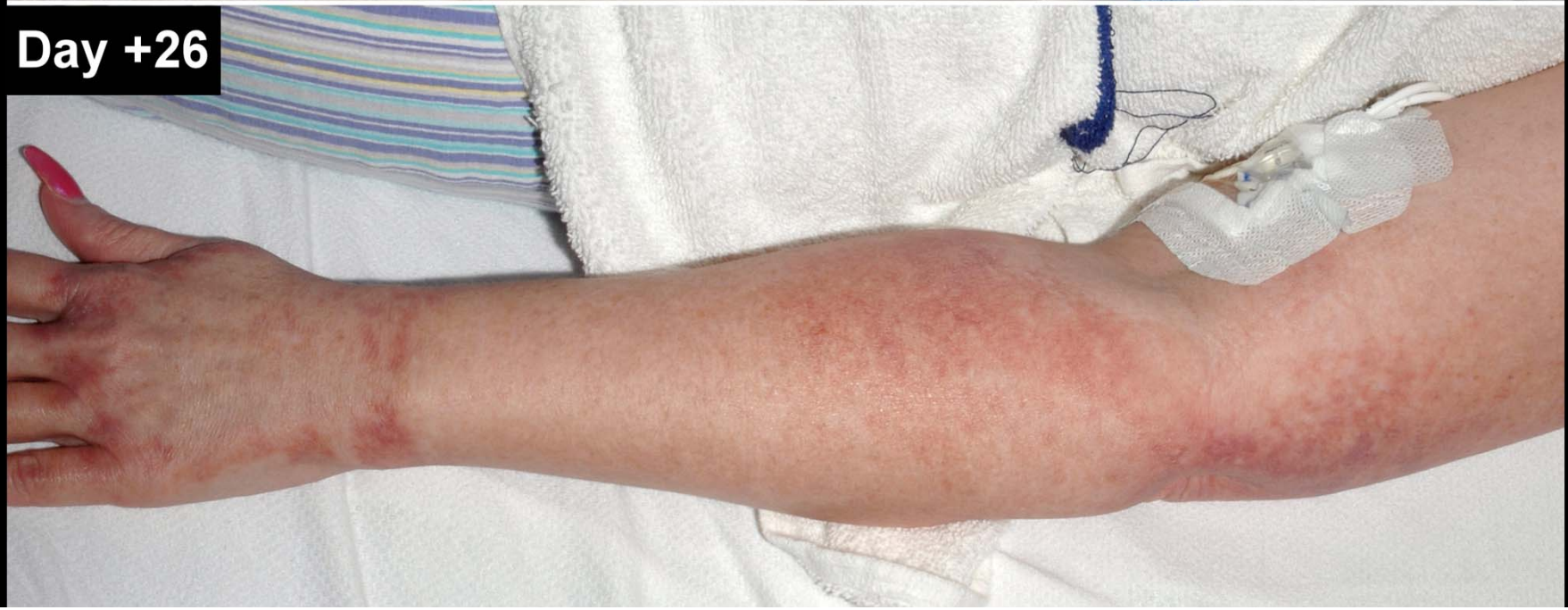


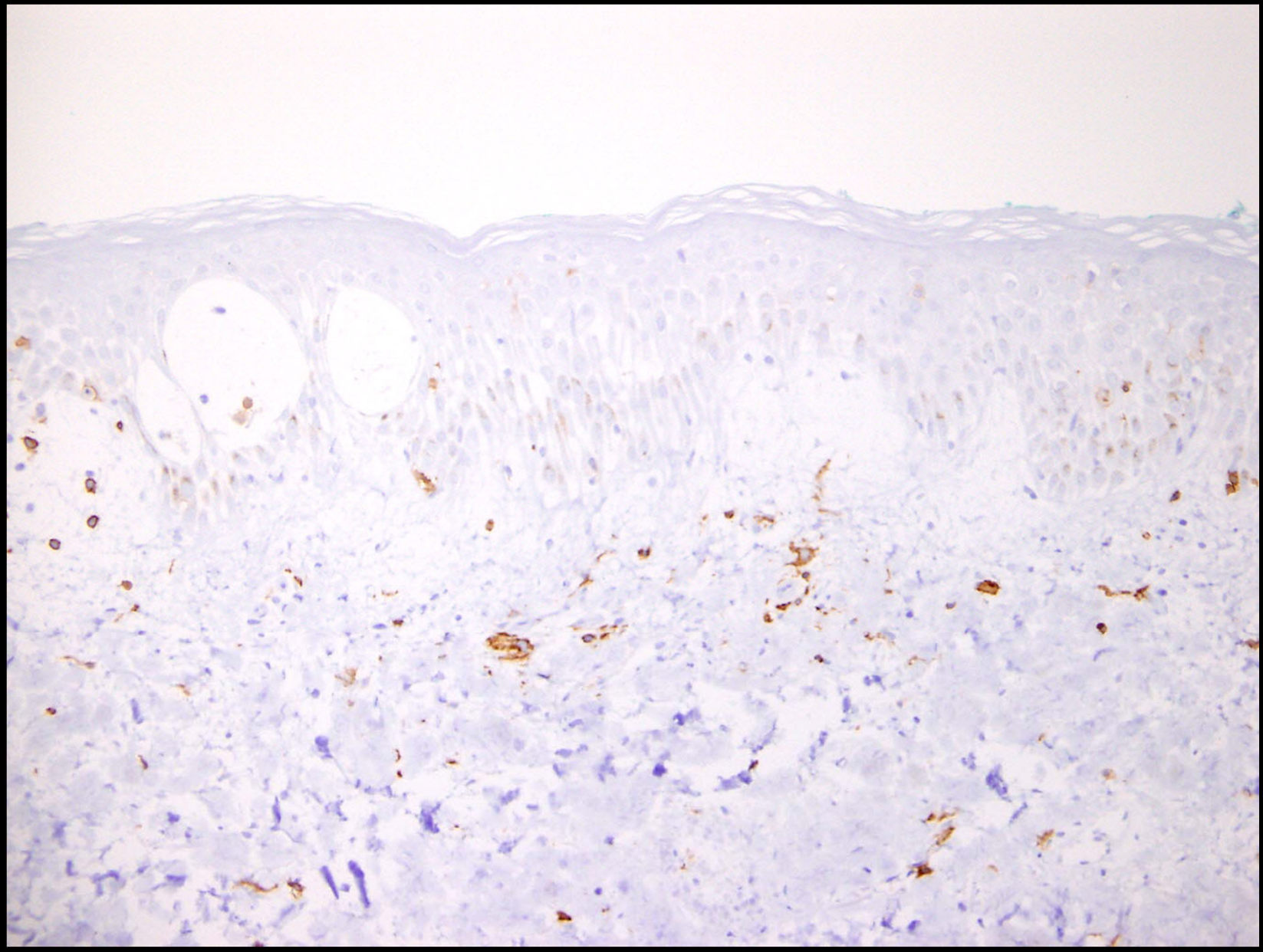
D.Tu. F5 TCR

Day +6



Day +26

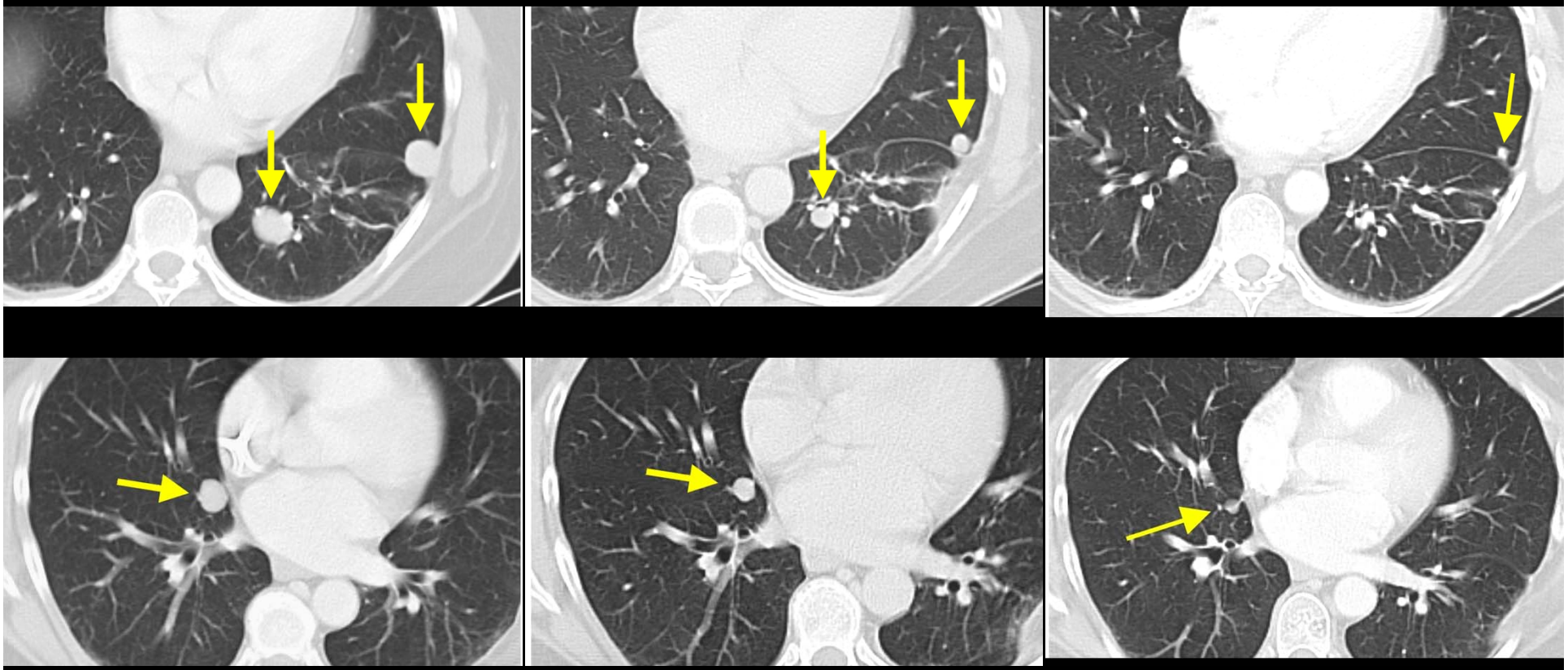




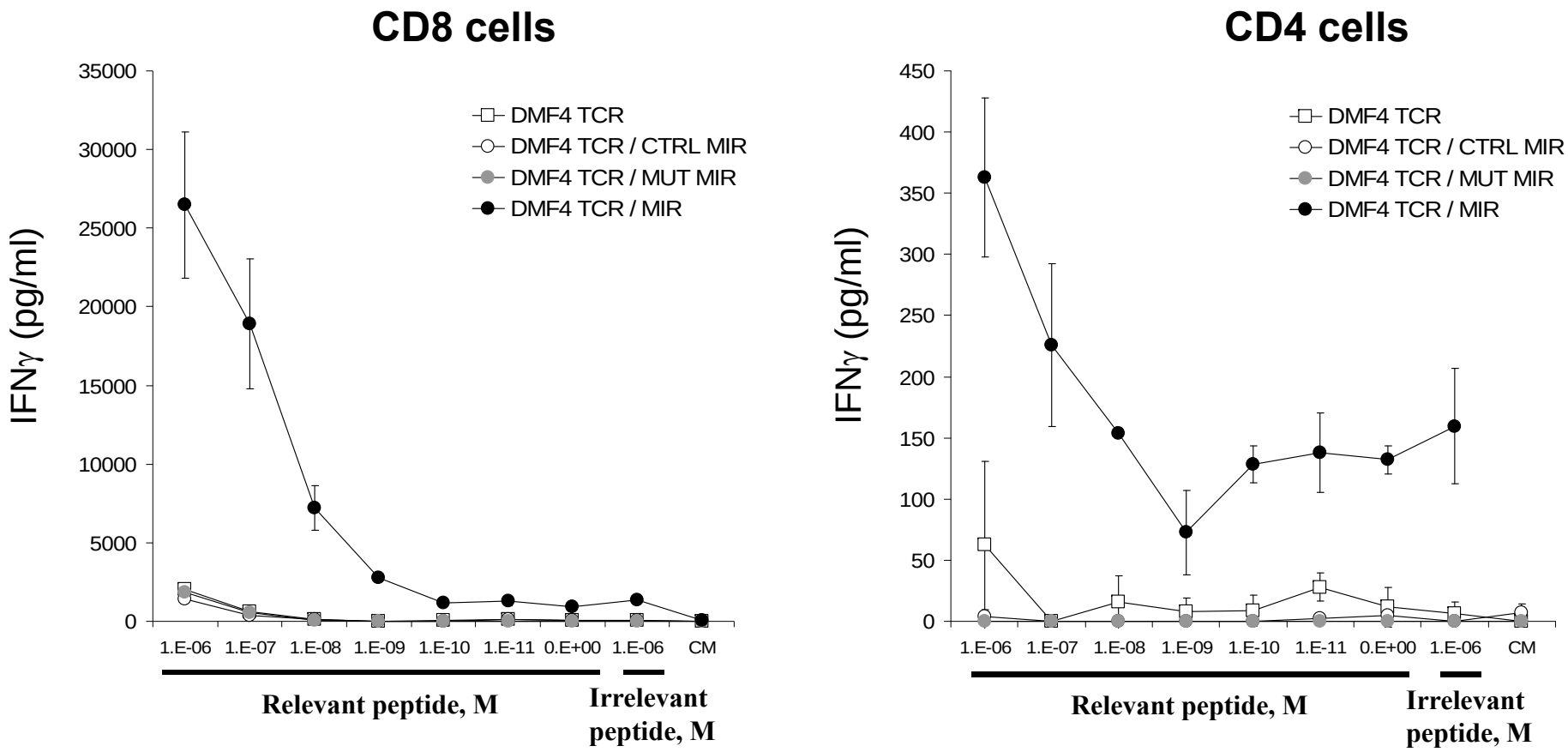
Day 5 post F5 TCR cell infusion (D. Tu)
Skin: CD4 positive cells

D.Tu. MART F5 TCR

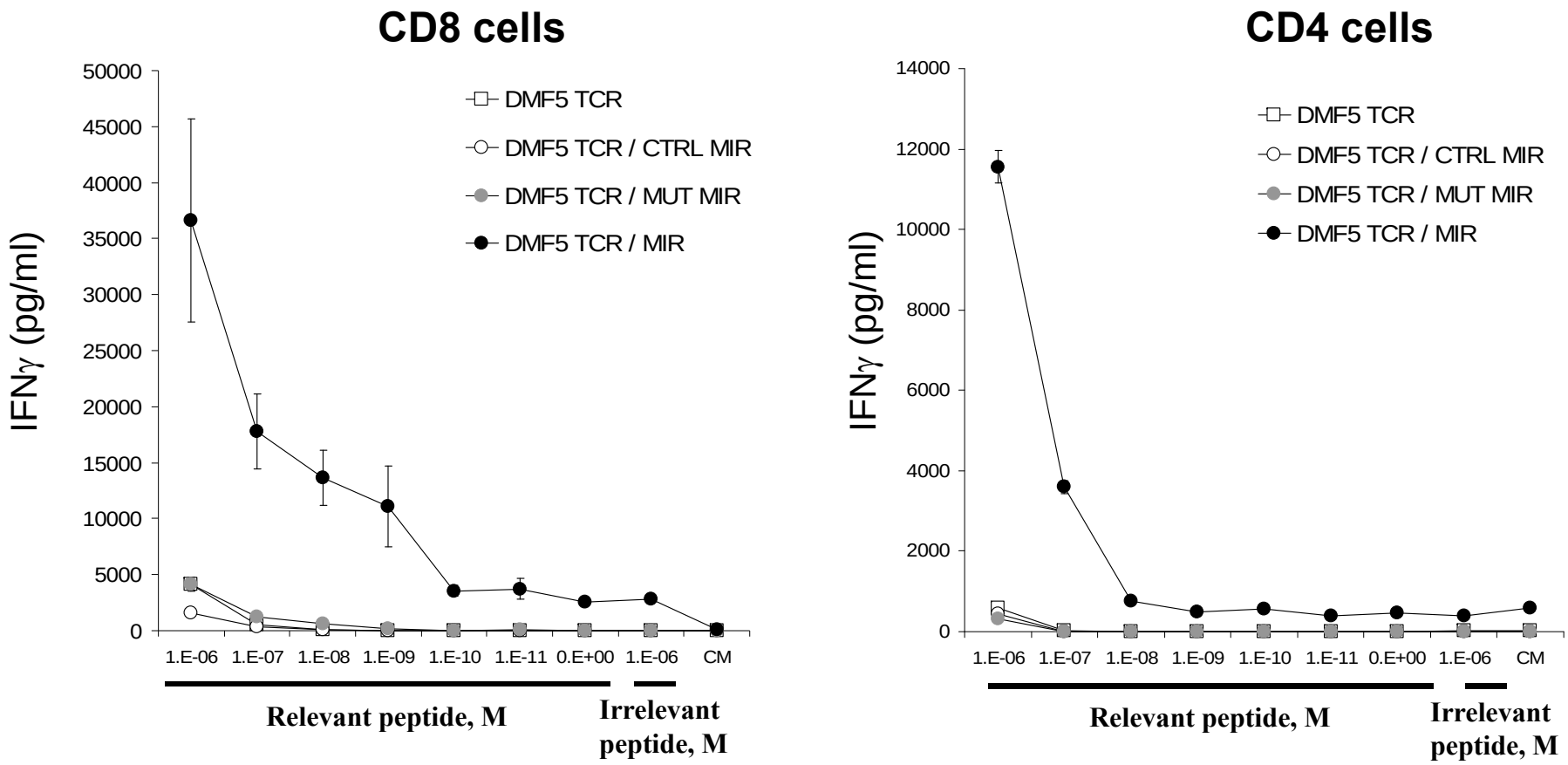
Pre-treatment | 1 month | 2 months



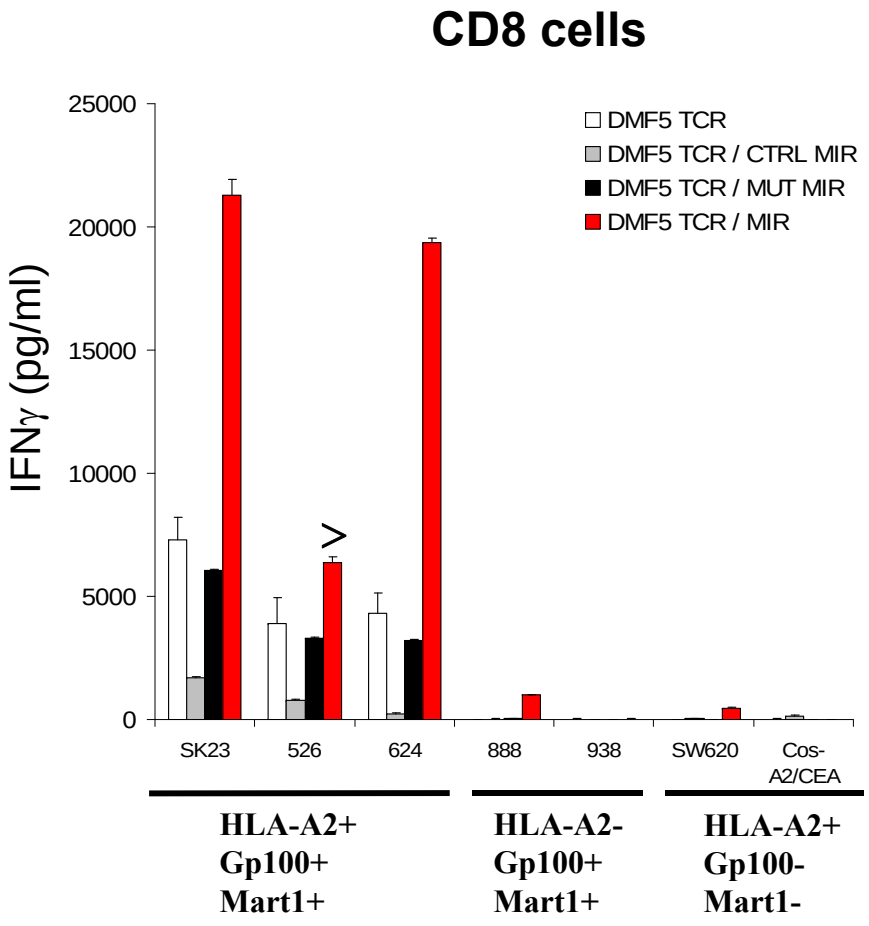
Mir-181a increases the functional avidity of human CD8 and CD4 cells expressing DMF4 TCR

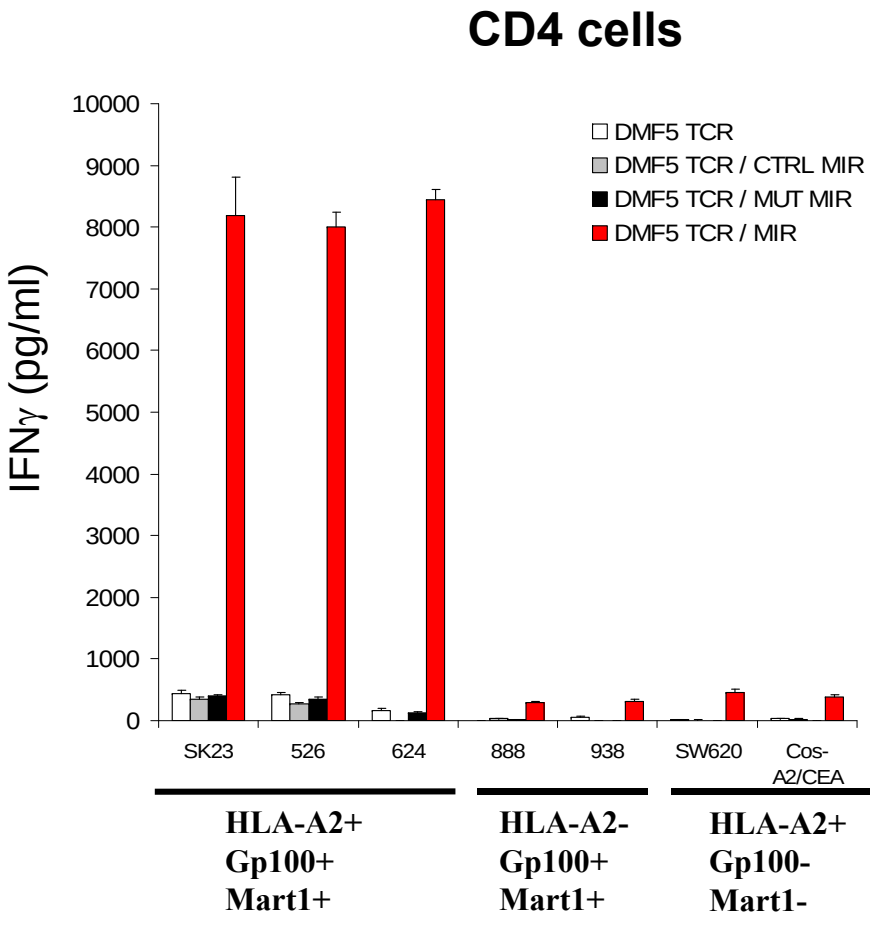


Mir-181a increases the functional avidity of human CD8 and CD4 cells expressing DMF5 TCR

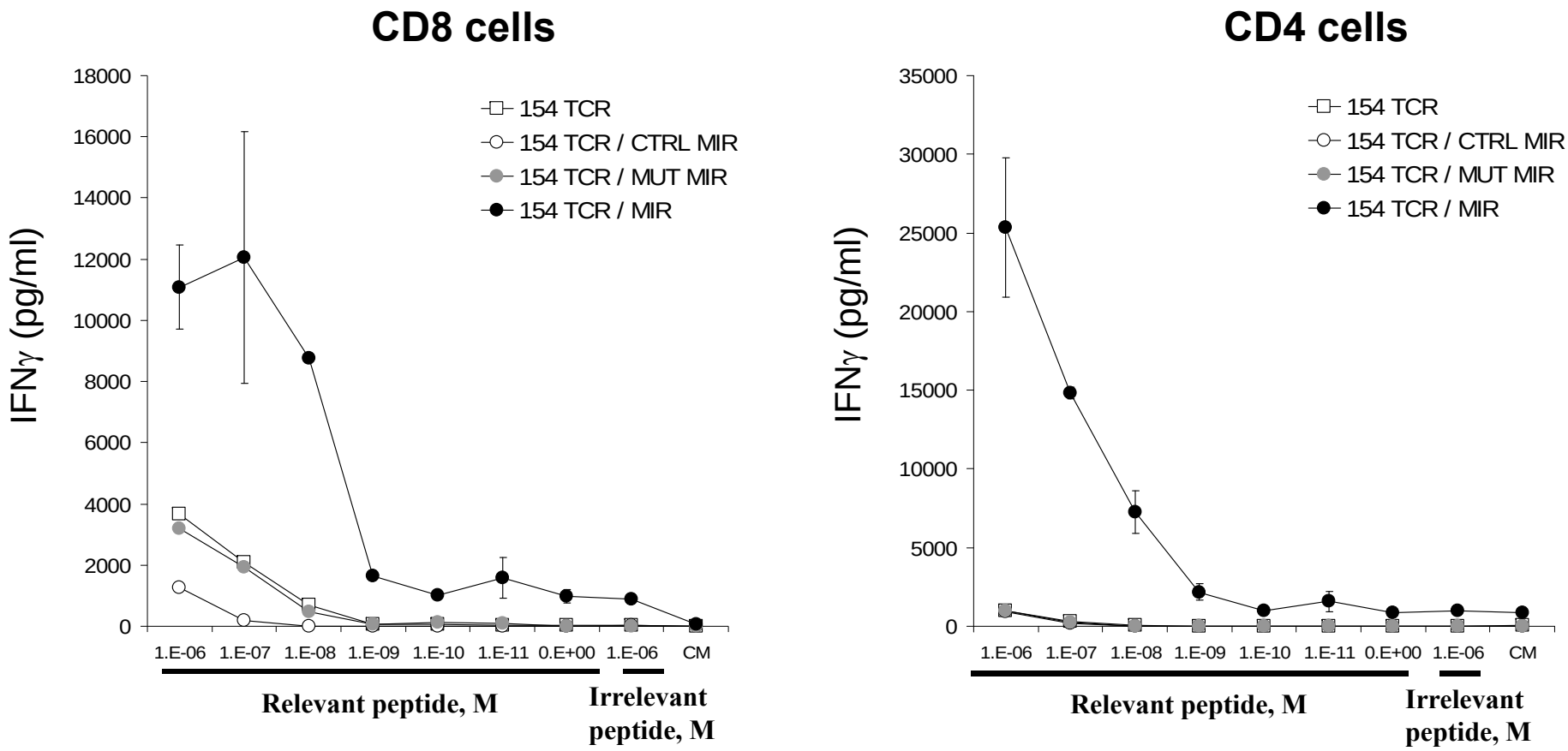


Mir-181a increases the tumor reactivity of human CD8 and CD4 cells expressing DMF5 TCR





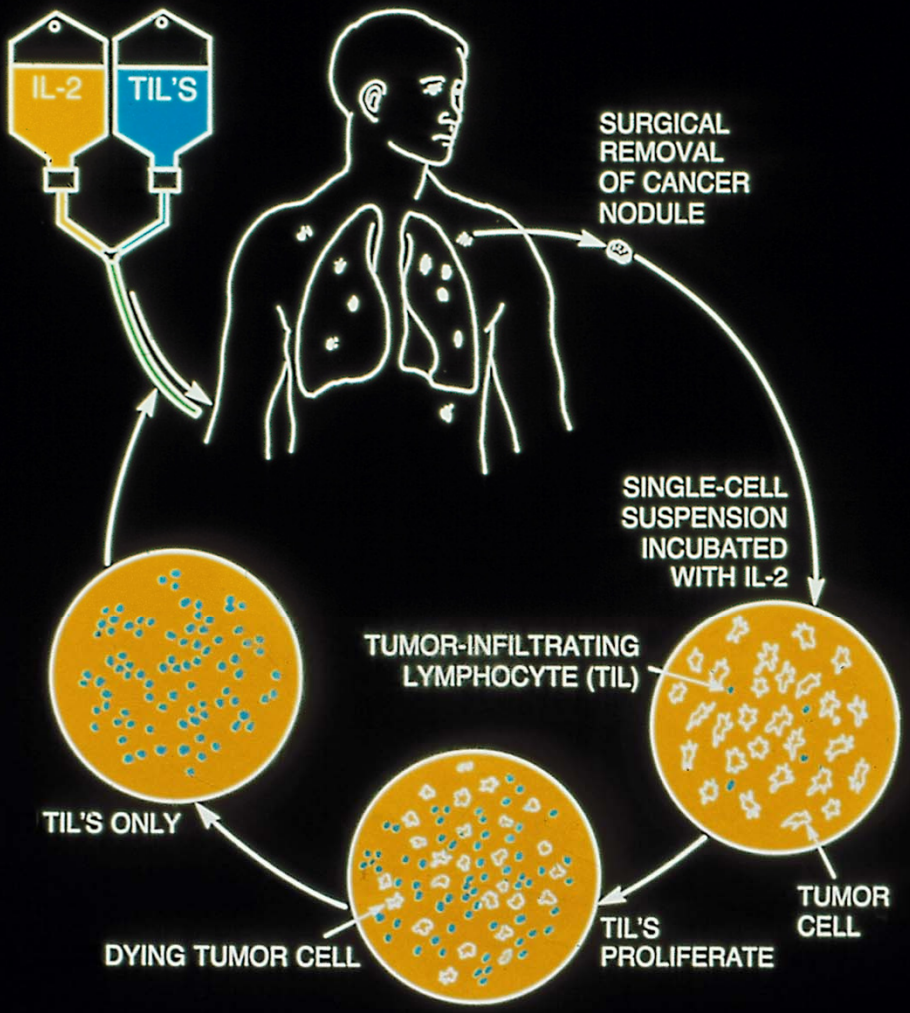
Mir-181a increases the functional avidity of human CD8 and CD4 cells expressing 154 TCR



QUESTION

What cells are responsible for the regression of melanoma in patients treated with IL-2 based immunotherapy?

TREATMENT WITH TUMOR INFILTRATING LYMPHOCYTES (TIL)



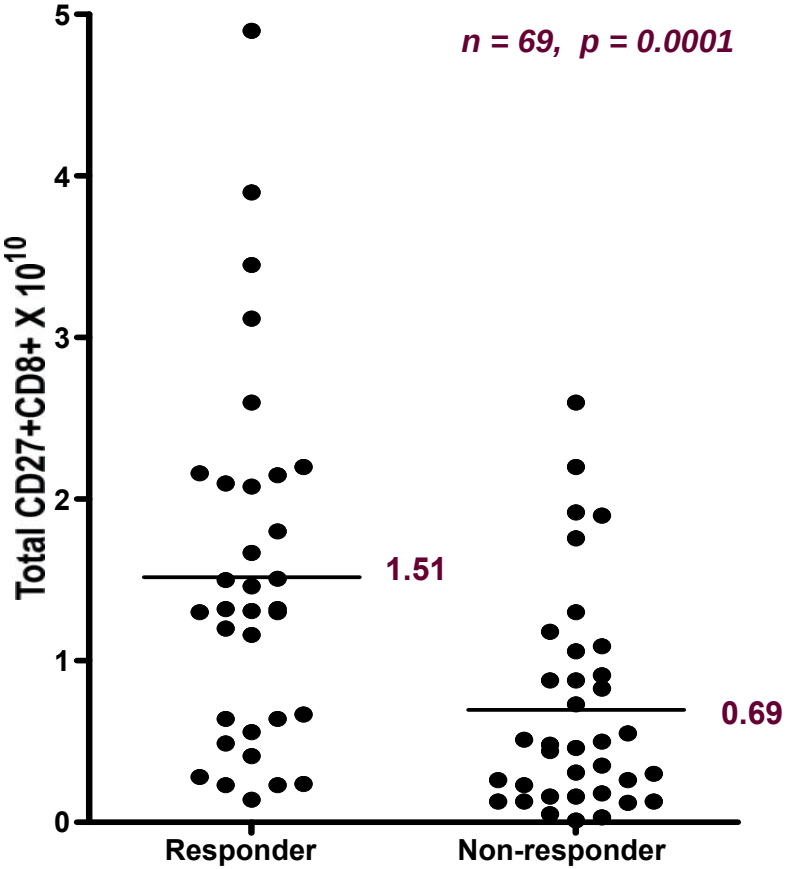
HUMAN CANCER ANTIGENS

In the Surgery Branch, NCI we have identified over 50 human cancer antigens and epitopes recognized by CD8+ cells (Class I) or by CD4+ cells (ClassII).

(reviewed in Nature 411:380-384, 2001)

Total number of $CD27^{+}CD8^{+}$ in infusion TIL highly correlates with response

NMA+TBI



* Infusion TIL cultured in IL-2 free medium for two days.

** The data were included only for the first treatment of each patient.

Approaches to the generation of high avidity, highly active anti-tumor T cell receptors

---

To identify high affinity anti-tumor TCR:

1) **Screen limiting dilution clones from tumor infiltrating lymphocytes or PBL from immunized patients**

2) **Immunize mice transgenic for human HLA molecules (avoid tolerance)**

Approaches to the generation of high avidity, highly active anti-tumor T cell receptors

---

Screen limiting dilution clones from tumor infiltrating lymphocytes or PBL from immunized patients

Immunize mice transgenic for human HLA molecules (avoid tolerance)

Modify the constant regions of the transduced TCR to minimize mispairing with endogenous TCR chains

Modify individual residues in the CDR2 and CDR3 antigen binding regions of the TCR to increase TCR affinity

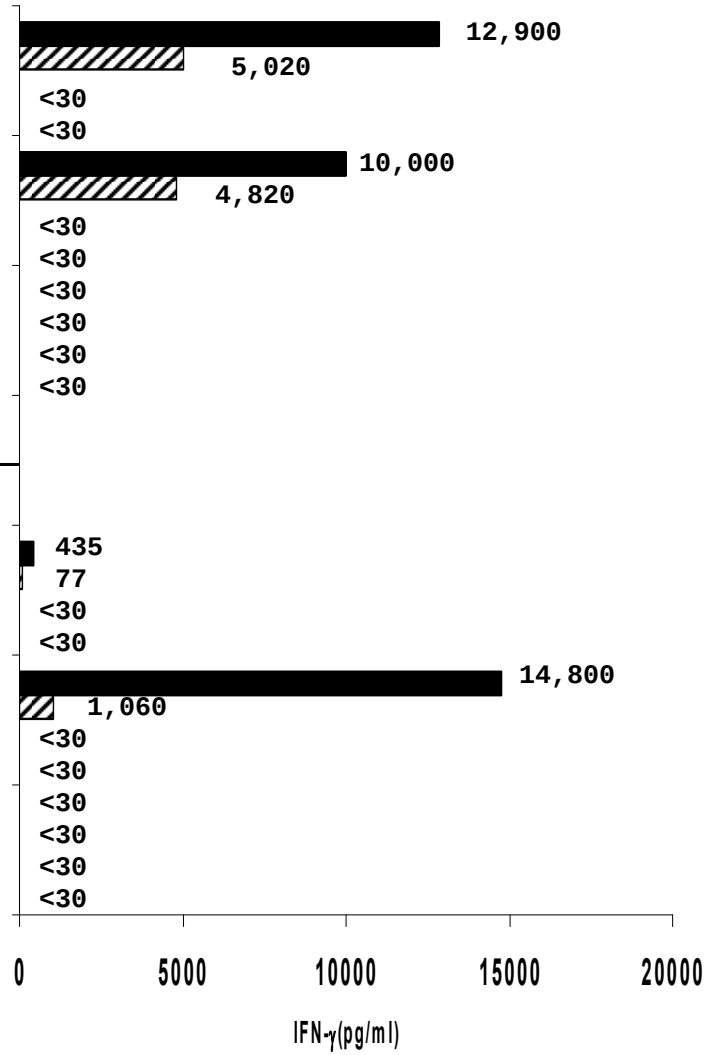
Reactivity of MART F5 TCR Transduced Cells

Effector	Target: None	888 mel ($A2^-$)	526 mel ($A2^+$)	264 mel ($A2^+$)
			(pg/ml IFN-g)	
AK1700	0	2	0	10
JKF6 (MART)	837	285	**22500**	**31400**
L2D9 (gp100)	0	0	**73800**	**83400**
D.Tu. F5 TCR (R2d7)	254	258	**32800**	**60200**
D.Tu. Untransduced	197	96	63	88

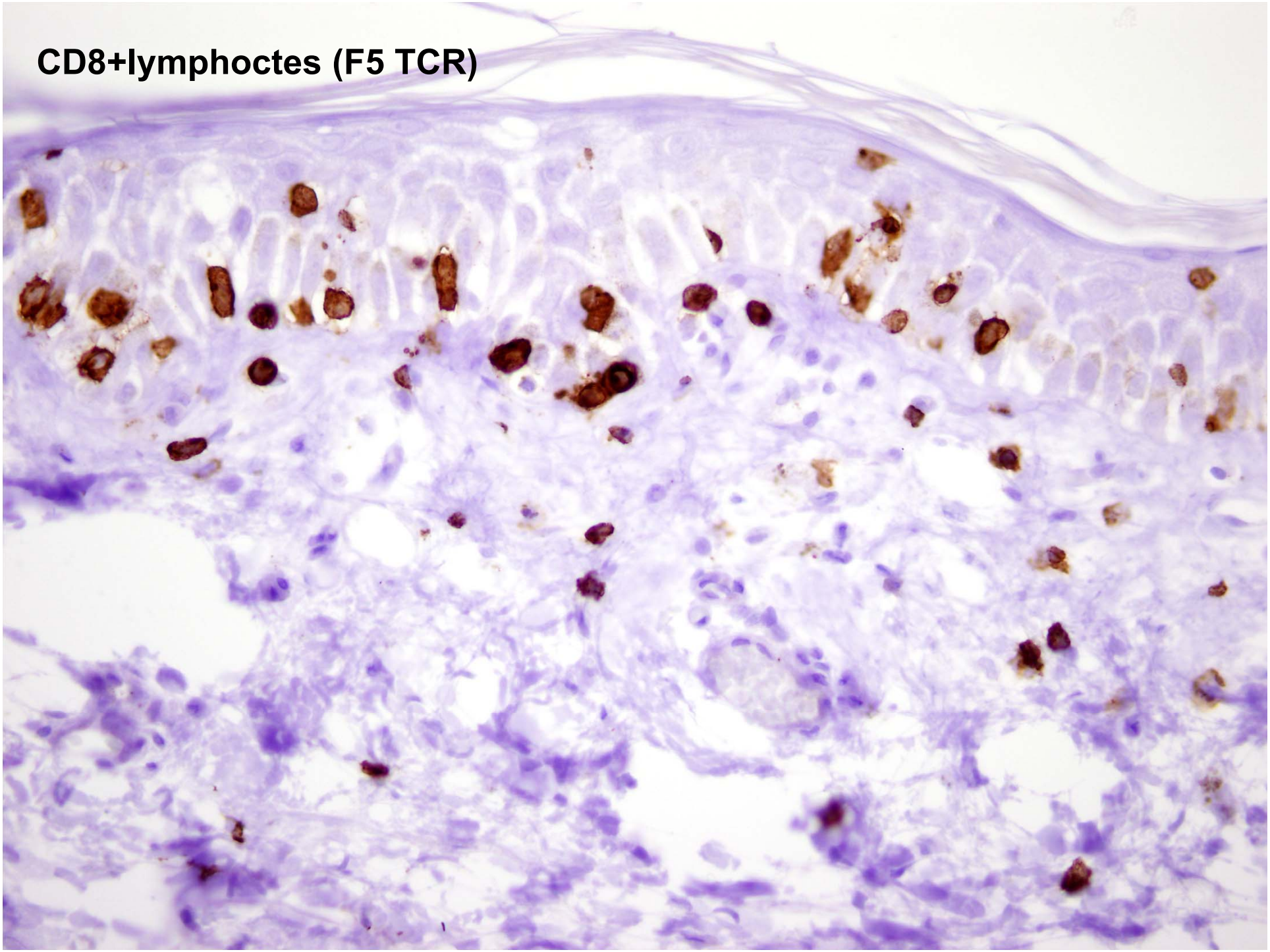
DMF4 MART-1 TCR

CDR2β (47-52)

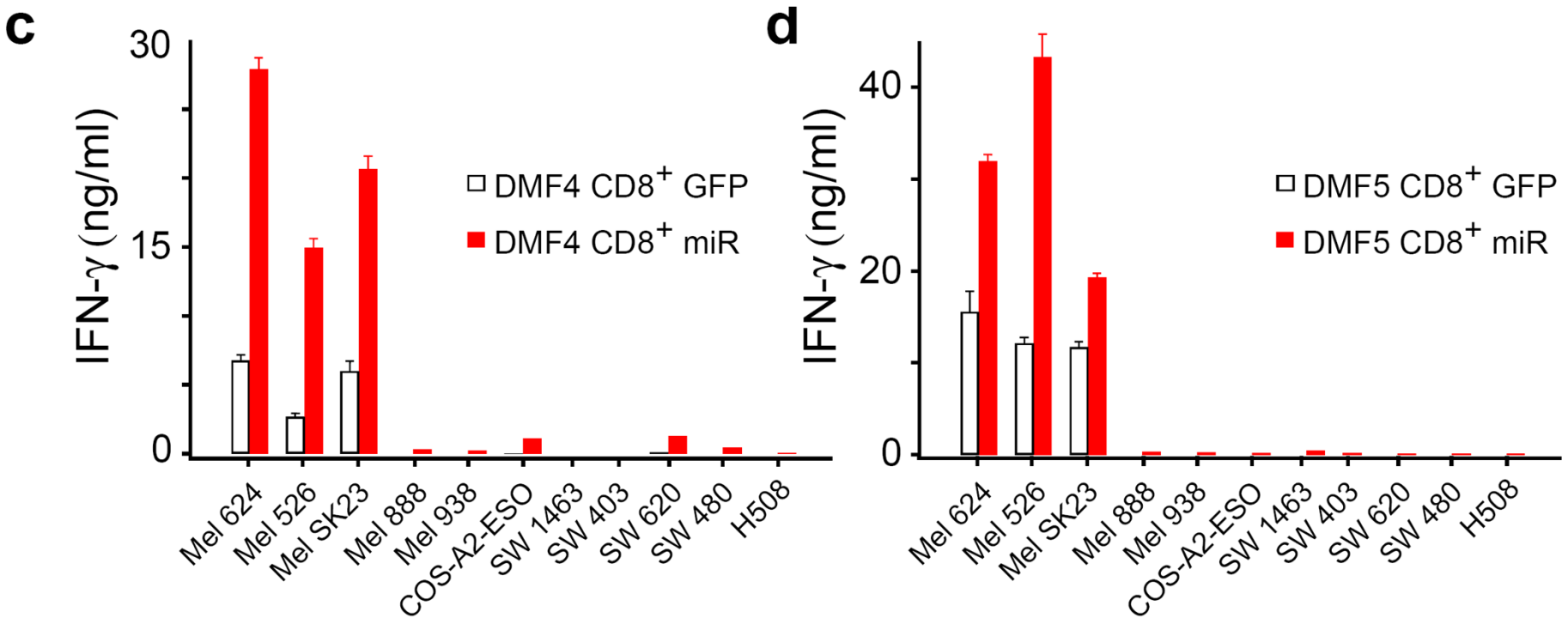
		49
CD8+	WT	G
		A
	None	
CD4+	WT	G
		A
	None	



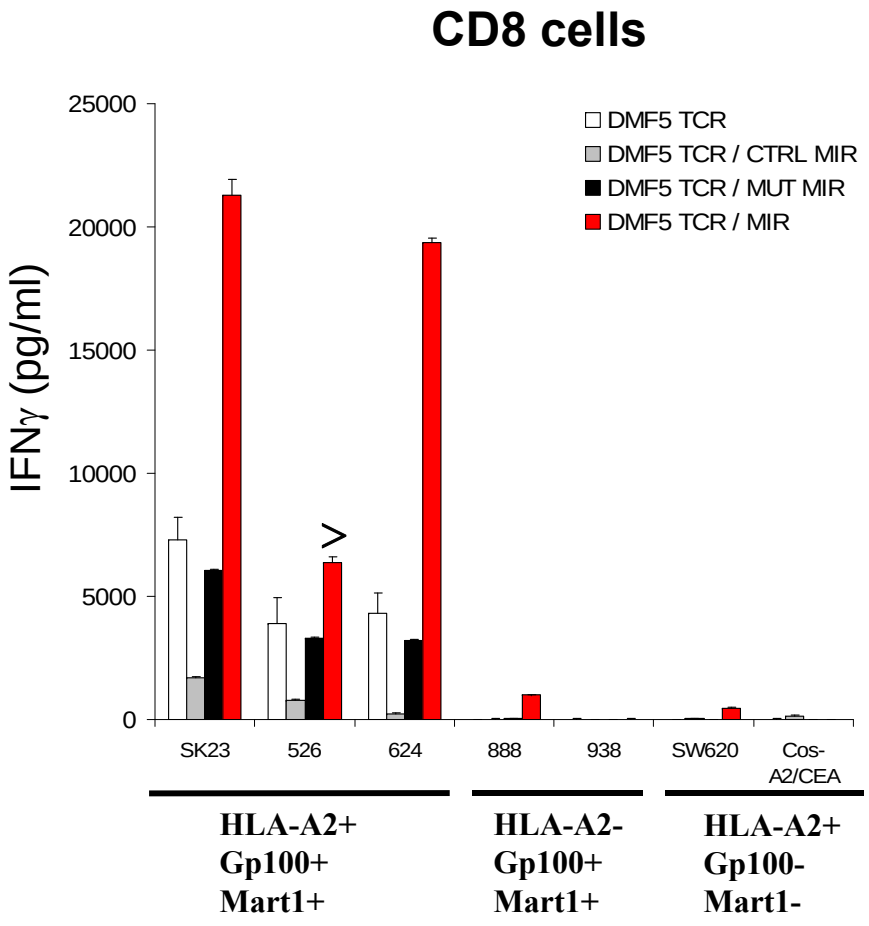
	A2	MART-1
1300 mel	+	+
624.38 mel	+	+
A375 mel	+	-
624.28 mel	+	-

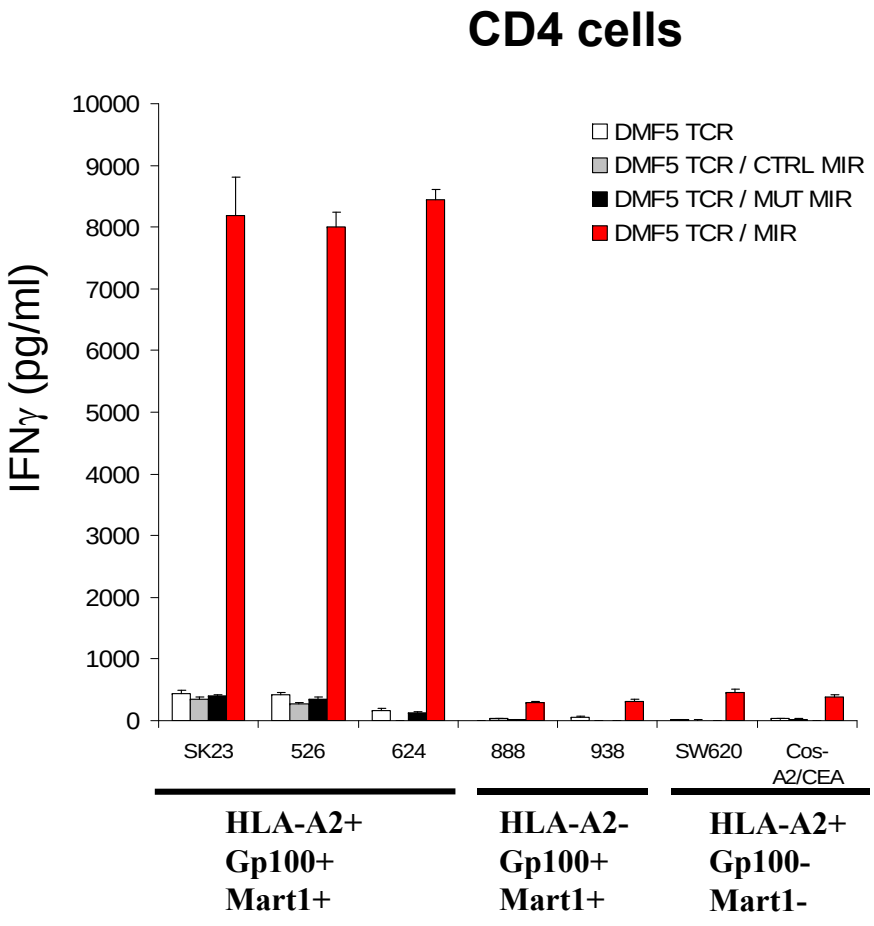

CD8+lymphoctes (F5 TCR)

Overexpression of miR181a increases the reactivity of genetically engineered human CD8+ T cells against tumors

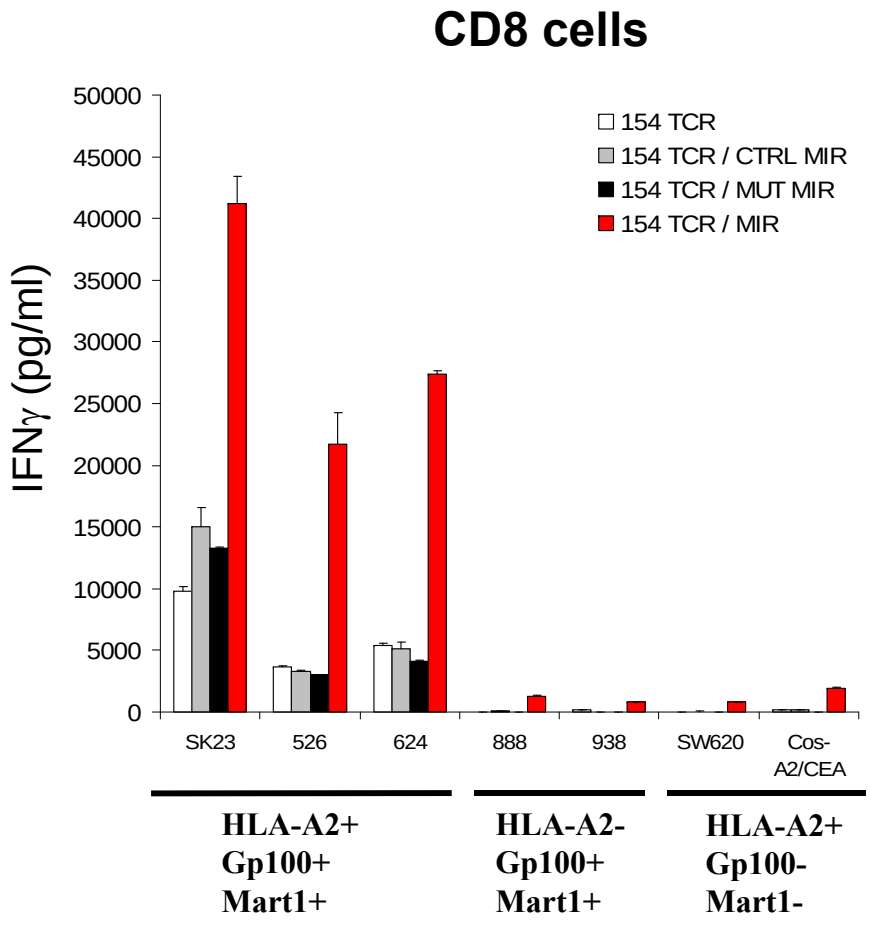


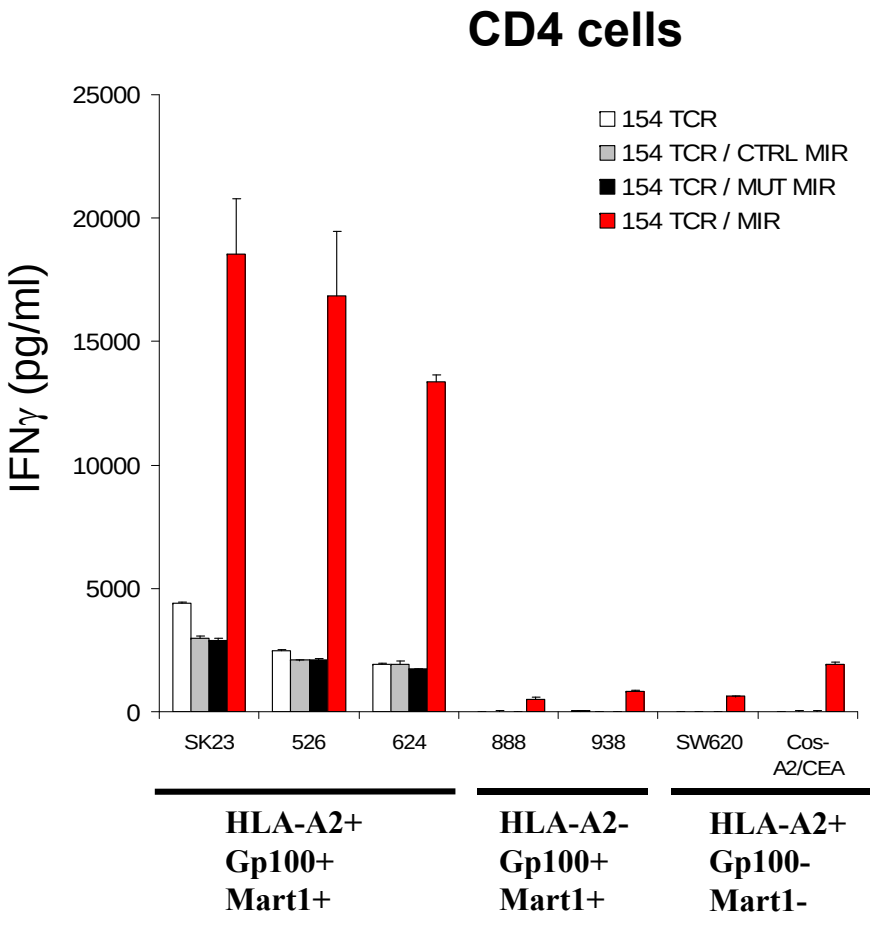
Mir-181a increases the tumor reactivity of human CD8 and CD4 cells expressing DMF5 TCR



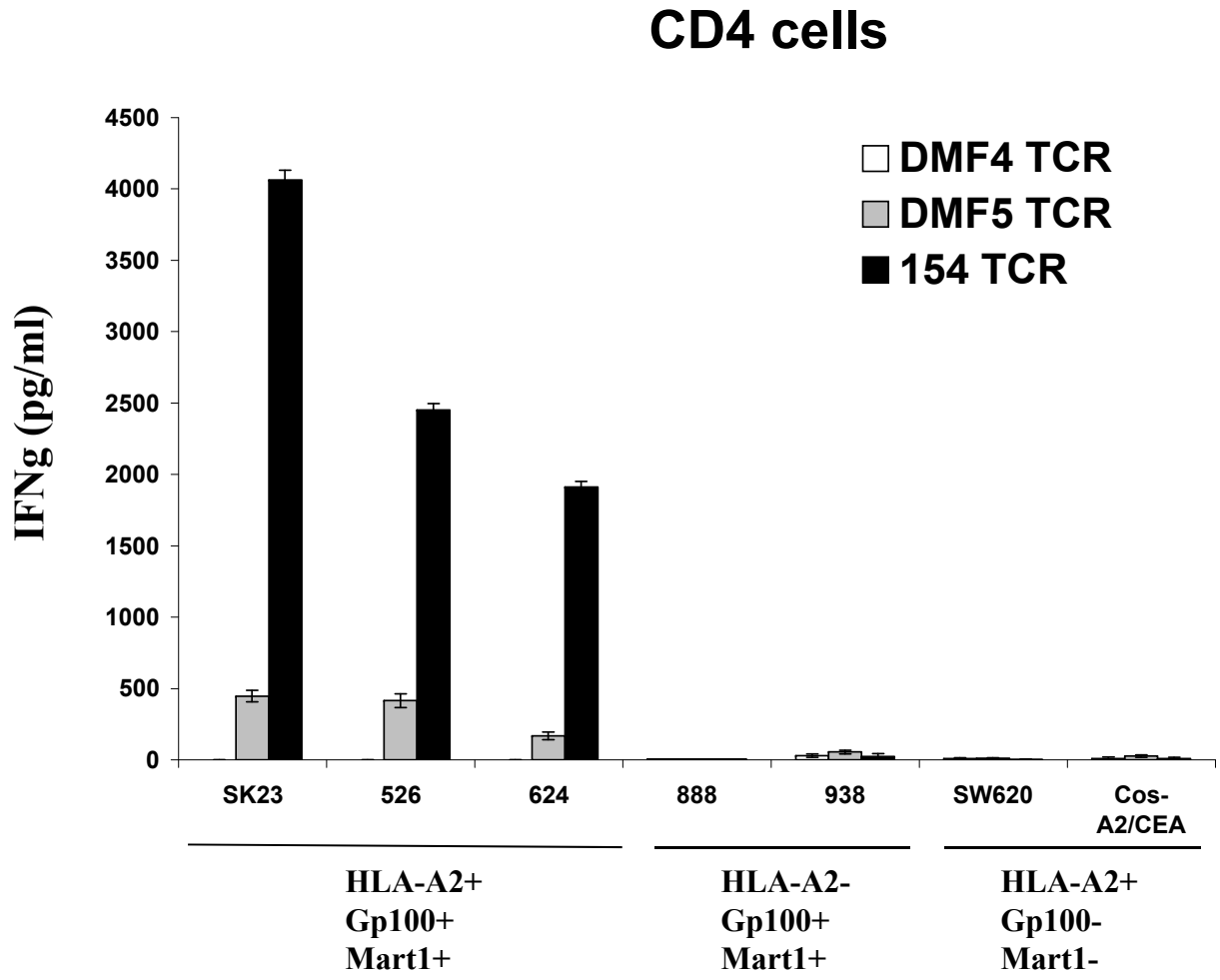


Mir-181a increases the tumor reactivity of human CD8 and CD4 cells expressing 154 TCR





Secretion of IFNg by CD4 cells transduced with DMF4, DMF5 or 154 TCR



PRINCIPLES OF THE DEVELOPMENT OF HUMAN CANCER IMMUNOTHERAPY

1. Stimulation of T cells with IL-2 can mediate regression of metastatic cancer in patients with melanoma and renal cancer.

2. Immune cells exist in cancer patients that recognize the cancer. These cells can be used to identify cancer antigens.

3. Blockade of lymphocyte inhibitory signals can mediate cancer regression (but also induces autoimmunity).

4. Prior depletion of the patient's natural immune system can improve the effectiveness of cell transfer immunotherapy.

5. Normal human peripheral lymphocytes can be genetically modified ex vivo for use in effective cancer immunotherapy. This approach is currently being explored for the immunotherapy of patients with melanoma and common epithelial cancers.

Melanoma Patients Receiving Anti-CTLA-4 Monoclonal Antibody

(FU: 3/15/07)

	Total	Objective response	Duration (months)
	(number of patients)		
No prior IL-2	72	15 (20.8%)	57+, 52+, 43, 34+, 32+, 29+, 25+, 22, 18+, 17+, 11, 10, 6, 5, 4
Prior low-dose IL-2	23	3 (13.0%)	53+, 17+, 6
Prior high-dose IL-2	44	5 (11.3%)	33+, 19, 10, 9, 7
Total	139	23 (16.5%)	(11 of 23 ongoing 17 to 57 months from 3/1/07)

Approaches to the generation of high avidity, highly active anti-tumor T cell receptors

---

Screen limiting dilution clones from tumor infiltrating lymphocytes or PBL from immunized patients

Immunize mice transgenic for human HLA molecules

(avoid tolerance)

SUMMARY

Immunotherapy can mediate the regression of bulky, invasive, metastatic cancer in humans.

Recent studies are providing approaches for the immunotherapy of patients with common epithelial cancers.

THREE MAIN APPROACHES TO CANCER IMMUNOTHERAPY

---

1. **Nonspecific stimulation of immune reactions**
   a) **Stimulate effector cells**
   b) **Inhibit suppressive factors**

2. Active immunization to enhance anti-tumor reactions (cancer vaccines)

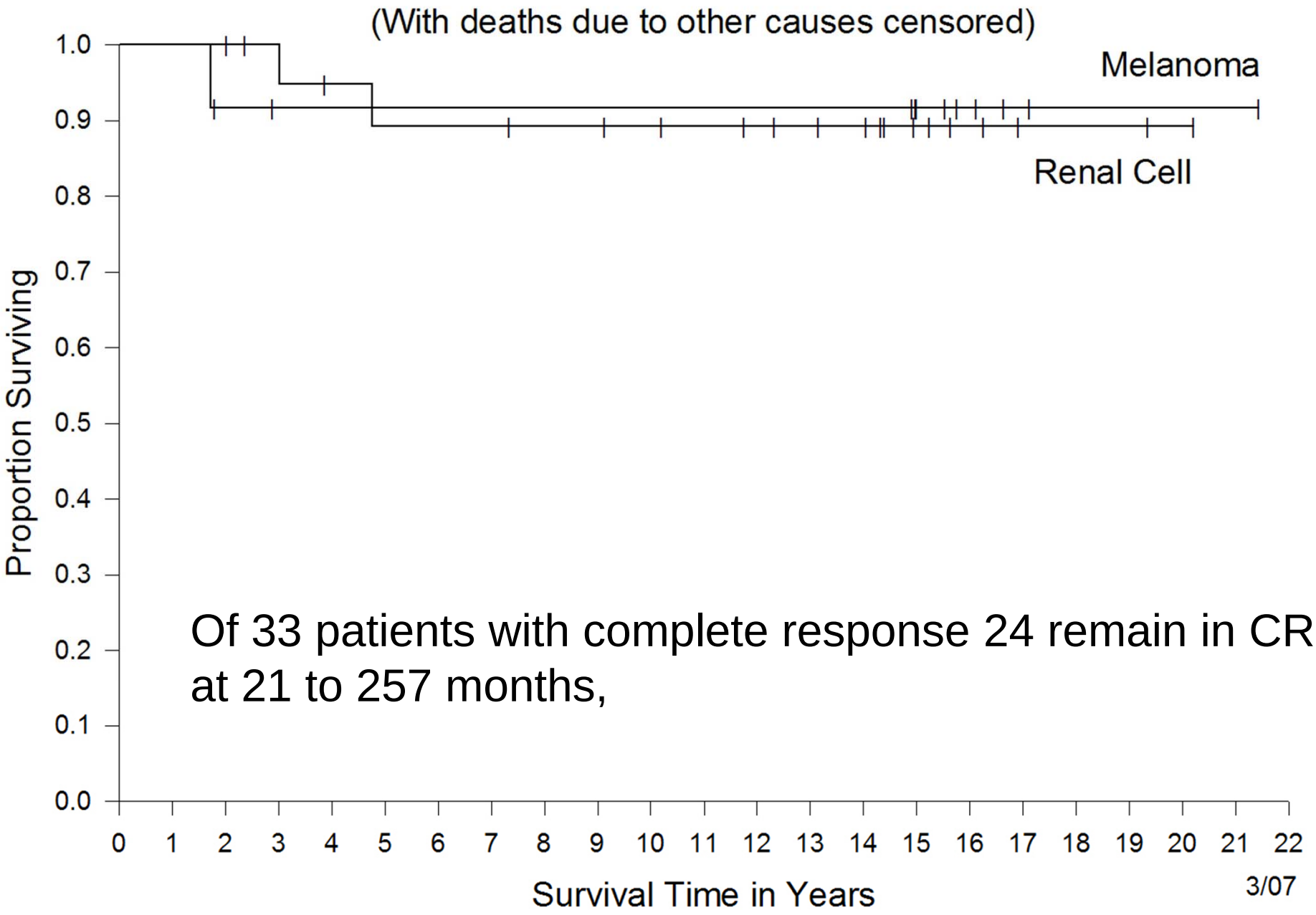
3. Passively transfer activated immune cells with anti-tumor activity (adoptive immunotherapy)

RESPONSE OF PATIENTS WITH METASTATIC CANCER TREATED USING HIGH-DOSE BOLUS INTERLEUKIN-2

Diagnosis	Total	CR	PR	CR + PR
		Number of patients (%)		
Melanoma	305	12 (4%)	27 (9%)	39 (13%)
Renal Cell Cancer	264	21 (8%)	32 (12%)	53 (20%)
Total	569	33 (6%)	59 (10%)	92 (16%)

Patients accrued between Sept. 1985 and Dec. 2005.
Follow-up as of March 15, 2007 (median follow-up 14.3 yrs)

Complete Response to Treatment with High-Dose IL-2



Of 33 patients with complete response 24 remain in CR at 21 to 257 months,

THREE MAIN APPROACHES TO CANCER IMMUNOTHERAPY

---

1. **Nonspecific stimulation of immune reactions**
   a) **Stimulate effector cells**
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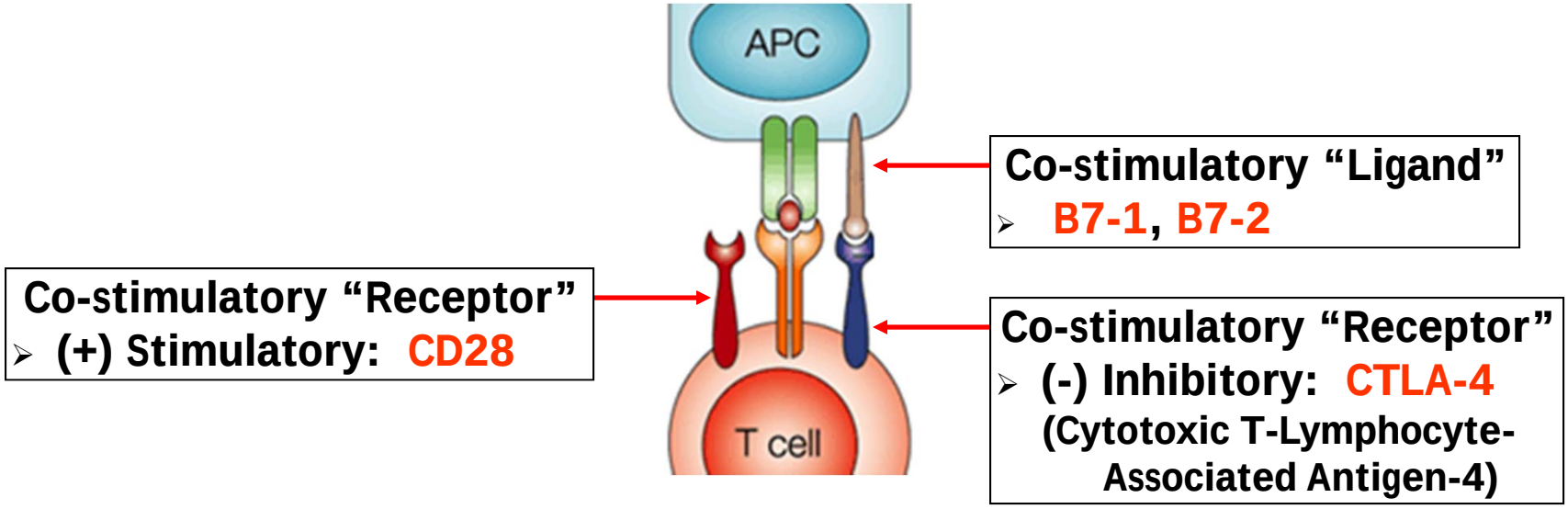
“Second Signal”

Additional signal(s) via co-stimulatory molecules



Treatment with Anti-CTLA 4 (MDX010)

(02/08)

Trial	Total	PR	CR	OR (%)
MDX010-05	54	5	2	7(13%)
(3 and 1mg/kg)		(61+,60+,42,5,4)	(66+,66+)	
MDX010-19	85	15	2	17(20%)
(Dose escalation; 3 to 9mg/kg)		(44+,38+,33+,27+, 27,26+,25,15,11, 10,9,9,7,6,5)	(42,41+)	
Total	139	20	4	24(17%)

GRADE 3/4 AUTOIMMUNE TOXICITIES IN PATIENTS RECEIVING ANTI-CTLA4

Total: 175 patients

Autoimmune toxicity	Number of patients (%)	
Colitis/enteritis	27	(15.4%)
Dermatitis	12	(6.9%)
Hypophysitis	8	(4.6%)
Uveitis	3	(1.7%)
Hepatitis	1	(0.6%)
Nephritis	1	(0.6%)
Arthritis	1	(0.6%)
Meningitis	1	(0.6%)

THREE MAIN APPROACHES TO CANCER IMMUNOTHERAPY

---

1. Nonspecific stimulation of immune reactions
   a) Stimulate effector cells
   b) Inhibit regulatory cells

2. **Active immunization to enhance anti-tumor reactions (cancer vaccines)**

3. Passively transfer activated immune cells with anti-tumor activity (adoptive immunotherapy)

REVIEW OF CLINICAL VACCINE STUDIES IN PATIENTS WITH METASTATIC SOLID CANCERS

	Number of trials	Total	Objective responders	%
Published				
		(number of patients)		
Peptide	11	175	7	4.0%
Pox virus	7	200	0	0
Tumor cells	5	142	6	4.2%
Dendritic cells	10	198	14	7.1%
Heat shock proteins	2	44	2	4.5%
Total	33	765	29	4.0%
Surgery Branch				
Peptide	15	366	9	2.9%
Virus or DNA	8	160	3	1.9%
Dendritic cells	2	15	2	13.3%
Total	25	541	14	2.6%
Overall:	58	1306	43	3.3%

FREQUENCY OF $CD8^+$ T-CELLS REACTIVE WITH NATIVE gp100:209-218 PEPTIDE (TETRAMER ASSAY)

Patient	After one course	After four courses
	(% of $CD8^+$ cells $tetramer^+$)	
1	2.57% (1/39)	13.2% (1/8)
2	0 (0)	6.4% (1/16)
3	0.12% (1/833)	4.8% (1/21)
4	1.80% (1/56)	19.1% (1/5)
5	0.54% (1/185)	38.1% (1/3)

THREE MAIN APPROACHES TO CANCER IMMUNOTHERAPY

1. Nonspecific stimulation of immune reactions
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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.

REACTIVITY OF DW CLONES TO g209 PEPTIDE

	lysis (%)	IFN-g	GM-CSF	TNF-a (pg/ml)	IL-2	IL-4	IL-10
W1C3	10	53	736	2	9	0	1
W1C6	11	658	>5000	31	90	-3	-22
W1D3	-1	432	920	0	16	0	3
W1D4	-2	0	2	-2	-1	-1	0
W1D7	37	351	1272	3	51	0	-2
W1D8	-1	143	>5000	1	14	-71	62
W2D12	-2	155	2364	0	50	-52	-23
W1F1	9	>10000	2720	36	124	0	29
W1F6	3	147	1310	0	ND	-2	ND
W1H6	9	463	436	84	ND	0	ND
W1G2	15	494	1244	14	ND	0	ND
W1G3	1	705	3164	12	ND	0	ND
W1G12	47	529	808	0	34	0	2
W2A5	5	54	415	0	70	0	0
W2A6	14	161	912	0	79	-3	-8
W2A8	-1	>10000	>5000	1	-40	0	1
W2C2	14	>10000	>5000	16	45	0	0
W2D3	-1	657	>5000	0	80	0	0
W2F1	8	>10000	>5000	144	257	0	2
W2F7	21	607	ND	27	73	0	0
W3B1	-1	>10000	>5000	79	669	0	4
W2C8	41	>10000	ND	619	>1500	2	27

PROTOCOL 99-C-95

Treatment of Patients with Metastatic Melanoma Using Cloned Lymphocytes Plus IL-2

Infuse up to 10^{11} cloned antitumor lymphocytes selected for high avidity for tumor recognition

Simultaneously begin IL-2 administration given either subcutaneously or intravenously

T-CELL CLONES USED FOR THE FIRST INFUSION CYCLE FOR EACH PATIENT SECRETE IFN-γ SPECIFICALLY TO g209 PEPTIDE AND HLA-A2$^+$ MELANOMAS

	Stimulator cell							
		T2 pulsed with peptide (μM)			Melanoma cell line			
Patient	g280 (1.0)	g209 (1.0)	g209 (0.01)	g209 (0.0001)	526-mel (A2$^+$)	624-mel (A2$^+$)	888-mel (A2$^-$)	938-mel (A2$^-$)
	(pg γ-IFN/ml)							
1	2	7633	1180	25	1000	1678	4	2
2	3	33025	3065	4	3263	6087	4	4
3	6	6190	4134	126	2040	4604	4	3
4	4	14103	5411	1096	5461	4078	12	0
5	6	>53402	18618	>5209	>49780	22835	0	0
6	0	5998	4609	164	973	940	0	0
7	10	17260	11229	816	3776	2760	7	4
8	72	7892	6321	988	2365	1624	0	0
9	1	15585	6624	840	92	401	0	0
10	9	33375	29720	>1259	699	2446	1	2
11	15	17435	20580	>1263	500	2848	1	0
12	1	9280	8051	>986	614	1596	ND	ND
13	35	2844	2175	148	2637	2155	16	24

PROTOCOL 99-C-0158

Treatment of patients with Metastatic Melanoma Using Cloned Lymphocytes Following Administration of a Non-Myeloablative but Lymphocyte Depleting Regimen

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a) Cyclophosphamide (30-60 mg/kg x2 days
Fludarabine (25 mg/m^2 x5 days)

b) After lymphocytes completely depleted (day 7):

Infuse up to 10^{11} cloned antitumor lymphocytes selected for high avidity for tumor recognition

Simultaneously (day 7) begin IL-2 administration

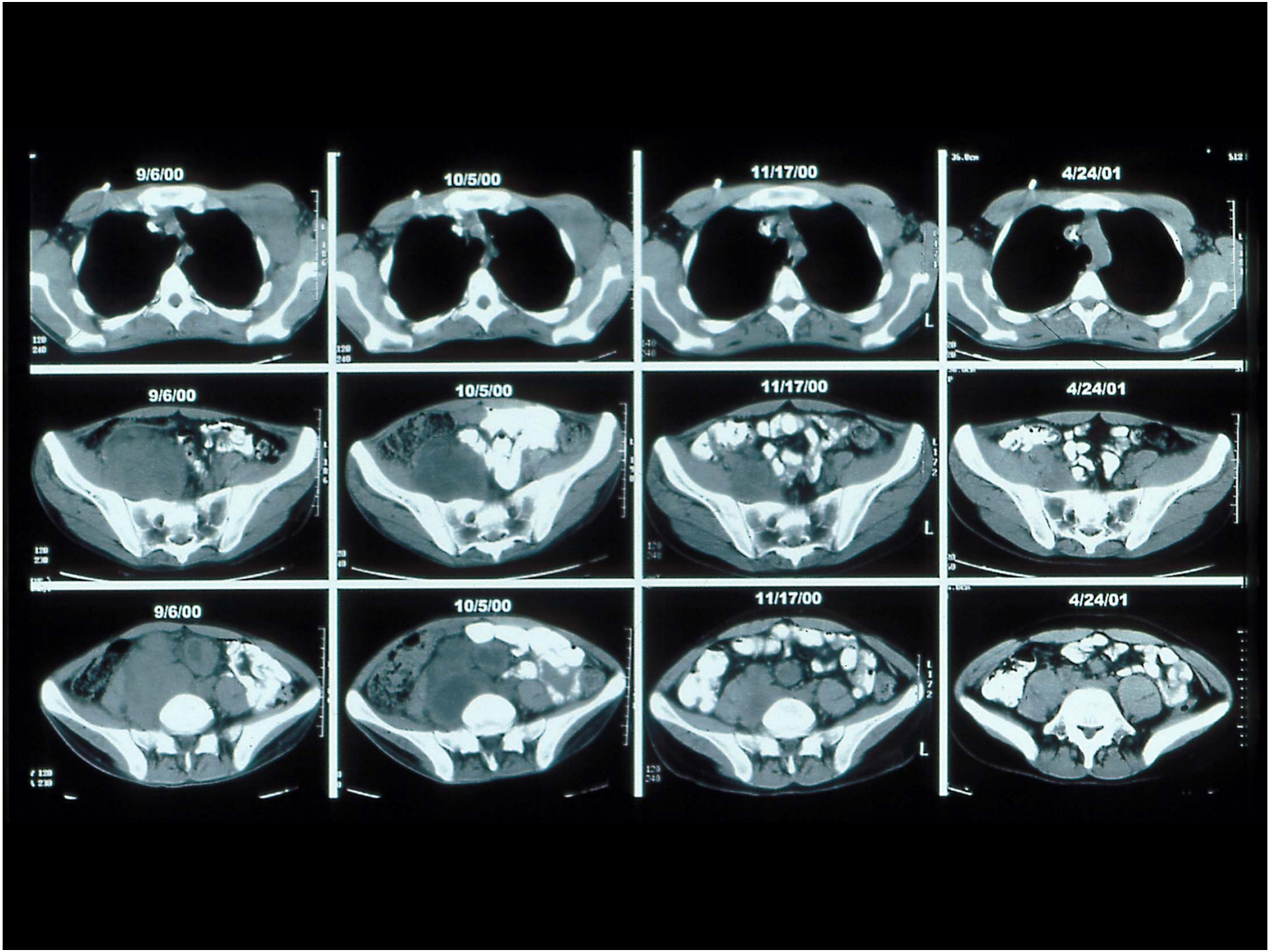
Phase I Study of Non-Myeloablative Chemotherapy Plus Transfer of Cloned T-Cells Plus IL-2

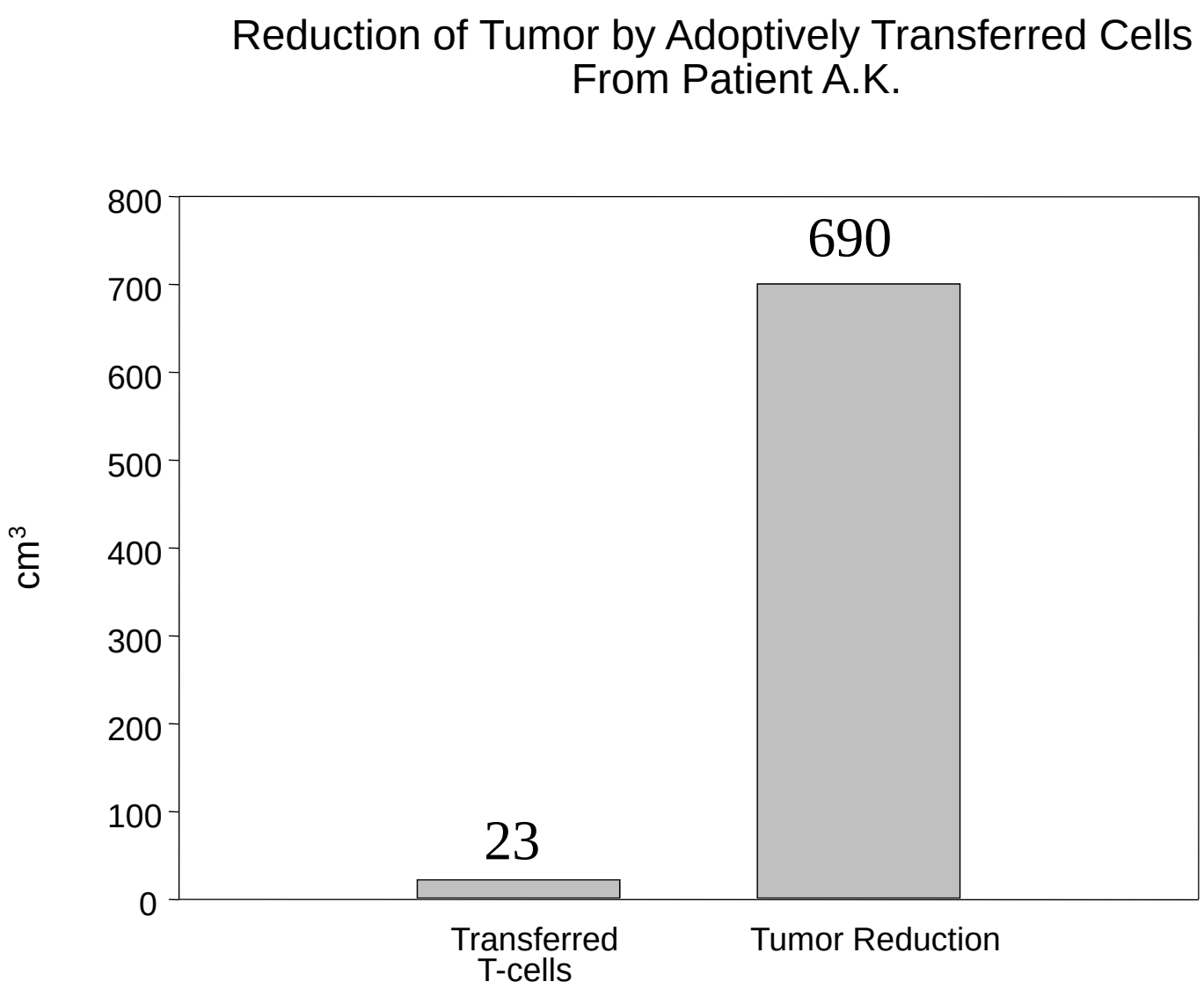
Patient	Cyclo-phosphamide (mg/M^2)	Fludarabine (IU/kg)	IL-2 ($x10^{-9}$)	Cloned Cells
LR	30	25	--	22.4
SC	30	25	--	21.5
FB	30	25	--	15.0
CK	60	25	--	9.3
JB	60	25	--	4.1
JH	60	25	--	5.5
BL	60	25	72,000	11.0
MD	60	25	72,000	6.8
CC	60	25	72,000	3.2
KS	60	25	720,000	2.8
AK	60	25	720,000	11.3
AT	60	25	720,000	0.9
CS	60	25	720,000	4.9
DK	60	25	720,000	12.6
ML	60	25	720,000	24.2

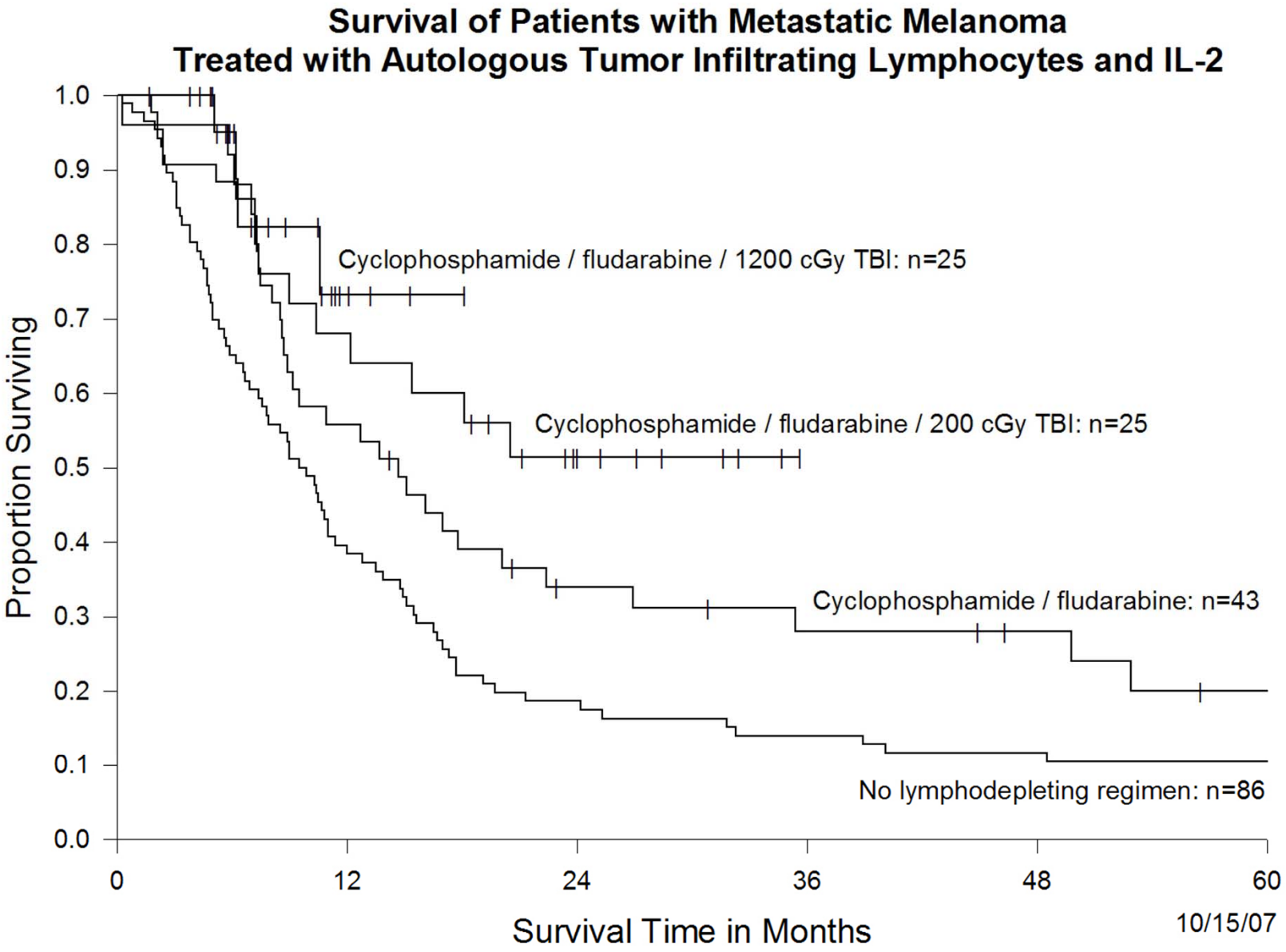
PATIENT A.K.

16 year old male referred to NCI with metastatic melanoma to multiple subcutaneous sites

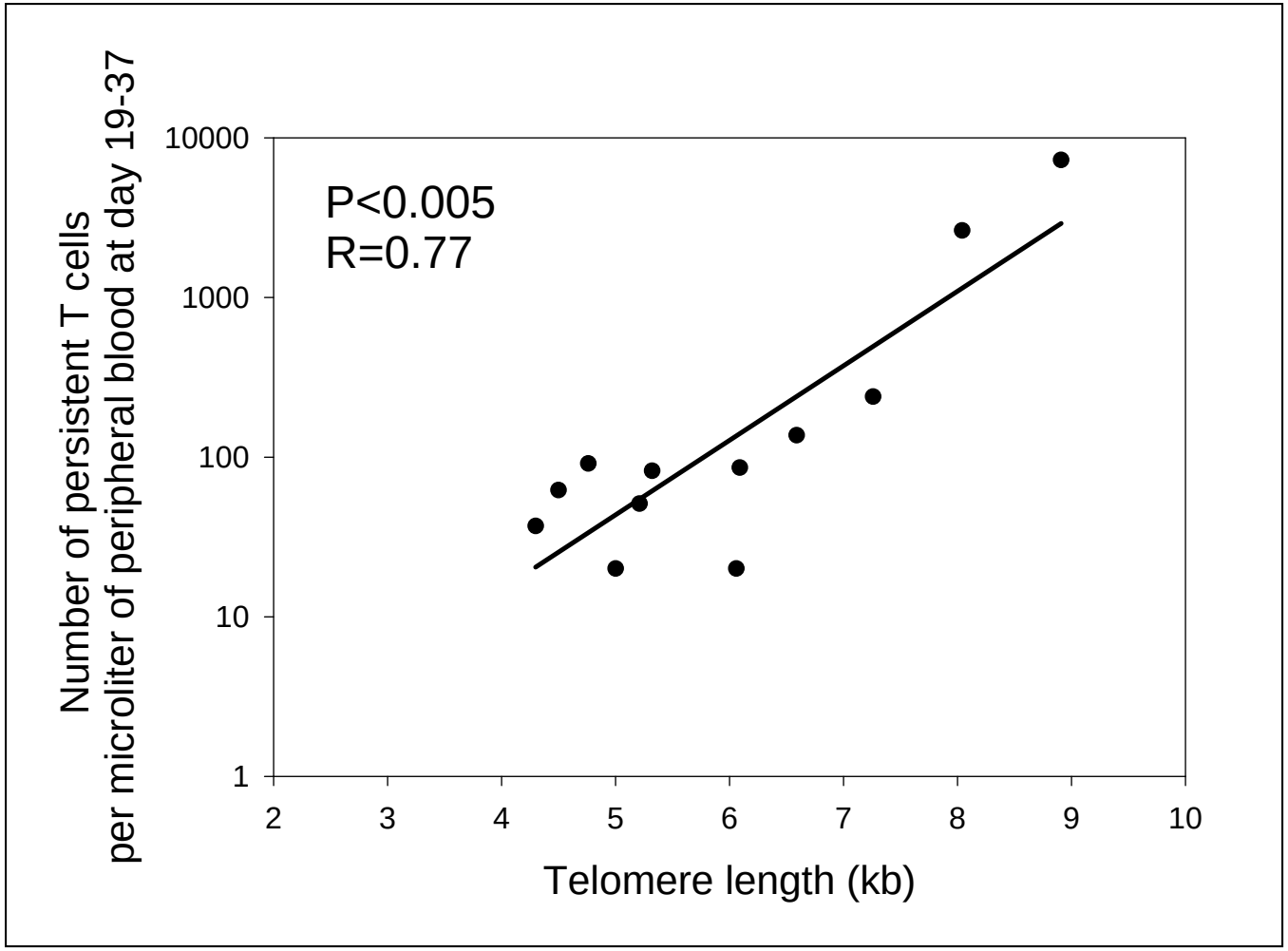
Oct. 1996	**Excision of a 3.4 mm deep melanoma of left knee**
March 1997	**Lymph node dissection, 1/9 positive; treated with alpha-interferon**
Oct. 1997	**Developed multiple subcutaneous metastases To NCI; treated with experimental 4 peptide vaccine; progressive disease**
Jan. 1998	**Multiple cycles of high-dose IL-2; progressive disease**
May 1998	**New subcutaneous masses; resected for TIL**
Jan. 2000	**Increasing subcutaneous, pelvic, axillary metastases Treated with cisplatin & dacarbazine; progressive disease**
March 2000	**Non-myeloablative chemotherapy plus cloned lymphocytes plus high-dose IL-2; progressive disease**
May 2000	**Brain metastasis; resected**
July 2000	**Non-myeloablative chemotherapy plus cloned lymphocytes (4 days) plus high-dose IL-2**
Sept. 2000	**Progressive bulky disease in axilla, pelvis and intraperitoneum; bedridden; narcotics for pain**


9/6/00
10/5/00
11/17/00
4/24/01
9/6/00
10/5/00
11/17/00
4/24/01
9/6/00
10/5/00
11/17/00
4/24/01


Reduction of Tumor by Adoptively Transferred Cells
From Patient A.K.
800
700
600
500
400
300
200
100
0
cm3
690
23
Transferred
T-cells
Tumor Reduction


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

Correlation between telomere length of persistent clonotypes and number of persistent T cells in blood around one month



CORRELATION OF CLINICAL RESPONSE WITH PERSISTENCE OF INDIVIDUAL TRANSFERRED T-LYMPHOCYTES AT GREATER THAN TWO WEEKS

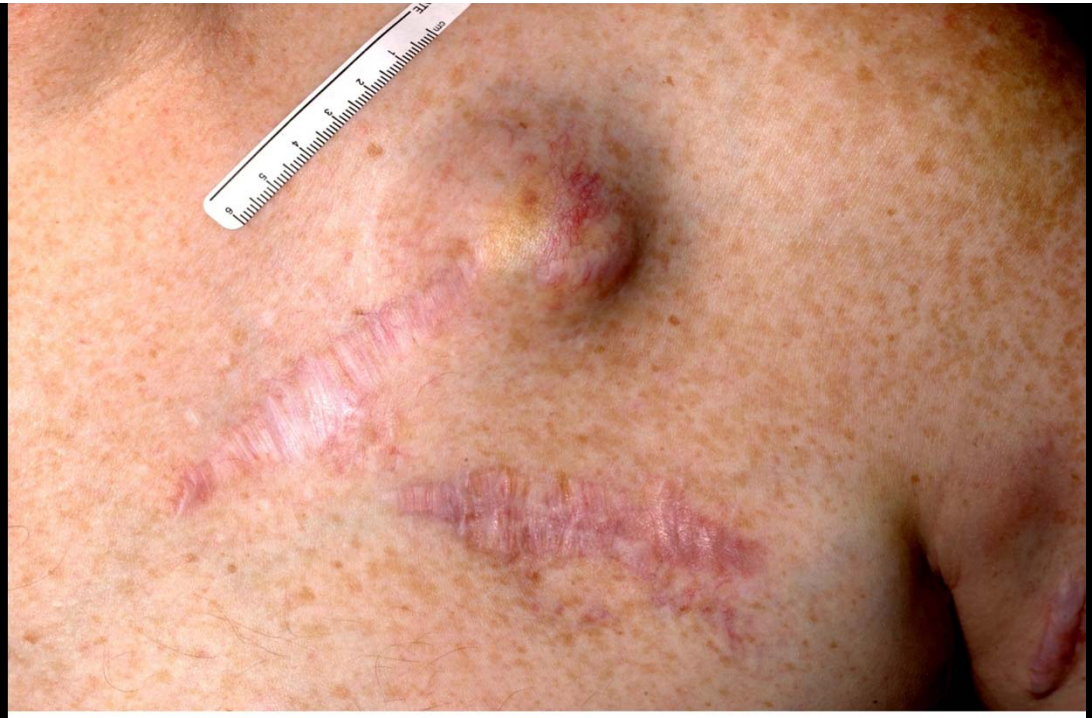
Responder		Non-Responder	
Patient	Persistence (≥ 5%)	Patient	Persistence (≥ 5%)
SF	16	VJ	---
CR	12	RS	---
DM	72	JK	---
BC	8	MP	---
AM	5	MM	---
RB	43	AW	---
EM	---	EW	---
MH	7	MB	---
HS	73	TR	---
LH(2)	7	RHE	---
RBR	---	RHO	---
PF	7	RHA	17
JM (2)	8	JM (1)	---
TOTAL	11/13		1/13

$p_2 = 0.0002$

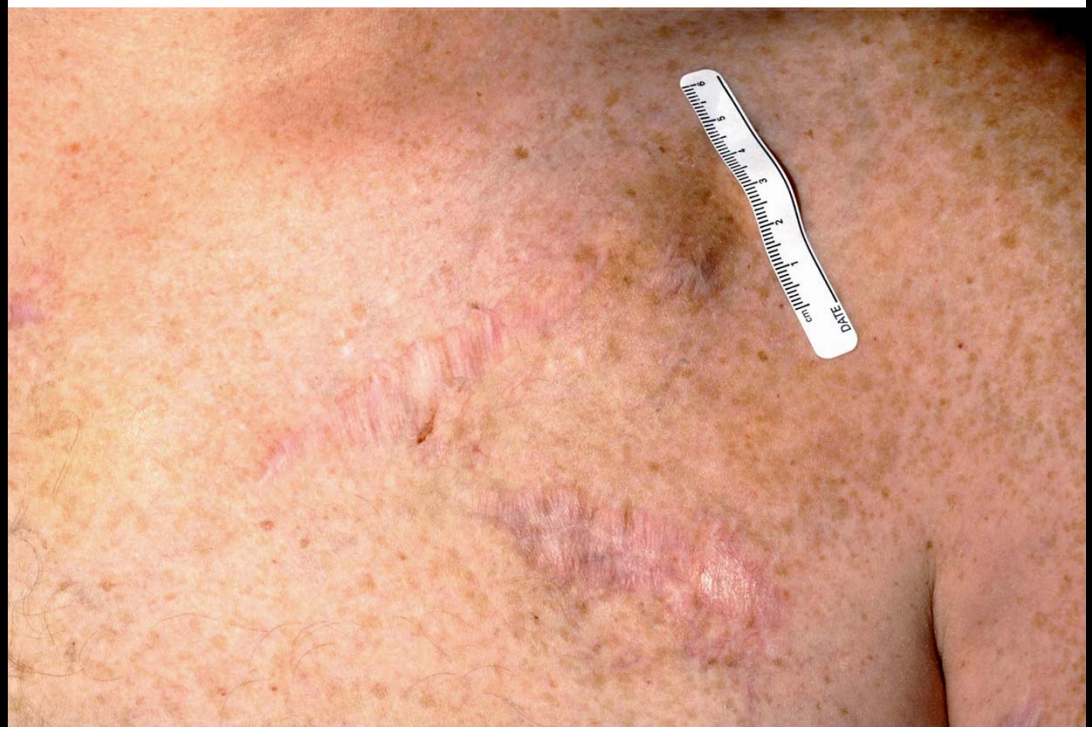
Pre-Treatment

C.K.
(200Gy)

Pre



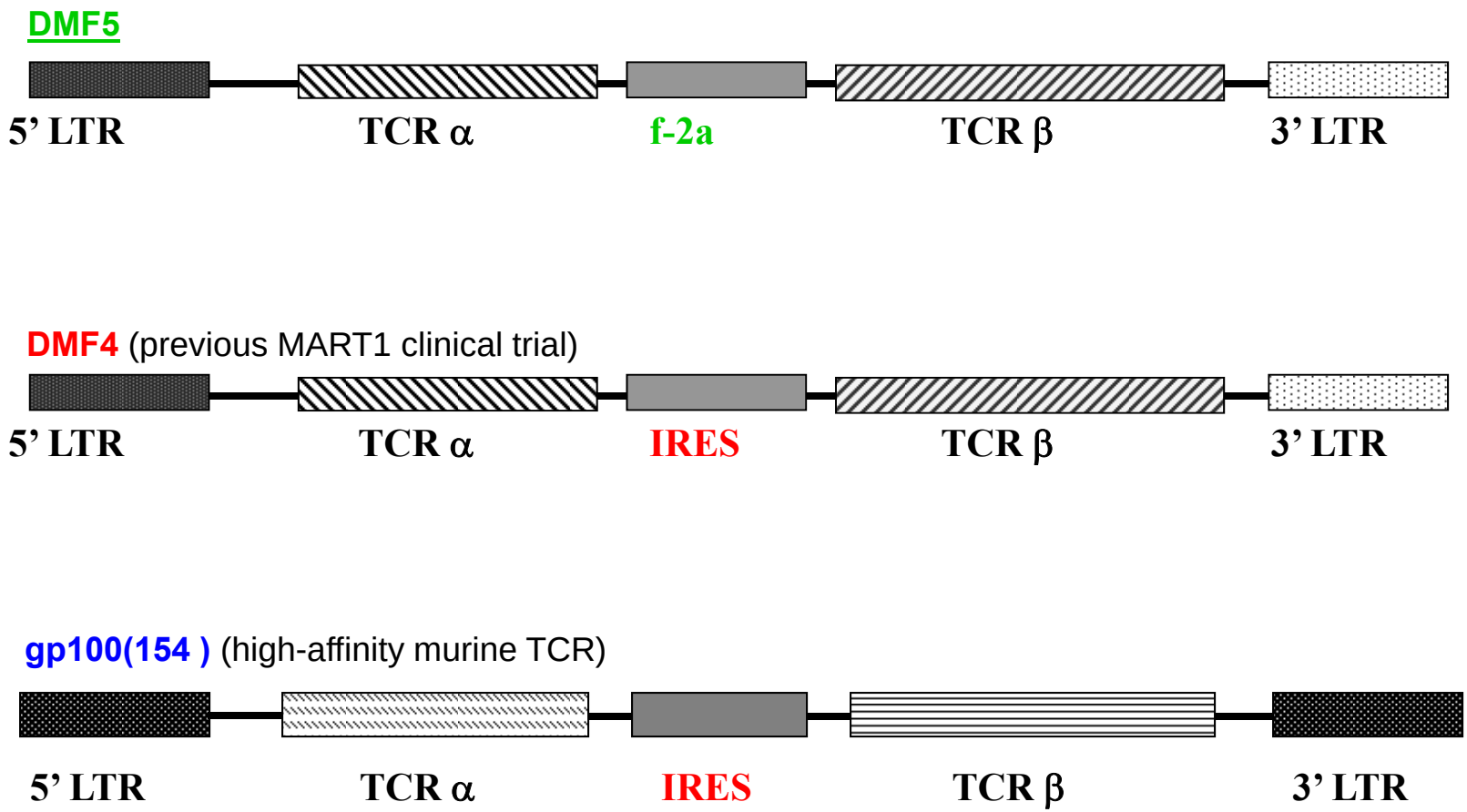
12 days



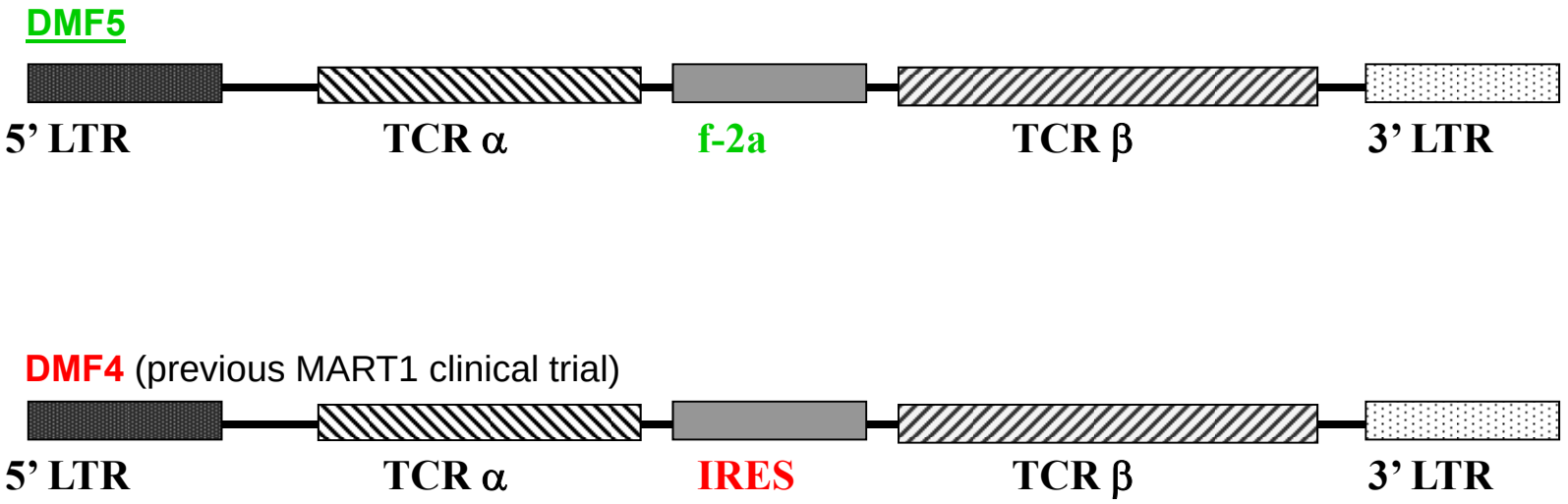
CONVERSION OF NORMAL PBL INTO ANTI-TUMOR CELLS BY TRANSDUCTION WITH GENES ENCODING THE GP100 T-CELL RECEPTOR

Responder Cells	Stimulators) None	526 Mel (A2+)	624 Mel (A2+)	888 Mel (A2-)	938 Mel (A2-)
			(pg/ml IFN-g released)		
No vector	9 (12)	3 0 (1.4)	10 1 (91)	9 1 (22)	2 8 (40)
YFP	8 (9.7)	3 5 (12)	5 6 (18)	15 0 (75)	8 0 (98)
TCR	80 (62)	2,528 (305)	1,614 (298)	24 5 (100)	6 3 (72)
TIL	56 (20)	2,713 (717)	2,240 (235)	1 0 (14)	2 1 (30)

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



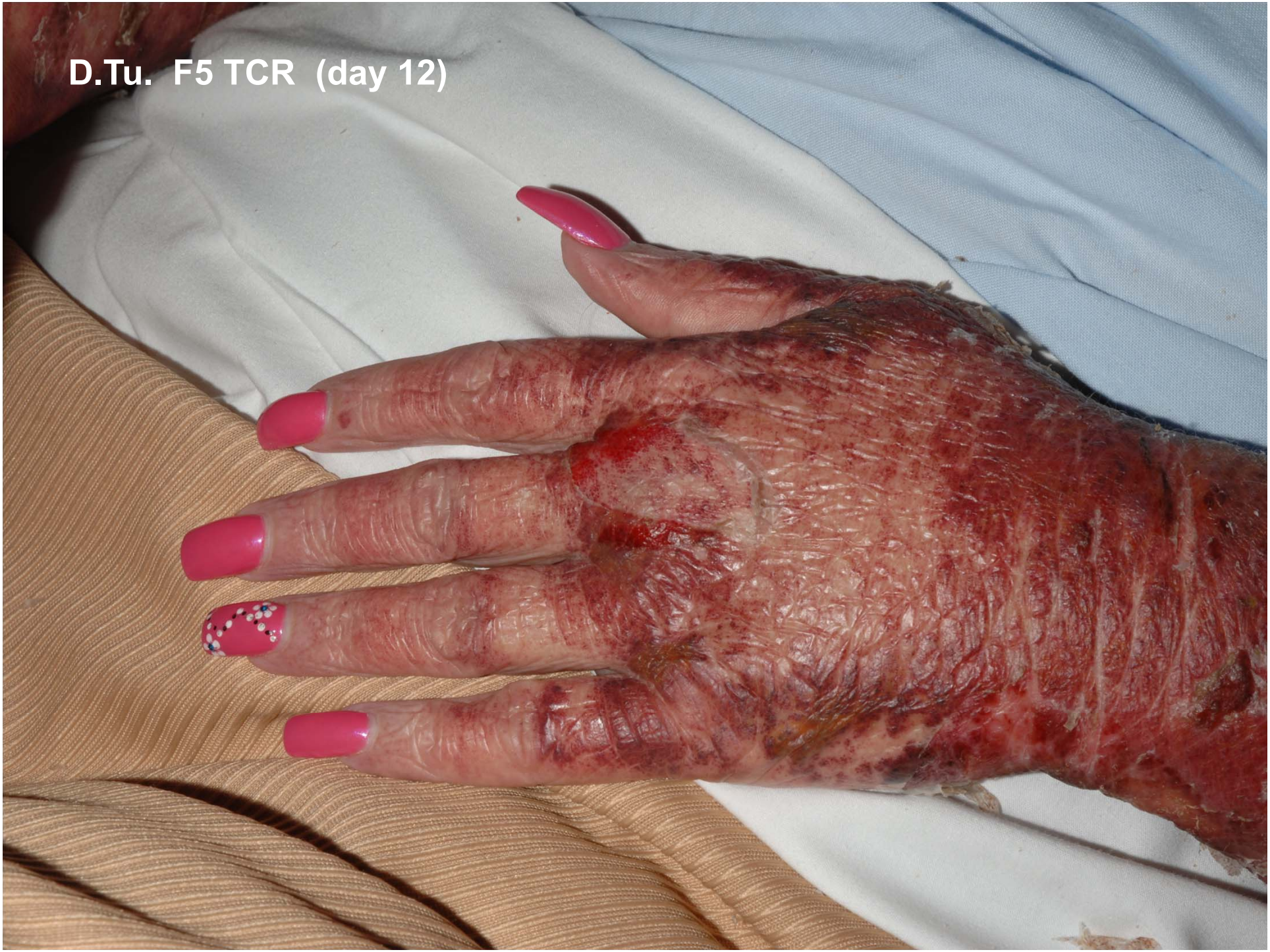
DMF4 and DMF5 MART1 TCR retroviral constructs



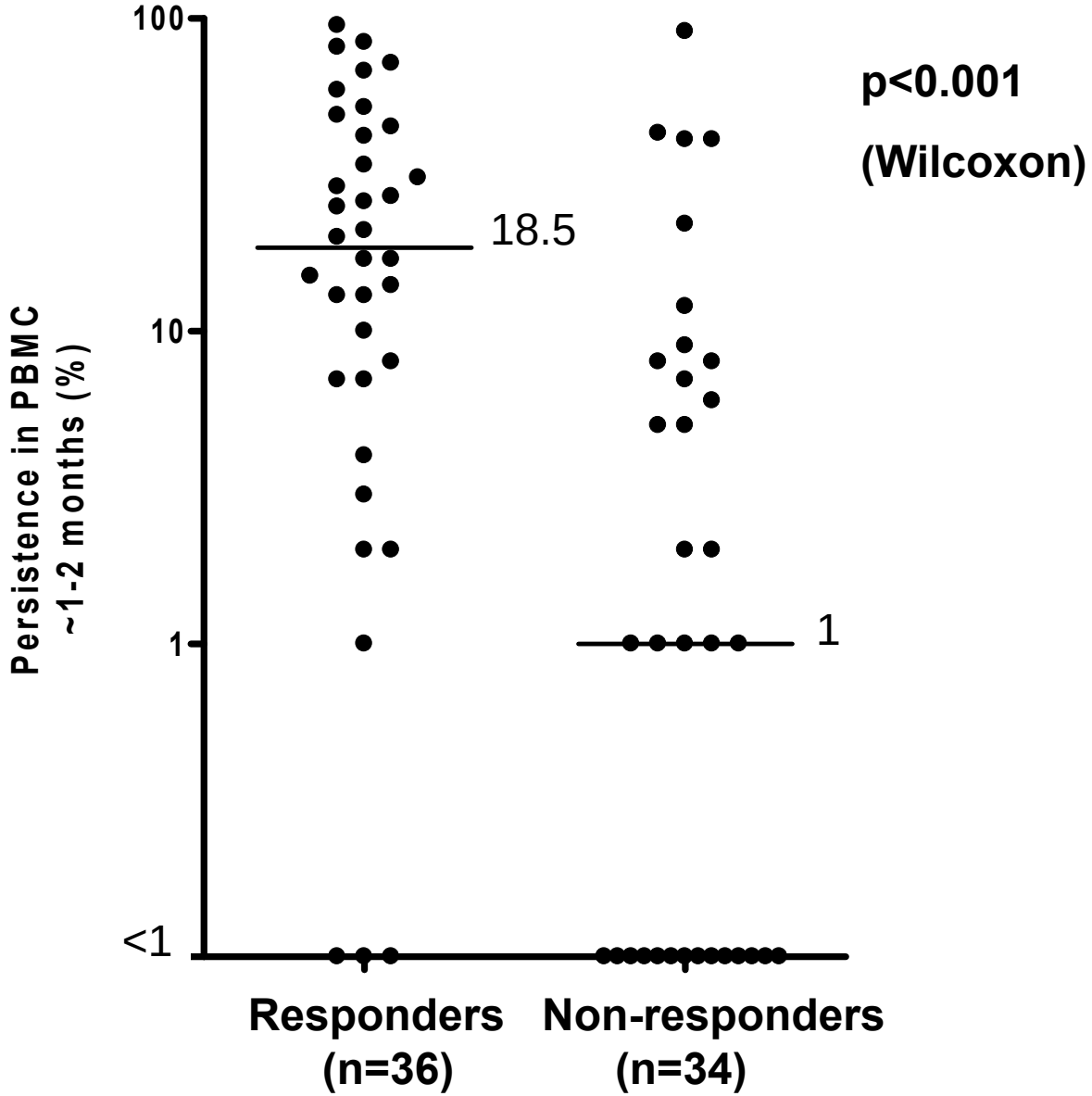
*4 out of 31 PR (13% response rate)**

How can we increase the effectiveness of TCR gene therapy for the treatment of cancer?

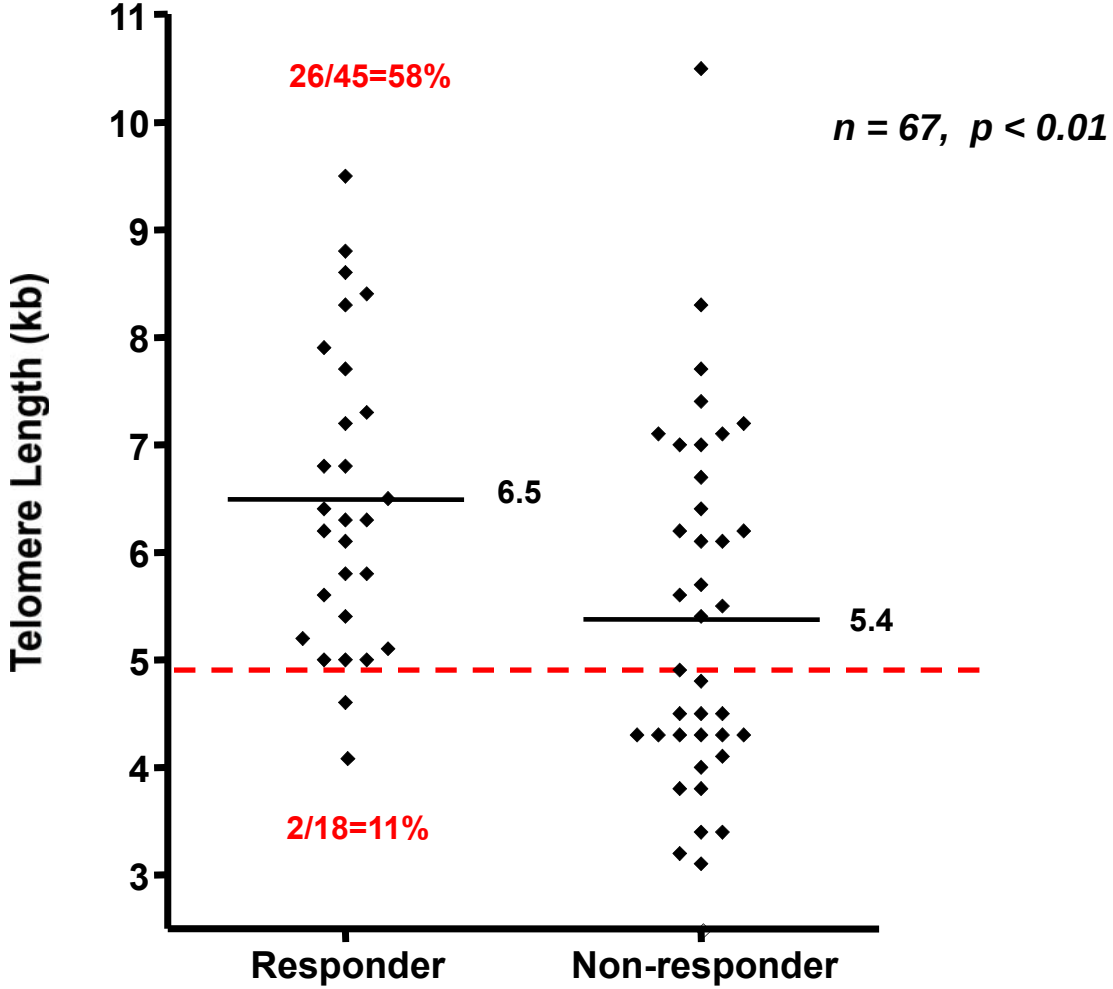
(Science 314:126, 2006)


D.Tu. F5 TCR (day 12)

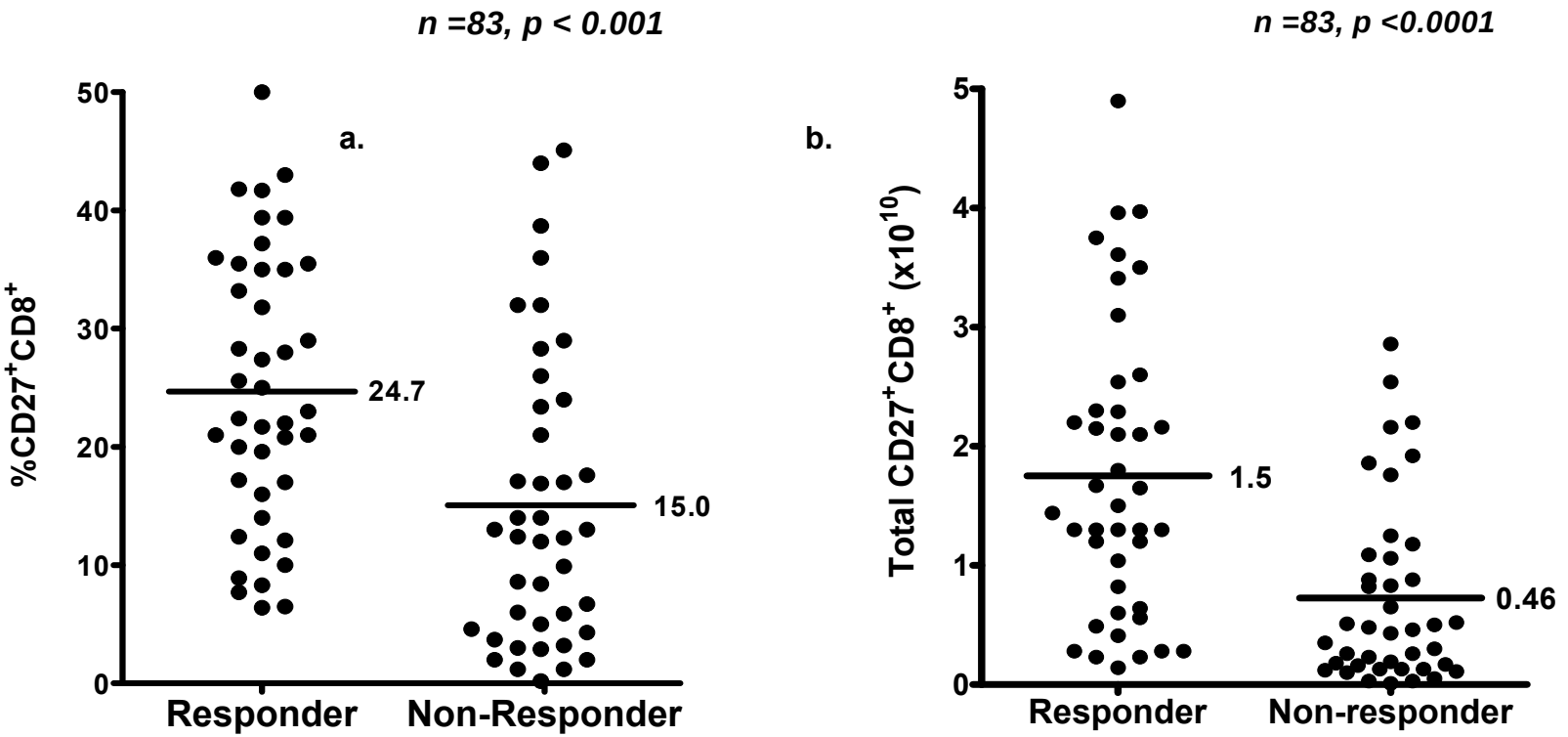
T cell persistence: NMA, 200 and 1200 TBI trials



Telomere Length of the infusion TIL significantly associated with response



Cellular differentiation associated with clinical response



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:

1) improve effectiveness

2) extend this approach to additional melanoma patients and patients with common epithelial cancers

Approaches to Improve the Effectiveness of Cell Transfer Therapy

1. Add vaccines
2. Stimulate antigen present cells (TLR agonists)
3. IL-15 (instead of IL-2)
4. IL-12 (plus IL-2)
5. Block immune inhibitory factors
    1. Selective removal of T regulatory cells
    2. anti-CTLA-4
    3. anti-PD1/PDL1
6. Combine with anti-angiogenic approaches to increase lymphocyte infiltration into tumors
7. Gene-modifying TIL
    1. cytokines (IL-2, IL-15)
    2. costimulatory molecules (41BB, CD28)
    3. homing molecules (CD62L, CCR7)
    4. anti-apoptosis molecules (Bcl-2)