Surgery Branch experience with administration of TCR gene-modified lymphocytes

Target cell	Transgene	Conditioning	Number of patients	TRM
PBL	MART-1 TCR (F4)	NMA	31	0
TIL	MART-1 TCR (F4)	NMA	3	0
PBL	MART-1 TCR (F4)	NMA+1200TBI	4	0
PBL	gp100 TCR (209)	NMA	11	0
TIL	gp100 TCR (209)	NMA	3	0
PBL	p53 TCR	NMA	11	0
PBL	MART-1 TCR (F5)	NMA	24	0
TIL	MART-1 TCR (F5)	NMA	3	0
PBL	gp100 TCR (154)	NMA	19	0
TIL	gp100 TCR (154)	NMA	2	0
PBL	gp100(154)+MART-1(F5)	NMA+600TBI	4	0
PBL	MART-1 TCR (F5)-ALVAC	NMA	4	0
PBL	gp100 TCR (154)-ALVAC	NMA	3	0
PBL	Her2/neu-CAR	NMA	1	1
PBL	NY-ESO-1 TCR	NMA	25	0
PBL	2G1-RCC TCR	NMA	2	0
PBL	CEA-TCR	NMA	3	0
PBL	CD19-CAR	NMA	4	1
		Total:	**157**	**2**

MART-1 and gp100

Intracellular molecules involved in melanin synthesis

Expressed in melanocytes in the skin, eye and ear

Expressed on ~ 90% of metastatic melanomas

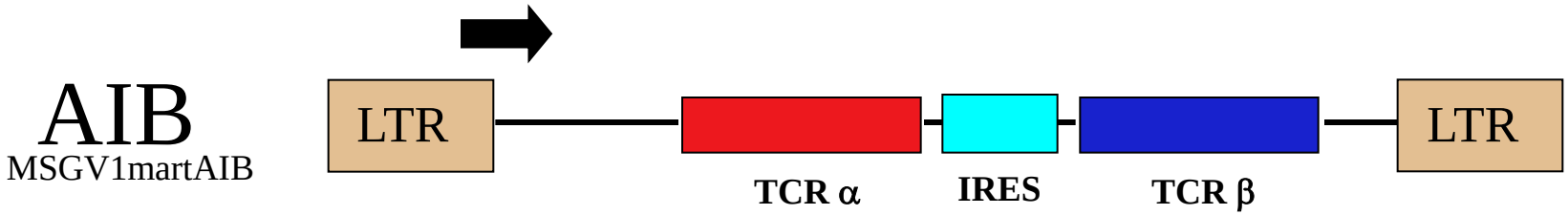
Epitopes recognized by T cells described in 1994 (PNAS, 91:3515 and 91:6458, 1994)

HLA-A2: MART-1, 1 predominant epitope, aa 27-35

gp100, 10 epitopes identified

- aa 154-162 (highest binder to A2)
- aa 209-217 (heteroclitic: 210M)
- aa 280-288 (heteroclitic: 288V)

anti-Mart-1 retroviral vector



(Science 314:126,2006)

Treatment with MART-1 TCR transduced autologous lymphocytes

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

(Science 314:126, 2006)

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)

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

16 patients with metastatic melanoma **(Science, 314:126-129,2006)**

2 (13%) with objective regressions

(both disease free over two years later)

15 additional patients treated

2 further objective regressions

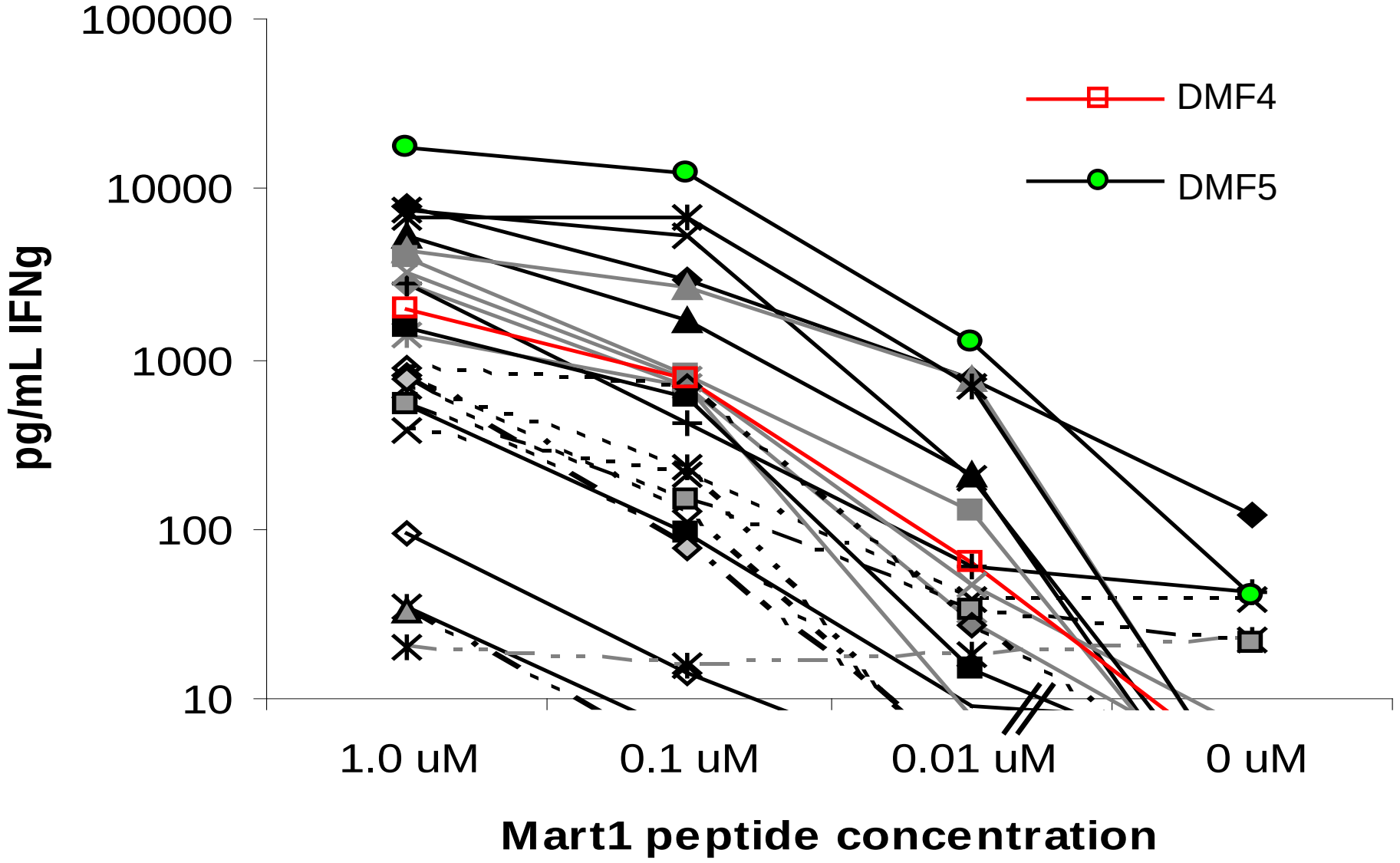
<u>Overall</u>: 4/31 (13%) objective regressions

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)

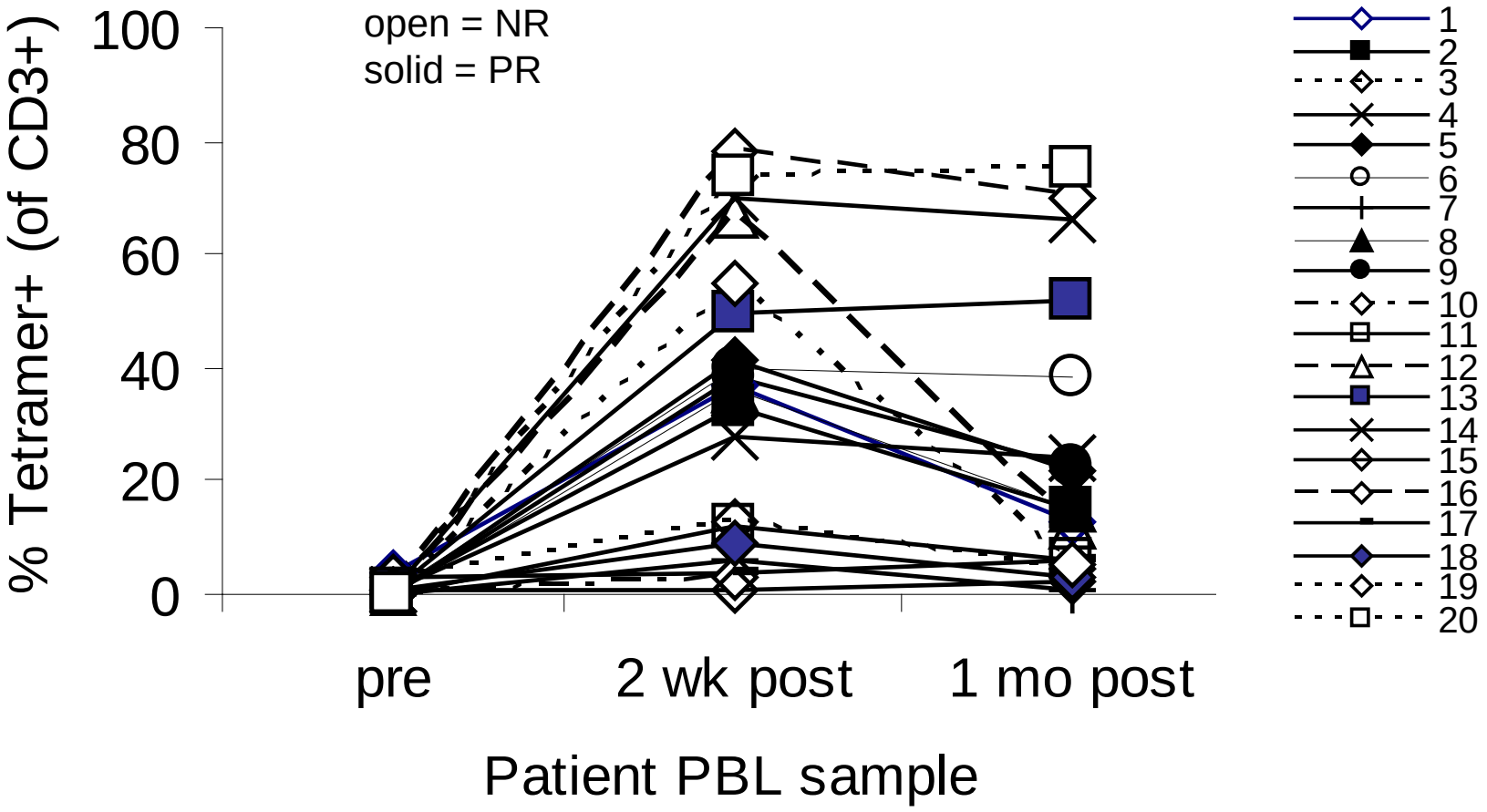
TCR Gene Therapy in Patients with Metastatic Melanoma

TCR	Response Total	Response OR	Toxicity Skin	Toxicity Uveitis	Toxicity Auditory
			(Grade 1/2/3)		
		(number of patients)			
MART-1TCR (DMF5)	20	6(30%)	11/3/0	2/9/0	2/0/7
gp100TCR (gp154)	16	3(19%)	11/4/0	0/4/0	2/2/3

	Grade 1	Grade 2	Grade 3
Skin	erythema	desquamation <50%	desquamation >50%
Eye	no symptoms	anterior, steriod drops	pan uveitis
Ear	15-25dB, 2 freq.	>25dB, 2 freq.	>25dB, 3 freq.

(Blood 114:535-546, 2009)

Persistence of infused cells in PBL at one month: Tetramer analysis



Persistence of infused cells in PBL at one month: Elispot analysis (IFNg)

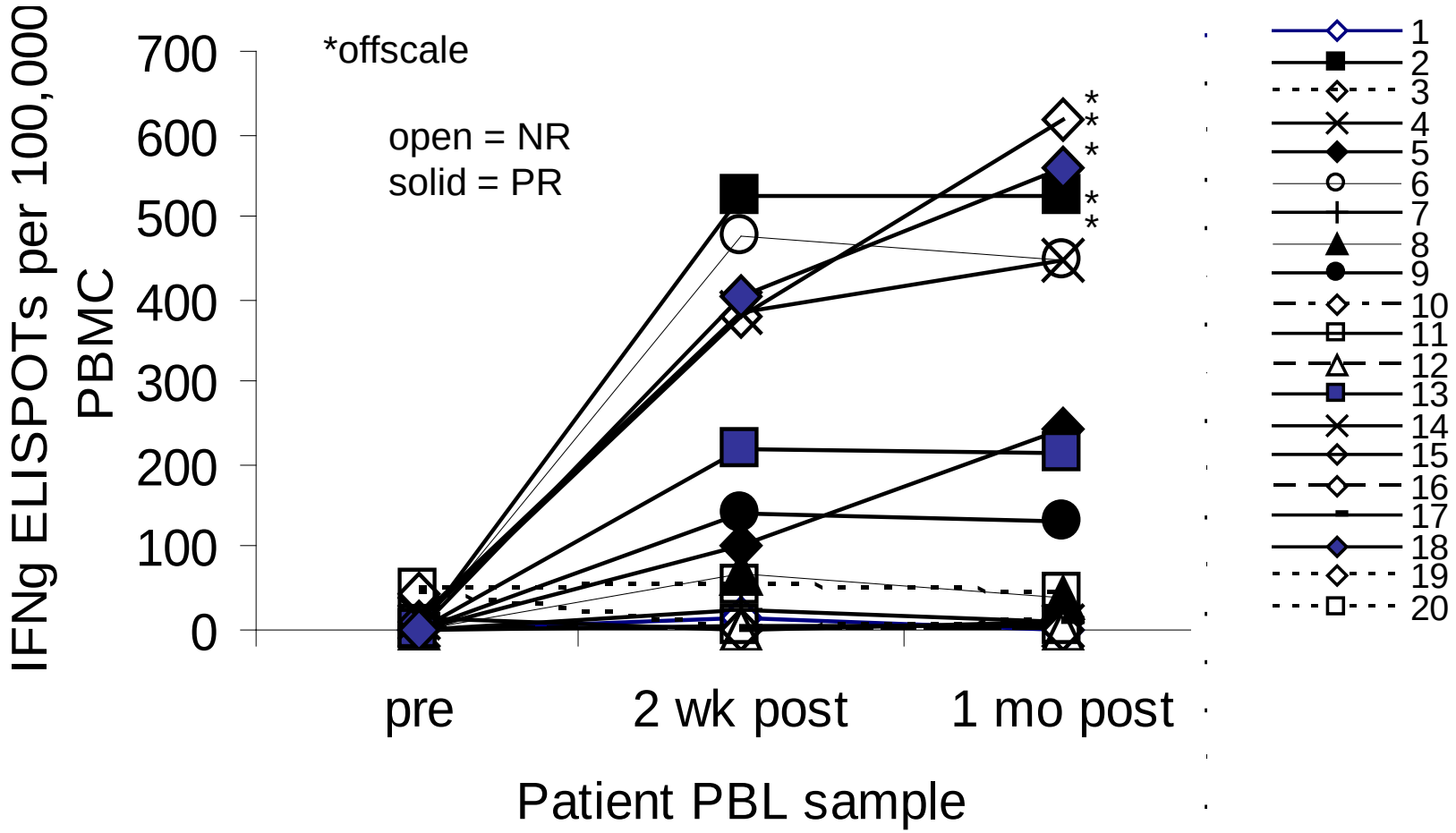
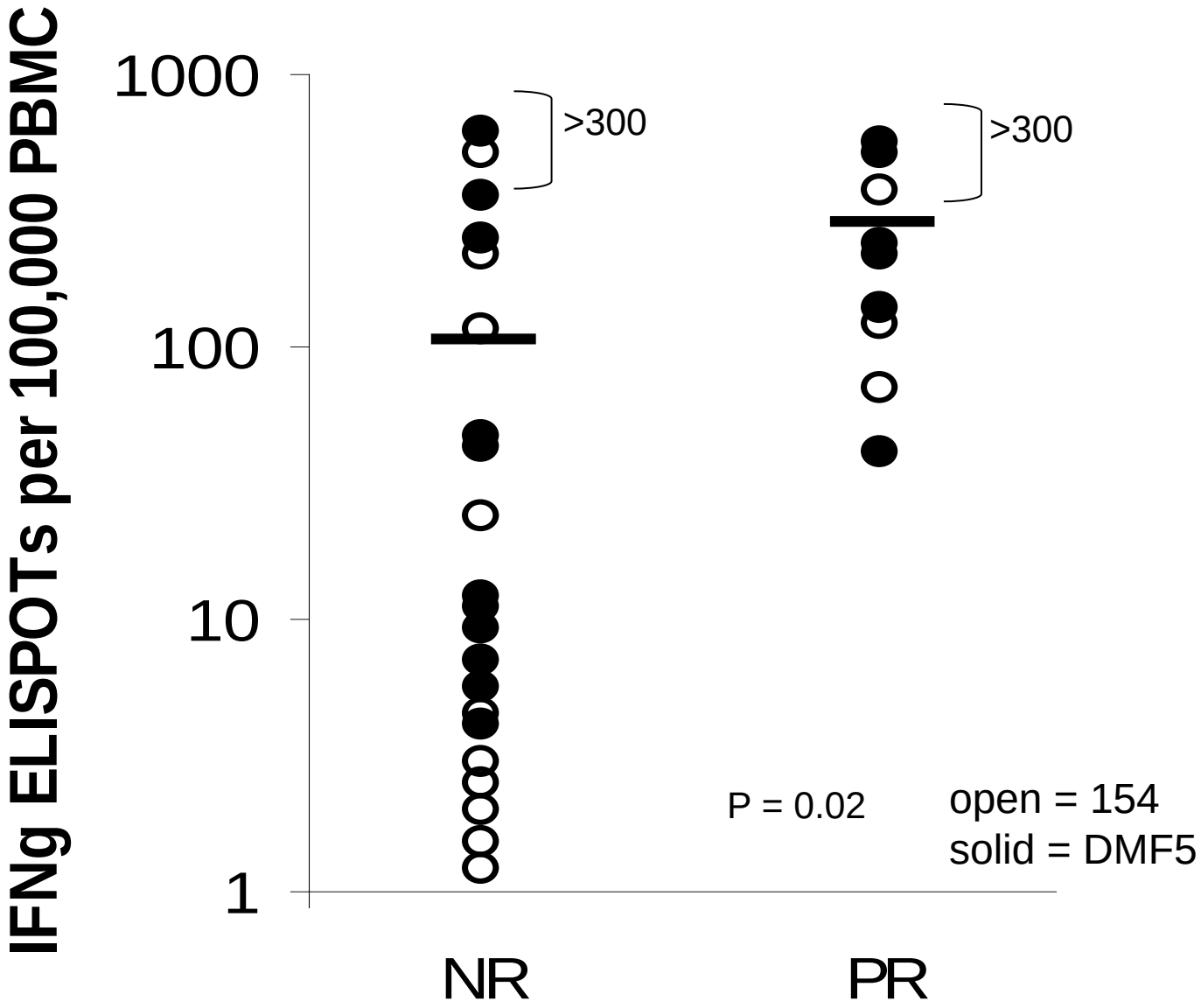
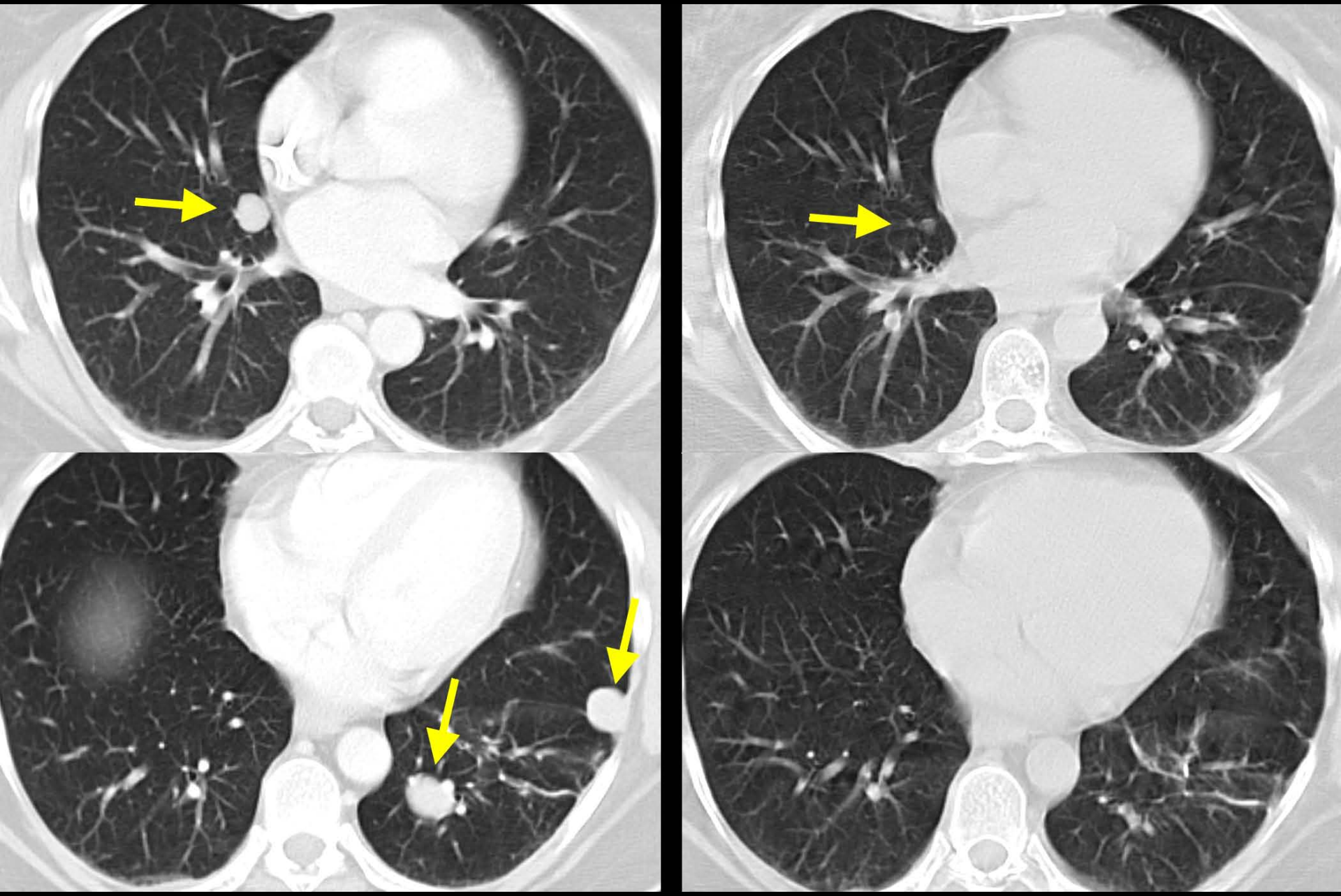


Figure 2D(L)


IFNg ELISPOTs per 100,000 PBMC
1000
100
10
1
>300
>300
P = 0.02
open = 154
solid = DMF5
NR
PR

D.T. DMF5 TCR

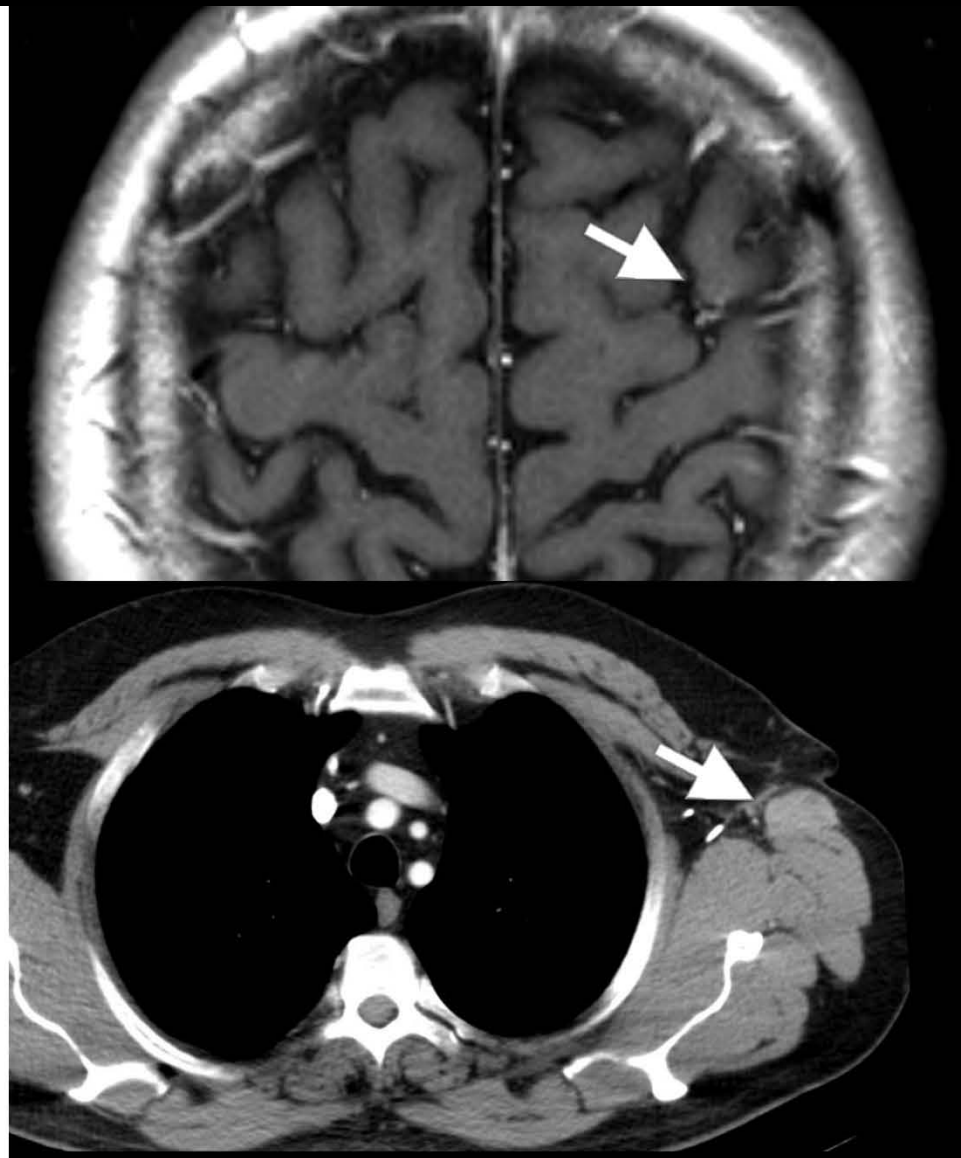


Pre-Treatment

5+ Months

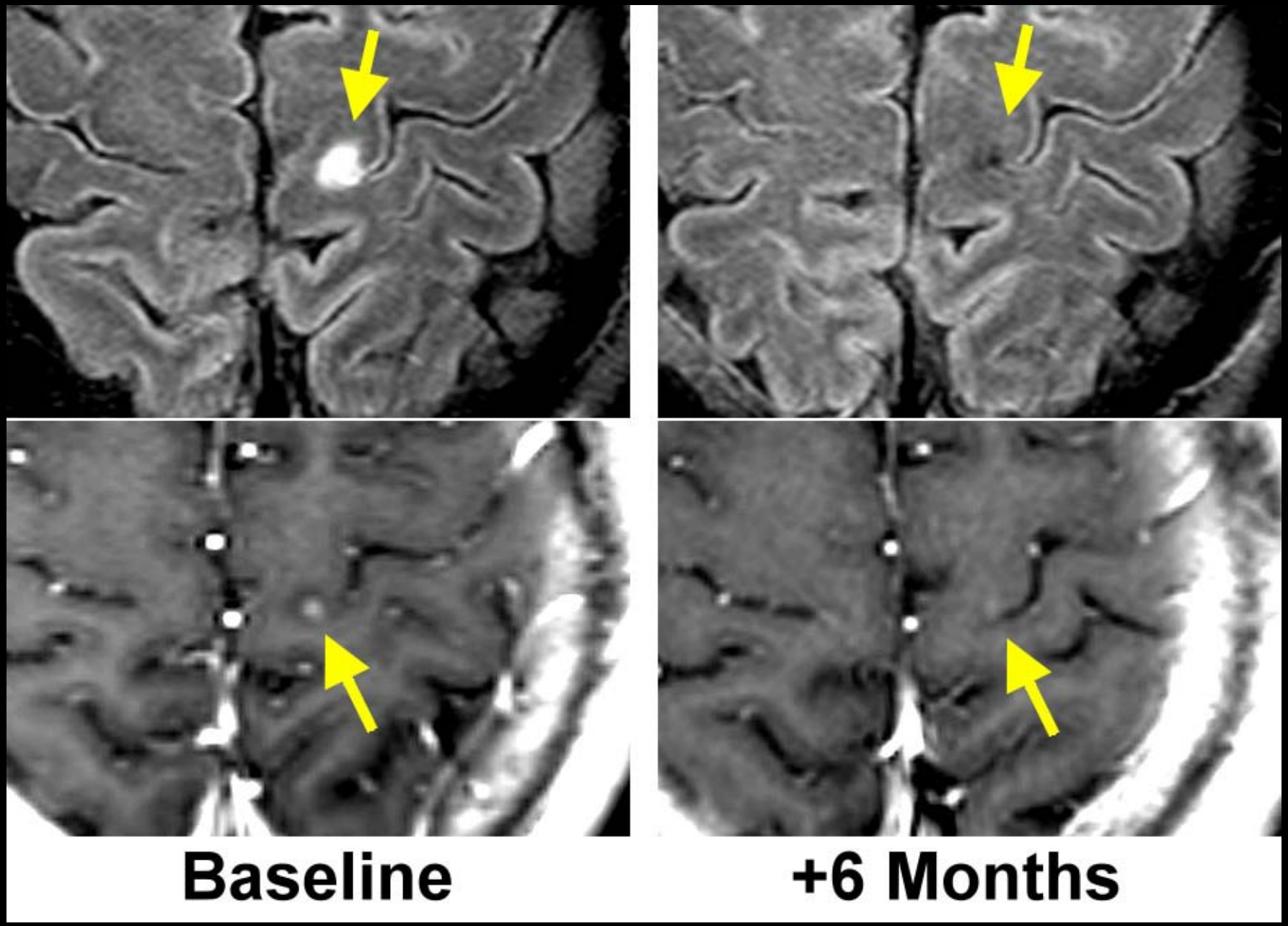
J.K. F5 TCR

Pre-Treatment

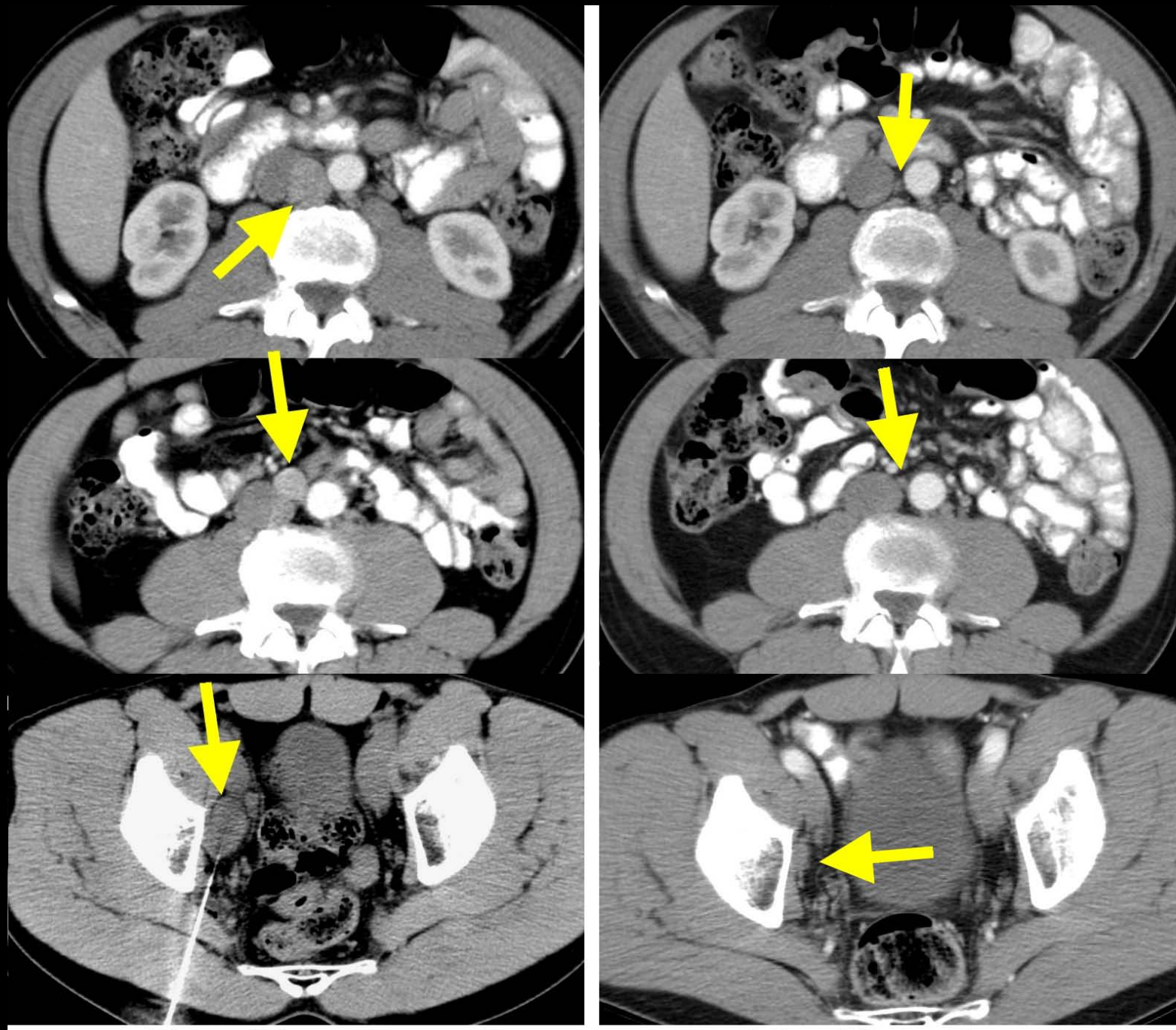


12+ Months

T.D. F5 TCR



S.S. 154 TCR

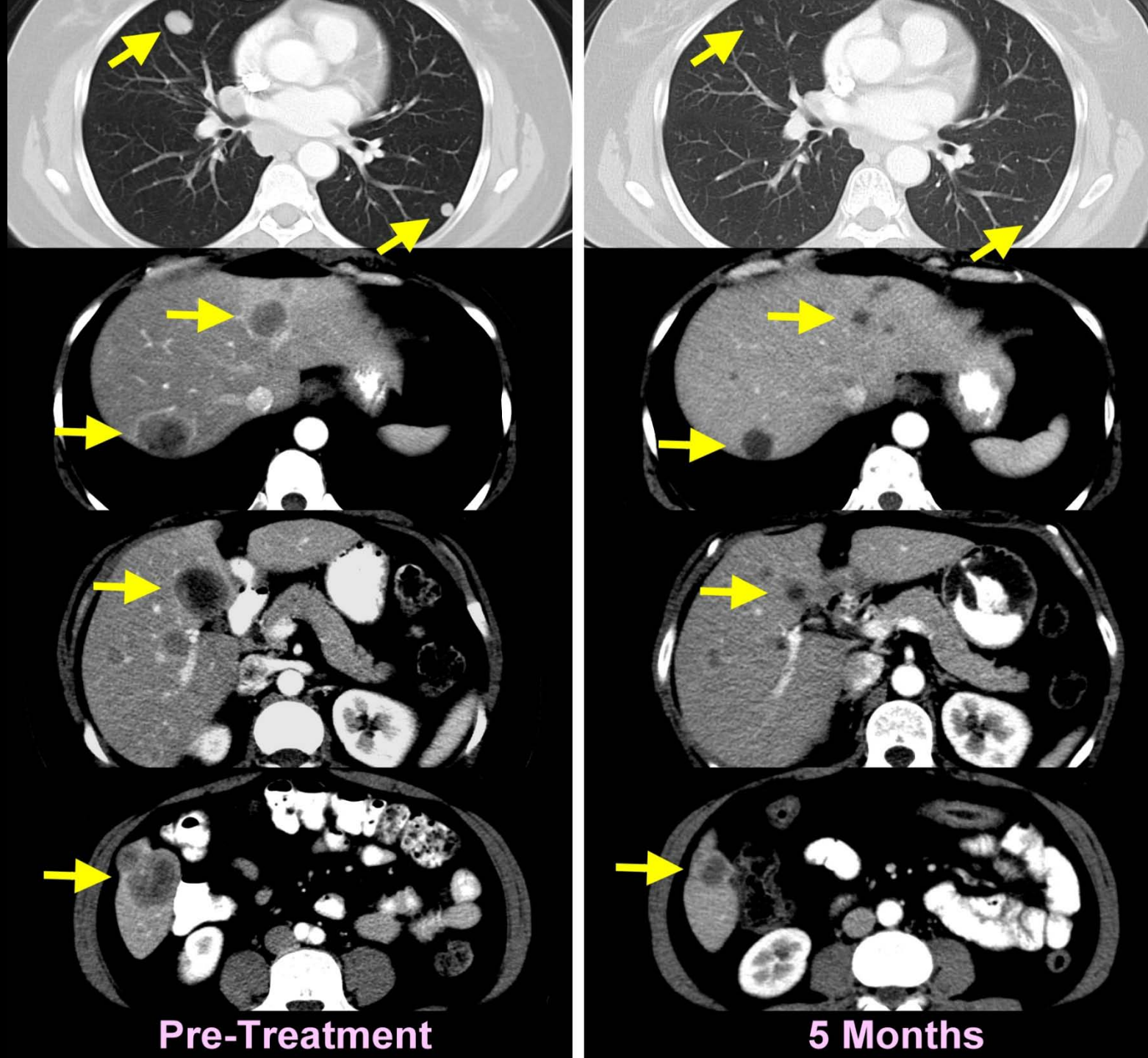


Pre-Treatment

10+ Months

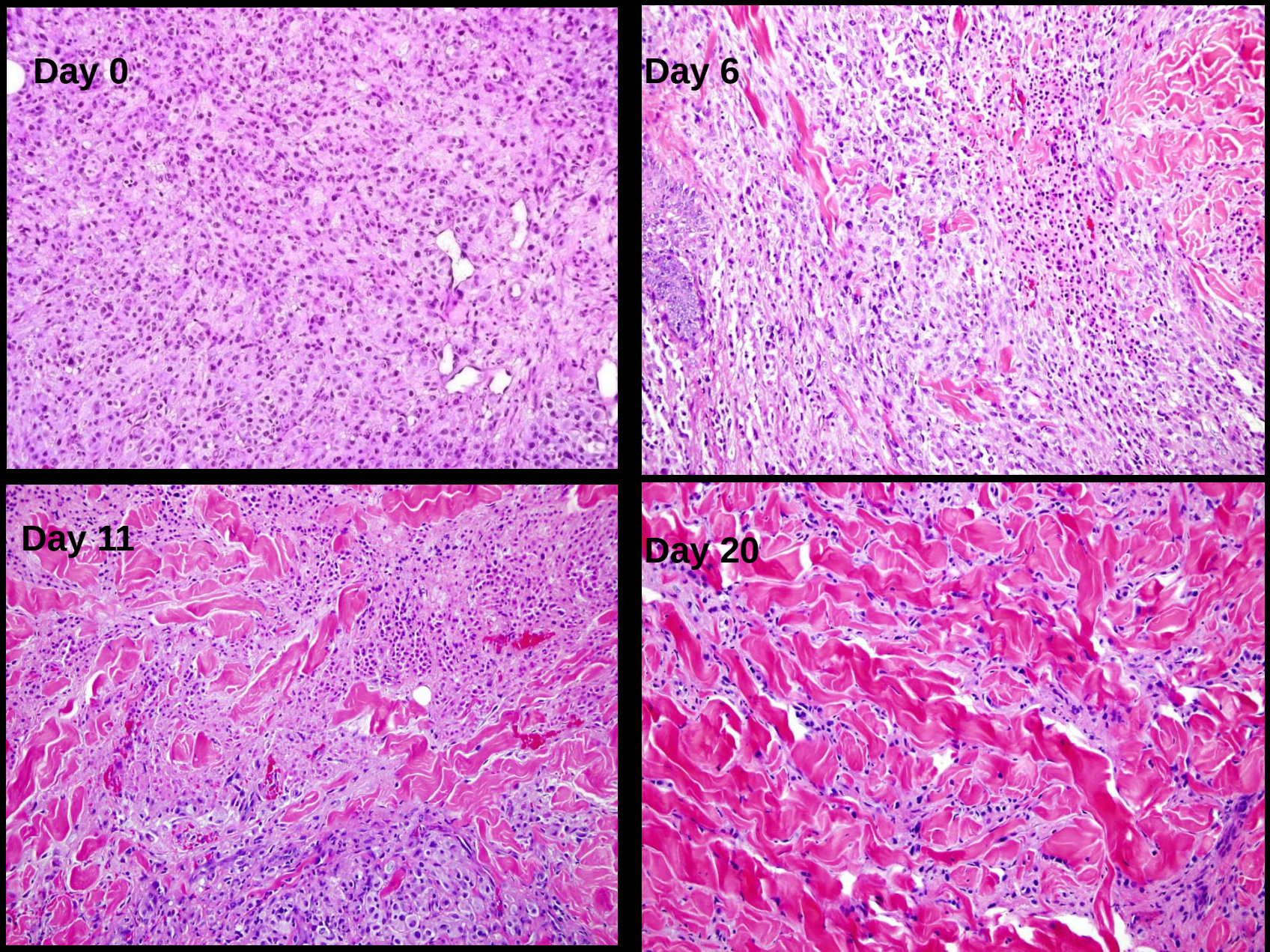
J.W.

154 TCR



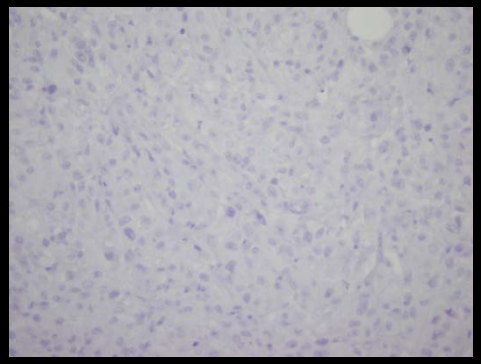
D.Th. F5 TCR Pretreatment

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

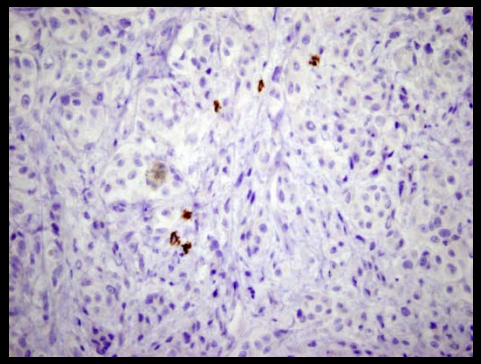


Sequential tumor biopsies (D.Th.): MART TCR (CD8, 40x)

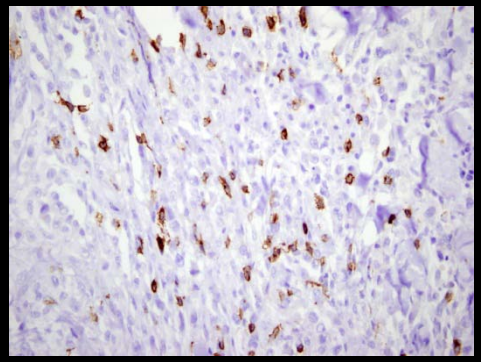
day 0



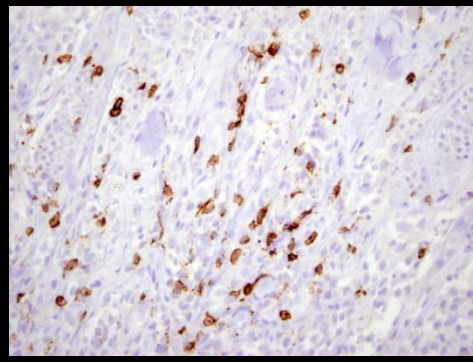
day 5



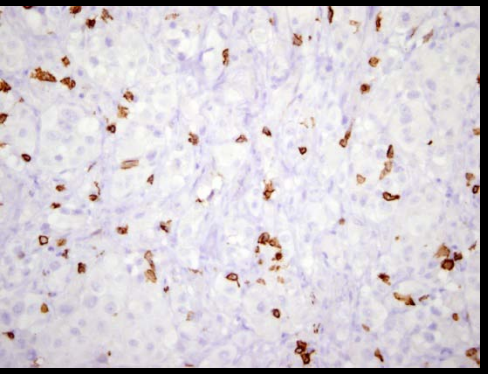
day 6



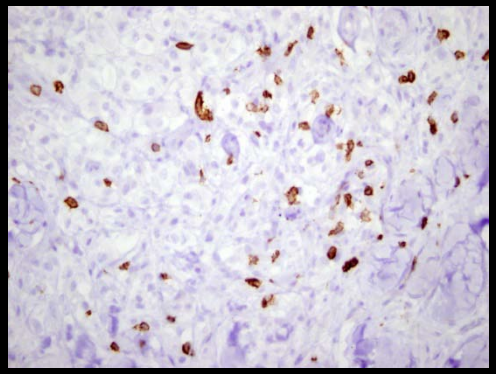
day 9



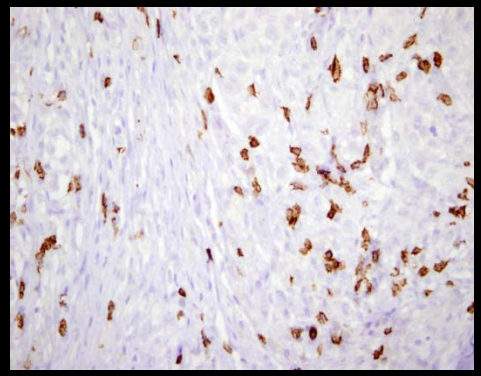
12 day



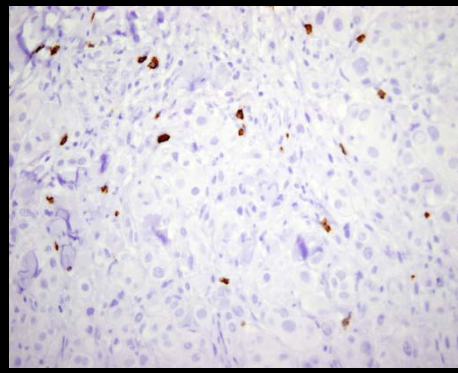
day 16



day 19



day 26



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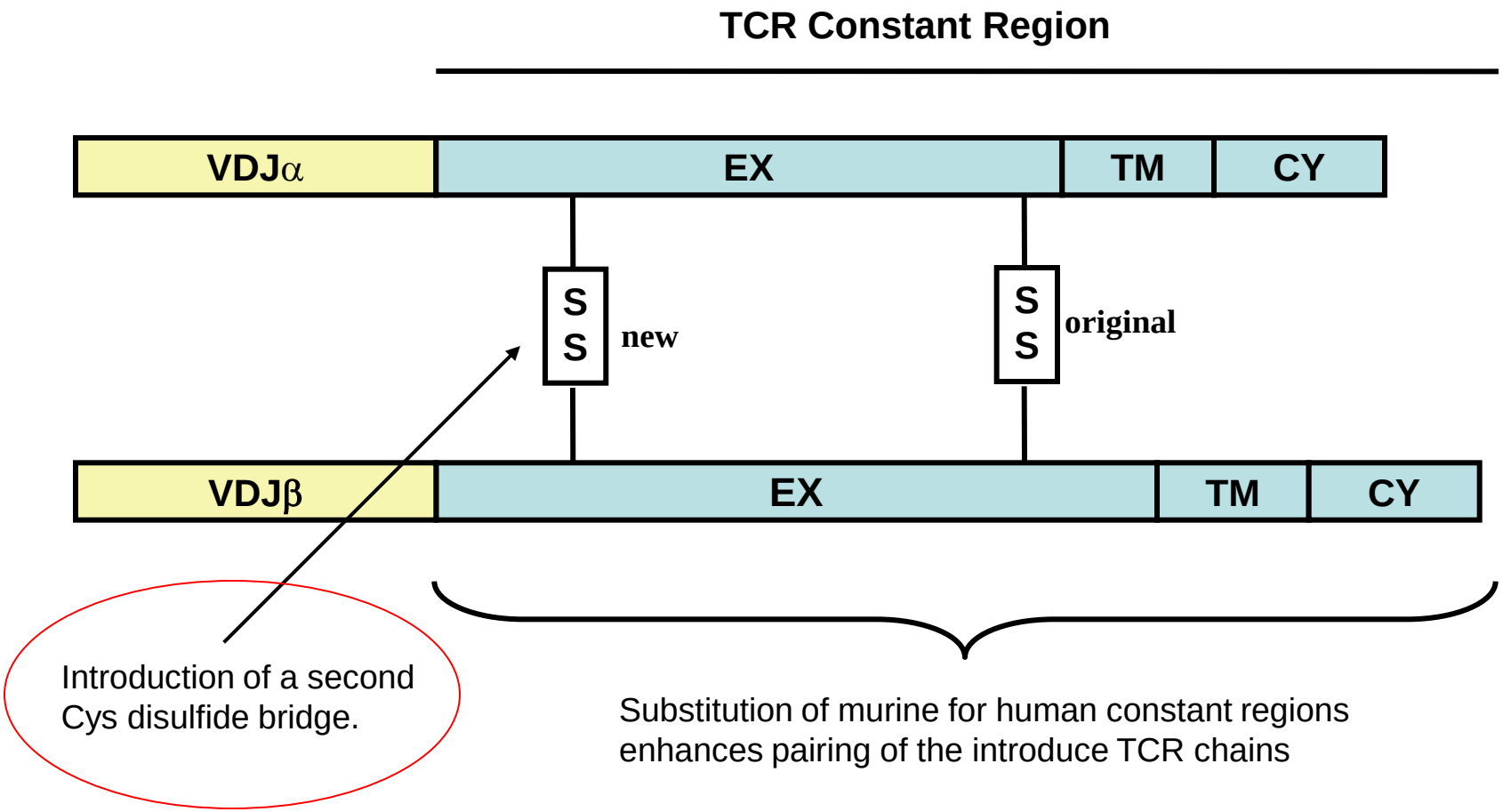
2. **Avoid mispairing of inserted and endogeneous alpha and beta chains**
    1. **mouse constant region chimeric TCR**
    2. **substitute additional cystines to form disulfide bond**

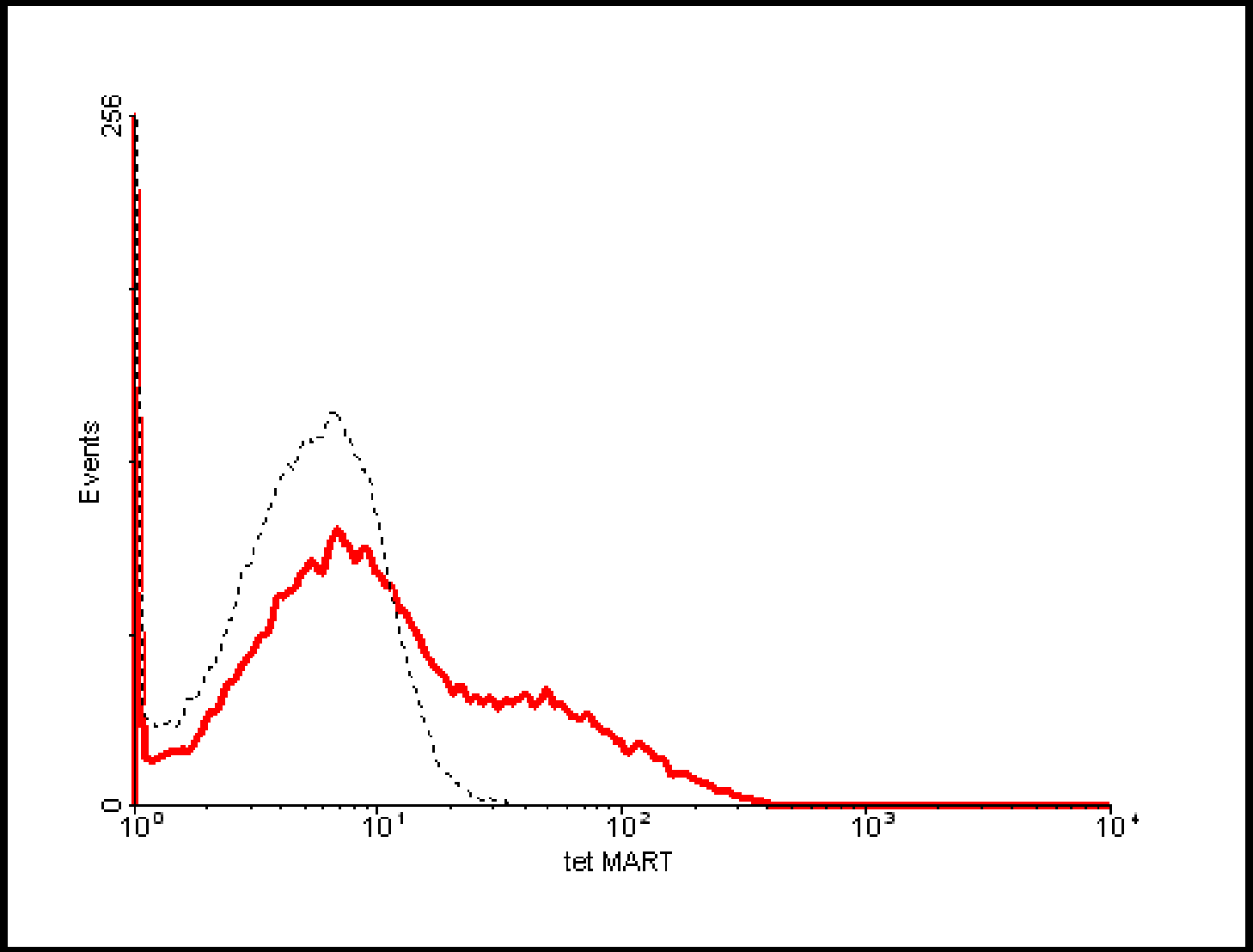
Optimizing Expression of the TCR Vector Constructs

Protein engineering of TCR chains



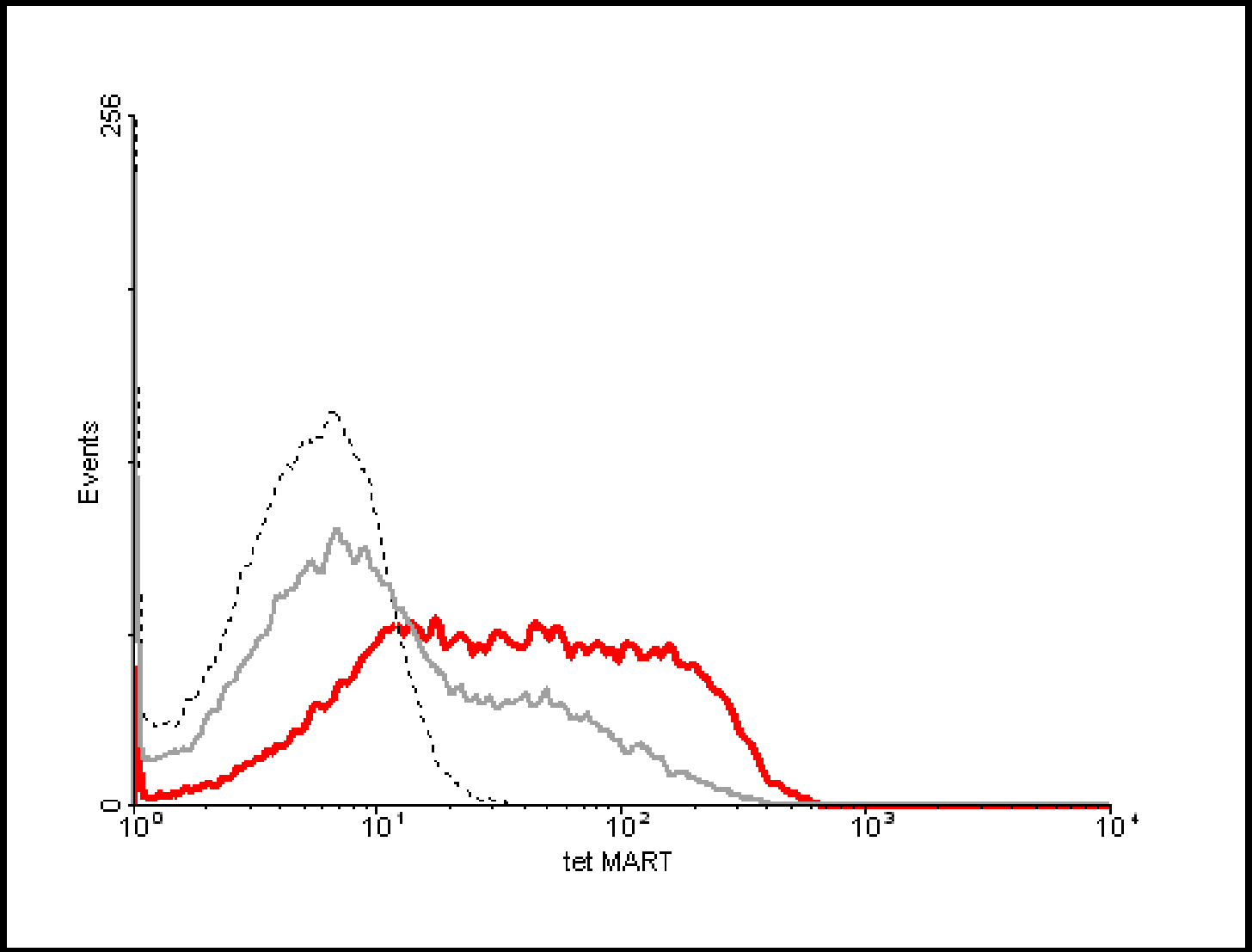
Developed by C. Cohen

Human Constant Regions



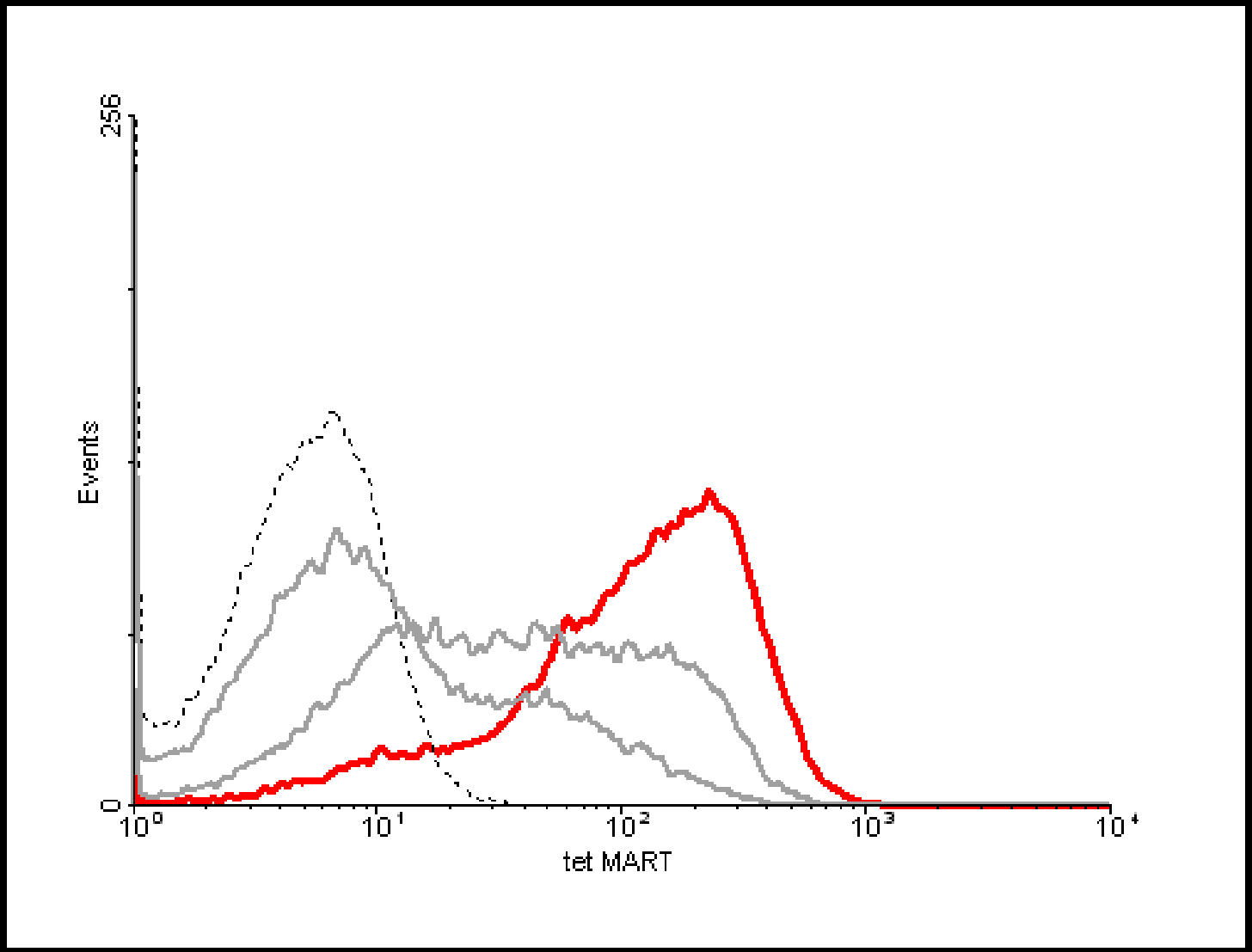
Constant Regions: H

Human CR+ Cysteines



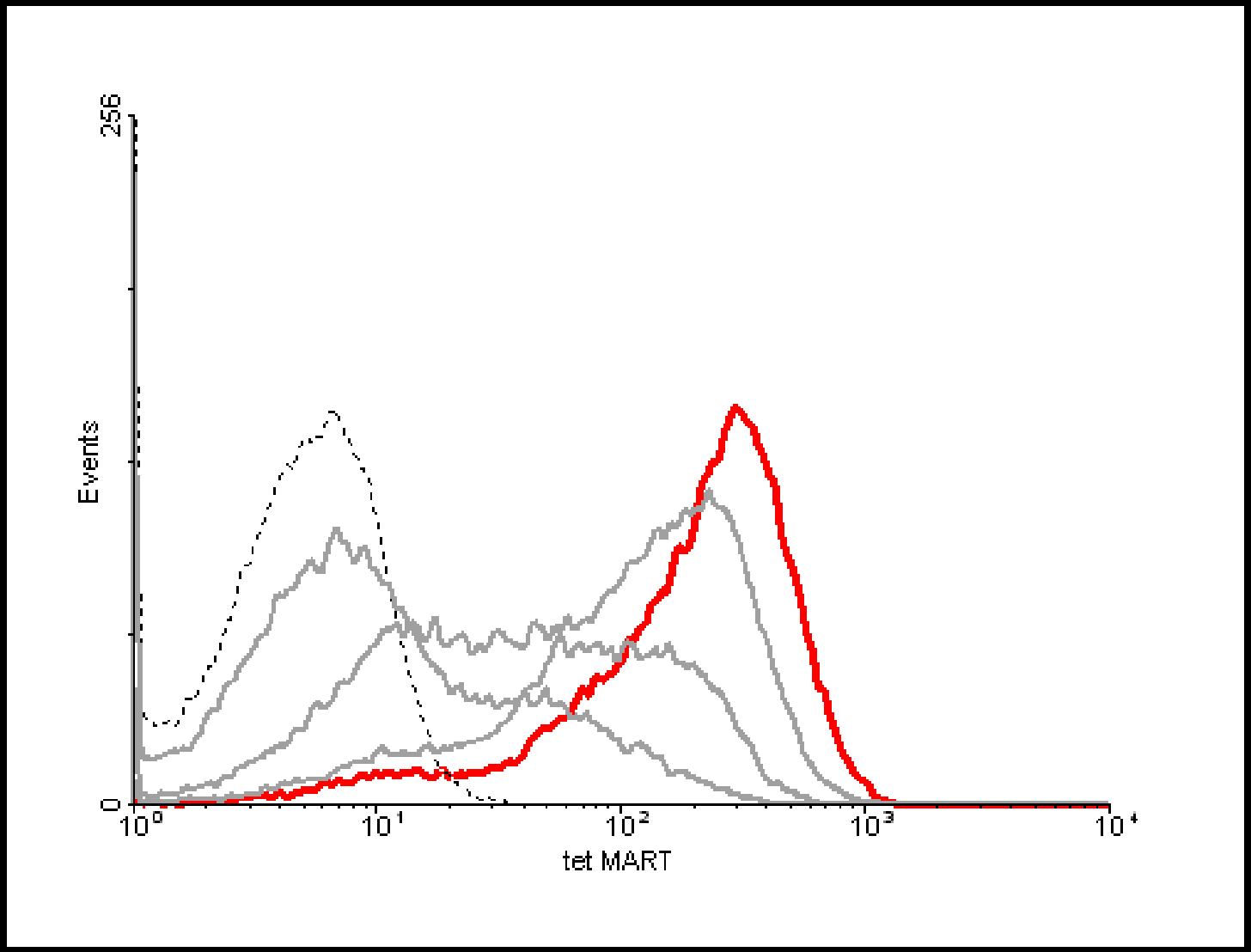
Constant Regions: H<H-Cys

Mouse Constant Regions



Constant Regions: H<H-Cys<M

Mouse CR + Cysteines



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

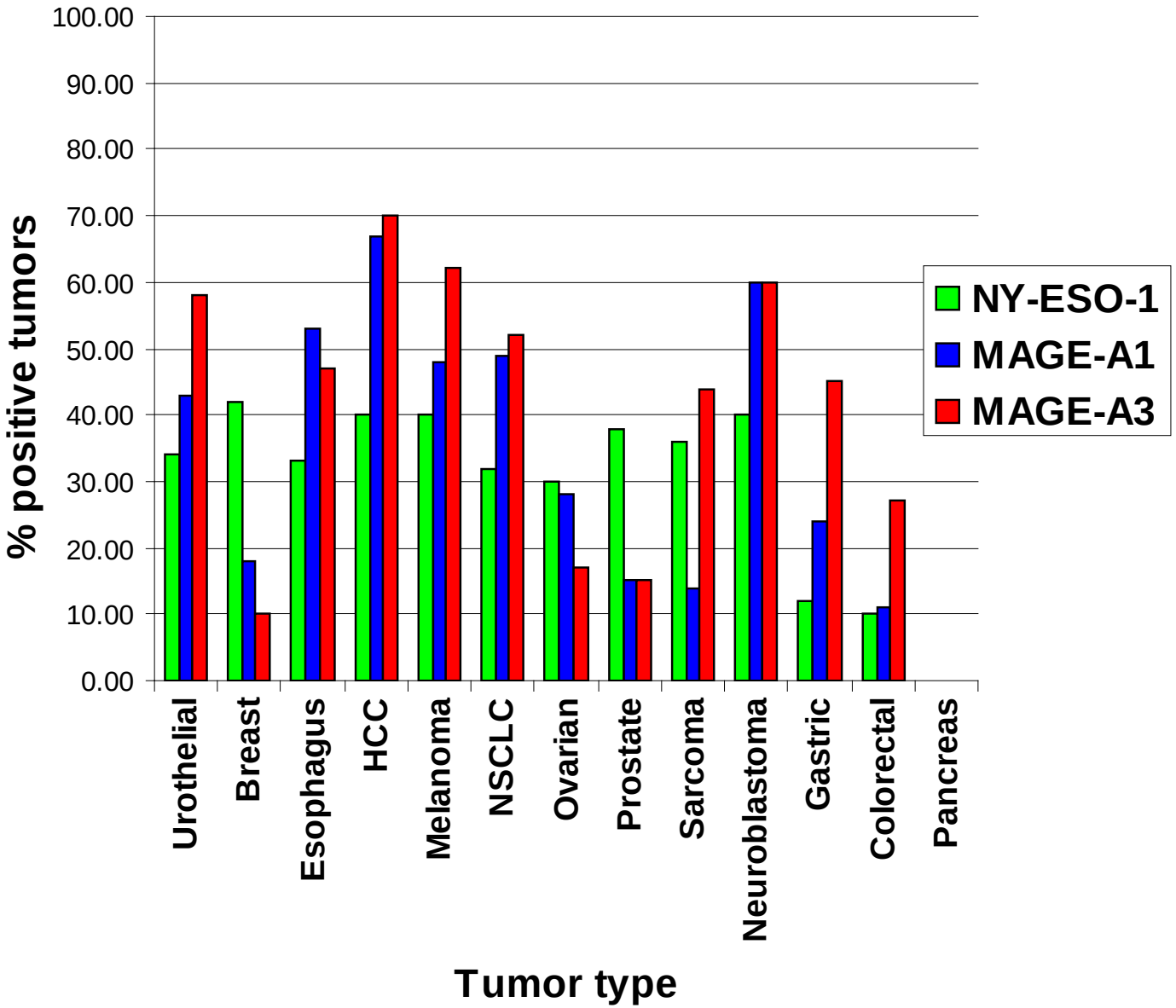
NY-ESO-1 CANCER ANTIGEN

No expression on adult human tissues except for testis

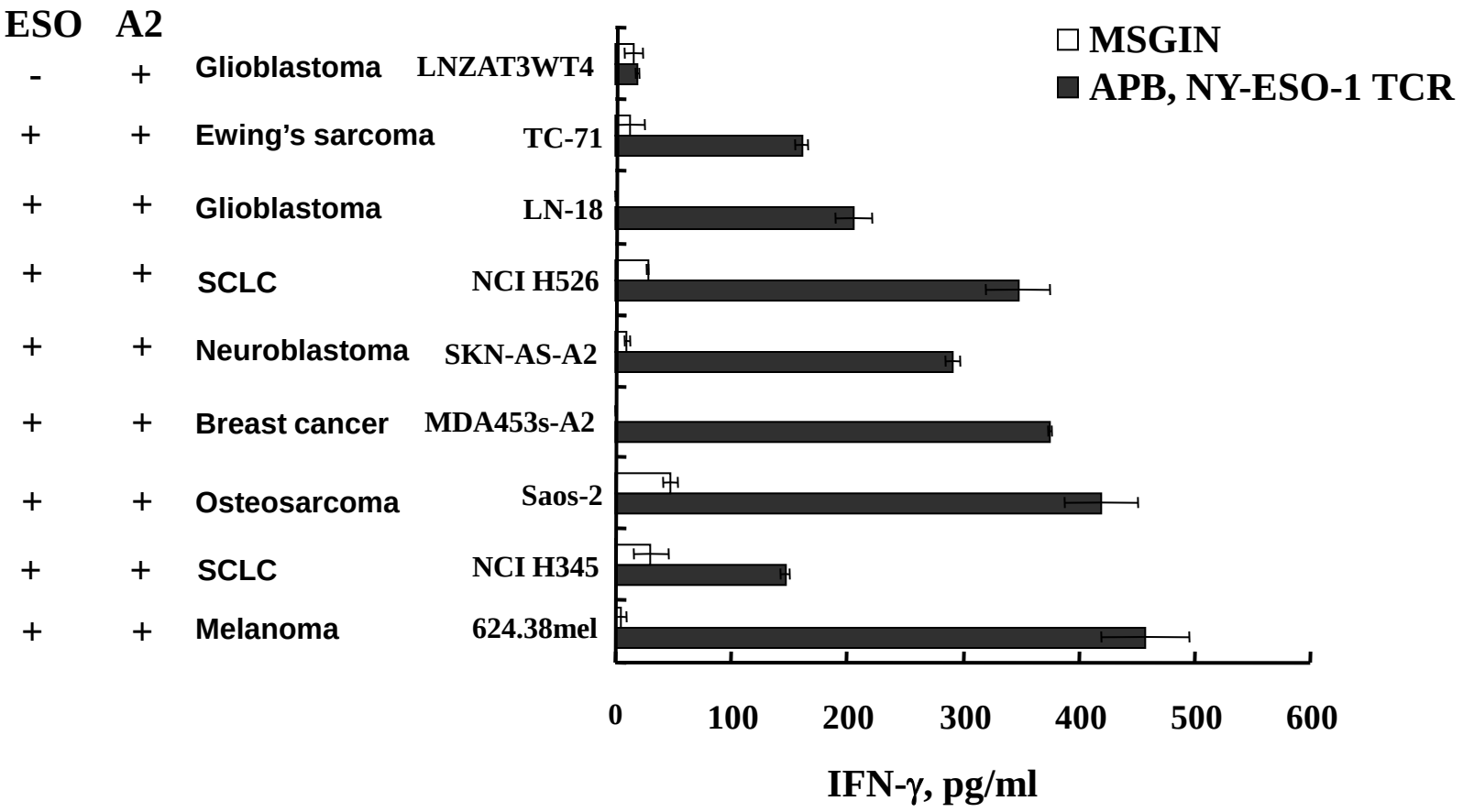
Expressed on about 25% of common epithelial cancers such as lung, breast, prostate

Anti NY-ESO-1 TCR inserted into autologous lymphocytes and used in cell transfer immunotherapy

Expression of CT family members: Variation between different tumor types



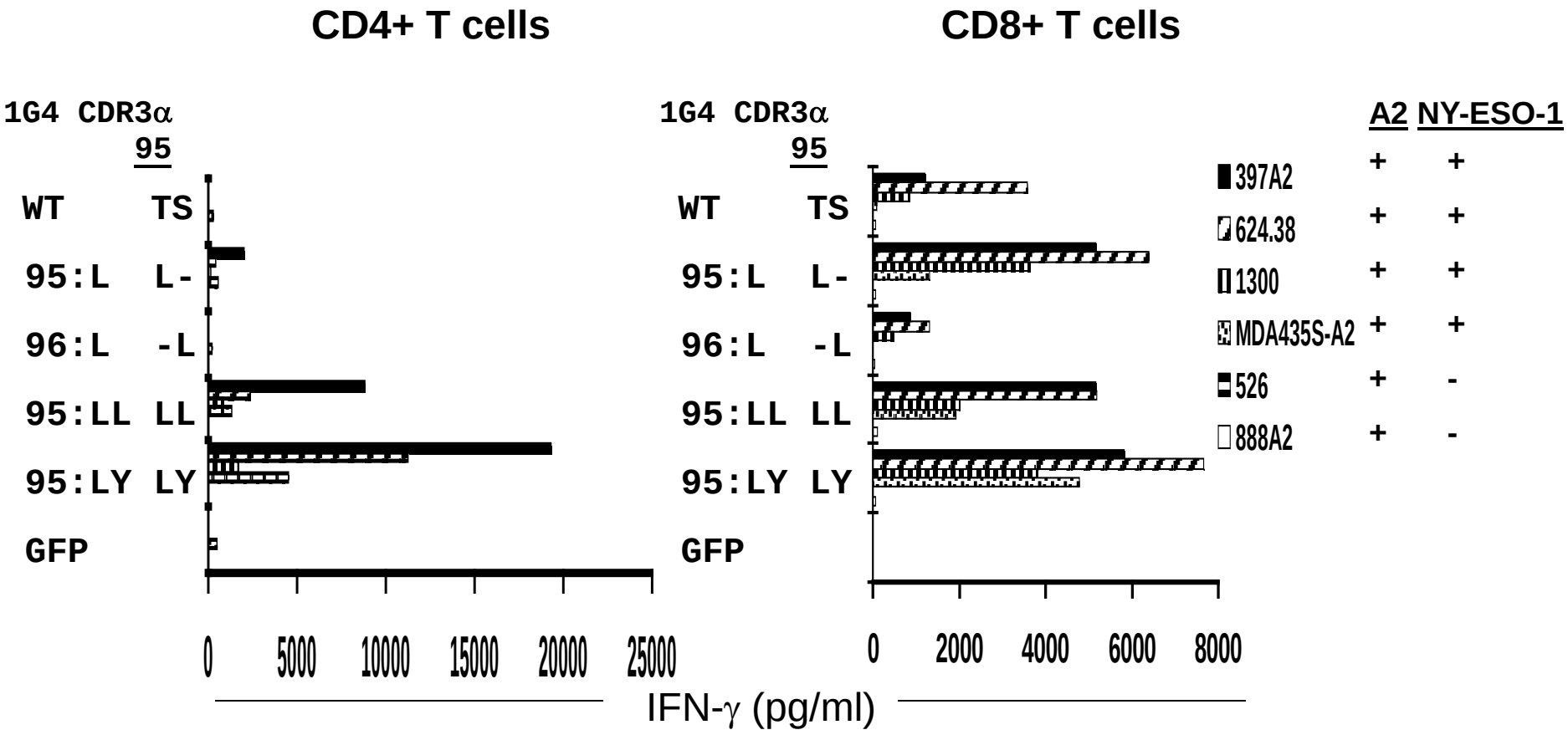
Recognition of Non-melanoma Tumors by NY-ESO-1 TCR Transduced PBL



Gene Therapy with Anti-NY-ESO-1 TCR Transduced Lymphocytes

Cancer	Number of patients (%) Total	Objective response	
Synovial cell sarcoma	6	4	(67%)
Melanoma	11	5	(45%)

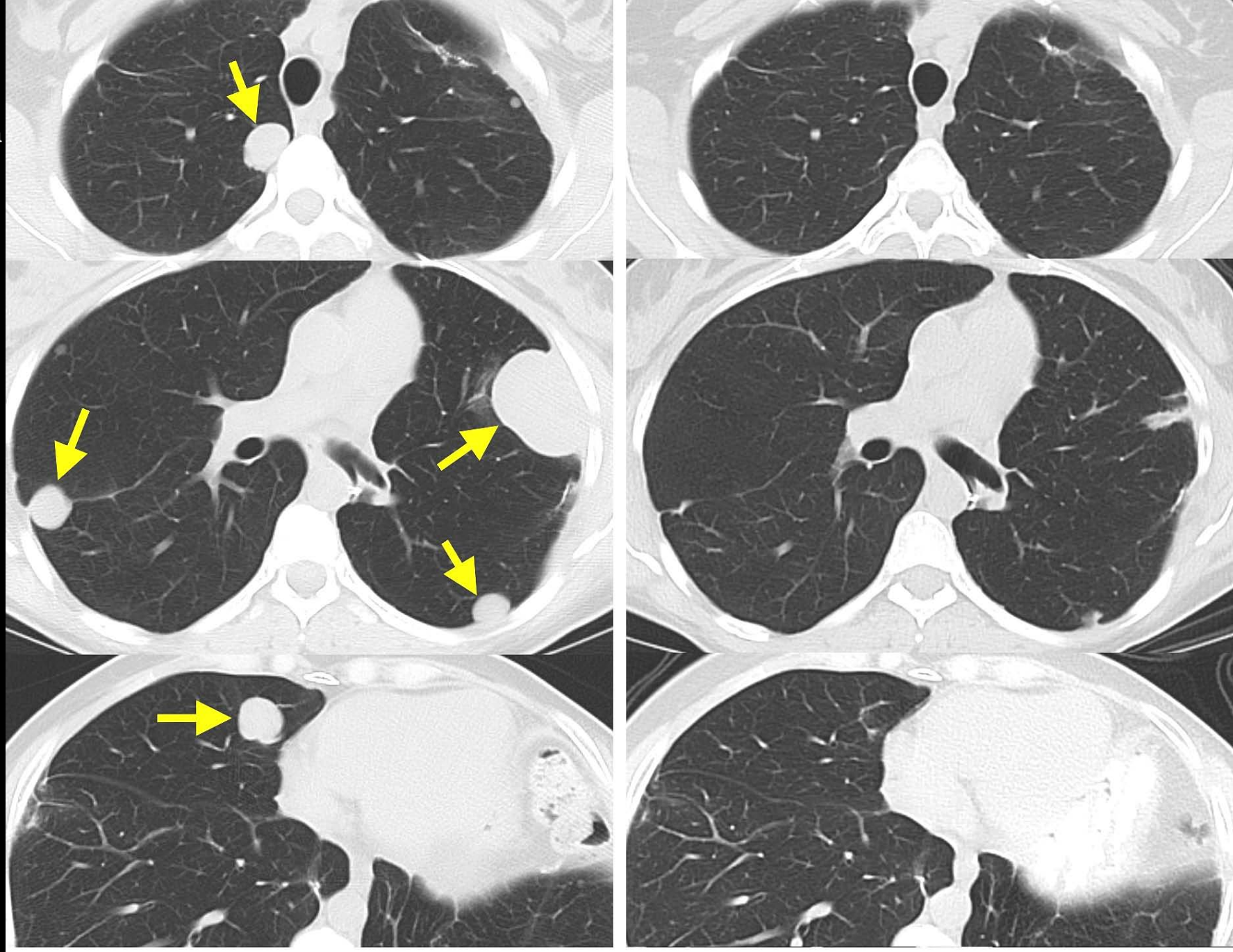
Reactivity of Wild-type and Substituted Anti-ESO T Cell Receptor



H.K.

Synovial Sarcoma

ESO TCR



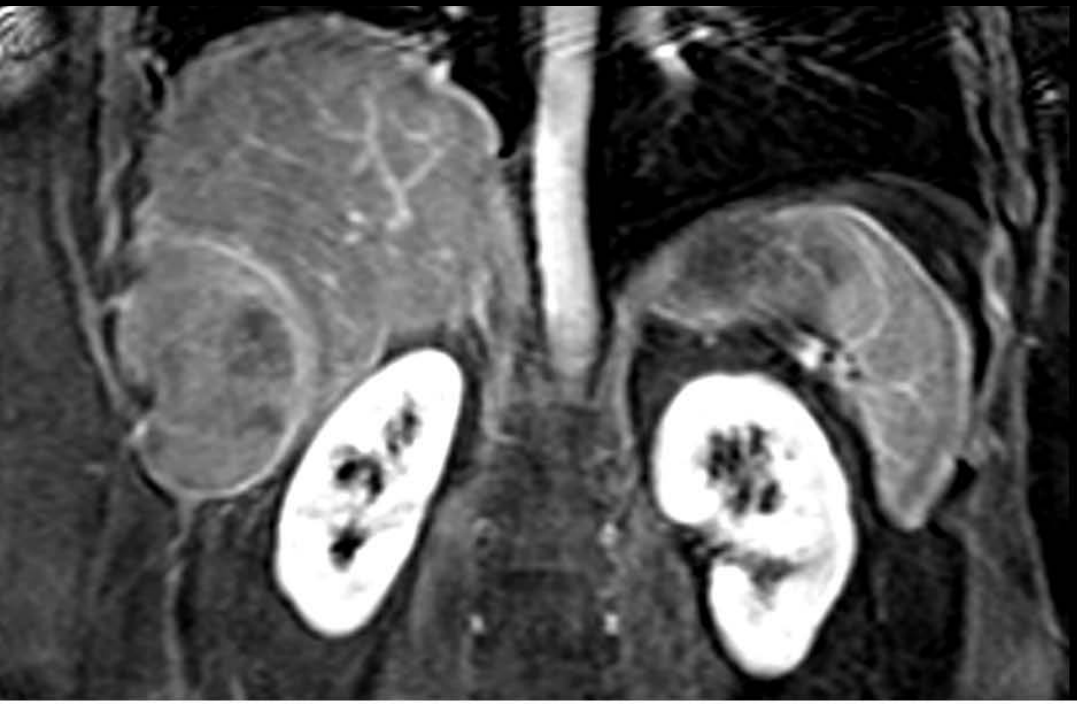
Pre-Treatment **14 Months**

M.C.
ESO-TCR
Synovial sarcoma

Pre

6 months

M.M. Synovial cell sarcoma ESO TCR



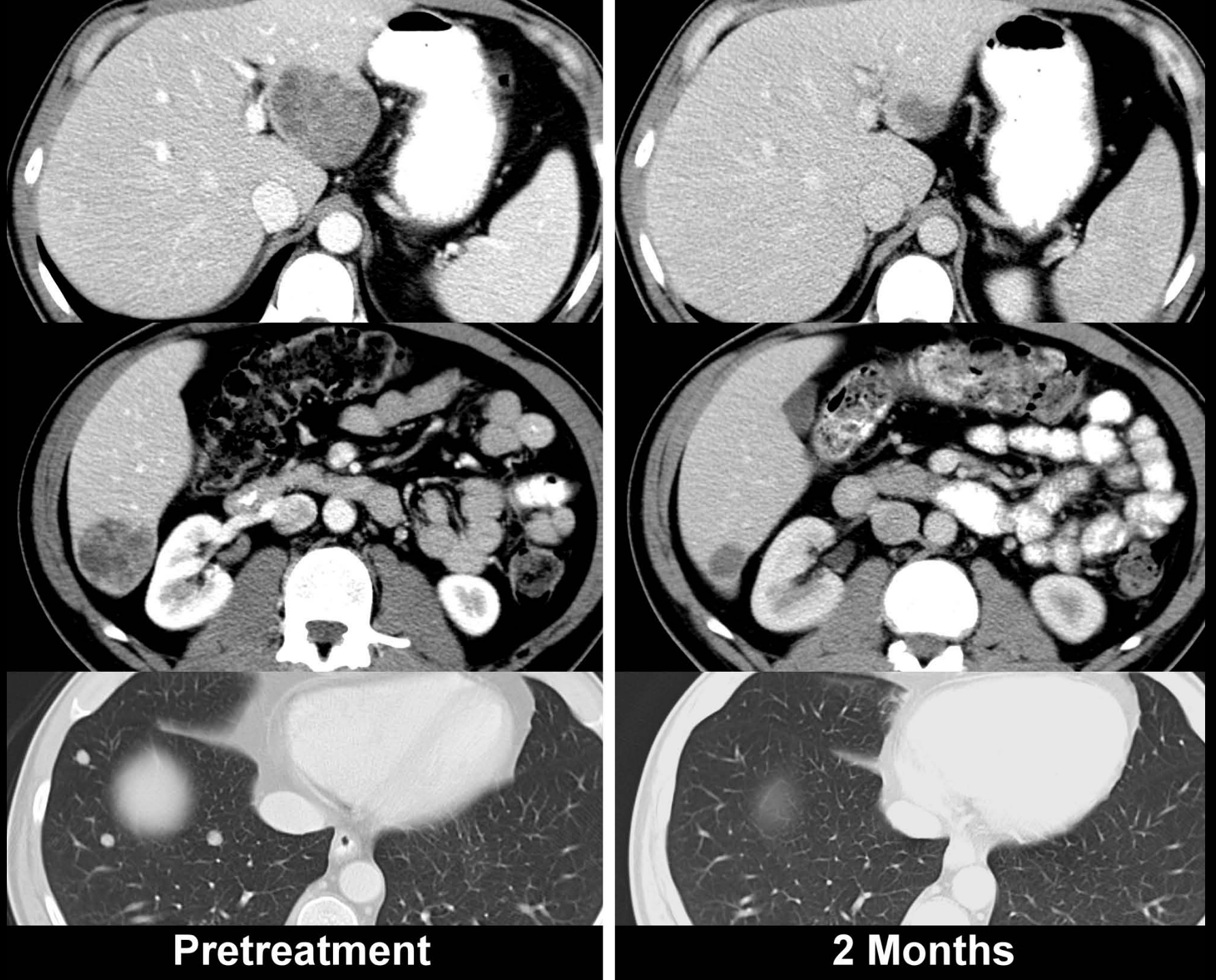
Pre-Treatment

6 Months

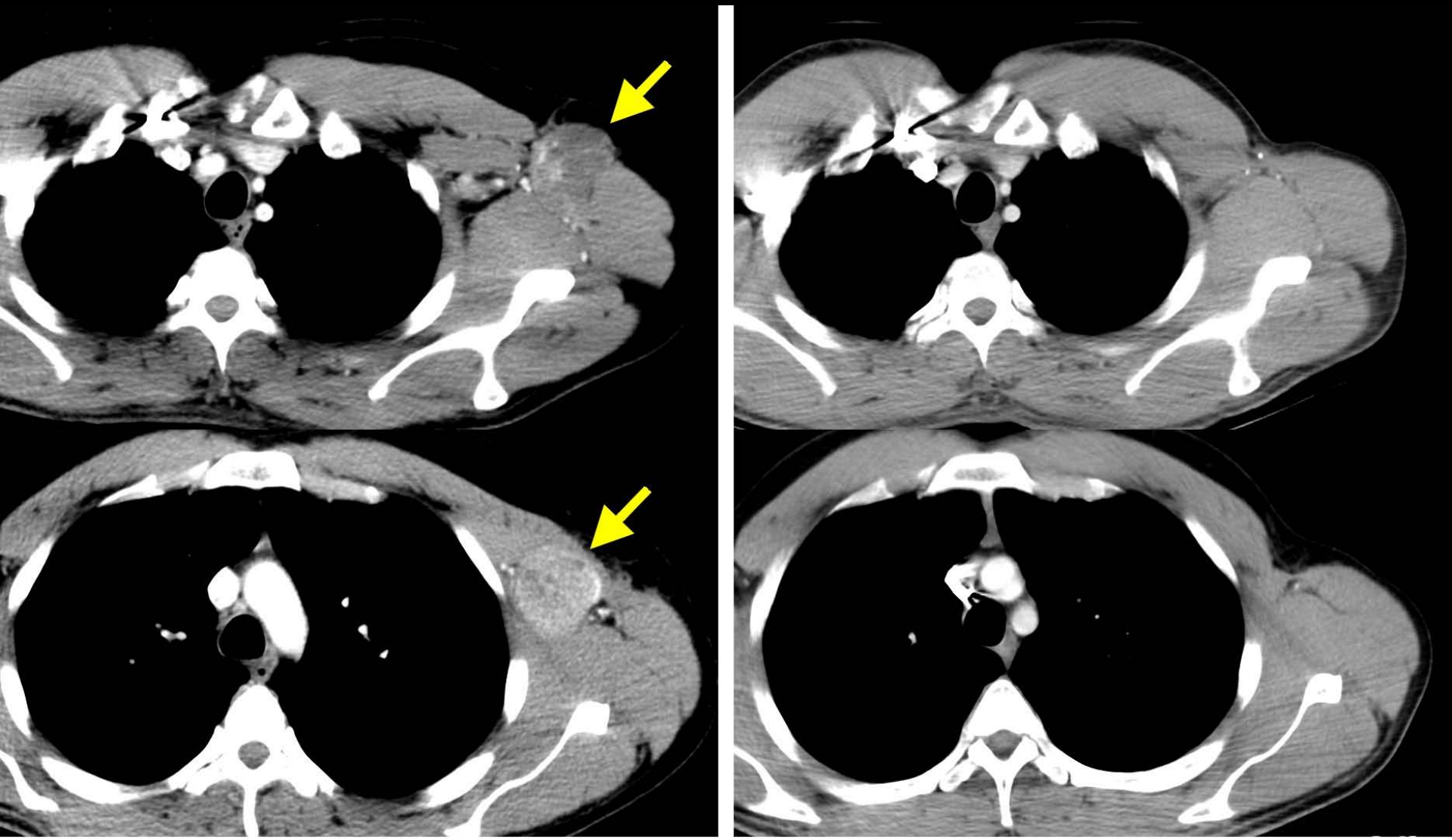
D.C.

Mel

ESO
TCR



M.M. ESo TCR Melanoma



Pre-Treatment

12 Months

Carcinoembryonic Antigen (CEA)

CEA is a highly glycosylated protein:

- **50% carbohydrate**
- **180 kDa**

Highly expressed in fetal development with very low expression in colonic epithelial cells, squamous epithelium in the esophagous and cervix, epithelial cells of cervix

High expression in some cancers:

- **colorectal**
- **breast**
- **lung**
- **cervix**
- **stomach**
- **pancreas**
- **and others**

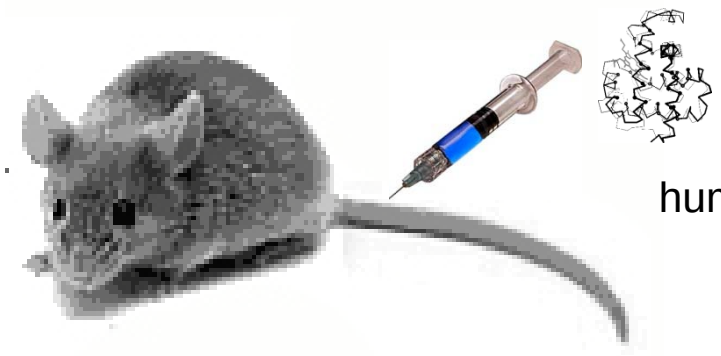
Sequence of the CEA:691-699 antigenic epitope

Human sequence: IMIGVLVGV

No corresponding murine sequences in gene databases

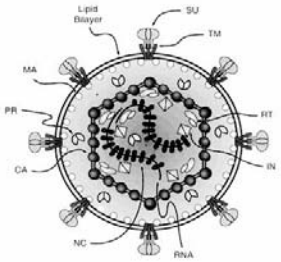
Using transgenic mice to generate T cells specific for human tumor antigens

Clone human MHC class I

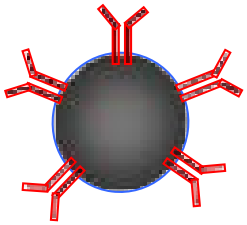


Immunize with human cancer antigen

Mouse transgenic for Human MHC class I



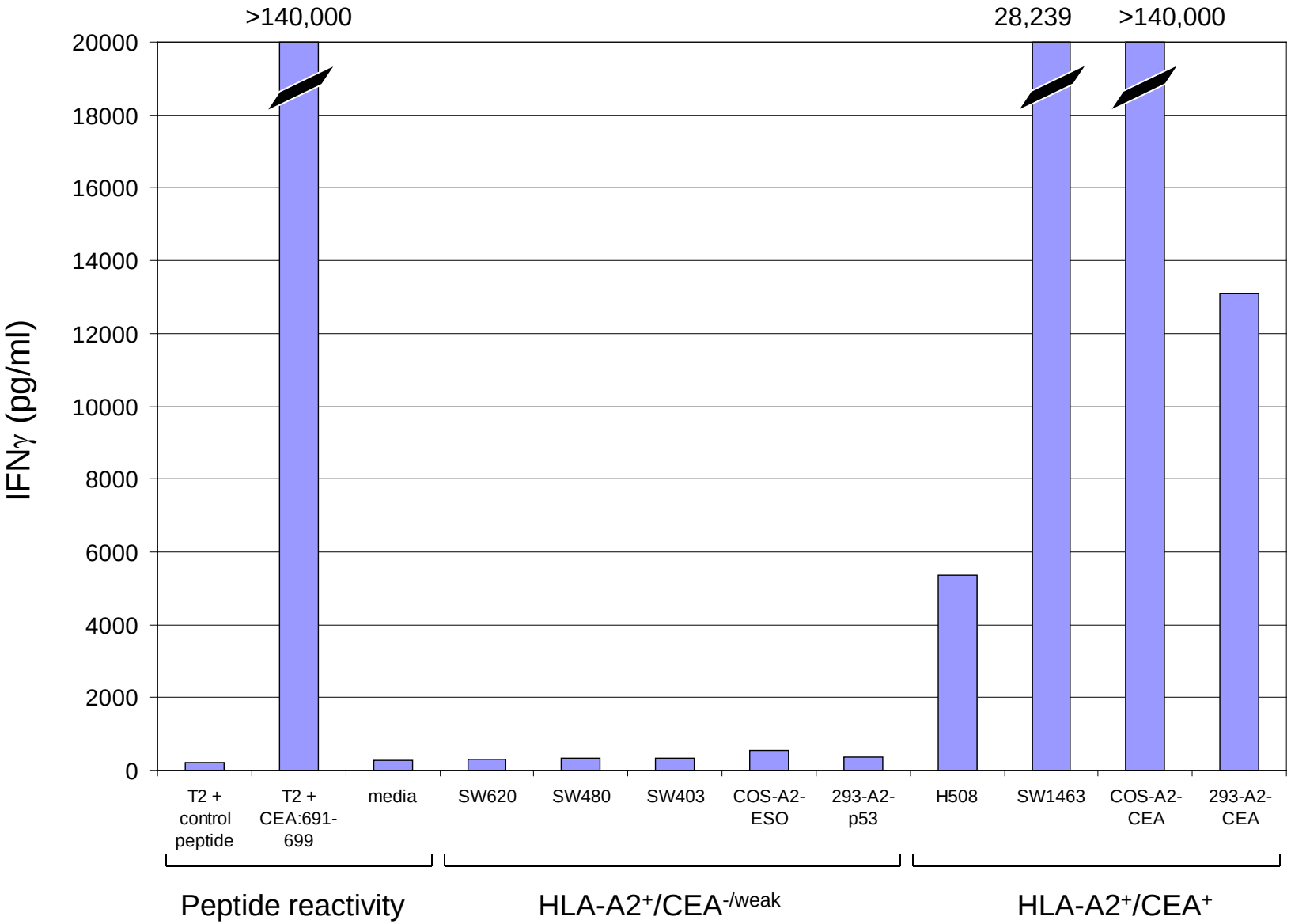
Clone mouse TCR into retroviral vector



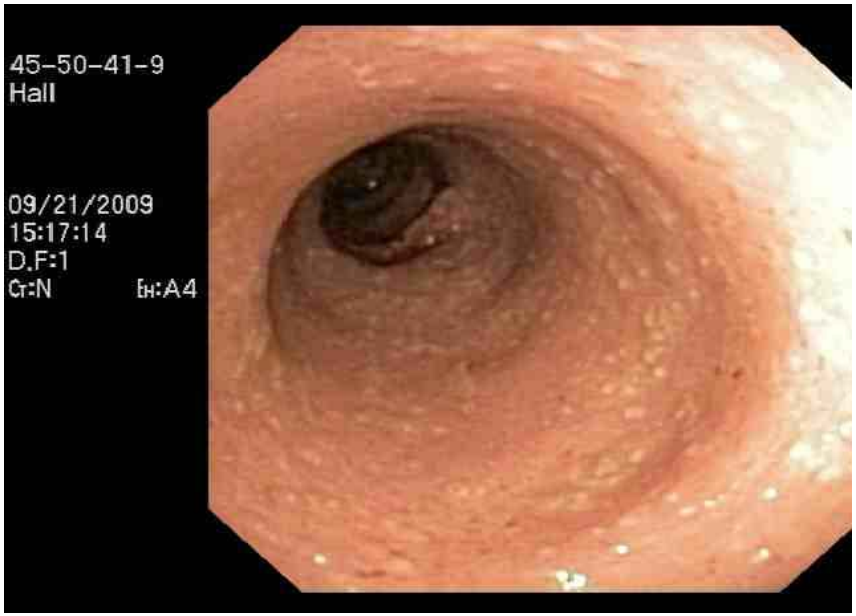
Human T cell expressing **mouse TCR**

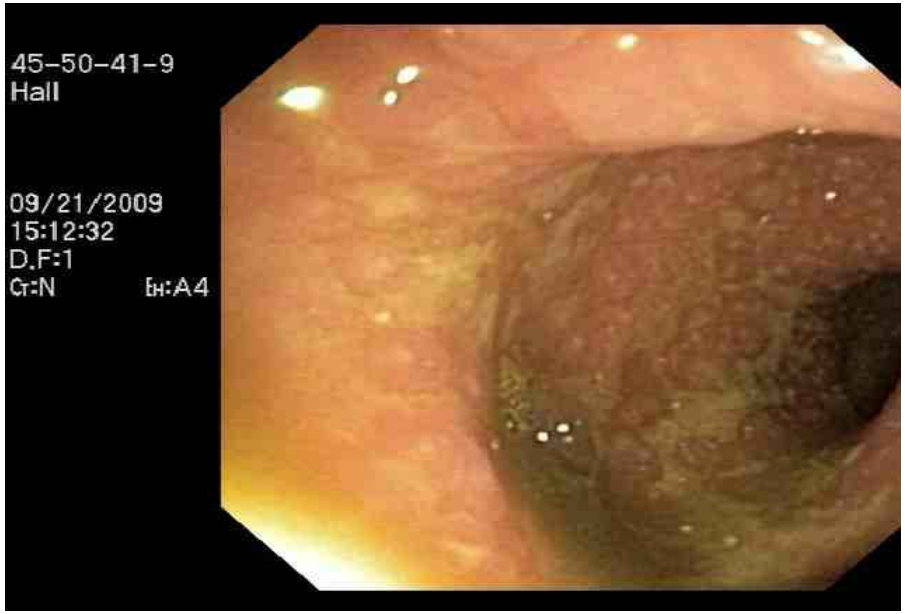
Treat humans with genetically engineered T cells

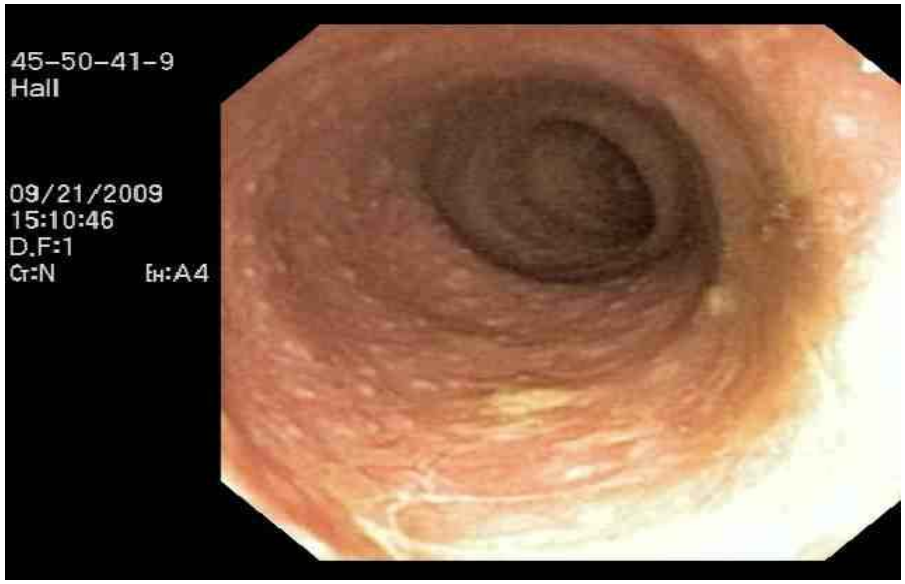
HLA-A2 transgenic mouse T cell clone specifically reactive with human CEA:691-699

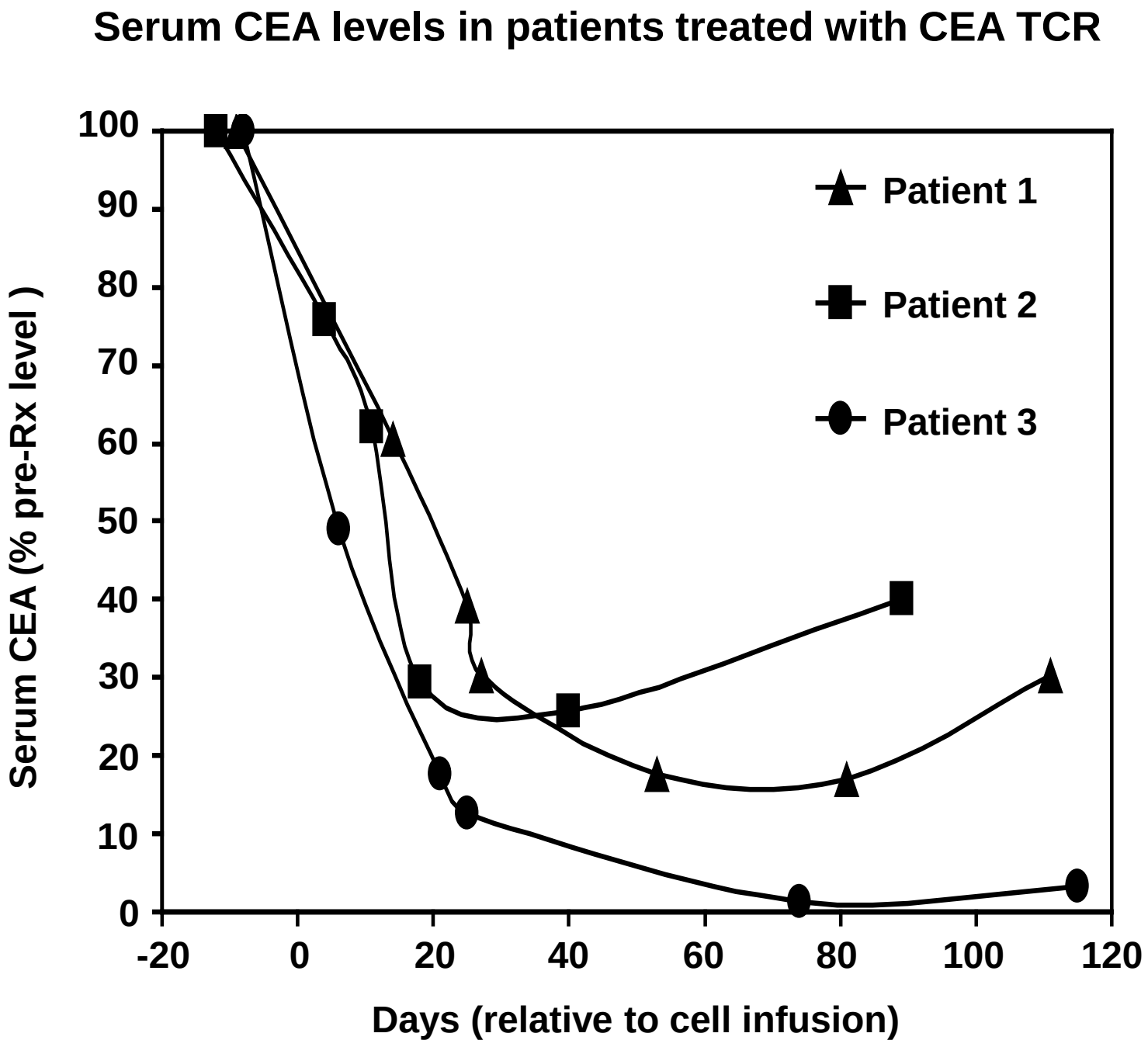


GI findings for Patient RH 17 days post-treatment





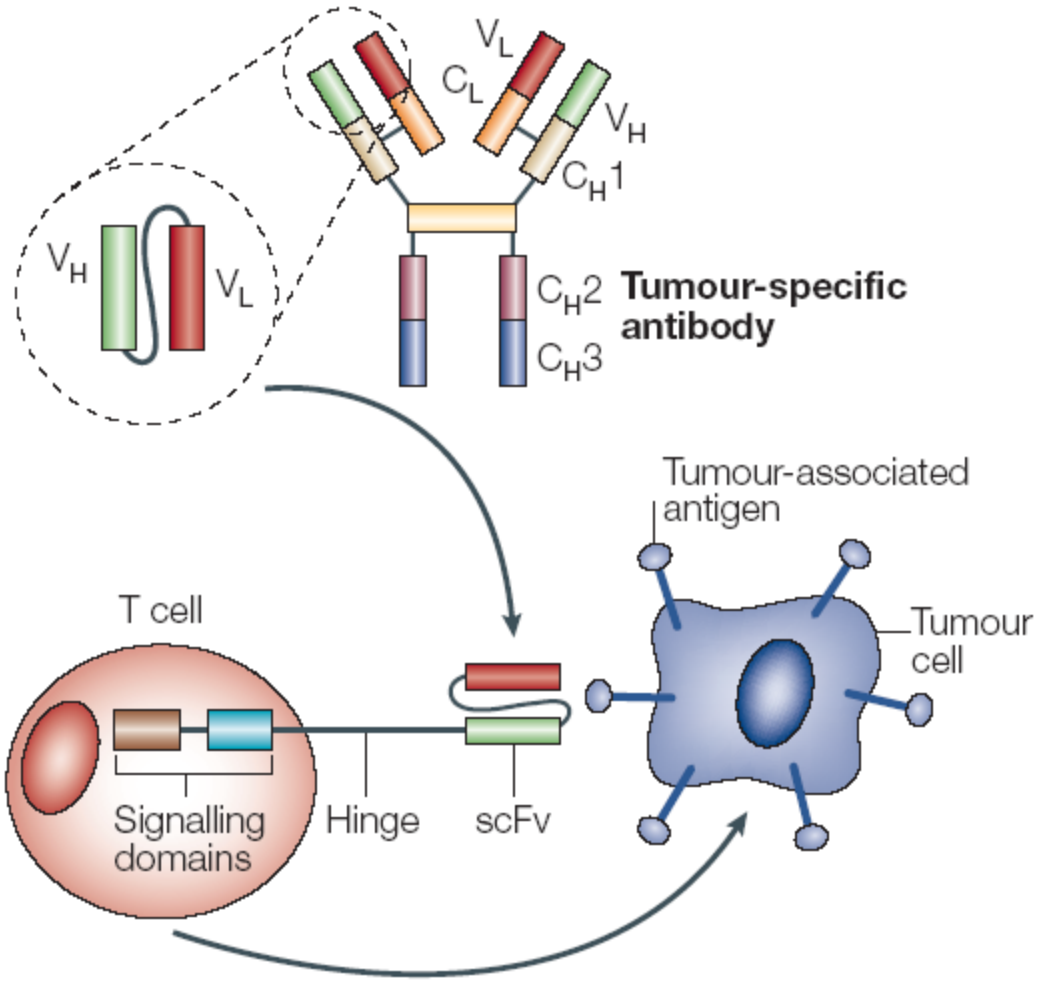



Serum CEA levels in patients treated with CEA TCR
Serum CEA (% pre-Rx level)
100
90
80
70
60
50
40
30
20
10
0
-20
0
20
40
60
80
100
120
Days (relative to cell infusion)
Patient 1
Patient 2
Patient 3

N.H.

CEA
TCR

Chimeric Antigen Receptors (CARs)



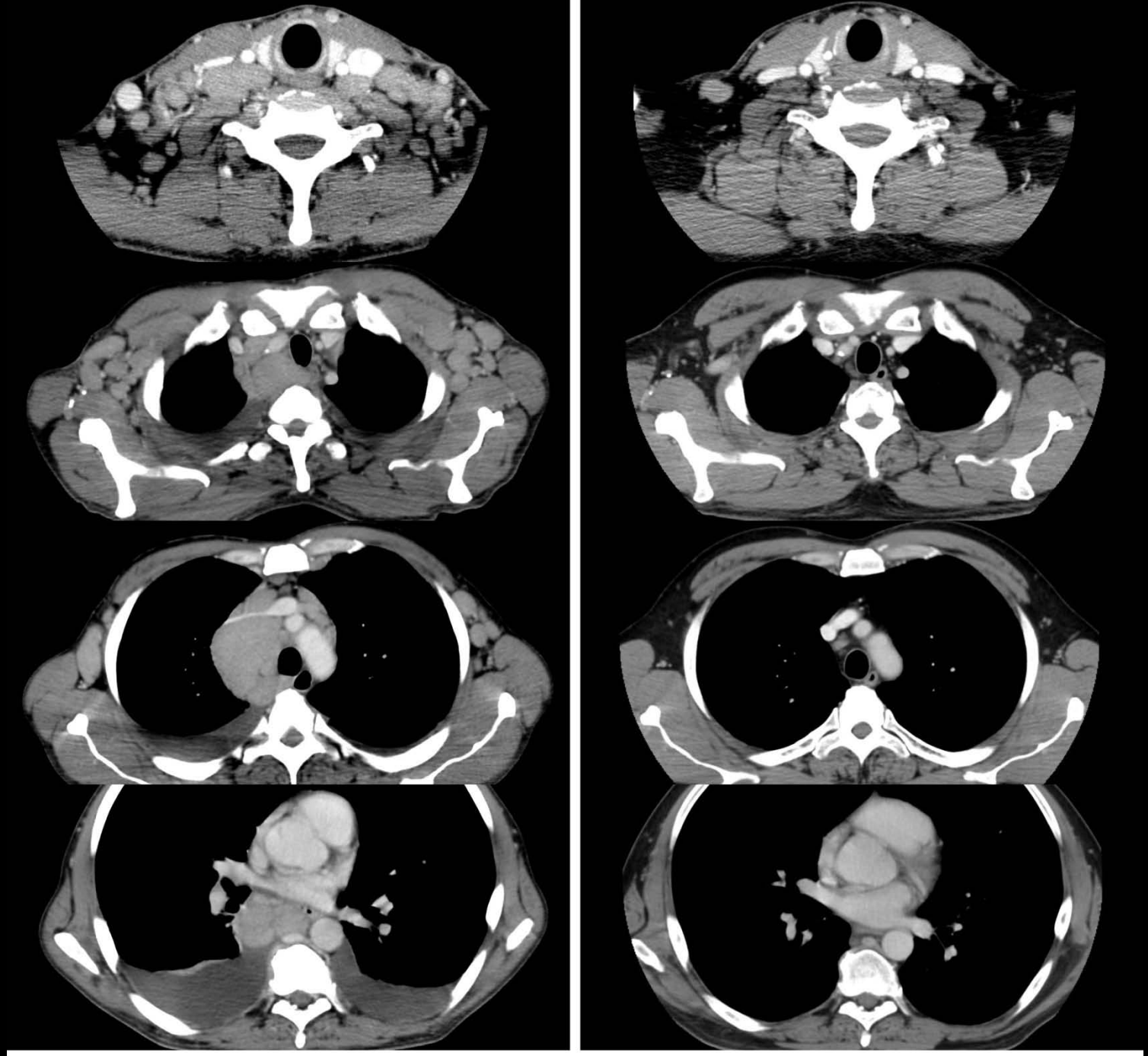
M.H. Kershaw et al. Nature Reviews Immunology Vol. 5 pg. 928-940 Nov. 2005

Patient 1: Pre-infusion INF–gamma ELISA

Effector cells	CD19-expressing targets			CD19-negative targets		Effectors alone
	Toledo	**Nalm6**	**CD19-K562**	**NGFR-K562**	**CCRL-CEM**	
Patient 1 anti-CD19 CAR-transduced	**2180**	**4765**	**48050**	581	193	110
Patient 1 Not transduced	63	70	59	66	66	31

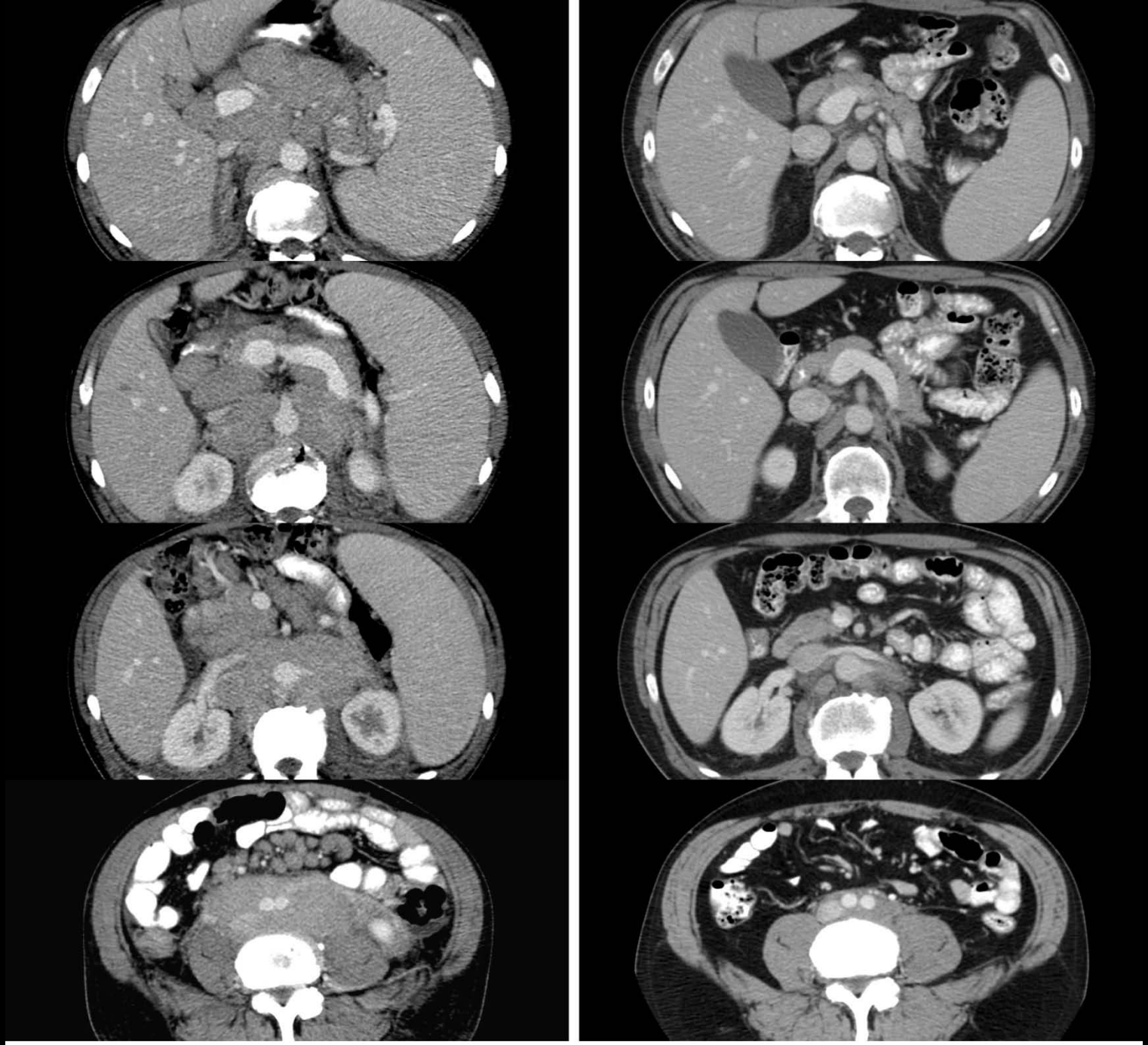
R.K.

CD19 TCR



R.K.

CD19
TCR



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.

Synovial Cell Sarcoma

High grade malignancy (5 – 10% of all soft tissue sarcomas)

Chromosomal rearrangement of:

SYT gene on chromosome 18

SSX genes (1 of 5) on chromosome X

80% of synovial sarcomas express high levels of NY-ESO-1

As of 3/1/10 four of six patients (67%) with heavily pretreated synovial cell sarcoma have had objective responses to treatment with autologous TCR gene-engineered T cells.

(5 of 11 (45%) of patients with metastatic melanoma responded)