Adoptive Immune Therapy: Lessons Learned in Trial Design

SITC (4/13)

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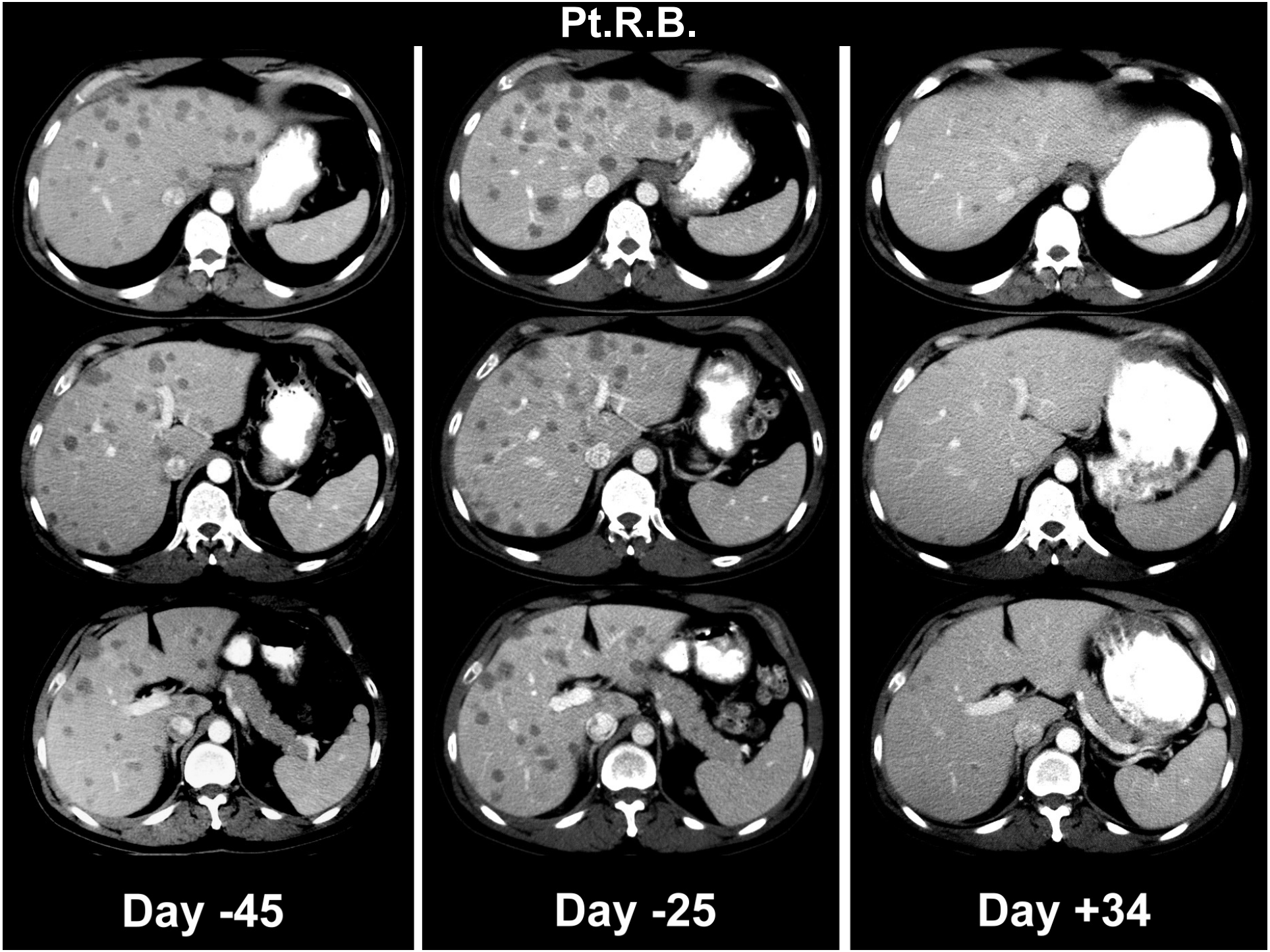
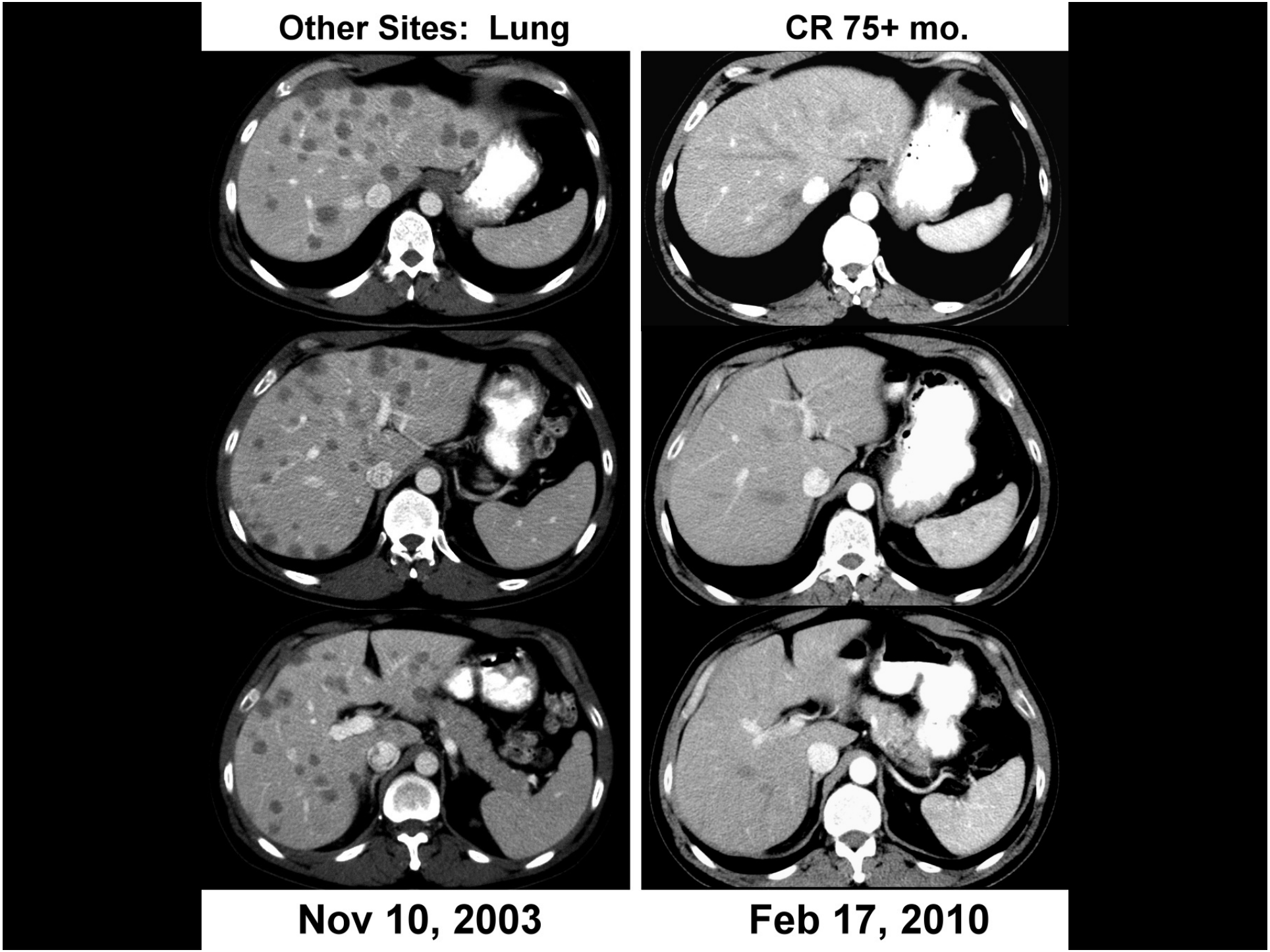
Surgery Branch, National Cancer Institute

Cell Transfer Therapy (11/1/12)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (109+, 106+, 105+, 93+, 88+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (95+, 91+, 87+, 84+, 81+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (75+, 73+, 71+, 71+, 66+, 65+, 65+, 65+, 64+, 19)	18(72%)

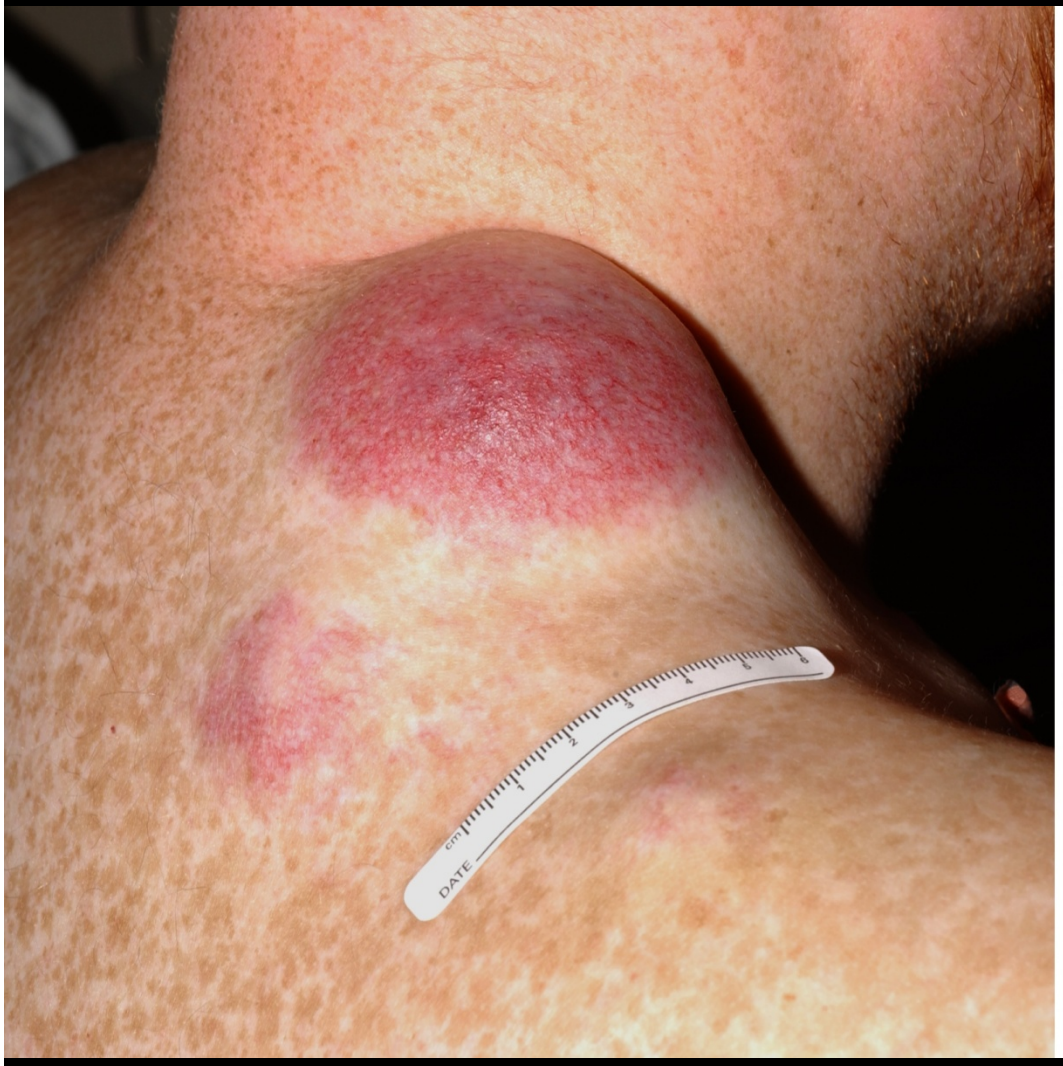
(20 complete responses: 19 ongoing at 64 to 109 months)

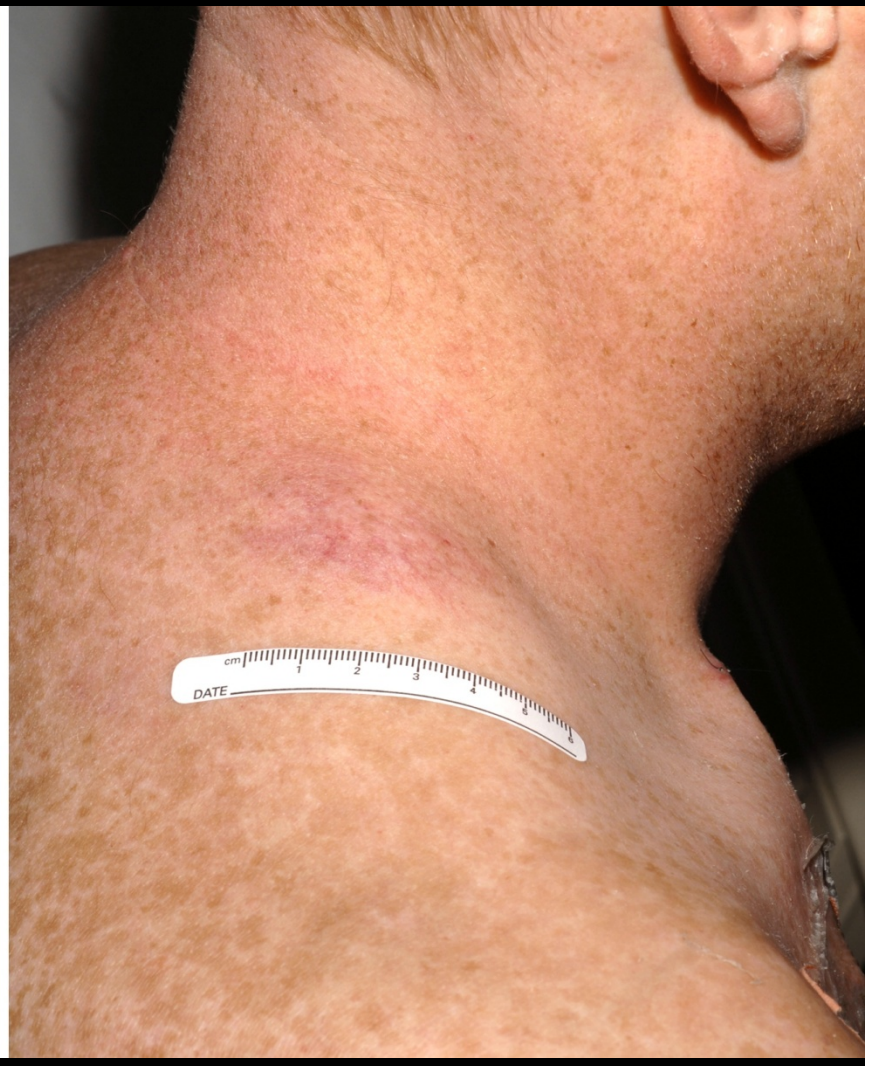

Pt.R.B.
Day -45
Day -25
Day +34


Other Sites: Lung
CR 75+ mo.
Nov 10, 2003
Feb 17, 2010

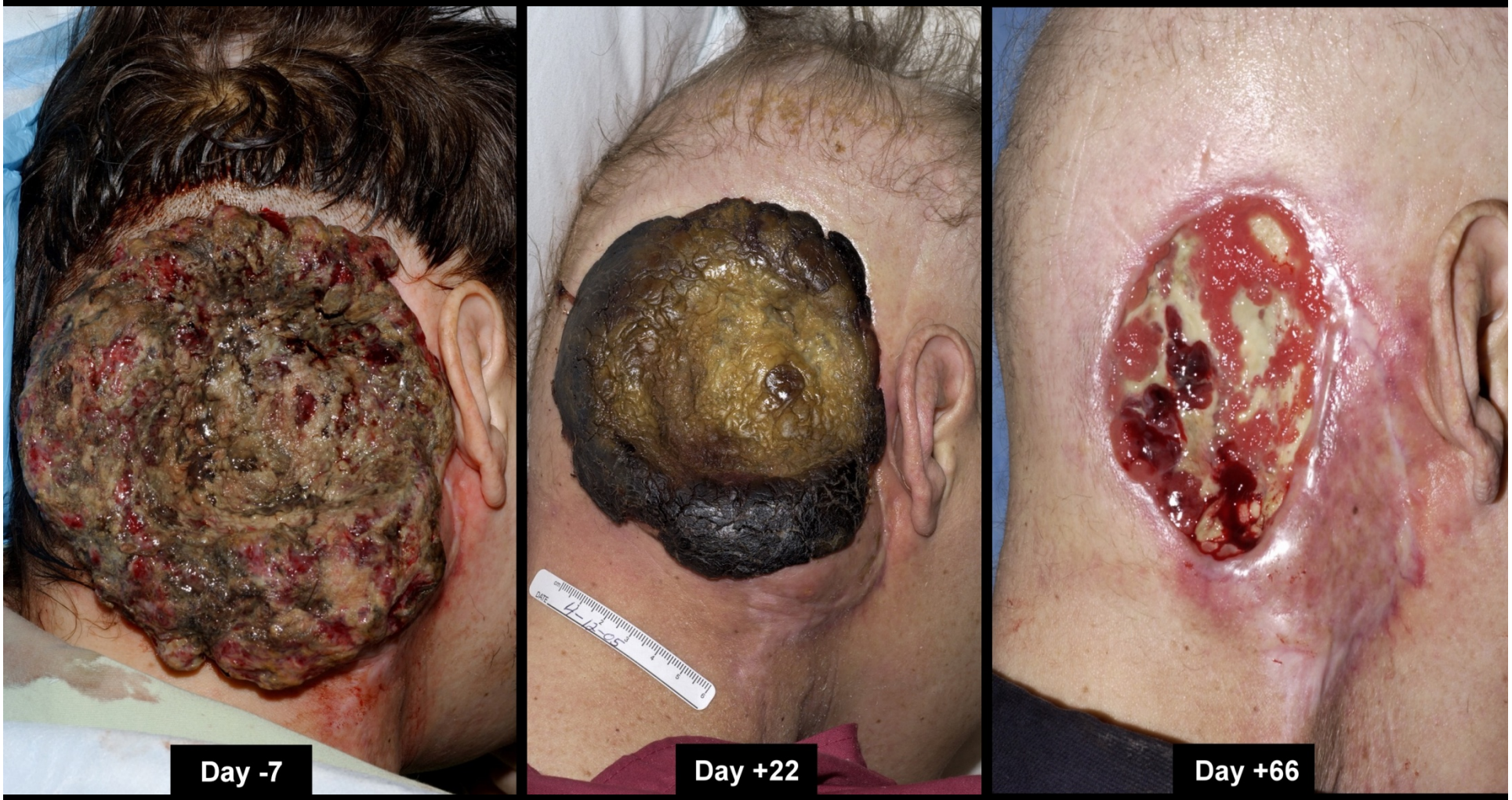
C.K. (200cGy) Pre

12 days



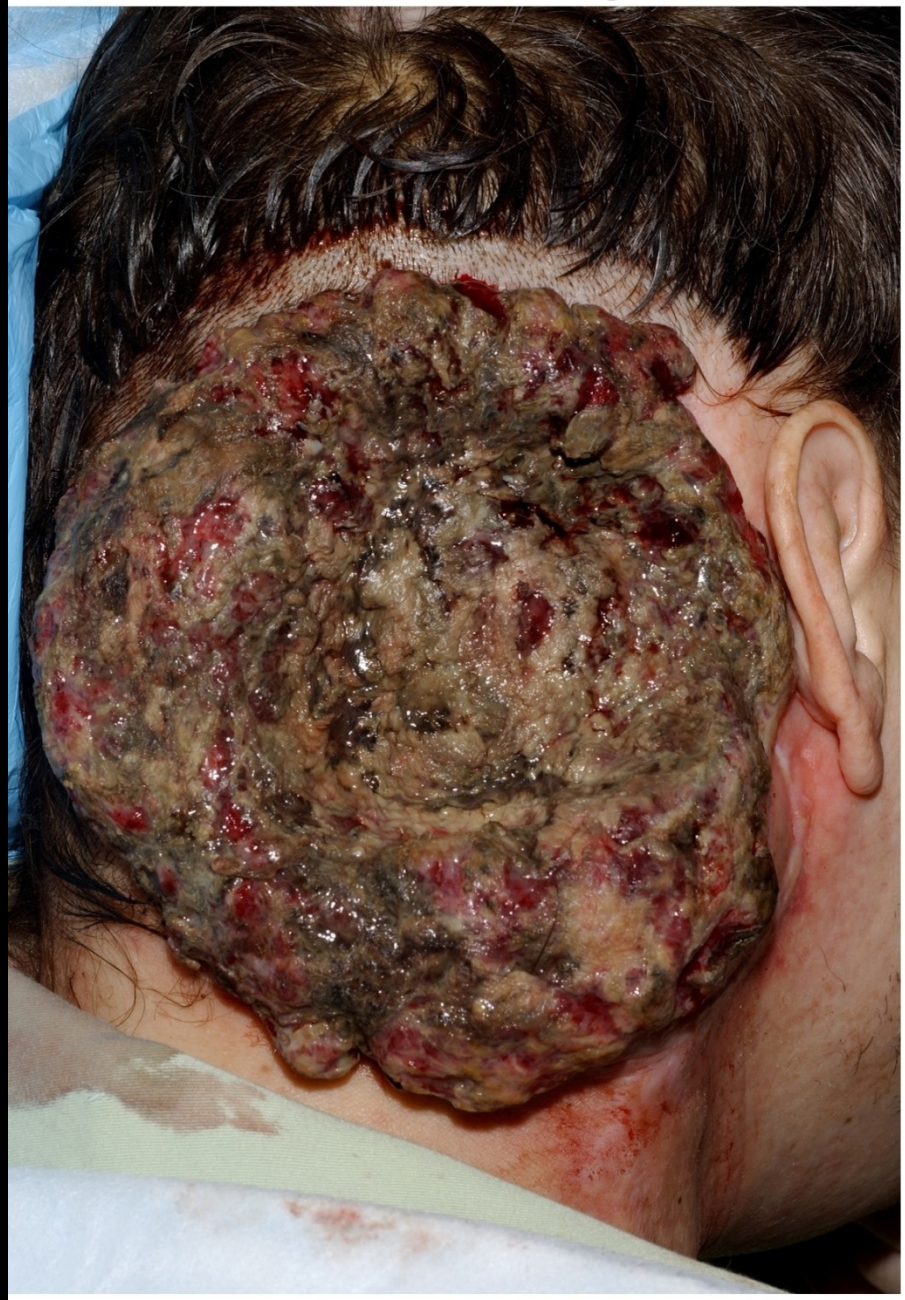


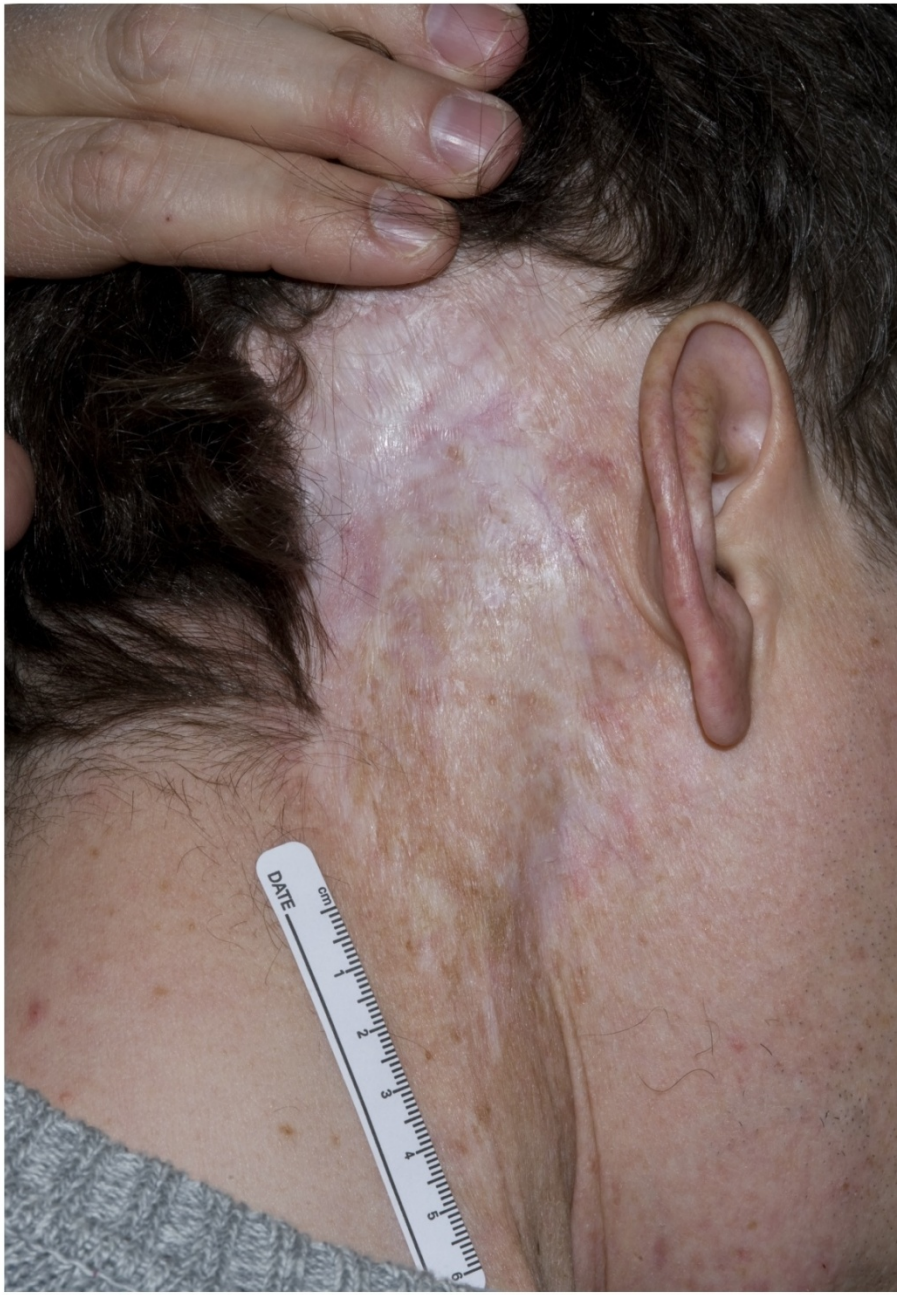
A.H.: N-M cell transfer



Other Sites: Lung

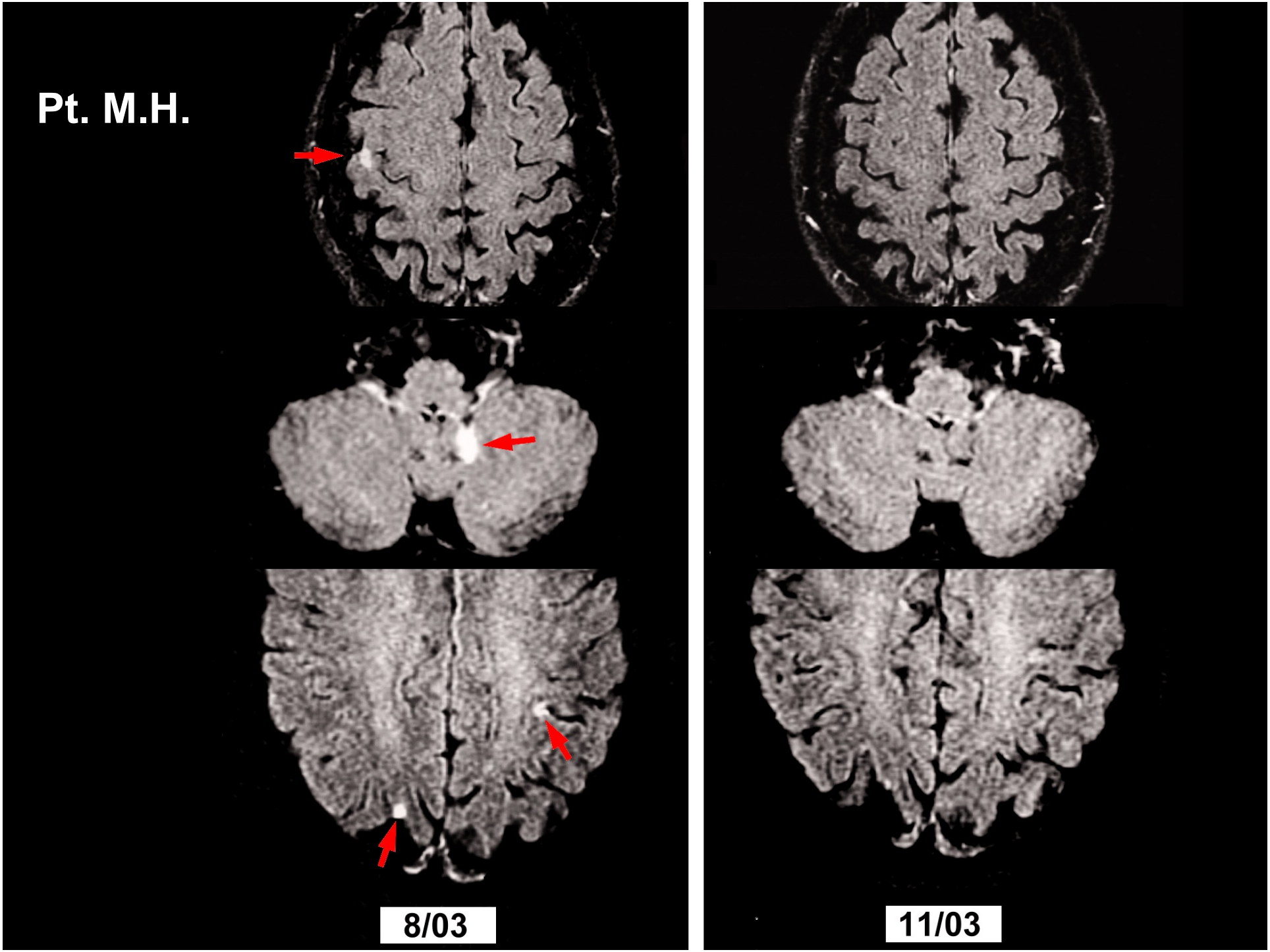
CR 59+ mo.

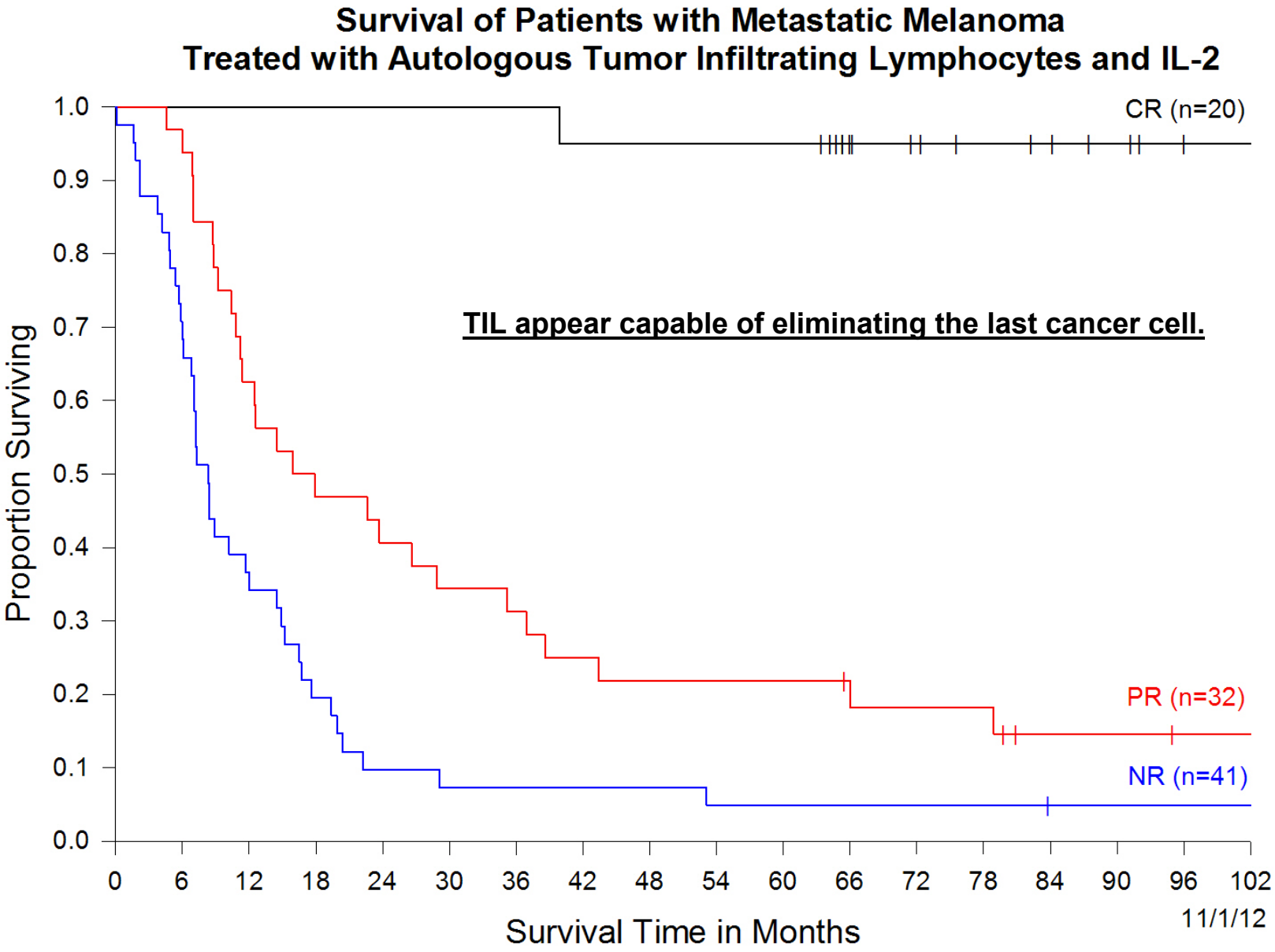




March 21, 2005

Feb 23, 2010


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


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
CR (n=20)
TIL appear capable of eliminating the last cancer cell.
PR (n=32)
NR (n=41)
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Survival Time in Months
11/1/12

Potential Gene Alterations to Improve the Efficacy of Cell Transfer Therapy

Expand tumor recognition	**T cell receptors or chimeric T cell receptors that recognize cancer antigens**
Cytokines	**IL-2, 12, 15, 17, 21, 23**
Costimulatory molecules	**CD8, CD27, CD80, 41BBL, OX-40L**
Antiapoptotic molecules	**Bcl-2, Bcl-xl, FLIP, TIPE-2**
Reverse inhibitory influences	**KO SHP-1, PD-1, CTLA-4, SOCS, CIS Dominant negative TGF-β, cbl-b**
Trafficking molecules	**CD62L, CCR7, CXCR2, CXCR4**
Improve survival	**Telomerase, KOp53**

Phase I Study of ACT Using TIL Transduced with Gene Encoding IL-12 (4/1/13)

TIL grown for 2-3 weeks
Stimulated with OKT-3, transduced and expanded
Infuse after Cy/flu preparative regimen
No IL-2 administered

Cohort (# cells x 10^{-9})	Number of patients	Result
0.001	1	1NR
0.003	1	1NR
0.01	7	7NR
0.03	5	1CR (24+ mos); 4NR
0.1	3	3NR
0.3	3	3PR (4, 6, 12+)
1.0	4	1PR (12+); 3NR
3.0	4	1CR (5+) 3PR (9+, 7, 5)

In first 5 cohorts 1 of 17 patients responded. At doses greater than $0.1X10^{-9}$ 8 of 11 patients responded.

POINTS TO CONSIDER IN IMMUNOTHERAPY TRIAL DESIGN

Tension in Phase I studies between safety and possible effectiveness in selection of the starting dose and mode of dose escalation.

Goal: Find a safe dose but minimize the number of patients receiving very low ineffective doses.

Potential Gene Alterations to Improve the Efficacy of Cell Transfer Therapy

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A critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

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1. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

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4. Mutations unique to each cancer (EGFRvIII)

5. Critical components of the tumor stroma (VEGFR2, FAP)

TCR Gene Therapy in Patients with Metastatic Melanoma

TCR	Response		Toxicity		
	Total	OR	Skin (Grade 1/2/3)	Uveitis (Grade 1/2/3)	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
(Total)	36	9(25%)	22/7/0 (81%)	2/13/0 (42%)	4/2/3 (25%)

	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.

(Science 314:126, 2006; Blood 114:535, 2009)

Carcinoembryonic Antigen (CEA)

CEA is a highly glycosylated protein:

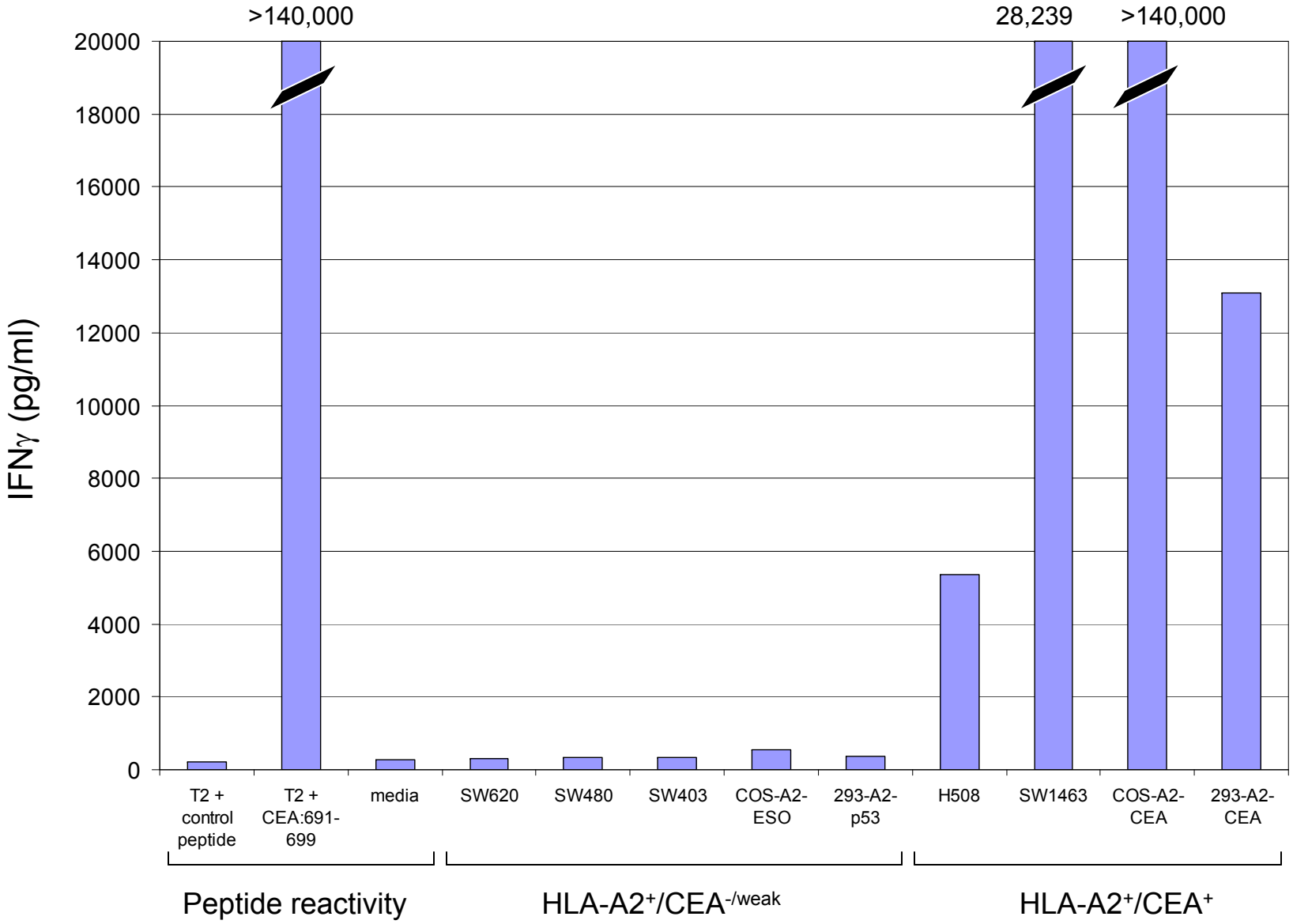
- **50% carbohydrate**
- **180 kDa**

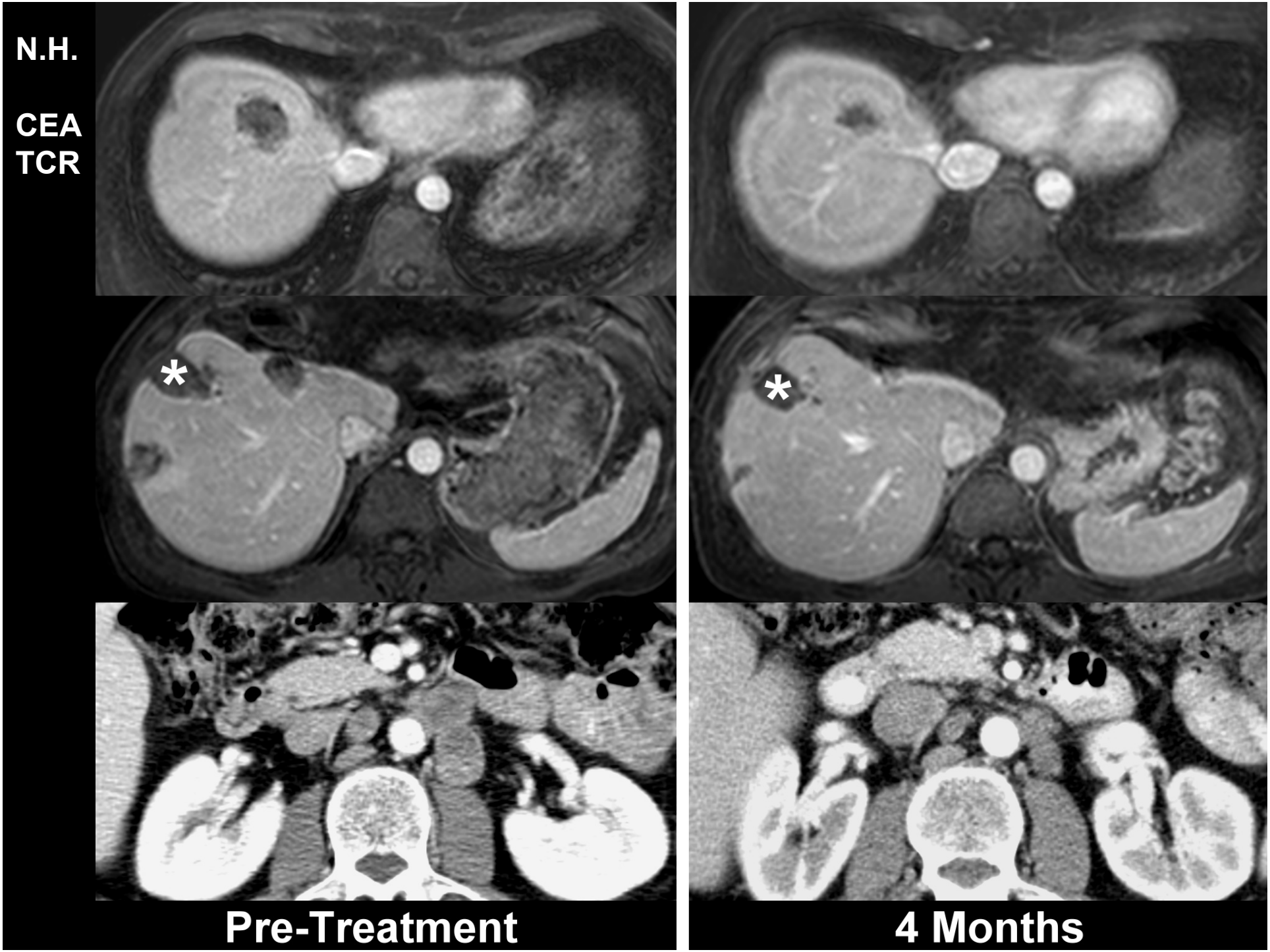
Highly expressed in fetal development with very low expression in colonic epithelial cells, squamous epithelium in the esophagous and cervix, epithelial cells of cervix

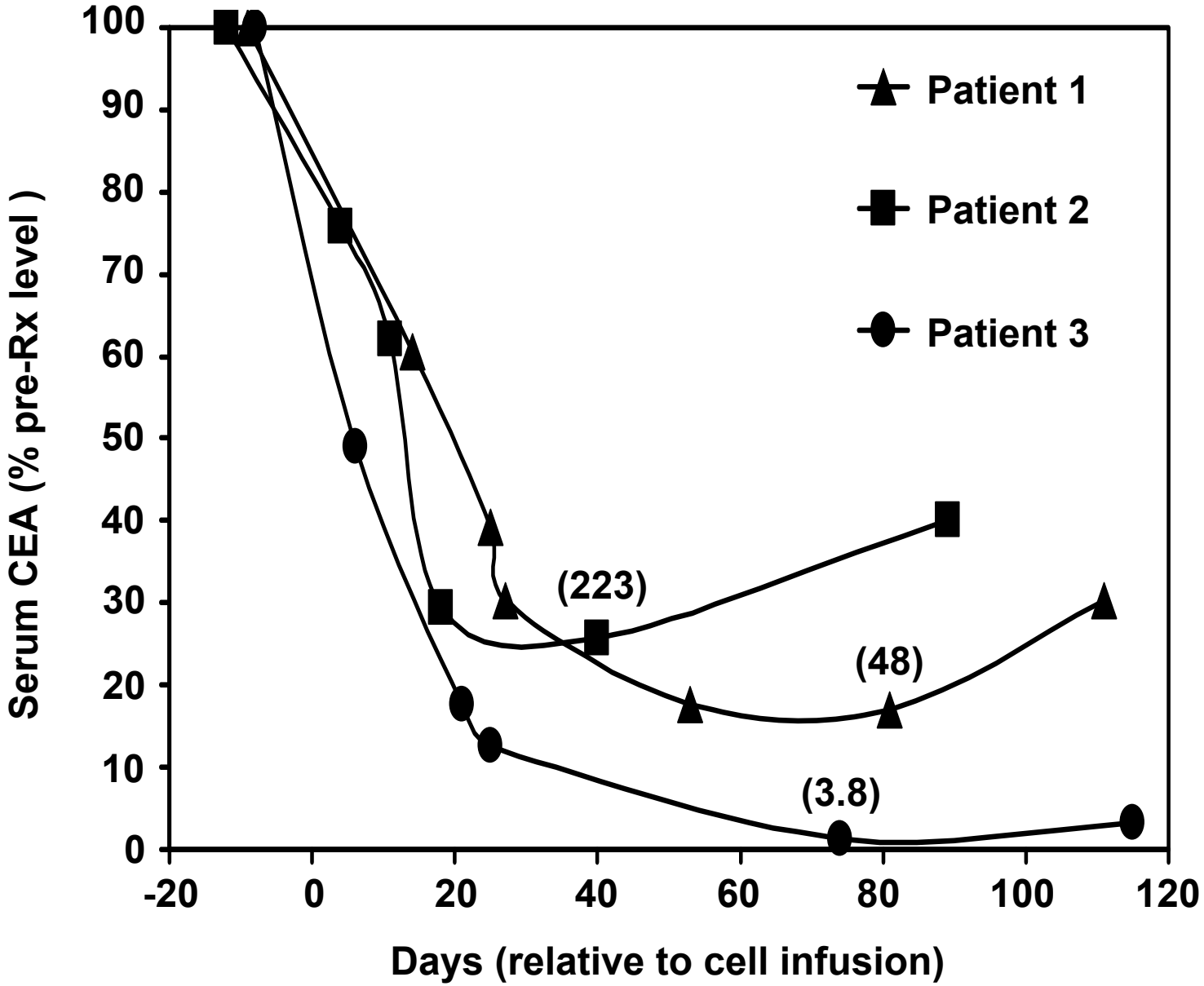
High expression in some cancers:

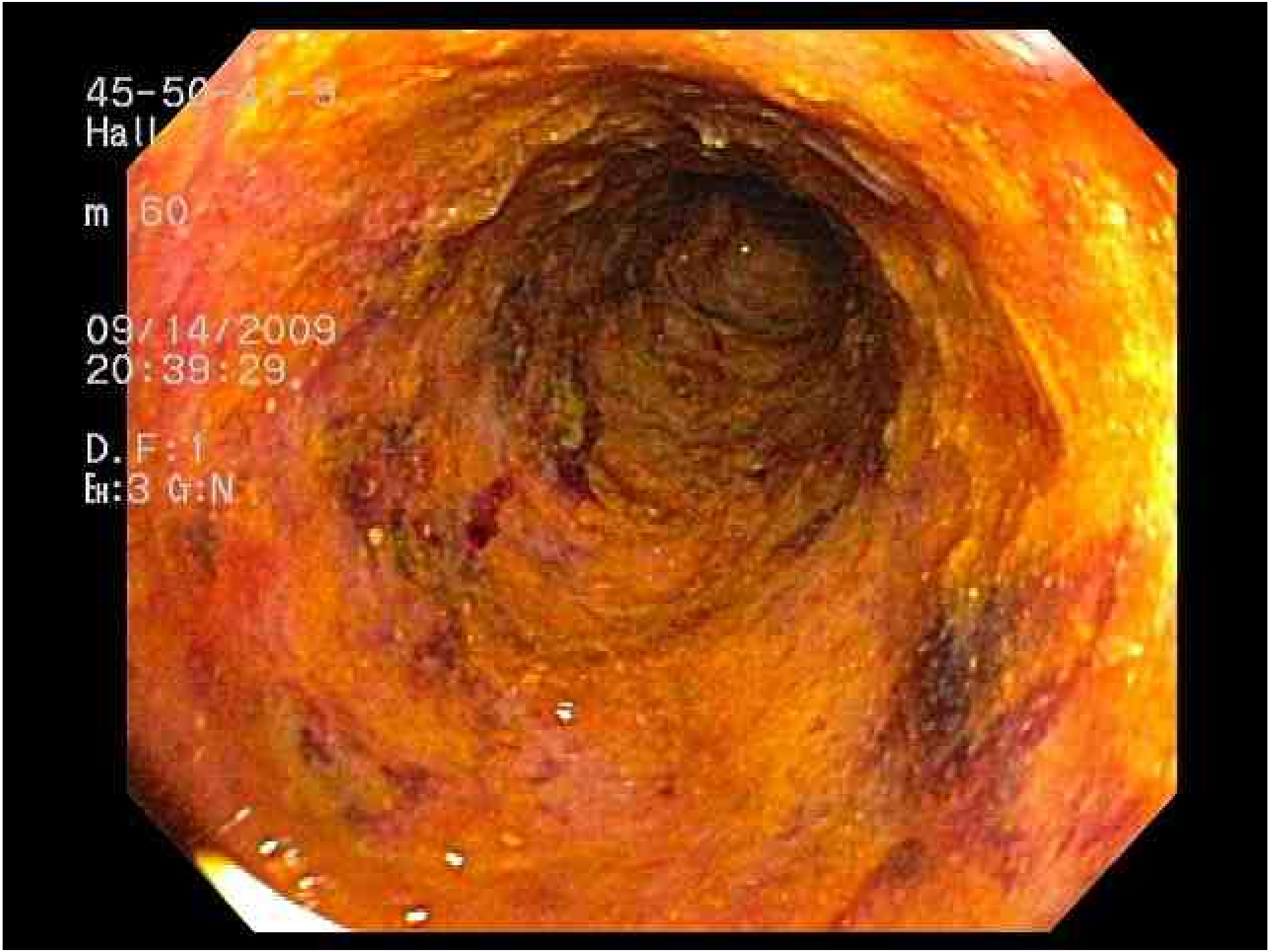
- **colorectal**
- **breast**
- **lung**
- **cervix**
- **stomach**
- **pancreas**
- **and others**

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




N.H.
CEA
TCR
*
*
Pre-Treatment
4 Months


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
(223)
(48)
(3.8)


45-5
Hall
m 60
09/14/2009
20:39:29
D.F:1
EH:3 G:N

POINTS TO CONSIDER IN IMMUNOTHERAPY TRIAL DESIGN

The exquisite sensitivity of high affinity T cells can target very low levels of antigen expressing cells.

Finding cell affinities and cell numbers in immunotherapy approaches that can exploit the differences of antigen expression on tumors versus normal tissue can be difficult as well as dangerous.

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B-cell Malignancies

Approximately 22,000 people die of B-cell malignancies annually in the U.S.

CD19 is expressed by more than 90% of B-cell malignancies.

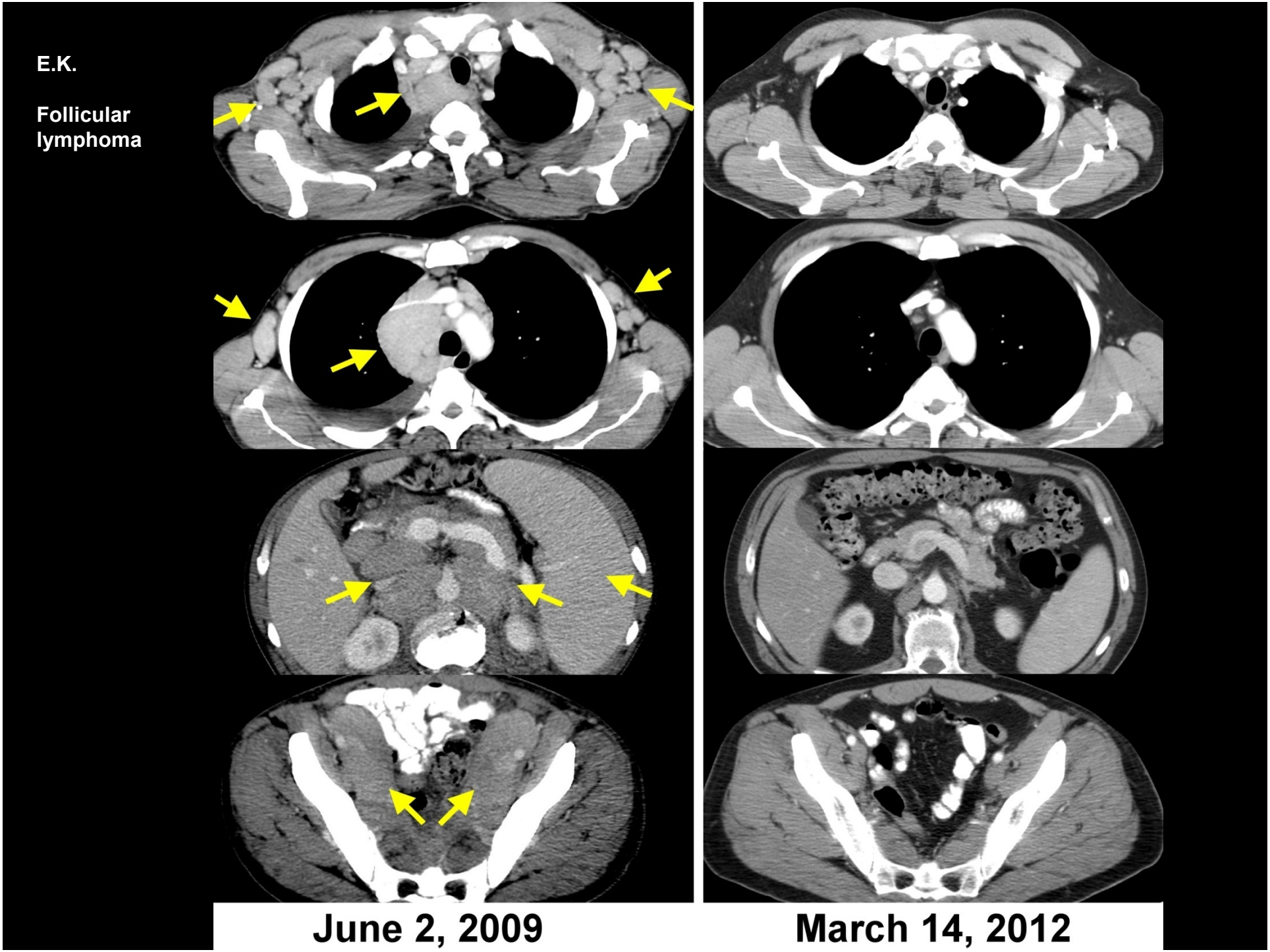
CD19 is expressed by mature B cells, B-cell precursors and plasma cells but not any other normal tissues.

T cells can be genetically engineered to express an anti-CD19 chimeric antigen receptor

We synthesized DNA encoding an anti-CD19 CAR and ligated it into the MSGV gammaretroviral backbone

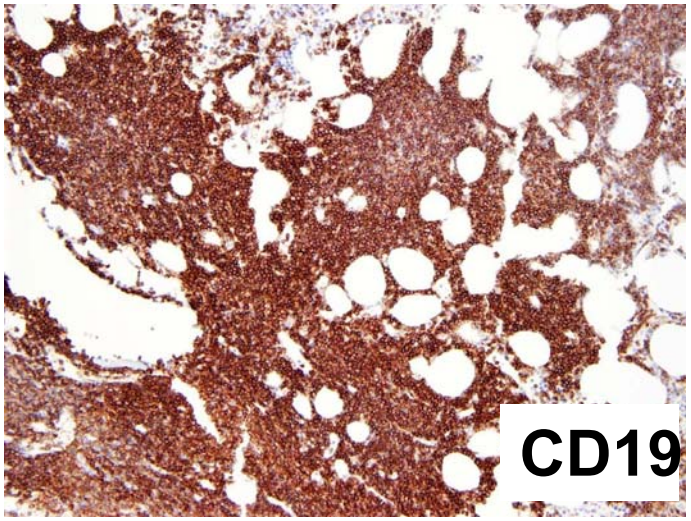
Anti-CD19 CAR

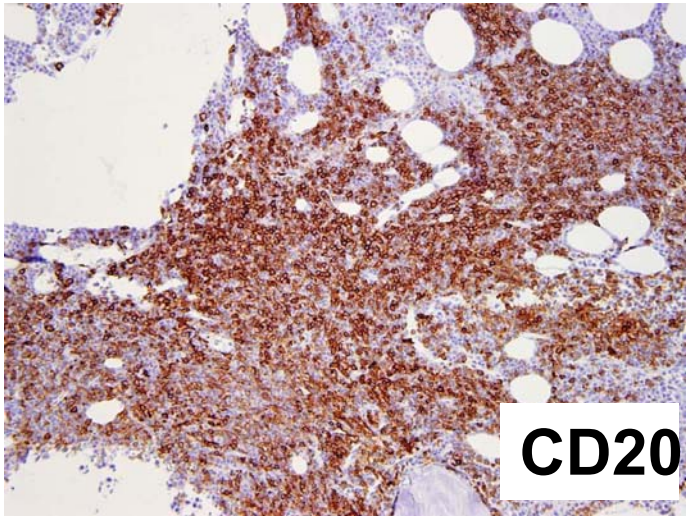
Retroviral supernatant for the trial was produced in the Surgery Branch Vector Production Facility


E.K.
Follicular lymphoma
June 2, 2009
March 14, 2012

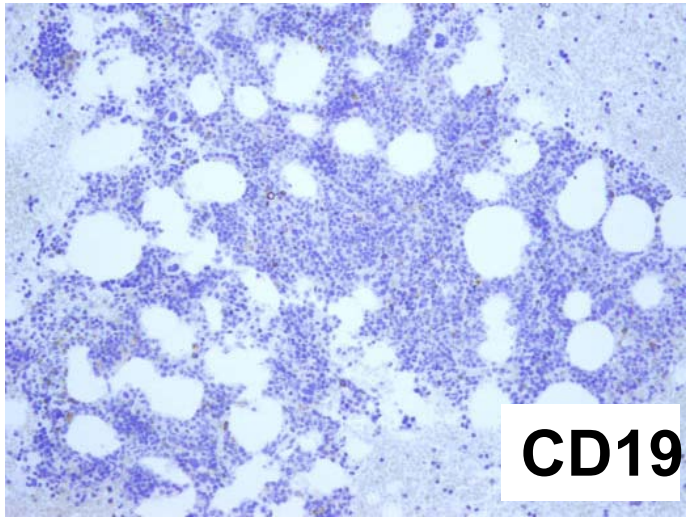
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment

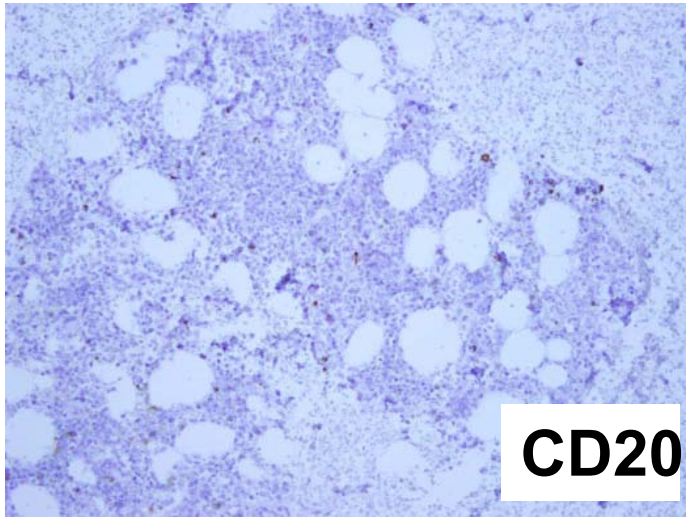
Before treatment



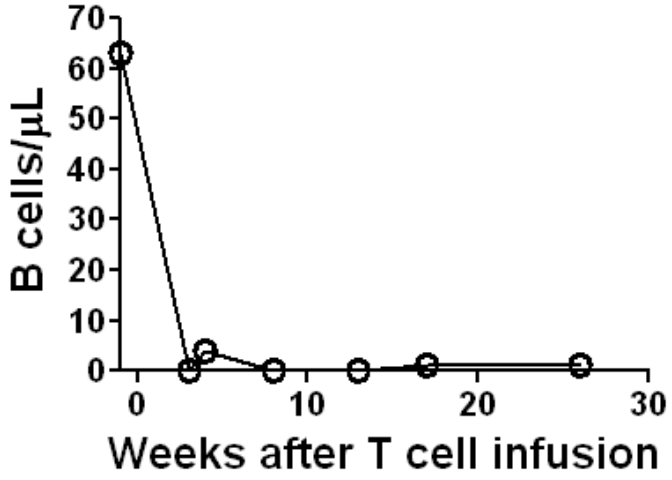


3 months after treatment

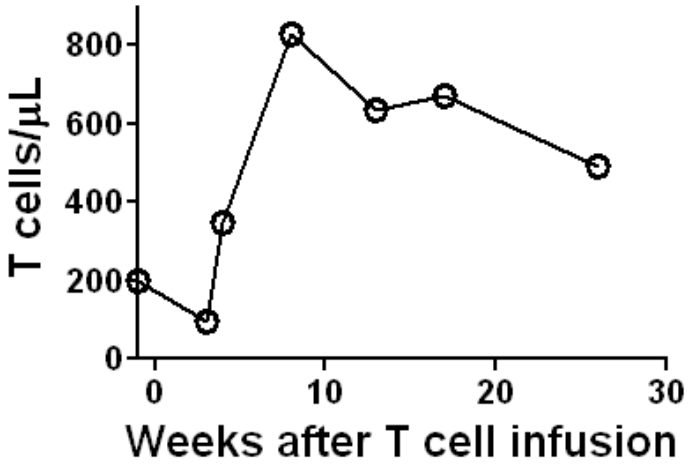


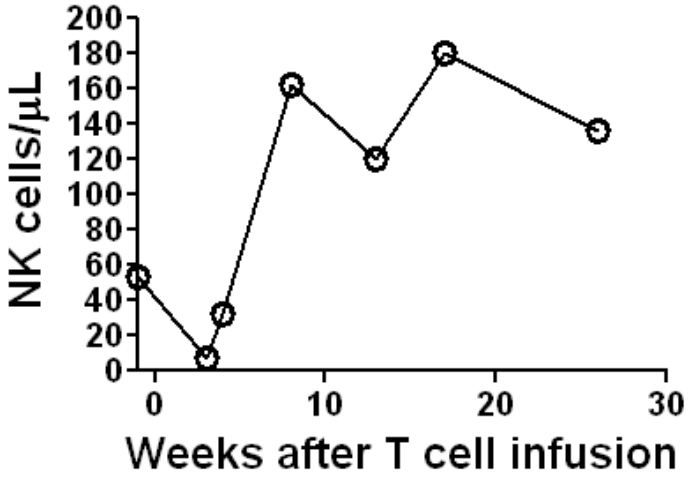


In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion



In contrast, T and NK cell counts rapidly recovered after treatment





Current anti-CD19 CAR protocol patient characteristics (4/13)

Patient	Age/sex	Disease	Number of prior therapies	Number of CAR+ cells infused/Kg	Response (duration in months)
1Ψ	56/M	Splenic Marginal Zone Lymphoma	4	$5x10^6$	PR (19+)
2*	43/F	DLBCL**	4	$5x10^6$	CR (16+)
3*	61/M	CLL (FR)	2	$4x10^6$	CR (14+)
4	30/F	DLBCL**	3	$2.5x10^6$	NE^^
5Ψ	63/M	CLL	4	$2.5x10^6$	PR (6+)
6*	48/M	CLL (FR)	1	$2.5x10^6$	CR (4+)
7	42/M	DLBCL**	4	$2.5x10^6$	CR (2+)
8	44/F	DLBCL	9	$2.5x10^6$	CR (2+)
9	38/M	DLBCL	3	$2.5x10^6$	TE

PR=partial remission, NE=not evaluable, CR=complete remission SD=stable disease FR=fludarabine-refractory

ΨPatient 1 and Patient 5 were previously treated with our original anti-CD19 CAR regimen

*Received two doses of 30 mg/kg cyclophosphamide due to low platelet count

^^Patient died before one month staging. DLBCL, diffuse large B-cell lymphoma; **Chemo-refractory

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Cancer/Testes Antigens - Shared Tumor Specific Antigens

Expressed during fetal development

Restricted in their expression in adult normal tissues to germ cells

Up-regulated in 10-80% of cancers from multiple tissues

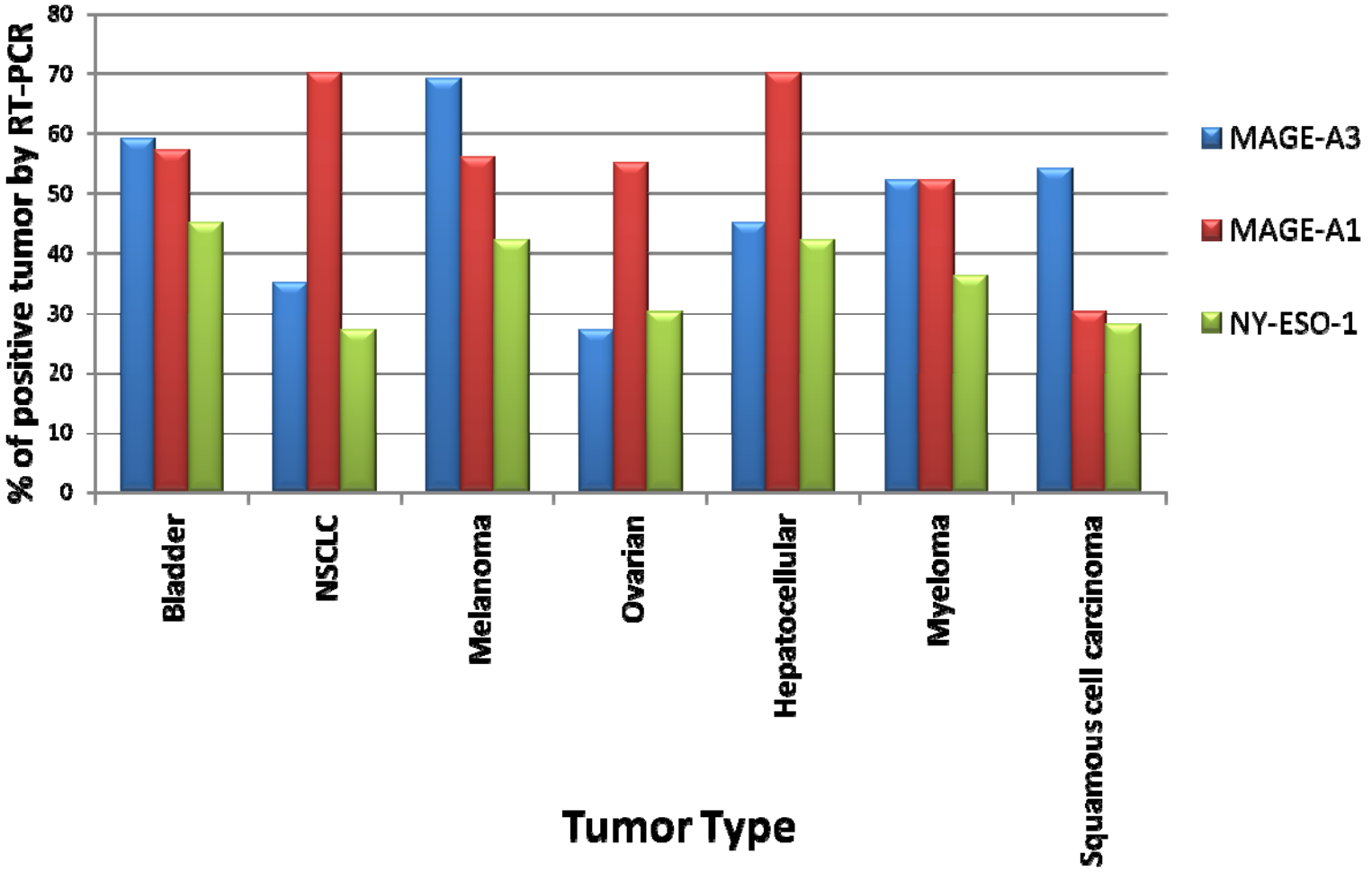
NY-ESO-1 Family

- **Small family of X-linked genes that includes NY-ESO-1 and LAGE-1**

MAGE Family

- **Family of ~ 45 X-linked genes**

Cancer/Testis Antigens Expressed in Multiple Tumor Types



Responses to Therapy with NY-ESO-1 TCR (2/1/13)

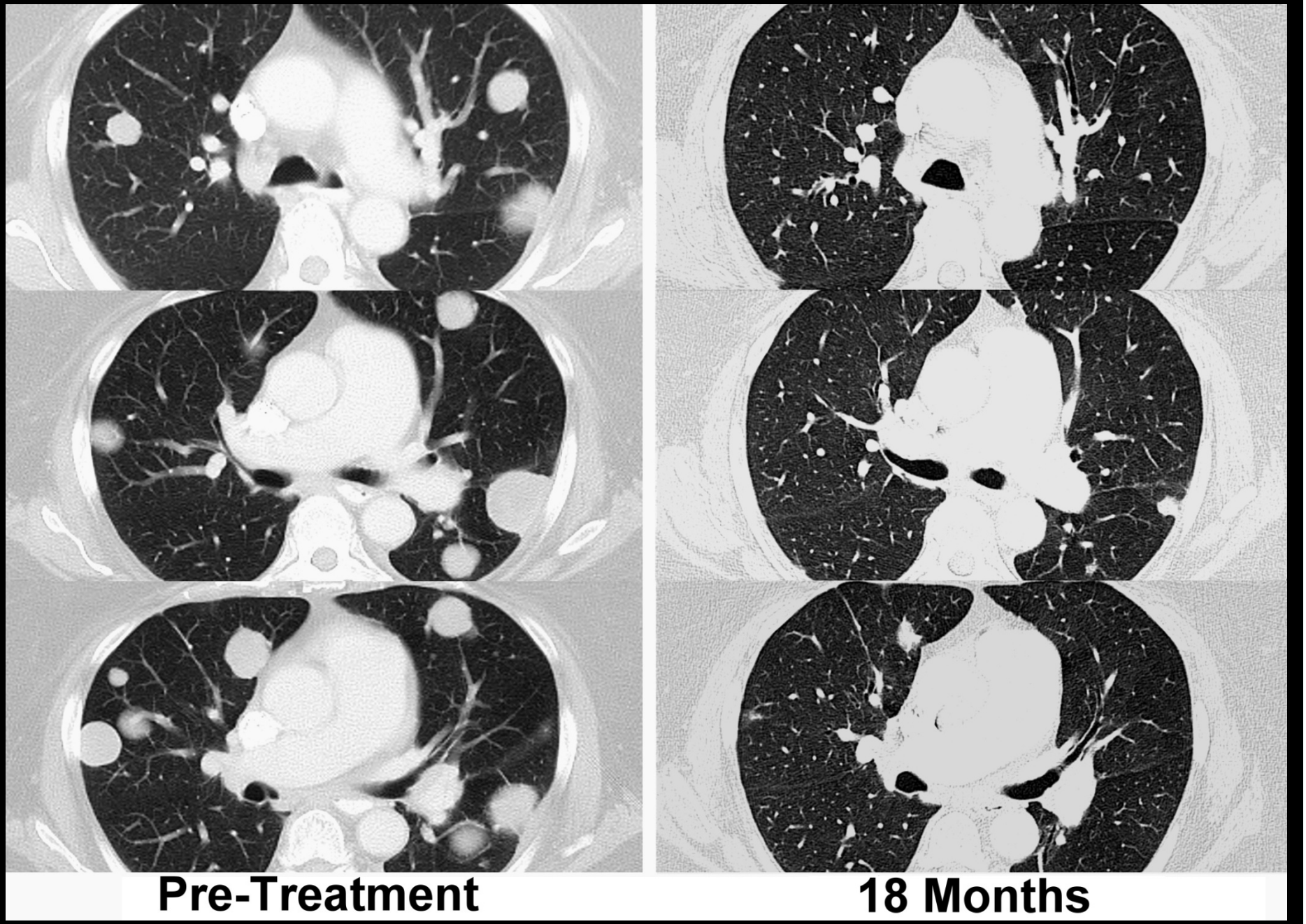
	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	18	5 (28%) (18+,10**, 8, 4, 3)	4 (22%) (48+, 37+, 25, 21+**)	9 (50%)
Synovial Cell Sarcoma	16	10 (63%) (29+**,14*, 12**,10, 8, 6+, 5, 4, 3**,2+)	0	10 (63%)

*treated twice

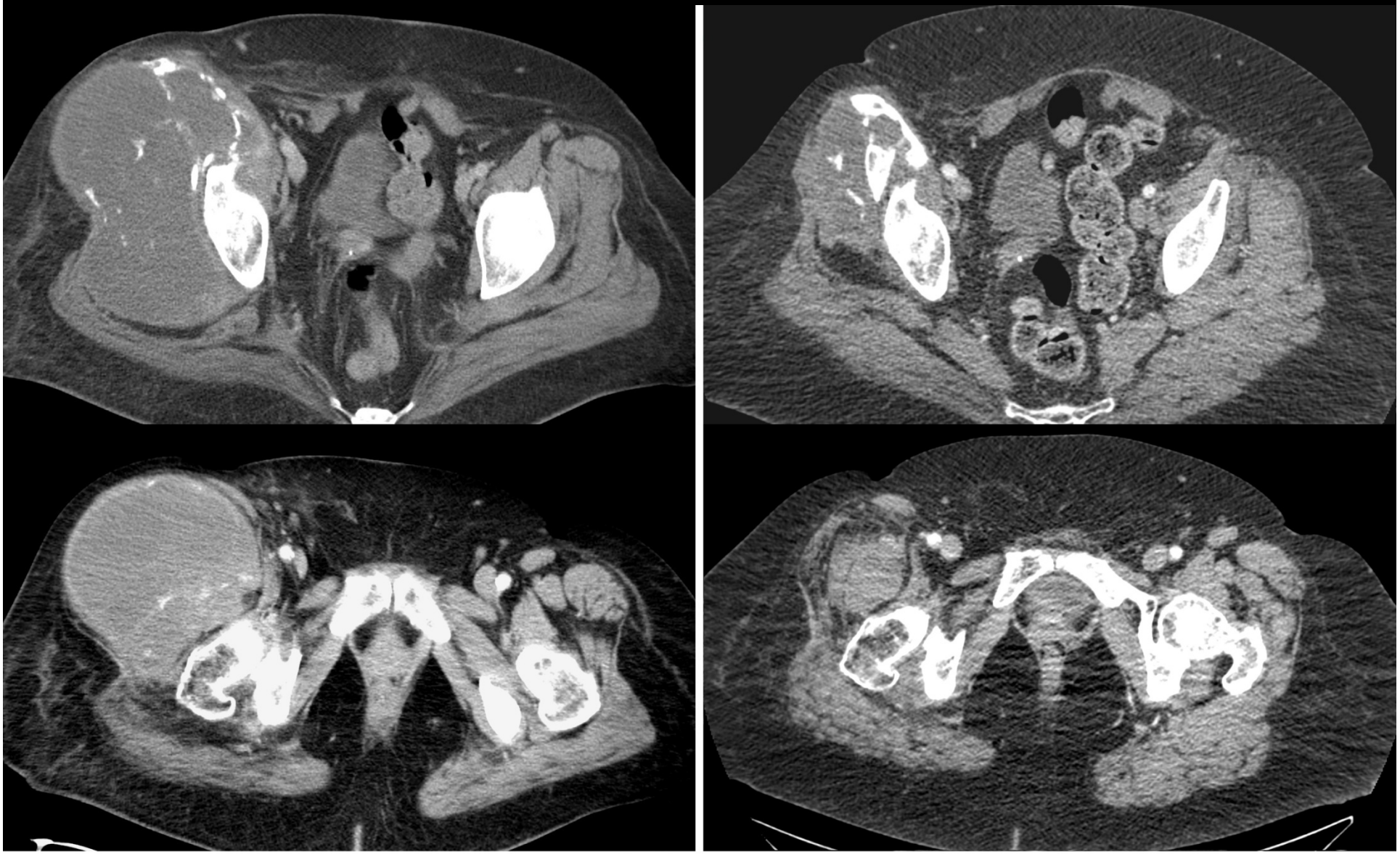
**plus ALVAC vaccine

(Robbins et al J Clin Oncol 29:917-924, 2011)

A.R. Synovial cell sarcoma NY-ESO-1 TCR



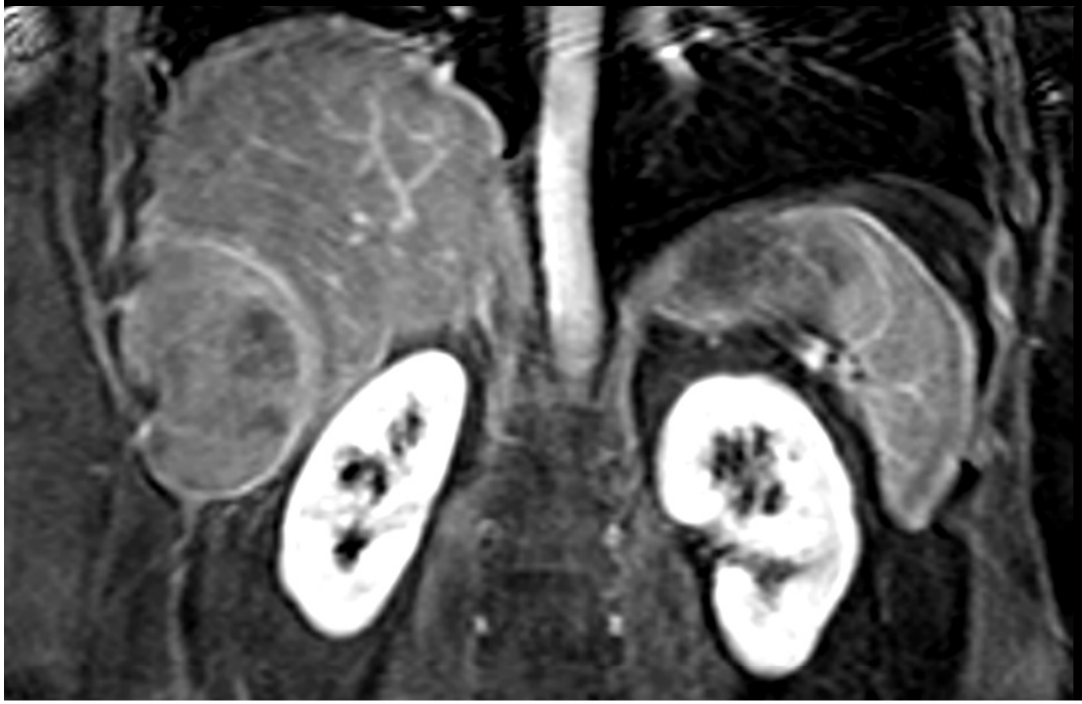
A.R. Synovial cell sarcoma NY-ESO-1 TCR



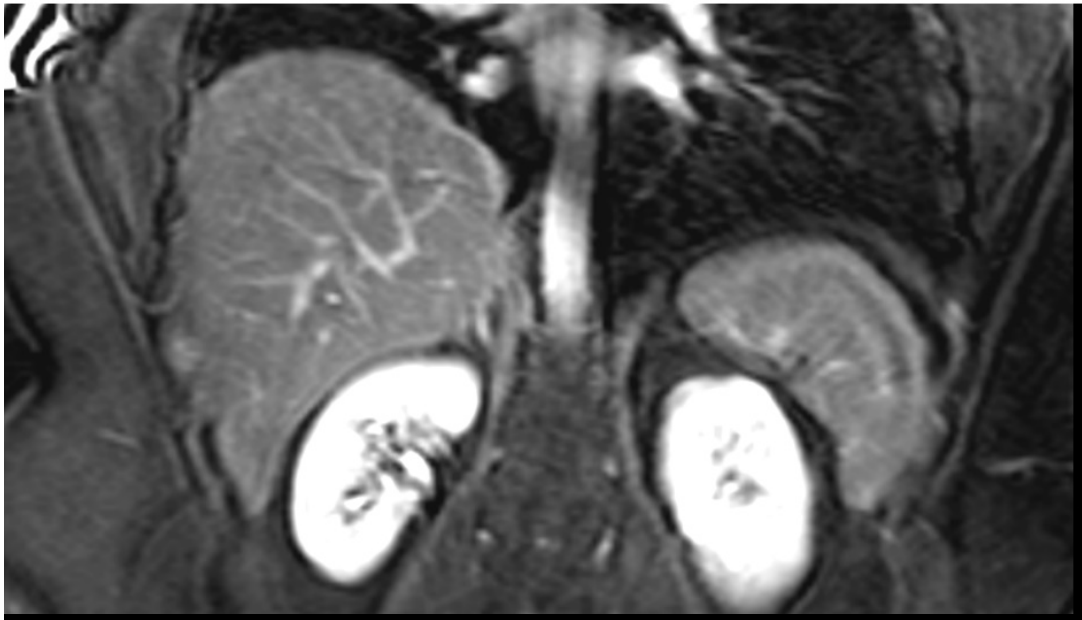
Pre-Treatment

18 Months

M.M. Synovial cell sarcoma ESO TCR



Pre-Treatment

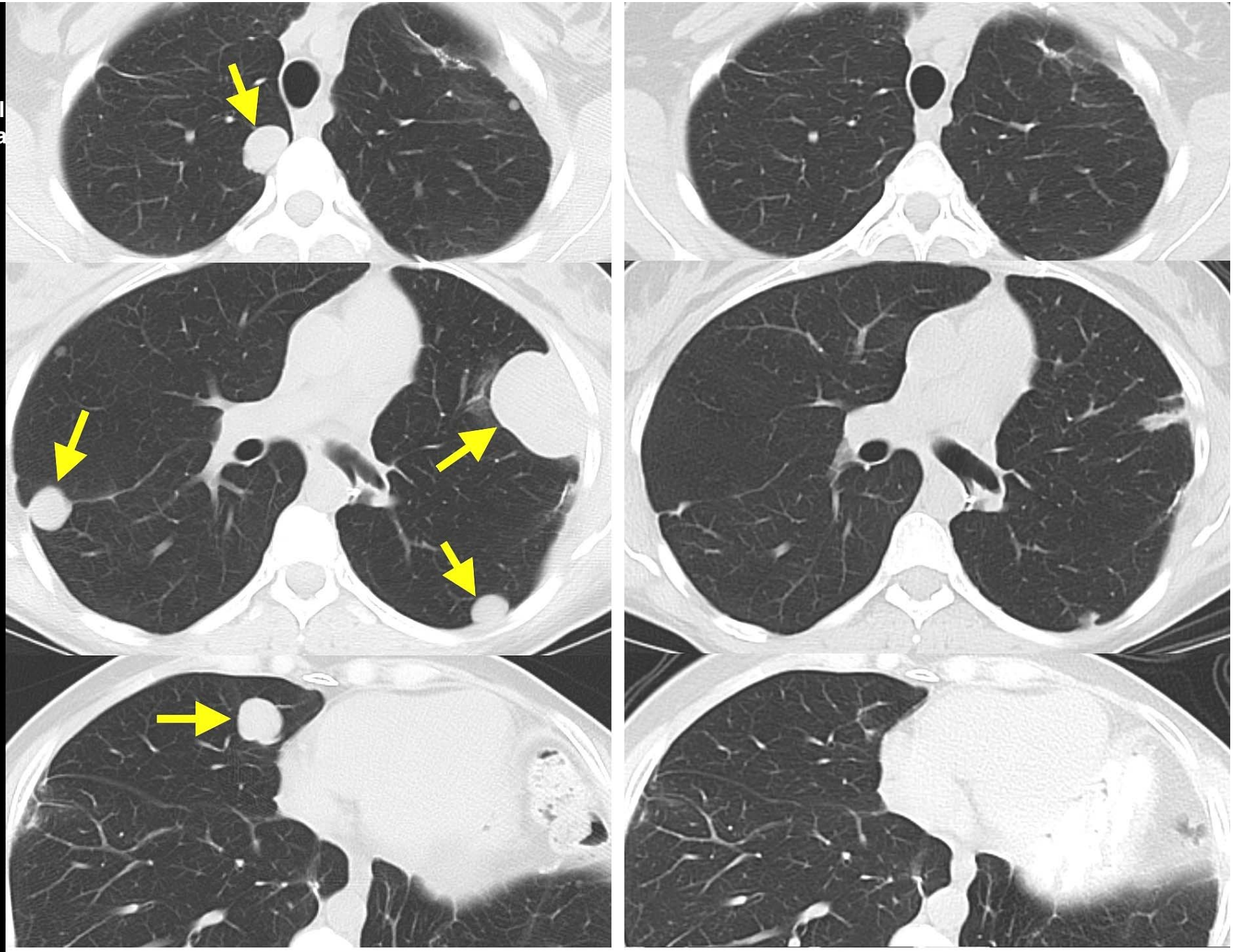


6 Months

H.K.

Synovial Sarcoma

ESO TCR



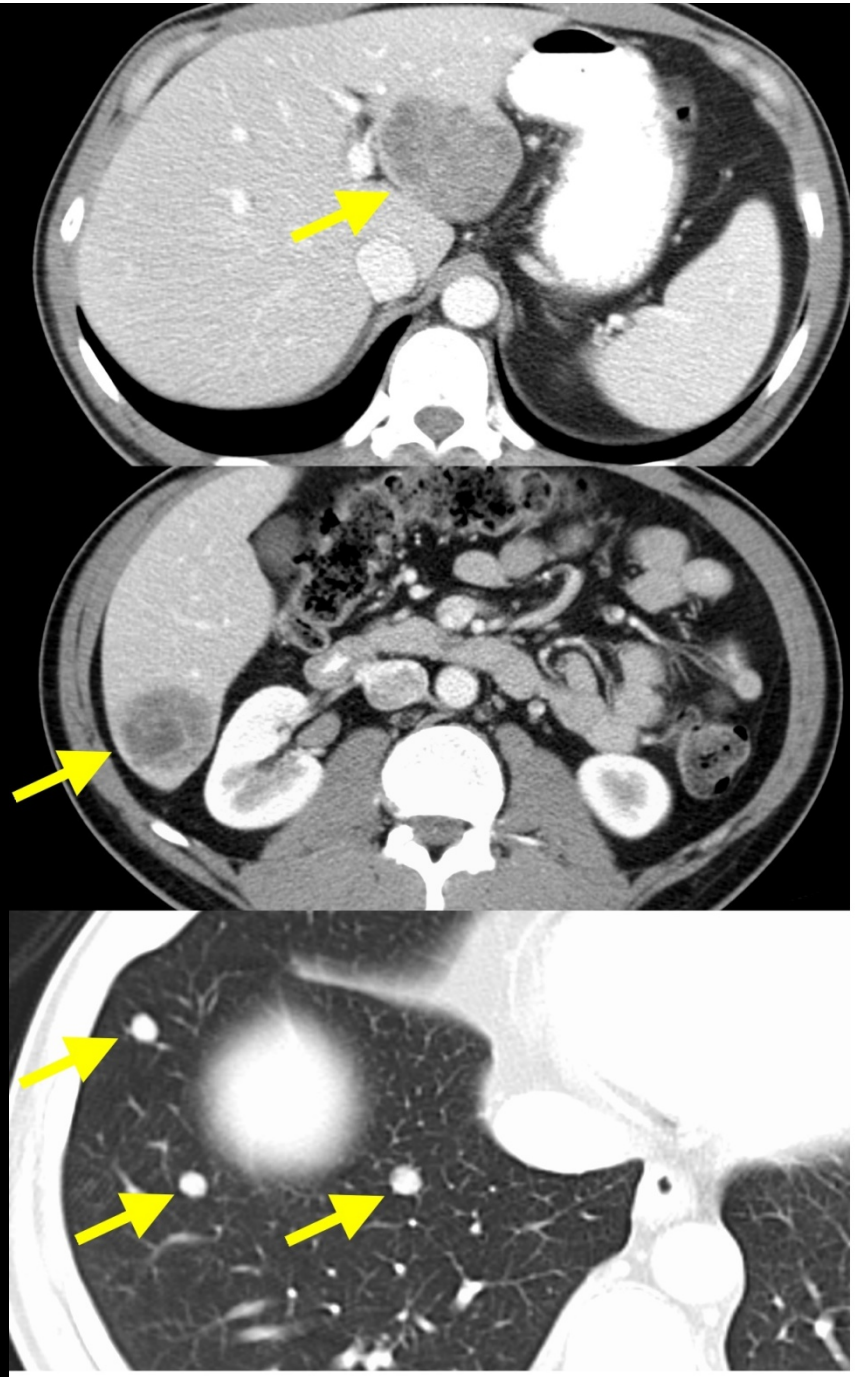
Pre-Treatment

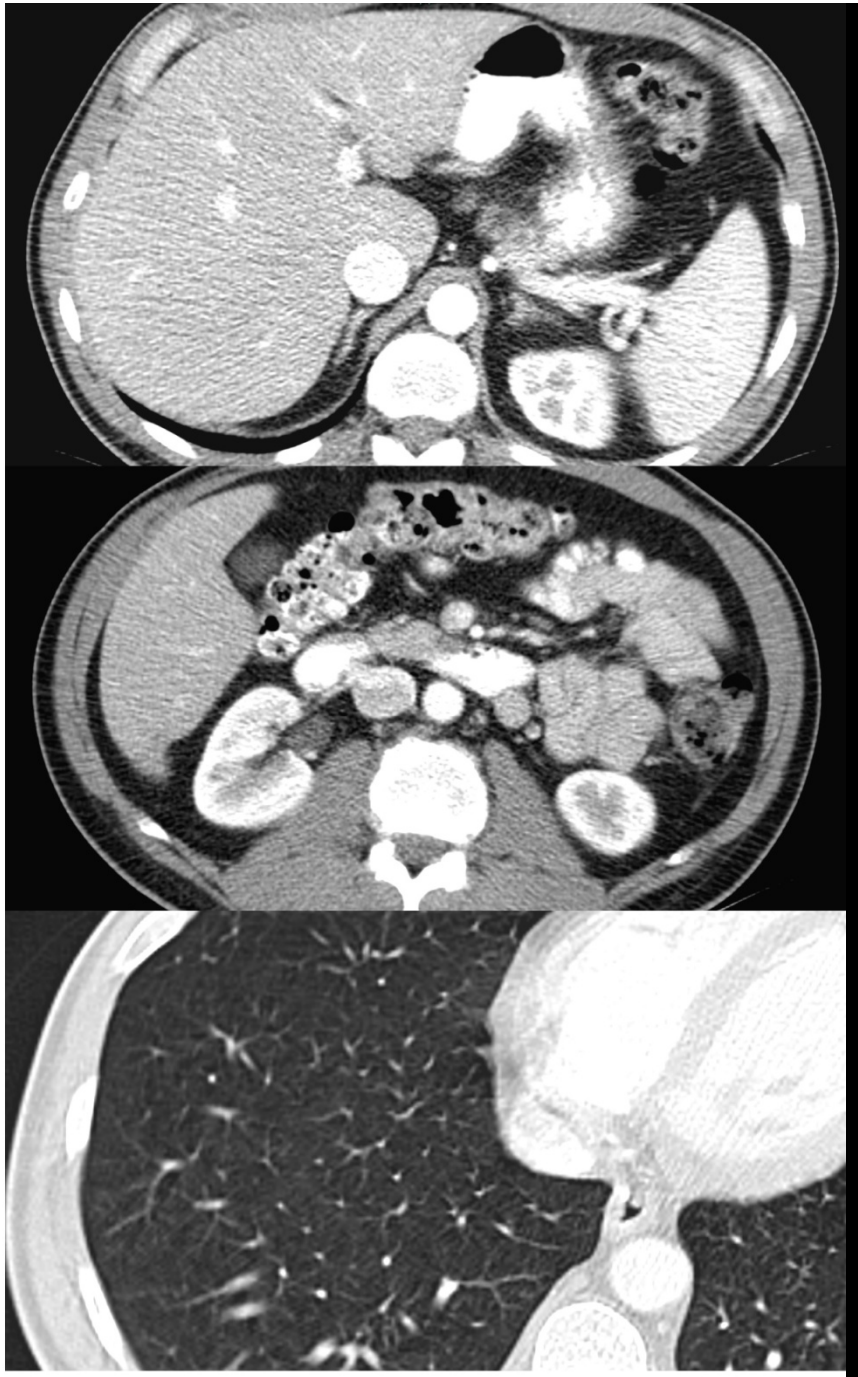
14 Months

D.C.

ESO TCR

Melanoma





December 2009

March 2012

Patients on MAGE-A3 TCR Protocol (F/U 3/1/12)

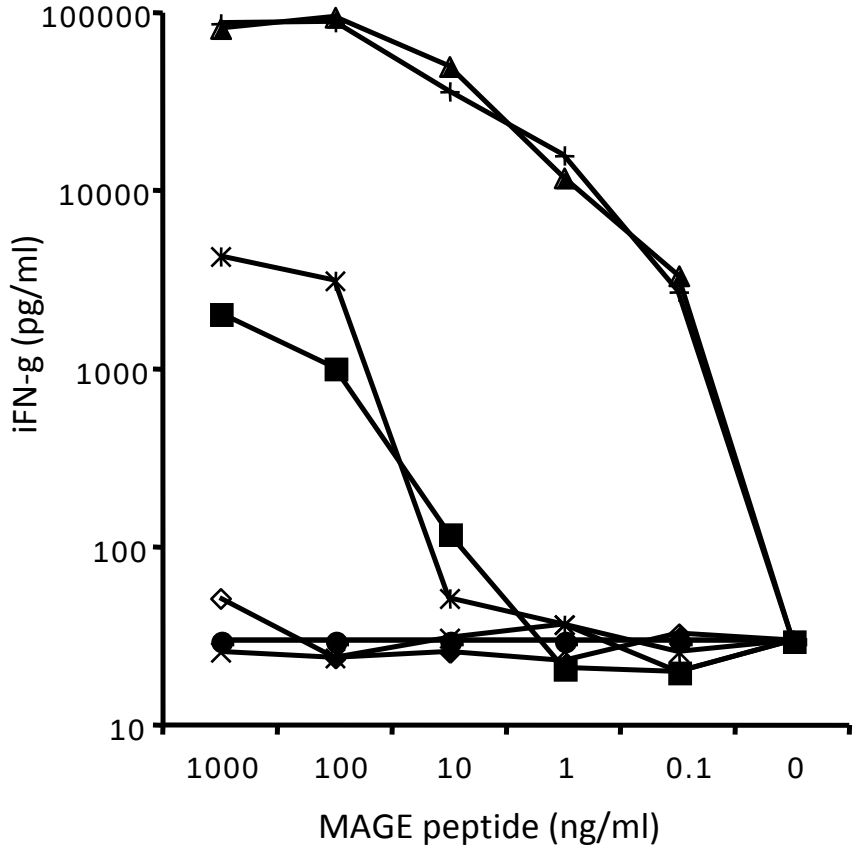
	Patient		Diagnosis	Date of cells	# cells ($x10^{-9}$)	#IL-2 doses	Response	Neurologic
1.	L.A.	59	Melanoma	2/24/11	28	6	CR(12+)	None
2.	J.P.	38	Melanoma	3/24/11	30	5	NR	None
3.	P.M.	56	Melanoma	5/5/11	30	7	PR(4)	None
4.	K.H.	21	Synovial Sarc.	6/10/11	41	1	PR(5)	None
5.	M.S.	54	Melanoma	7/22/11	79	5	PR(4)	Coma (white matter)
6.	J.M.	44	Melanoma	8/5/11	53	4	NR	None
7.	F.B.	62	Melanoma	8/17/11	62	6	CR(6+)	Seizure (normal MRI; recovered completely)
8.	G.T.	71	Esophageal	8/18/11	61	1	NR	Coma (white matter)
9.	J.S.	62	Melanoma	8/31/11	30	0	NR	TIA (Normal MRI; recovered completely)

Can this MAGE-A3 TCR recognize other related MAGE peptides?

MAGE peptides

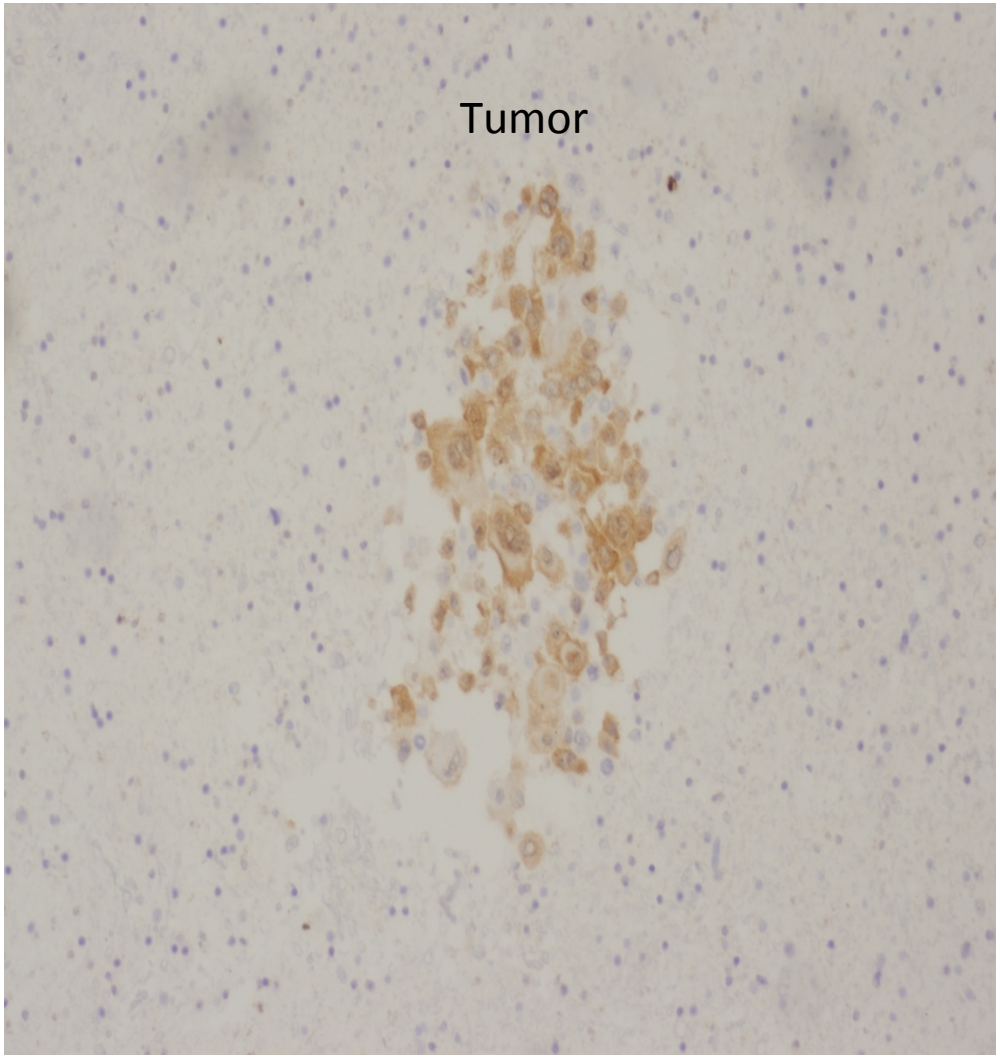
MAGE A1	KVA**D**LV**G**FL	
MAGE A2	K**MV**ELVHFL	√
MAGE A3	KVAELVHFL	
MAGE A4	KV**D**EL**A**HFL	
MAGE A6	KVA**K**LVHFL	√
MAGE A8	KVAELV**R**FL	
MAGE A12	K**M**AELVHFL	
MAGE C2	KVAELV**E**FL	

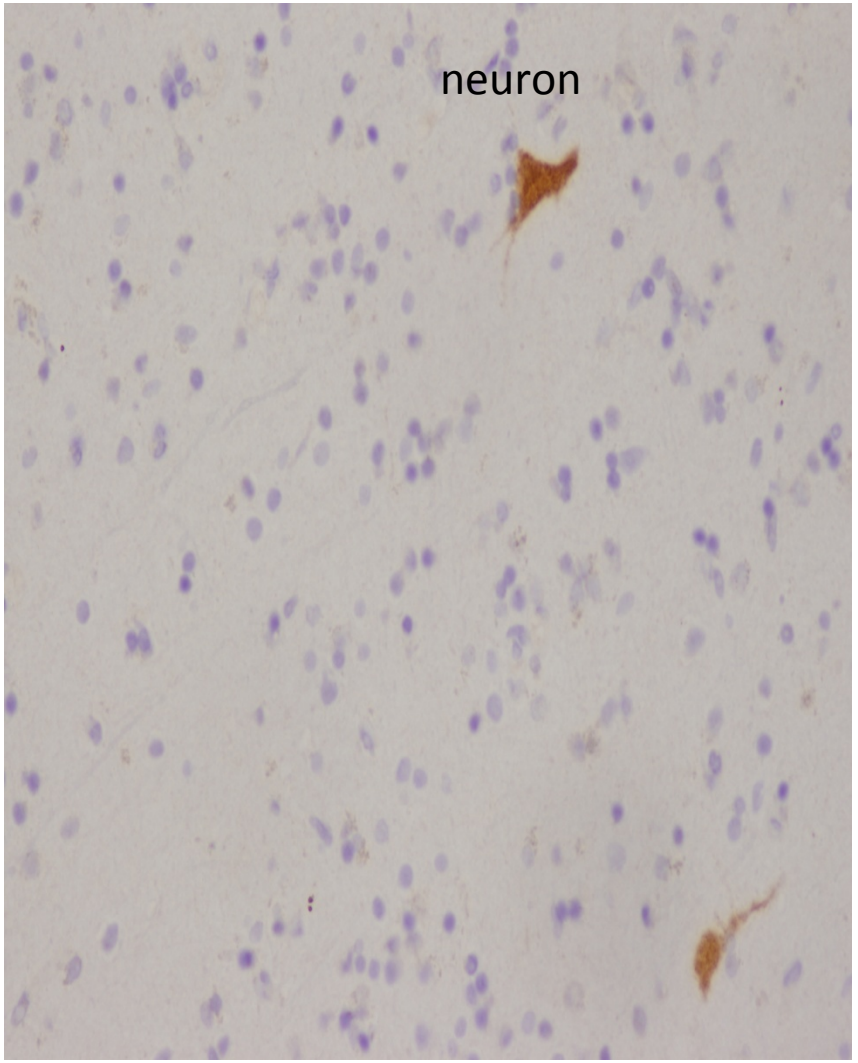
T2 cell co-culture assay



MAGE-A3 TCR transduced PBL also recognized MAGE-A12 peptide efficiently and MAGE-A2 and A6 peptides at higher concentrations

Autopsy tissue from an esophageal cancer patient showing MAGE-A staining





11-43; metastatic poorly differentiated SCC (positive for MAGE); neurons nearby are also positive for MAGE

POINTS TO CONSIDER IN IMMUNOTHERAPY TRIAL DESIGN

Even if the target is not on the tumor cell, cross-reactive antigens can lead to substantial toxicity.

A critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

---

1. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

3. Shared antigens unique to cancer (cancer-testes antigens)

4. Mutations unique to each cancer (EGFRvIII)

5. Critical components of the tumor stroma (VEGFR2, FAP)

EGFRvIII Activating Mutation is an Excellent Target for the Treatment of Glioblastoma

---

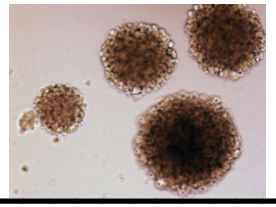
Expressed in 30-50% of glioblastomas

Not expressed in normal tissues

Likely essential for the malignant phenotype so loss variants are unlikely

Highly specific antibodies that recognize EGFRvIII are available to produce CAR for use in cell transfer therapy

Recognition of Glioblastoma by T-cells Expressing an anti-EGFRvIII Chimeric Antigen Receptor

		Targets		Glioblastoma Stem Cell Lines*		
Transduction	Media	U251 EGFRwt	U251 EGFRvIII	1228 	308	882
			(pg/ml IFN-g)			
None	0	0	0	0	0	80
GFP	0	0	0	0	0	180
EGFRvIII CAR	384	331	**4523**	**3306**	**3351**	**4406**

*All lines express EGFRvIII

(R. Morgan, H. Fine et al)

Phase I dose escalation trial in patients with recurrent glioblastoma (collaboration with Neurooncology Branch, NCI)

Two groups:

a) receiving steroids

b) no steroids

Escalation cohorts: 1 patient per cohort (1st three cohorts) unless DLT; then 3 patients per cohort

Dose Escalation Schedule

Dose Level	**Dose of Anti-EGFRvIII CAR T cells**	
Cohort 1 (group a & b)	10^7	1 patient **(5/16/12)**
Cohort 2 (group a & b)	$3x10^7$	1 patient
Cohort 3 (group a & b)	10^8	1 patient
Cohort 4 (group a & b)	$3x10^8$	3 patients
Cohort 5 (group a & b)	10^9	3 patients
Cohort 6 (group a & b)	$3x10^9$	3 patients
Cohort 7 (group a & b)	10^{10}	3 patients
Cohort 8 (group a & b)	$3 – 6 \times 10^{10}$	3 patients

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3. Shared antigens unique to cancer (cancer-testes antigens)

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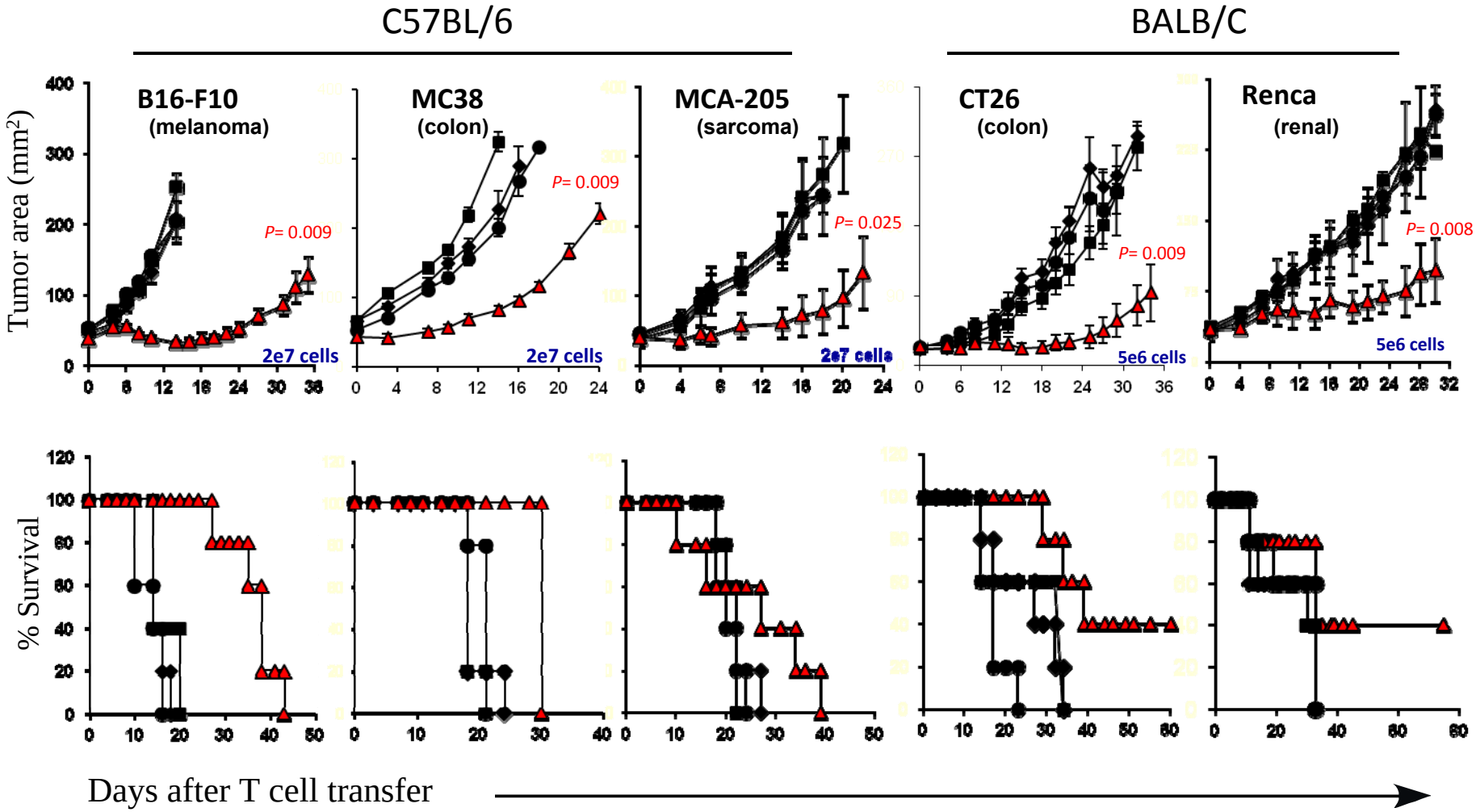
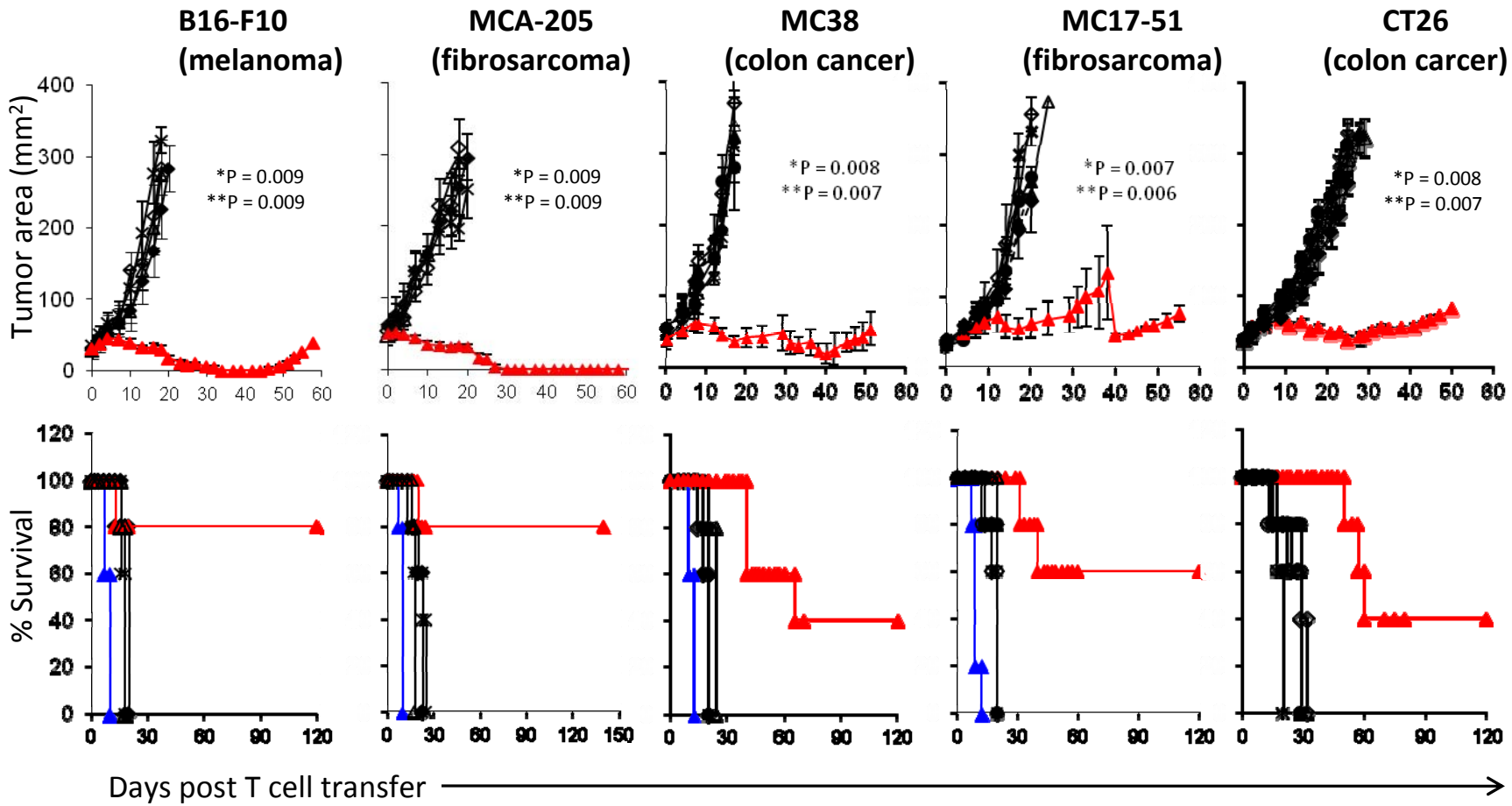
5. Critical components of the tumor stroma (VEGFR2, FAP)

Adoptively transferred VEGFR-2 CAR engineered syngeneic T cells induced regression of multiple established solid tumors in two strains of mice



(D. Chinnasamy et al , J Clin Invest 120:3953, 2010)

Anti-VEGFR2 CAR and IL-12 cotransduced mouse T cells induced regression of multiple types of vascularized tumors *in mice* without exogenous IL-2 administration



P values: * DC101 CAR/Flexi-IL12 vs no treatment group; ** DC101 CAR/Flexi-IL12 vs DC101 CAR alone

(D. Chinnasamy et al, Clin Cancer Res 18:1672-83, 2012)

Program for the Application of Cell Transfer Therapy to a Wide Variety of Human Cancers

Receptor	Type	Cancers	Status
MART-1	TCR	Melanoma	Closed
gp100	TCR	Melanoma	Closed
NY-ESO-1	TCR	Epithelial & Sarcomas	Accruing
CEA	TCR	Colorectal	Closed
CD19	CAR	Lymphomas	Accruing
VEGFR2	CAR	All cancers	Accruing
2G-1	TCR	Kidney	Accruing
IL-12	Cytokine	Adjuvant for all receptors	Accruing
MAGE-A3*	TCR	Epithelial	in development
EGFRvIII	CAR	Glioblastoma	Accruing
SSX-2	TCR	Epithelial	in development
Mesothelin	CAR	Pancreas & mesothelioma	Accruing
CSP4 (HMWAg)	CAR	Melanoma, Tnbreast, Panc	in development

*(MAGE-A3 TCRs; restricted by HLA-A2, A1, Cw7, DP4 – covers 80% of patients)

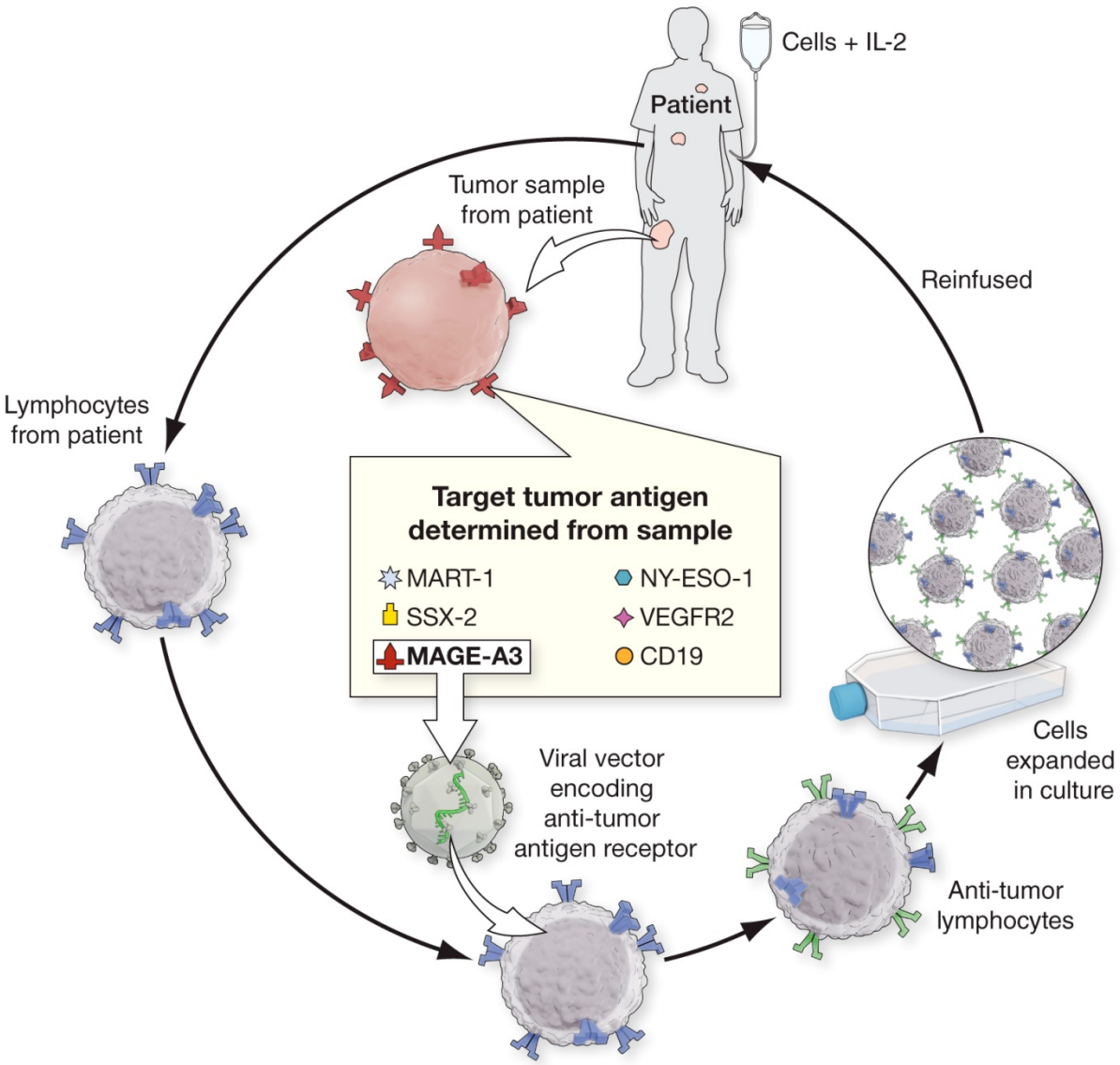
Conclusion

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.

Personalized immunotherapy using anti-tumor receptor gene-modified lymphocytes



The adoptive transfer of anti-tumor T cells can mediate the durable complete regression of large, invasive metastatic cancers in patients with metastatic melanoma.

All tumor sites are susceptible (including brain)

No relationship between the bulk of tumor and the likelihood of achieving a complete response

Prior treatment has no impact on the likelihood of response

Cells with high proliferative potential (long telomeres and less differentiated phenotype) and cells with long in vivo persistence are associated with objective responses

A critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

---

1. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

2. Shared antigens unique to cancer (cancer-testes antigens)

3. Mutations unique to each cancer (EGFRvIII)

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5. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

Melanoma/Melanocyte Differentiation Antigens

1994 MART-1 and gp100 cloned by Kawakami et al.

1996 TRP-2 cloned by Wang et al.

---

16 of 36 (42%) patients treated with PBL transduced with high-affinity anti-MART-1 or gp100 had eye and/or ear toxicity (25% objective response rate)

1 of 93 patients treated with TIL had severe eye and ear toxicity

These M/M antigens do not appear responsible for tumor rejection

Expression cloning of antigens recognized by TIL responders (since 1994) have identified 8 mutated antigens restricted by Class I

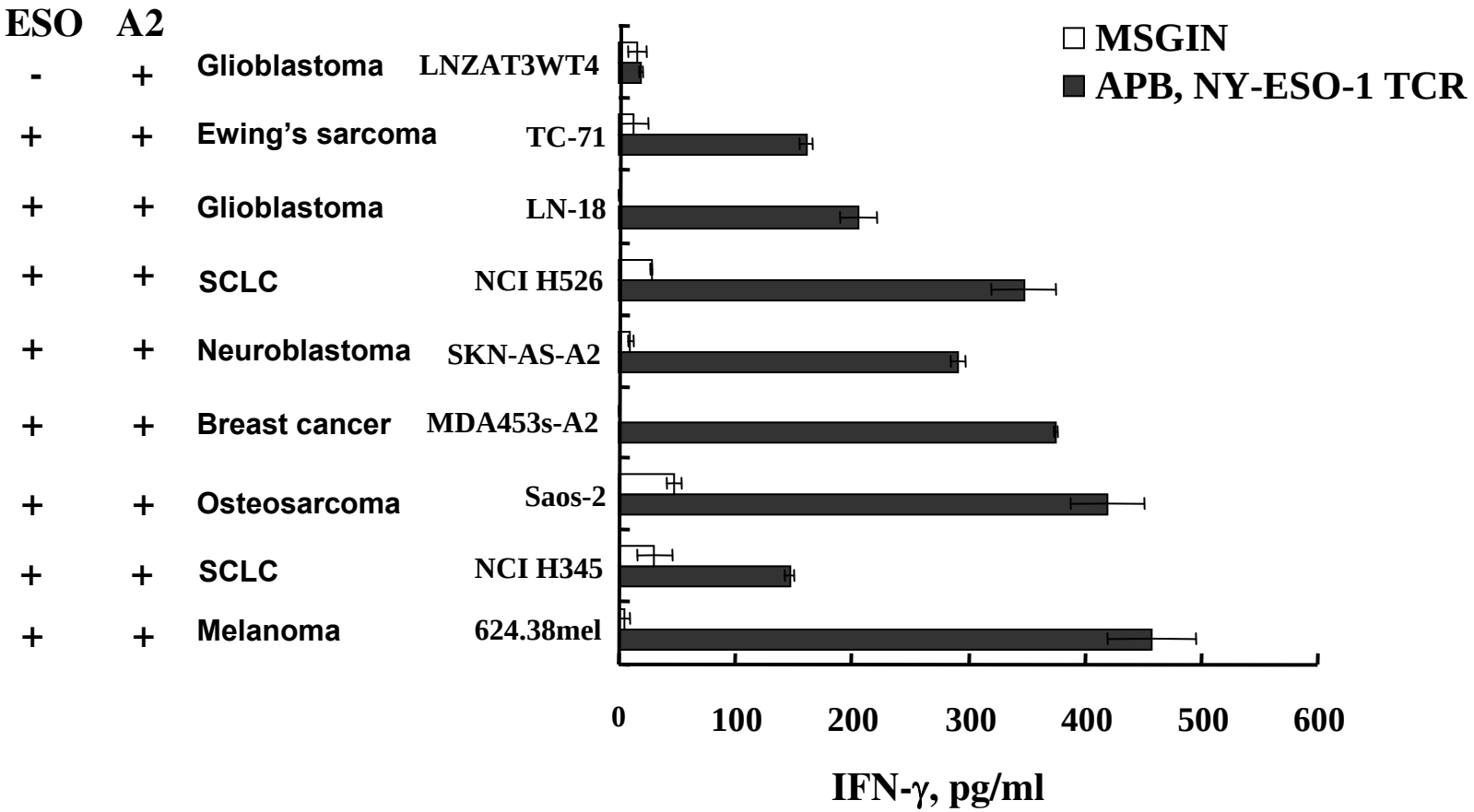
Patient A.R.

65 year old female with metastatic synovial sarcoma

Oct. 2008	**resection of primary sarcoma right iliac wing**
Nov. 2008	**3 cycles ifosphamide**
Feb. 2009	**2 cycles ifosphamide, doxorubicin**
April 2009	**ifosphamide**
Aug. 2009	**gemcitabine, taxotere**
Nov. 2009	**sorafenib**
Dec. 2009	**radiation therapy right hip**
Aug. 2010	**To NCI; autologous cells transduced with anti-NY-ESO-1 T cell receptor**

(Ongoing response as of Feb. 2013 30+ months)

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



Treatment of Refractory B-cell Lymphomas and Chronic Lymphocytic Leukemia with anti-DC19 CAR (3/13)

		PR	CR	Total
		numbers (duration in months)		
Lymphoma	8	3 (37.5%) (42*,29+*,25+)	3 (37.5%) (15+,1+,1+)	6 (75%)
Chronic lymphocytic leukemia	6	2 (33%) (25+*,6)	3 (50%) (25+,13+,1+)	5 (83%)

*treated twice

Patient E.K.

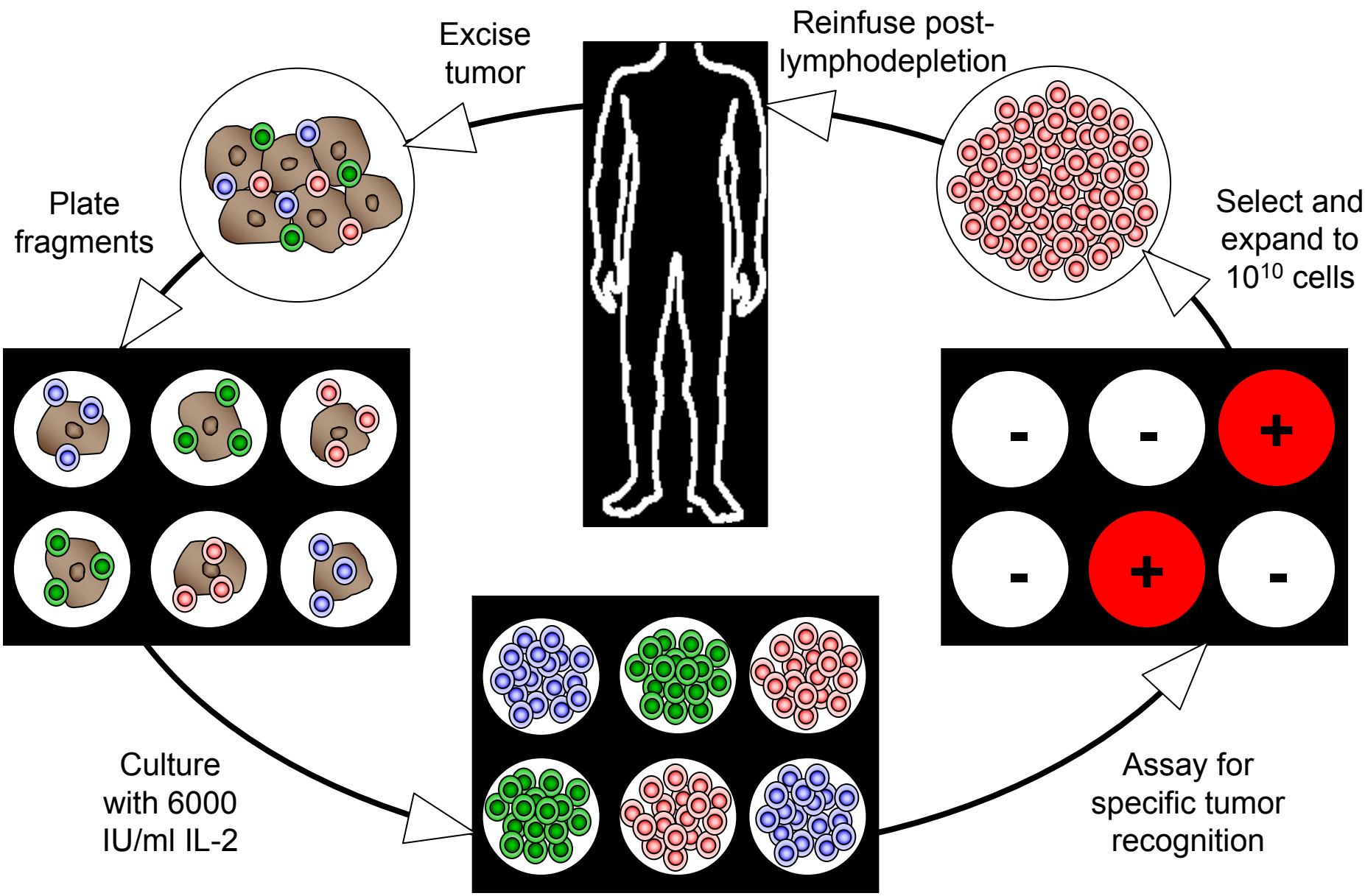
48 year old male with follicular non-Hodgkin lymphoma

Aug. 2002	**diagnosed with stage IV lymphoma** **7 cycles PACE chemotherapy (cisplatin, doxorubicin, cyclophosphamide, etoposide)**
April 2004	**idiotypic/KLH vaccine (5 doses)**
Sept. 2007	**ipilimumab**
Nov. 2007	**6 cycles EPOCH-R chemotherapy (etoposide, predisone, vincristine, cyclophosphamine, rituximab)**
May 2009	**To NCI for treatment with autologous anti-CD19 CAR transduced T cells**

In ongoing regression as of November 2012 (42+ months).

(Blood 116:3875-86, 2010; 119:2709-20, 2012)

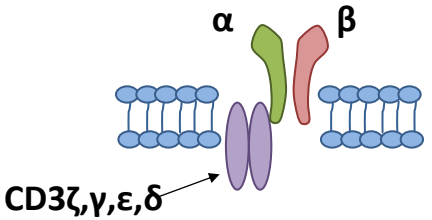
Adoptive transfer of tumor infiltrating lymphocytes (TIL)



Construction of T-cell Receptors (TCR) and Chimeric Antigen Receptors (CAR)

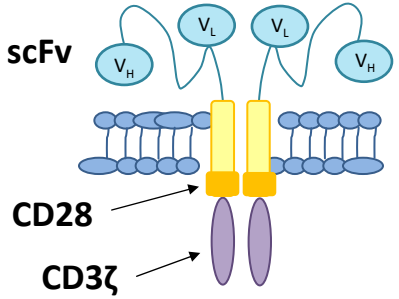
TCR Vector (eg, MART1, NY-ESO)

TCR receptor



CAR Vector (eg, CD19)

CAR receptor



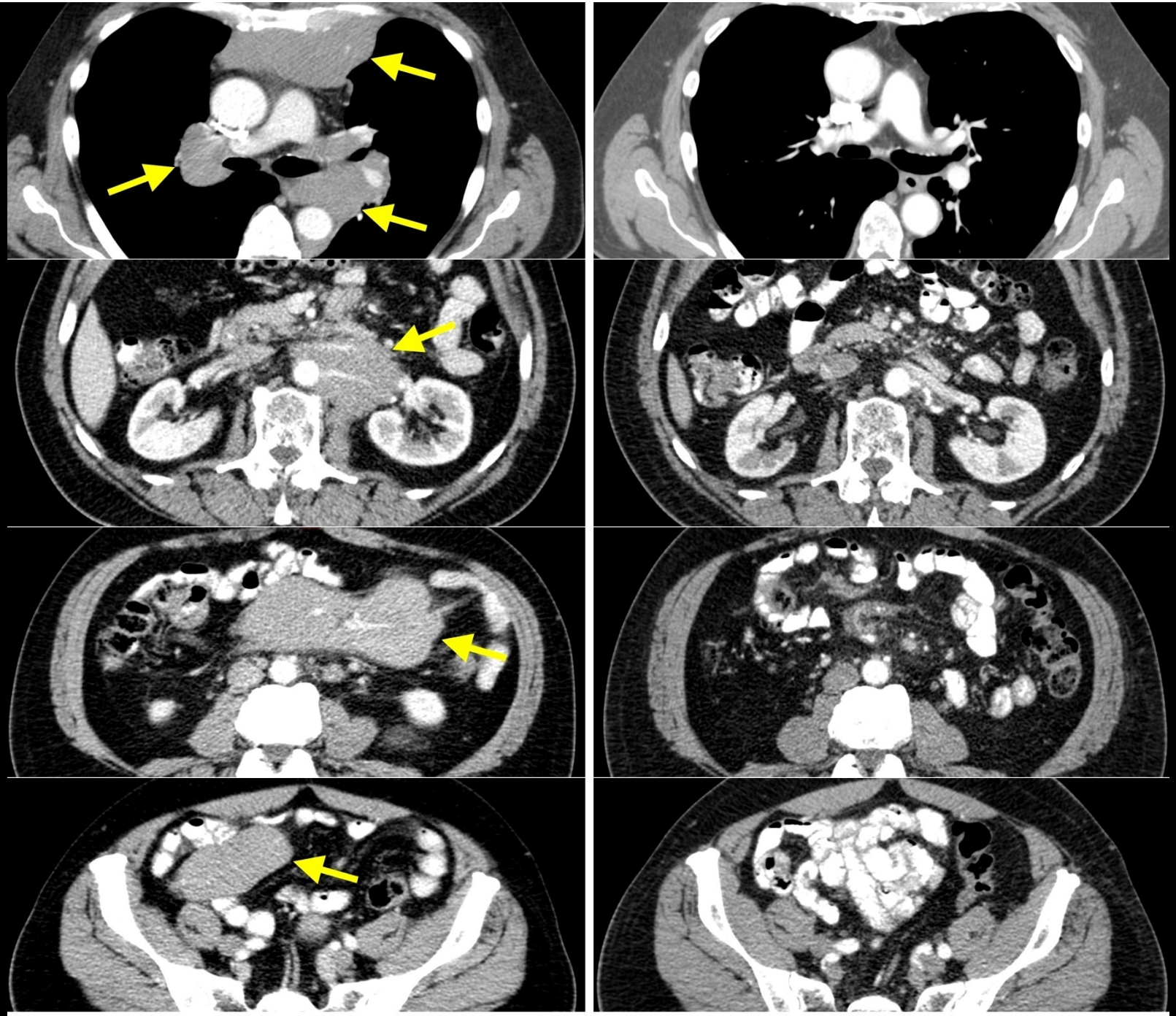
Melanoma is the only cancer that easily and reproducibly gives rise to tumor infiltrating lymphocytes with anti-tumor activity.

Thus, to use cell transfer therapies against other cancer types, anti-tumor T cells must be generated in vitro by genetic engineering to insert either conventional alpha-beta T cell receptors (TCR) or chimeric antigen receptors (CAR)

Patient	Age/ Sex	Dx	Sites of Disease	#Cells	Pre-Rx CEA (μg/L)	% CD8 CD4	% TCR+	T2+ HBVc: 18	T2+ CEA: 691	SW480 A2+ CEA-	SW620 A2+ CEA-	H508 A2+ CEA+	SW1463 A2+ CEA+	SW480 A2+ CEA-	H508 A2+ CEA+
								IFNγ (pg/ml)						IFNγ ELISPOTS	
1	55/M	Rectal Ca	Adrenal, Lung	$4x10^8$	281	29.7 71.0	79.2	109	44786	37	67	1529	10124	5	3700
2	43/M	Rectal Ca	Lung, LN	$2x10^8$	865	63.7 34.2	89.8	435	54782	58	72	8731	6718	34	5220
3	52/M	Colon Ca	Liver, LN, Lung	$2x10^8$	226	64.3 33.6	90.1	59	22527	21	40	10292	5848	130	7900

D.G.

Follicular lymphoma



Pre-Treatment

14 Months

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+		

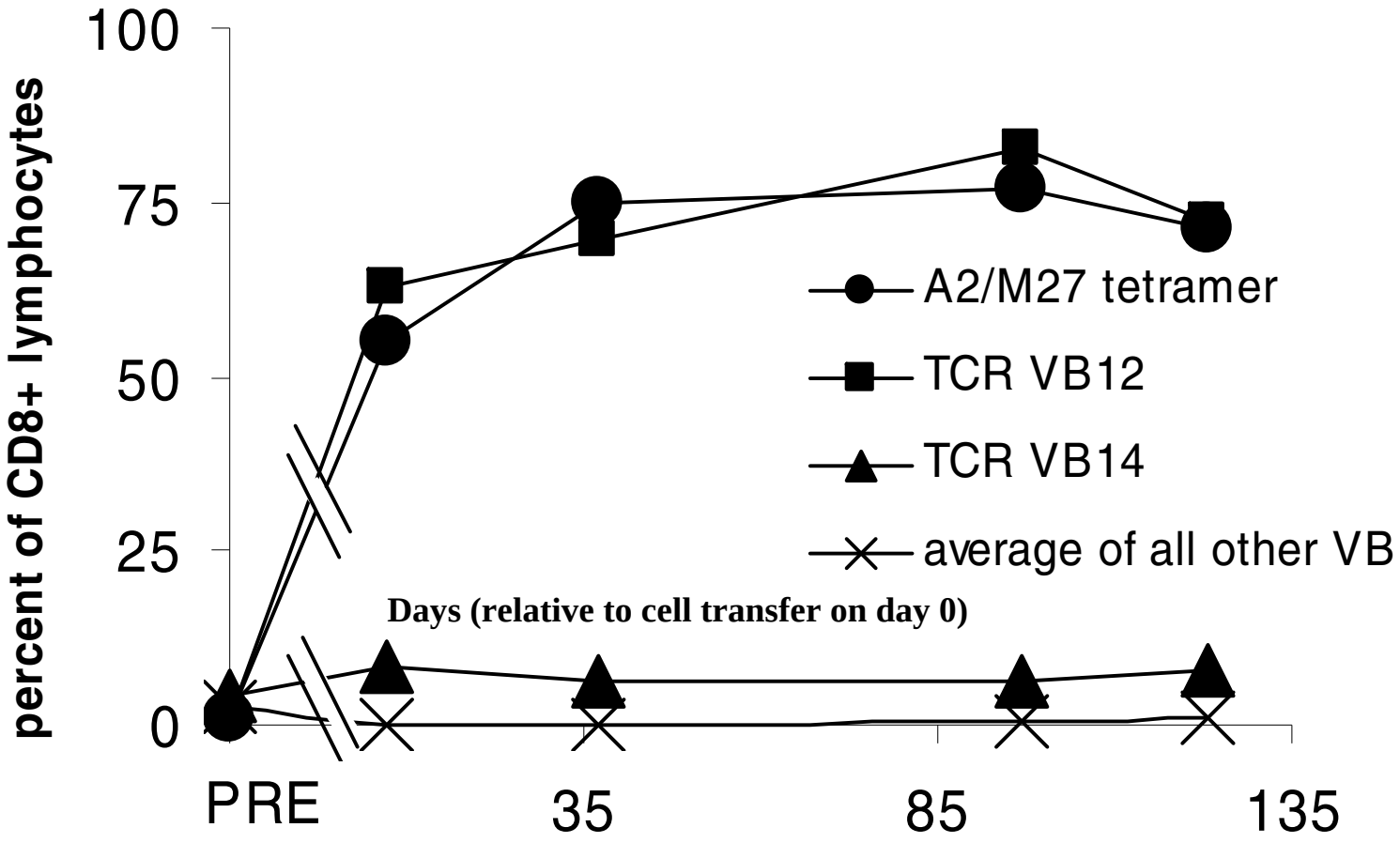
Adoptive Cell Therapy (ACT) is a Powerful Approach to Cancer Immunotherapy

1. Large numbers of antitumor cells can be grown in vitro.

2. High avidity anti-tumor cells can be selected using in vitro assays or created in vitro by genetic engineering

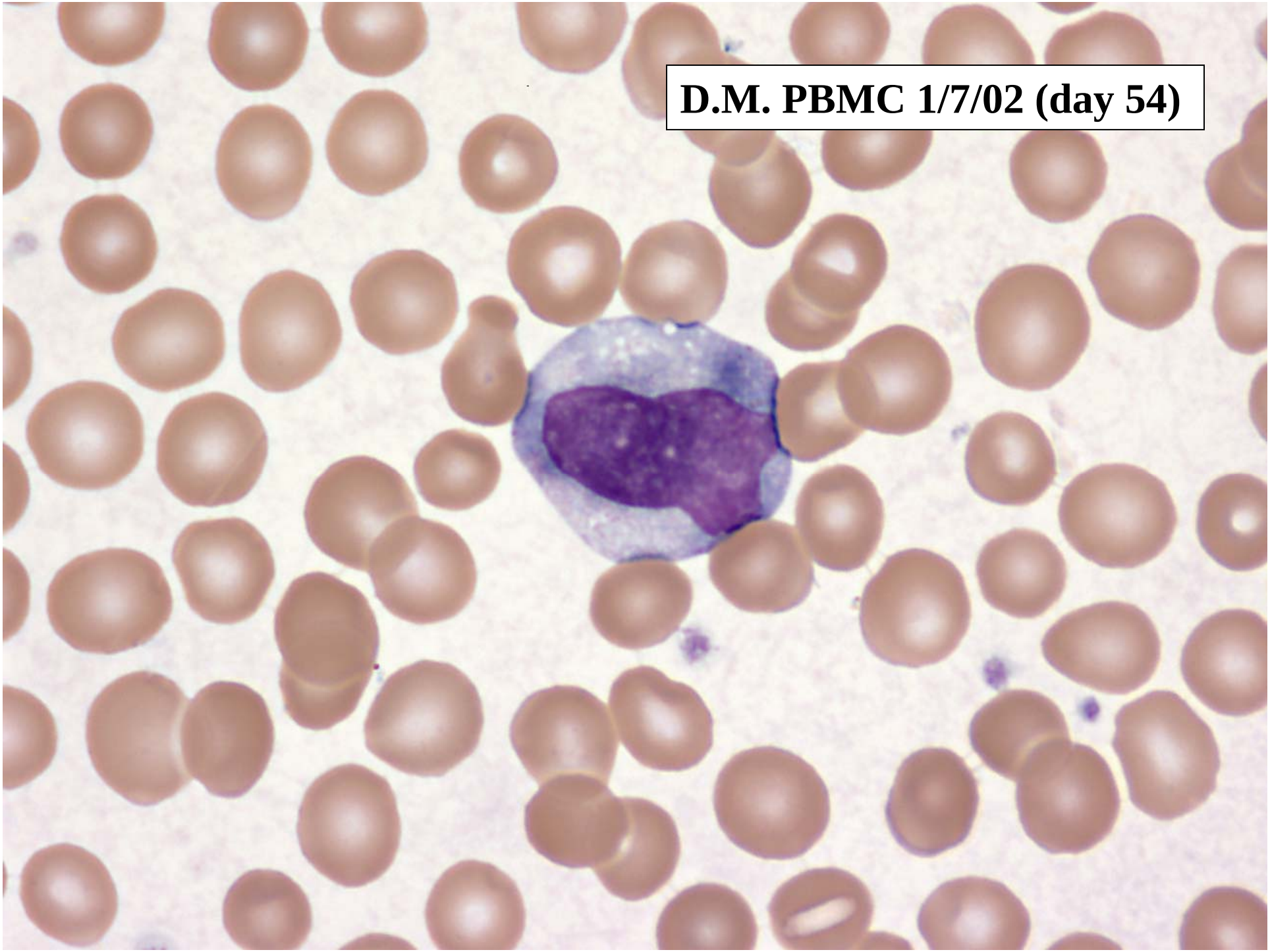
3. The host can be manipulated to provide a favorable tumor microenvironment prior to administering the cells

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)

Predicted binding of mutated epitopes recognized by TIL

Protein	Length(aa)	Peptide(length)	HLA allele	NetMHCpan Rank (%)
β-catenin	781	SYLDSGHIF(9)	A*2402	1 (0.8)
PPP1R3B	285	YTDFHCQYVK(10)	A*01	1 (0.25)
CDKN2A(p16INK4A-ORF-3)	144	AVCPWTWLR(9)	A*11	1 (0.4)
CDKN2A(p14ARF-ORF3)	199	AVCPWTWLR(9)	A*11	2 (0.4)
GAS7	412	SLADEAEVYL(10)	A*0201	1 (0.5)
GAPDH	335	GIVEGLITTV(10)	A*0201	1 (0.8)
NOP56	594	VIAEILREV(9)	A*0201	1 (0.8)
MART-2	483	FLEGNEVGKTY(11)	A*01	15 (1.5)

Mutated epitopes recognized by TIL were most often the highest binding peptides in that protein.

This implied that to be recognized by TIL, mutations occurred in or created high binding peptides.

Cytokine Genes Transduced into Mouse Anti-tumor Cells to Evaluate Therapeutic Effectiveness

---

Interferon – alpha

Interferon – beta

Interferon – gamma

IL-1 beta

IL-2

IL-12

IL-15

IL-17

IL-21

IL-23

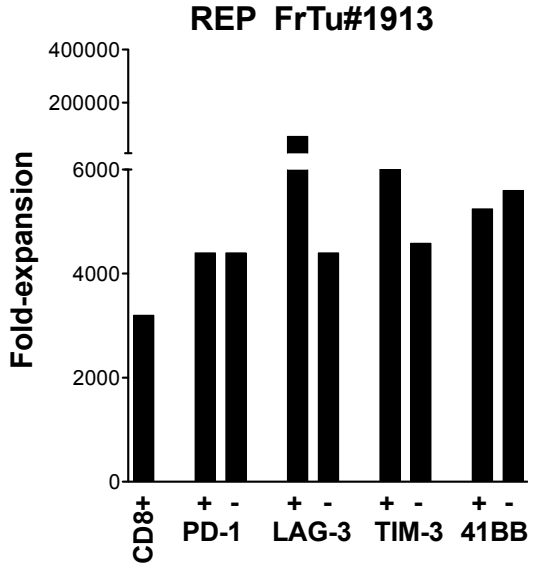
IL-27

Sort FrTu#1913 according to marker expression and expand the cells *in vitro*

FACS-sorted populations:

CD8+
CD3+ CD8+ PD-1+/-
CD3+ CD8+ TIM-3+/-
CD3+ CD8+LAG-3+/-
CD3+ CD8+ 41BB+/-

REP 14 days

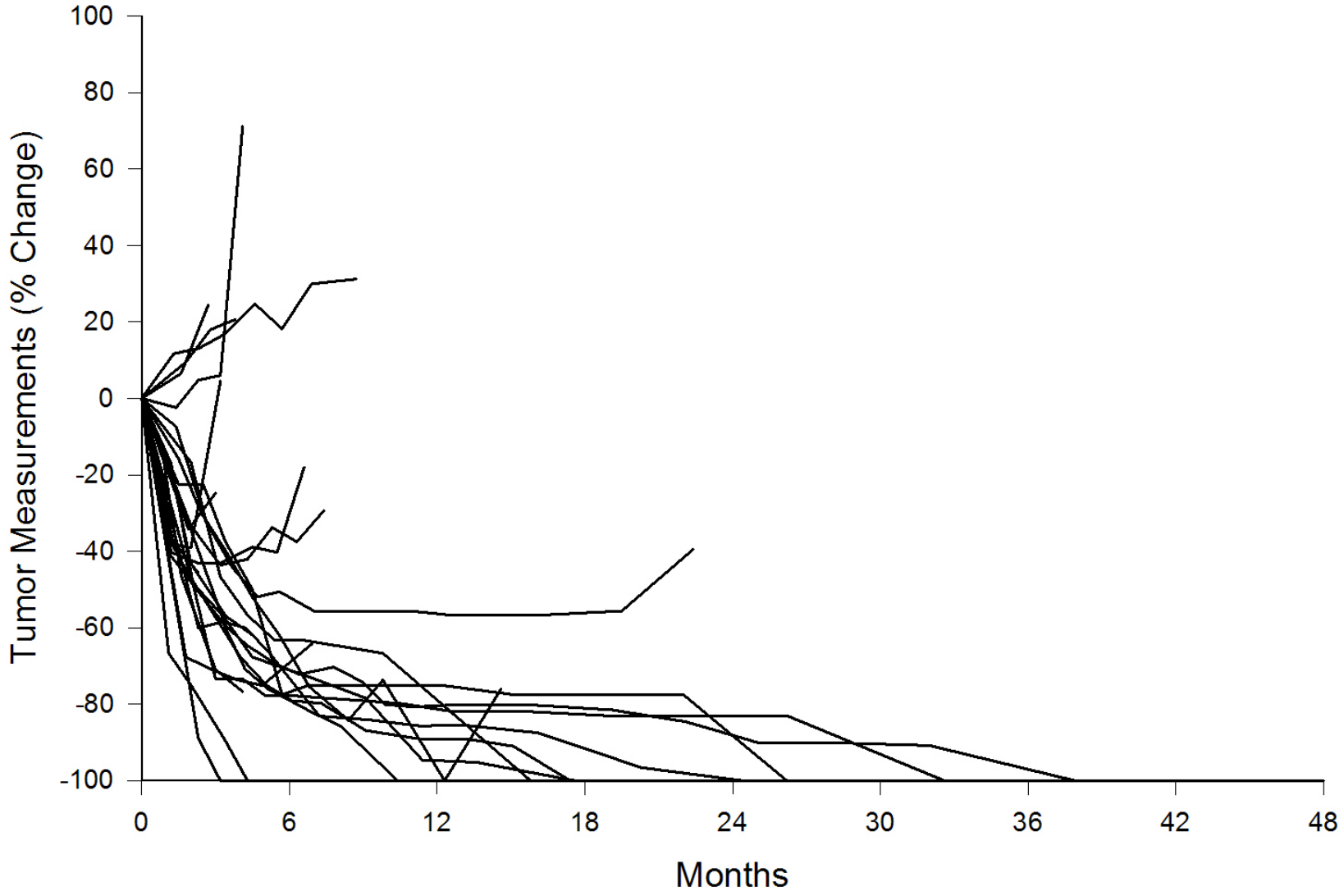


Test reactivity against autologous tumor and specific peptide recognition ($CDKN2A_{mut}$):

- IFN γ release
- 41BB up-regulation (24h)
- %$CDKN2A_{mut}$ Tetramer+ cells

TBI 1200 cGy + TIL + HD IL-2

Protocol 06-C-0136: All Patients



Four Factors that Correlate with Cancer Regression Following Cell Transfer Therapy

1. Long persistence of the transferred cells

2. Long telomere length

3. High expression of CD27 (less differentiated cells)

4. Decreased recovery of endogenous CD4/Foxp3 T regulatory cells

Melanoma/Melanocyte Differentiation Antigens

1994 MART-1 and gp100 cloned by Kawakami et al.

1996 TRP-2 cloned by Wang et al.

---

16 of 36 (42%) patients treated with PBL transduced with high-affinity anti-MART-1 or gp100 had eye and/or ear toxicity (25% objective response rate)

1 of 93 patients treated with TIL had severe eye and ear toxicity

These M/M antigens do not appear responsible for tumor rejection

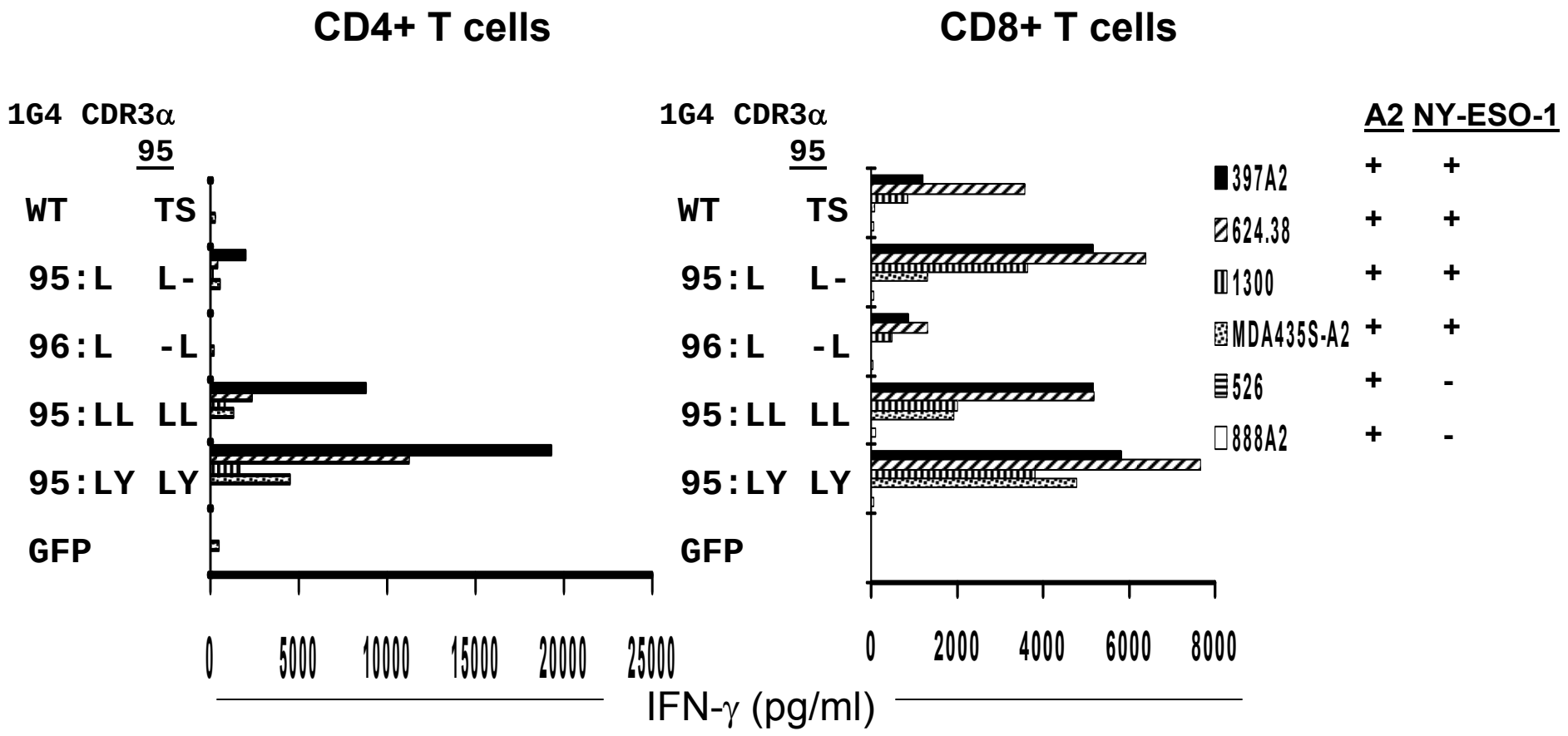
Expression cloning of antigens recognized by TIL responders (since 1994) have identified 8 mutated antigens restricted by Class I

Impact of Prior Treatment on Response to Cell Transfer Therapy Using Selected TIL

	Total	CR	PR	OR
		number (%)*		
All Patients	93	20(22%)	32(34%)	52(56%)
Prior Treatment				
None	5(5%)	2(40%)	1(20%)	3(60%)
IL-2	77(83%)	14(18%)	28(36%)	42(54%)
Chemotherapy	40(43%)	7(18%)	16(40%)	23(39%)
Interferon	52(56%)	11(21%)	17(33%)	28(54%)
Anti-CTLA4	11(12%)	5(45%)	2(18%)	7(64%)
IL-2+ Chemotherapy	37(40%)	6(16%)	16(43%)	22(59%)
IL-2+ Anti-CTLA4	8(9%)	3(38%)	1(13%)	4(50%)
IL-2+ Anti-CTLA4+ Chemotherapy	6(7%)	2(33%)	1(17%)	3(50%)

*This refers to the percent of patients with a CR, PR or OR in each group that had received the prior treatment.

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



Prospective Randomized Trial Evaluating Two Lymphodepleting Regimens Prior to Cell Transfer in Patients with Metastatic Melanoma

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

Cyclophosphamide 60mg/kg
Fludarabine: 25mg/m^2
TBI: 2Gy bid

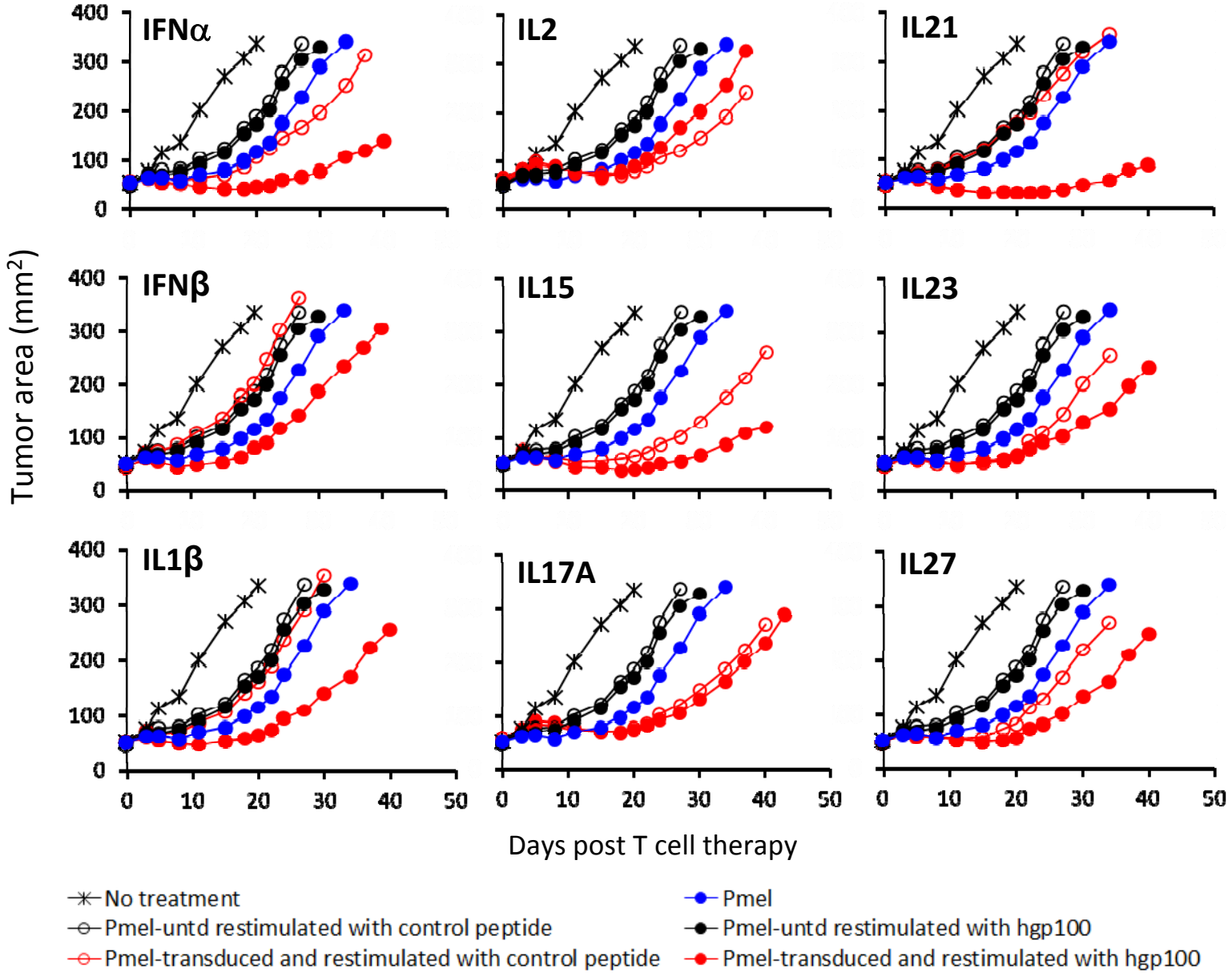
TIL Protocol: NMA vs. NMA/TBI

(8/12)

Treatment	Total	PR	CR	OR(%)	(TE)
		number of patients (duration in months)			
NMA	30	11 (41%) 12+,10+,10+,7+,5+, 4+,4+,3+,3+,3+,2+	1(4%) 7+	12(44%)	(3)
NMA/TBI	28	12 (52%) 12+,2,13+,13+,11+, 8,7+,6+,4+3+,2+,2+	3(13%) 14+ 10+ 8+	15(65%)	(5)

(1st patient randomized 3/24/11)

Anti-tumor efficacy of Pmel T cells transduced with NFAT driven cytokine genes on established B16 melanoma in immunocompetent B6 mice



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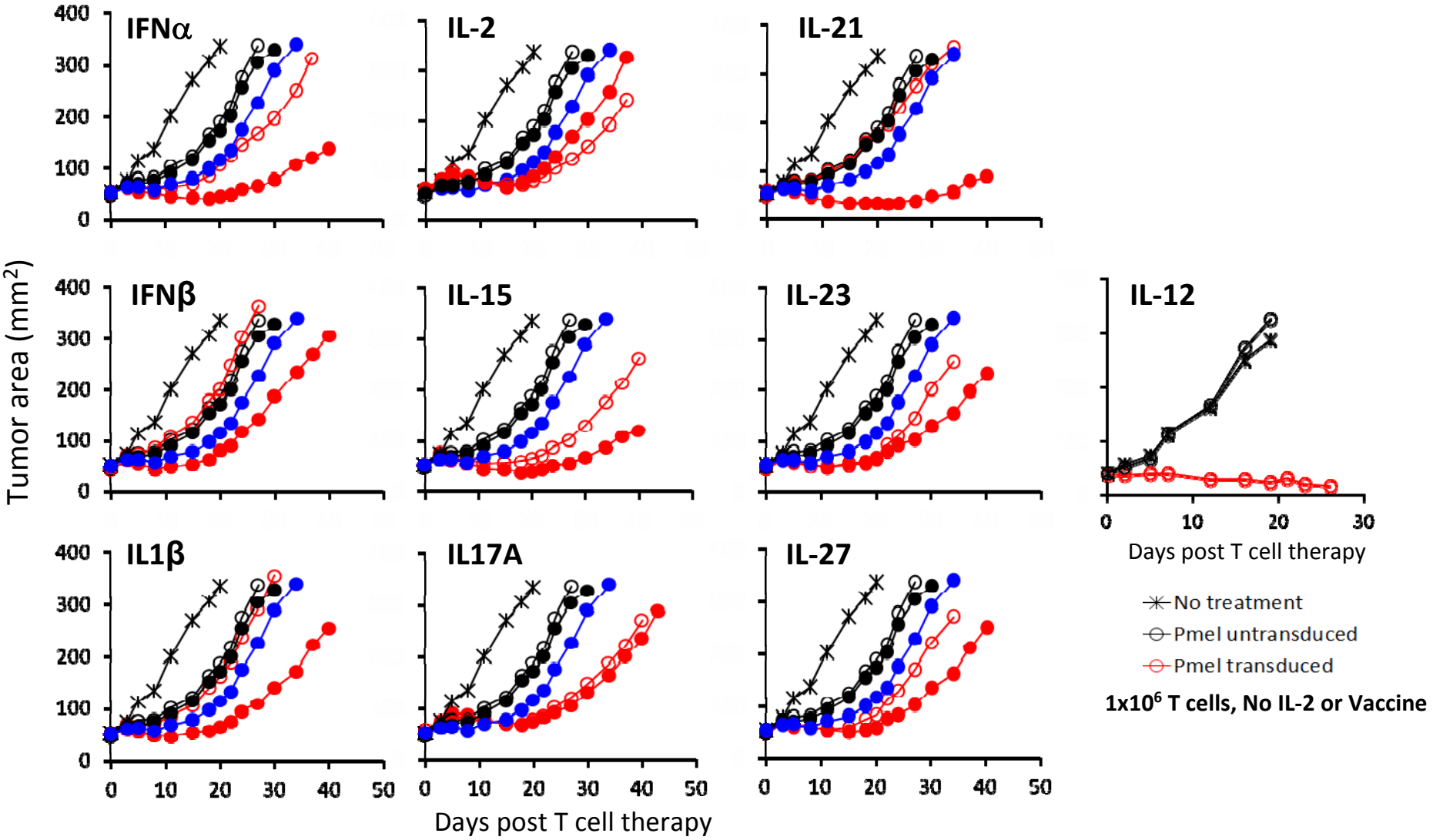

All treatment groups received 1x 10^6 T cells, 3.3x 10^4 CU IL-2/dose x 6 doses, and vaccine

Summary

Selection of PD-1, TIM-3, LAG-3 and 41BB in the fresh melanoma digest enriched for tumor-reactive cells including mutated epitopes

TCR-β clonotype analysis suggests PD-1+ derived cells have undergone clonal expansion and the most frequently expressed clonotypes contain the anti-tumor reactivity

Thus:

Sorting the original preparation for expression of these markers could enrich for multiple tumor-reactive cells (including mutated epitopes), without screening for autologous tumor recognition, and shorten the period of cell culture before transfer

Response of Patients with Metastatic Melanoma Treated with anti-CTLA4 Monoclonal Antibody in Surgery Branch, NCI (2002-2005)

Ipilimumab dose	Total	CR	PR	CR+PR
		(number of patients)		
3 mg	56	4 (7%)	3 (6%)	7 (13%)
3 - 9 mg	85	5 (6%)	12 (14%)	17 (20%)
Total	141	9 (6%)	15 (11%)	24 (17%)

(Proc. Natl. Acad. Sci. 100:8372-7, 2003)
(Clin. Cancer Res. 18:2039-47, 2012)

Patient A.R.

65 year old female with metastatic synovial sarcoma

Oct. 2008	**resection of primary sarcoma right iliac wing**
Nov. 2008	**3 cycles ifosphamide**
Feb. 2009	**2 cycles ifosphamide, doxorubicin**
April 2009	**ifosphamide**
Aug. 2009	**gemcitabine, taxotere**
Nov. 2009	**sorafenib**
Dec. 2009	**radiation therapy right hip**
Aug. 2010	**To NCI; autologous cells transduced with anti-NY-ESO-1 T cell receptor**

(Ongoing response as of Nov. 2012; 27+ months)

Rationale for the anti-VEGFR2 Gene Therapy Protocol

Vascular endothelial growth factor (VEGF) stimulates tumor angiogenesis by binding to its receptor, VEGFR2

Antibodies to VEGF (bevacizumab) interferes with tumor angiogenesis and improves survival in several metastatic cancers (FDA approved)

Redundancy in angiogenic pathways limits the effectiveness of VEGF

Destruction of cells bearing VEGFR2 may more effectively destroy tumor vasculature and result in effective cancer treatment

TCR Gene Therapy in Patients with Metastatic Melanoma

TCR	Response		Toxicity		
	Total	OR	Skin (Grade 1/2/3)	Uveitis (Grade 1/2/3)	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
(Total)	36	9(25%)	22/7/0 (81%)	2/13/0 (42%)	4/2/3 (25%)

	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.

(Science 314:126, 2006; Blood 114:535, 2009)

Simplified Screening Procedure for TIL Fragments

Overnight co-culture (γ-IFN) of individual TIL wells vs. suspension of autologous tumor

- **Alone**
- **Tumor**
- **Tumor and anti-Class I Ab**

	Total	OR
Reactive and Class I blocked	17	13 (76%)
Not reactive or not blocked	10	0
		(p-0.0002)

Of 39 patients deemed eligible and resected 32 (82%) were treated.
(1 refused, 2 no growth, 4 disease progression)

Other patients resected to NED, TIL cryopreserved and at recurrence were randomized and treated

Potential Improvements in TIL Therapy for Cancer

Test minimally cultured, unselected TIL

Pre-select subpopulations of TIL with anti-tumor activity

Genetically modify TIL to improve anti-tumor efficacy

Identify the mutated antigens in melanoma that are recognized by TIL and selectively target them

Generate anti-tumor reactive TIL from non-melanoma histologies

Antigens Recognized by TIL Responsible for Cancer Regressions

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PBMC genetically modified to recognize melanoma/melanocyte antigens mediated severe eye, ear and vestibular toxicity (these organs contain melanocytes)

TIL mediated durable, complete melanoma regressions in the absence of toxicity to any normal organs

It thus appears that TIL recognized mutated antigens on melanomas

Since all cancers contain mutations, what accounts for the unique response of melanoma to immunotherapy? (TIL, IL-2 and CTLA-4)

Predicted binding of mutated epitopes recognized by TIL

Protein	Length(aa)	Peptide(length)	HLA allele	NetMHCpan Rank (%)	Rank of WT peptide(%)	Pred. Aff. (nm)
					WT peptide	
β-catenin	781	SYLDSGHIF(9)	A*2402	1 (0.8)	107(32)	18,750
PPP1R3B	285	YTDFHCQYVK(10)	A*01	1 (0.25)	1(0.3)	136
CDKN2A(p16INK4A-ORF-3)	144	AVCPWTWLR(9)	A*11	1 (0.4)	-	-
CDKN2A(p14ARF-ORF3)	199	AVCPWTWLR(9)	A*11	2 (0.4)	-	-
GAS7	412	SLADEAEVYL(10)	A*0201	1 (0.5)	2(1.5)	39
GAPDH	335	GIVEGLITTV(10)	A*0201	1 (0.8)	1(1)	27
NOP56	594	VIAEILREV(9)	A*0201	1 (0.8)	1(0.8)	0.7
MART-2	483	FLEGNEVGKTY(11)	A*01	15 (1.5)	31(4)	4500

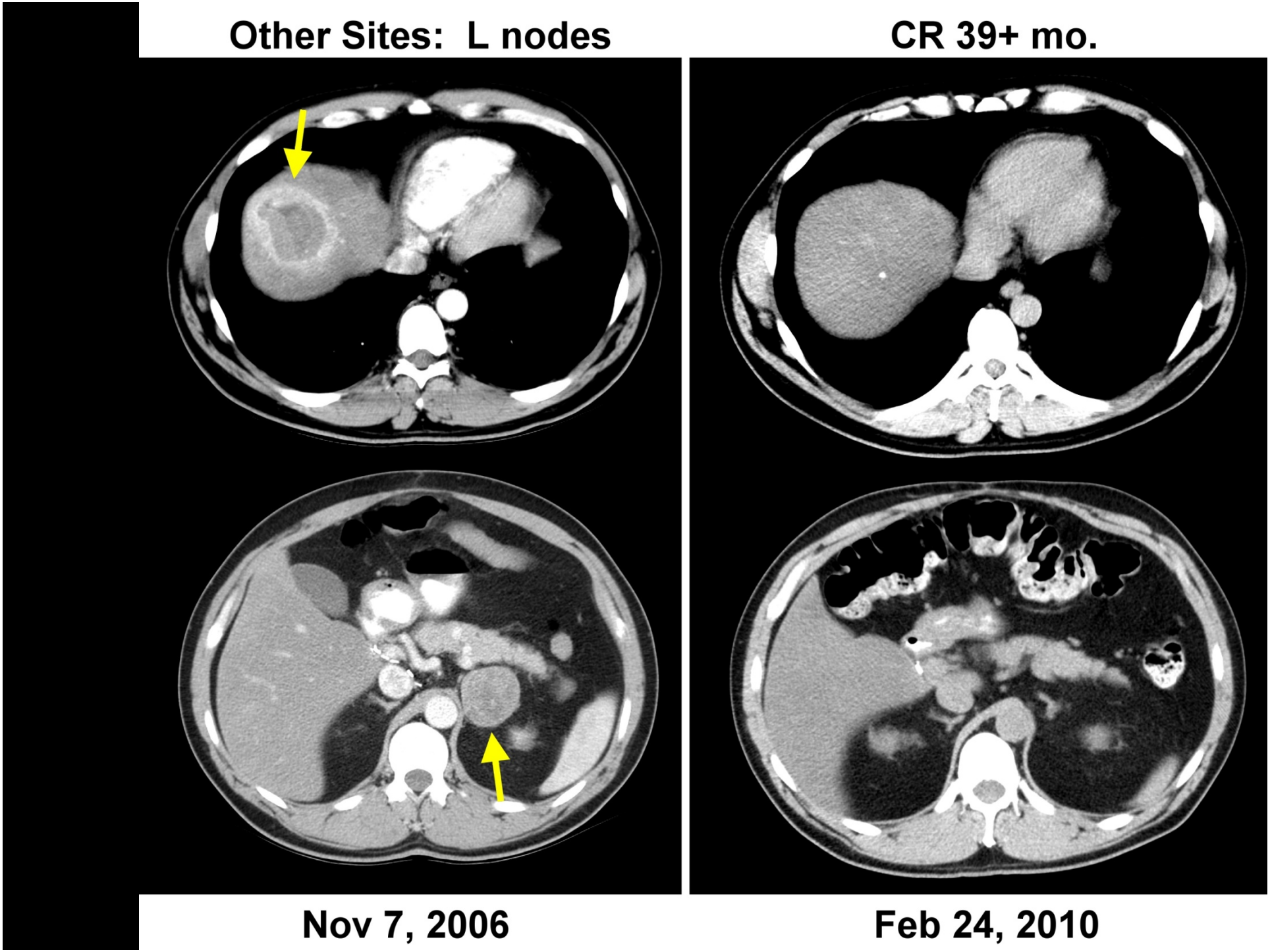
Mutated epitopes recognized by TIL were most often the highest binding peptides in that protein.

This implied that to be recognized by TIL, mutations occurred in or created high binding peptides.

This led to the hypothesis that cancers with high levels of mutations were more likely to give rise to immunogenic epitopes.

Other Sites: L nodes

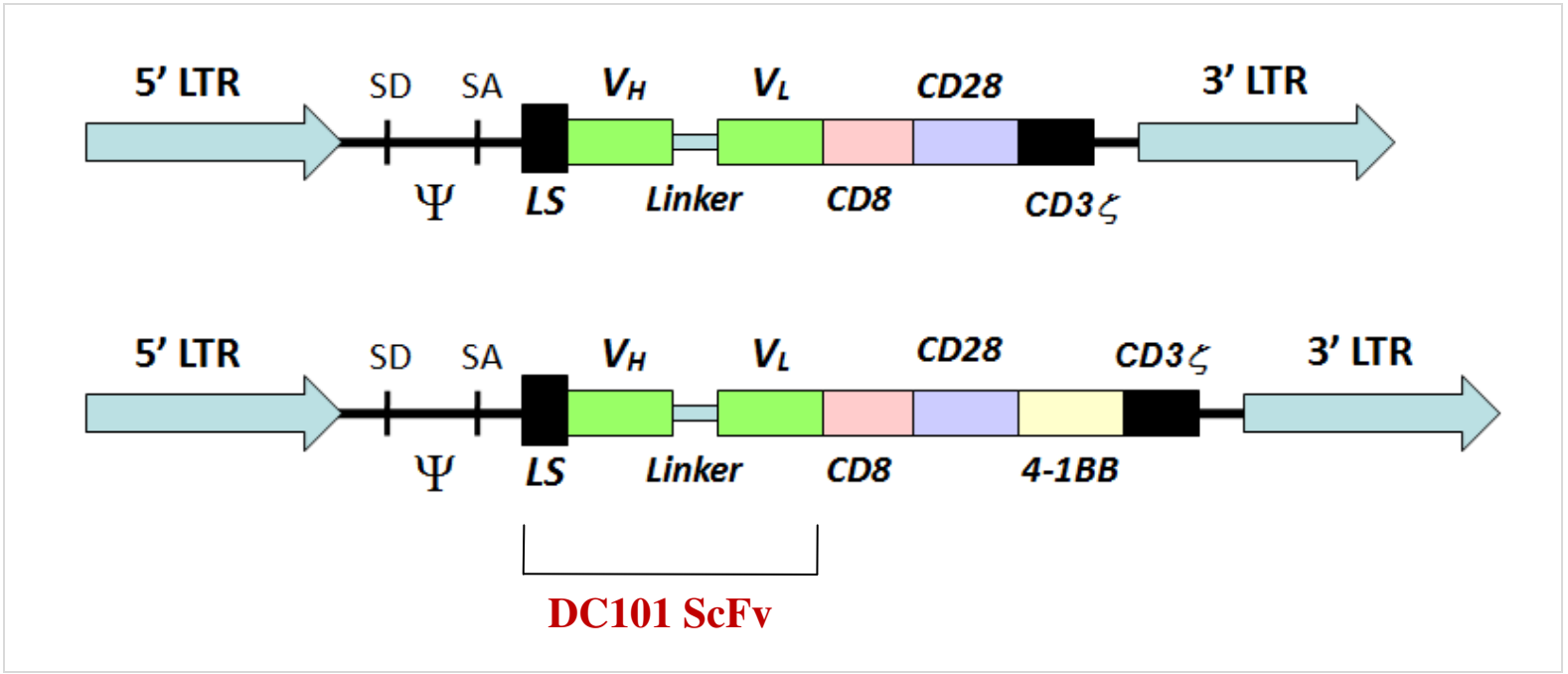
CR 39+ mo.



Nov 7, 2006

Feb 24, 2010

Design of DC101 Anti-VEGFR2 CAR Retroviral Vectors



(D. Chinnasamy, J Clin Invest 120:3953, 2010)

Adoptive Cell Therapy of Refractory Metastatic Melanoma

Depending on the intensity of prior lymphodepletion

Objective response 49 – 72%

Complete response 12 – 40%

19 of 20 complete responses ongoing at 56 to 101 months

No relationship between bulk and sites of disease or prior treatment and the likelihood of a complete response

ACT APPEARS CAPABLE OF ELIMINATING THE LAST CANCER CELL

(Clin Cancer Res 17:4550, 2011.)

Ongoing anti-CD19 CAR Gene Therapy Protocol (5/1/12)

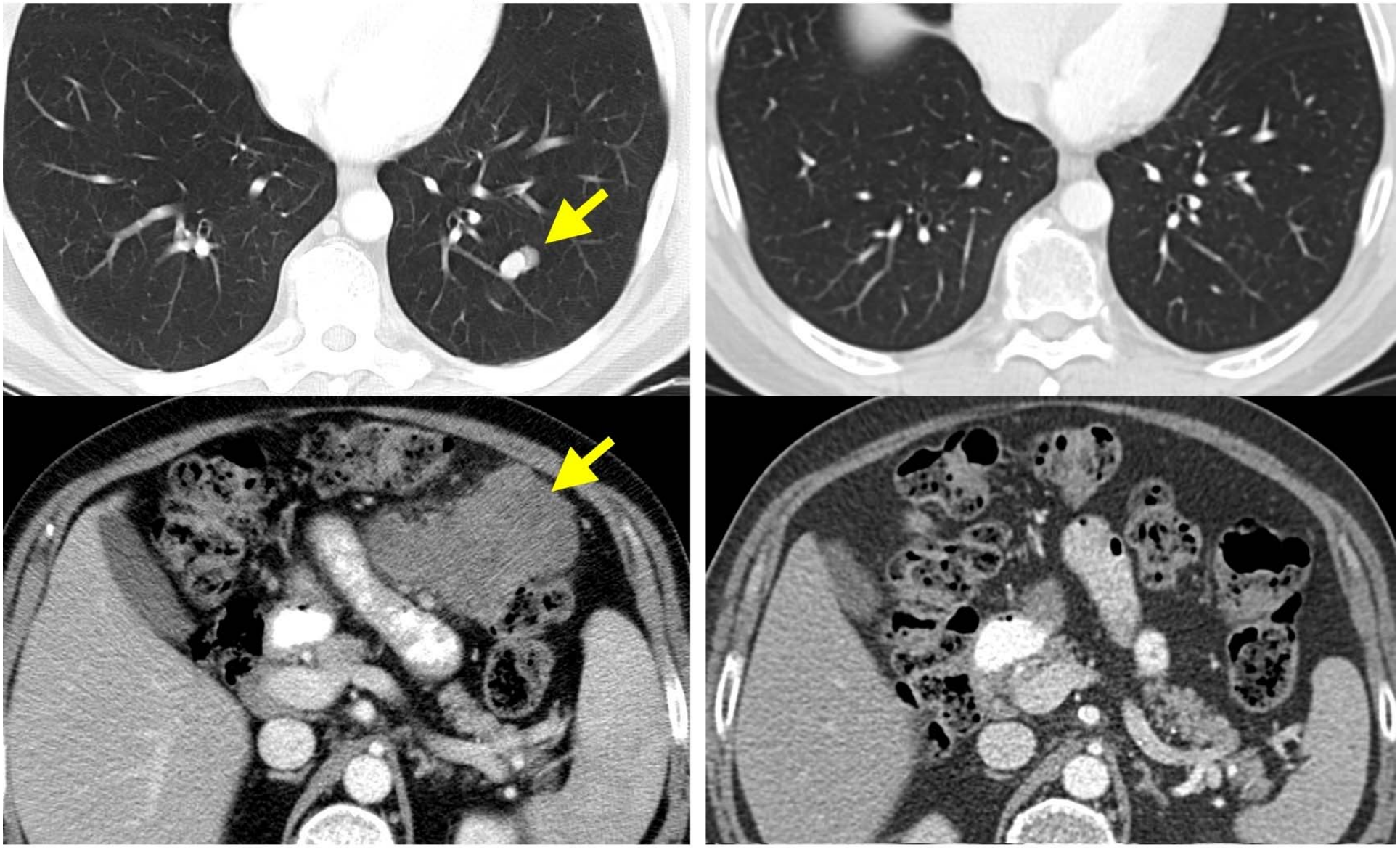
Patient	Diagnosis	Response	Duration (months)
1	Follicular lymphoma	PR (90%)*	34+
2	Follicular lymphoma	N.E. (died H1N1 pneumonia)	
3	Chronic lymphocytic leukemia	CR	20
4	Splenic marginal zone lymphoma	PR (95%)*	23+
5	Chronic lymphocytic leukemia	NR	--
6	Chronic lymphocytic leukemia	PR	6
7	Chronic lymphocytic leukemia	CR	15+
8	Follicular lymphoma	PR (95%)	15+
9	Large B cell lymphoma	CR	5+
10	Chronic lymphocytic leukemia	PR (78%)	3+

*These two patients treated twice (recurred after 1st treatment; ongoing after 2nd)

(Kochenderfer et al, Blood 116:4099, 2010; 119:2709,2012.)

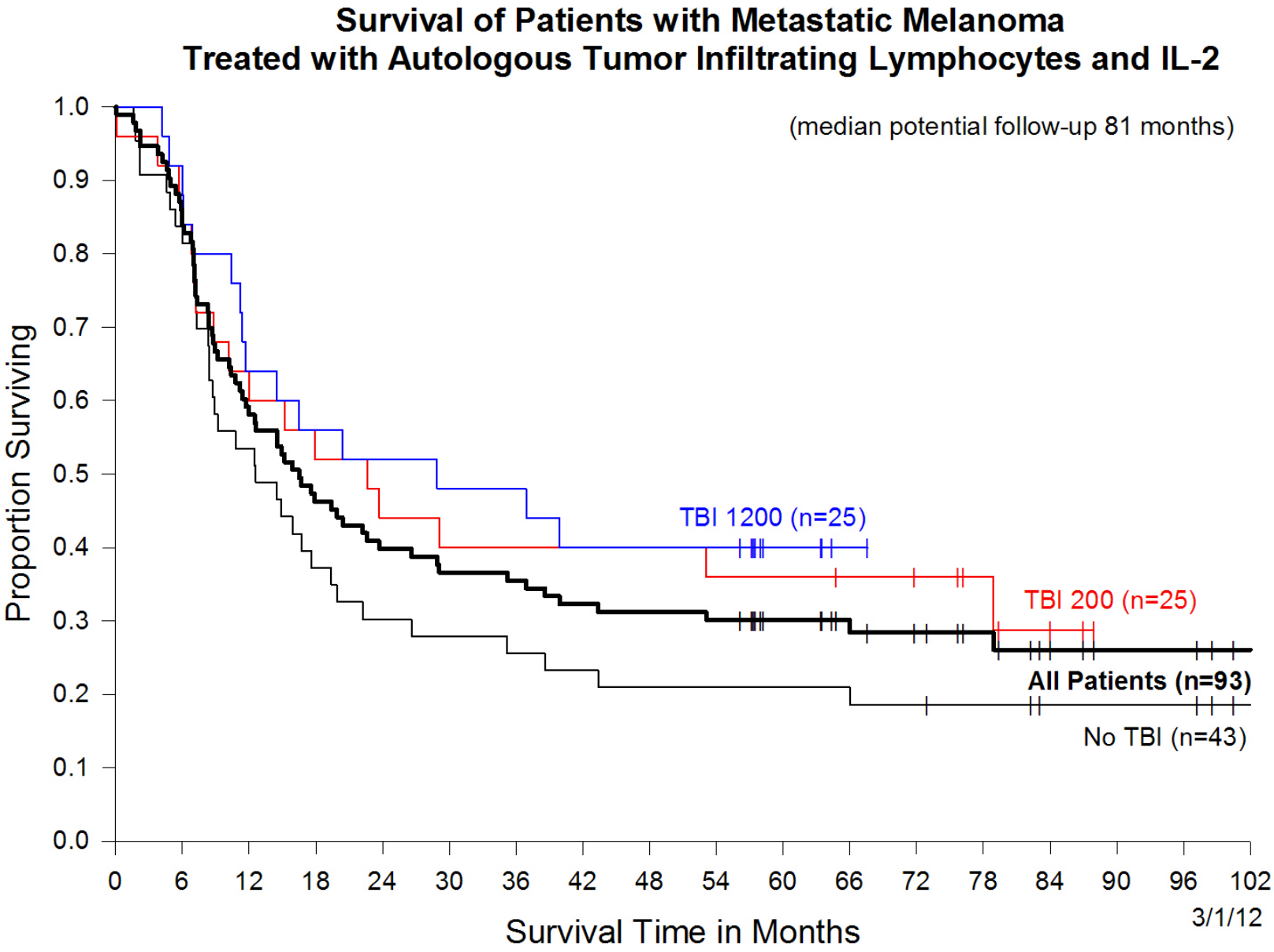
Other Sites: Sm bowel, S.C., LN

CR 58+ mo



Nov 19, 2005

Aug 18, 2010


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(median potential follow-up 81 months)
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)
All Patients (n=93)
No TBI (n=43)
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Survival Time in Months
3/1/12

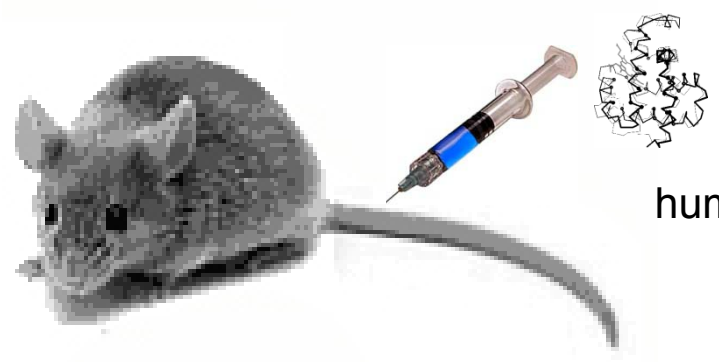
Objective Responses in Patients with Metastatic Melanoma

	Total	CR	PR	OR
		number of patients (%)		
Dacarbazine[1,2]	220	0	12(5.5%)	12(5.5%)
Interleukin-2[3,4]	270	17(6.3%)	26(9.6%)	43(15.9%)
	305	13(4.3%)	26(8.5%)	39(12.8%)
Ipilimumab[5]	540	3(0.6%)	35(6.4%)	38(7.0%)
Vemurafenib[2]	219	2(0.9%)	104(47.5%)	106(48.4%)
Cell Transfer[6]	93	20(21.5%)	32(34.4%)	52(55.9%)

1) Middleton et al JCO, 18:158, 2000
2) Chapman et al NEJM, 364:2507, 2010
3) Atkins et al JCO, 17:2105, 1999
4) Smith et al CCR, 14:5610, 2008
5) Hodi et al NEJM, 363:711, 2010
6) Rosenberg et al CCR, 17:1-8, 2011

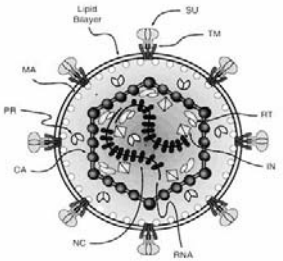
Using transgenic mice to generate T cells specific for human tumor antigens

Clone human MHC class I

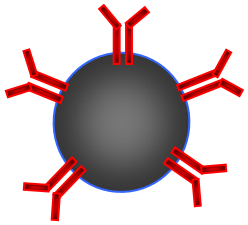


Immunize with human cancer antigen

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

Patients on MAGE-A3 TCR Protocol (F/U 3/1/12)

	Patient		Diagnosis	Date of cells	# cells ($x10^{-9}$)	#IL-2 doses	Response	Neurologic
1.	L.A.	59	Melanoma	2/24/11	28	6	CR(12+)	None
2.	J.P.	38	Melanoma	3/24/11	30	5	NR	None
3.	P.M.	56	Melanoma	5/5/11	30	7	PR(4)	None
4.	K.H.	21	Synovial Sarc.	6/10/11	41	1	PR(5)	None
5.	M.S.	54	Melanoma	7/22/11	79	5	PR(4)	Coma (white matter)
6.	J.M.	44	Melanoma	8/5/11	53	4	NR	None
7.	F.B.	62	Melanoma	8/17/11	62	6	CR(6+)	Seizure (normal MRI; recovered completely)
8.	G.T.	71	Esophageal	8/18/11	61	1	NR	Coma (white matter)
9.	J.S.	62	Melanoma	8/31/11	30	0	NR	TIA (Normal MRI; recovered completely)

The most critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

---

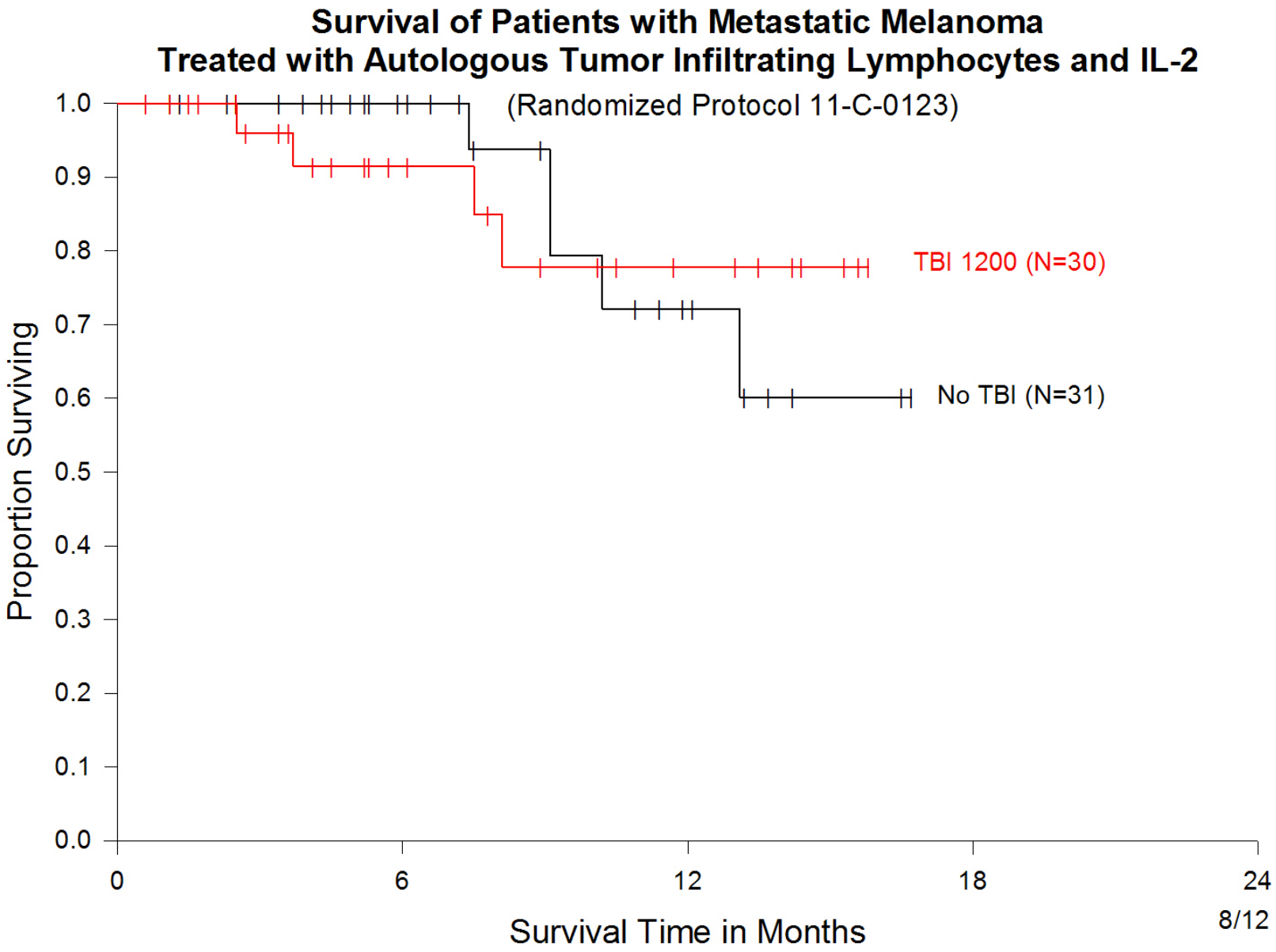
1. Shared antigens unique to cancer (cancer-testes antigens)

2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

3. Mutations unique to each cancer (EGFRvIII)

4. Critical components of the tumor stroma (VEGFR2)

5. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(Randomized Protocol 11-C-0123)
TBI 1200 (N=30)
No TBI (N=31)
Proportion Surviving
1.0
0.9
0.8
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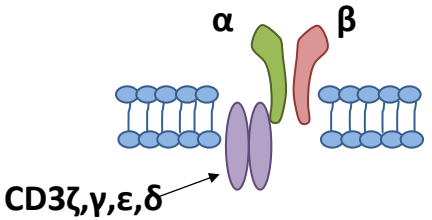
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
Survival Time in Months
8/12

Construction of T-cell Receptors (TCR) and Chimeric Antigen Receptors (CAR)

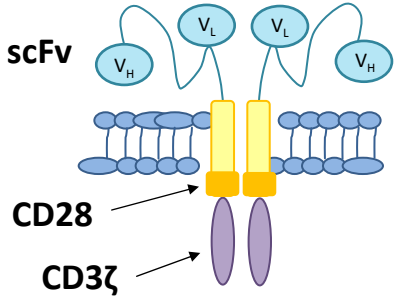
TCR Vector (eg, MART1, NY-ESO)

TCR receptor



CAR Vector (eg, CD19)

CAR receptor



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

(Kochenderfer et al, Blood 116:4099, Dec. 2010.)

Ongoing anti-CD19 CAR Gene Therapy Protocol (3/15/12)

Patient	Diagnosis	Response	Duration (months)
1	Follicular lymphoma	PR (90%)*	31+
2	Follicular lymphoma	N.E. (died H1N1 pneumonia)	
3	Chronic lymphocytic leukemia	CR	22+
4	Splenic marginal zone lymphoma	PR (91%)*	22+
5	Chronic lymphocytic leukemia	NR	--
6	Chronic lymphocytic leukemia	PR	6
7	Chronic lymphocytic leukemia	CR (98%)	14+
8	Follicular lymphoma	PR (97%)	13+
9	Large B cell lymphoma	CR	4+
10	CLL	TE	(76% decr. 1mo)

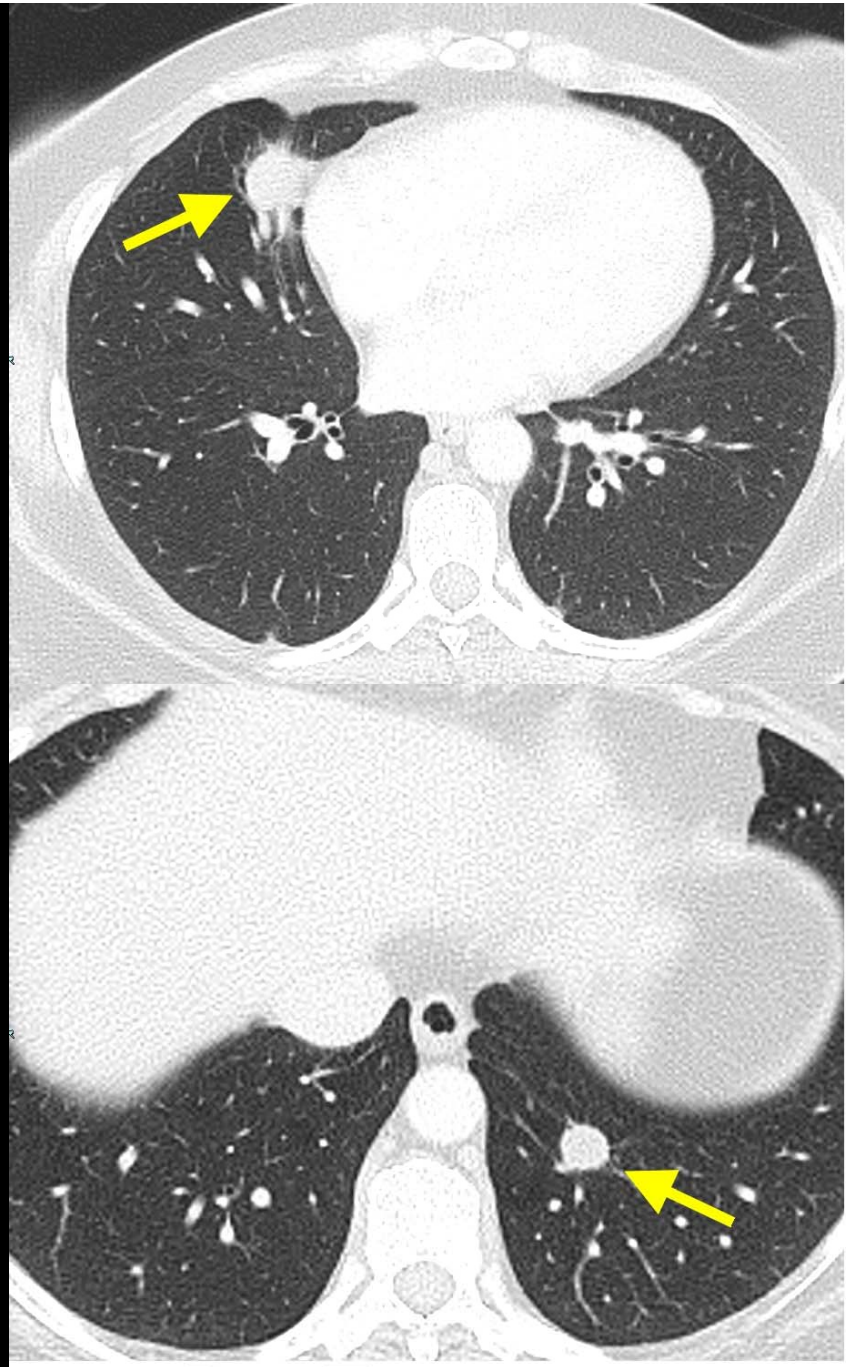
*These two patients treated twice

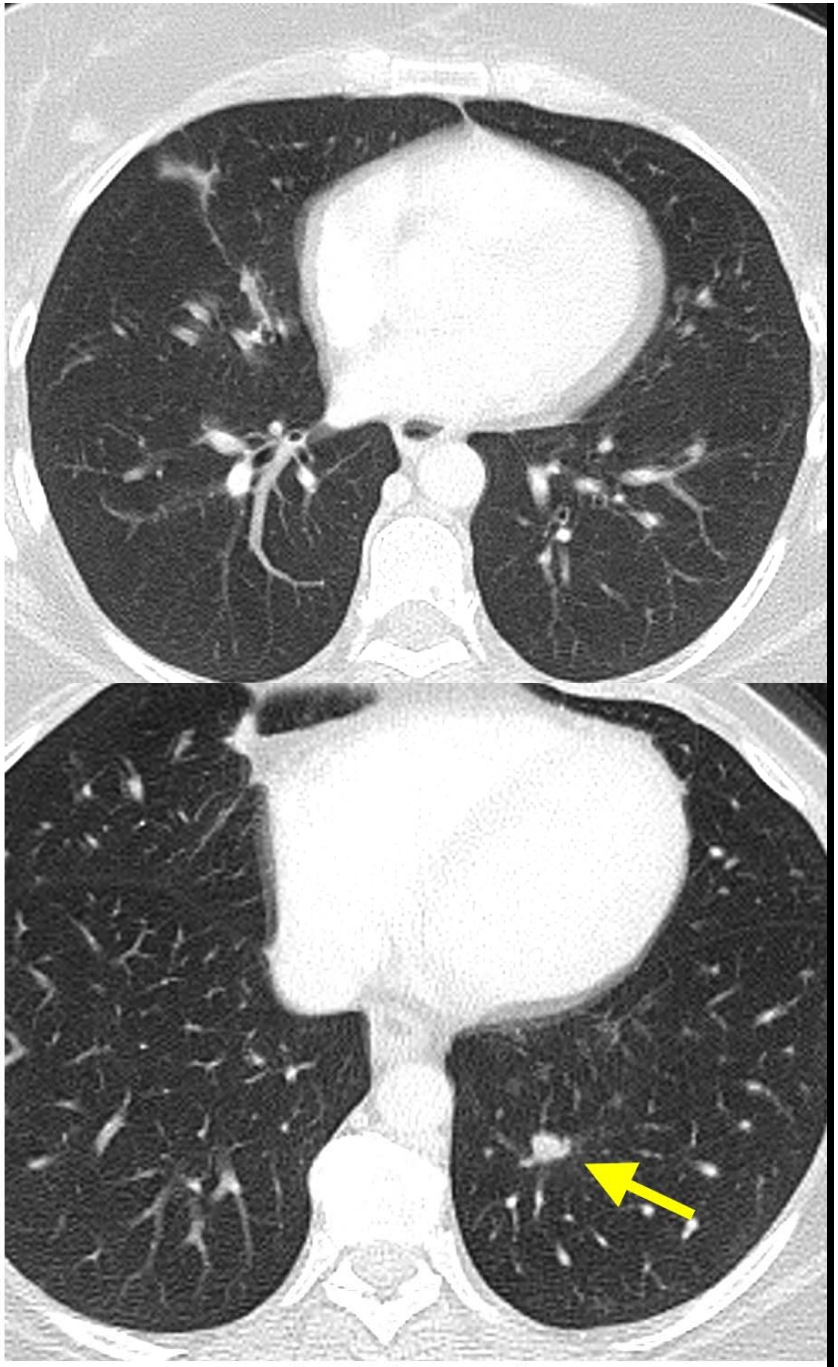
(Kochenderfer et al, Blood 116:4099, Dec. 2010)

C.W.

1200TBI rand

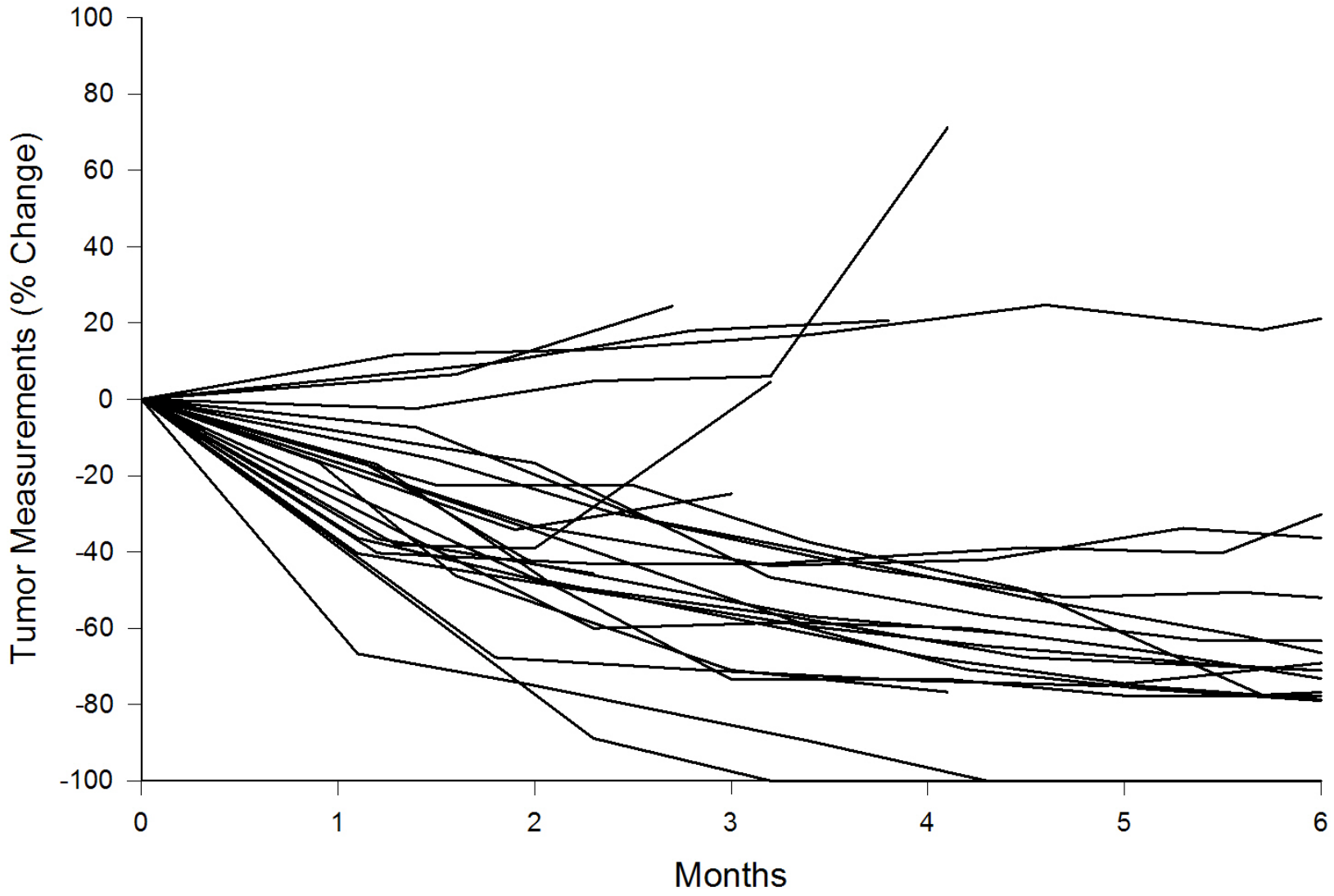
Prior VEM





Pre-Treatment

1 Month


TBI 1200 cGy + TIL + HD IL-2
Protocol 06-C-0136: All Patients
Tumor Measurements (% Change)
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
1
2
3
4
5
6
Months

New Approach to Rapidly Identify Cancer Antigens Associated with Complete Cancer RegressionsBased on Exomic Sequencing

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Exomic sequencing of fresh tumor or early cultured lines and normal tissue to determine the number of exomic mutations

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Obtain sequence of 9aa on either side of aa mutation

New Approach to Rapidly Identify Cancer Antigens Associated with Complete Cancer RegressionsBased on Exomic Sequencing

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Exomic sequencing of fresh tumor or early cultured lines to determine the number of exomic mutations

Obtain sequence of 9aa on either side of aa mutation

Use algorithm to predict best binders to that patient's HLA antigens

Synthesize best binding peptides

Test for recognition of pulsed peptides by TIL

Patient B.C. (TIL 2098)

53 year old female with metastatic melanoma

July 1995: excision of leg melanoma
Dec. 1995: diffuse leg recurrence
Feb. 1996: isolated limb perfusion; progressive disease
May 1999: below-knee amputation
Dec. 1999: recurrence in stump and lungs
May 2000: chemotherapy with cisplatin; progressive disease
Jan. 2003: TIL/NMA/IL-2

Complete regression until December 2009 when she died of an unrelated ovarian cancer; no melanoma present

(Exomic analysis revealed 300 nonsynonomous mutations)

Listing of the 20-mer peptides surrounding the central mutated amino acid (#2098)
(300 nonsynonomous mutations)

Gene name	cDNA change	protein change	ref AA	var AA
ZNF559	c.A299T	p.Q100L	KWSAPQQNFLQGKTSSVVEM	KWSAPQQNFL**L**GKTSSVVEM
TRPC6	c.C271T	p.R91C	NRGPAYMFSDRSTSLSIEEER	NRGPAYMFSD**C**STSLSIEEER
SERPINB11	c.C95T	p.S32L	NNIGDNIFFSSLSLLYALSMV	NNIGDNIFFS**L**LSLLYALSMV
HTR1F	c.G226A	p.V76M	AVTDFLVAVLVMPFSIVYIVR	AVTDFLVAVLMMPFSIVYIVR
UNC13A	c.C3842T	p.S1281F	LFSCSVVDVFSQLNQSFEIIK	LFSCSVVDVFFQLNQSFEIIK
PXDNL	c.C1838T	p.S613F	RQAGDDFVESSILDAVQRVDS	RQAGDDFVESFILDAVQRVDS
KCNK5	c.G335A	p.R112H	GNVAPKTPAGRLFCVFYGLFG	GNVAPKTPAGHLFCVFYGLFG
CNKSR1	c.C929T	p.A310V	SLSLAPLSPRAPSEDVFAFDL	SLSLAPLSPRVPSEDVFAFDL
MARCH11	c.C762G	p.F254L	QMIAVILGSLFLIASVTWLLW	QMIAVILGSLLLIASVTWLLW
C1S	c.A1672C	p.N558H	ICLPGTSSDYNLMDGDLGLIS	ICLPGTSSDYHLMDGDLGLIS
KCNA6	c.C214T	p.R72W	FPDTLLGDPGRRVRFFDPLRN	FPDTLLGDPGWRVRFFDPLRN
GPR174	c.C751T	p.P251S	ICFAPYHFSFPLDFLVKSNEI	ICFAPYHFSFSLDFLVKSNEI
WDR47	c.C247T	p.R83C	CMEKFDKKRFRYIILKQKFLE	CMEKFDKKRFCYIILKQKFLE
GAS7	85C>T	p.H289Y	KKSLADEAEVHLKFSAKLHSE	KKSLADEAEVYLKFSAKLHSE
GSTA4	c.G547A	p.E183K	NILSAFPFLQEYTVKLSNIPT	NILSAFPFLQKYTVKLSNIPT
OR8D4	c.G98A	p.G33E	LQLPLFCLFLGIYTVTVVGNL	LQLPLFCLFLEIYTVTVVGNL
BRCA2	c.C3116T	p.P1039L	MFFKDIEEQYPTSLACVEIVN	MFFKDIEEQYLTSLACVEIVN
RRP1B	c.C568T	p.L190F	GGKELLADQNLKFIDPFCKIA	GGKELLADQNFKFIDPFCKIA
CNTN5	c.C3251T	p.S1084L	QSTLHSLSTSSSSVTLLLALM	QSTLHSLSTSLSSVTLLLALM
C4orf15	c.A478G	p.T160A	QSQGILNAMITKISNELQALT	QSQGILNAMIAKISNELQALT
NOTCH2	c.C4729G	p.L1577V	RALGTLLHTNLRIKRDSQGEL	RALGTLLHTNVRIKRDSQGEL
KCNA6	c.C214T	p.R72W	FPDTLLGDPGRRVRFFDPLRN	FPDTLLGDPGWRVRFFDPLRN
UNC13A	c.C3842T	p.S1281F	LFSCSVVDVFSQLNQSFEIIK	LFSCSVVDVFFQLNQSFEIIK
C15orf32	c.G193A	p.G65R	CEMLSILALVGVLHPFYRSNN	CEMLSILALVRVLHPFYRSNN
C4orf15	c.A478G	p.T160A	QSQGILNAMITKISNELQALT	QSQGILNAMIAKISNELQALT
MYH4	c.G2968A	p.D990N	KNLTEEMAGLDETIAKLTKEK	KNLTEEMAGLNETIAKLTKEK
A2BP1	c.C133T	p.H45Y	NGIPAEYTAPHPHPAPEYTGQ	NGIPAEYTAPYPHPAPEYTGQ
SCN3A	c.T2315A	p.L772*	VDLAITICIVLNTLFMAMEHY	VDLAITICIV*NTLFMAMEHY
OR8D4	c.G98A	p.G33E	LQLPLFCLFLGIYTVTVVGNL	LQLPLFCLFLEIYTVTVVGNL
GALNT14	c.A236G	p.H79R	YQRGHLPTGGHLAVCHFPCLL	YQRGHLPTGGRLAVCHFPCLL

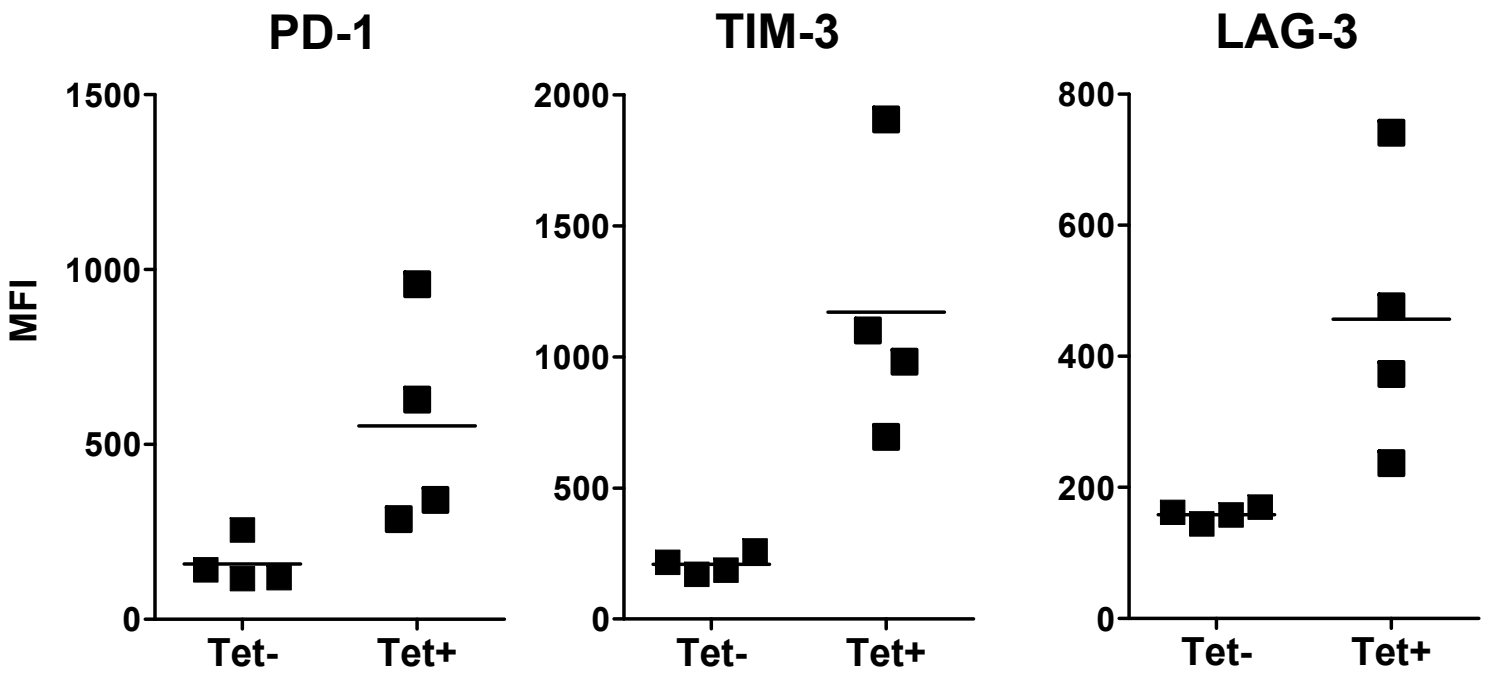
Treatment of patients in the randomized protocol

Of 39 patients deemed eligible and resected 32 (82%) were treated.

- 1 refused
- 2 no growth
- 4 disease progression

Other randomized patients resected to NED, TIL cryopreserved and at recurrence were randomized and treated.

Mart-1_{27-35} reactive cells express higher levels of PD-1, TIM-3 and LAG-3



Research Goal

To identify a specific marker/markers expressed by CD8 T cells in the fresh tumor digest that could be used to select tumor reactive cells

1- Characterization of the phenotype of CD8 T cells in the initial fresh tumor digest

Positive co-stimulatory molecules: 41BB
Negative co-stimulatory molecules: PD-1, TIM-3 and LAG-3

2- Assessment of the use of the markers to enrich for tumor-reactive cells

Exome sequencing of melanoma 2369

	Peptide[!]	Mutation[#]	Affinity (nM)	Gene	Transcript	IFN-γ (pg/ml)
1	FSDYYDL**S**Y	117 G to S	2	C22orf33	uc003aqe.1	<30
2	LTDDRLFTC**Y**	1005 H to Y	3	PLEKHM2	uc001axa.2	10400
3	YSSAL**D**LCY	621 N to D	5	GRIN3B	uc002lqo.1	<30
4	**F**SDKKVGTY	688 L to F	5	PLCB1	uc002wna.1	<30
5	HSEYSSFF**Y**	603 H to Y	6	HEG1	uc003ehs.2	<30
6	CSNF**L**LLAY	84 S to L	7	BAI3	uc003pev.2	<30
7	ESDKEE**L**VGY	332 F to L	7	MPP4	uc002uyj.2	<30
8	CTDT**Y**MLELF	191 H to Y	8	OR4C46	uc001nhj.1	<30
9	FTGTI**S**VMY	60 P to S	12	UEVLD	uc001mot.1	<30
10	QTQSVVF**L**Y	156 S to L	13	COL9A1	uc003pfg.2	<30
11	MSSYIAS**F**TY	356 L to F	14	LST-3TM12	uc001ren.1	<30
12	CTDT**Y**MLEL	191 H to Y	22	OR4C46	uc001nhj.1	<30
13	LL**D**LMAYDRY	117 G to D	22	OR2T2	uc001iek.1	<30
14	SSDSQE**E**NY	117 G to E	23	MEOX2	uc003stc.1	<30
15	LTSMAYD**C**Y	122 R to C	31	OR8B3	uc001qac.1	<30
16	YTDF**H**CQYV	176 P to H	49	PPP1R3B	uc003wsn.2	13400
17	WADWGHR**T**Y	3344 A to T	51	LRP2	uc002ues.1	<30
18	FTMVI**L**YVVY	219 S to L	54	LRRC3B	uc003cdp.1	<30
19	CVDSPPPL**F**F	528 S to F	71	C15orf2	uc001ywo.1	<30
20	V**S**DGFTAVM	198 P to S	85	RNPEP	uc001gxd.1	<30
21	**W**SCLGHLGY	267 R to W	86	MIRO-2	uc002ciq.1	<30
22	YTDF**H**CQYVK	176 P to H	100	PPP1R3B	uc003wsn.2	22000
23	YTFLI**F**SDY	849 S to F	104	BCR	uc002zww.1	<30
24	ISANSPYI**S**Y	86 P to S	124	ABCA12	uc002vev.1	<30
25	**S**SFLVPSLPY	796 P to S	125	KIAA1211	uc003hbk.2	<30

The most critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

---

1. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

2. Shared antigens unique to cancer (cancer-testes antigens)

3. Mutations unique to each cancer (EGFRvIII)

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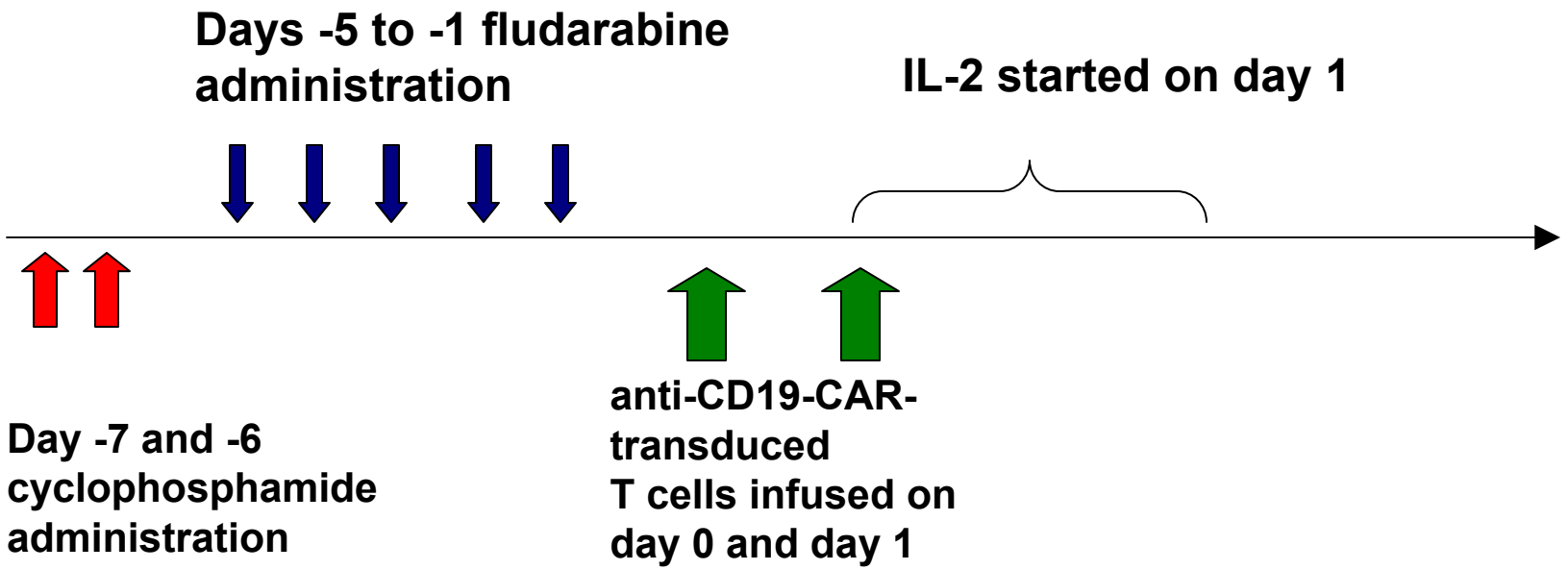
5. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

Treatment plan



Cyclophosphamide dose was 60 mg/kg per day

Fludarabine dose was 25 mg/m^2 per day

Anti-CD19 CAR T-cell dose was 1x10^8 cells on day 0 and 3x10^8 cells on day 1

IL-2 dose and schedule: 720,000 IU/kg every 8 hours (8 total doses)

Prior Treatment of Patients Treated with the anti-CD19 CAR

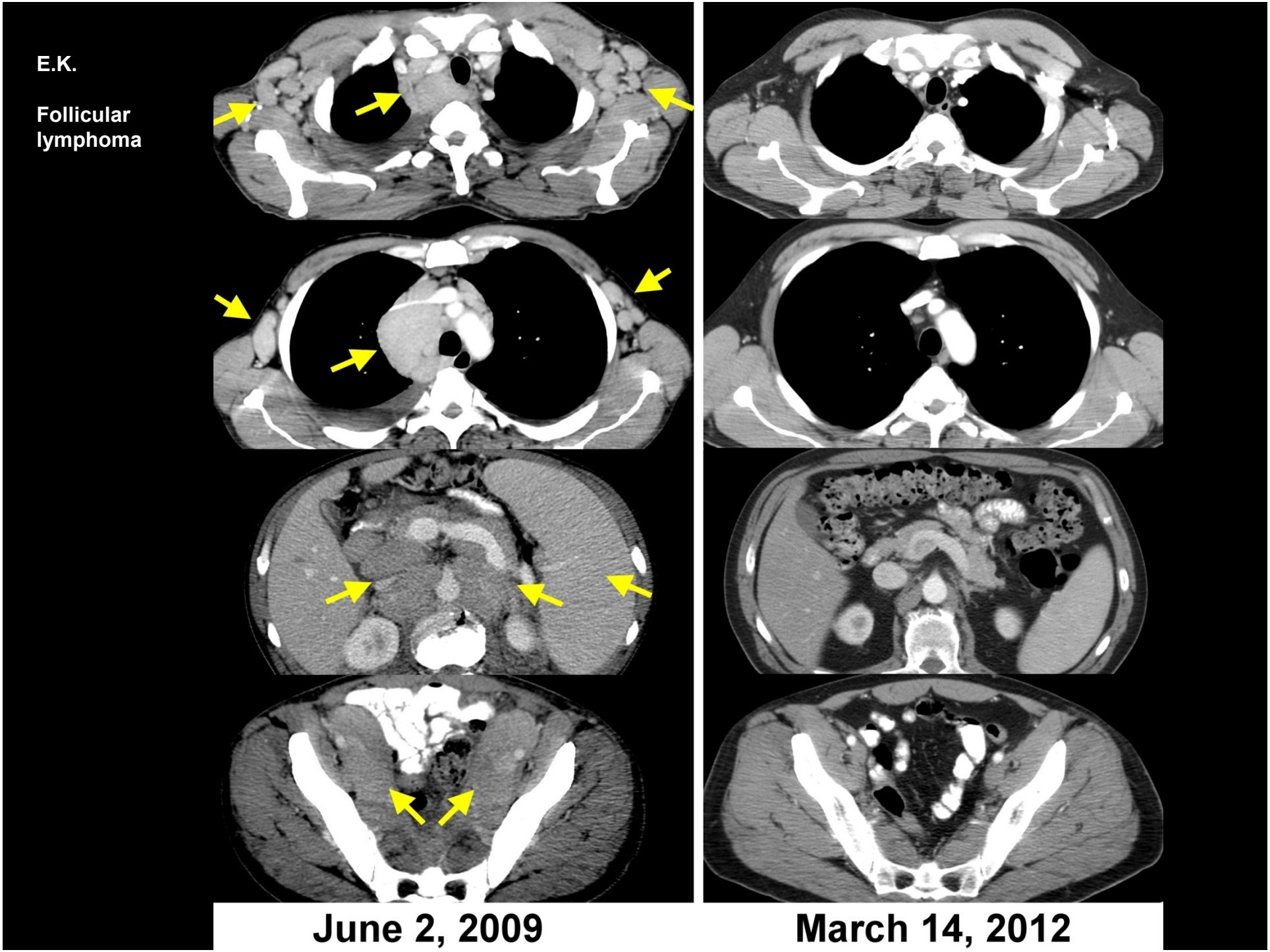
Pt	Inits	Date Cells	N	Agent	Schedule	Date Started
1	D.S.	04/21/2010	1)	FLUDARABINE	6 cycles	XX/XX/2003
			2)	CYCLOPHOSPHAMIDE	7 cycles	03/XX/2006
				FLUDARABINE	7 cycles	
				MIXOXANTRONE	7 cycles	
			3)	METHYLPREDNISONE	21 days	XX/XX/2008
			4)	SOLUMEDROL	11 cycles	05/XX/2009
2	R.L.	01/13/2011	1)	CYCLOPHOSPHAMIDE	12 cycles	05/XX/1999
				VINCRISTINE	12 cycles	
				PREDNISONE	12 cycles	
			2)	CHLORAMBUCIL		XX/XX/2005
				PREDNISONE		
			3)	CYCLOPHOSPHAMIDE	7 cycles	XX/XX/2008
				FLUDARABINE	7 cycles	
			4)	SOLU-MEDROL	PULSED	08/XX/2010
				OFATUMUMAB		
3	S.T.	11/04/2011	1)	R-CHOP	6 cycles	09/16/2010
				RITUXIMAB		
				CYCLOPHOSPHAMIDE		
				DOXORUBICIN		
				ONCOVIN		
				PREDISOLONE		
			2)	IFOSFAMIDE/CARBOPLATIN	2 cycles	07/07/2011
				RITUXIMAB/ETOPOSIDE	2 cycles	
			3)	HYPER-CVAD	1 cycle	09/12/2011
				RITUXIMAB	1 cycle	

Treatment of Refractory B-cell Lymphomas and Chronic Lymphocytic Leukemia with anti-DC19 CAR

		PR	CR	Total
		numbers (duration in months)		
Lymphoma	5	3 (60%) (34+,23+,15+)	1 (20%) (5+)	4 (80%)
Chronic lymphocytic leukemia	5	2 (40%) (6,3+)	2 (40%) (20,15+)	4 (80%)

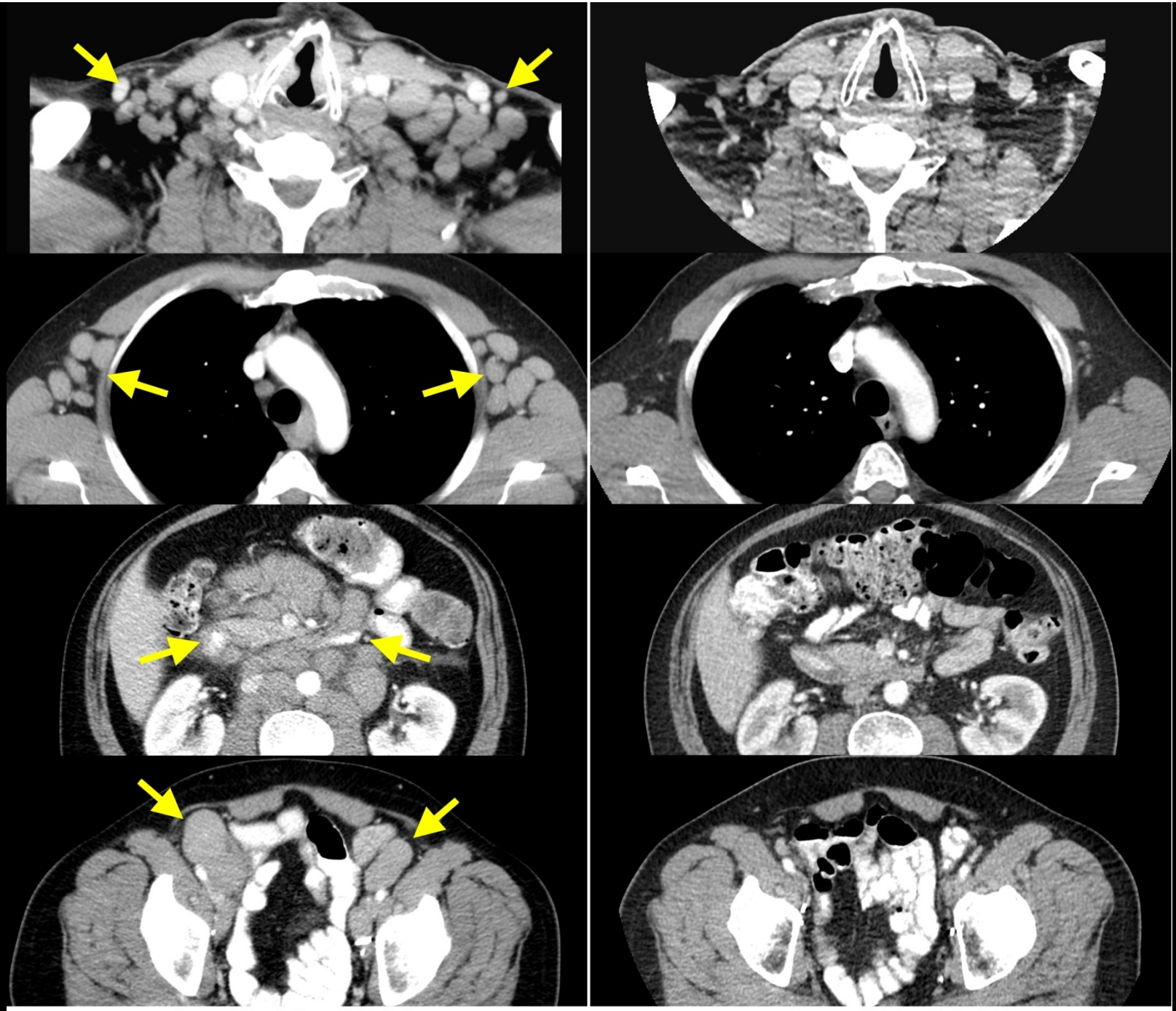
E.K.

Follicular lymphoma



D.S.

Chronic lymphocytic leukemia



April 13, 2010

Jan 25, 2012

The most critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

---

1. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

2. Shared antigens unique to cancer (cancer-testes antigens)

3. Mutations unique to each cancer (EGFRvIII)

4. Critical components of the tumor stroma (VEGFR2, FAP)

5. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

Melanoma/Melanocyte Differentiation Antigens

1994 MART-1 and gp100 cloned by Kawakami et al.
1996 TRP-2 cloned by Wang et al.

---

26 of 50 consecutive TIL used for therapy recognized these antigens
no correlation with response:
14 objective responders
12 non-responders

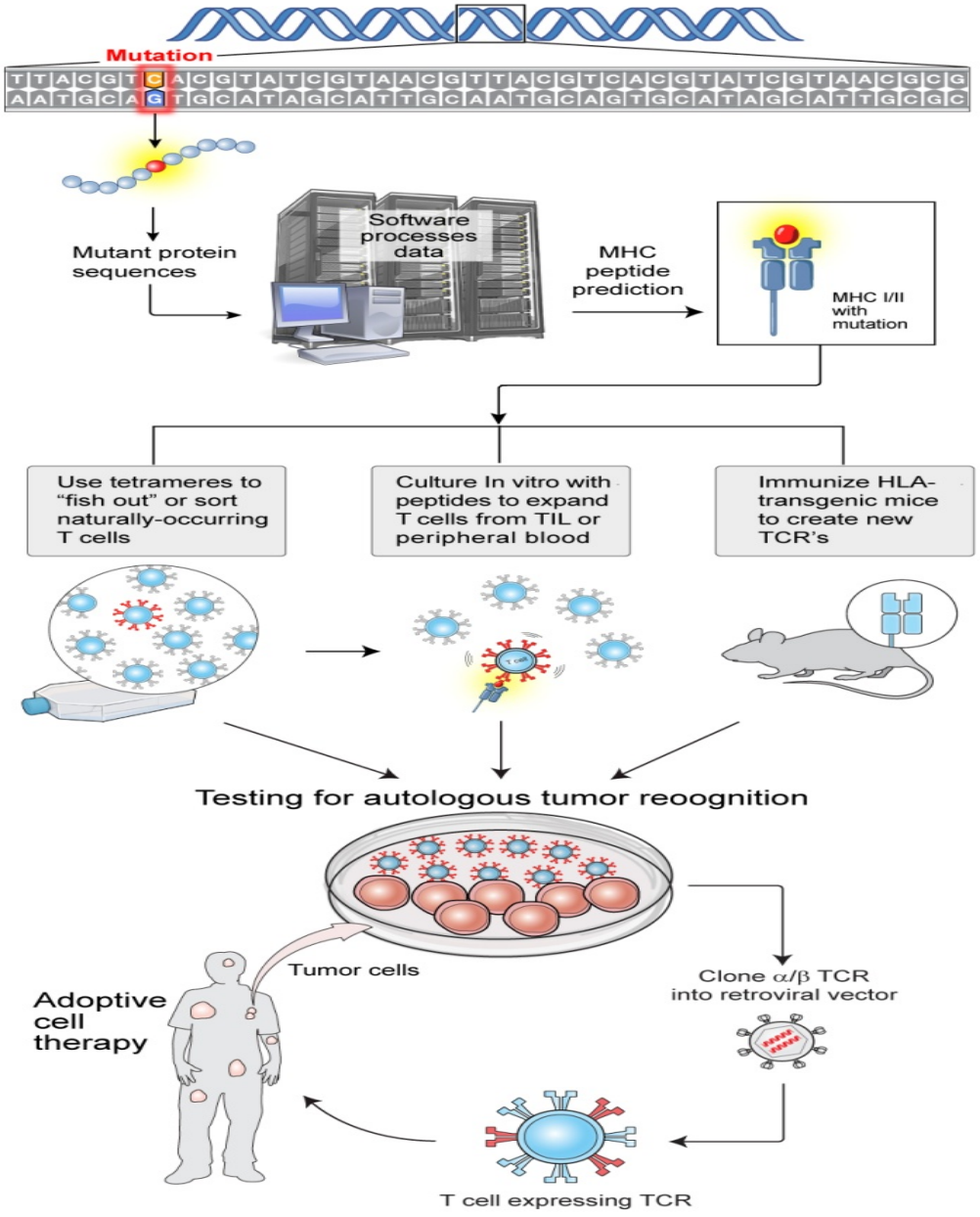
1 of 93 patients treated with TIL had severe eye and ear toxicity

16 of 36 (42%) patients treated with PBL transduced with high-affinity anti-MART-1 or gp100 had eye and/or ear toxicity

These M/M antigens do not appear responsible for tumor rejection

Expression cloning of antigens recognized by TIL responders (since 1994) have identified 8 mutated antigens restricted by Class I

Opportunities for personalized immunotherapy based on exomic identification of mutations



Cells reactive with autologous tumor are highly enriched in the subsets expressing PD-1, LAG-3, TIM-3 and 41BB

Populations scrted FrTu#1913	T cells	Aut. TC1913 A 0201	Aut. TC1913 + W6/32	Allo. TC2301	COS A11 1 µM Irrel. Pept	COS A11 1 µM $CDKN2A_{mut}$
PD1+	0 (2.1)	9633 (46.1)	57 (12.9)	268 (3.1)	9 (1.3)	17762 (30.3)
PD1-	0 (0.5)	0 (1.0)	0 (0.9)	176 (3.2)	69 (0.7)	68 (0.6)
LAG-3+	0 (1.3)	15290 (61.2)	221 (16.5)	0 (0.6)	0 (1.0)	23587 (55.7)
LAG-3-	0 (1.7)	0 (1.2)	0 (1.4)	632 (4.2)	363 (1.3)	427 (1.4)
TIM-3+	0 (1.2)	11954 (58)	102 (11.4)	1190 (2.9)	0 (0.5)	21140 (56.3)
TIM-3-	0 (0.8)	0 (1.0)	0 (0.9)	79 (3.0)	100 (0.5)	92 (0.4)
41BB+	55 (10.3)	6418 (39.6)	44 (10.0)	1767 (11.2)	11 (1.7)	12557 (19.5)
41BB-	0 (0.6)	0 (1.0)	0 (0.8)	106 (2.7)	1874 (1.3)	2026 (1.4)

pg/ml IFN γ (% 41BB+)
>200 pg/ml and >twice background is positive

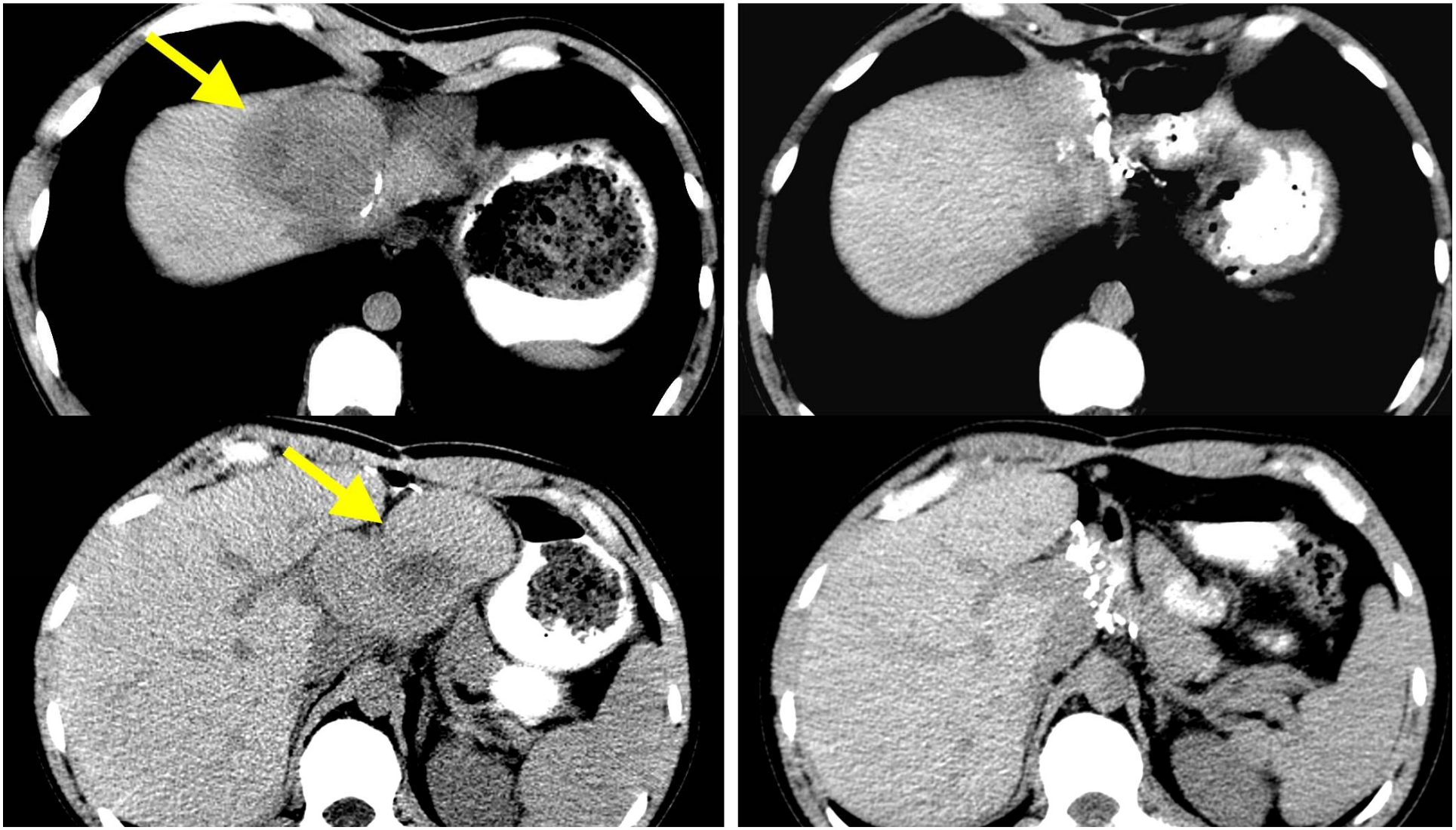
Objective Responses in Patients with Metastatic Melanoma

	Total	CR	PR	OR
		number of patients (%)		
Dacarbazine[1,2]	220	0	12(5.5%)	12(5.5%)
Interleukin-2[3,4]	270	17(6.3%)	26(9.6%)	43(15.9%)
	305	13(4.3%)	26(8.5%)	39(12.8%)
Ipilimumab[5]	540	3(0.6%)	35(6.4%)	38(7.0%)
Vemurafenib[2]	219	2(0.9%)	104(47.5%)	106(48.4%)
Cell Transfer[6]	93	20(21.5%)	32(34.4%)	52(55.9%)

1) Middleton et al JCO, 18:158, 2000
2) Chapman et al NEJM, 364:2507, 2010
3) Atkins et al JCO, 17:2105, 1999
4) Smith et al CCR, 14:5610, 2008
5) Hodi et al NEJM, 363:711, 2010
6) Rosenberg et al CCR, 17:1-8, 2011

Other Sites: Portal LN, brain

CR 57+ mo.



July 12, 2005

April 12, 2010

(595 synonymous mutations)

Melanoma/Melanocyte Differentiation Antigens

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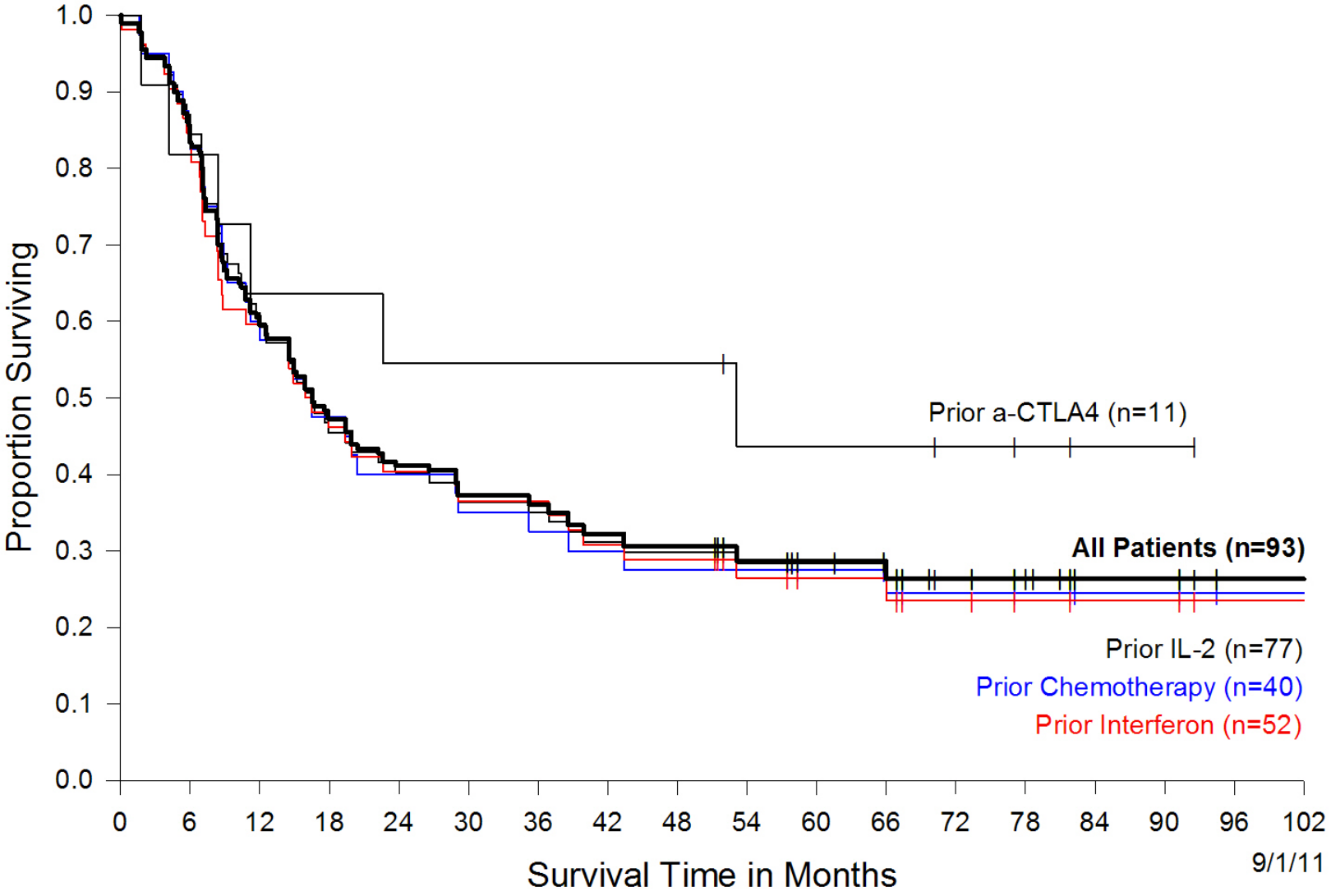
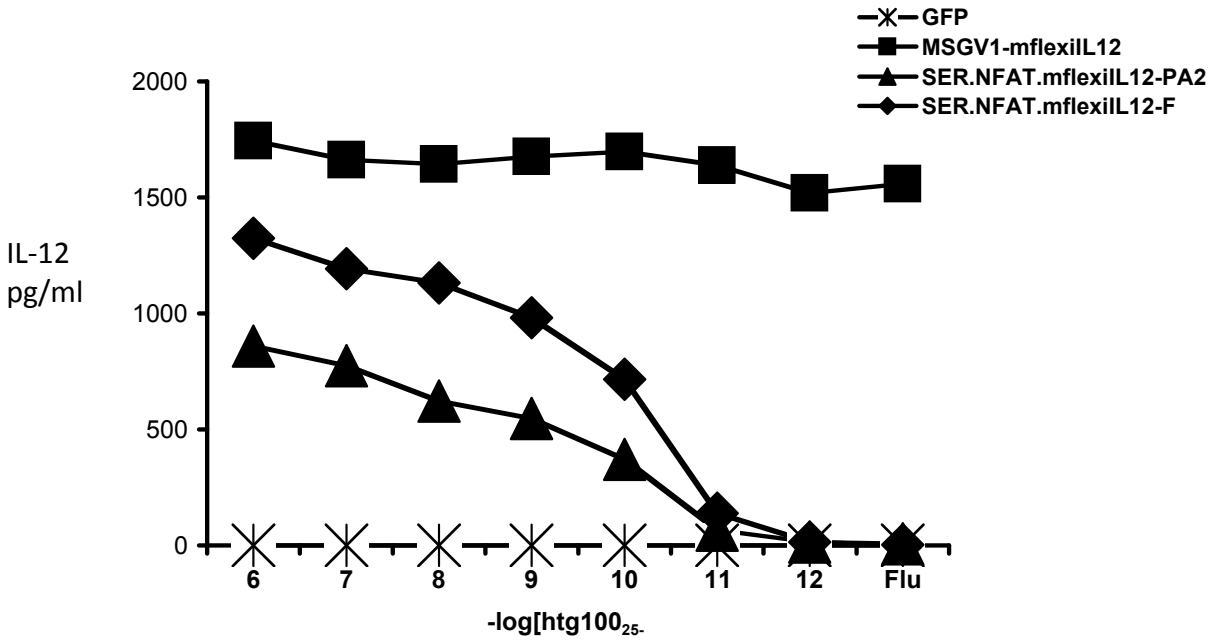
Impact of Prior Therapy on Response to Adoptive Cell Therapy using Selected Young TIL

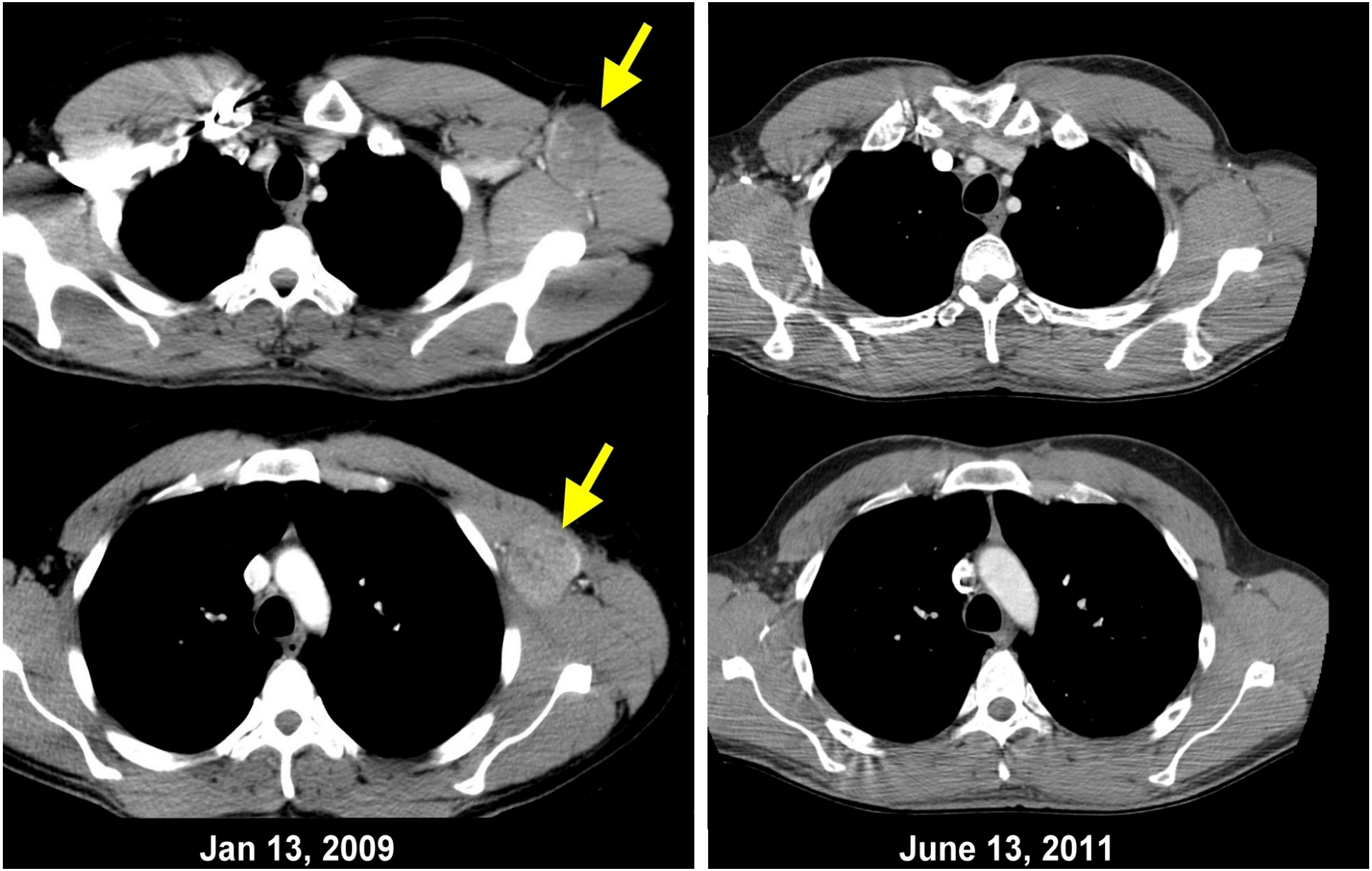


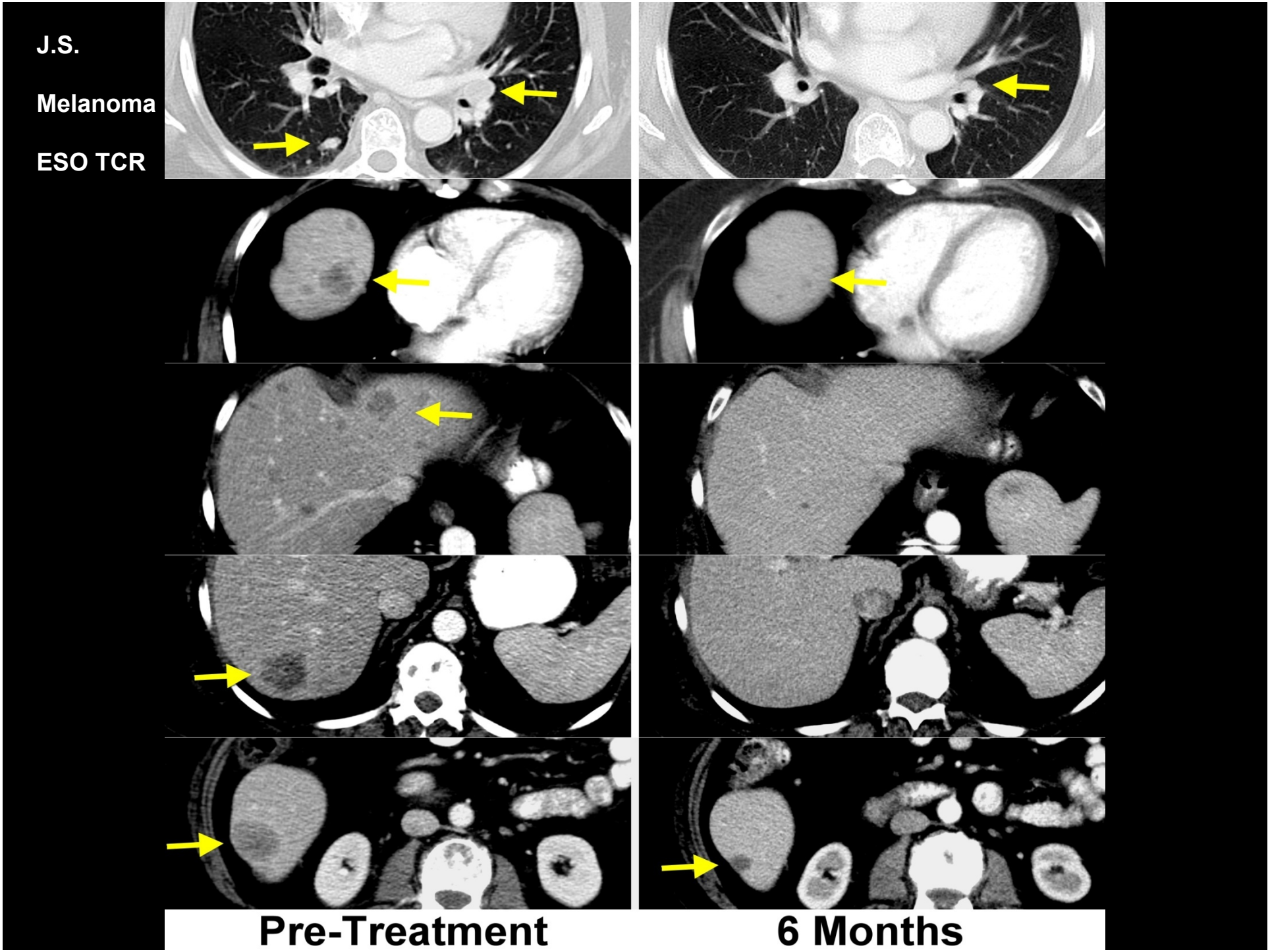
Development of Retrovirus NFAT-mflexiIL12 vector



(L. Zhang, R. Morgan, Surgery Branch, NCI)

M.M.-- Metastatic Melanoma




J.S.
Melanoma
ESO TCR
Pre-Treatment
6 Months

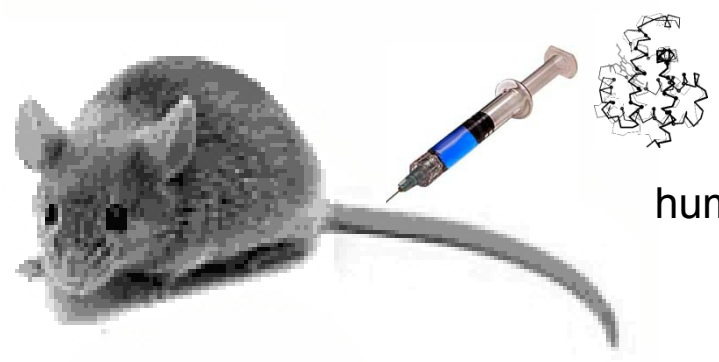
MAGE-A3 (Melanoma Antigen Gene)

- ➢ **MAGE-A3** is a member of the MAGE super family
- ➢ MAGE-A3 is expressed in tumor cells and not in normal tissue except testicular germ cells that are non-MHC-expressing cells
- ✓ Therefore, the **risk of toxicity to normal tissue may be very low**
- ✓ MAGE is also expressed in **non-melanoma epithelial malignancies, thus has potential to treat other types of cancer**

Therefore, MAGE-A3 may be an attractive target for TCR based gene therapy

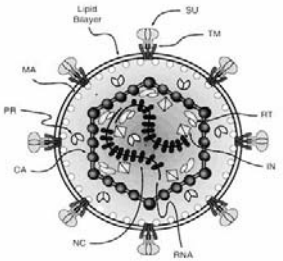
Using transgenic mice to generate T cells specific for human tumor antigens

Clone human MHC class I

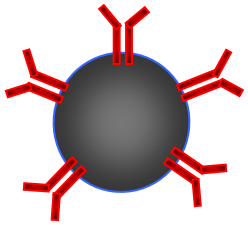


Immunize with human cancer antigen

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

Interferon-γ production by the MAGE-TCR transduced PBL following co-culture with tumor cell lines

Cell line	Histology	Interferon-γ (pg/ml) MAGE-A3: 112-120	MAGE-A3: 271-279
2361	RCC	<30	<30
H2721	SCLC	<30	<30
1088	Mel	<30	<30
888	Mel	<30	<30
H1299	NSCLC	<30	<30
H2122	SCLC	<30	<30
938	Mel	<30	<30
397-A*0201	Mel	25077	1992
526	Mel	17142	1996
1300	Mel	68746	7212
2984	Mel	36330	4605
624.38	Mel	29090	4131
1359-A*0201	Mel	32166	5759
H1299-A*0201	NSCLC	26229	475
H1250	SCLC	7730	231

MAGE-A3: 112-120 TCR recognized tumor cells more efficiently

Patients on MAGE-A3 TCR Protocol (F/U 3/1/12)

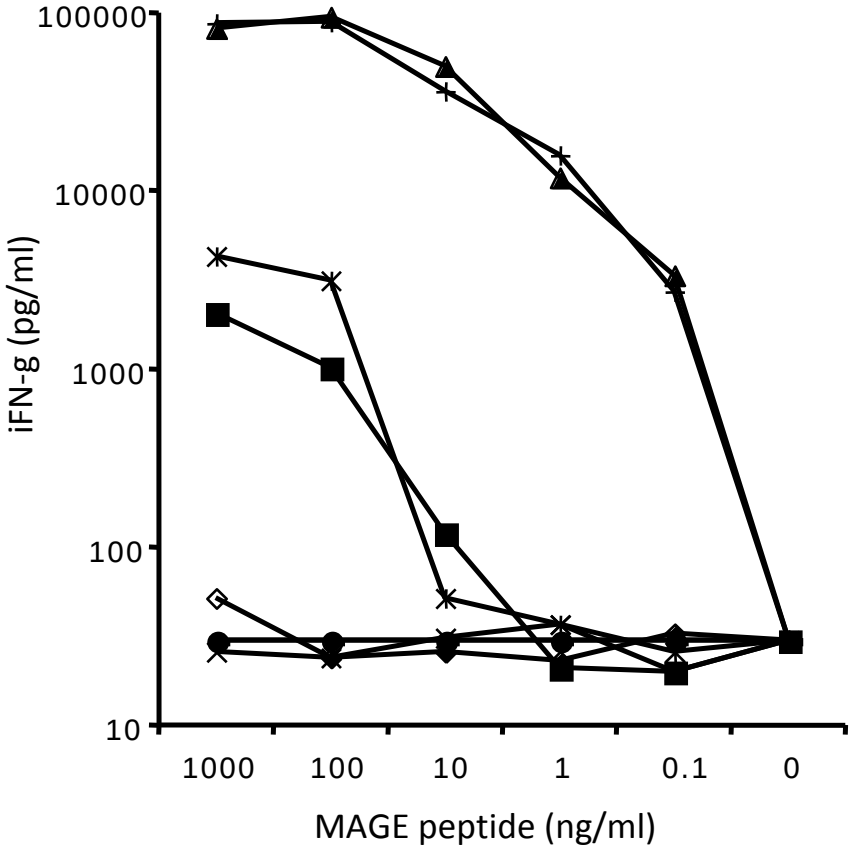
	Patient		Diagnosis	Date of cells	# cells ($x10^{-9}$)	#IL-2 doses	Response	Neurologic
1.	L.A.	59	Melanoma	2/24/11	28	6	CR(12+)	None
2.	J.P.	38	Melanoma	3/24/11	30	5	NR	None
3.	P.M.	56	Melanoma	5/5/11	30	7	PR(4)	None
4.	K.H.	21	Synovial Sarc.	6/10/11	41	1	PR(5)	None
5.	M.S.	54	Melanoma	7/22/11	79	5	PR(4)	Coma (white matter)
6.	J.M.	44	Melanoma	8/5/11	53	4	NR	None
7.	F.B.	62	Melanoma	8/17/11	62	6	CR(6+)	Seizure (normal MRI; recovered completely)
8.	G.T.	71	Esophageal	8/18/11	61	1	NR	Coma (white matter)
9.	J.S.	62	Melanoma	8/31/11	30	0	NR	TIA (Normal MRI; recovered completely)

Can this MAGE-A3 TCR recognize other related MAGE peptides?

MAGE peptides

MAGE A1	KVA**D**LV**G**FL	
MAGE A2	K**MV**ELVHFL	√
MAGE A3	KVAELVHFL	
MAGE A4	KV**D**EL**A**HFL	
MAGE A6	KVA**K**LVHFL	√
MAGE A8	KVAELV**R**FL	
MAGE A12	K**M**AELVHFL	
MAGE C2	KVAELV**E**FL	

T2 cell co-culture assay



MAGE-A3 TCR transduced PBL also recognized MAGE-A12 peptide efficiently and MAGE-A2 and A6 peptides at higher concentrations

Construction and evaluation of retroviral vectors encoding CAR against human VEGFR-2 (KDR)

KDR-1121 (IMC-1121B): A fully human anti-human VEGFR-2 (KDR) antibody - currently being evaluated in Phase II/III clinical trials.

KDR1121-hCD828BBZ) (KDR CAR)

KDR ScFv

TM

Human T cell signaling domains

Phase I dose escalation trial ongoing.

The most critical challenge confronting the development of human cancer immunotherapy is the identification of antigens to target

---

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2. Shared antigens unique to cancer (cancer-testes antigens)

3. Mutations unique to each cancer (EGFRvIII)

4. Critical components of the tumor stroma (VEGFR2, FAP)

5. Differentiation antigens overexpressed on cancers compared to normal tissue (MART-1, gp100, CEA, Her-2)

Interleukin-12

1989 **Discovered by G. Trinchieri**

- **two chains p35 & p40**
- **produced by dendritic cells and macrophages**
- **central role in innate and adaptive immunity**

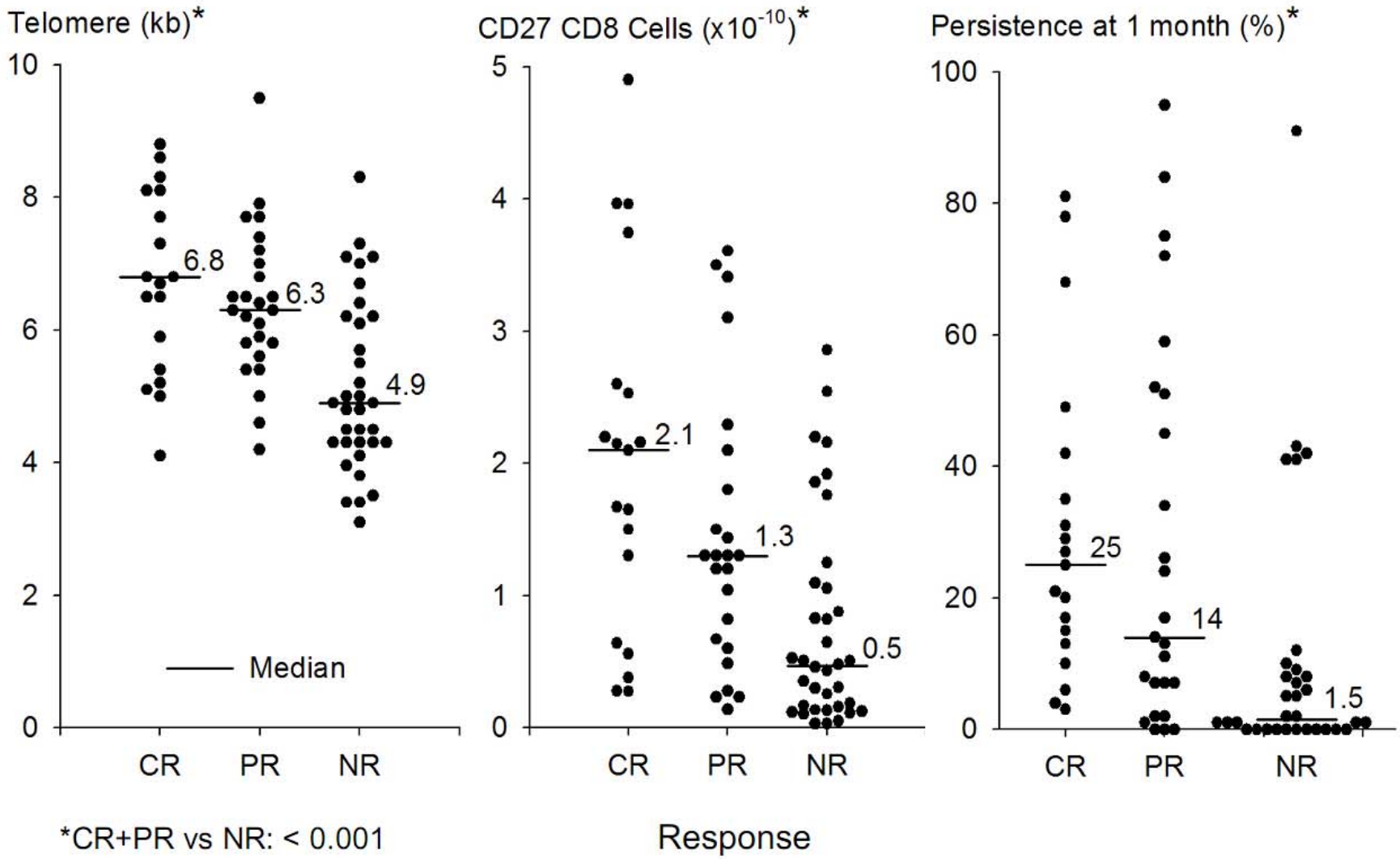
1995 **Highly toxic when administered systemically to cancer patients**

PLAN: Use TIL to deliver IL-12 directly to the tumor site.

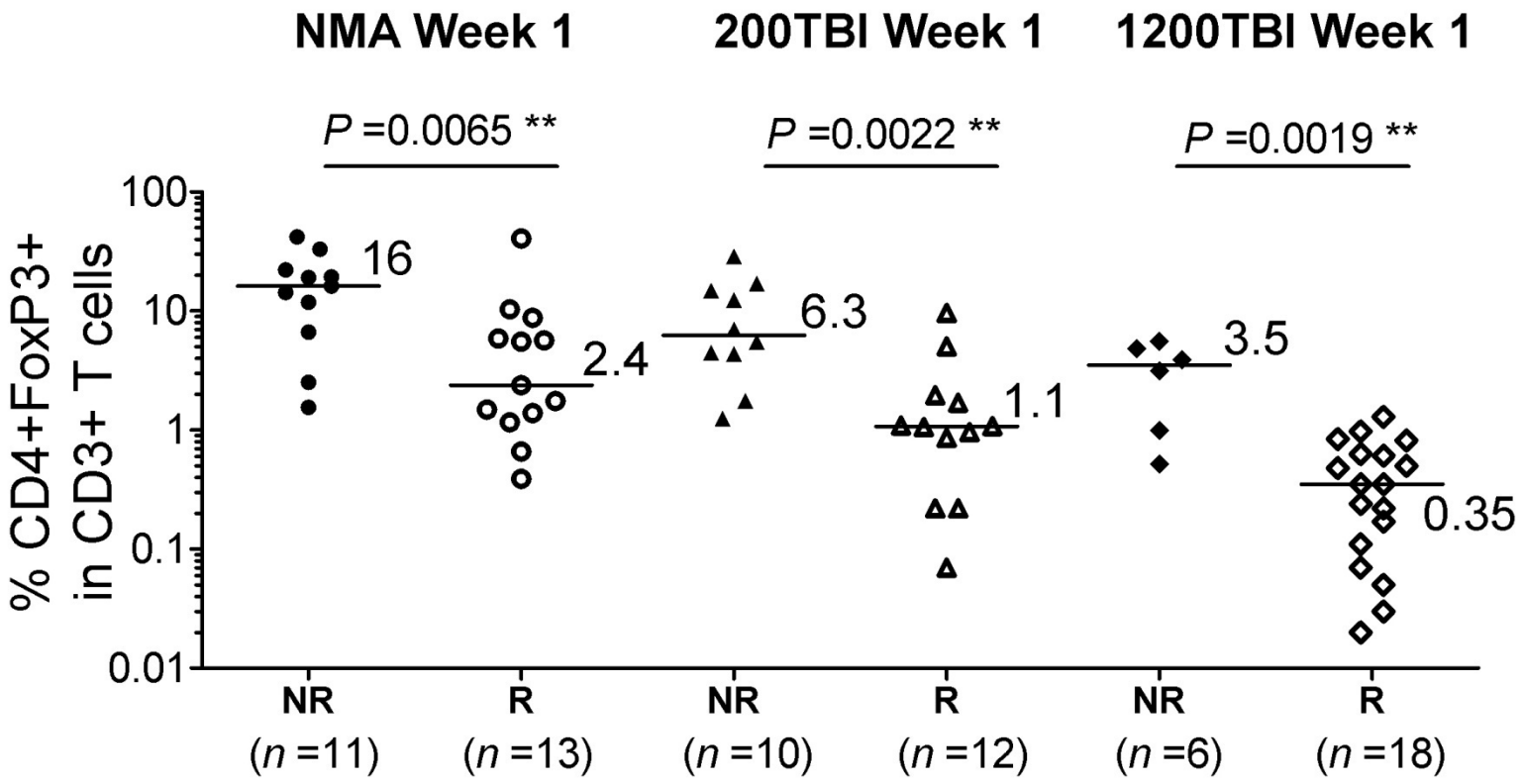
No Impact of Prior Treatment on the Incidence of Complete Regressions Using Selected TIL

	Total	CR number (%)
All Patients	93	20(22%)
Prior Treatment		
None	5(5%)	2(40%)
IL-2	77(83%)	14(18%)
Chemotherapy	40(43%)	7(18%)
Interferon	52(56%)	11(21%)
Anti-CTLA4	11(12%)	5(45%)
IL-2+ Chemotherapy	37(40%)	6(16%)
IL-2+ Anti-CTLA4	8(9%)	3(38%)
IL-2+ Anti-CTLA4+ Chemotherapy	6(7%)	2(33%)

Cell and Host Factors Associated with Response to Cell Transfer Therapy



Reconstitution with CD4+Foxp3+ cells is negatively correlated with clinical response



(Xin Yao, Surgery Branch, NCI)

Hypothesis of Mechanism of Cancer Regression Following Cell Transfer

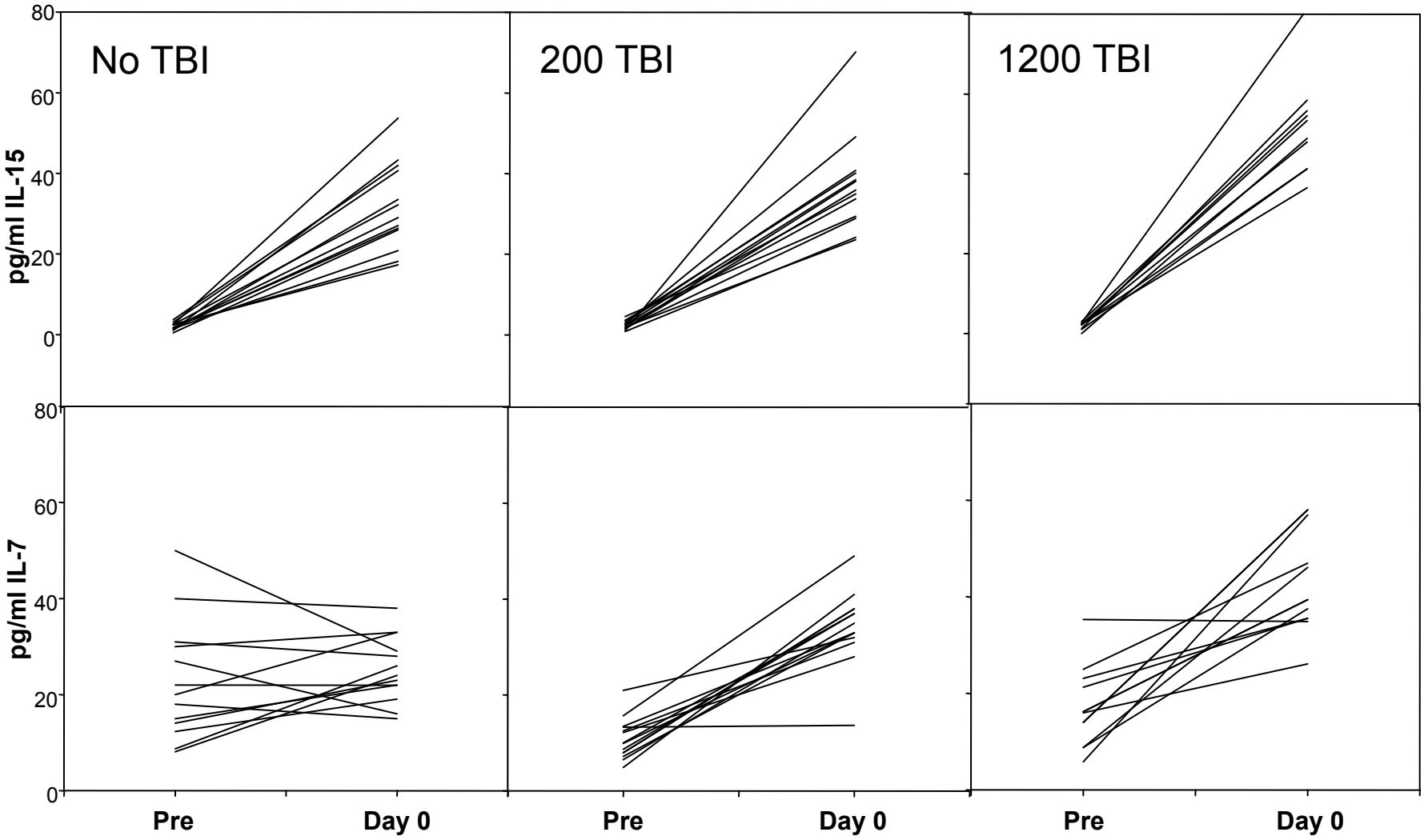
The lymphophenic environment

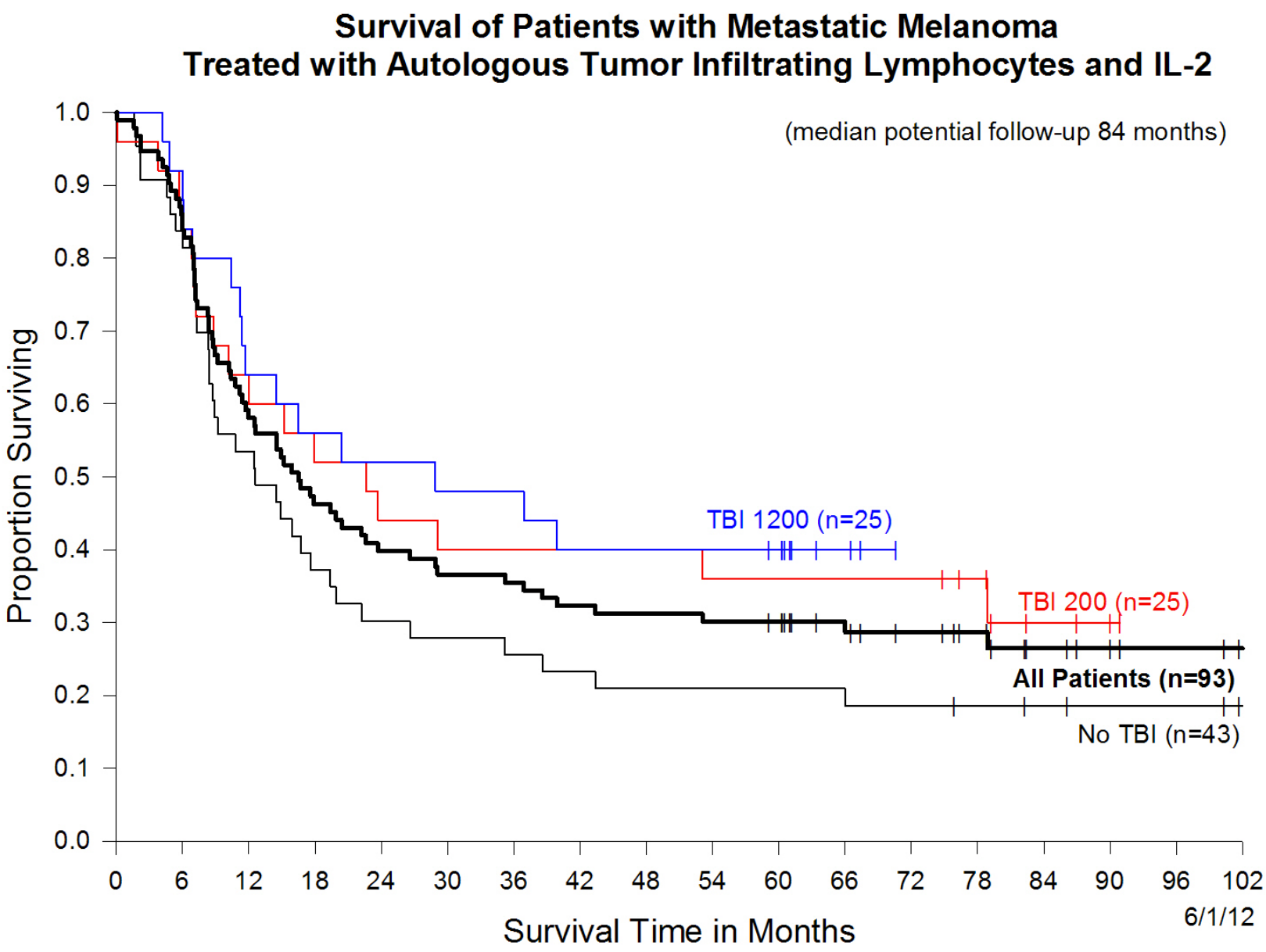
1) eliminates T regulatory (suppressor) cells

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




Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(median potential follow-up 84 months)
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)
All Patients (n=93)
No TBI (n=43)
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Survival Time in Months
6/1/12

The Puzzle of Melanoma Immunogenicity

Melanoma, among the many cancer histologies appears to be unique in:

1. **susceptibility to treatment with immune modulators**
   - **e.g. IL -2**
   - **anti-CTLA4**
   - **anti-CD40**
   - **anti-PD1**

2. **generating infiltrating lymphocytes (TIL) that recognize cancer-associated antigens**

Two intertwining questions:

Why is melanoma uniquely immunogenic?

What do TIL recognize that enables the in vivo destruction of the last cancer cell?

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1996 TRP-2 cloned by Wang et al.

---

16 of 36 (42%) patients treated with PBL transduced with high-affinity anti-MART-1 or gp100 had eye and/or ear toxicity (25% objective response rate)

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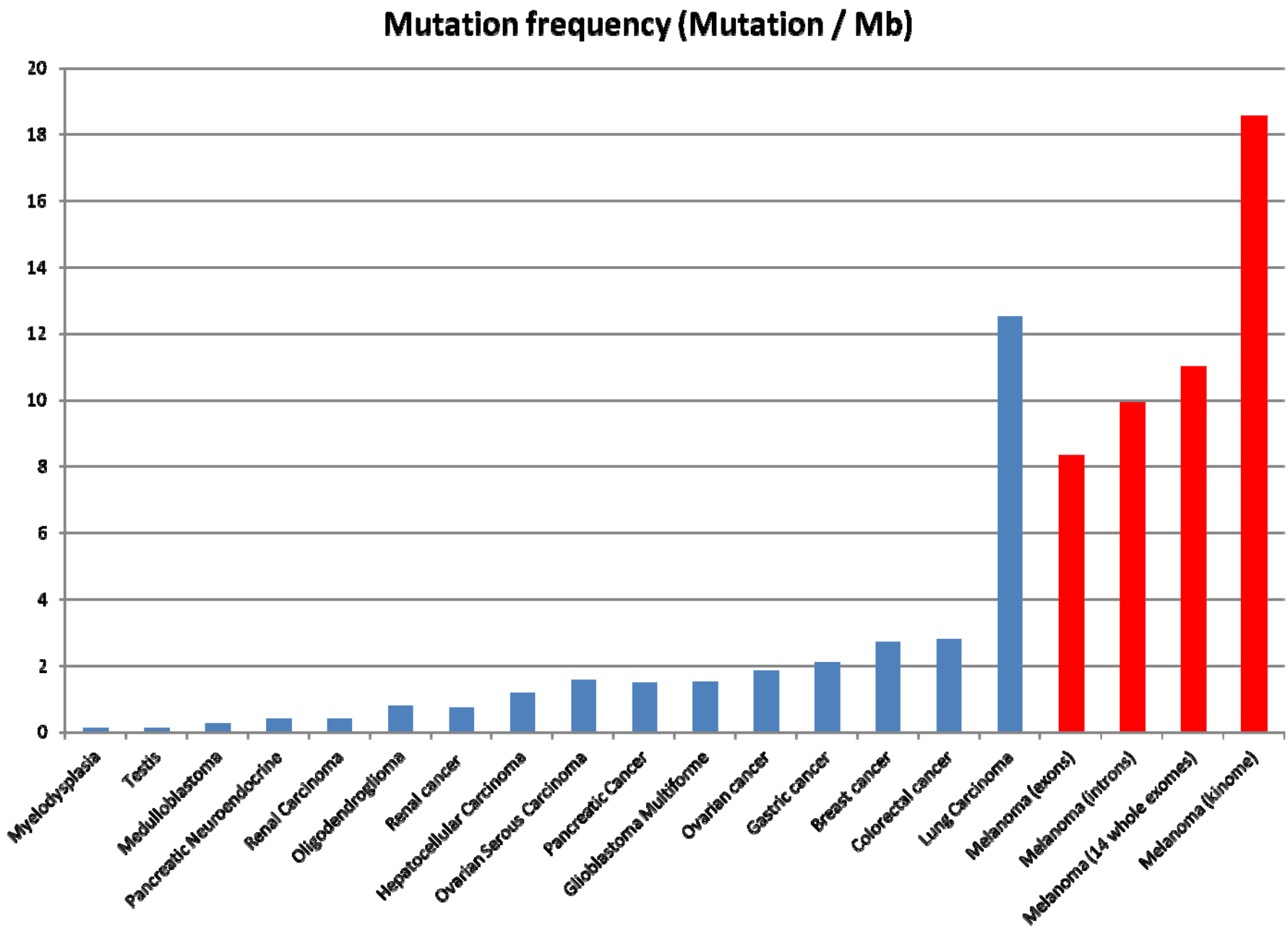
These M/M antigens do not appear responsible for tumor rejection

Wide variation in # of non-synonymous mutations detected in melanomas

Tumor	Date	Patient	# of Nonsynonymous mutations
TC 1477	11/2/1994	BA	142
TC 2589	8/17/2005	TB	308
TC 3309-3	7/8/2009	RC	303
TC 2098	12/12/2002	BC	279
TC 2167a	4/30/2003	PD	415
TC 2265	12/16/2003	ED	32
TC 2555-2	11/16/2005	GJ	1,637
TC 2202	7/22/2003	EG	121
TC 2224	8/28/2003	AH	105
TC 2159	4/1/2003	KH	95
TC 2272	1/22/2004	DH	114
TC 2427	12/29/2004	AH	5839
TC 2598	10/16/2006	JS	234
TC 2146	3/20/2003	AK	83
TC 2197	7/16/2003	JK	578
TC 2535-2a	9/23/2005	DL	579
TC 1946-3	7/10/2001	MM	108
TC 2479-2	5/26/2005	LN	234
TC 2531	9/16/2005	LN	182
TC 2183	6/10/2003	CP	646
TC 3338	8/12/2009	LP	337
TC 3396	11/16/2009	LP	302
TC 2614-1	3/15/2006	JR	306
TC 2232-L	9/16/2003	MR	350
TC 2650-2	6/30/2006	ER	219
TC2359	9/8/2004	DS	552
TC 2698	10/11/2006	SS	124
TC 2199	7/21/2003	TT	110
TC 2238	9/30/2003	AW	107
TC 2133-1	2/25/2003	EW	139
TC 2244	10/23/2003	CW	157
TC2591	2/8/2006	DW	235
TC 2207	7/24/2003	SW	116

Non-synonymous mutations

Average – 457 (289 w/o AH)
Median – 234
(n=33)


Mutation frequency (Mutation / Mb)
20
18
16
14
12
10
8
6
4
2
0
Myelodysplasia
Testis
Medulloblastoma
Pancreatic Neuroendocrine
Renal Carcinoma
Oligodendroglioma
Renal cancer
Hepatocellular Carcinoma
Ovarian Serous Carcinoma
Pancreatic Cancer
Glioblastoma Multiforme
Ovarian cancer
Gastric cancer
Breast cancer
Colorectal cancer
Lung Carcinoma
Melanoma (exons)
Melanoma (introns)
Melanoma (14 whole exomes)
Melanoma (kinome)

New Approach to Rapidly Identify Cancer Antigens Associated with Complete Cancer RegressionsBased on Exomic Sequencing

---

Exomic sequencing of fresh tumor or early cultured lines to determine the number of exomic mutations

Obtain sequence of 9 – 10 aa on either side of aa mutation

1) Use algorithm to predict best binders to that patient's HLA antigens

Synthesize best binding peptides

Test for recognition of pulsed peptides by TIL

2) Synthesize a minigene encoding the 19aa peptide containing each mutation

Transfect the minigene into autologous antigen presenting cells

Test for recognition by TIL

Patient D.S. (TIL 2369)

---

32 year old male with metastatic melanoma

Jan. 1996: excision of neck melanoma; positive lymph nodes
Feb. 1996: 1 year alpha interferon
July 2004: liver and brain metastases
Oct. 2004: High-dose IL-2; progressive disease
Jan. 2005: SRS to brain met; then excision
July 2005: TIL/200TBI/IL-2 with lymphodepletion

Complete regression ongoing as of March, 2012

(Exomic analysis revealed 595 nonsynonomous mutations)

Listing of the 20-mer peptides surrounding the central mutated amino acids (#2369)

Gene	cDNA change	protein change	nM	ref aa	var aa
C22orf33	c.G349A	p.G117S	2.3497	SSVFSDYYDLGYNMRSNLFRG	SSVFSDYYDL**S**YNMRSNLFRG
PLEKHM2	c.C3013T	p.H1005Y	3.206	VLTDDRLFTCHEDCQTSFFRS	VLTDDRLFTC**Y**EDCQTSFFRS
GRIN3B	c.A1861G	p.N621D	4.7952	STVFSYSSALNLCYAILFRRT	STVFSYSSAL**D**LCYAILFRRT
PLCB1	c.C2062T	p.L688F	5.2812	LSVKIISGQFLSDKKVGTYVE	LSVKIISGQFFSDKKVGTYVE
HEG1	c.C1807T	p.H603Y	5.9497	SSHSEYSSFFHAQTERSNISS	SSHSEYSSFFYAQTERSNISS
BAI3	c.C251T	p.S84L	6.9239	SKKDLSCSNFSLLAYQFDHFS	SKKDLSCSNFLLLAYQFDHFS
MPP4	c.T994C	p.F332L	7.2822	EETFESDKEEFVGYGQKFFIA	EETFESDKEELVGYGQKFFIA
OR4C46	c.C571T	p.H191Y	8.0429	PLLNLACTDTHMLELFIAANS	PLLNLACTDTYMLELFIAANS
UEVLD	c.C178T	p.P60S	12.364	KDLLNFTGTIPVMYQGNTYNI	KDLLNFTGTISVMYQGNTYNI
COL9A1	c.C467T	p.S156L	13.3332	INGQTQSVVFSYKGLDGSLQT	INGQTQSVVFLYKGLDGSLQT
LST-3TM12	c.C1066T	p.L356F	14.0447	LLHMSSYIASLTYIIKMVEQQ	LLHMSSYIASFTYIIKMVEQQ
OR4C46	c.C571T	p.H191Y	21.9828	PLLNLACTDTHMLELFIAANS	PLLNLACTDTYMLELFIAANS
OR2T2	c.G350A	p.G117D	22.418	TLIGGEFFLLGLMAYDRYVAV	TLIGGEFFLLDLMAYDRYVAV
MEOX2	c.G530A	p.G177E	23.3779	RKSDSSDSQEGNYKSEVNSKP	RKSDSSDSQEENYKSEVNSKP
OR8B3	c.C364T	p.R122C	31.391	CYMLTSMAYDRYVAICNPLLY	CYMLTSMAYDCYVAICNPLLY
PPP1R3B	c.C527A	p.P176H	48.7889	FDTWKSYTDFPCQYVKDTYAG	FDTWKSYTDFHCQYVKDTYAG
LRP2	c.G10030A	p.A3344T	51.034	YLYWADWGHRAYIGRVGMDGT	YLYWADWGHRTYIGRVGMDGT
LRRC3B	c.C656T	p.S219L	53.9571	TMFGWFTMVISYVVYYVRQNQ	TMFGWFTMVILYVVYYVRQNQ
C15orf2	c.C1583T	p.S528F	71.4286	SMCVDSPPPLSFLTLLPVPST	SMCVDSPPPLFFLTLLPVPST
RNPEP	c.C592T	p.P198S	85.0666	KYKYSALIEVPDGFTAVMSAS	KYKYSALIEVSDGFTAVMSAS
MIRO-2	c.C799T	p.R267W	86.0664	QWTLVTYLDVRSCLGHLGYLG	QWTLVTYLDVWSCLGHLGYLG
PPP1R3B	c.C527A	p.P176H	100.1711	FDTWKSYTDFPCQYVKDTYAG	FDTWKSYTDFHCQYVKDTYAG
BCR	c.C2546T	p.S849F	103.6965	RNGKSYTFLISSDYERAEWRE	RNGKSYTFLIFSDYERAEWRE
ABCA12	c.C172T	p.P58S	124.4896	LNISANSPYIPYLACVRNVTD	LNISANSPYISYLACVRNVTD
KIAA1211	c.C2386T	p.P796S	125.3308	TEGCKFAKDLPSFLVPSLPYP	TEGCKFAKDLSSFLVPSLPYP
SYPL2	c.C374T	p.S125F	126.6517	AEFFVTLGIFSFFYTMAALVI	AEFFVTLGIFFFFYTMAALVI
PLCB1	c.C2062T	p.L688F	134.0483	LSVKIISGQFLSDKKVGTYVE	LSVKIISGQFFSDKKVGTYVE
PPP4R4	c.G953A	p.G318E	165.2447	SILISLSFHLGKLCHGLYGIF	SILISLSFHLEKLCHGLYGIF
FLRT2	c.C1330T	p.L444F	167.9839	DTSIQVSWLSLFTVMAYKLTW	DTSIQVSWLSFFTVMAYKLTW
HHLA2	c.C806T	p.S269F	171.8293	TWSRMKSGTFSVLAYYLSSSQ	TWSRMKSGTFFVLAYYLSSSQ
HHLA2	c.C806T	p.S269F	172.6459	TWSRMKSGTFSVLAYYLSSSQ	TWSRMKSGTFFVLAYYLSSSQ
CDH5	c.C1106T	p.P369L	180.1531	VIINITDVDEPPIFQQPFYHF	VIINITDVDELPIFQQPFYHF

Exome sequencing of melanoma 2369

	Peptide[!]	Mutation[#]	Affinity (nM)	Gene	IFN-γ (pg/ml)
1	FSDYYDL**S**Y	117 G to S	2	C22orf33	<30
2	LTDDRLFTC**Y**	1005 H to Y	3	PLEKHM2	10400
3	YSSAL**D**LCY	621 N to D	5	GRIN3B	<30
4	**F**SDKKVGTY	688 L to F	5	PLCB1	<30
5	HSEYSSFF**Y**	603 H to Y	6	HEG1	<30
6	CSNF**L**LLAY	84 S to L	7	BAI3	<30
7	ESDKEE**L**VGY	332 F to L	7	MPP4	<30
8	CTDT**Y**MLELF	191 H to Y	8	OR4C46	<30
9	FTGTI**S**VMY	60 P to S	12	UEVLD	<30
10	QTQSVVF**L**Y	156 S to L	13	COL9A1	<30
11	MSSYIAS**F**TY	356 L to F	14	LST-3TM12	<30
12	CTDT**Y**MLEL	191 H to Y	22	OR4C46	<30
13	LL**D**LMAYDRY	117 G to D	22	OR2T2	<30
14	SSDSQE**E**NY	117 G to E	23	MEOX2	<30
15	LTSMAYD**C**Y	122 R to C	31	OR8B3	<30
16	YTDF**H**CQYV	176 P to H	49	PPP1R3B	13400
17	WADWGHR**T**Y	3344 A to T	51	LRP2	<30
18	FTMVI**L**YVVY	219 S to L	54	LRRC3B	<30
19	CVDSPPPL**F**F	528 S to F	71	C15orf2	<30
20	V**S**DGFTAVM	198 P to S	85	RNPEP	<30
21	**W**SCLGHLGY	267 R to W	86	MIRO-2	<30
22	YTDF**H**CQYVK	176 P to H	100	PPP1R3B	22000
23	YTFLI**F**SDY	849 S to F	104	BCR	<30
24	ISANSPYI**S**Y	86 P to S	124	ABCA12	<30
25	**S**SFLVPSLPY	796 P to S	125	KIAA1211	<30

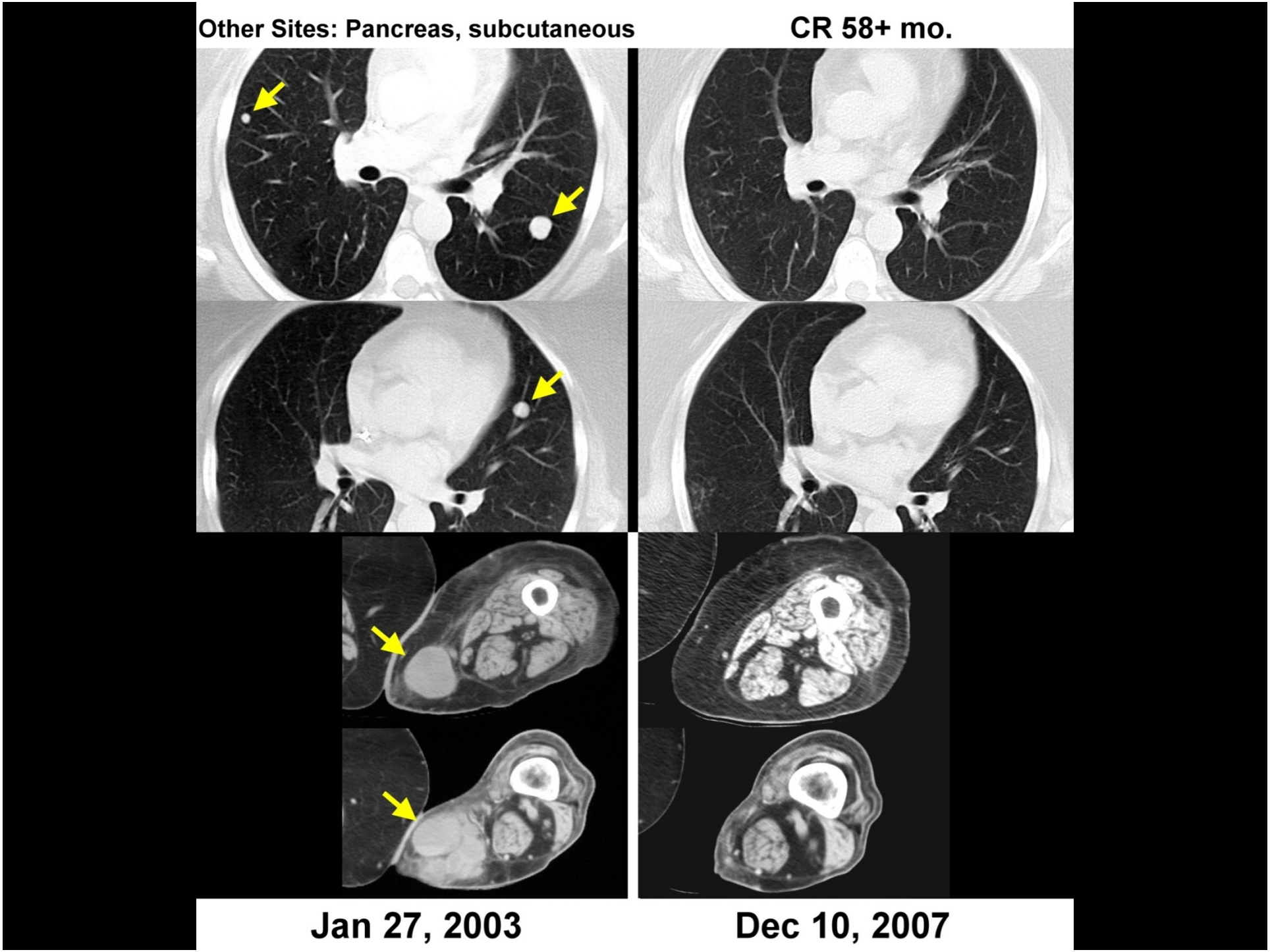
Mutated Antigens in Autologous Tumor Recognized by TIL 2369

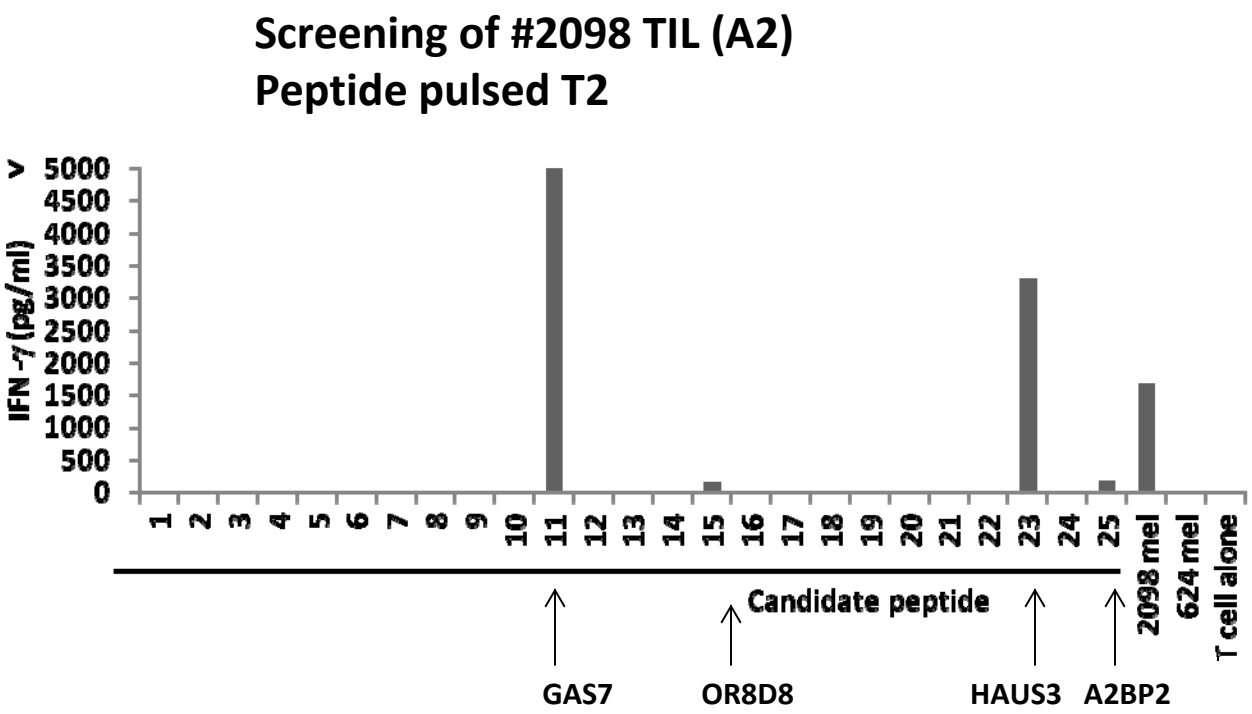
PLEKHM2: Pleckstrin homology domain – containing family member M2

interacts with kinesin and plays a role in microtubule formation

PP1R3B: Protein phosphatase 1 regulating subunit Ga protein

regulates glycogenesis in myotubes


Other Sites: Pancreas, subcutaneous
CR 58+ mo.
Jan 27, 2003
Dec 10, 2007



No.	Peptide	Sub.	Predicted affinity (nM)	Gene	IFN-γ (pg/ml)
1	FL**L**GKTSSV	136 Q to L	4	ZNF559	<30
2	YMFSD**C**STSL	91 R to C	4	TRPC6	<30
3	S**L**LSLLYAL	32 S to L	4	SERPINB11	<30
4	L**M**MPFSIVYI	76 V to M	4	HTR1F	<30
5	**F**QLNQSFEI	1281 S to F	6	UNC13A	<30
6	**F**ILDAVQRV	613 S to F	6	PXDNL	<30
7	SLAPLSPR**V**	310 A to V	7	CNKSR1	<30
8	LLGDPG**W**RV	72 R to W	11	KCNA6	<30
9	FSF**S**LDFLV	251 P to S	11	GPR174	<30
10	MLFLRF**C**YI	55 R to C	12	WDR47	<30
11	SLADEAEV**Y**L	229 H to Y	12	GAS7	47288
12	FLQ**K**YTVKL	183 E to K	13	GSTA4	<30
13	CLFL**E**IYTV	33 G to E	13	OR8D4	<30
14	TMS**F**SHLFYL	13 S to F	16	IGF1	<30
15	FL**E**IYTVTV	33 G to E	17	OR8D4	156
16	Y**L**TSLACVEI	1039 P to L	17	BRCA2	<30
17	LLADQN**F**KFI	190 L to F	20	RRP1B	<30
18	SLSTS**L**SSV	1084 S to L	21	CNTN5	<30
19	AMI**A**AKISNEL	160 T to A	22	C4orf15	<30
20	ALGTLLHTN**V**	1577 L to V	23	NOTCH2	<30
21	SVVDVF**F**QL	1281 S to F	28	UNC13A	<30
22	MLSILALV**R**V	65 G to R	31	C15orf32	<30
23	ILNAMI**A**KI	160 T to A	34	HAUS3	3300
24	GL**N**ETIAKL	990 D to N	35	MYH4	<30
25	YTAP**Y**PHPA	45 H to Y	36	A2BP1	181

Mutated Antigens in Autologous Tumor Recognized by TIL 2098

GAS7: Member of the Pombe Cdc 15 homology protein family

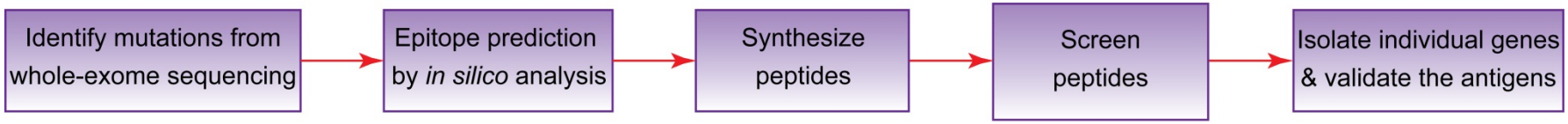
expressed primarily in growth arrested cells

HAUS3: augmin-like complex, subunit 3

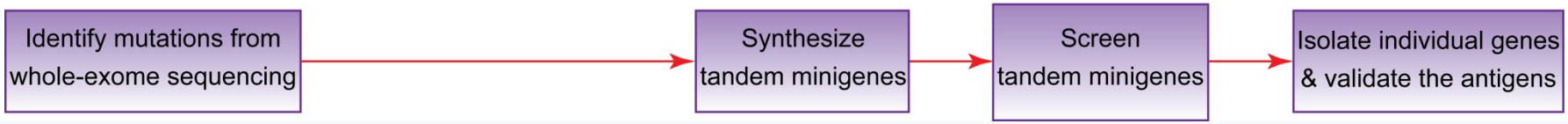
plays a role in microtuble formation within the mitotic spindle

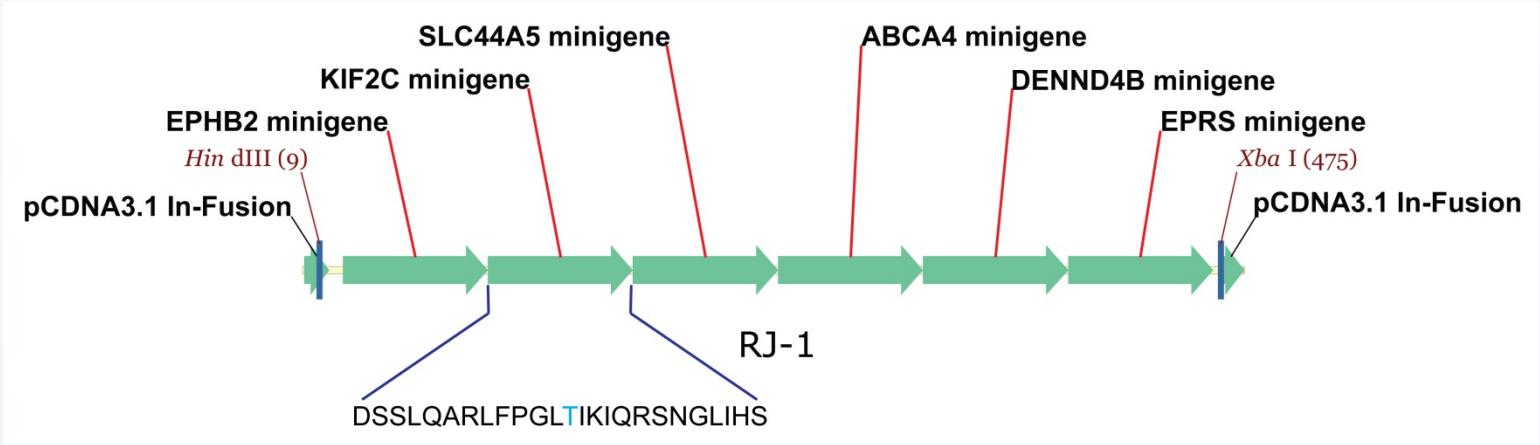
Exome sequencing-based antigen discovery

A. Peptide-based approach



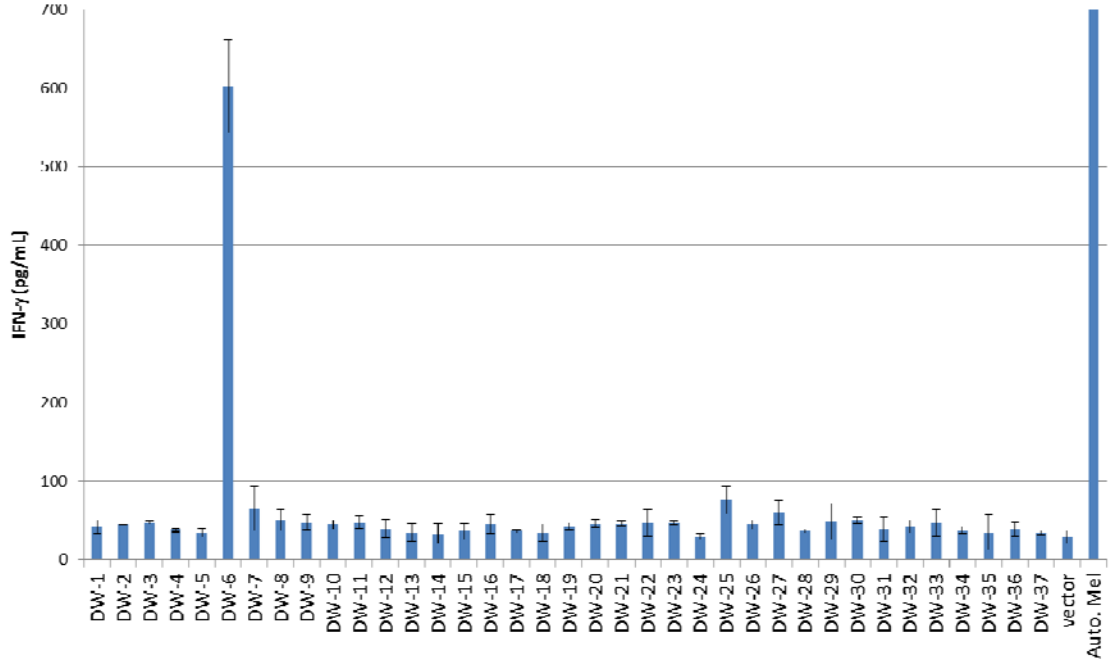
B. Minigene-based approach





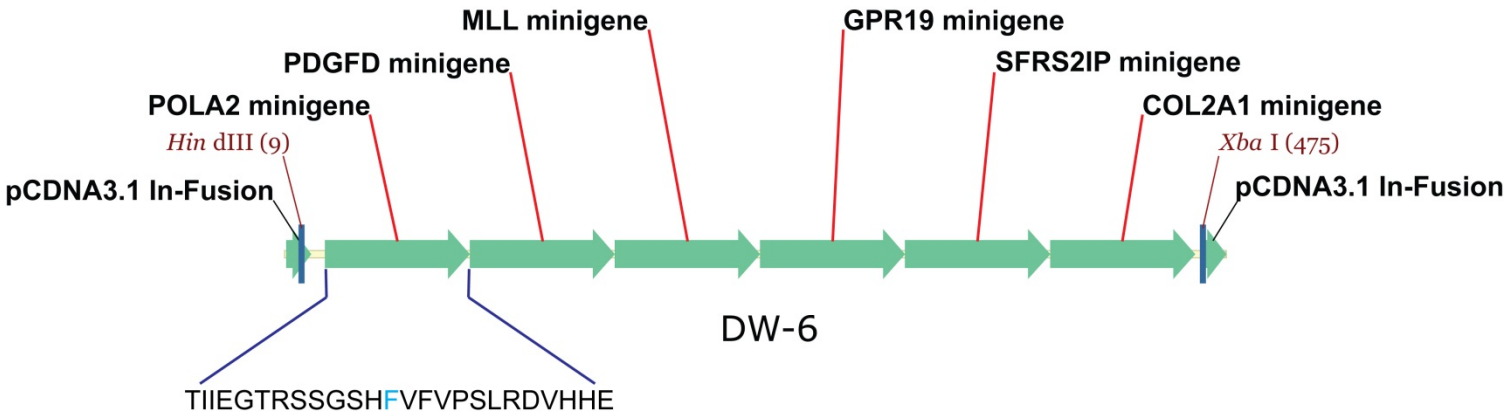
Minigene library approach: Tandem minigene DW-6 recognized by D. Wilson TILs

A



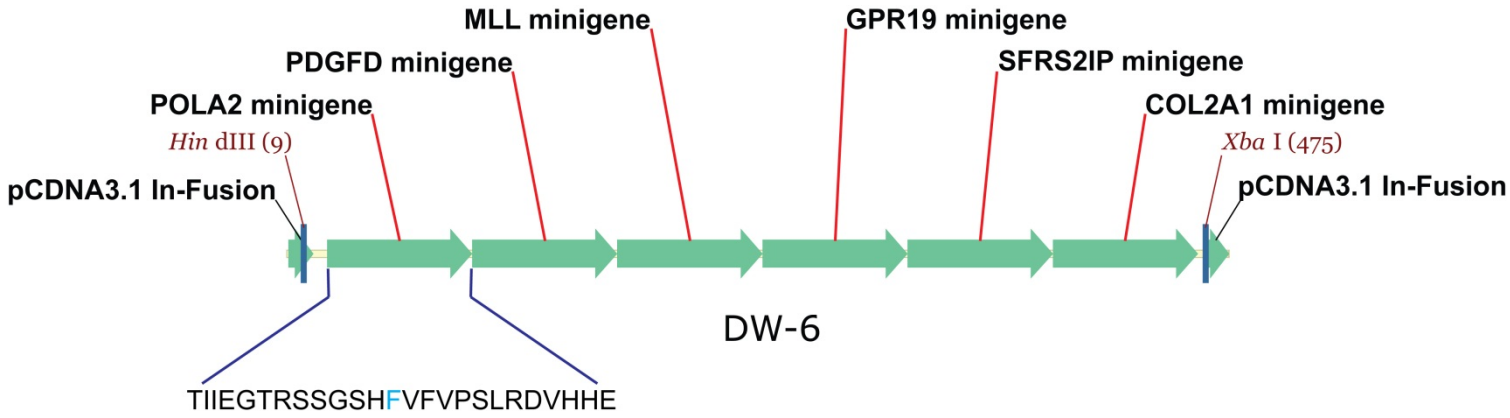
218 non-synonymous mutations
37 tandem minigene constructs
6 HLAs

B

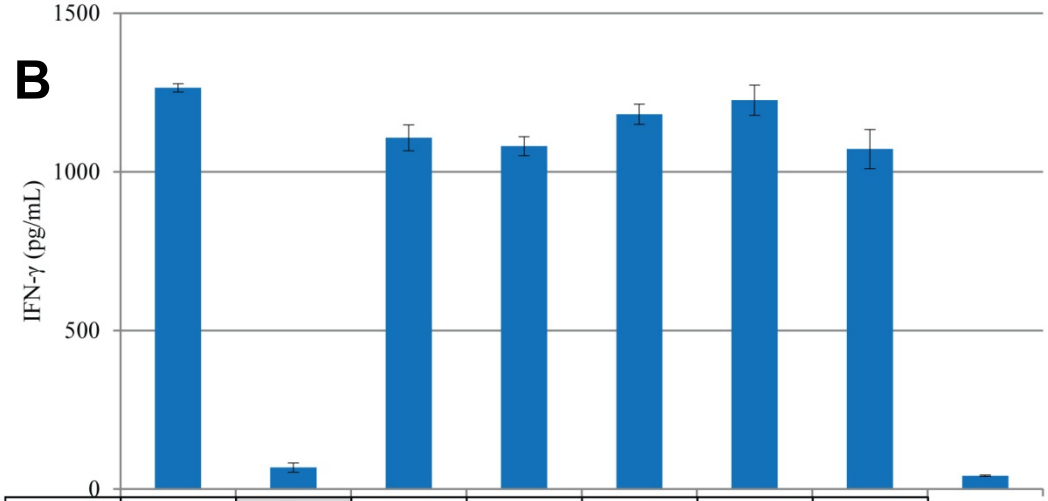


Mutated antigen POLA2 [polymerase (DNA directed), alpha 2, accessory subunit] recognized by D. Wilson TILs

A

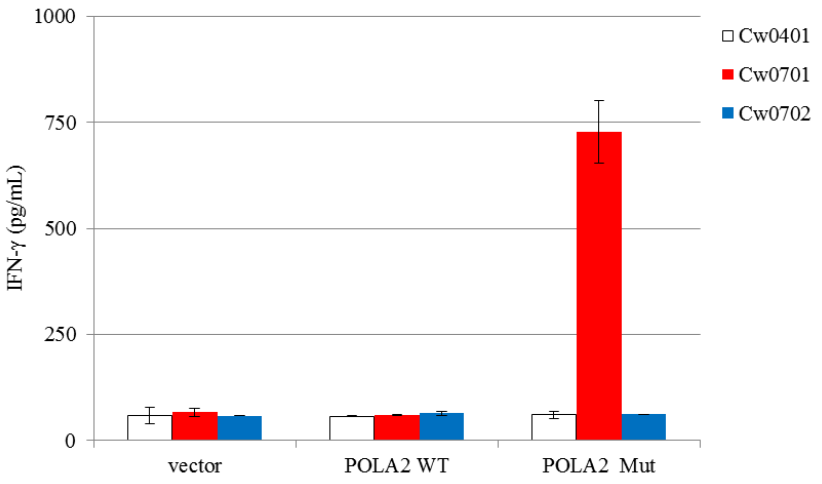


B



POLA2	mut	wt	mut	mut	mut	mut	mut
PDGFD	mut	mut	wt	mut	mut	mut	mut
MLL	mut	mut	mut	wt	mut	mut	mut
GPR19	mut	mut	mut	mut	wt	mut	mut
SFRS2IP	mut	mut	mut	mut	mut	wt	mut
COL2A1	mut	mut	mut	mut	mut	mut	wt

C



Full-length WT or Mutated POLA2 cDNA were co-transfected with HLA into COS-7 cells.

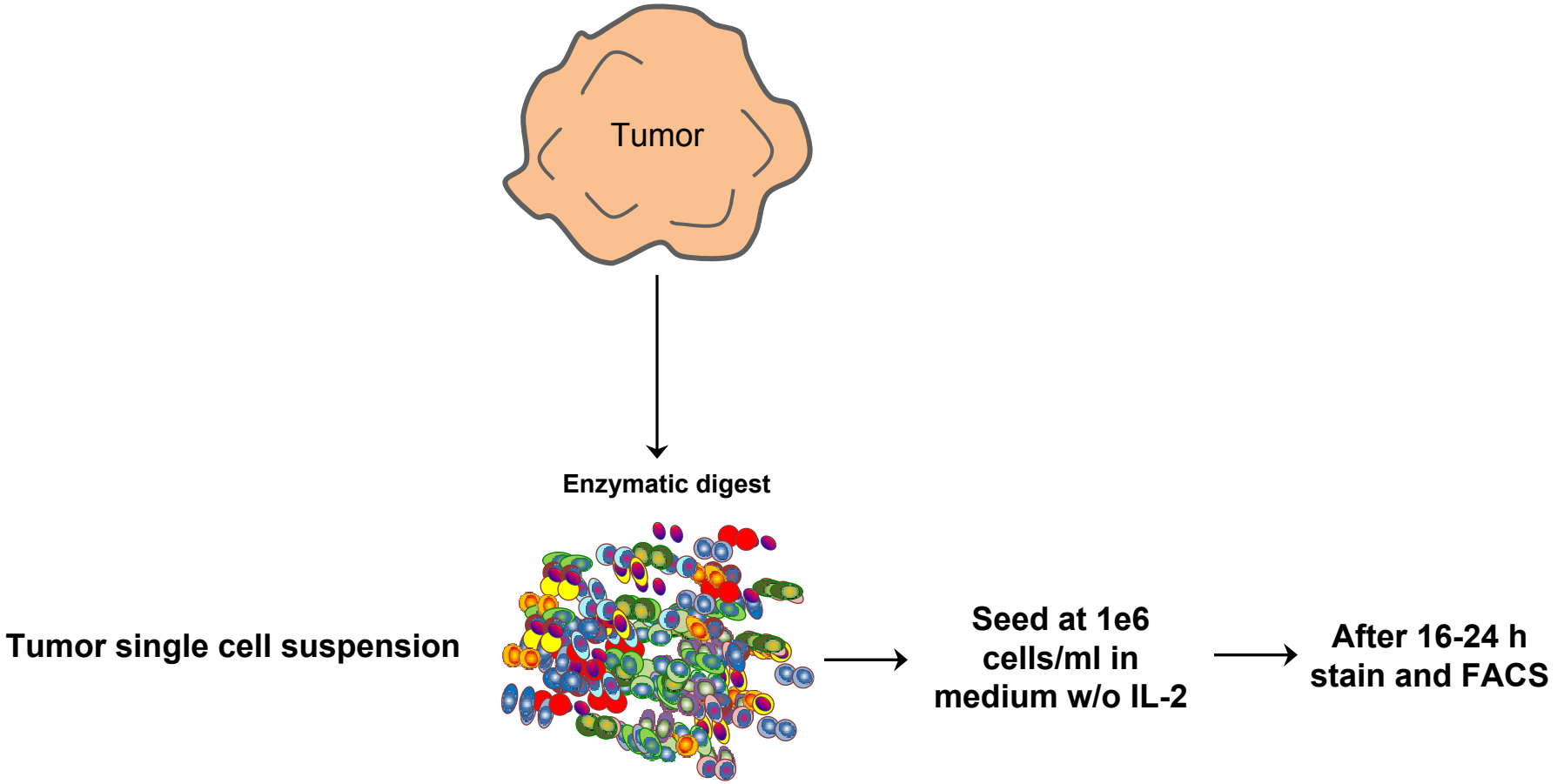
Potential Improvements in TIL Therapy for Melanoma

Identify the mutated antigens in melanoma that are recognized by TIL and selectively target them (tetramer selection of the reactive cells or IVS to generate reactive lympocytes)

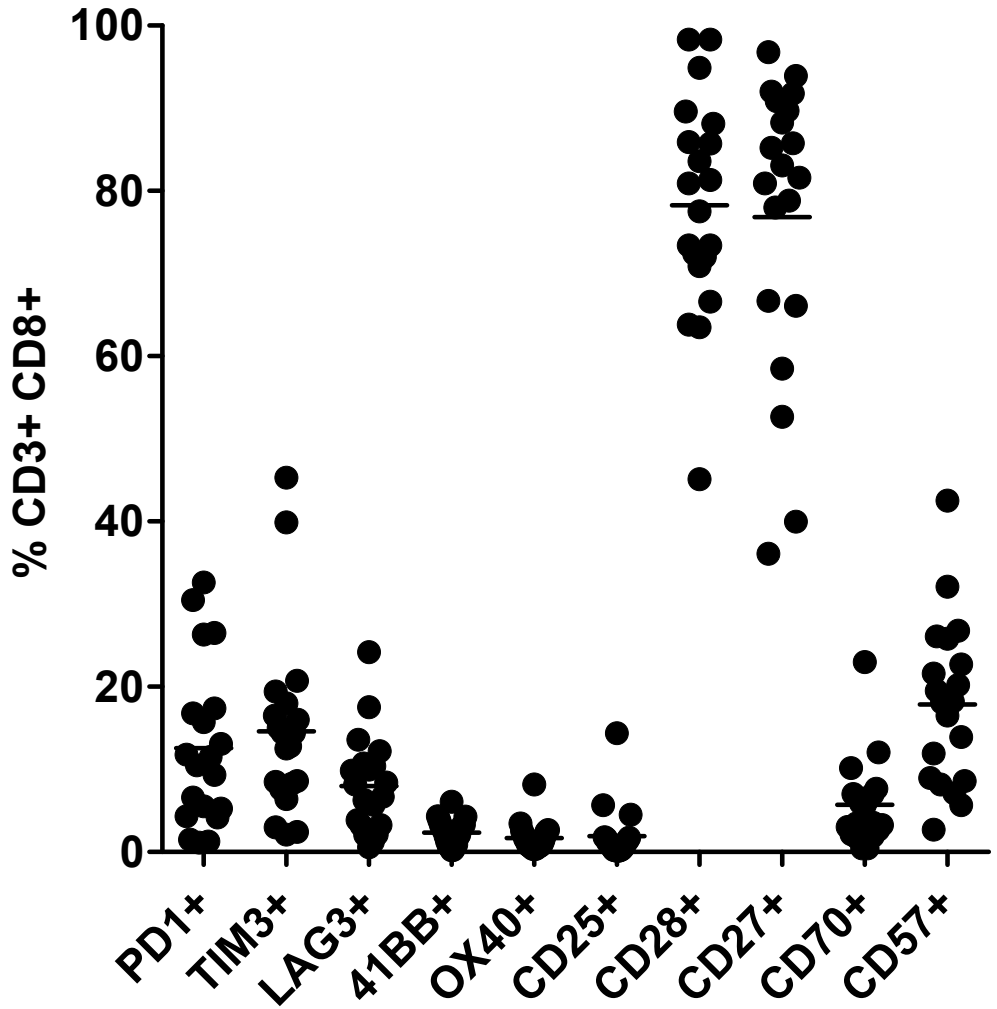
Pre-select subpopulations of TIL with anti-tumor activity

Genetically modify TIL to improve anti-tumor efficacy

Characterization of the phenotype of CD8 T cells in the fresh tumor digest



There is high variability in the levels of expression of the activation markers studied in the fresh tumor digest



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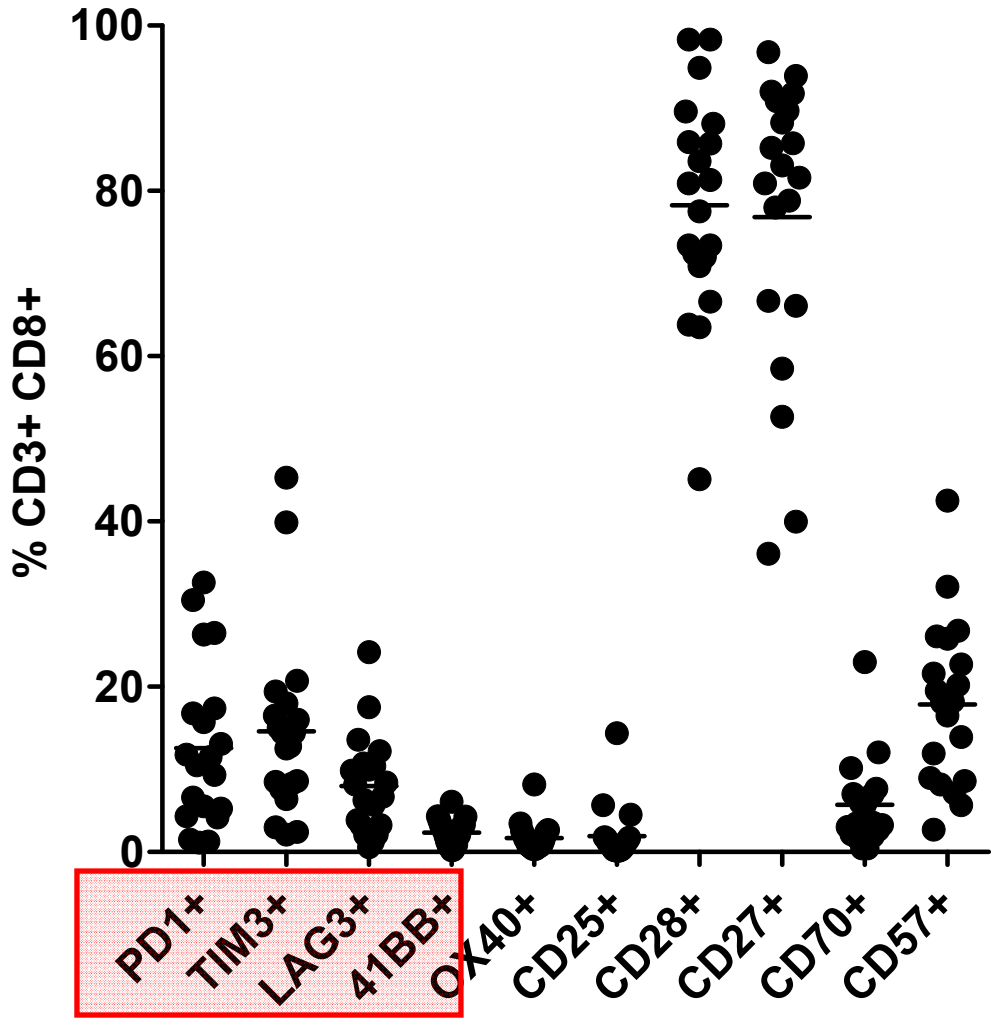
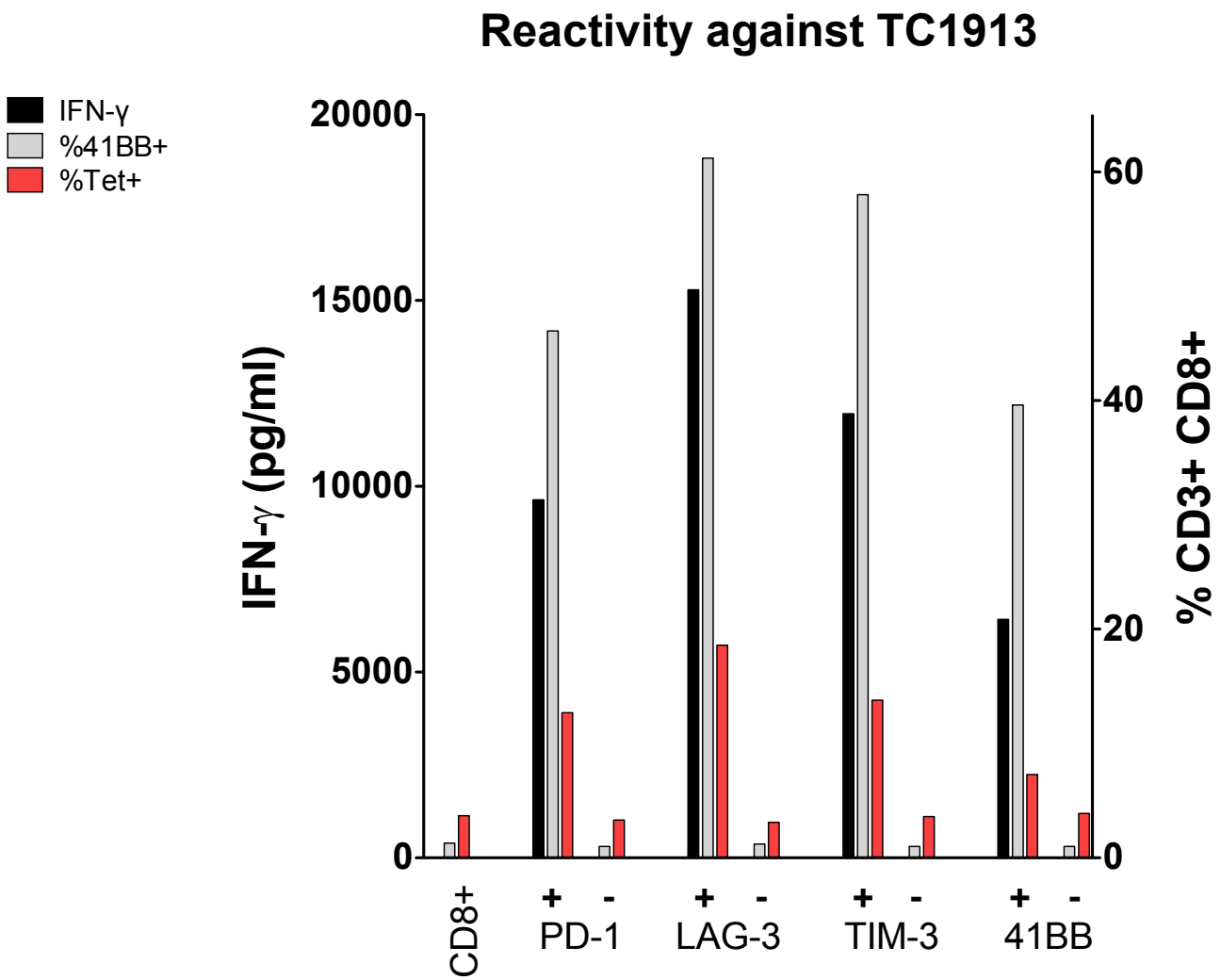


41BB < LAG-3 < PD-1 ≤ TIM-3

Cells reactive with autologous tumor are highly enriched in subsets expressing PD-1, LAG-3, TIM-3 and 41BB



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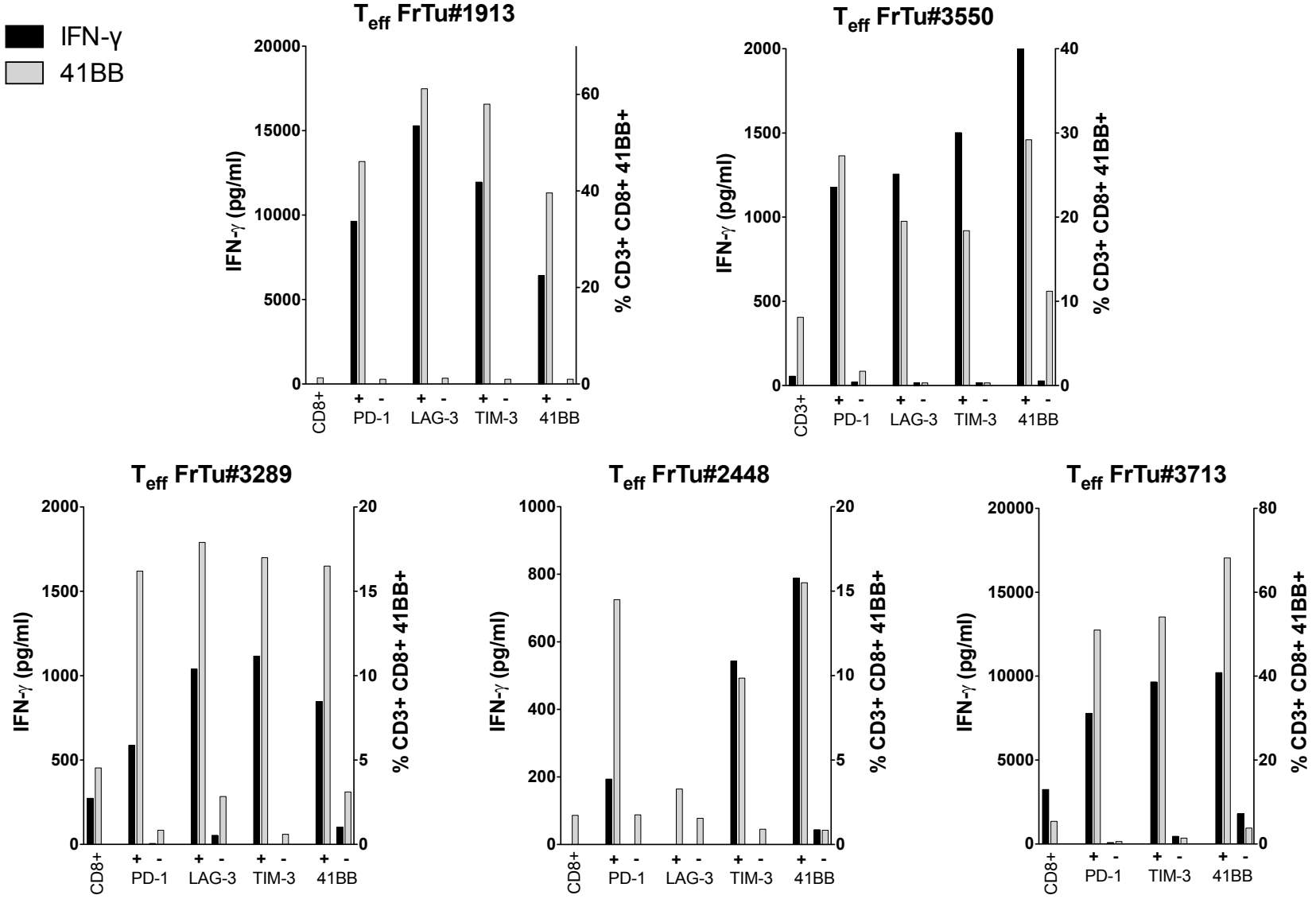
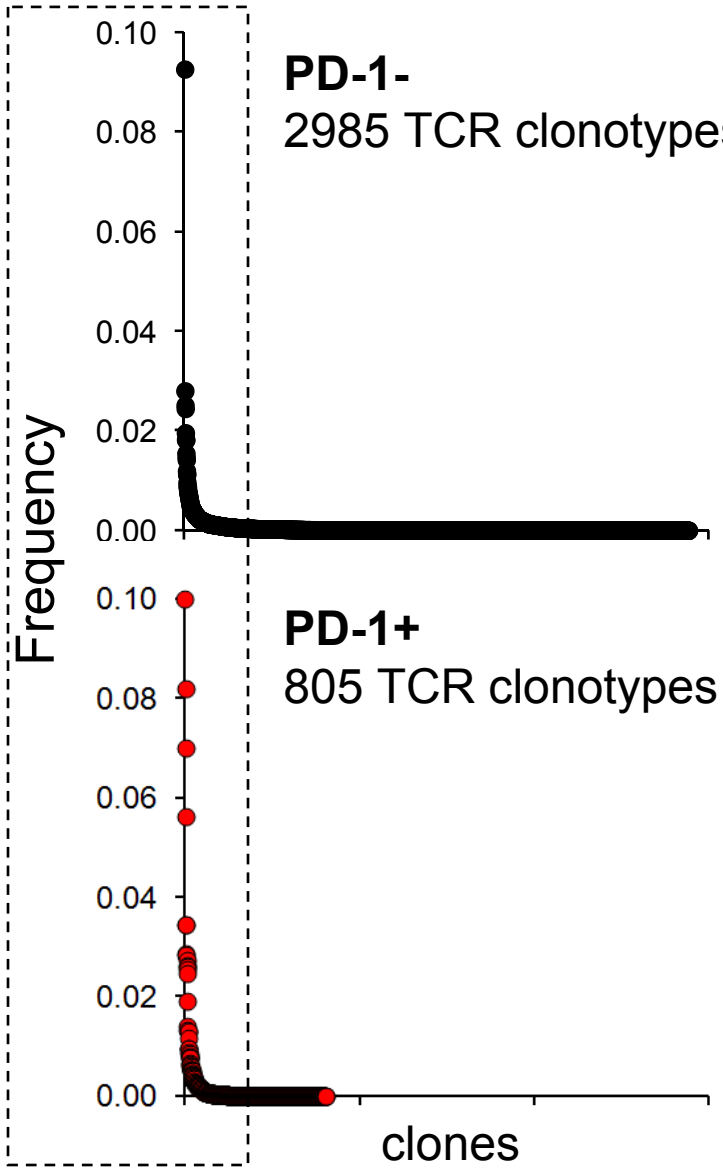
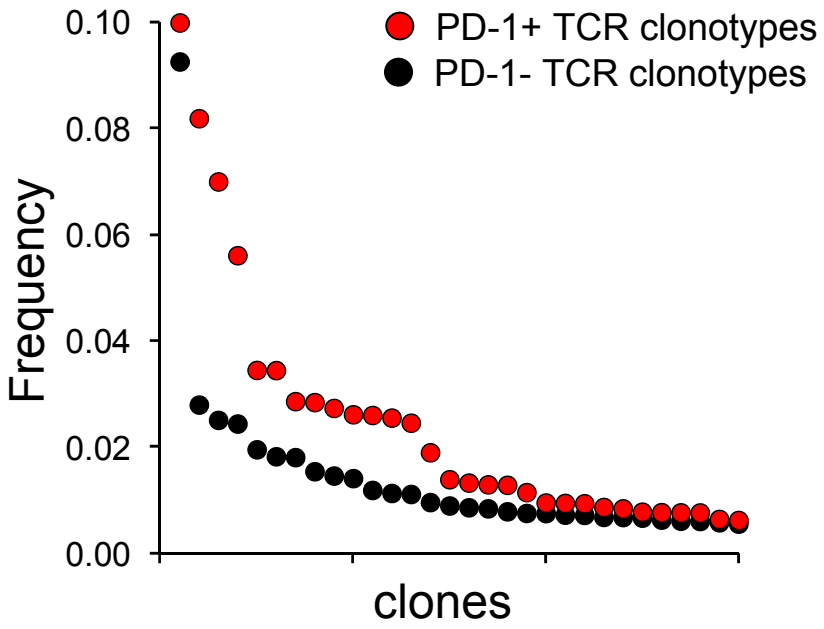
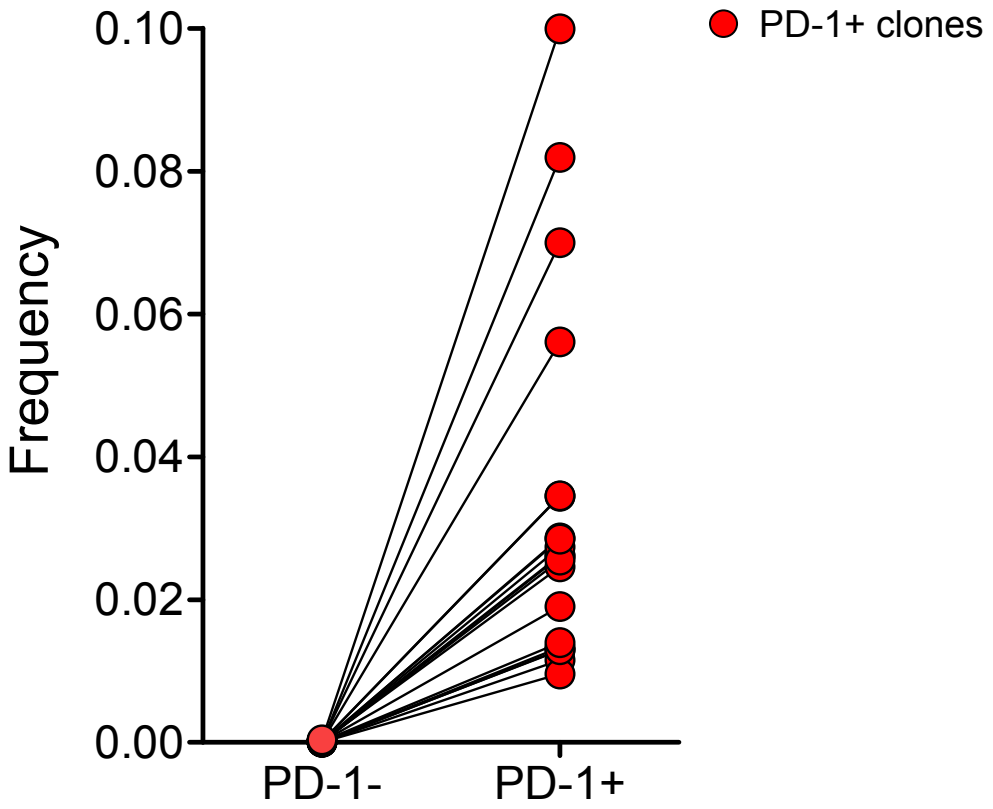


PD-1+ derived cells are more oligoclonal than PD-1- derived cells

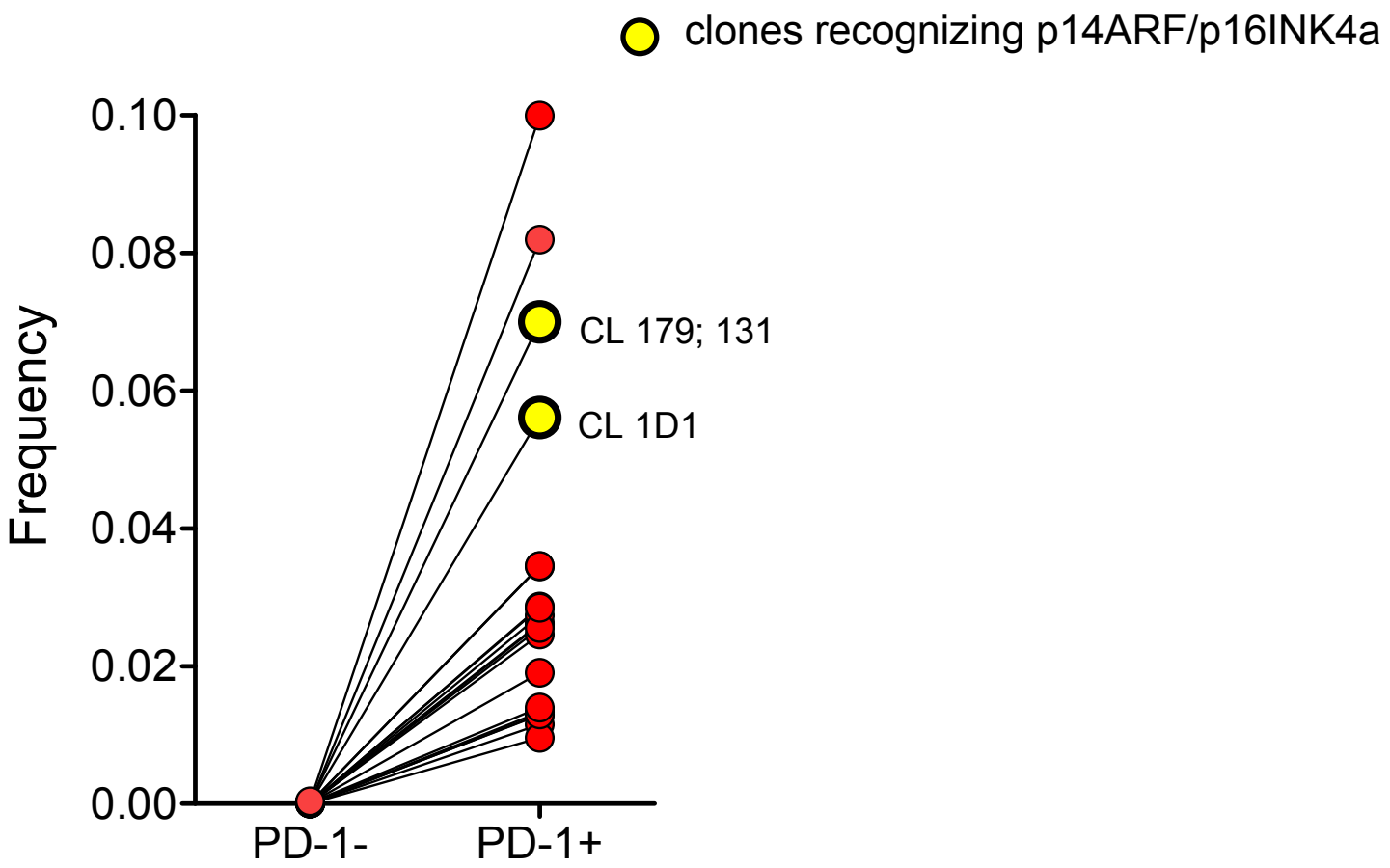




The most frequent TCR beta clonotypes in the PD-1+ cells were seen at very low frequency in the PD-1- fraction



Clones targeting mutated epitopes were found within the 20 most frequent clones in the PD-1+ derived population



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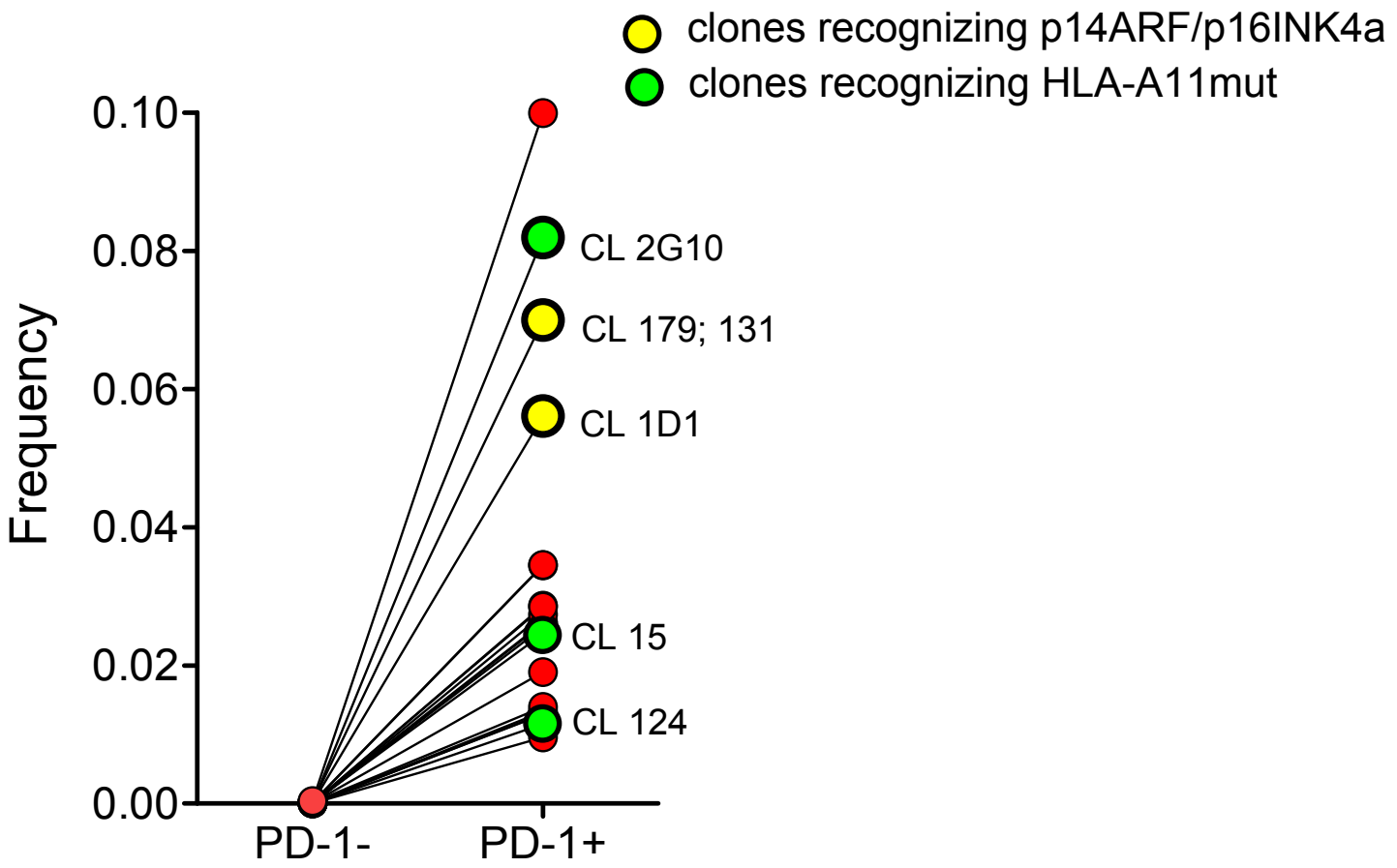

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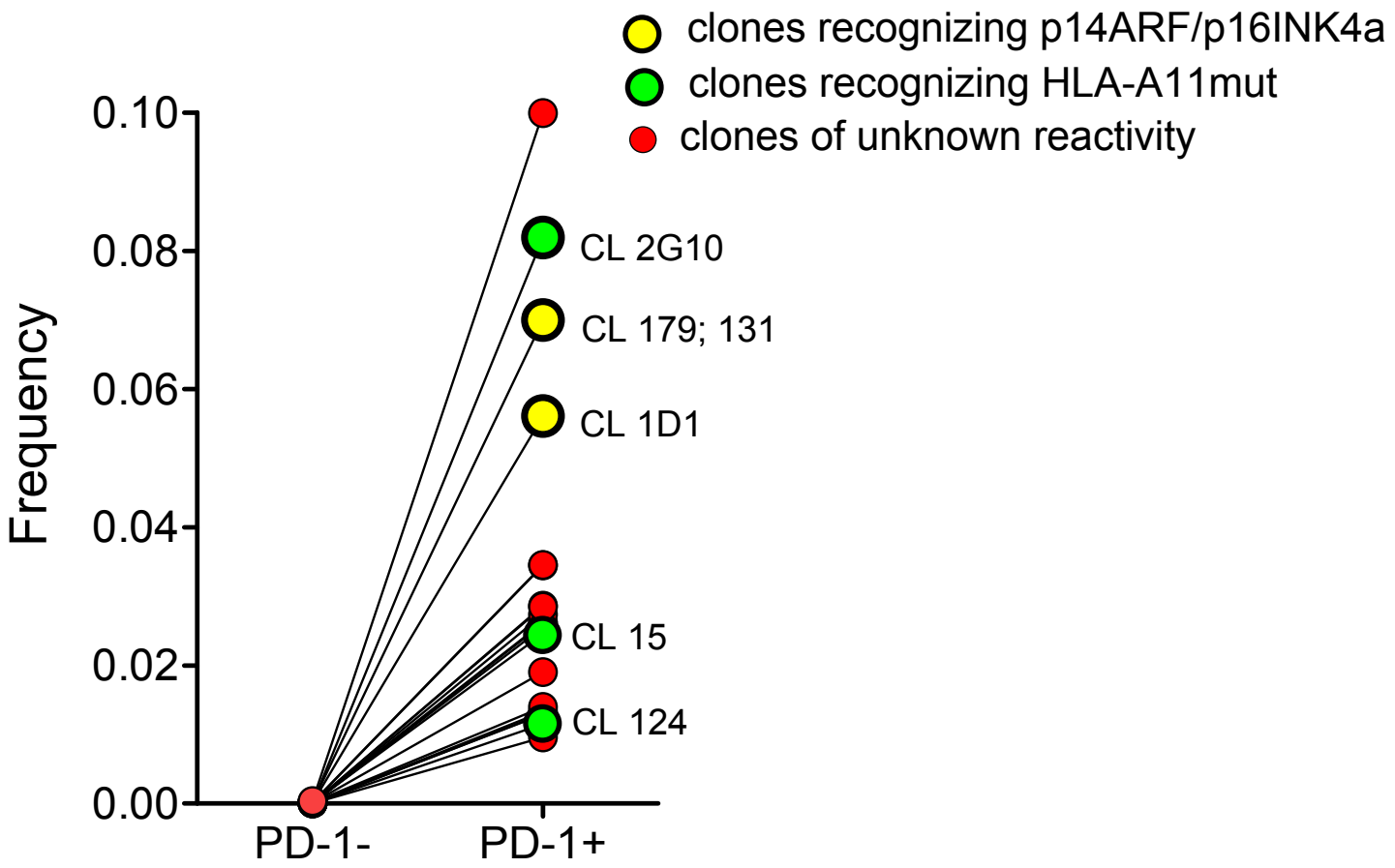


Improvement in TIL Technology

Sort single cell suspension of tumor for $41BB^+$ or $PD1^+$ cells

1. **enriches reactivity against multiple tumor antigens**
2. **no screening of anti-tumor activity necessary**
3. **shortens the time of cell culture so cells are more proliferative**

CONCLUSION

T cell based immunotherapy is capable of mediating long-term durable regressions of large vascularized, invasive metastatic melanoma in humans.

CHALLENGE

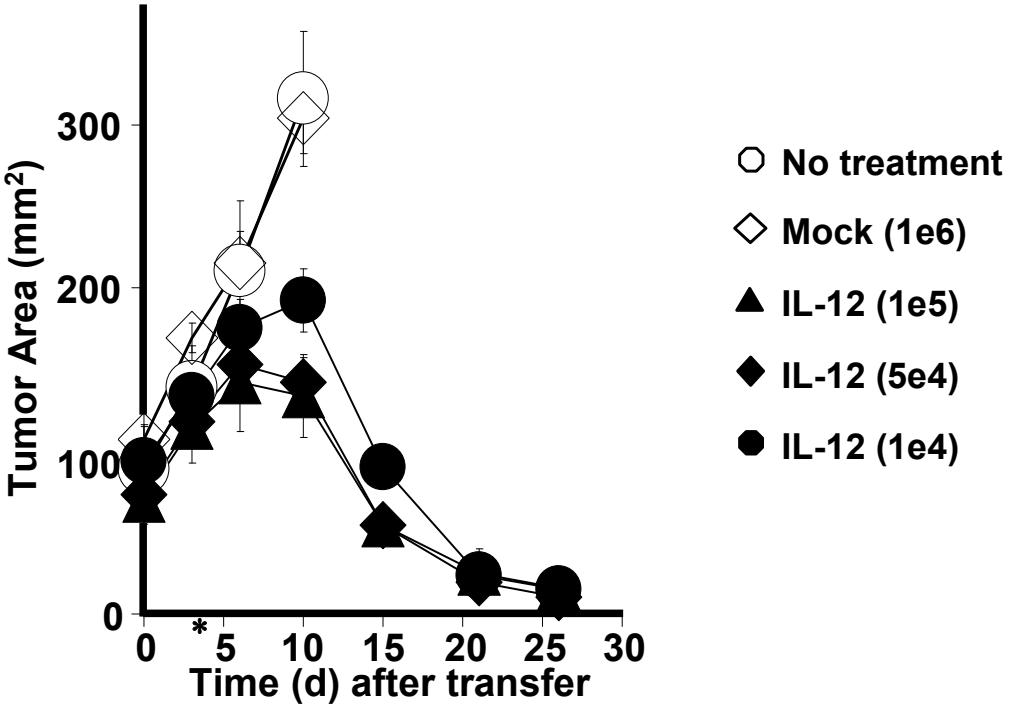
1) **Improve TIL treatment for melanoma**

2) **Extend cell transfer therapy to additional cancer types**

Potential Gene Alterations to Improve the Efficacy of Cell Transfer Therapy

Expand tumor recognition	T cell receptors or chimeric T cell receptors that recognize cancer antigens
Cytokines	IL-2, 12, 15, 17, 21, 23
Costimulatory molecules	CD8, CD27, CD80, 41BBL, OX-40L
Antiapoptotic molecules	Bcl-2, Bcl-xl, FLIP, TIPE-2
Reverse inhibitory influences	KO SHP-1, PD-1, CTLA-4, SOCS, CIS Dominant negative TGF-β, cbl-b
Trafficking molecules	CD62L, CCR7, CXCR2, CXCR4
Improve survival	Telomerase, KOp53

Pmel-1 CD8+ T cells engineered to produce IL-12 enhance anti-tumor responses without exogenous IL-2 and vaccine



But constitutive production of IL-12 was toxic to mice.

(Kerkar et al, Cancer Res, 2010)

Development of an Inducible Vector to Mediate IL-12 Production Only in the Tumor Microenviroment

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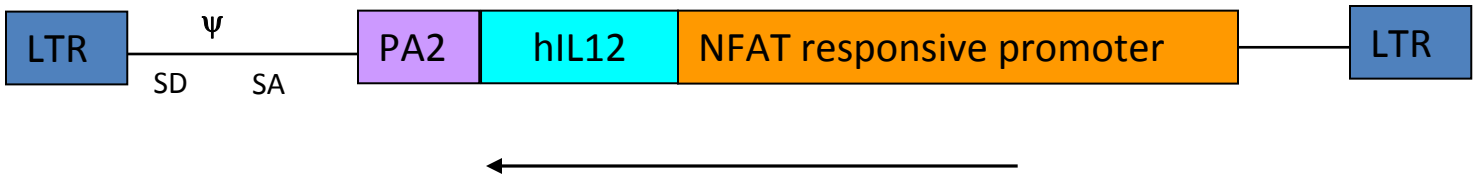
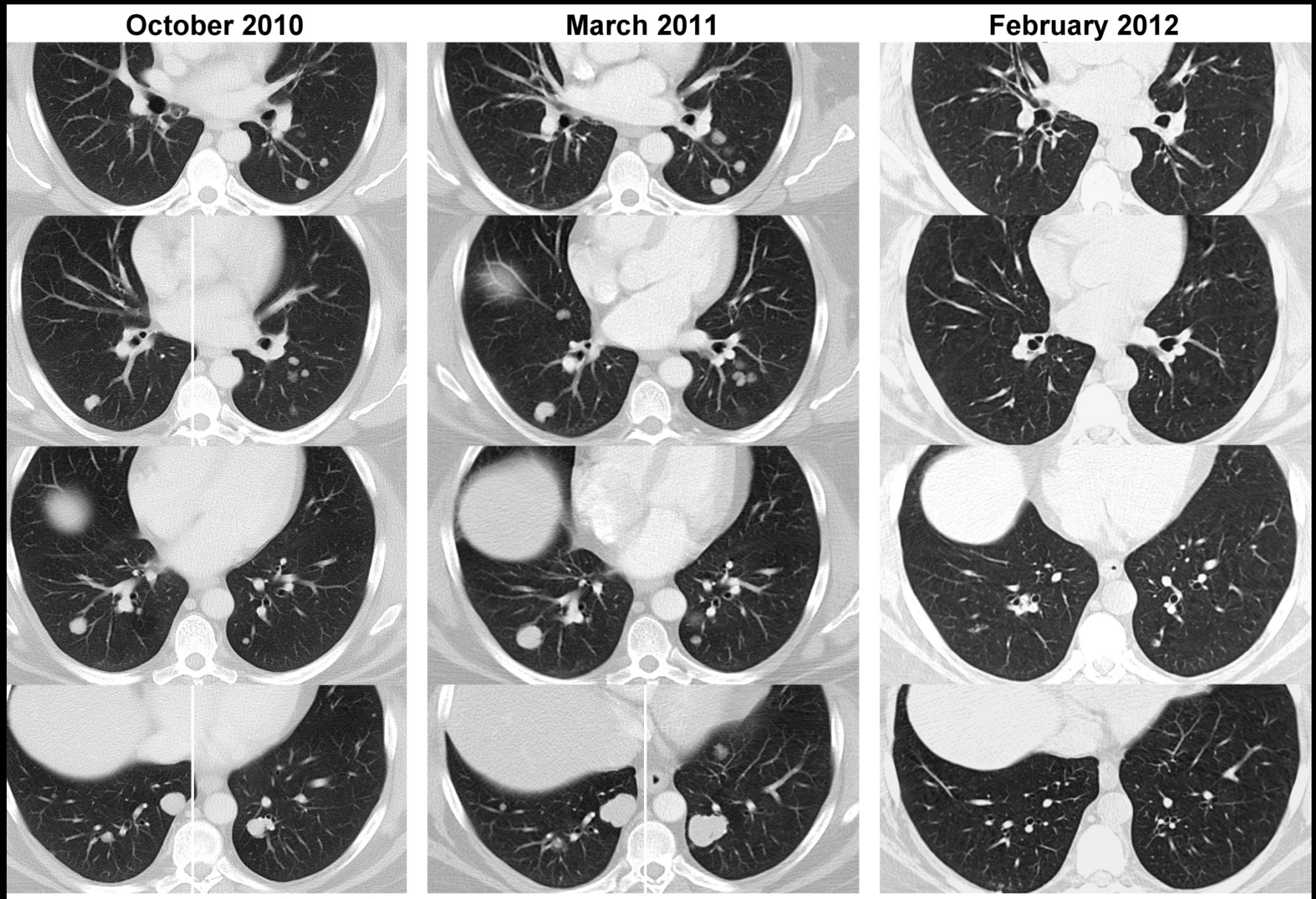
NFAT is a transcription factor produced in T cells activated by antigen specific triggering of the T cell receptor

An NFAT responsive promoter (NFAT.hIL-12) is used to drive single chain IL-12 production by T cells only when encountering specific antigen stimulation



(L. Zhang, R. Morgan, Surgery Branch, NCI)

M.S. Melanoma TIL/IL-12




Nunc

M.C. TIL/IL-12