Autologous T Cells as a Personalized Treatment for Patients with Cancer (10/12)

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Surgery Branch, National Cancer Institute

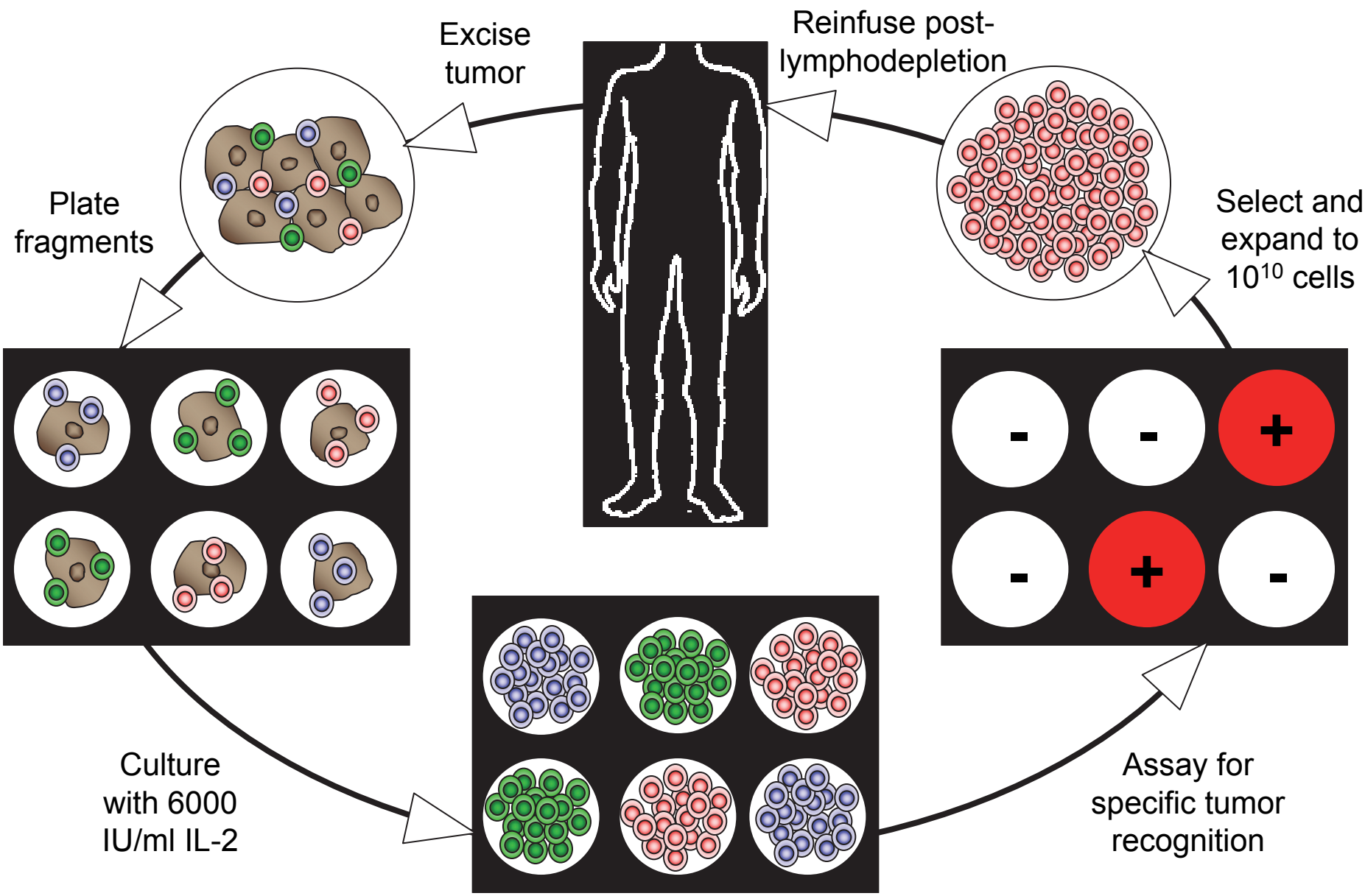
Adoptive Cell Therapy (ACT) is a Powerful Approach to Cancer Immunotherapy

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

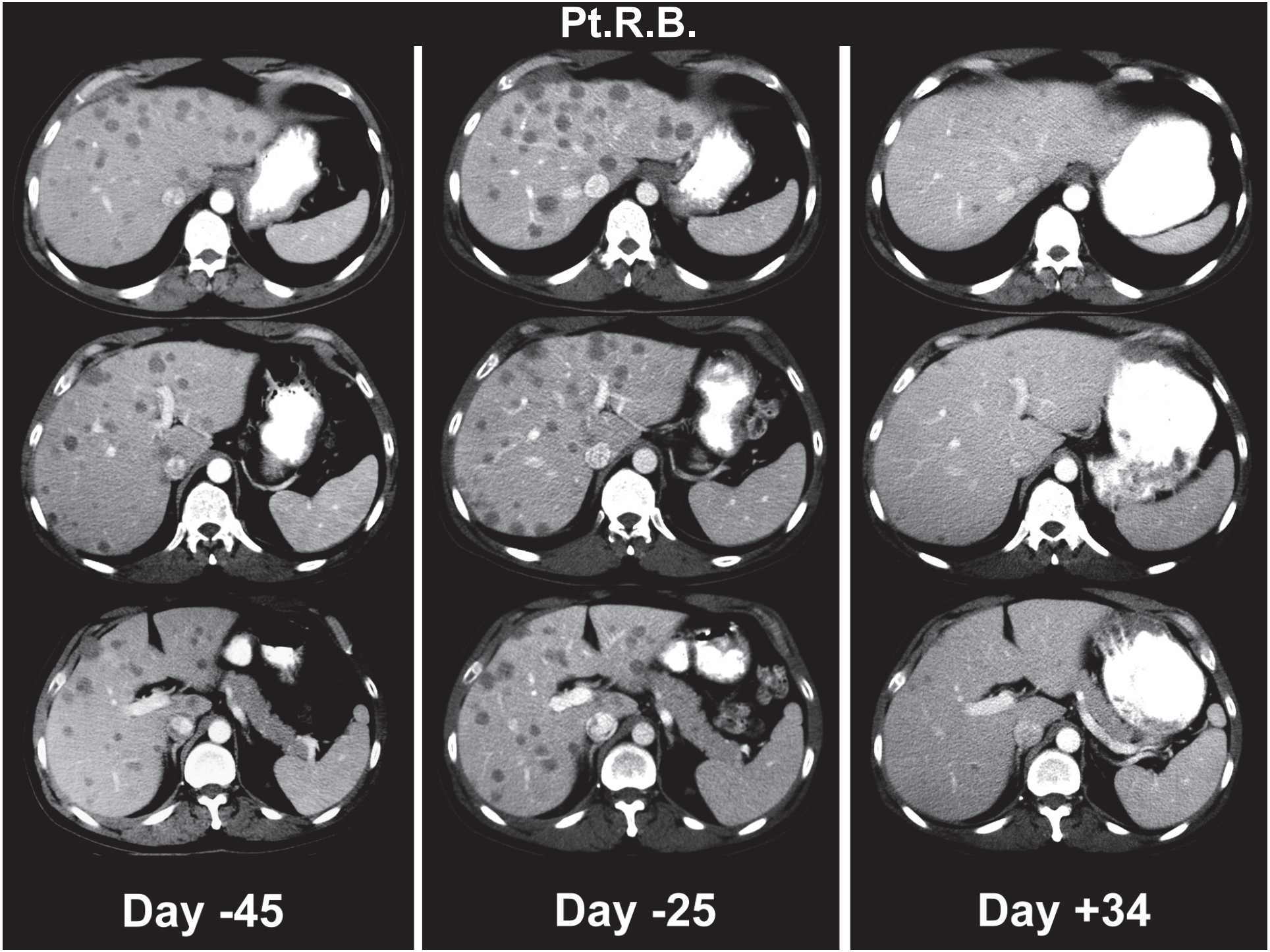
Adoptive transfer of tumor infiltrating lymphocytes (TIL)



Cell Transfer Therapy (6/1/12)

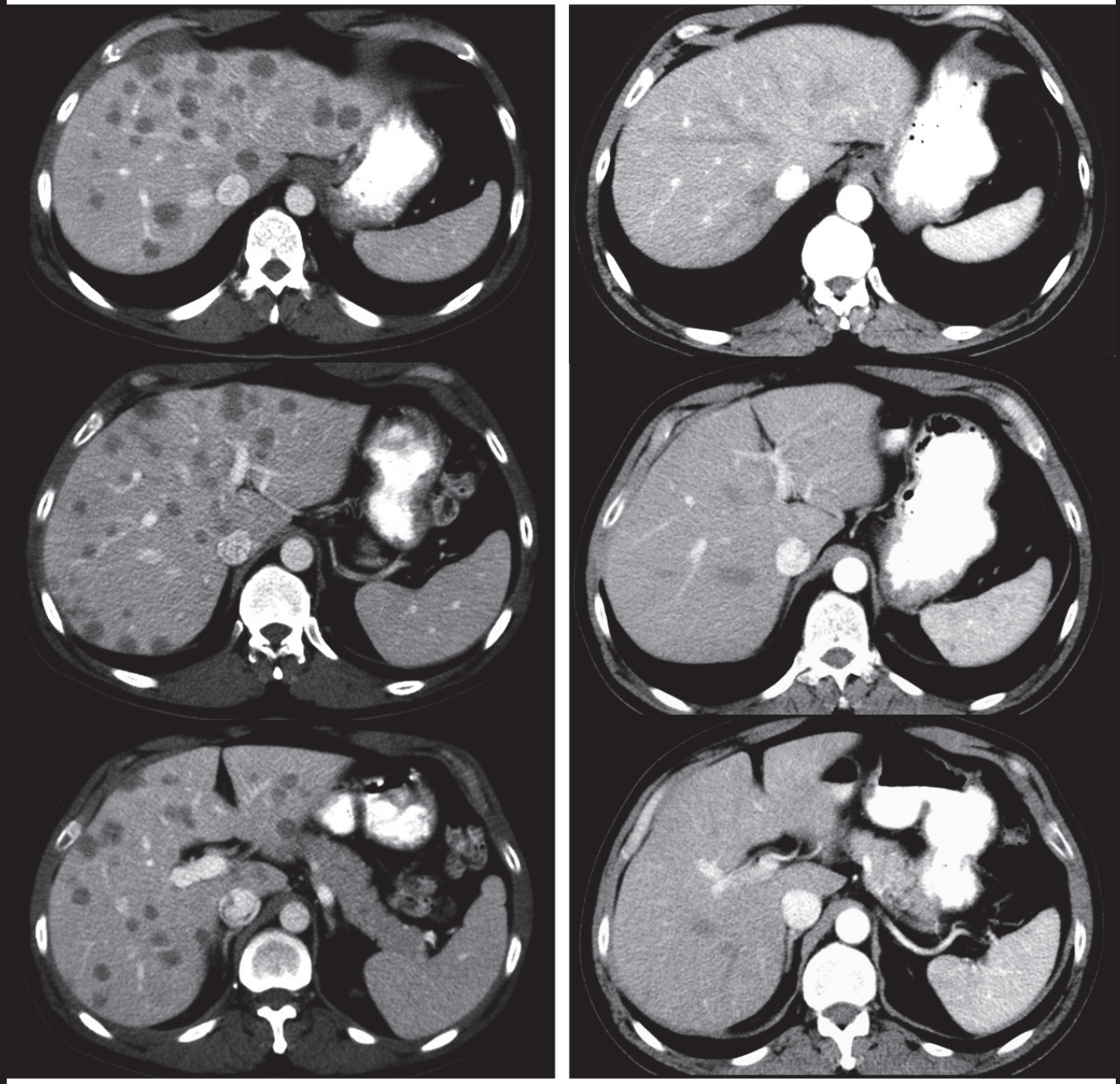
Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (104+, 101+, 100+, 88+, 83+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (90+, 86+, 82+, 79+, 76+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (70+, 68+, 66+, 66+, 61+, 60+, 60+, 60+, 59+, 19)	18(72%)

(20 complete responses: 19 ongoing at 59 to 104 months)


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

Other Sites: Lung

CR 75+ mo.

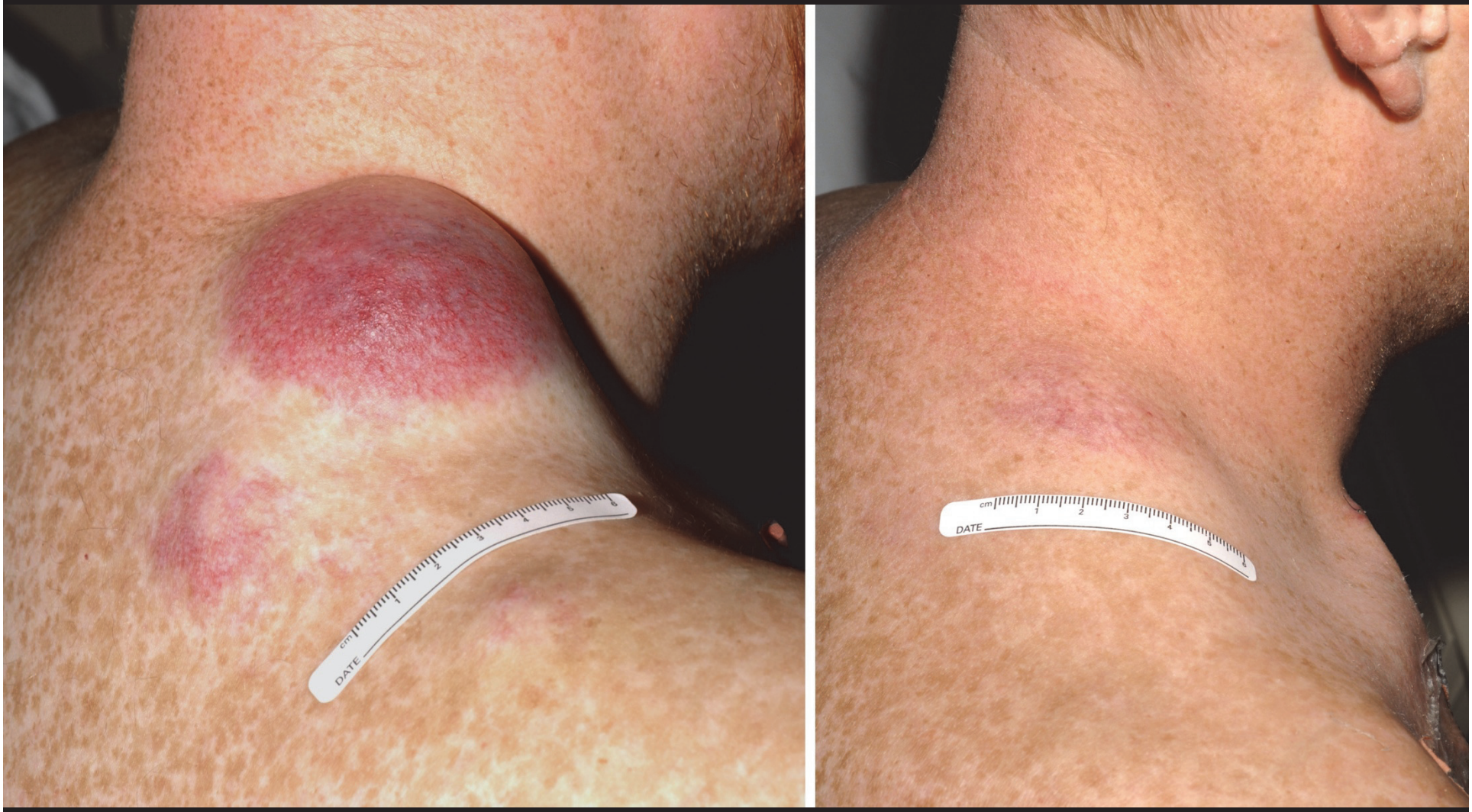


Nov 10, 2003

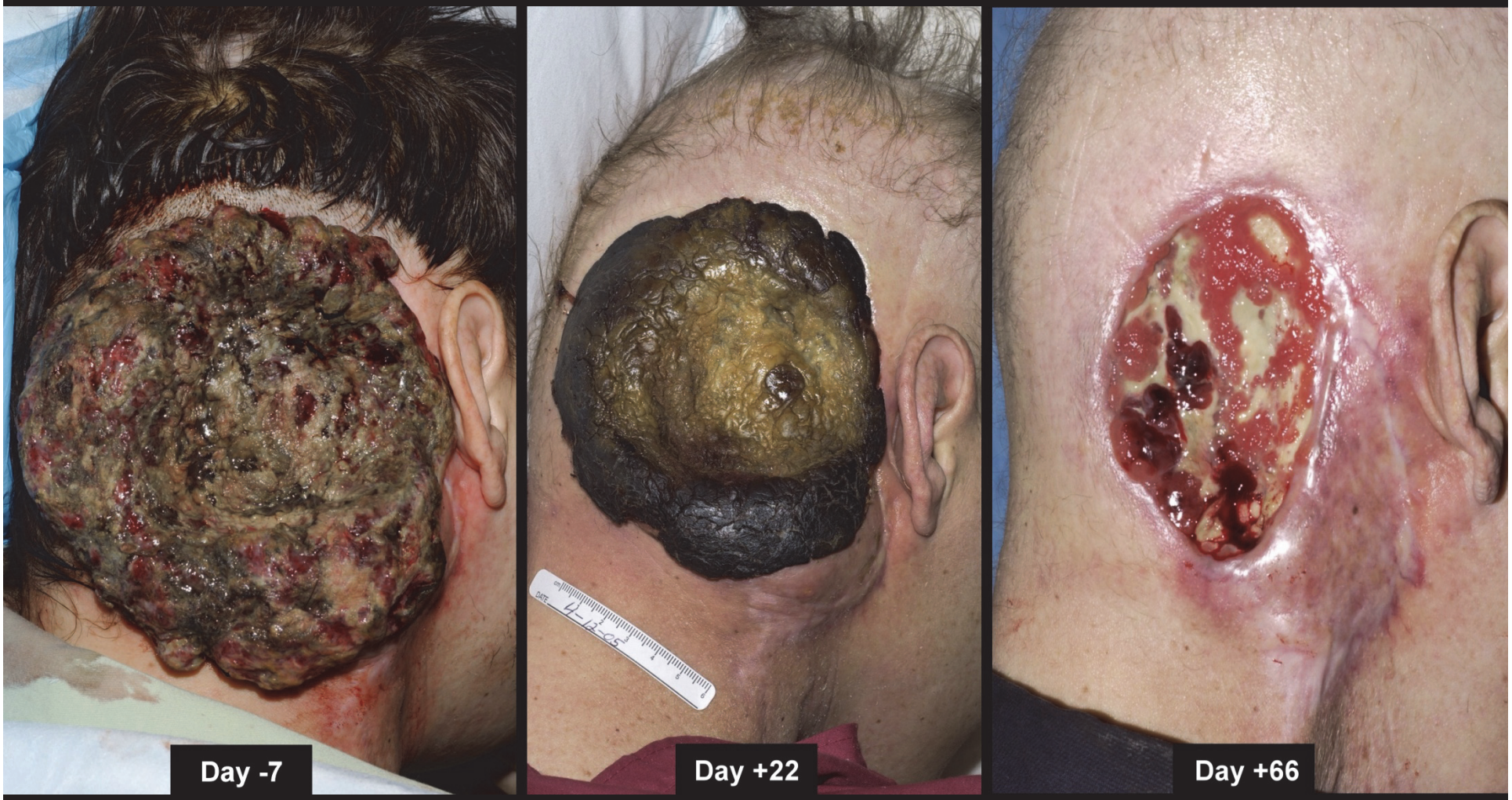
Feb 17, 2010

C.K. (200cGy) Pre

12 days

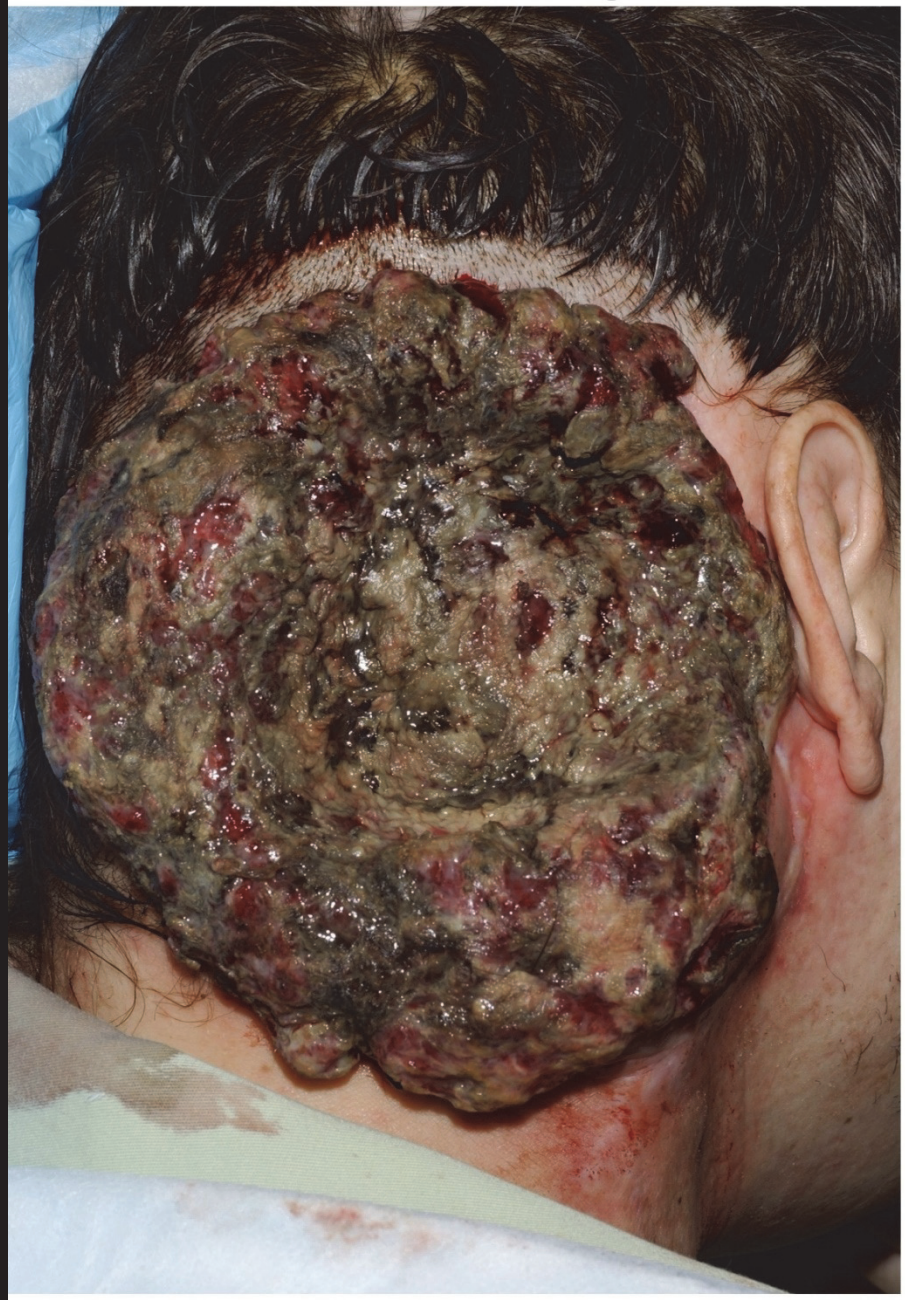


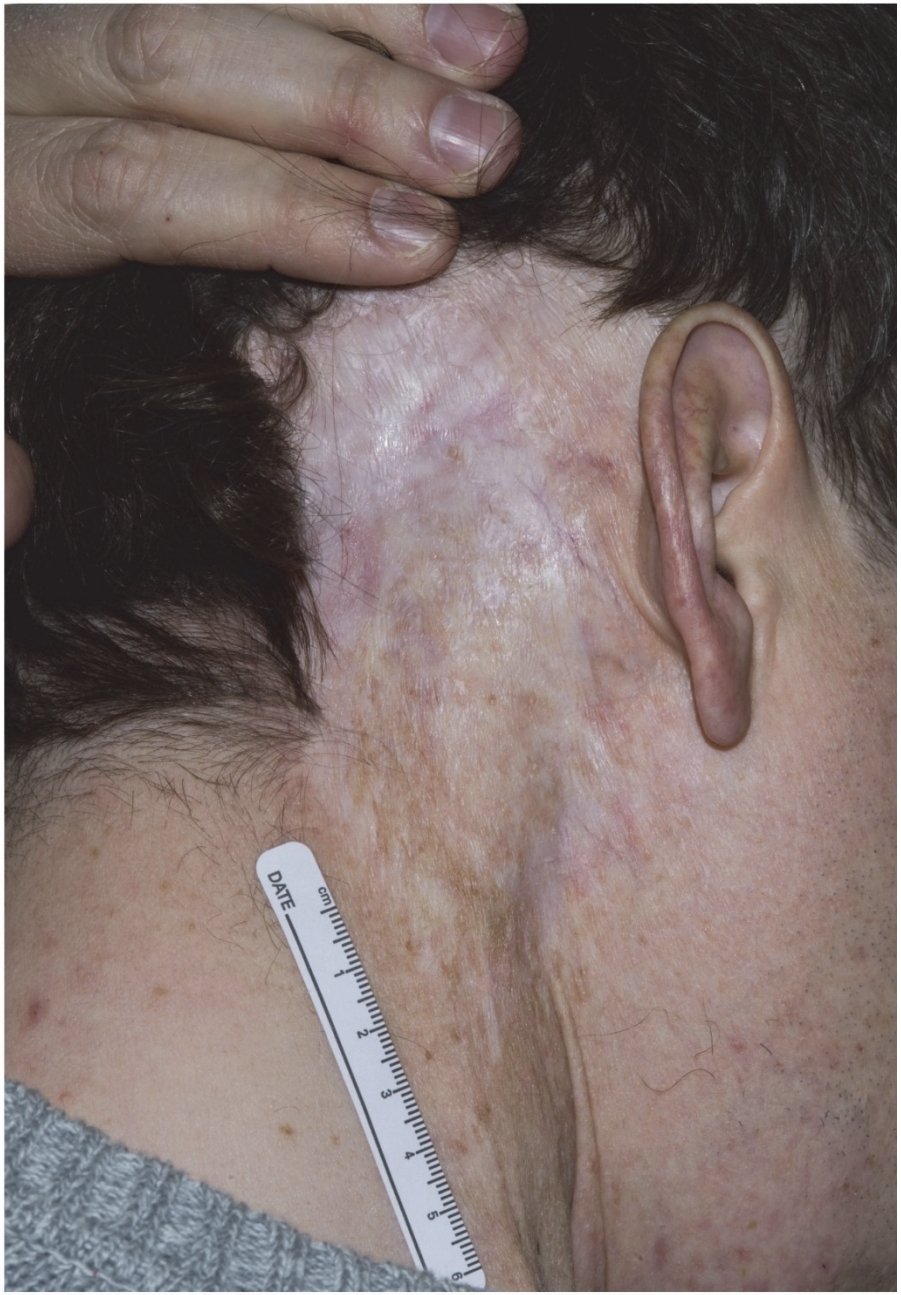
A.H.: N-M cell transfer



Other Sites: Lung

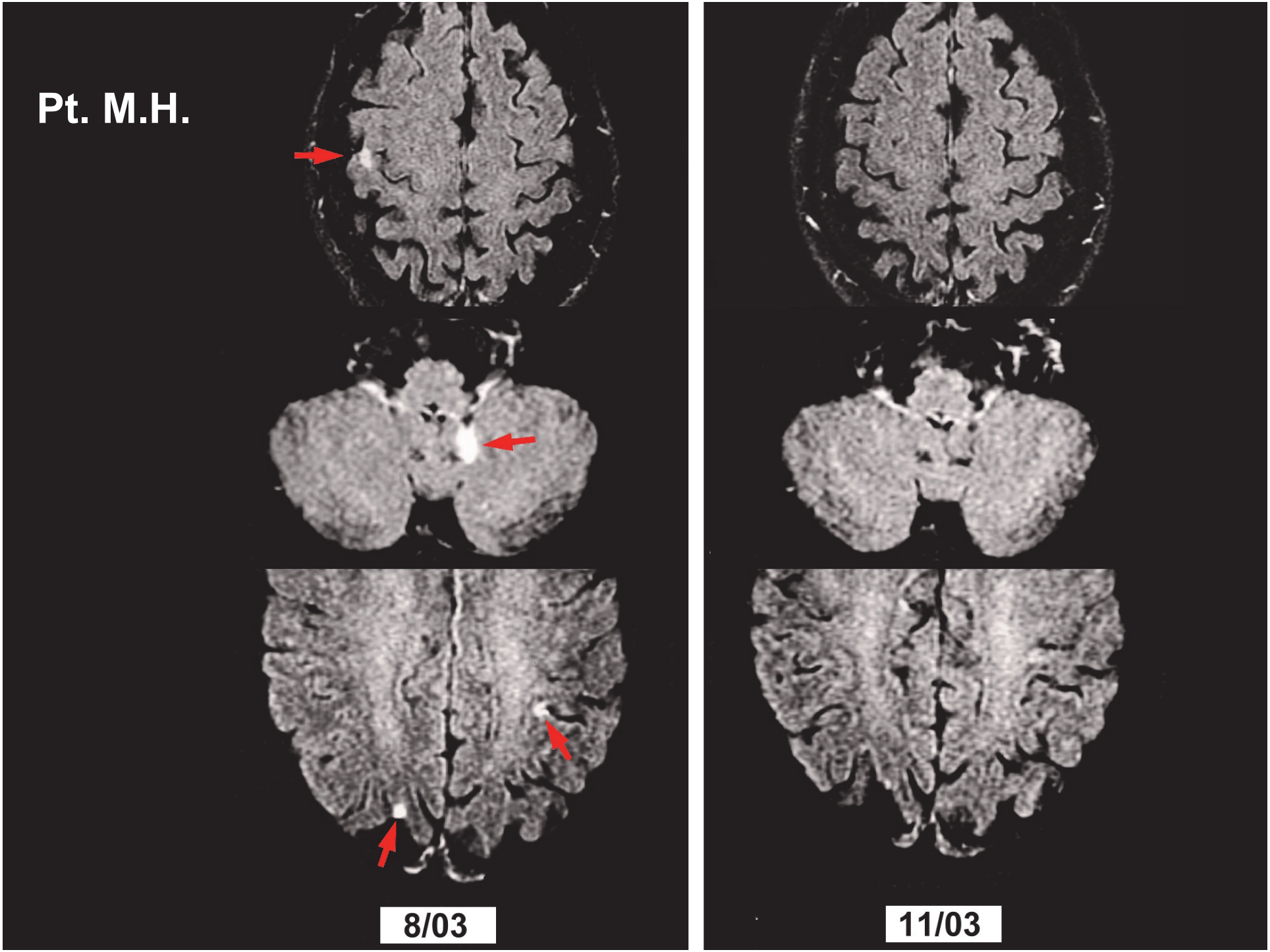
CR 59+ mo.



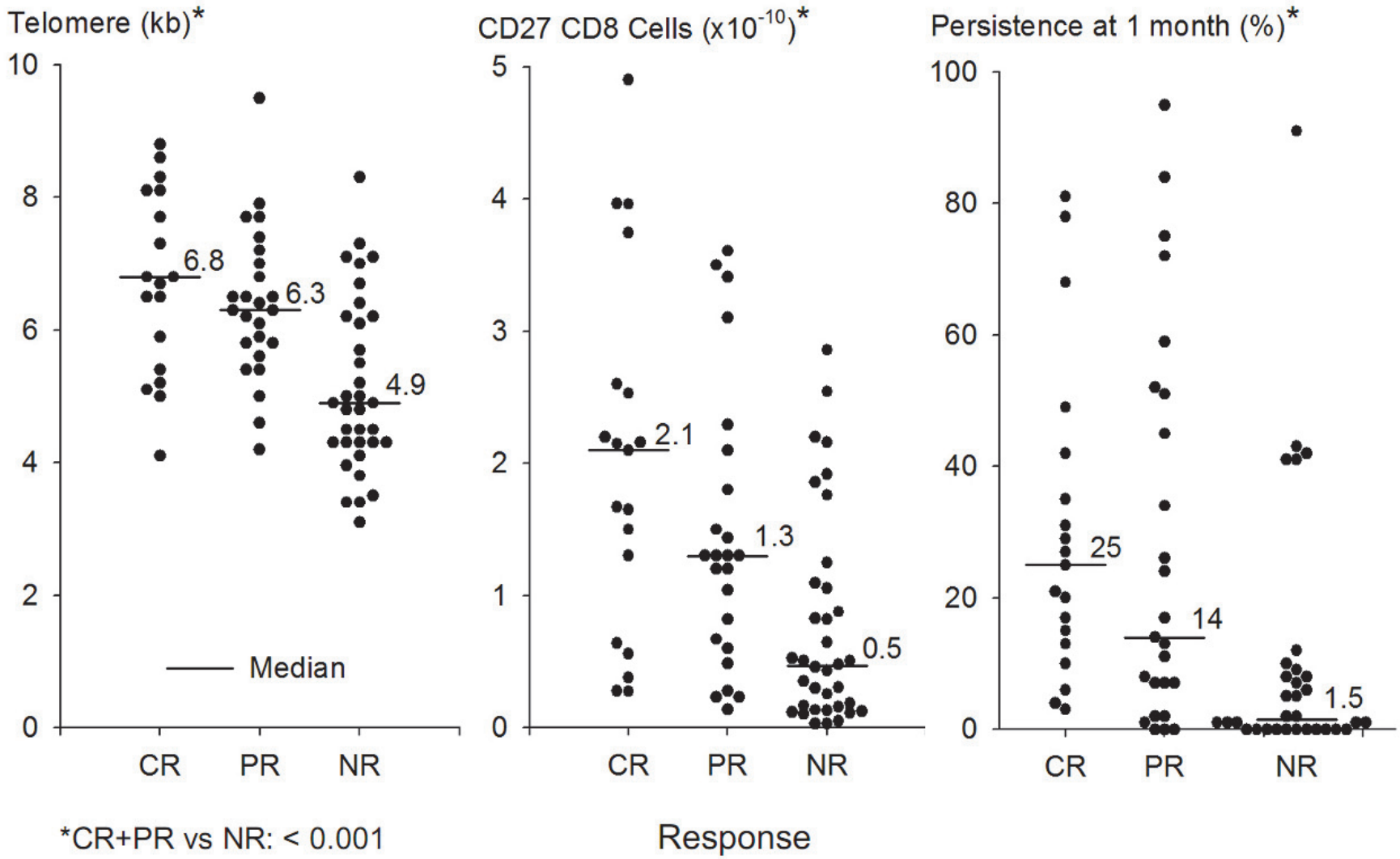


March 21, 2005

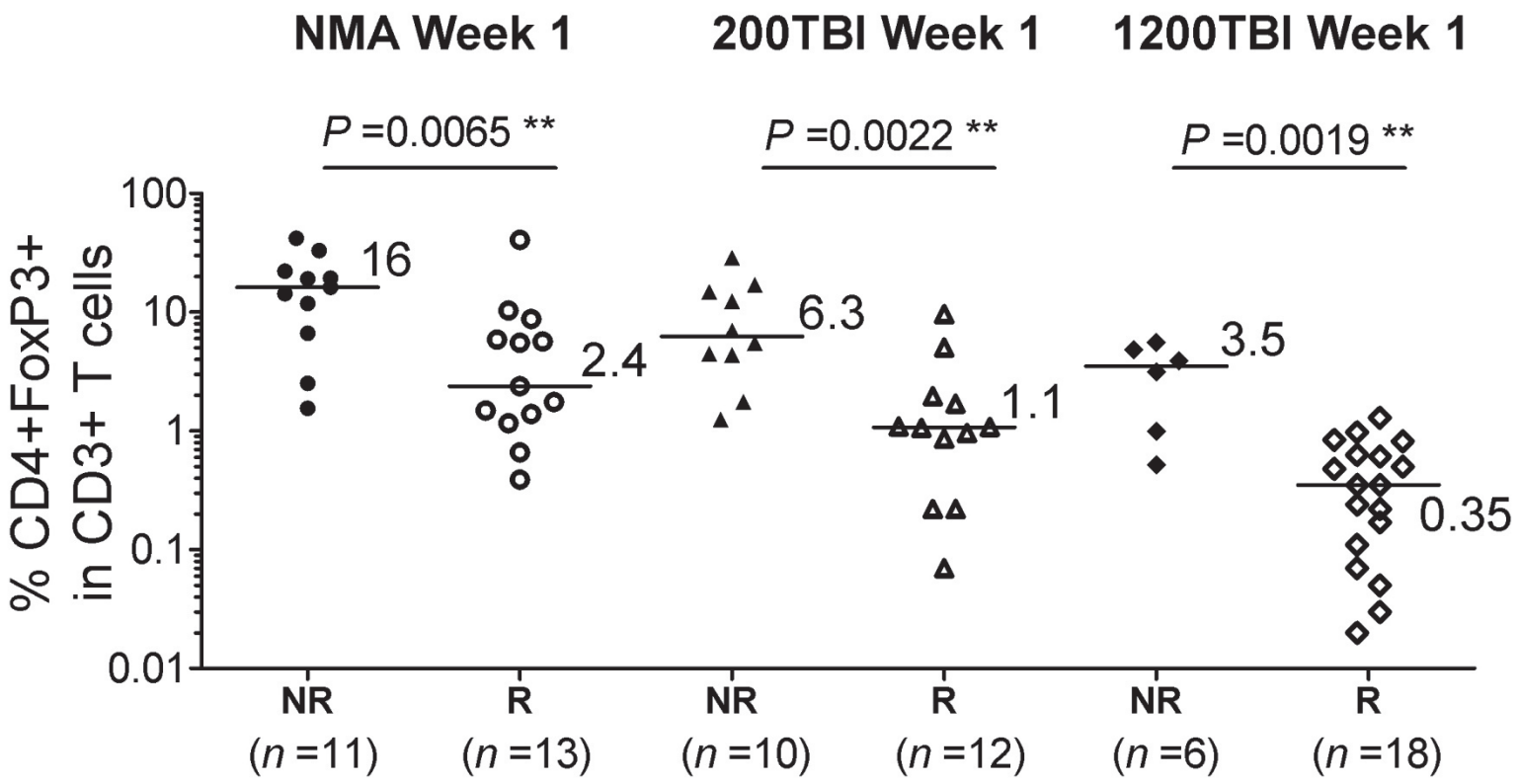
Feb 23, 2010


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

Cell and Host Factors Associated with Response to Cell Transfer Therapy



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

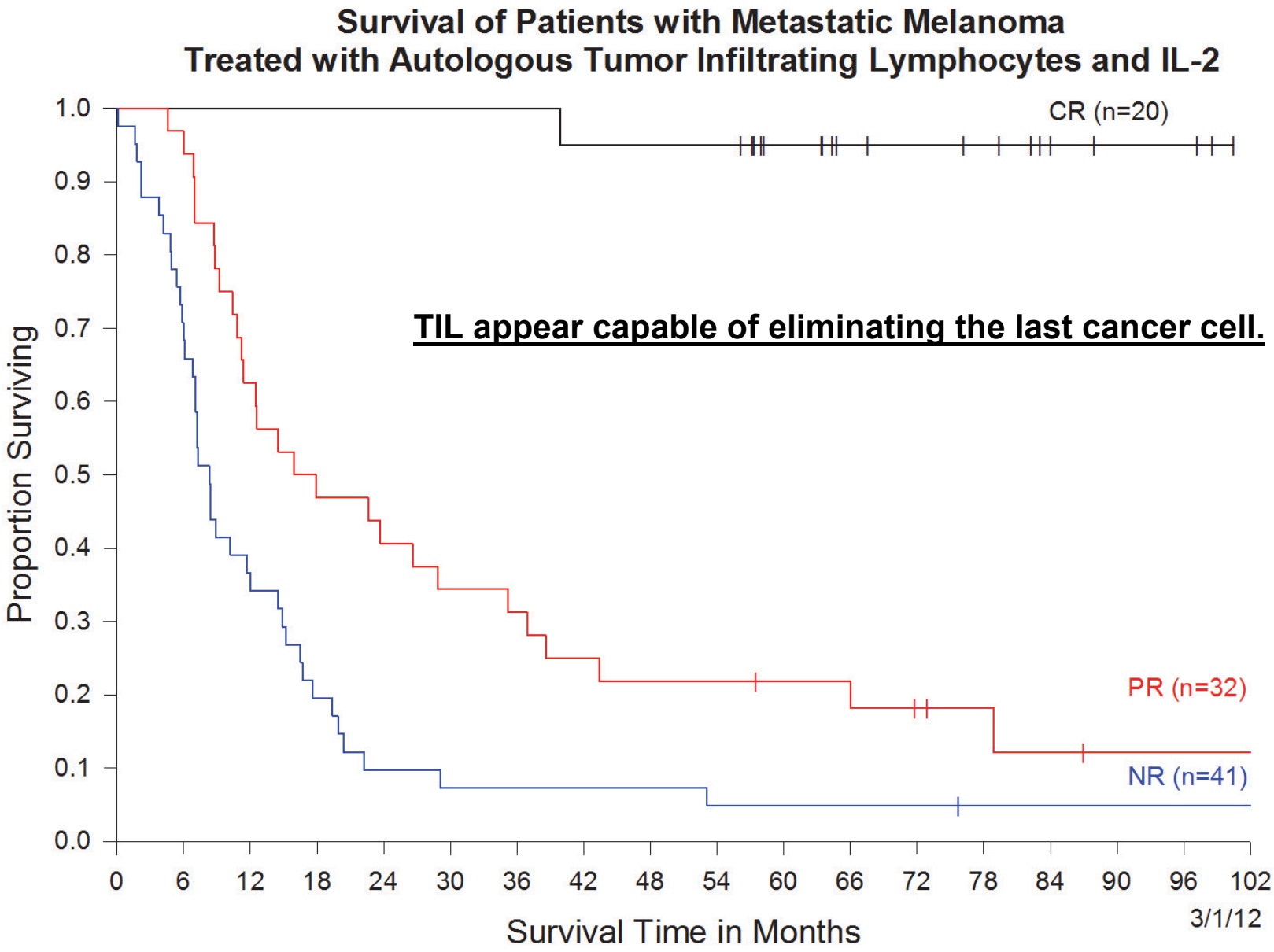


(Xin Yao, Surgery Branch, NCI)

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


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
3/1/12

Hypothesis of Mechanism of Cancer Regression Following Cell Transfer

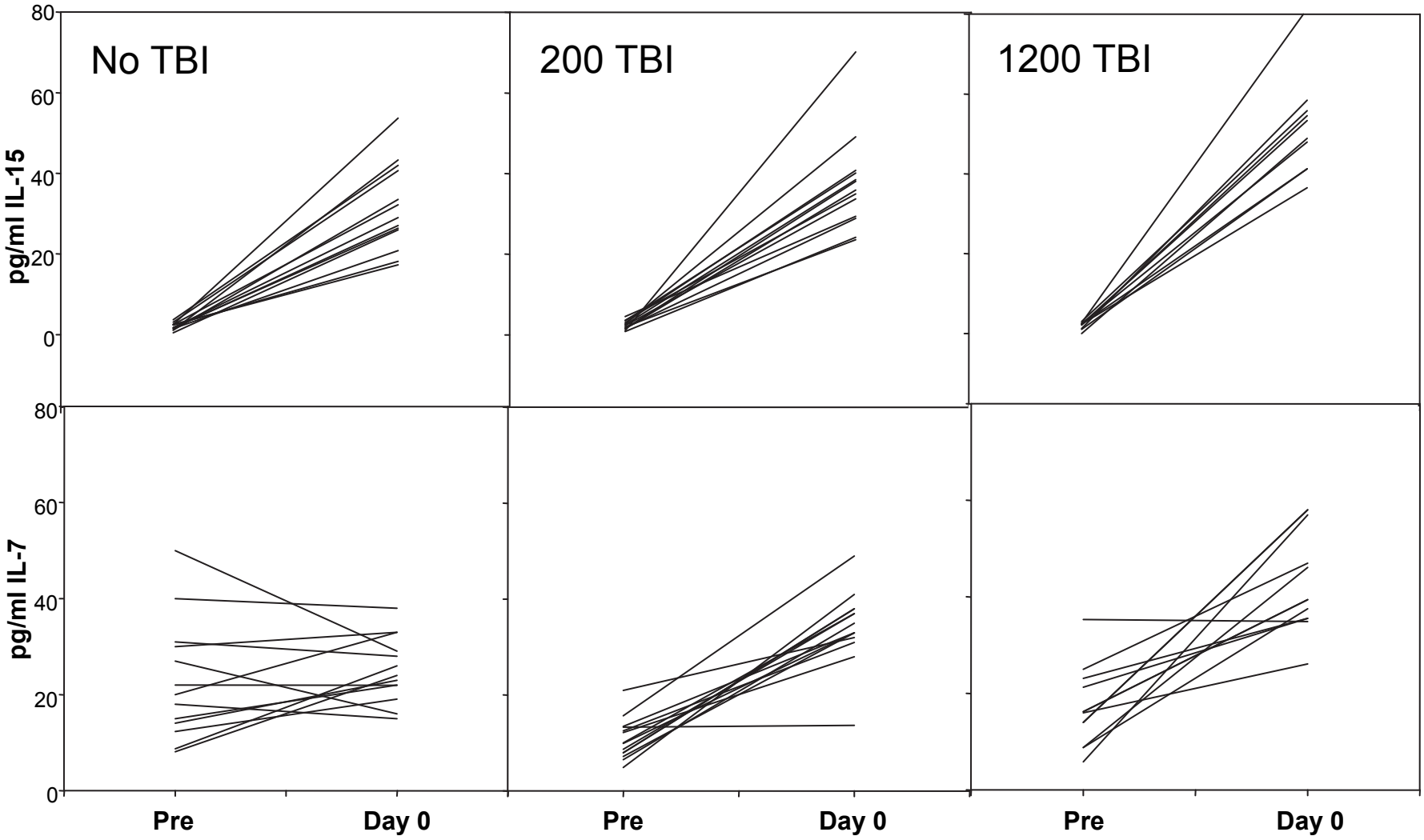
The lymphophenic environment

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



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

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

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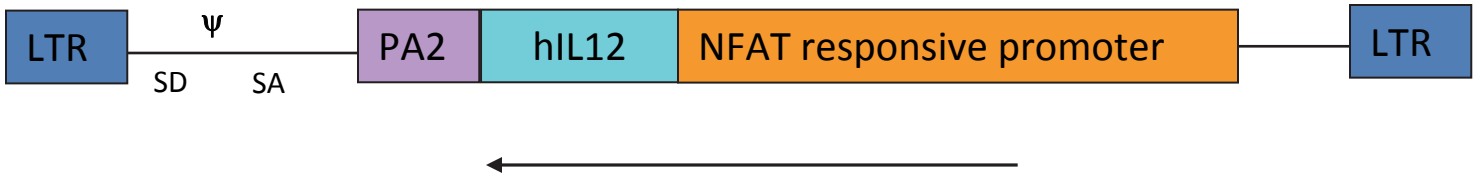
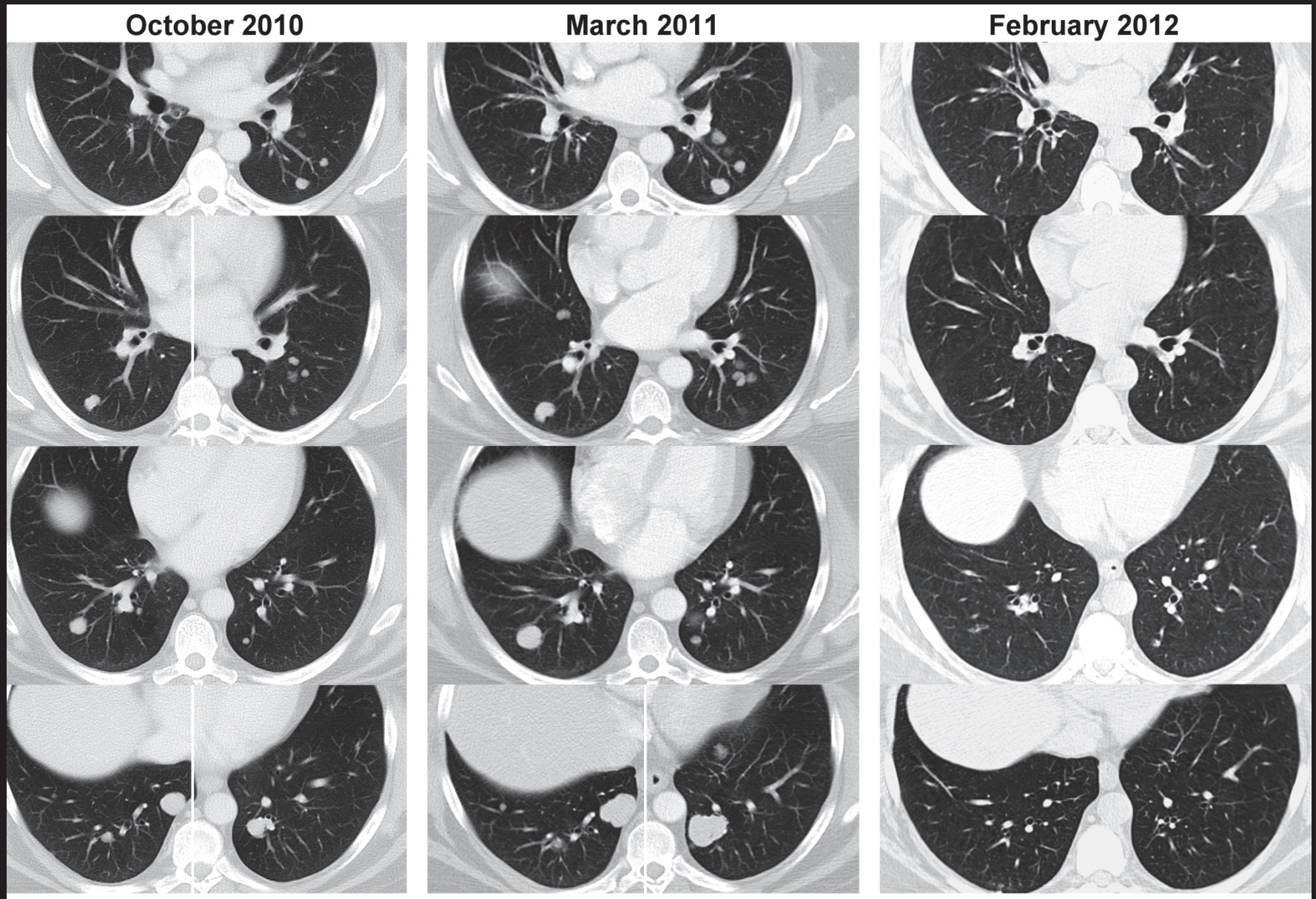
NFAT is a transcription factor produced in T cells activated by antigen specific triggering of the T cell receptor

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
178883

ACT Using TIL Transduced with Gene Encoding IL-12 (10/1/12)

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 (18+ mos); 4NR
0.1	3	3NR
0.3	3	3PR (4, 6, 7+)
1.0	3	1PR (7+)
3.0	2	2TE (decr 37% and 45% at 2mo)

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Expand tumor recognition	T cell receptors or chimeric T cell receptors that recognize cancer antigens
Cytokines	IL-2, 12, 15, 17, 21, 23
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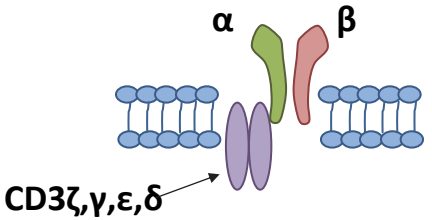
Trafficking molecules	CD62L, CCR7, CXCR2, CXCR4
Improve survival	Telomerase, KOp53

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

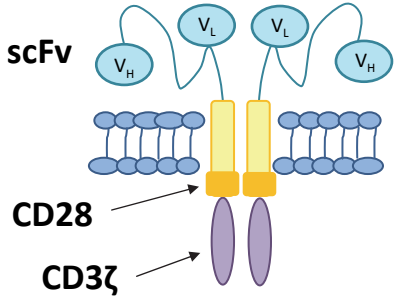
TCR Vector (eg, MART1, NY-ESO)

TCR receptor



CAR Vector (eg, CD19)

CAR receptor



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

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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)

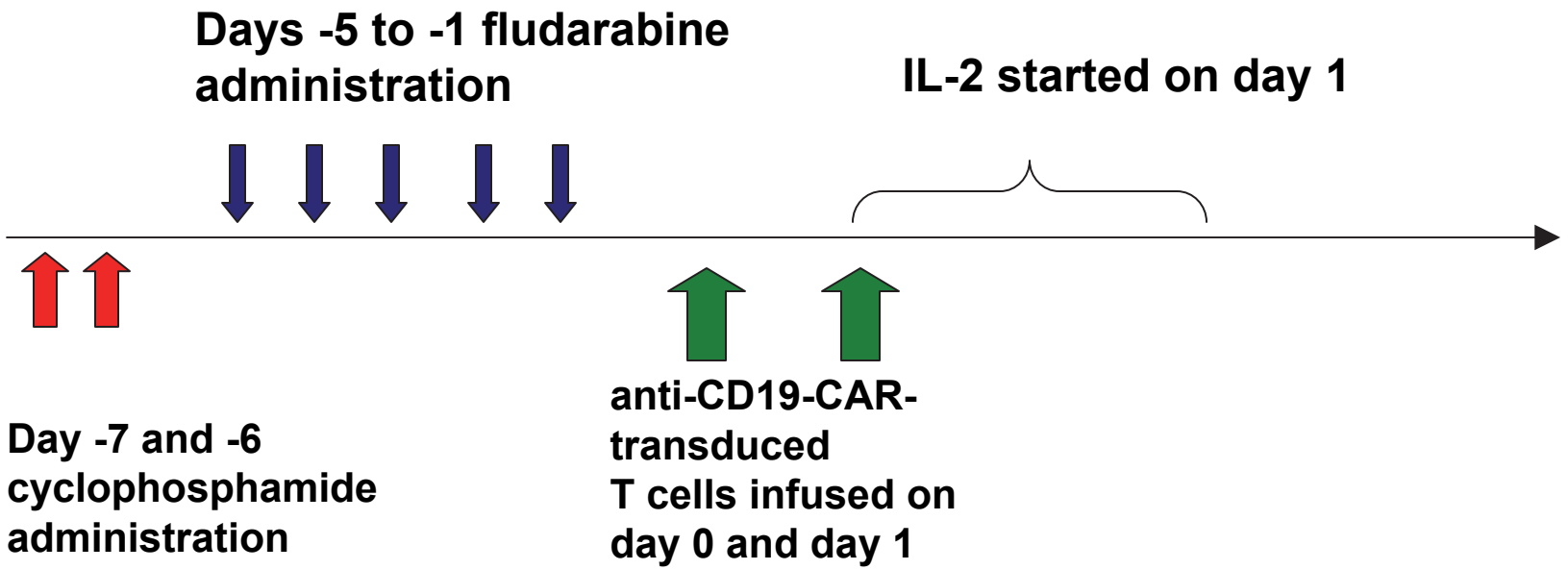
T cells can be genetically engineered to express an anti-CD19 chimeric antigen receptor

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

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

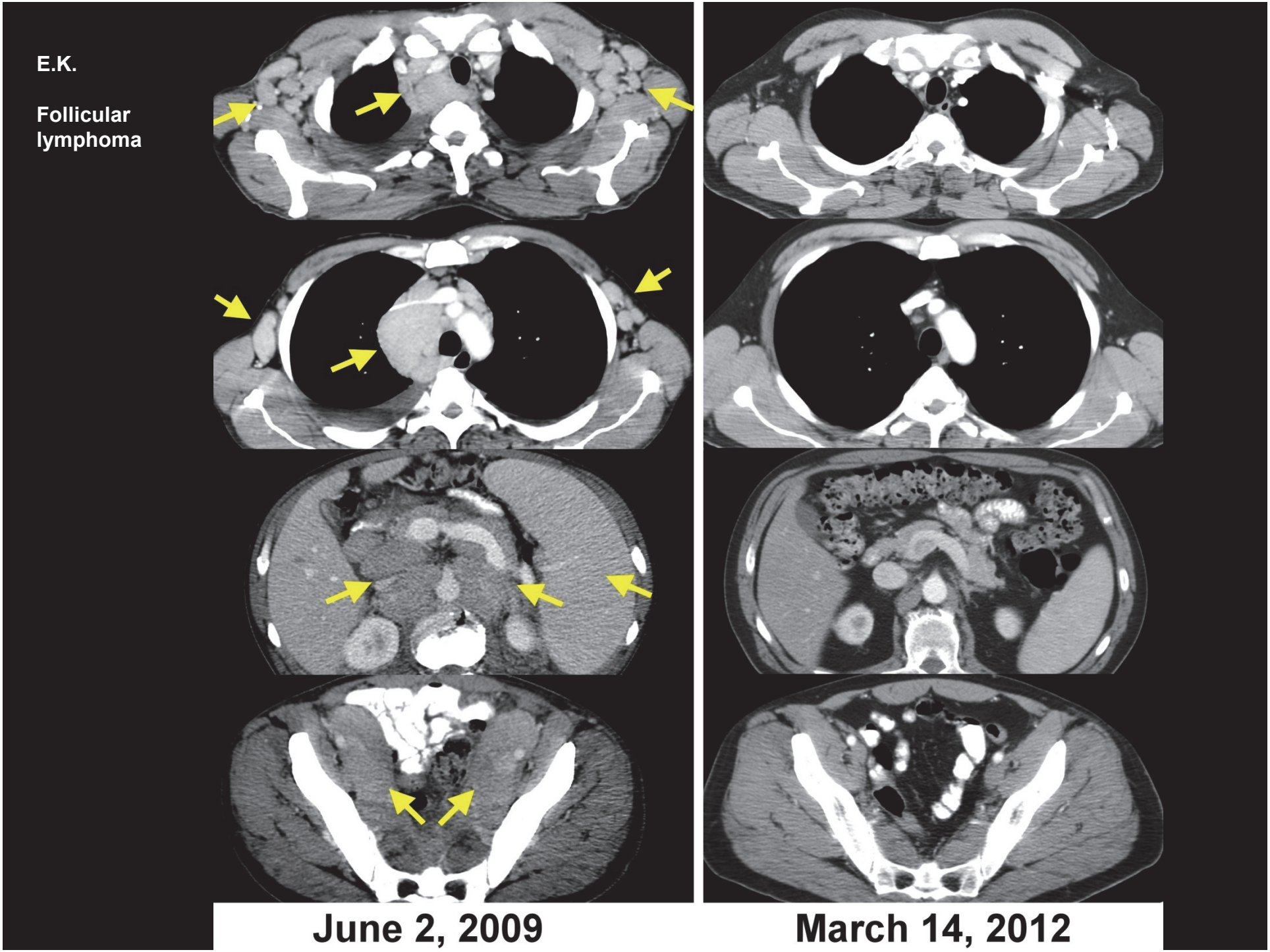
Pt	Inits	Date Cells	N	Agent	Schedule	Date Started
1	D.S.	04/21/2010	1)	FLUDARABINE	6 cycles	XX/XX/2003
			2)	CYCLOPHOSPHAMIDE	7 cycles	03/XX/2006
				FLUDARABINE	7 cycles	
				MIXOXANTRONE	7 cycles	
			3)	METHYLPREDNISONE	21 days	XX/XX/2008
			4)	SOLUMEDROL	11 cycles	05/XX/2009
2	R.L.	01/13/2011	1)	CYCLOPHOSPHAMIDE	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%)

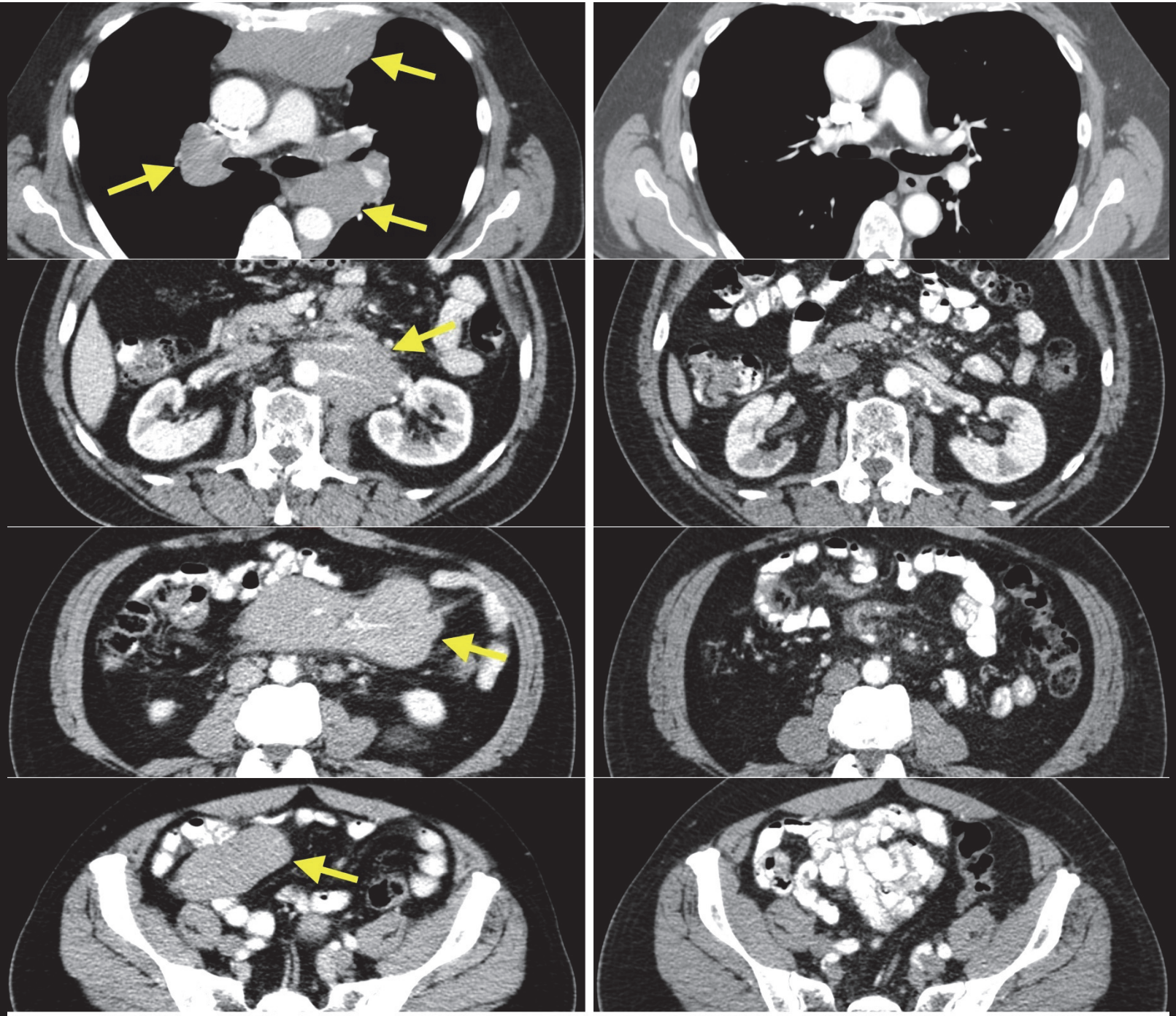
E.K.

Follicular lymphoma



D.G.

Follicular lymphoma

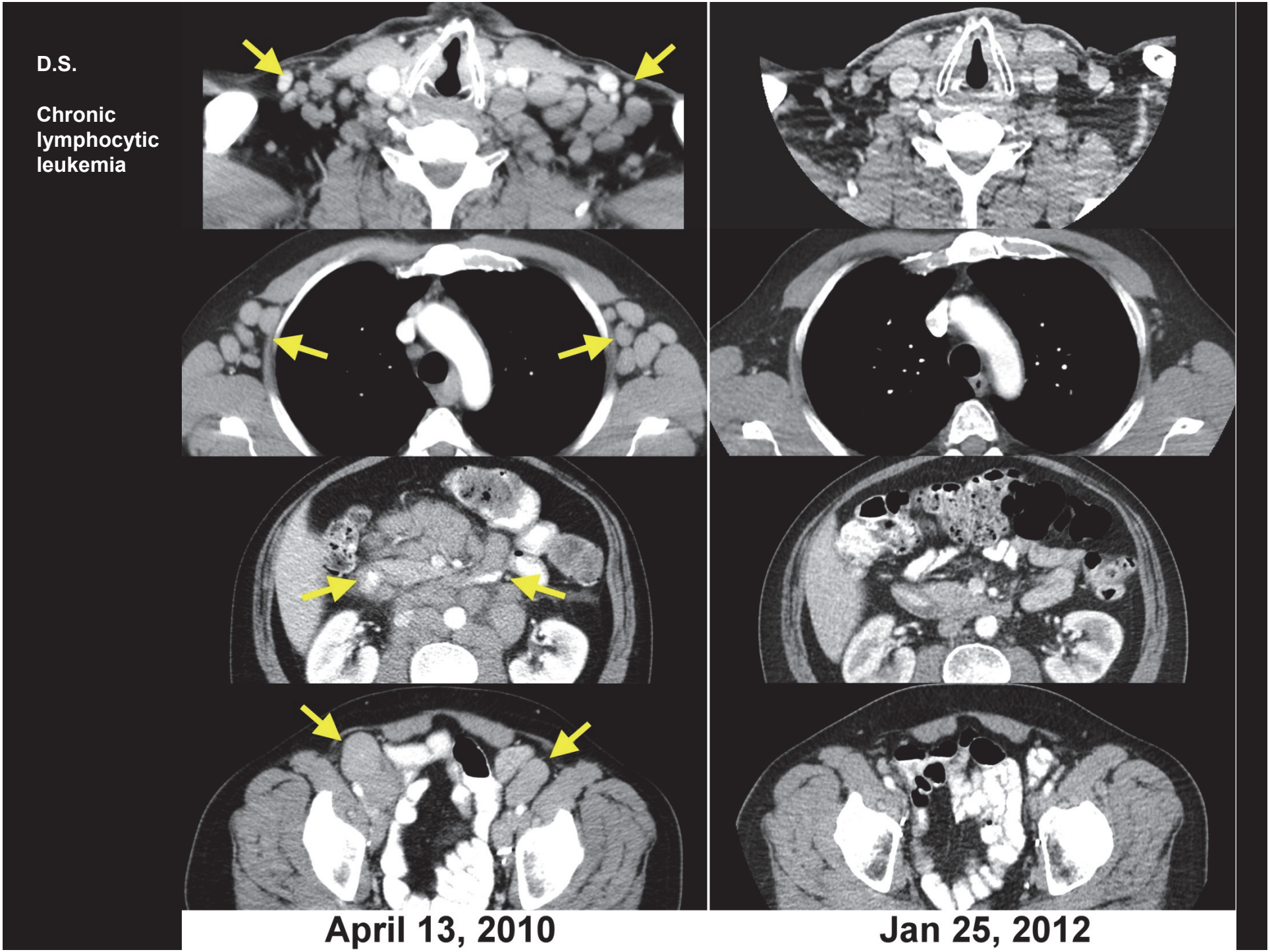


Pre-Treatment

14 Months

D.S.

Chronic lymphocytic leukemia

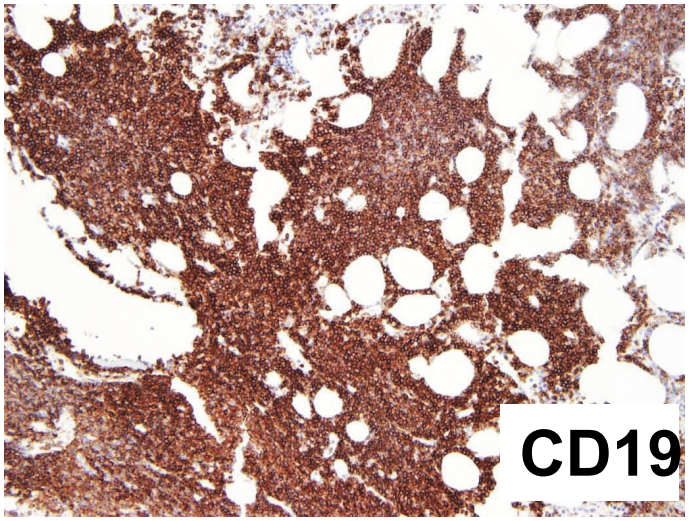


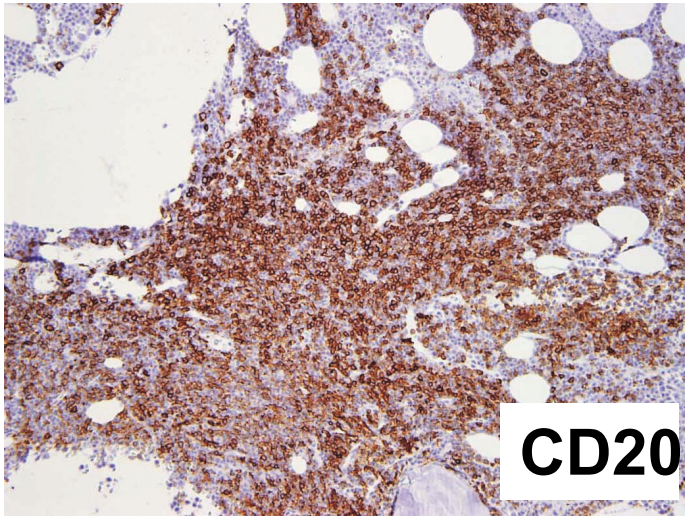
April 13, 2010

Jan 25, 2012

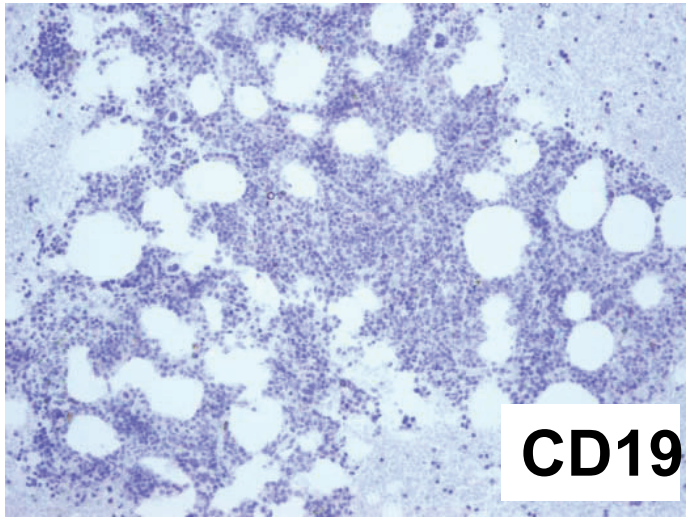
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment

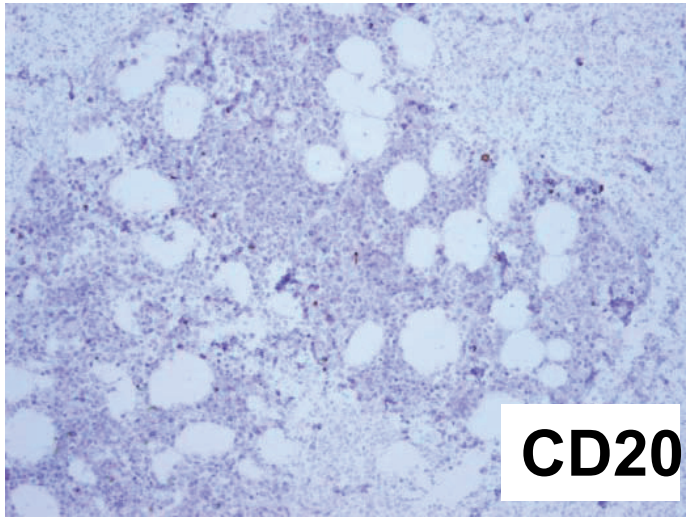
Before treatment



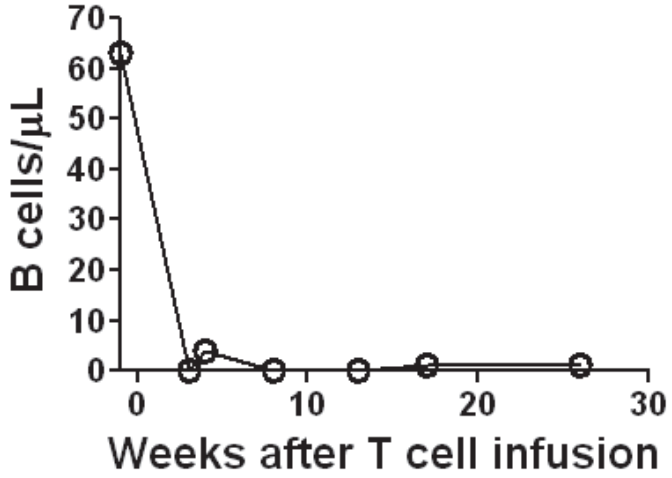


3 months after treatment

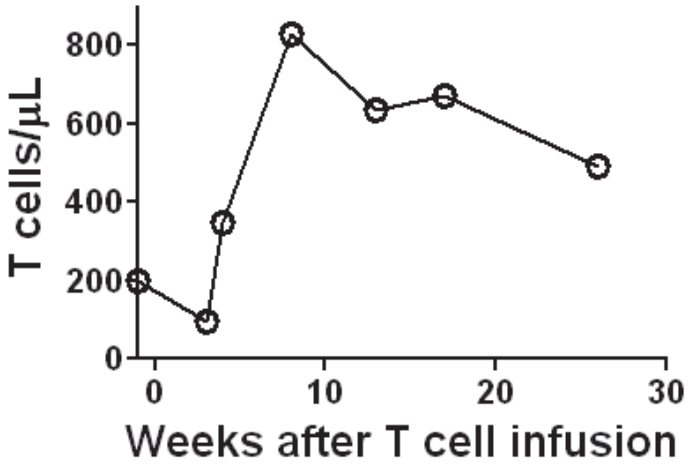


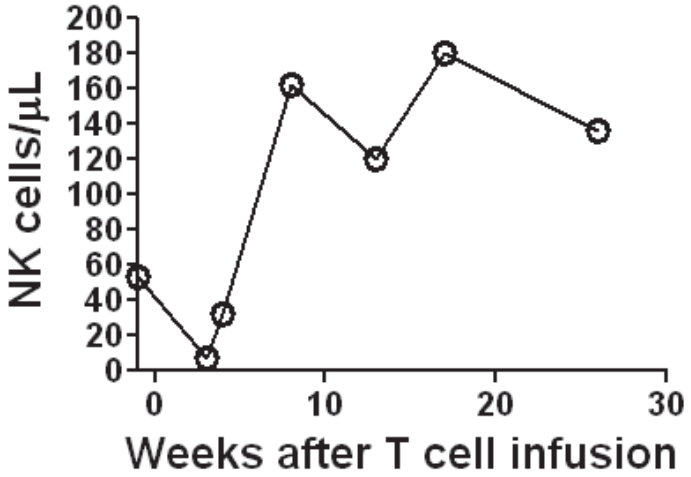


In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion



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





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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

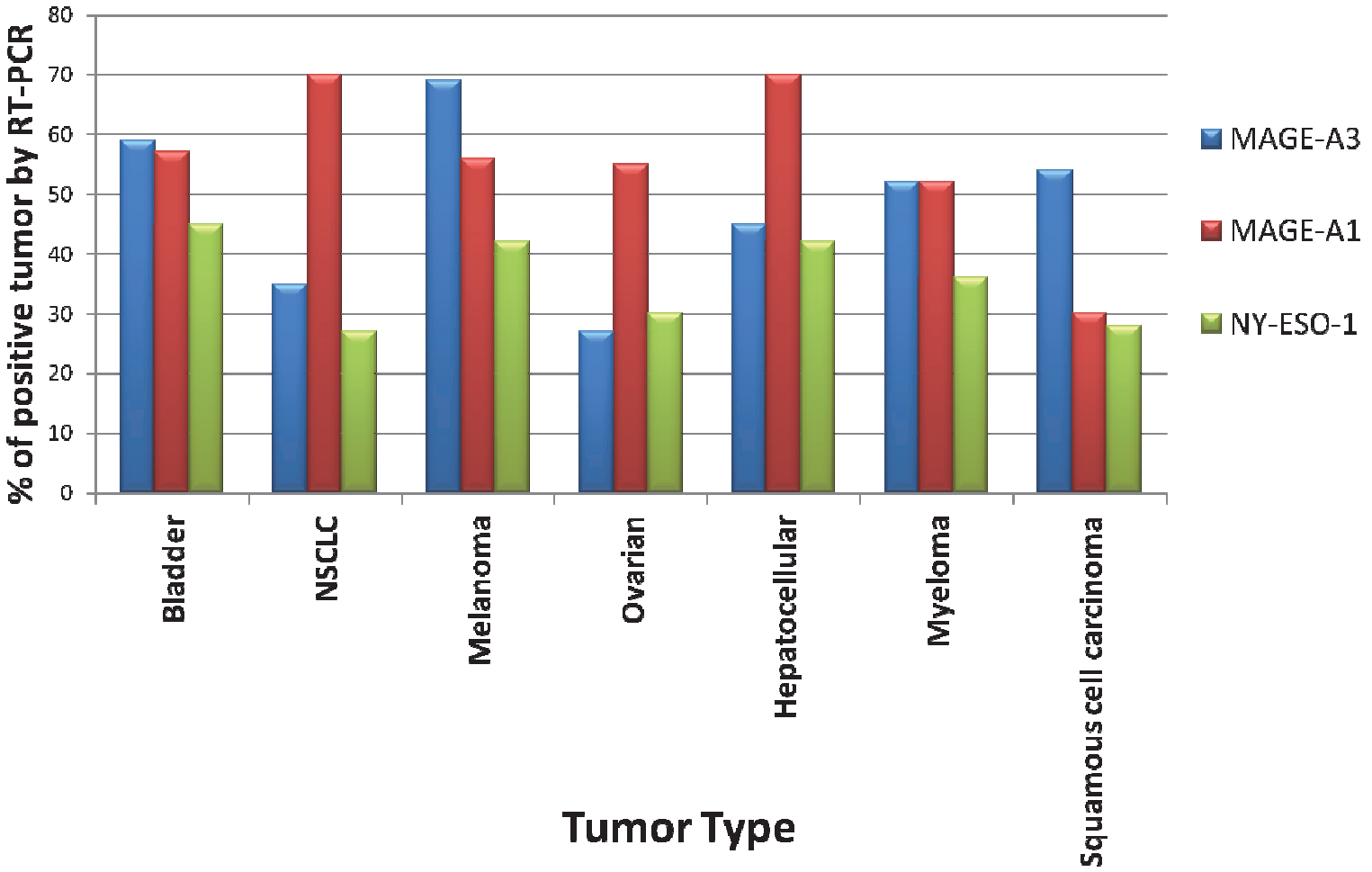
NY-ESO-1 Family

- **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



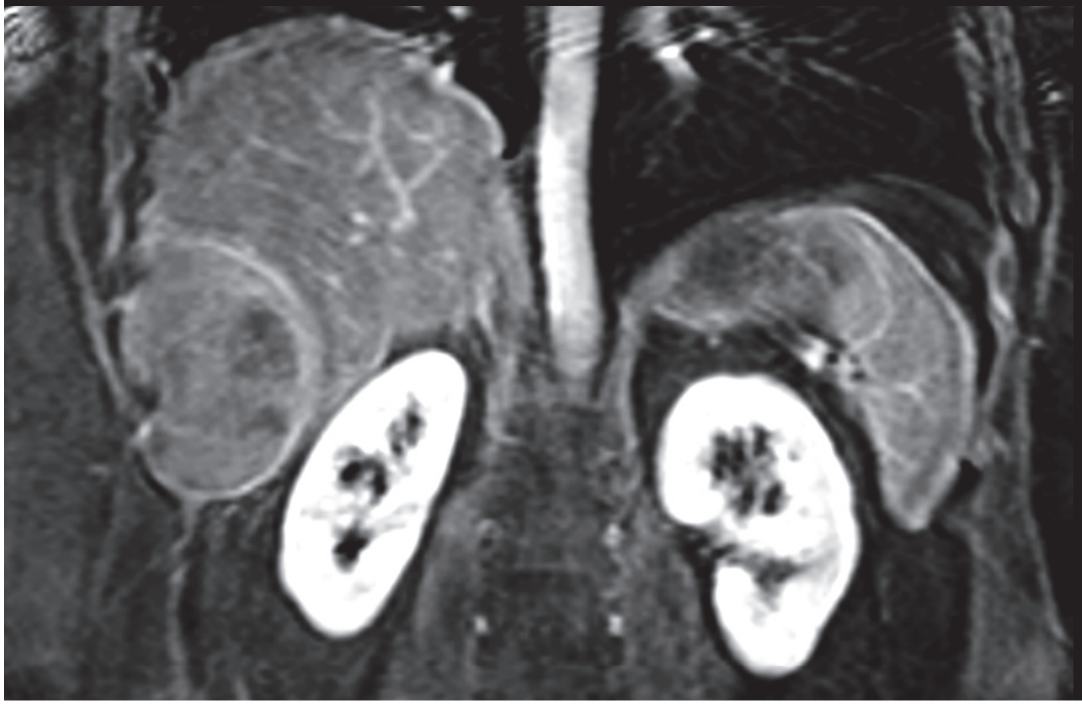
Responses to Therapy with NY-ESO-1 TCR (8/1/12)

	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	16	4 (25%) (12+, 10, 8, 3)	4 (25%) (42+, 31+, 25, 15+)	8 (50%)
Synovial Cell Sarcoma	11	8 (73%) (23+, 14*, 12, 10, 8, 5, 4, 3)	0	8 (73%)

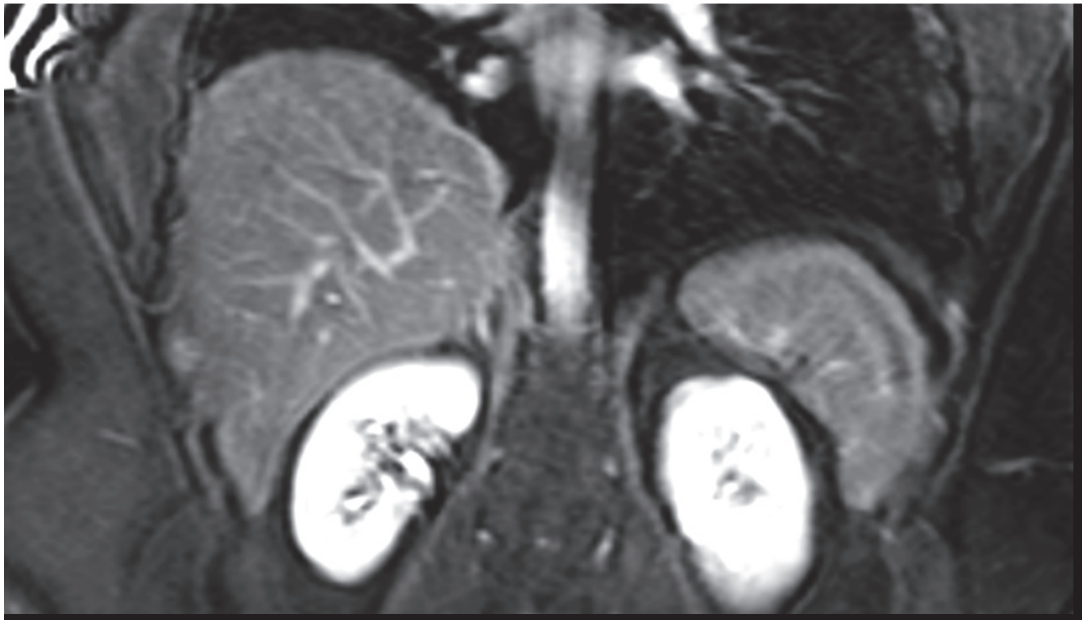
*treated twice

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

M.M. Synovial cell sarcoma ESO TCR



Pre-Treatment

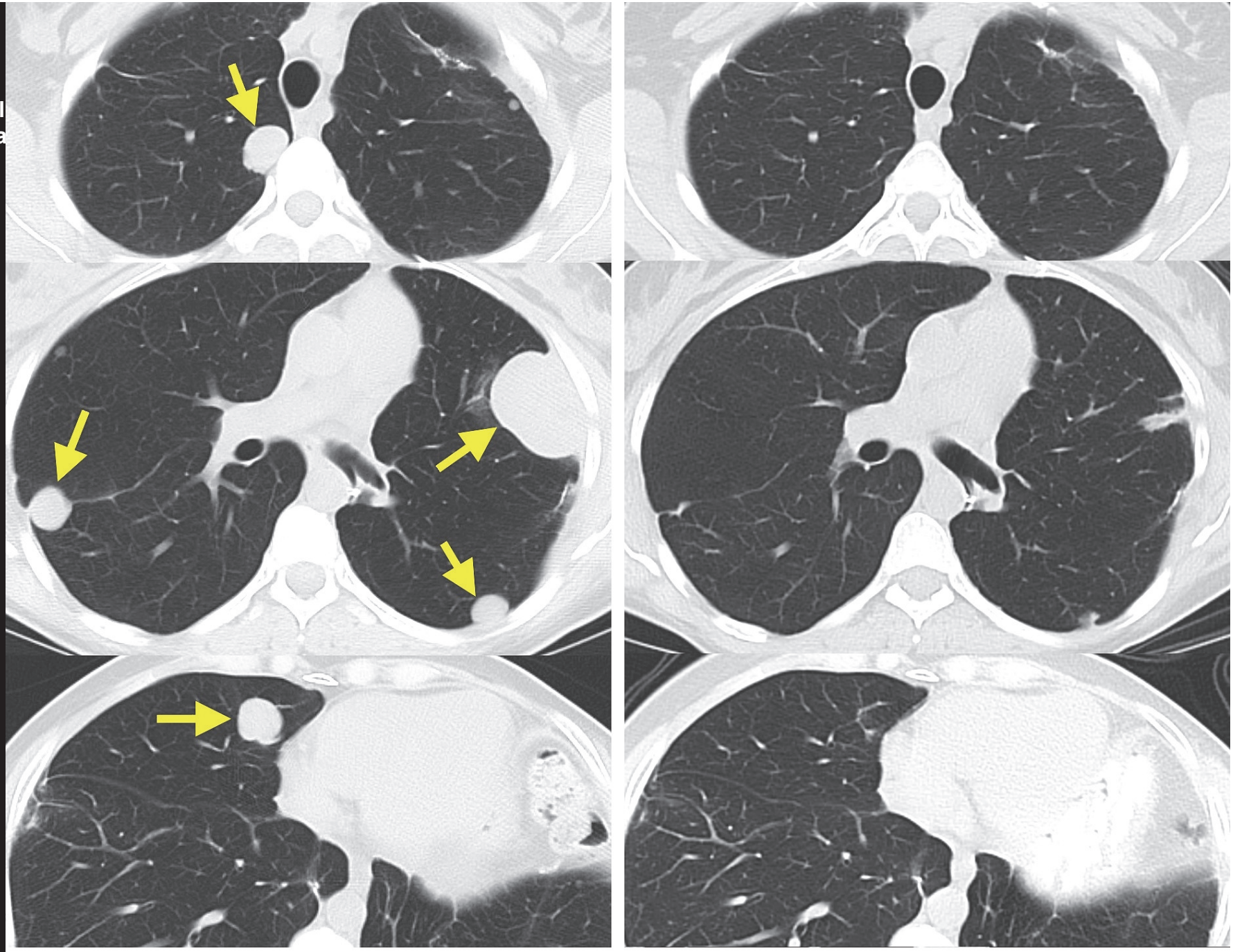


6 Months

H.K.

Synovial Sarcoma

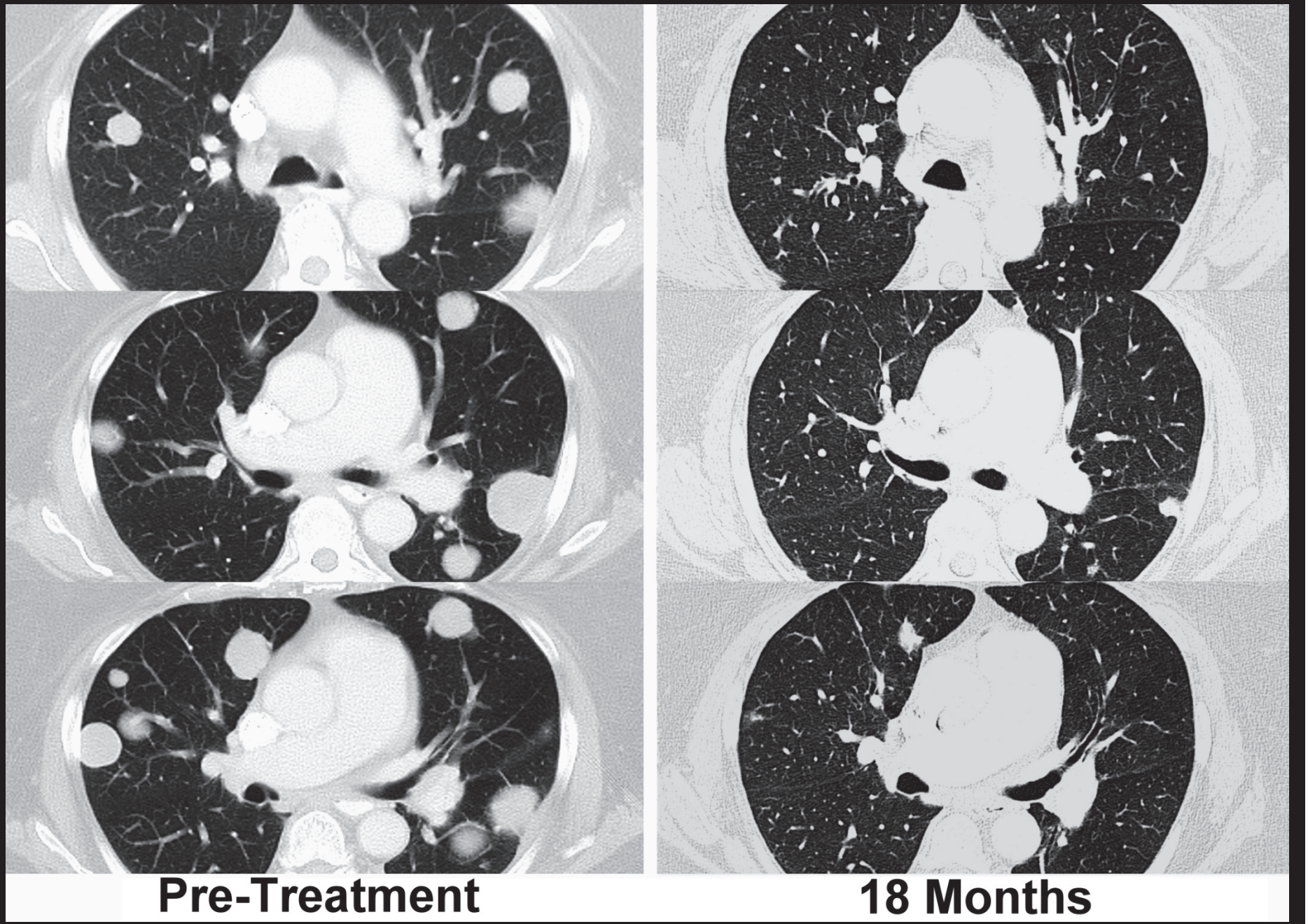
ESO TCR



Pre-Treatment

14 Months

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



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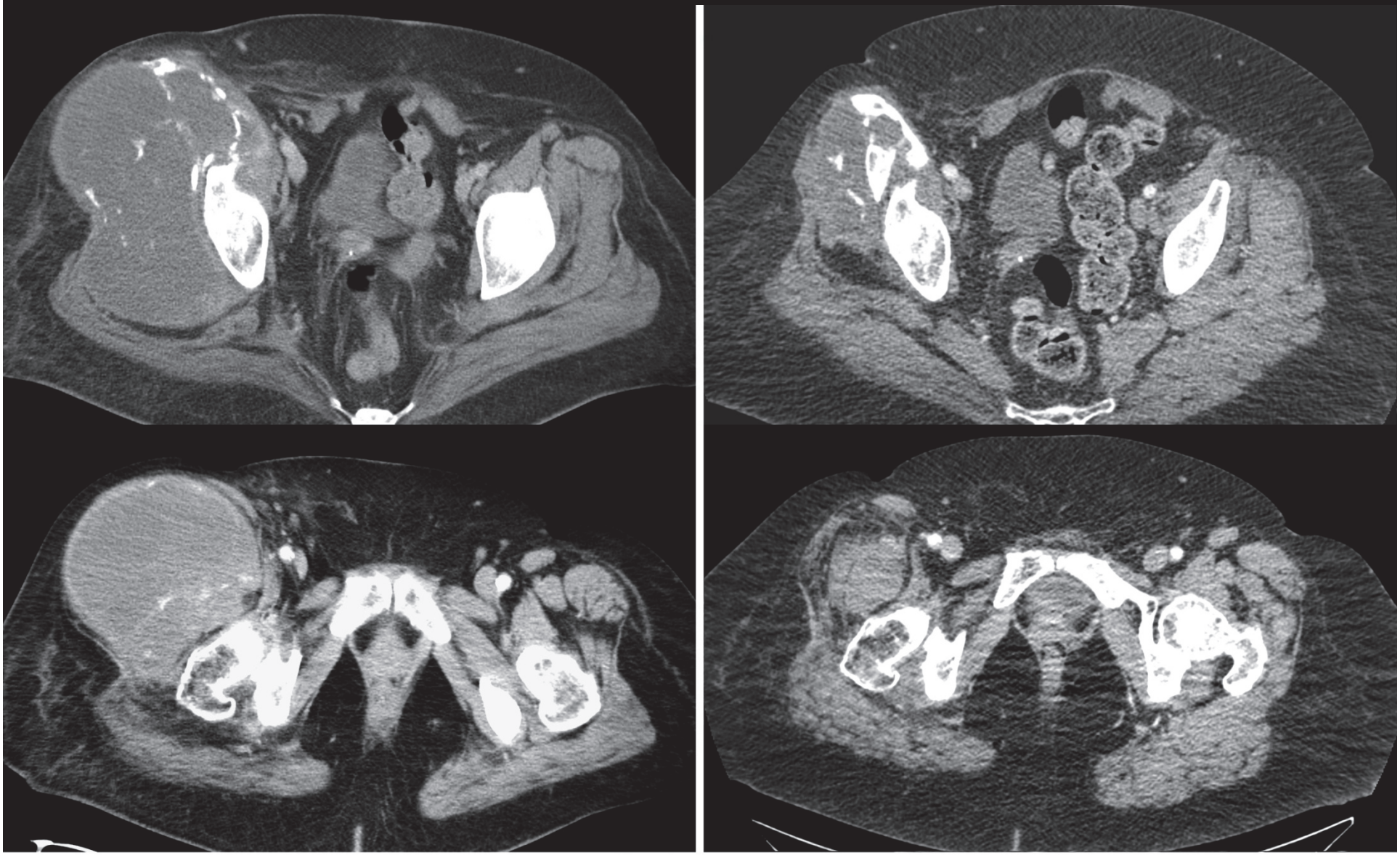

Pre-Treatment

18 Months

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

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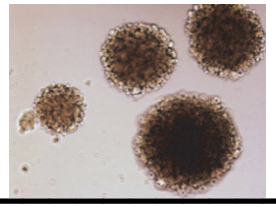
Expressed in 30-50% of glioblastomas

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

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)

Rationale for the anti-VEGFR2 Gene Therapy Protocol

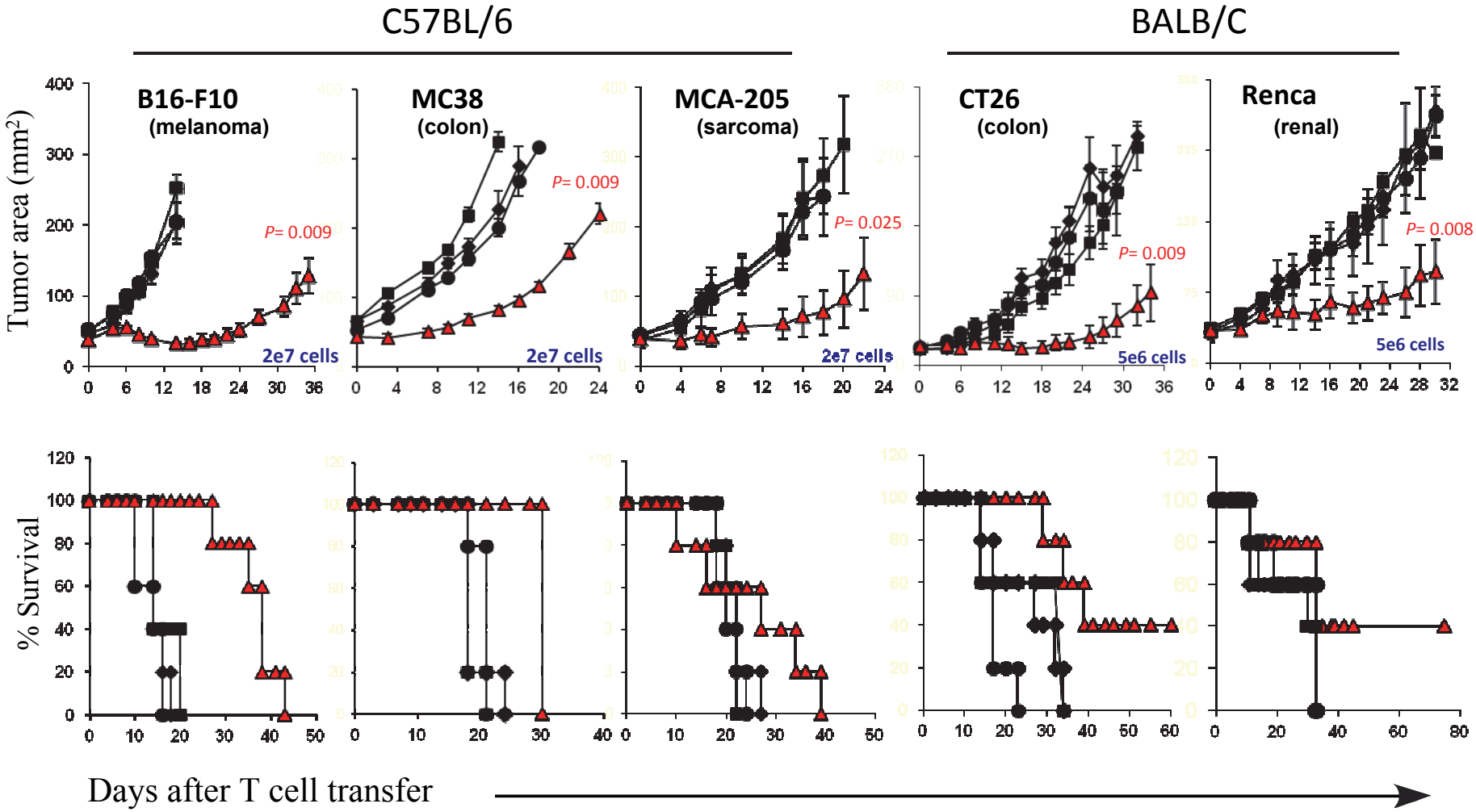
Vascular endothelial growth factor (VEGF) stimulates tumor angiogenesis by binding to its receptor, VEGFR2

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

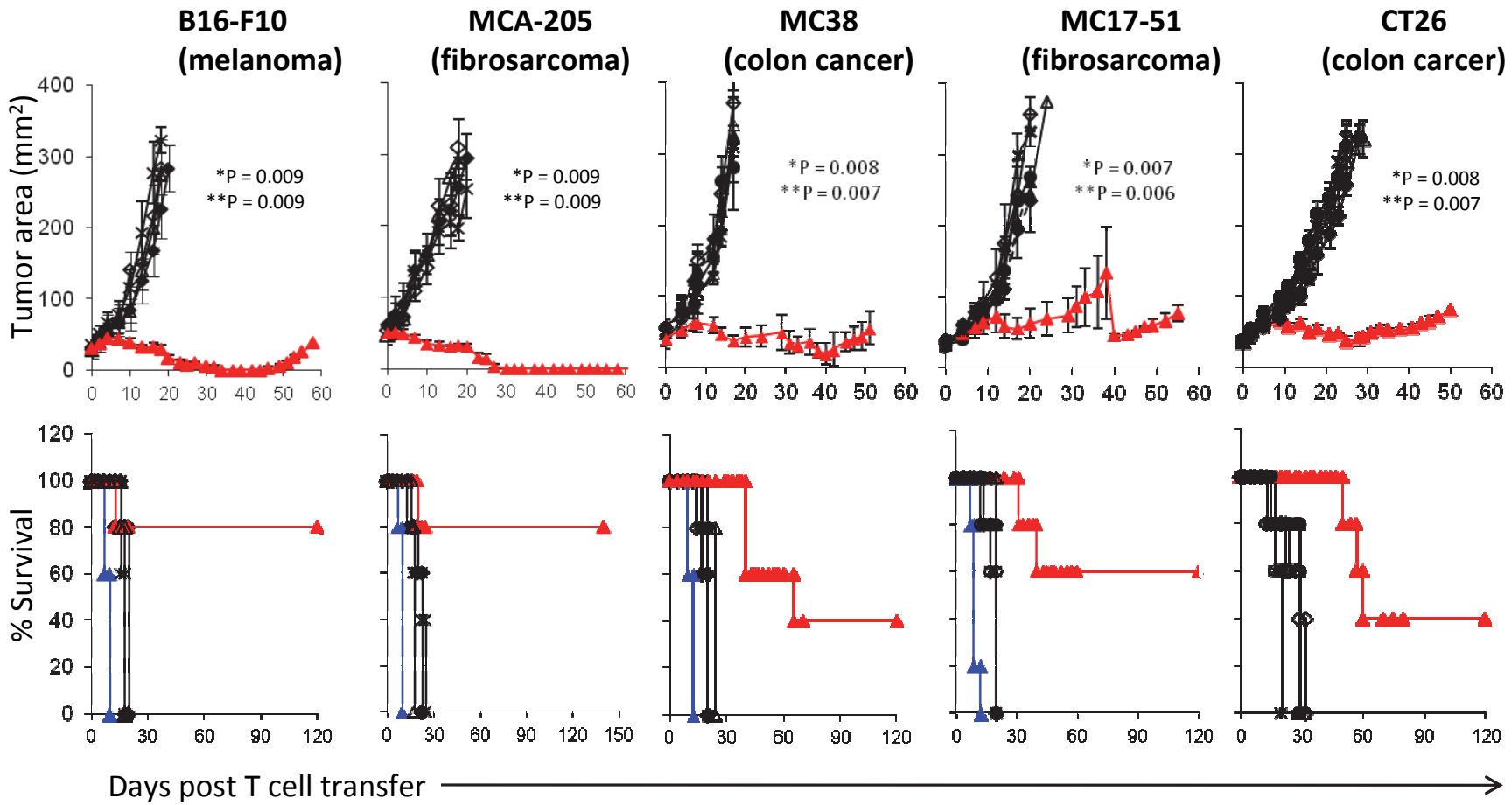
Destruction of cells bearing VEGFR2 may more effectively destroy tumor vasculature and result in effective cancer treatment

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)

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

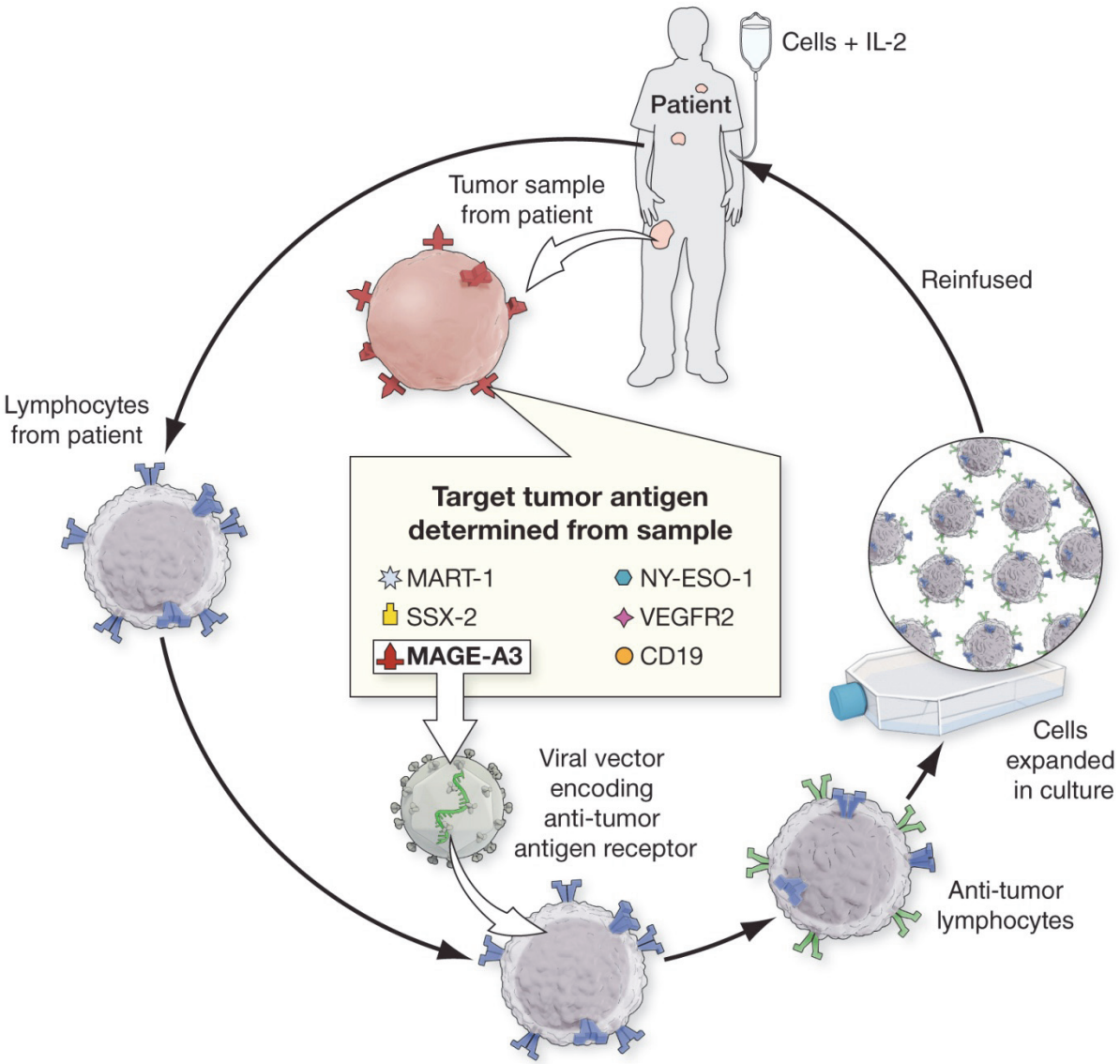
Phase I dose escalation trial ongoing.

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

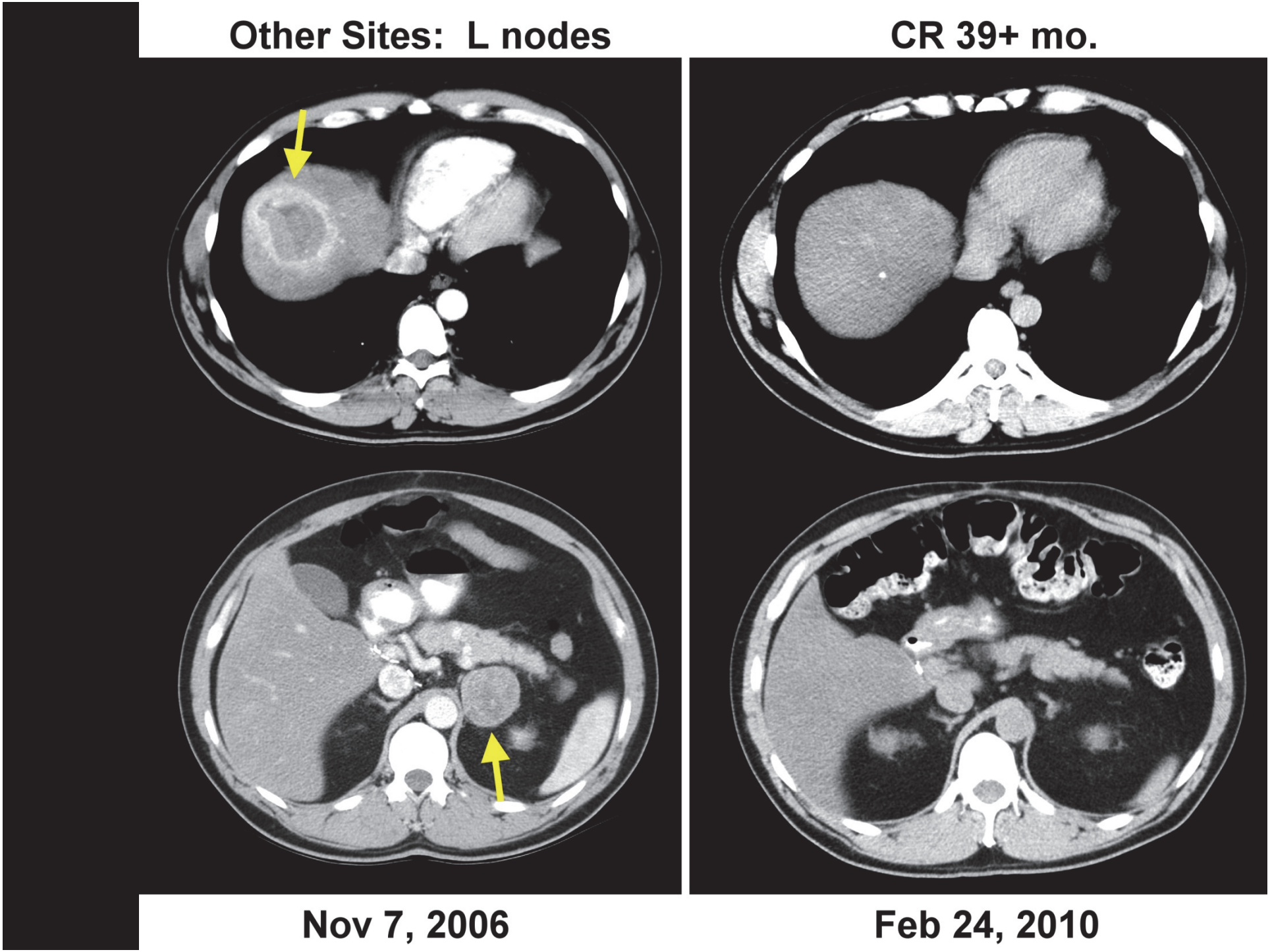
*(MAGE-A3 TCRs; restricted by HLA-A2, A1, Cw7, DP4 – covers 80% of patients)

Personalized immunotherapy using anti-tumor receptor gene-modified lymphocytes



Other Sites: L nodes

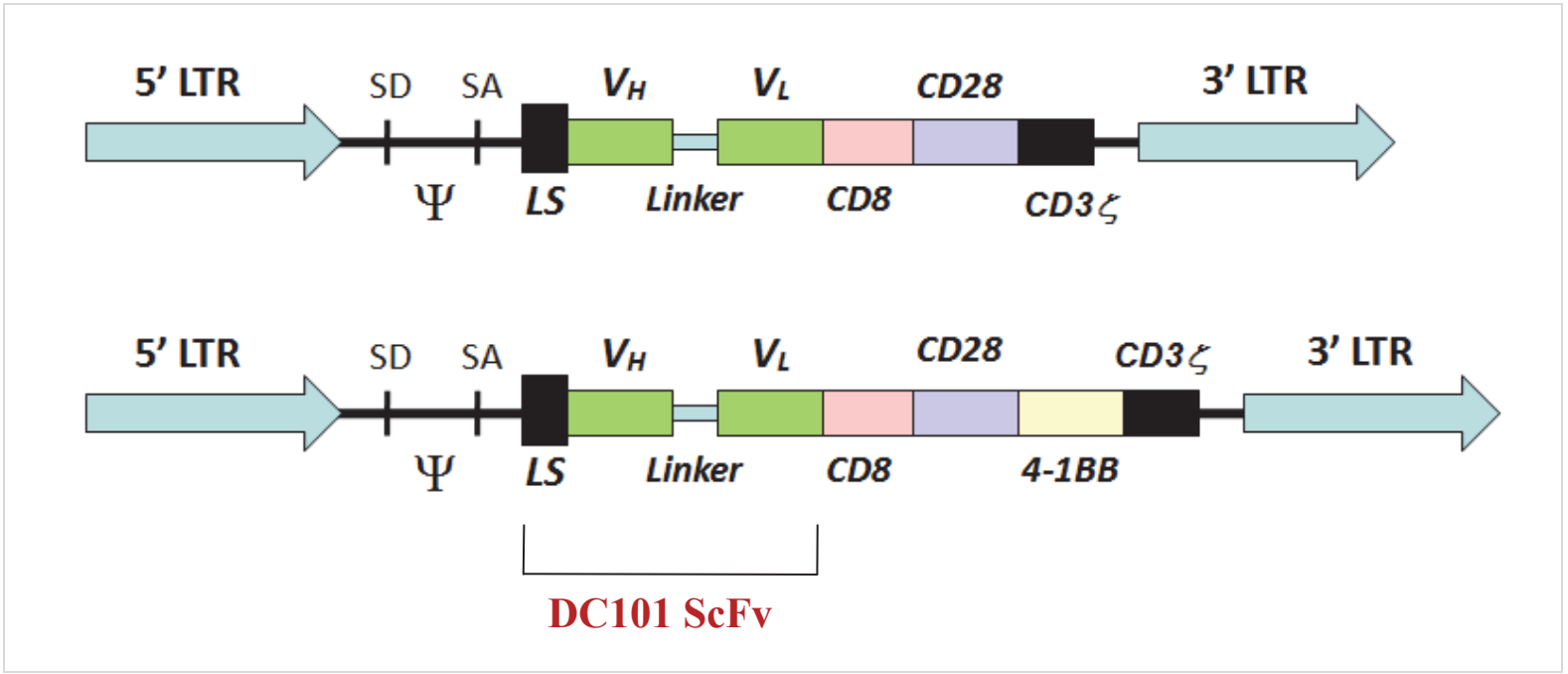
CR 39+ mo.



Nov 7, 2006

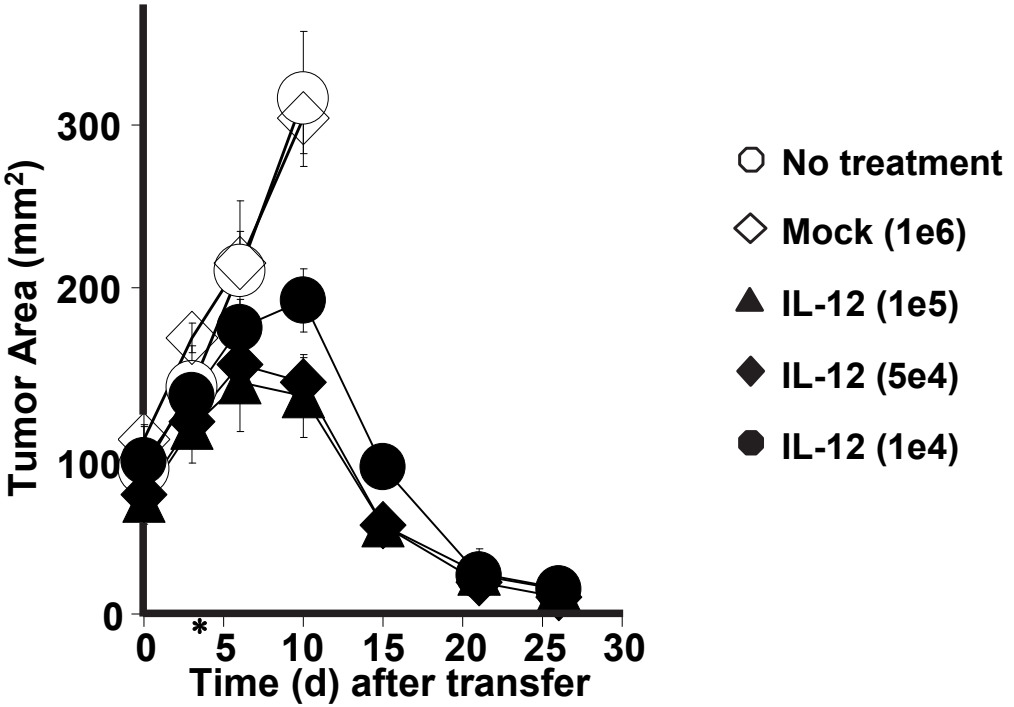
Feb 24, 2010

Design of DC101 Anti-VEGFR2 CAR Retroviral Vectors



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

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)

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.)

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)

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

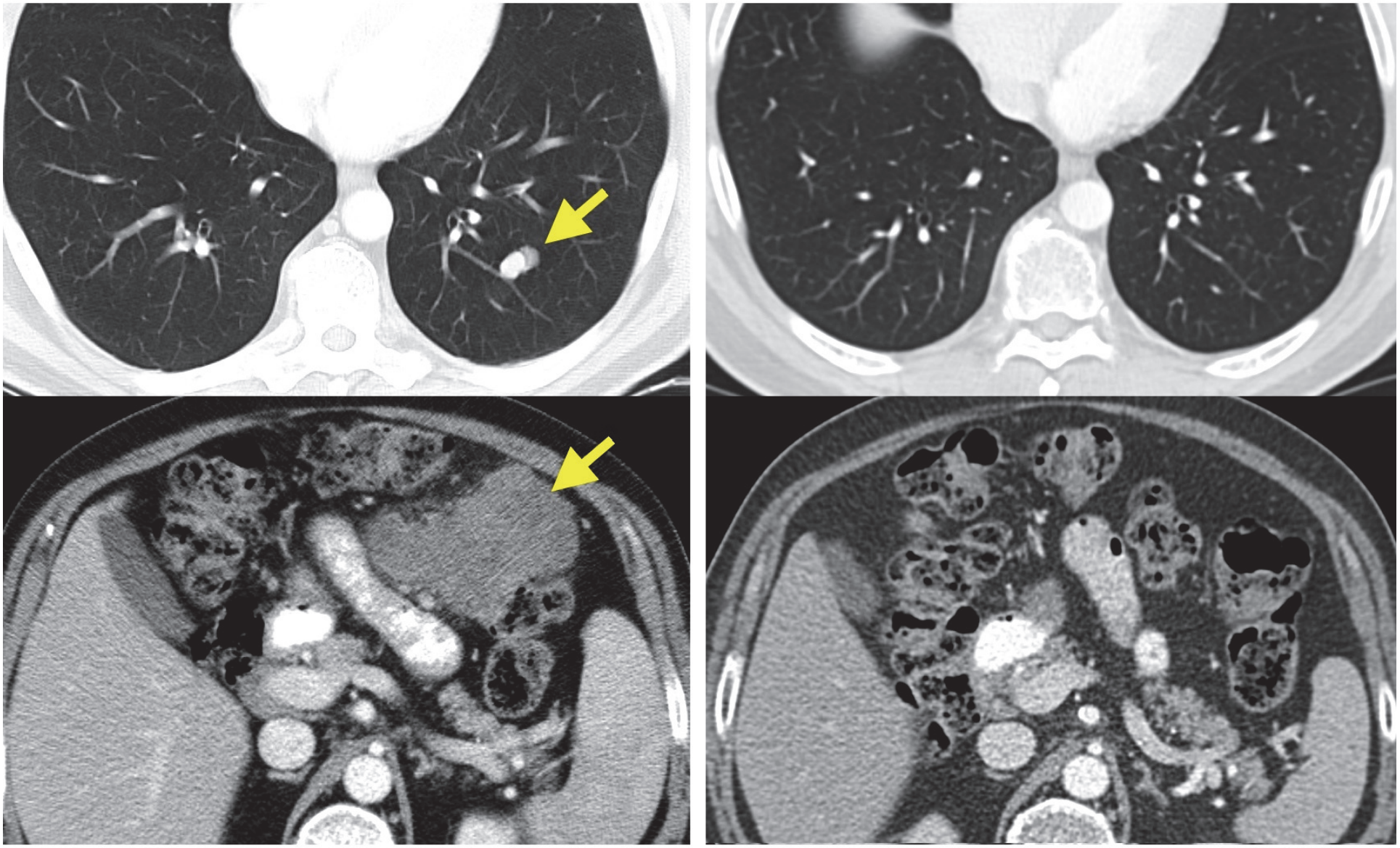
Patient	Diagnosis	Response	Duration (months)
1	Follicular lymphoma	PR (90%)*	34+
2	Follicular lymphoma	N.E. (died H1N1 pneumonia)	
3	Chronic lymphocytic leukemia	CR	20
4	Splenic marginal zone lymphoma	PR (95%)*	23+
5	Chronic lymphocytic leukemia	NR	--
6	Chronic lymphocytic leukemia	PR	6
7	Chronic lymphocytic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

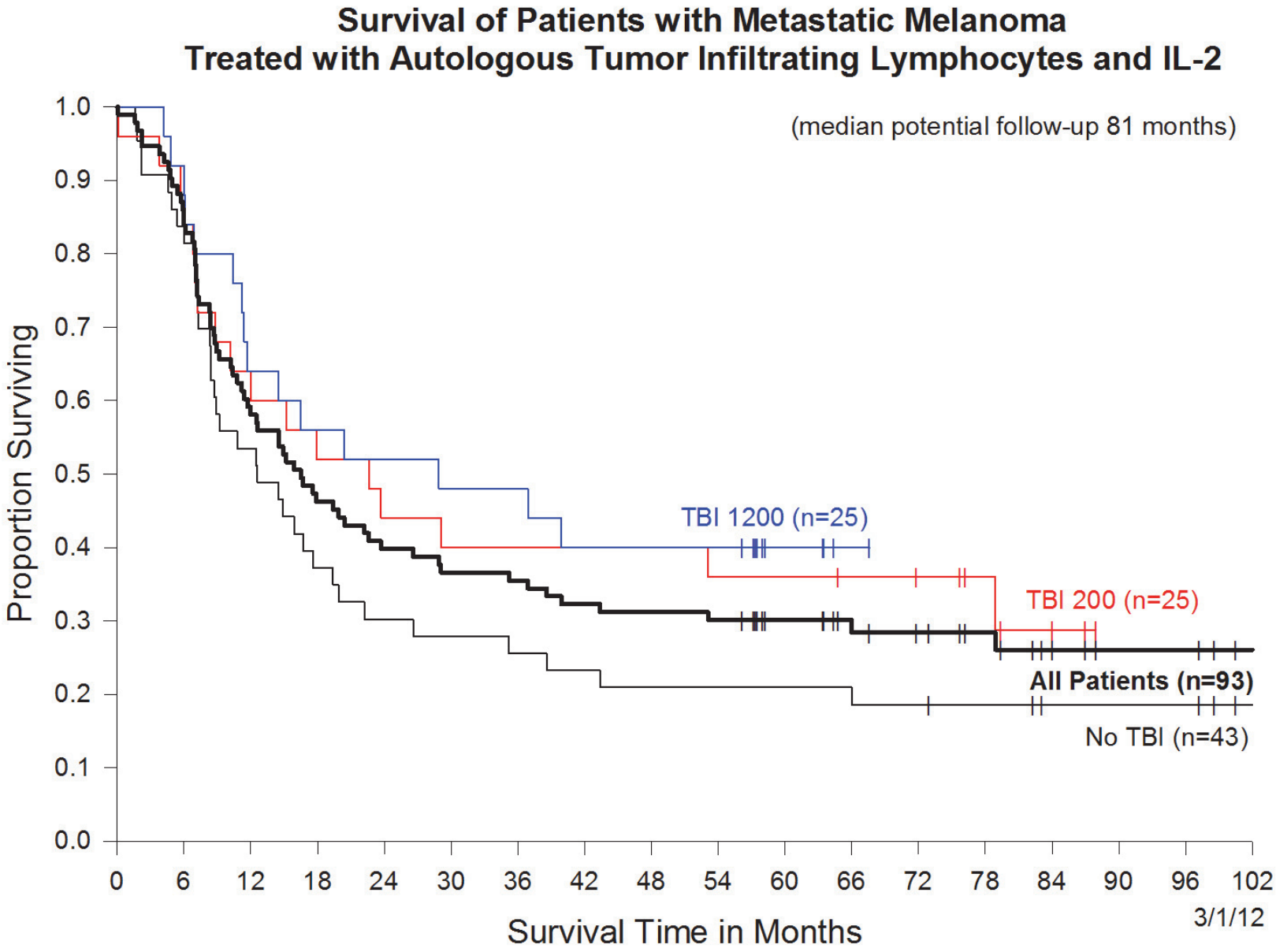
Other Sites: Sm bowel, S.C., LN

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

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%)

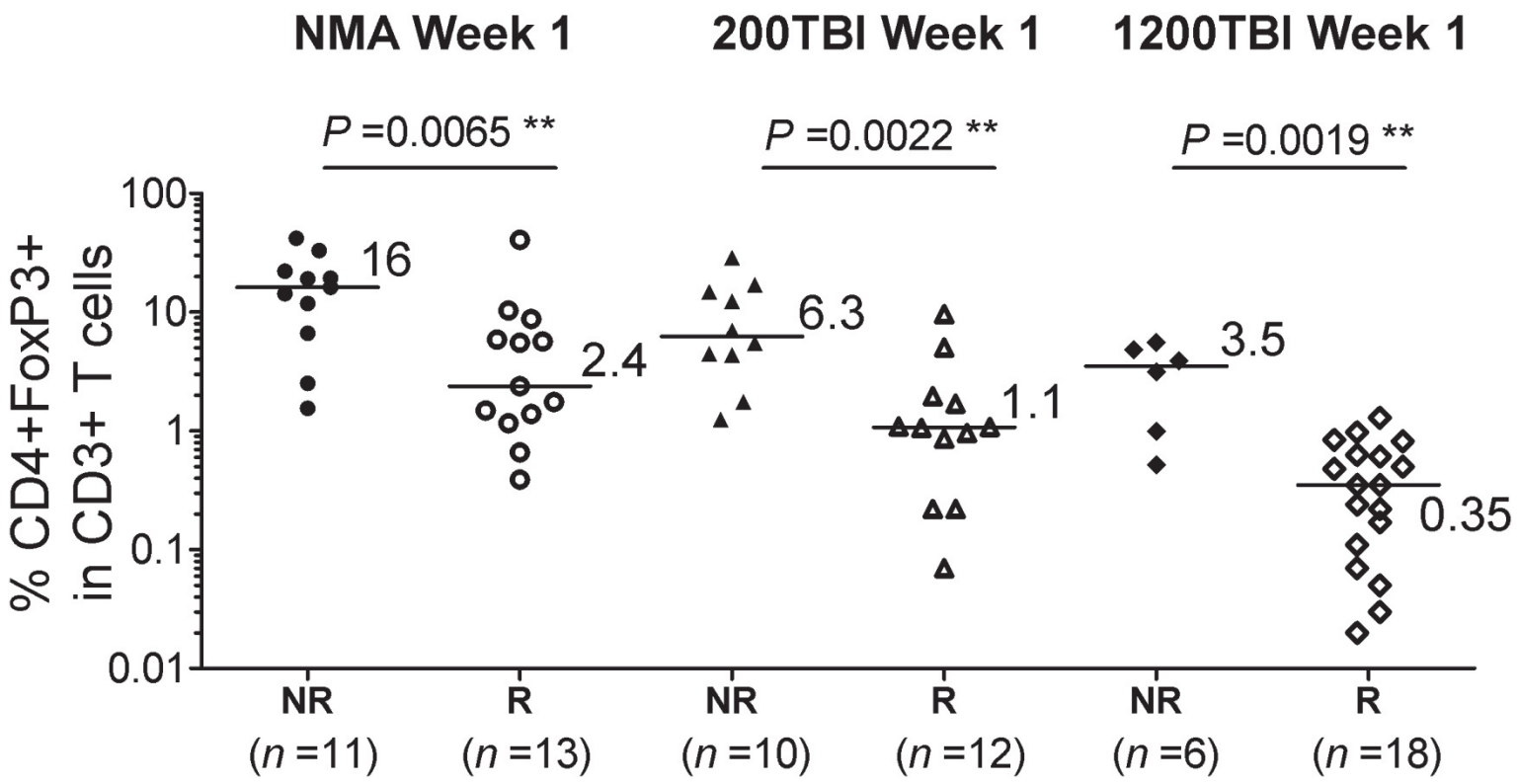
*This refers to the percent of patients with a CR, PR or OR in each group that had received the prior treatment.

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

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



(Xin Yao, Surgery Branch, NCI)

The Puzzle of Melanoma Immunogenicity

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

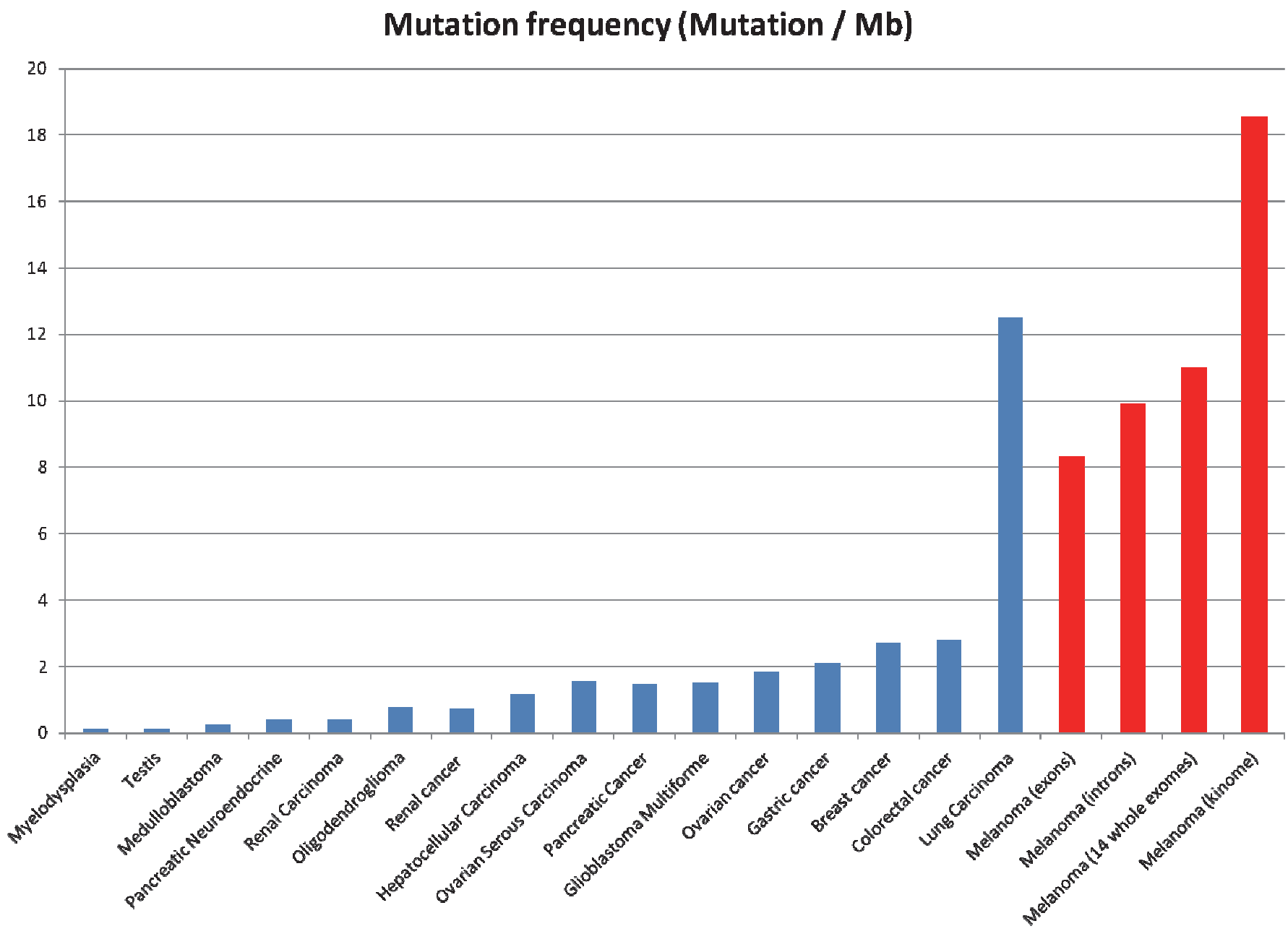
1. **susceptibility to treatment with immune modulators**
   - **e.g. IL -2**
   - **anti-CTLA4**
   - **anti-CD40**
   - **anti-PD1**

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?


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 and normal tissue to determine the number of exomic mutations

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
May 2005: increasing liver and periportal lymph node metastases
TIL/IL-2 but no lymphodepletion; progressive disease
July 2005: TIL/200TBI/IL-2 with lymphodepletion

Complete regression ongoing as of March, 2012

(Exomic analysis revealed 595 nonsynonomous mutations)

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

---

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

Obtain sequence of 9aa on either side of aa mutation

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

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 9aa on either side of aa mutation

Use algorithm to predict best binders to that patients HLA antigens

Synthesize best binding peptides

Test for recognition of pulsed peptides by TIL

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

Mutated Antigens in Autologous Tumor Recognized by TIL 2369

PLEKHM2: Pleckstrin homology domain – containing family member M2

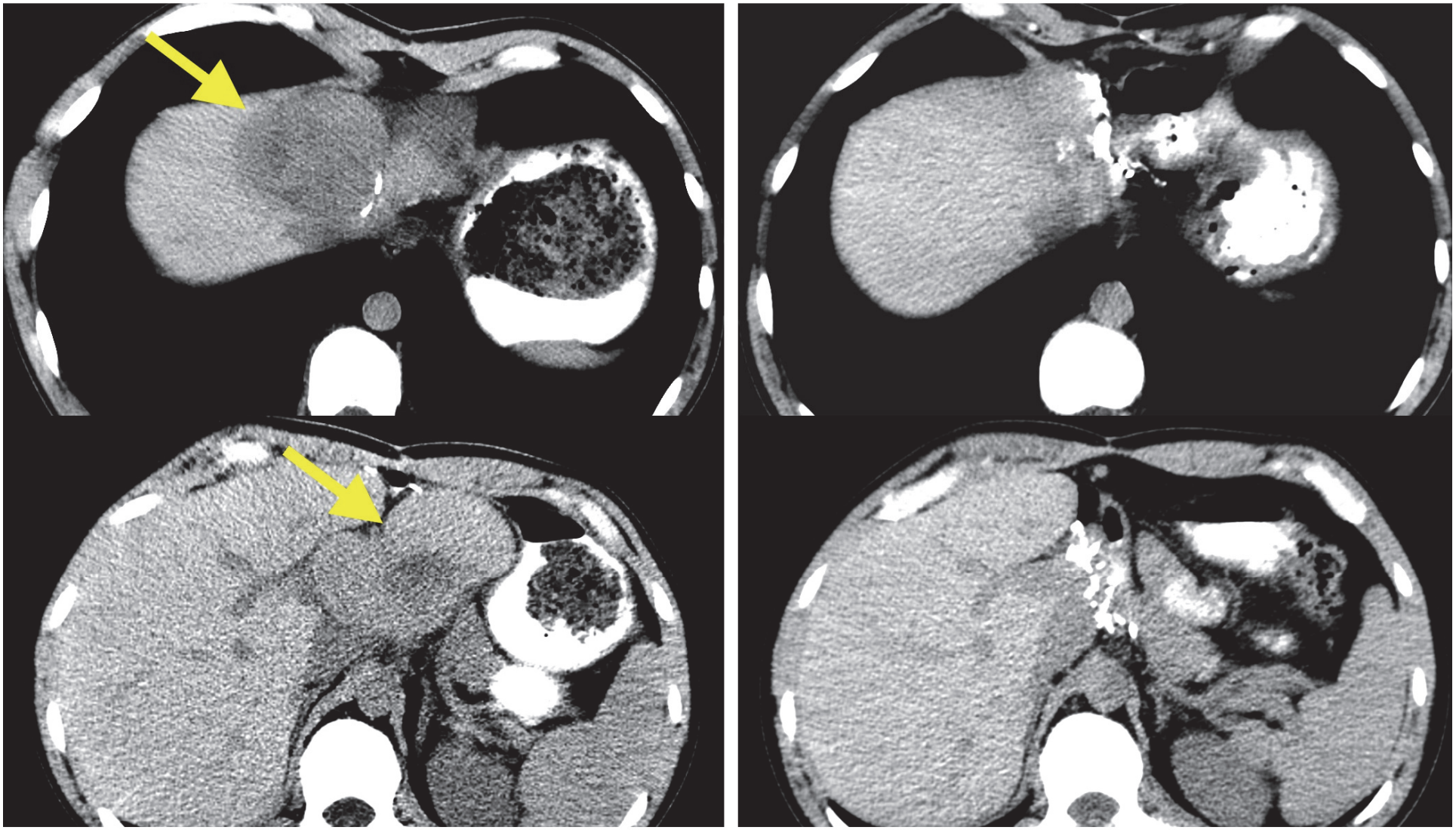
interacts with kinesin and plays a role in microtubule formation

PP1R3B: Protein phosphatase 1 regulating subunit Ga protein

regulates glycogenesis in myotubes

Other Sites: Portal LN, brain

CR 57+ mo.



July 12, 2005

April 12, 2010

Patient B.C. (TIL 2098)

53 year old female with metastatic melanoma

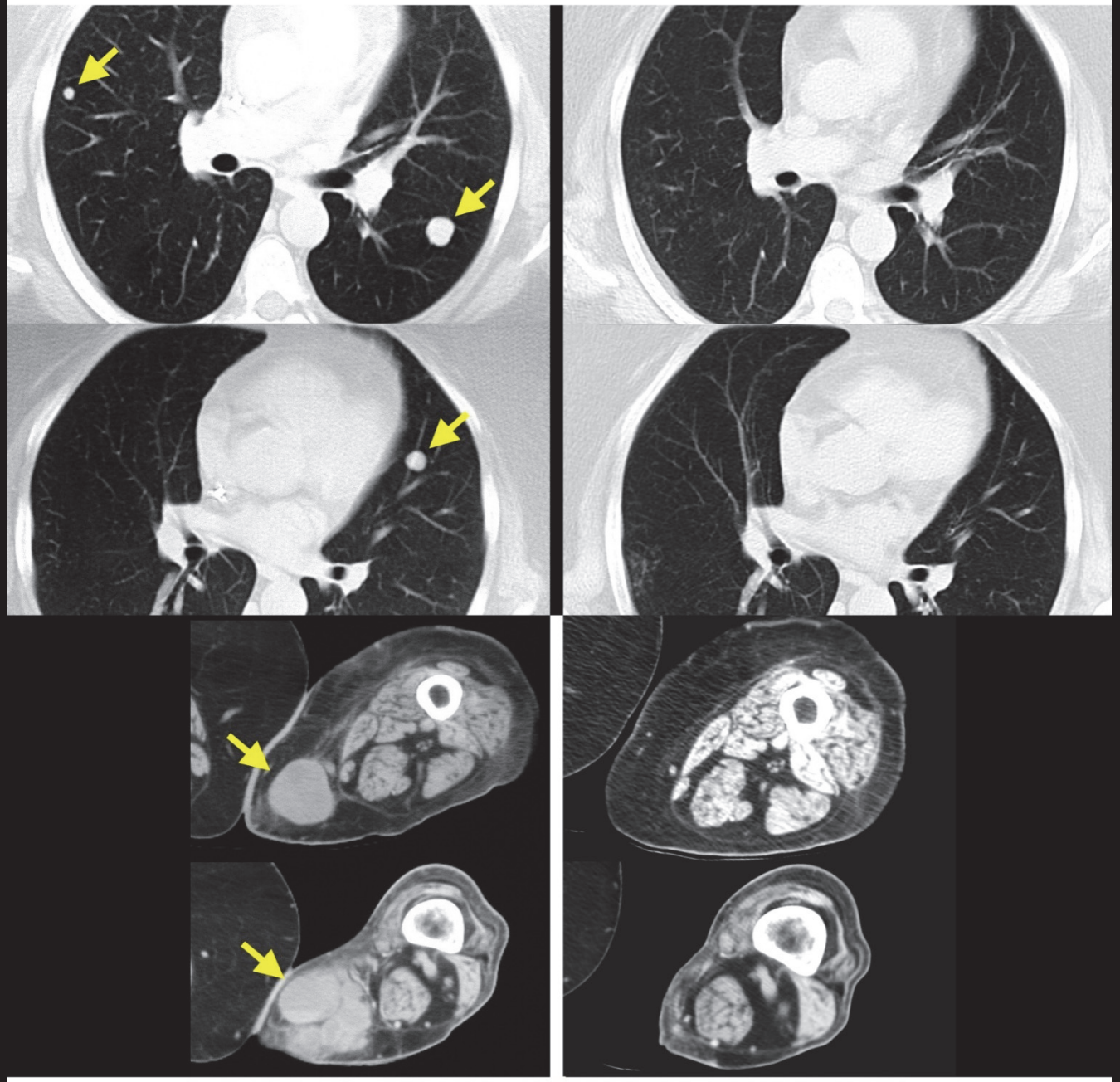
July 1995: excision of leg melanoma
Dec. 1995: diffuse leg recurrence
Feb. 1996: isolated limb perfusion; progressive disease
May 1999: below-knee amputation
Dec. 1999: recurrence in stump and lungs
May 2000: chemotherapy with cisplatin; progressive disease
Jan. 2003: TIL/NMA/IL-2

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

(Exomic analysis revealed 300 nonsynonomous mutations)

Other Sites: Pancreas, subcutaneous

CR 58+ mo.

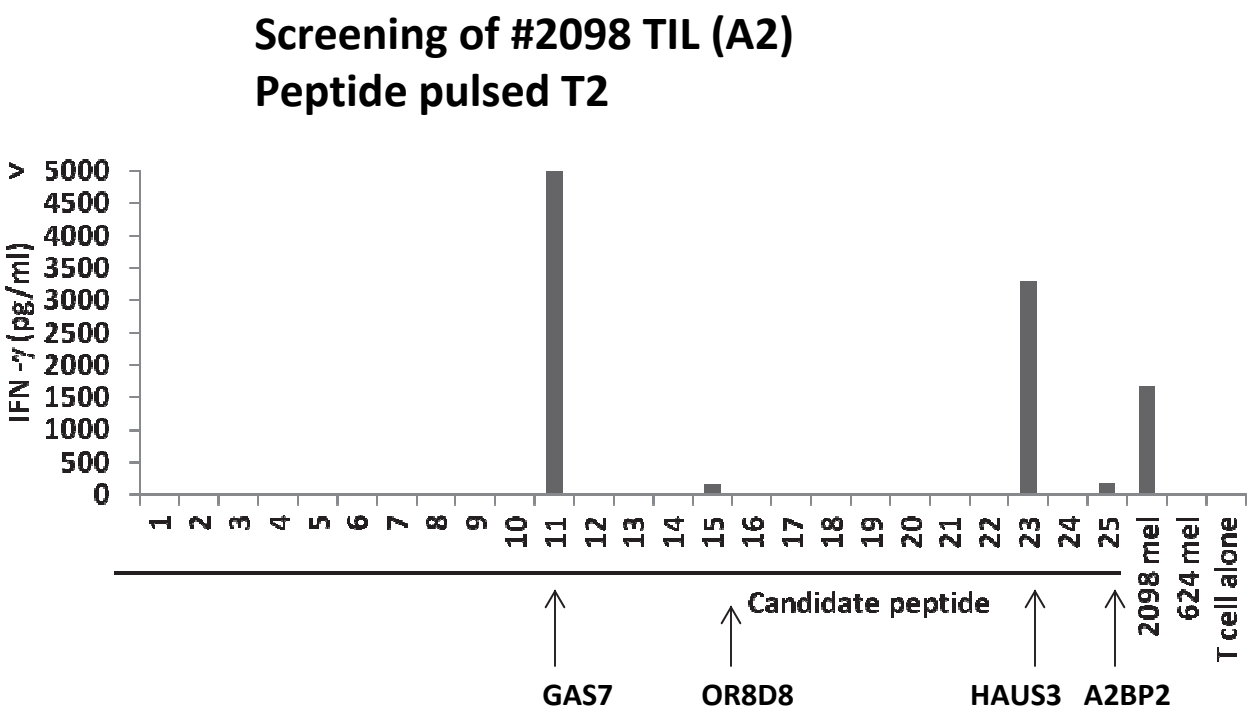


Jan 27, 2003

Dec 10, 2007

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



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

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

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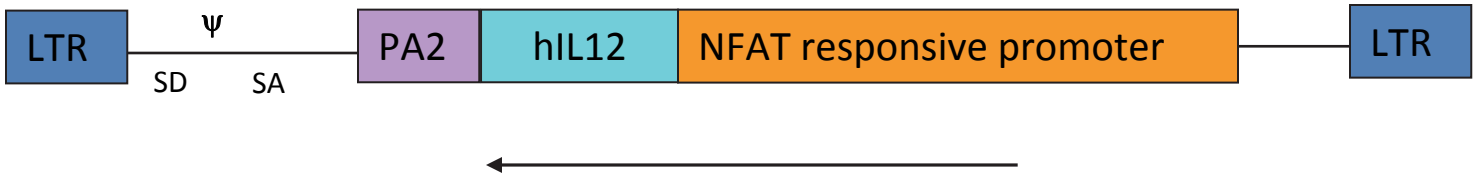
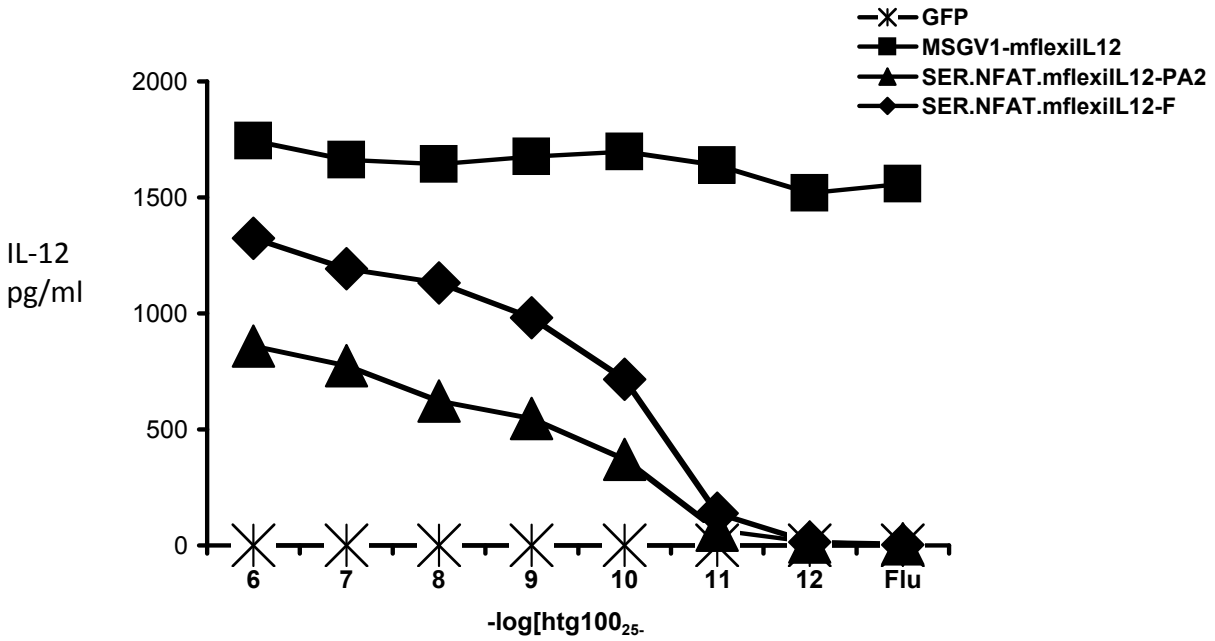
NFAT is a transcription factor produced in T cells activated by antigen specific triggering of the T cell receptor

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)

Development of Retrovirus NFAT-mflexiIL12 vector



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

IL-12 Gene Therapy: Treatment Regimen

TIL grown for 2-3 weeks

Stimulated with OKT-3, transduced and expanded

Infusion:

d-7 to d-1: Cy/flu preparative regimen

d0: single infusion (1-3 patients/cohort)

Cohort 1: 10^6 cells (first patient 11/4/10)

2: 3 X10^6

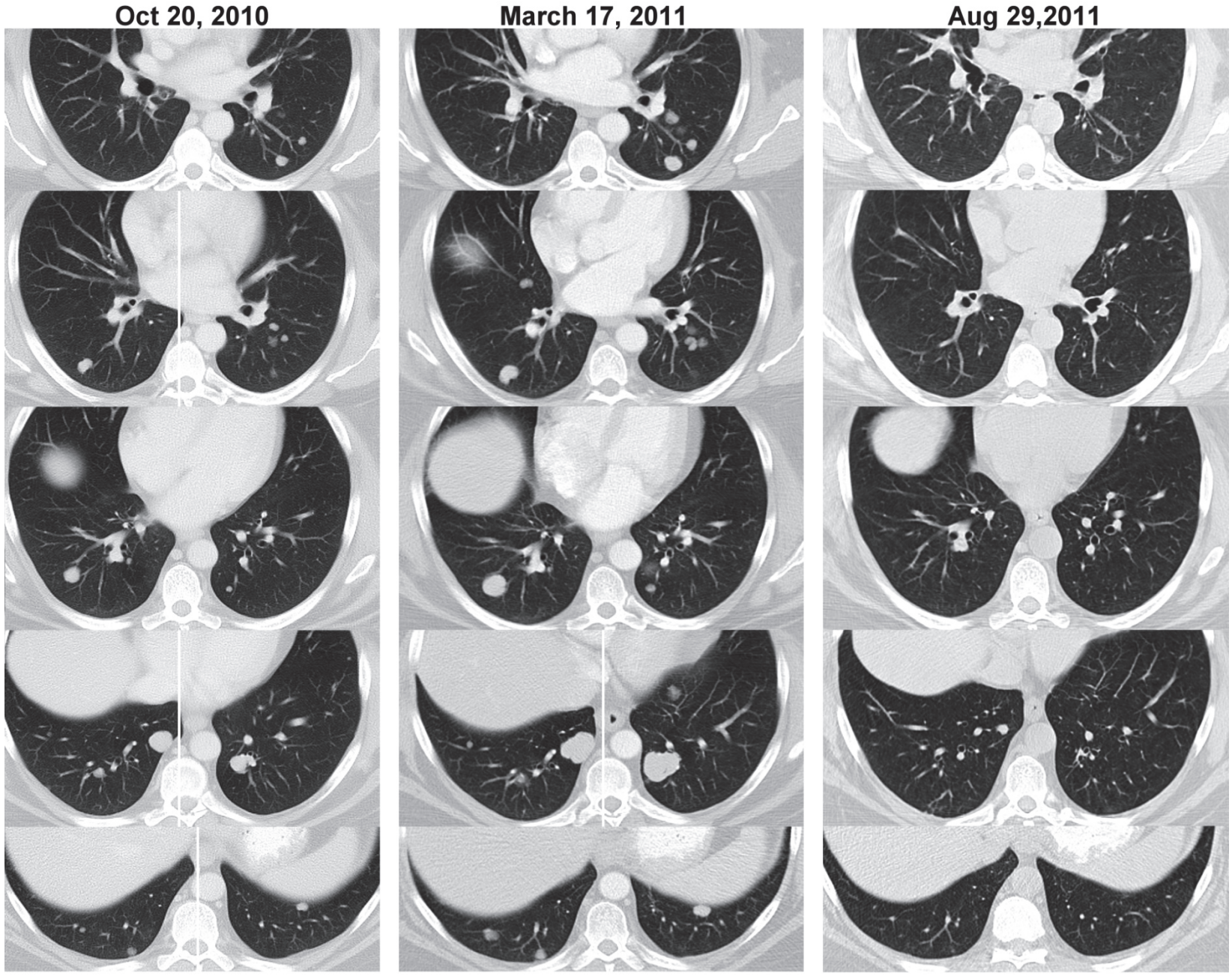
3: 10^7

4: 3 X 10^7

5: 10^8 (first patient at 10^8 cells treated on 9/15/2011.)

6: 3 X10^8 and on

No IL-2 is administered.


Oct 20, 2010
March 17, 2011
Aug 29,2011
Pre-Treatment
After 3x10E10 TIL
and 7 Doses IL-2
After 3x10E7 IL-12 TIL
and No IL-2

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

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)

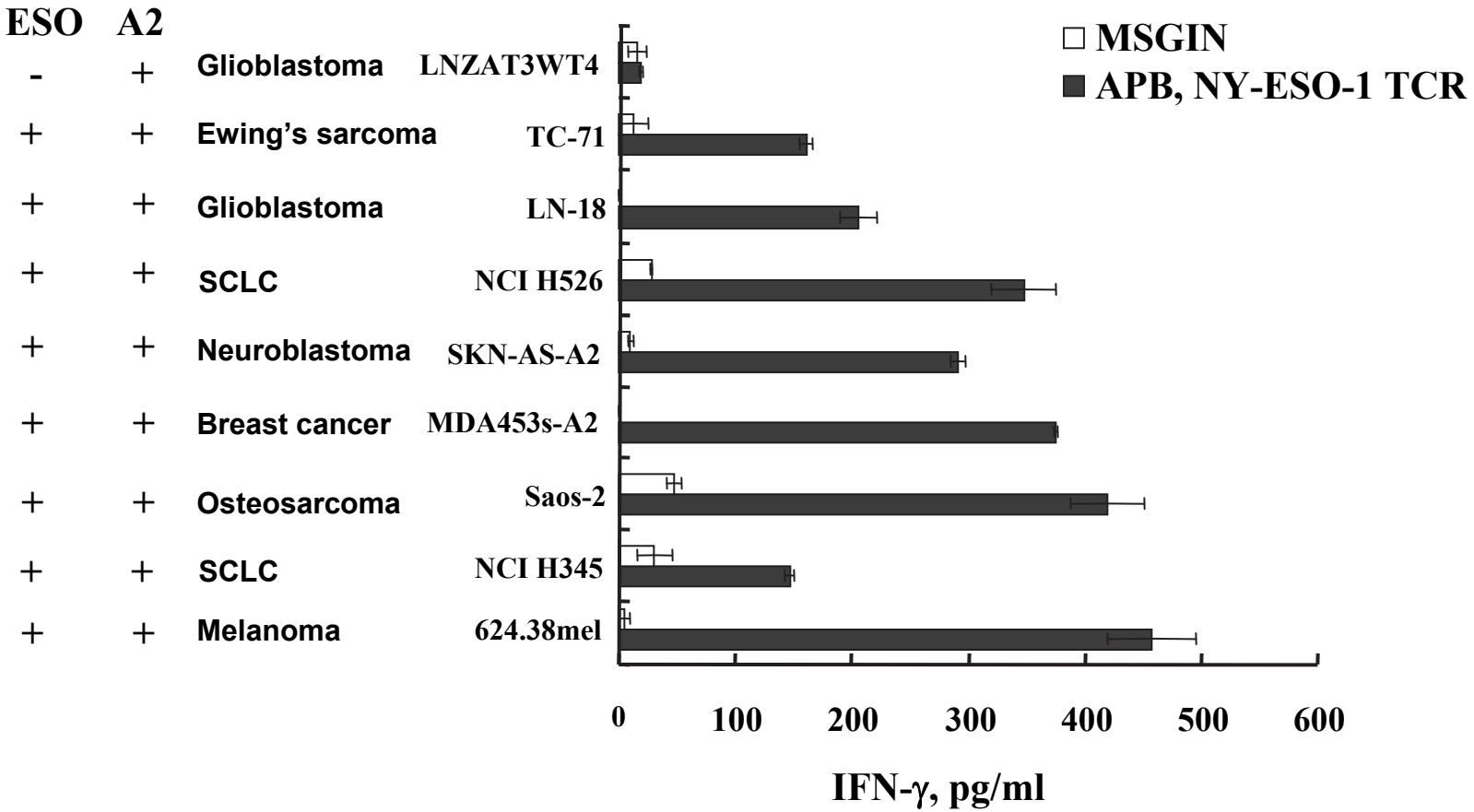
2. Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)

3. Mutations unique to each cancer (EGFRvIII)

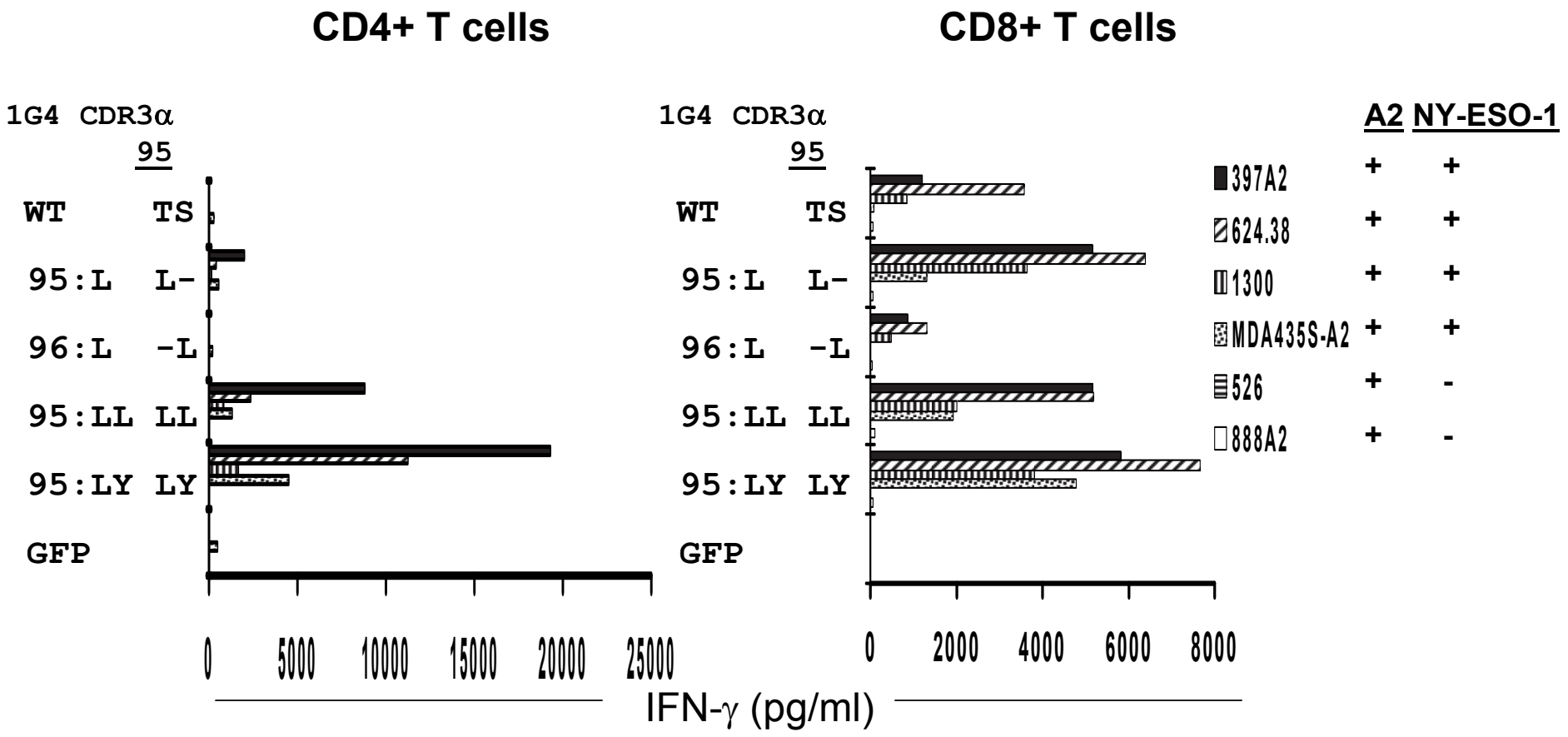
4. Critical components of the tumor stroma (VEGFR2, FAP)

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

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



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



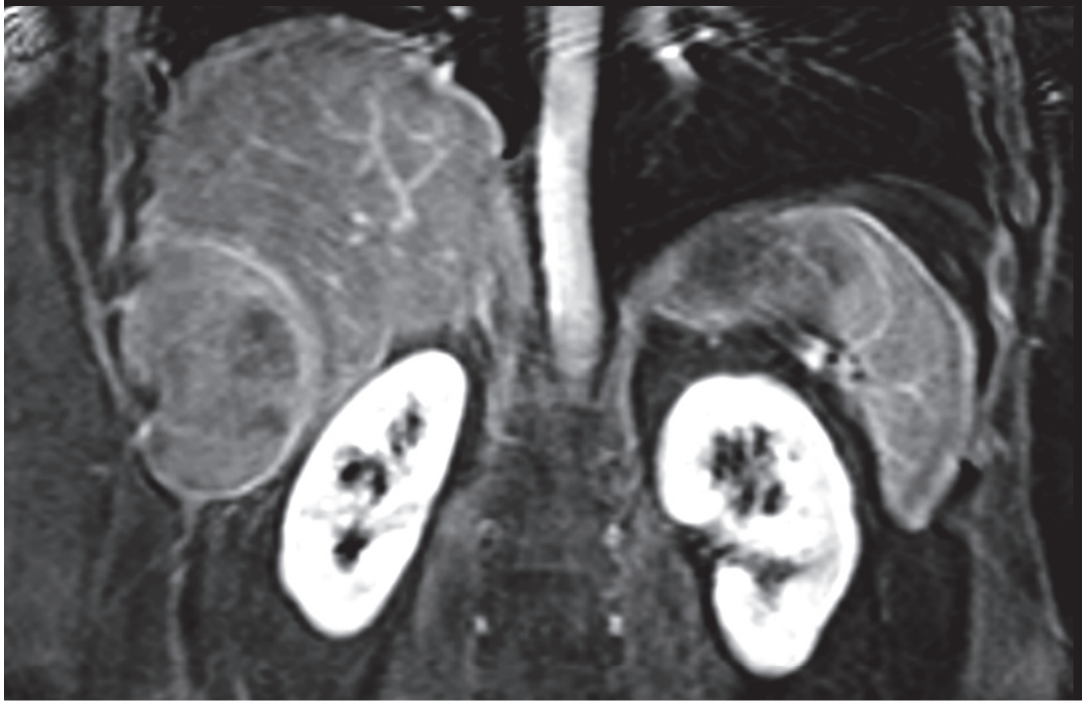
Responses to Therapy with NY-ESO-1 TCR

	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	16	4 (25%) (20+, 10, 8, 3)	3 (19%) (31+, 24, 5+)	7 (44%)
Synovial Cell Sarcoma	11	8 (73%) (14*, 13+, 12, 10, 8, 4, 3+, 3)	0	8 (73%)

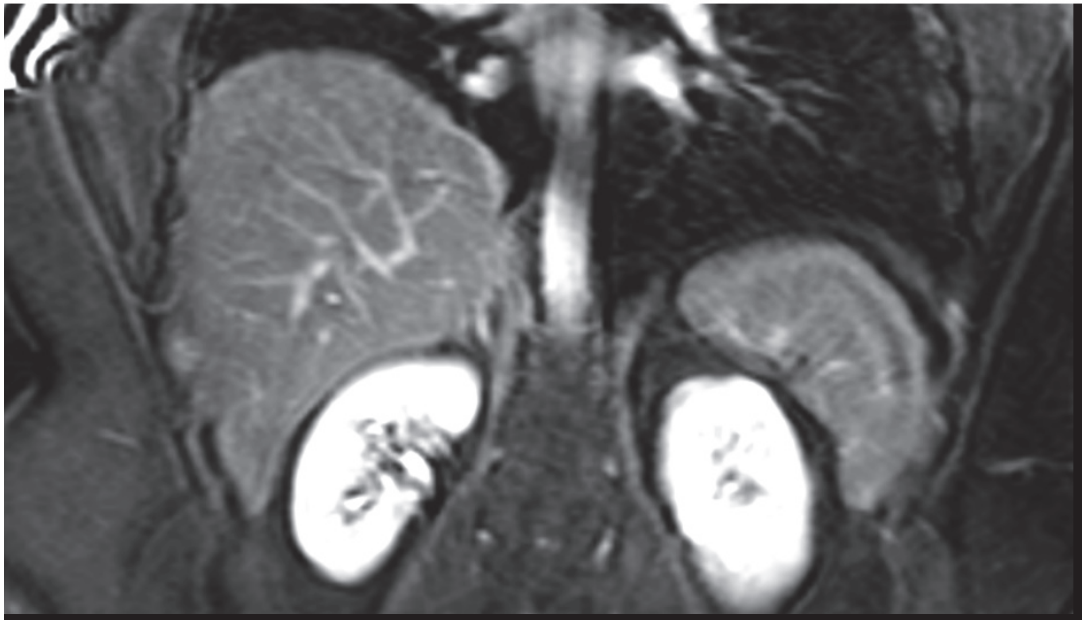
*treated twice

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

M.M. Synovial cell sarcoma ESO TCR



Pre-Treatment

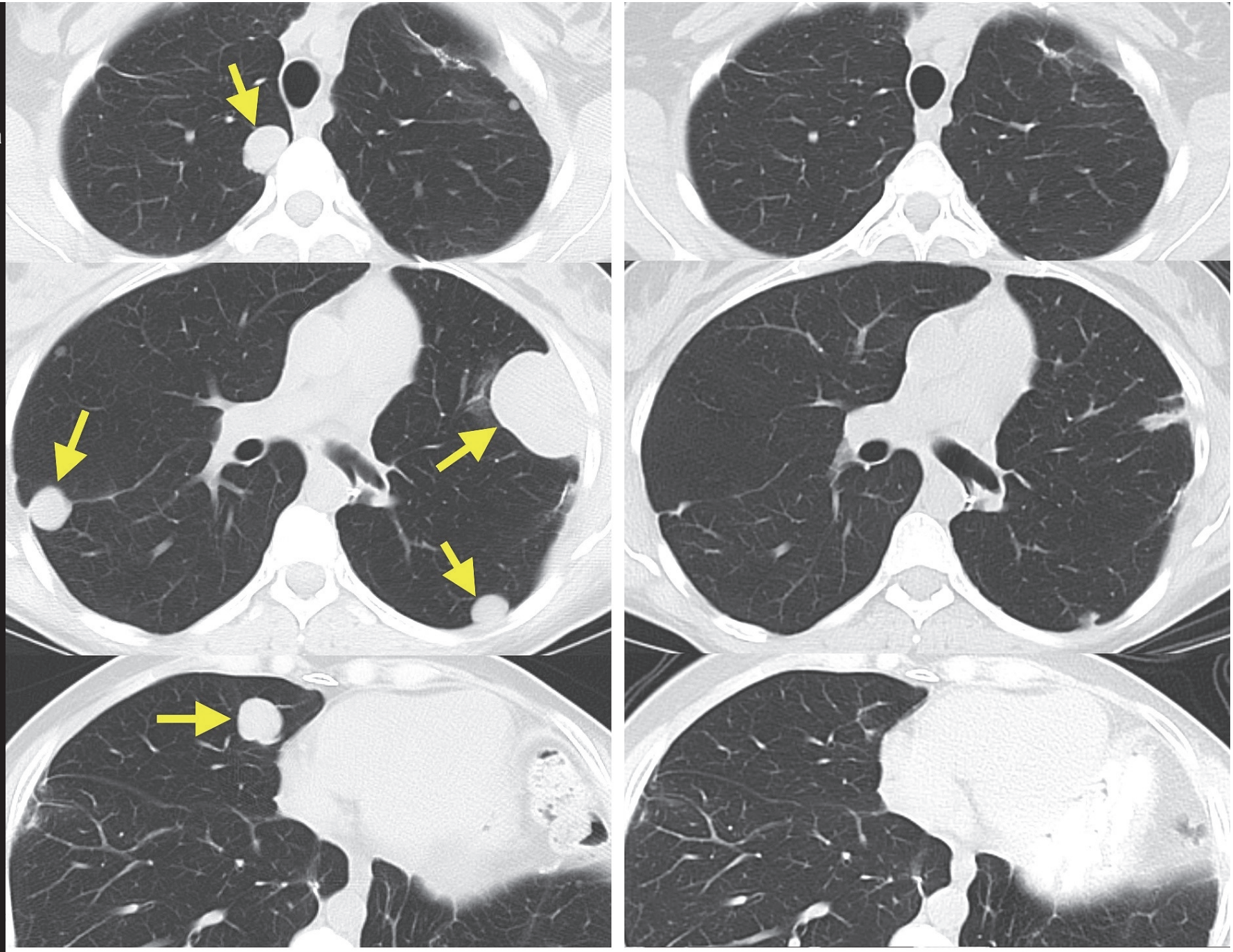


6 Months

H.K.

Synovial Sarcoma

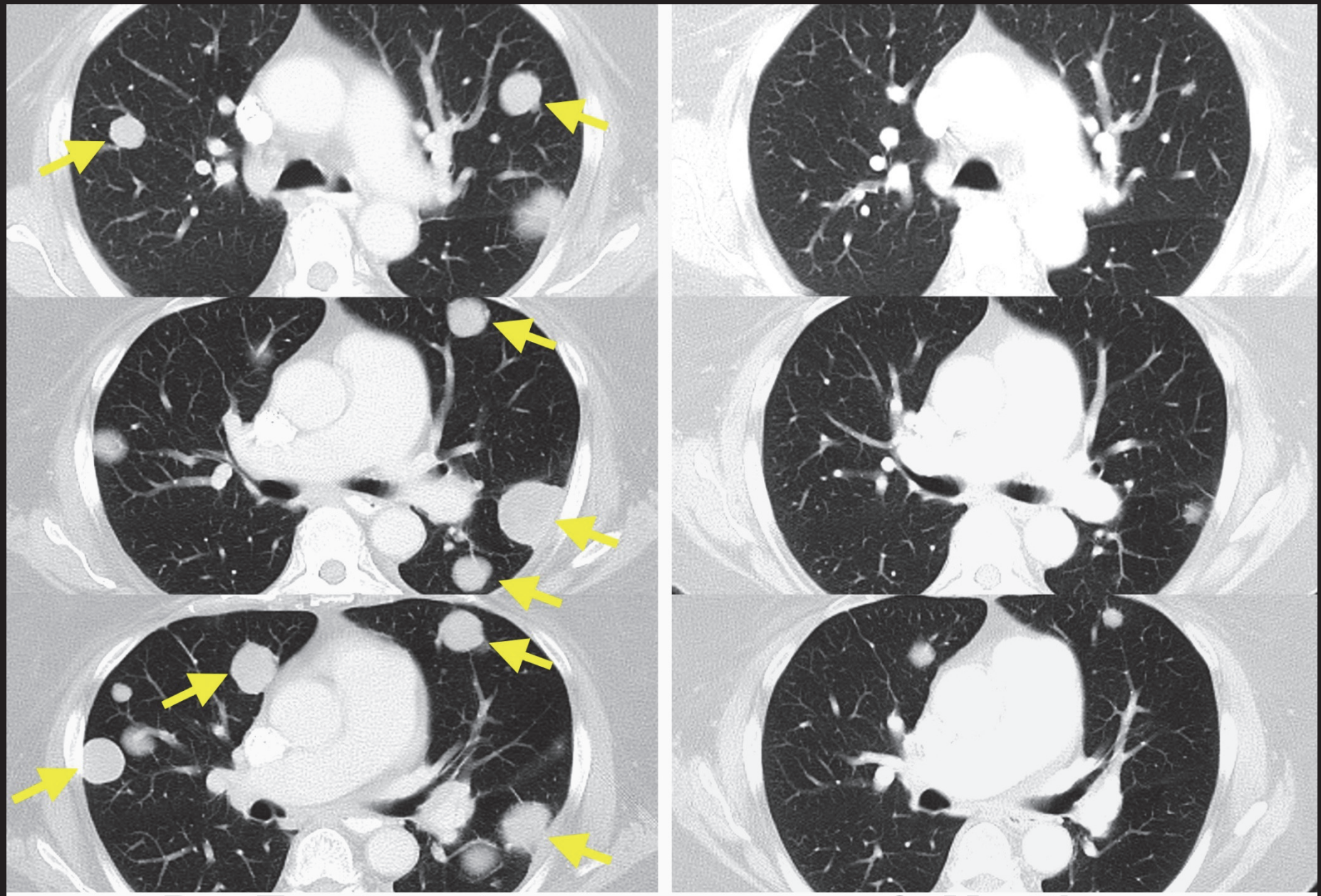
ESO TCR



Pre-Treatment

14 Months

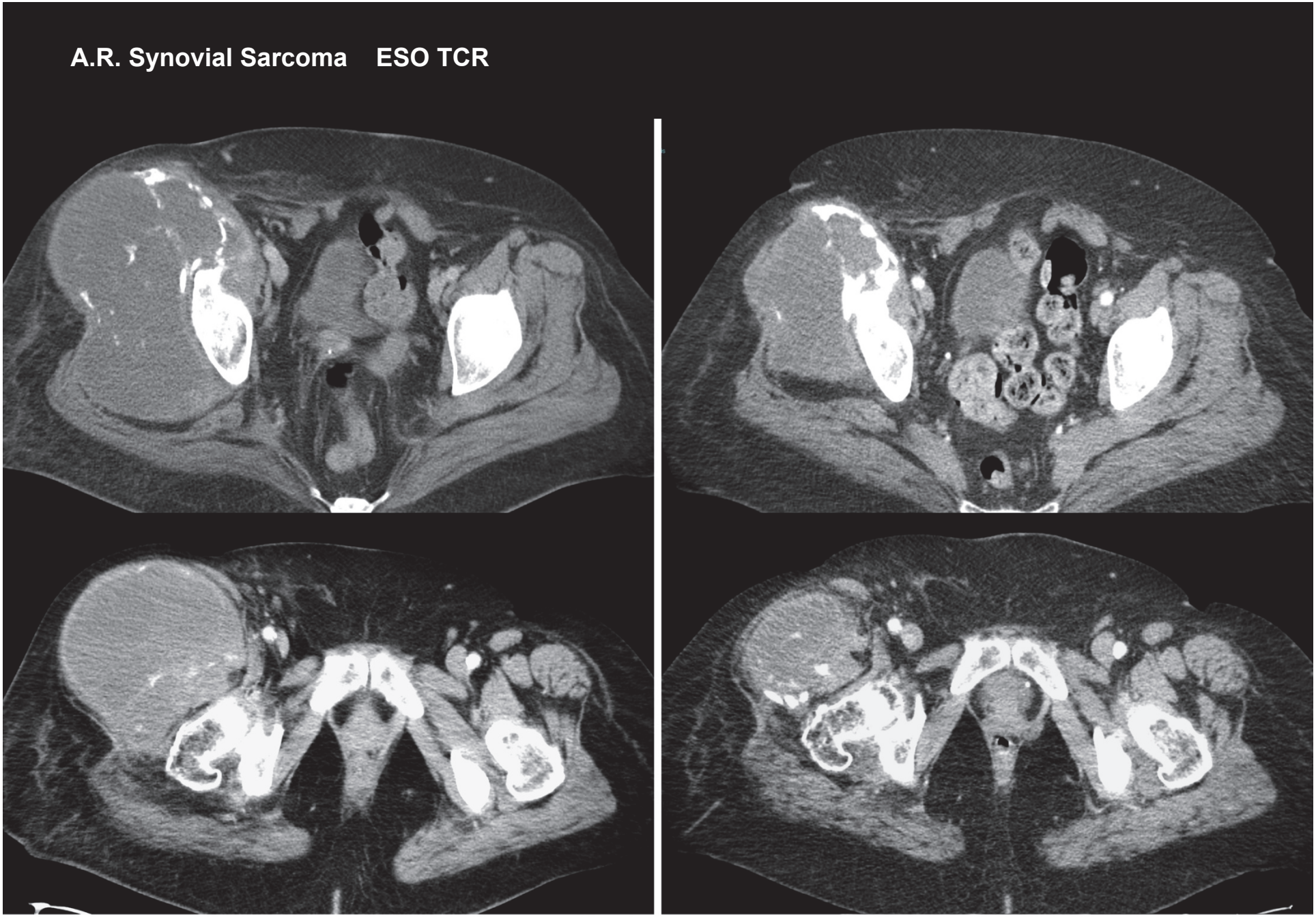
A.R. Synovial Sarcoma ESO TCR



Pre-Treatment

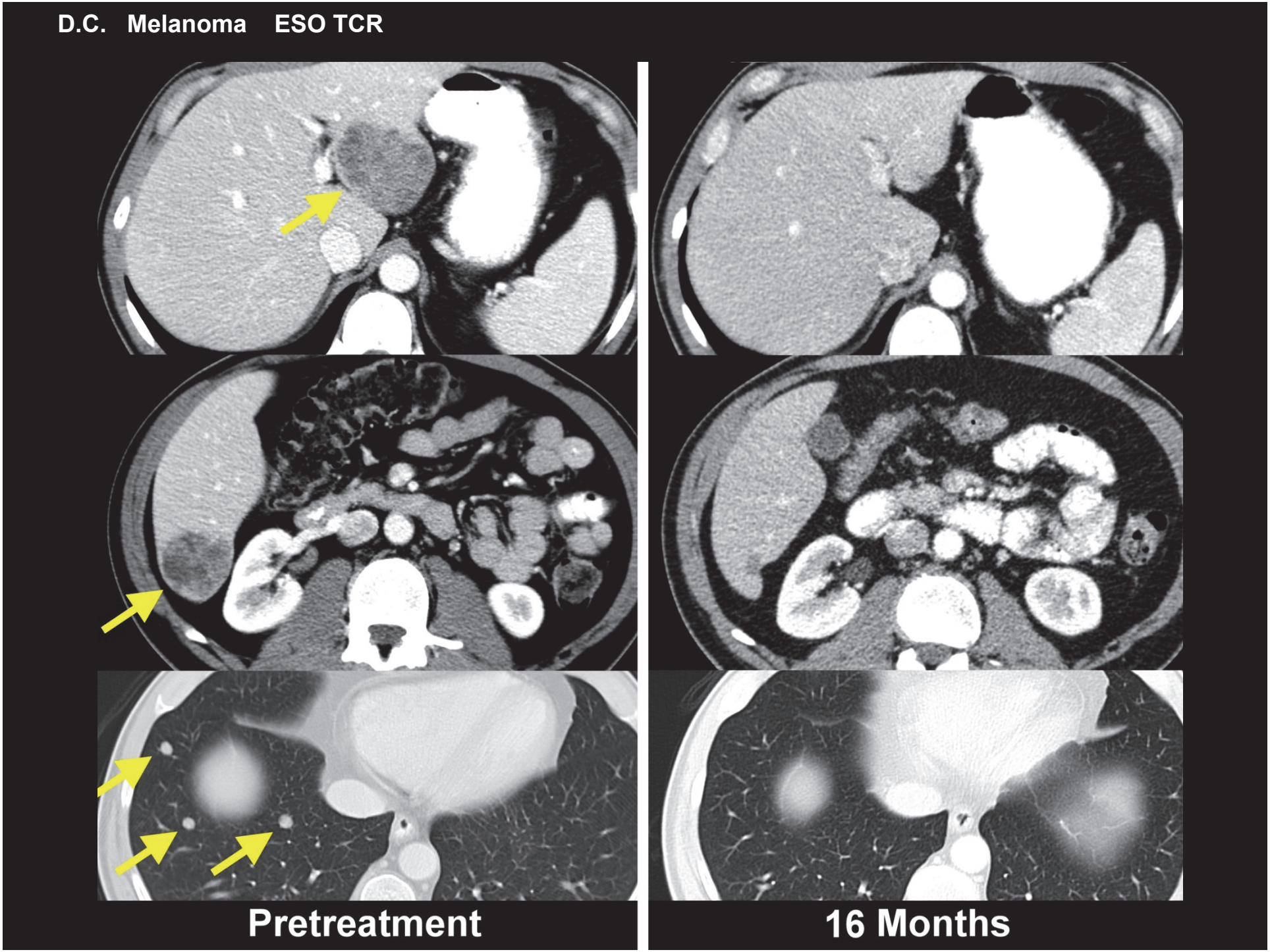
9 Months

A.R. Synovial Sarcoma ESO TCR

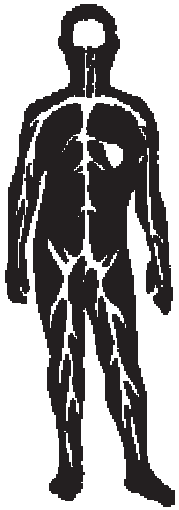


Pre-Treatment

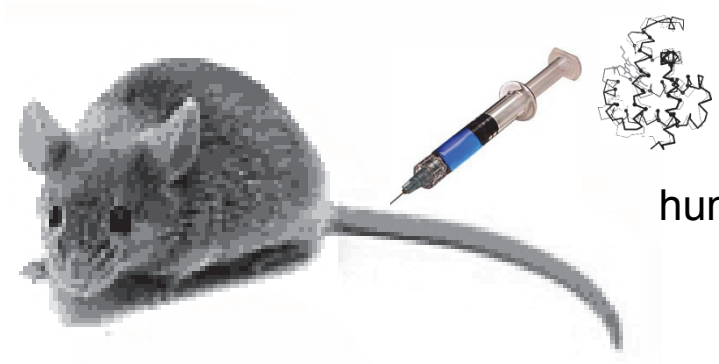
9 Months


D.C. Melanoma ESO TCR
Pretreatment
16 Months

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

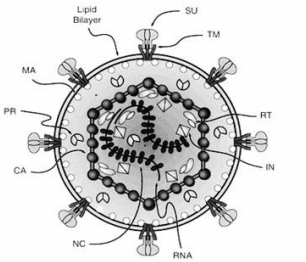


Clone human MHC class I

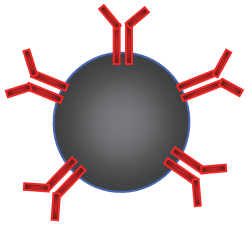


Immunize with human cancer antigen

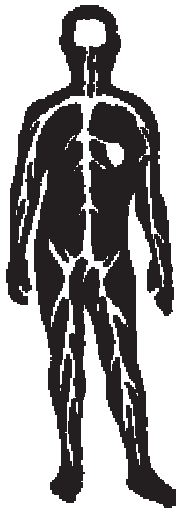
Mouse transgenic for Human MHC class I



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)

B-cell Malignancies

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

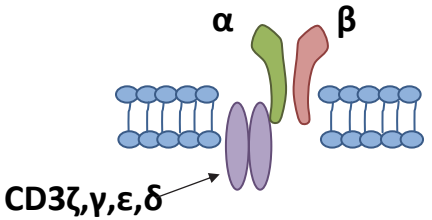
CD19 is expressed by more than 90% of B-cell malignancies.

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

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

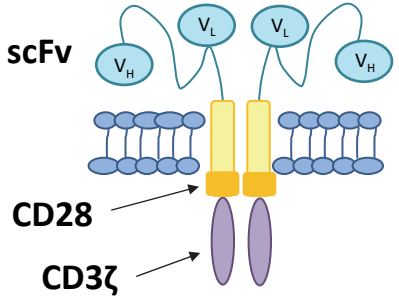
TCR Vector (eg, MART1, NY-ESO)

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)

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.