ADVANTAGES OF CELL TRANSFER IMMUNOTHERAPY

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

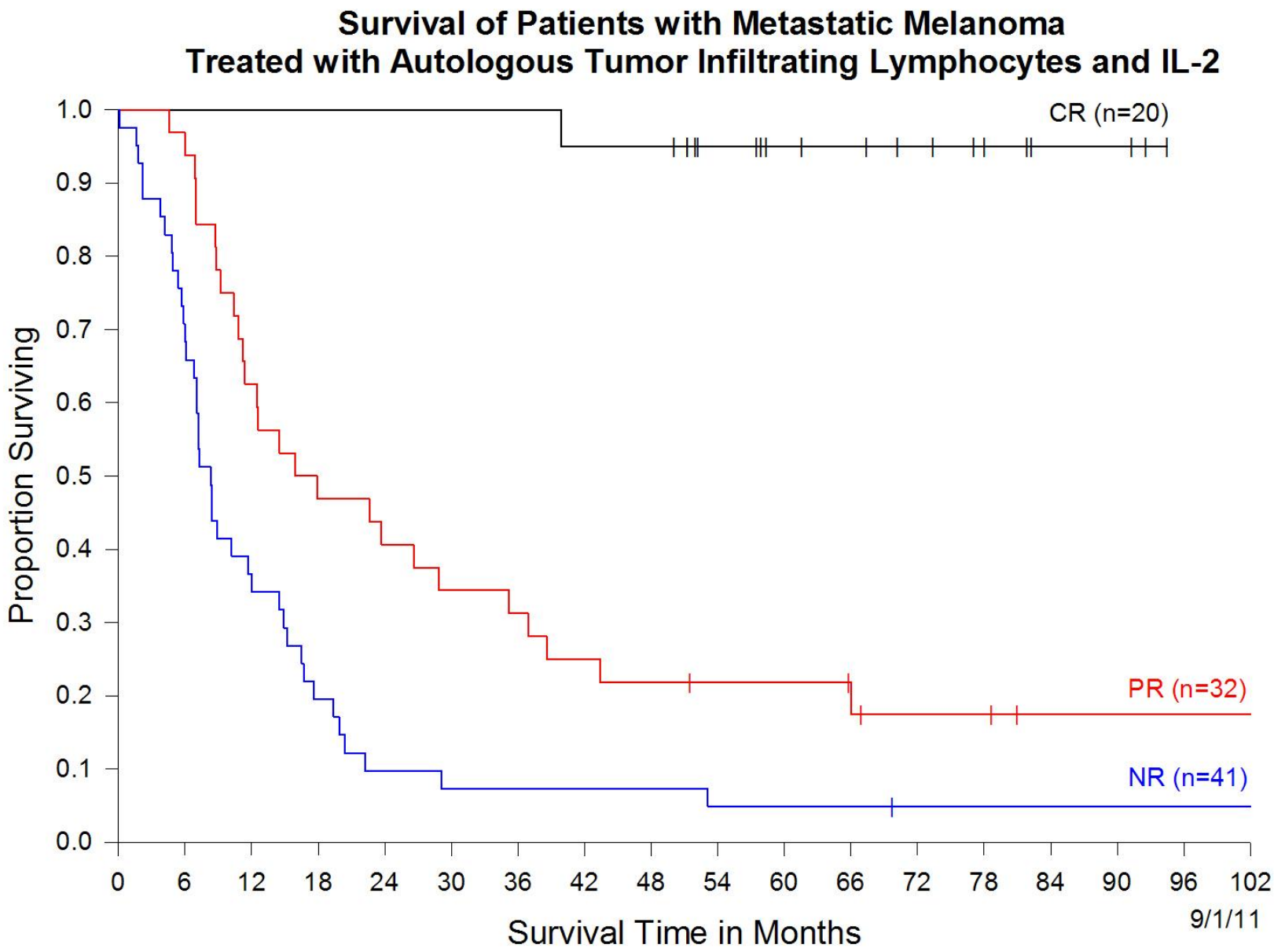
2. High avidity anti-tumor cells can be selected using in vitro assays

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

Cell Transfer Therapy (9/1/11)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (94+, 92+, 91+, 82+, 77+)	21 (49%)
200 TBI	25	8 (14, 9, 6, 6, 5, 4, 3, 3)	5 (81+, 77+, 73+, 70+, 67+)	13 (52%)
1200 TBI	25	8 (21, 13, 7, 6, 6, 5, 3, 2)	10 (61+, 58+, 57+, 57+, 52+, 51+, 51+, 51+, 50+, 19)	18(72%)

(52 responding patients: 42 had prior IL-2; 22 had prior IL-2 + chemotherapy)
(20 complete responses: 19 ongoing at 50 to 94 months)


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
CR (n=20)
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
9/1/11

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.

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**

Interleukin-12

1989 Discovered by G. Trinchieri

two chains p35 & p40

produced by dendritic cells and macrophages

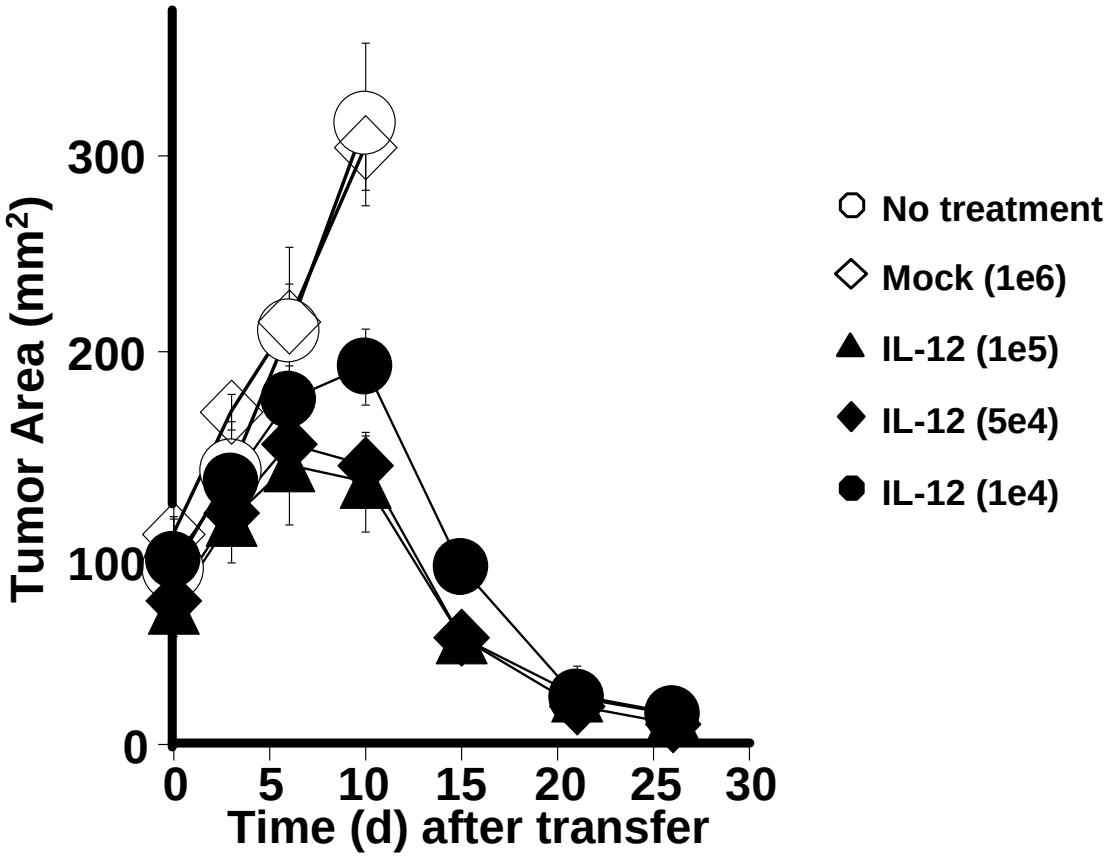
central role in innate and adaptive immunity

1995 Highly toxic when administered systemically to cancer patients

2010 Single chain IL-12 enhanced tumor rejection of anti B16 melanoma specific mouse T cells (Kerkar et al. Cancer Res 70:6725, 2010).

But constitutive IL-12 secretion by T cells was toxic

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)

Interleukin-12

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- two chains p35 & p40
- produced by dendritic cells and macrophages
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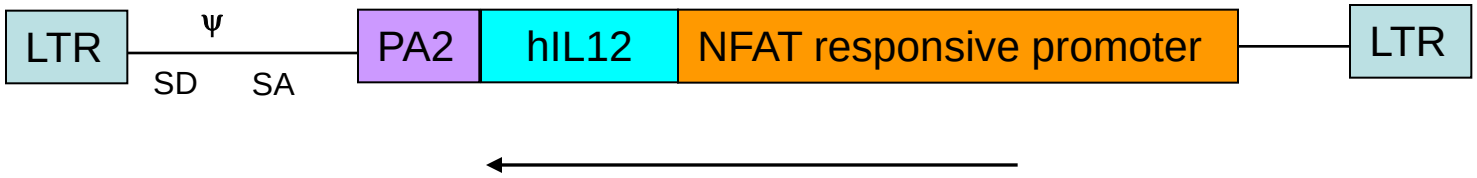
But constitutive IL-12 secretion by T cells was toxic

2011 Clinical trial in metastatic melanoma of human TIL transduced with single chain IL-12 using an inducible promoter

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

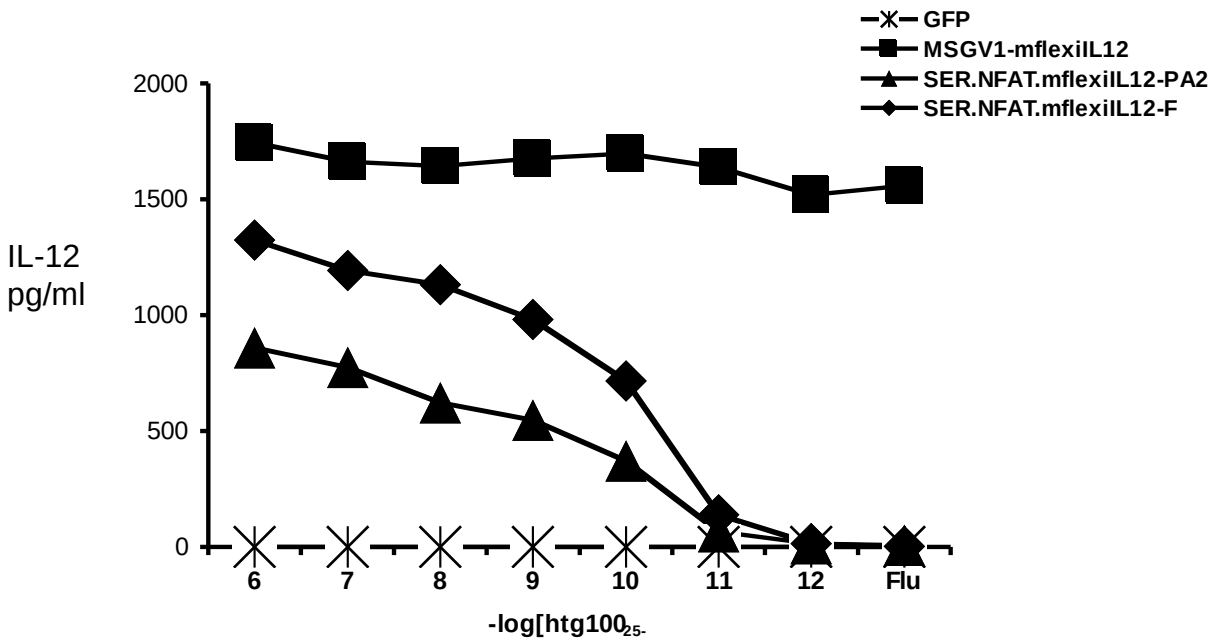
NFAT is a transcription factor produced in T cells activated by antigen specific triggering of the T cell receptor

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

(6: 3 X10^8 and on)

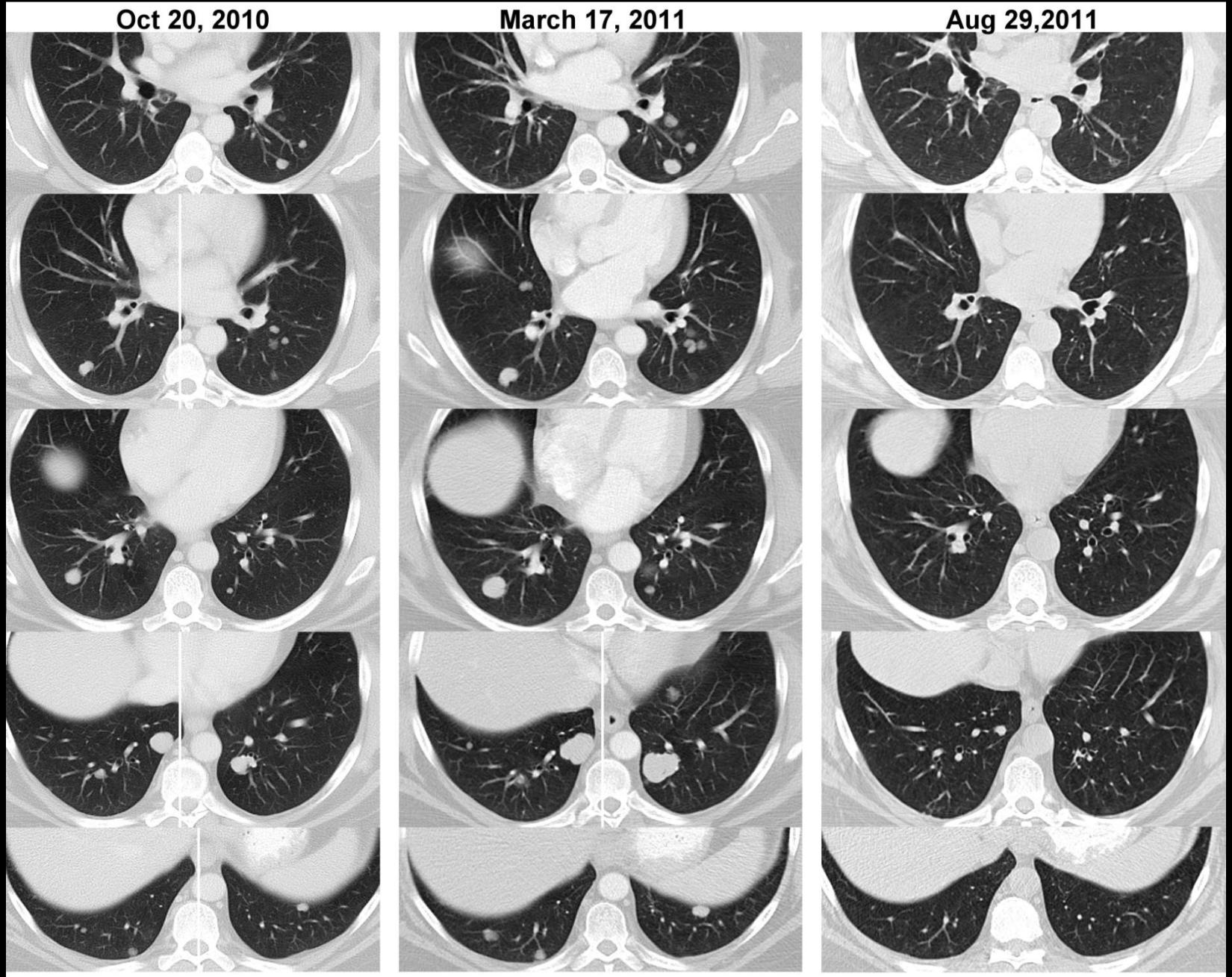
No IL-2 is administered.

M.S.

TIL/IL-12

3e7

No IL-2



Pre-Treatment

After 3x10E10 TIL and 7 Doses IL-2

After 3x10E7 IL-12 TIL and No IL-2

Nunc
178883

CONCLUSION

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

CHALLENGE

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

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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)

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

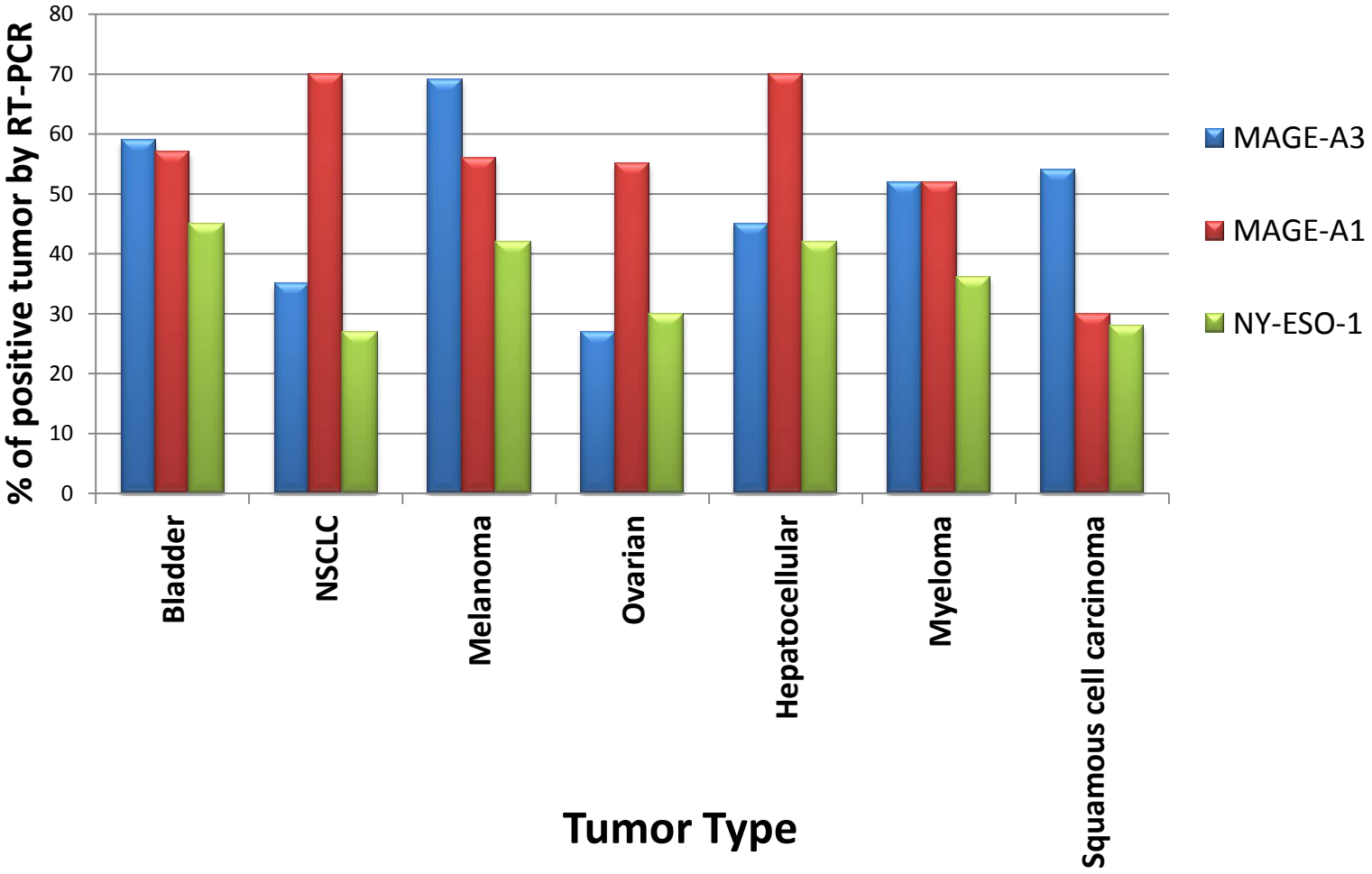
NY-ESO-1 Family

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

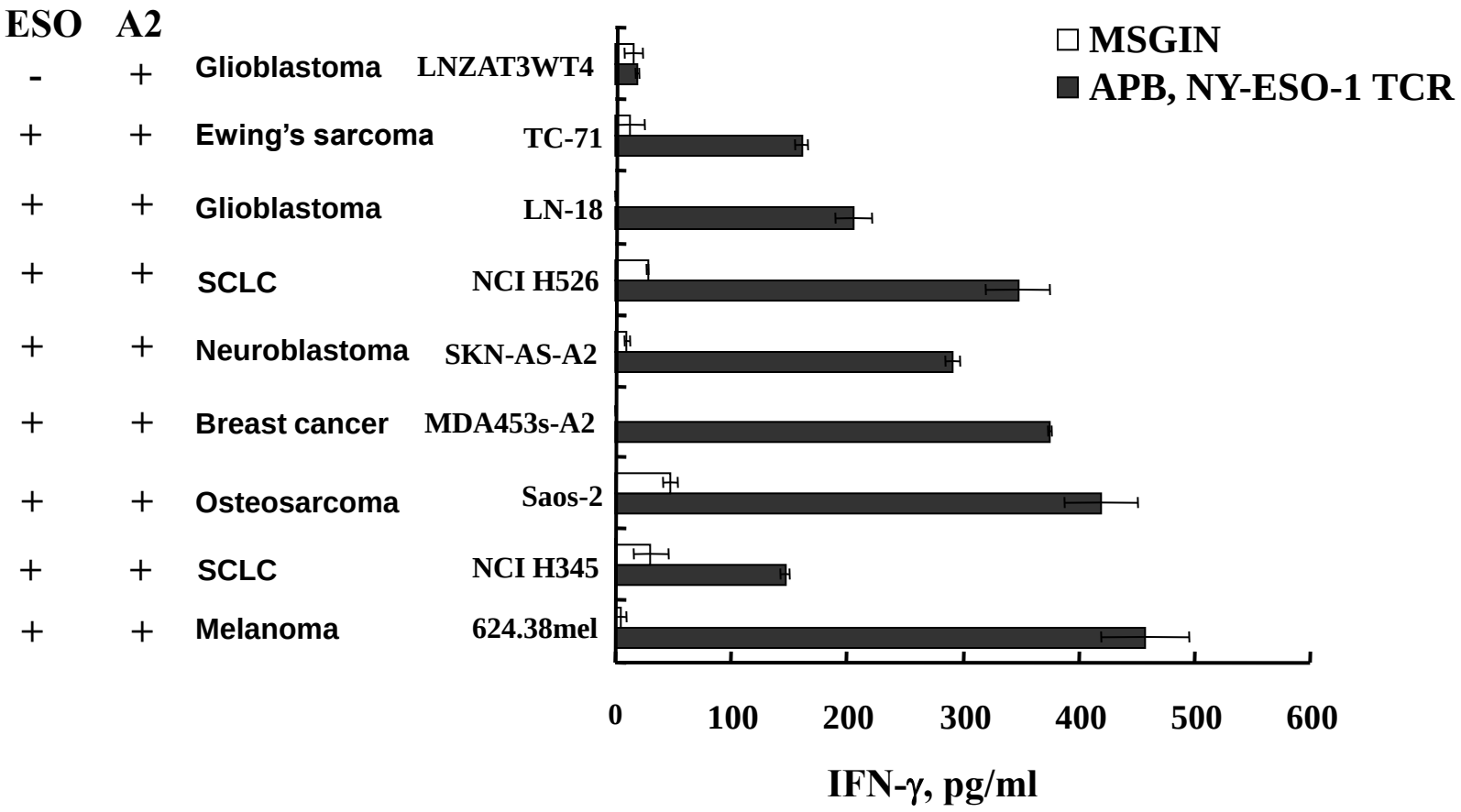
MAGE Family

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

Cancer/Testis Antigens Expressed in Multiple Tumor Types



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



Treatment of Metastatic Melanoma Using an NY-ESO-1 TCR

	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	17	5 (29%) (23+, 10, 8, 4+, 3)	3 (18%) (36+, 34+, 7+)	8 (47%)

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

Treatment of Metastatic Synovial Sarcoma Using an Anti-NY-ESO-1 TCR

Patient	Age	Number of cells infused ($x10^{-9}$)	Doses IL-2	Responses (months F/U - 12/1/11)
1	20	83.3	5	PR (10)
2*	37	49.6	8	PR (14)
3	47	55.6	8	PR (4)
4	19	15.6	5	NR
5	30	58.6	5	PR (8)
6	52	52.0	5	NR
7	31	30.3	5	PR (5)
8	64	39.3	5	PR (15+)
9	37	22.0	6	PR (12)
10	21	77.0	1	PR (3)

*Treated twice

M.M. Synovial cell sarcoma ESO TCR

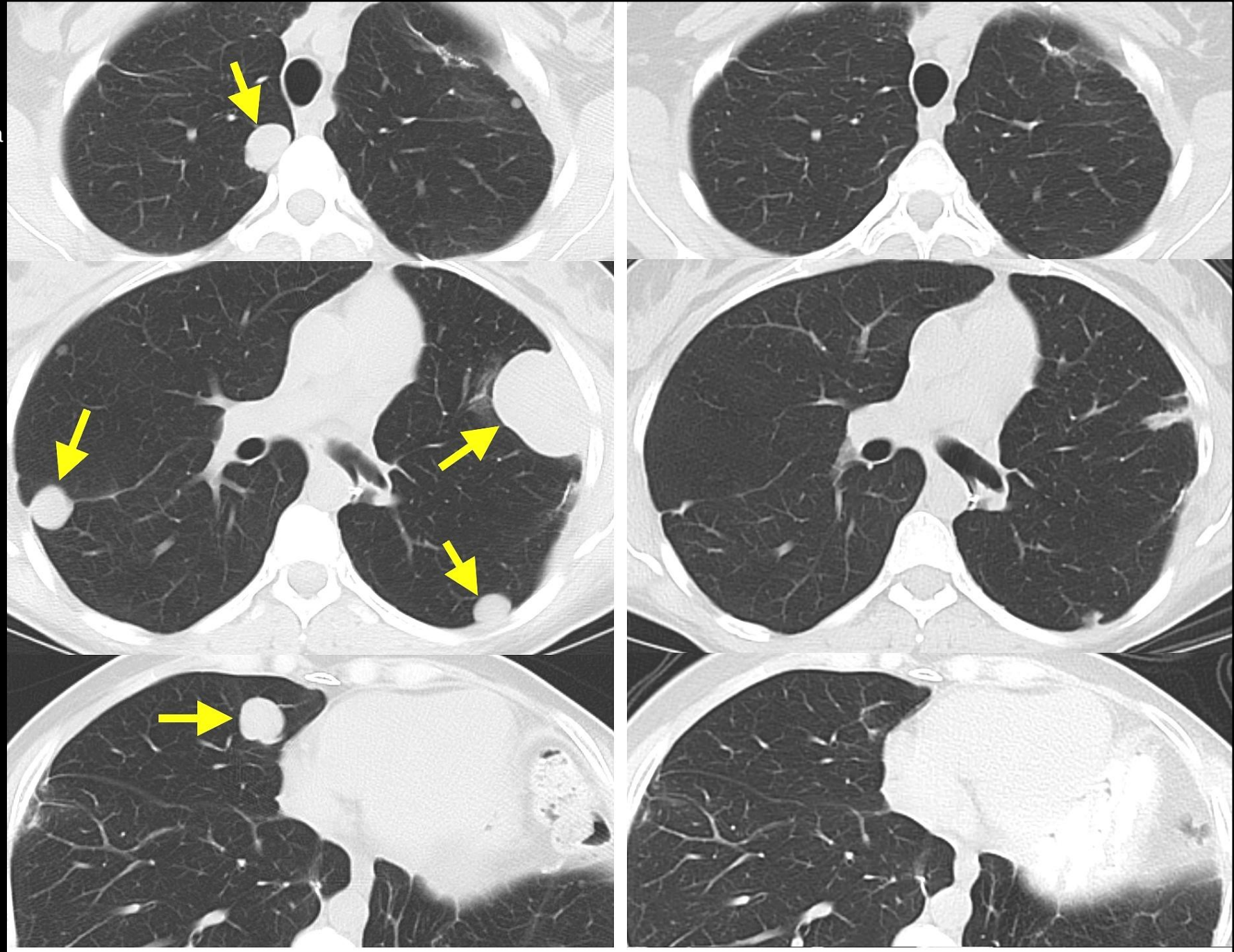
Pre-Treatment

6 Months

H.K.

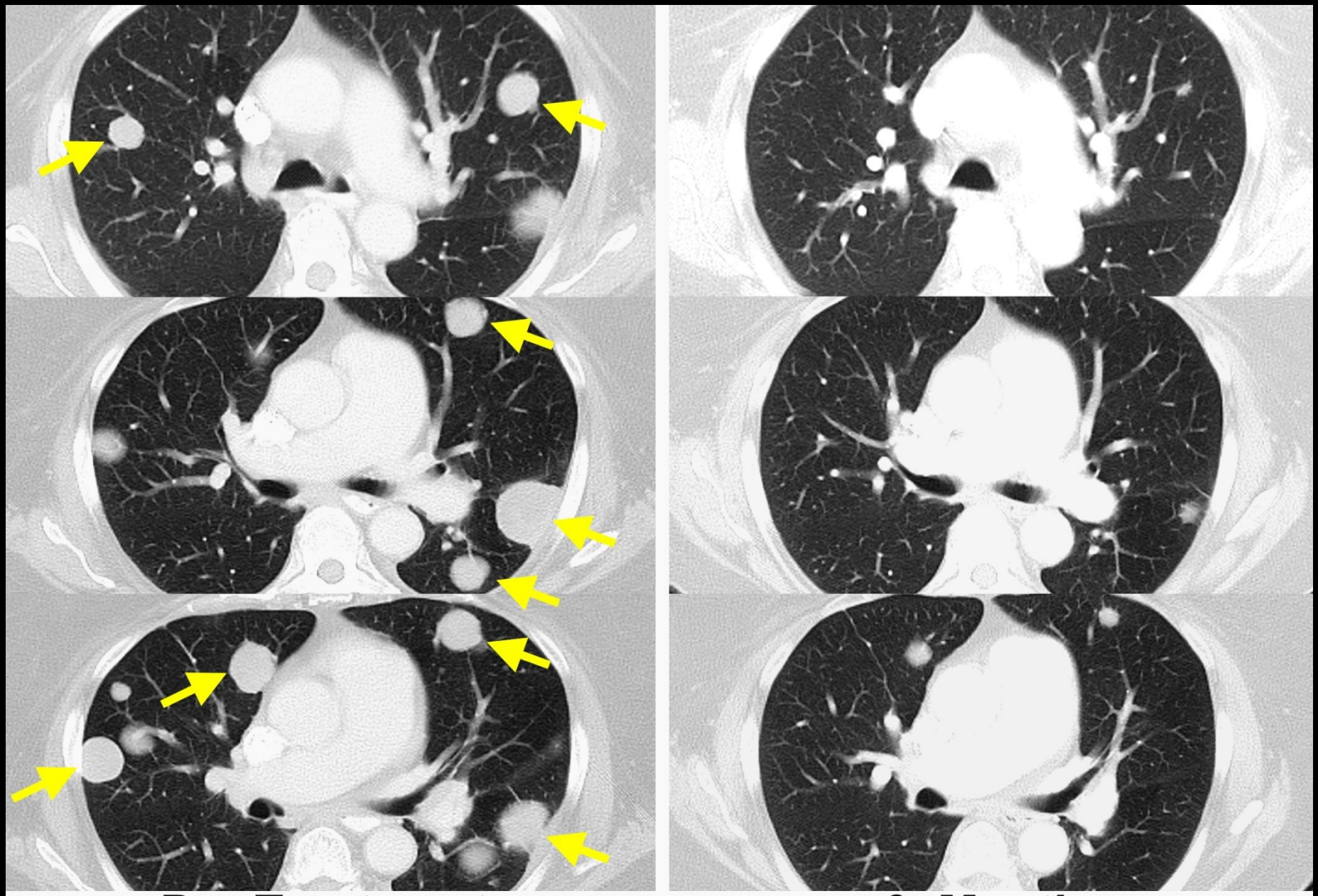
Synovial Sarcoma

ESO TCR

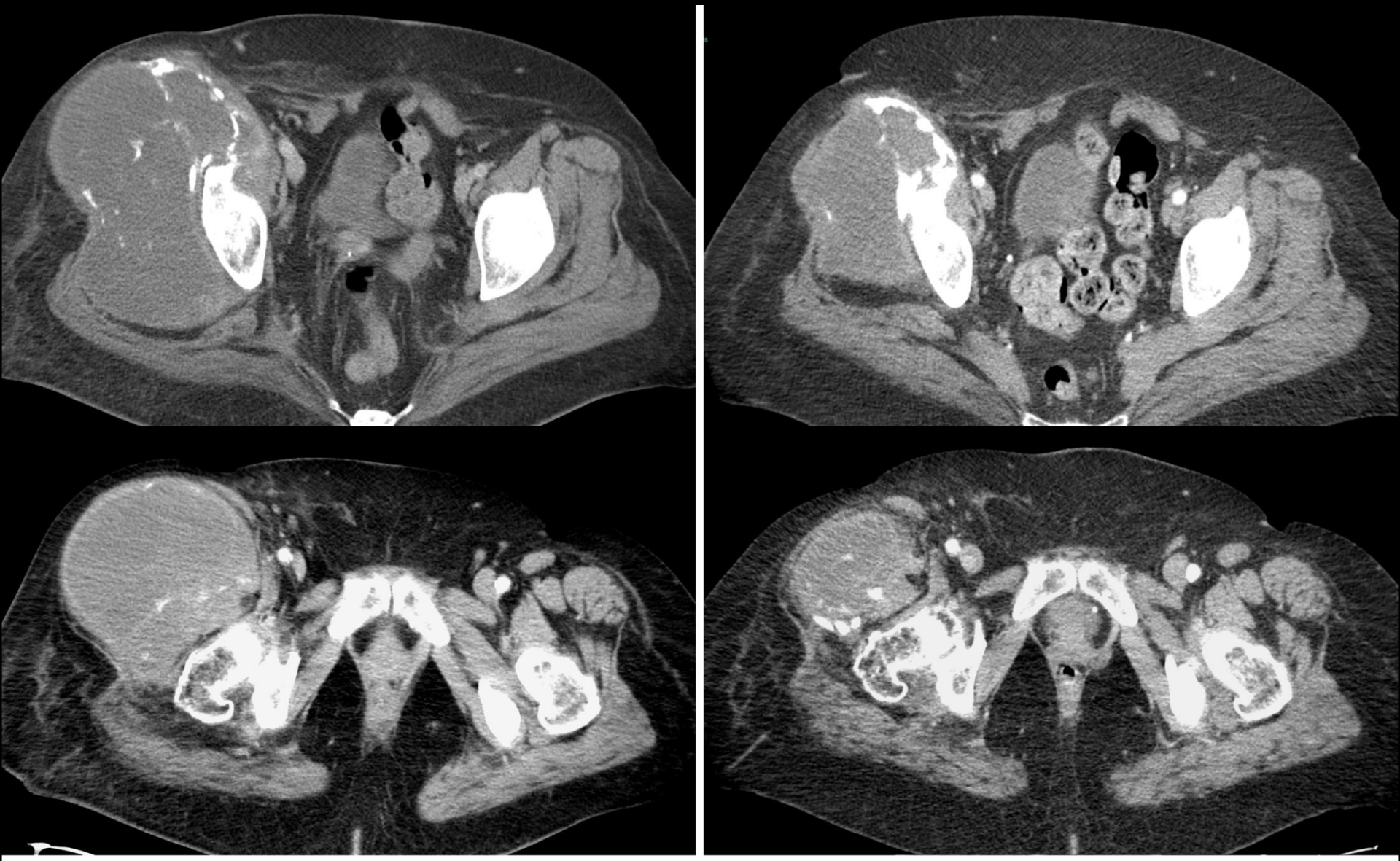


Pre-Treatment **14 Months**

A.R. Synovial Sarcoma ESO TCR

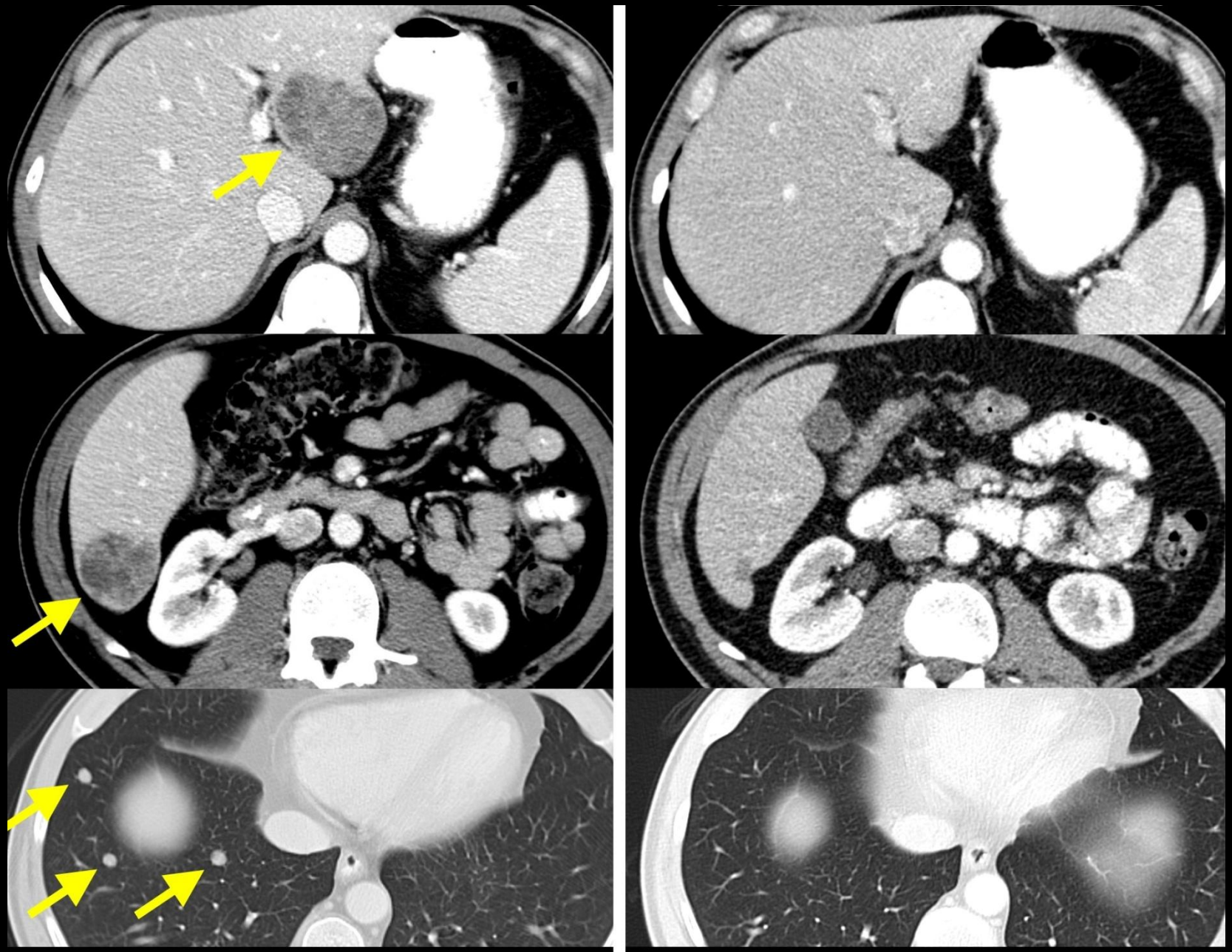


A.R. Synovial Sarcoma ESO TCR



Pre-Treatment 9 Months

D.C. Melanoma ESO TCR



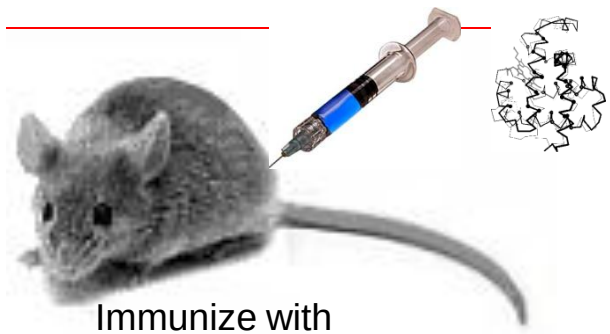
Abbreviated Title:IL-12 PLUS ANTI-ESO-1 TCR PBL
OBA: 1103-1097
IBC: RD-RD-11-III-02
CC Protocol Number:12-C-0006A
NCI Protocol Number: P11832
PROTOCOL TITLE

Phase I/II Study of Metastatic Cancer that Expresses NY-ESO-1 Using Lymphodepleting Conditioning Followed by Infusion of Gene Engineered Lymphocytes Cotransduced with Genes Encoding IL-12 and Anti-NY ESO-1 TCR

Phase 1 - Dose Escalation:
Cohort 1 107 cells
Cohort 2 3 X 107 cells
Cohort 3 108 cells
Cohort 4 3 X 108 cells
Cohort 5 109 cells
Cohort 6 3 X 109 cells
Cohort 7 1010 cells
Cohort 8 3 X 1010 cells

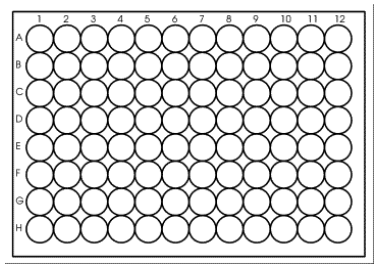
Development of TCR based gene therapy targeting MAGE-A3

Mouse transgenic for
Human MHC class I



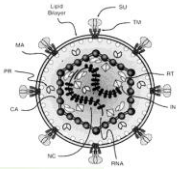
Immunize with
MAGE-A3: 112-120
peptide

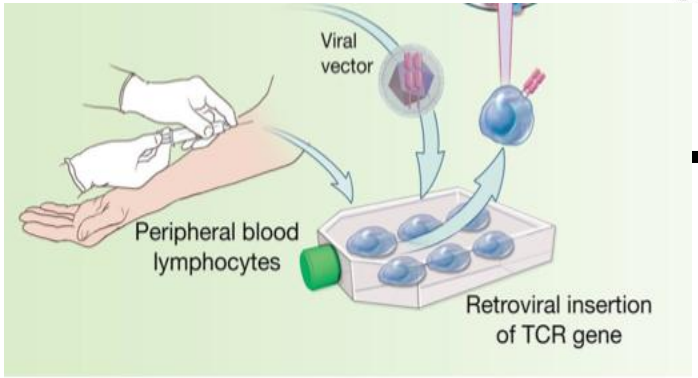
Clone mouse T cells

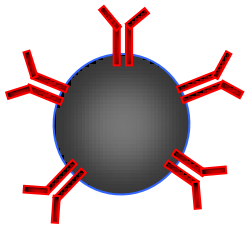


Isoate RNA
Identify TCRs
Clone TCRs
Test for tumor recognition

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

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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.

Treatment of B-cell Lymphomas and CLL using an Anti-CD19 CAR

Patient	Age	Malignancy	Number of prior therapies	Number of infused CAR+ cells/kg	Doses of IL-2 administered	Response and time since treatment (months; f/u 12/1/11)
1*	47	Follicular Lymphoma	4	0.3×10^7	8	PR (29+; 90%decr)
2	48	Follicular Lymphoma	5	0.3×10^7	9	NE (died H1N1 flu)
3	61	CLL	3	1.1×10^7	2	CR (18+)
4	55	Splenic Marginal Zone Lymphoma	3	1.1×10^7	4	PR (16+; 81% decr)
5	54	CLL	4	0.3×10^7	2	SD (6)
6	57	CLL	7	1.7×10^7	1	PR (7)
7	61	CLL	4	2.8×10^7	2	PR (10+; 98% decr)
8	63	Follicular Lymphoma	7	3.0×10^7	5	PR (10+; 97% decr)

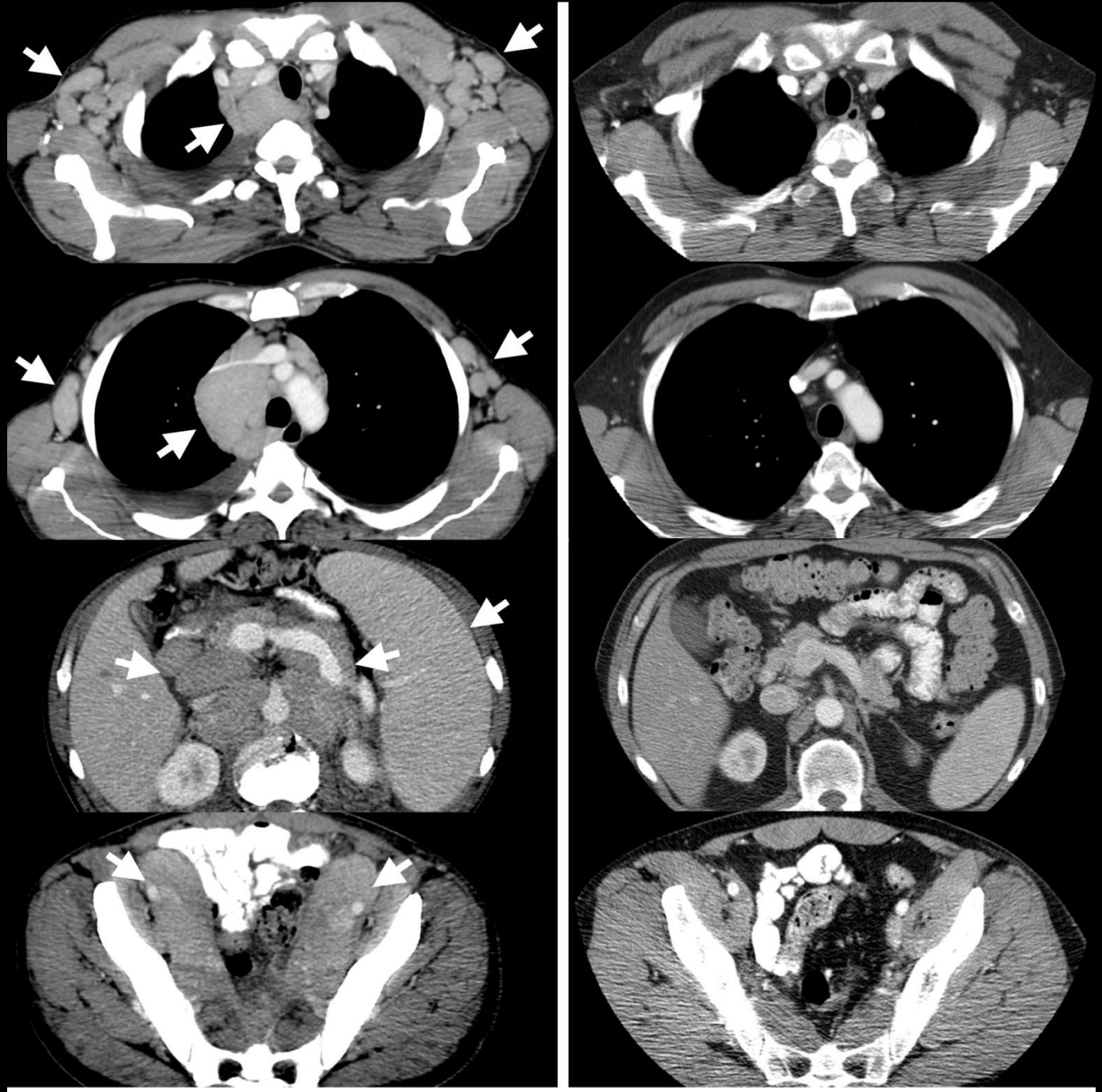
*Patient 1 recurred at 7 months and was retreated. Patient 4 recurred at 11 months and was retreated).

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

R.K.

CD19 CAR

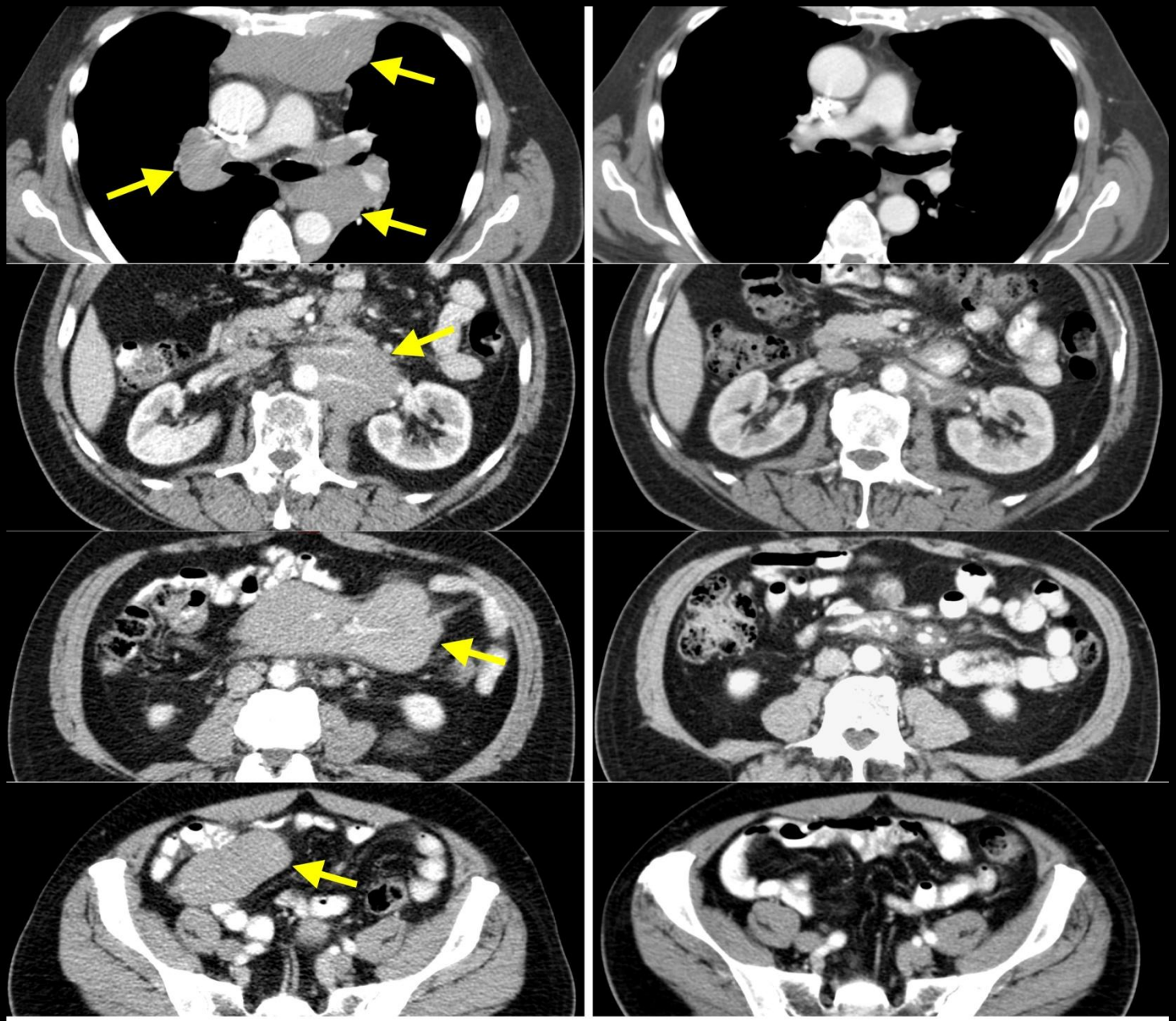
(after 2 Rx)



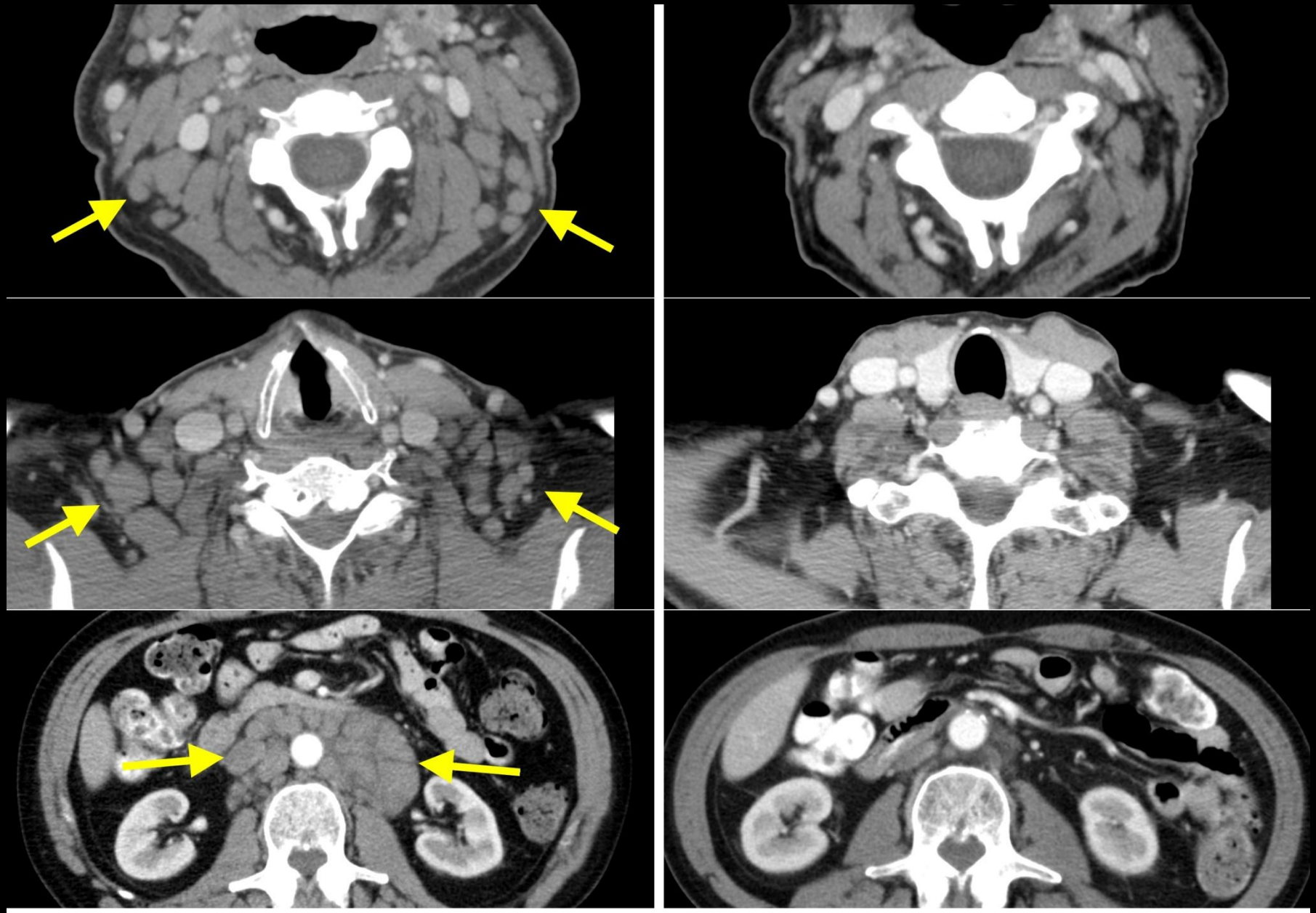
Pre-Treatment

25 Months

D.G. CD19 TCR Follicular lymphoma



R.L. CD19 TCR Chronic lymphocytic leukemia

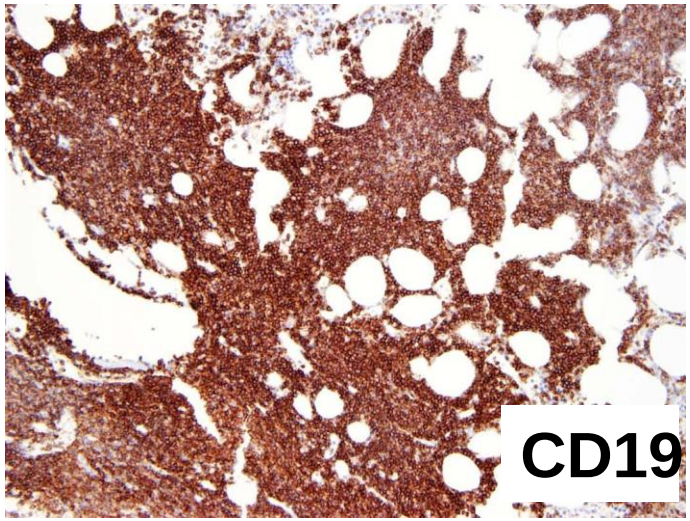


Pre-Treatment

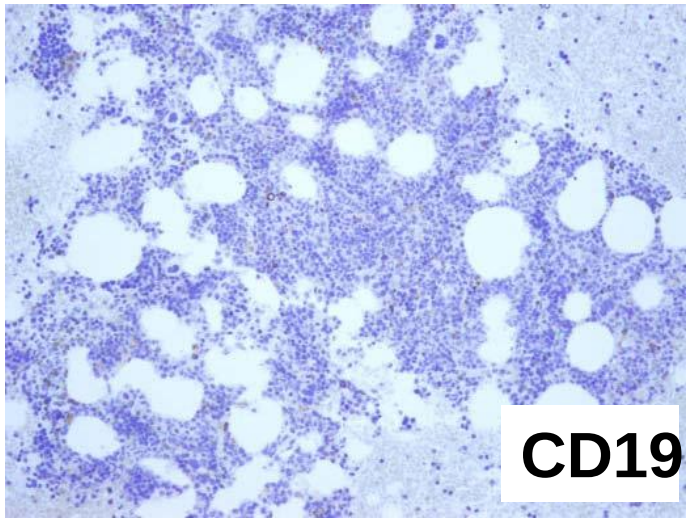
2 Months

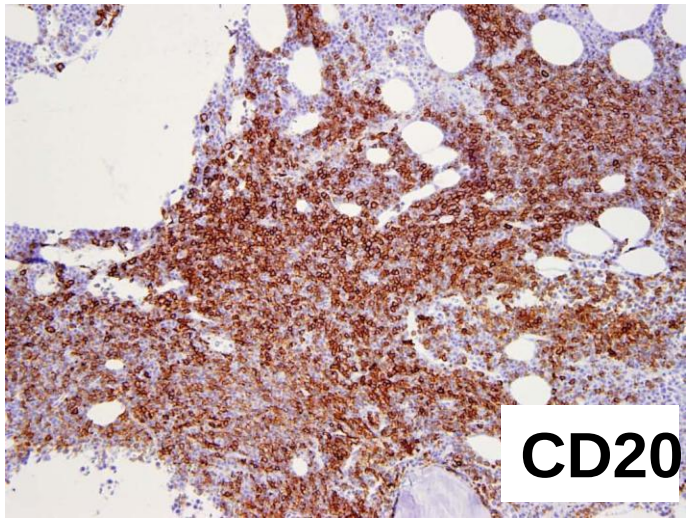
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment

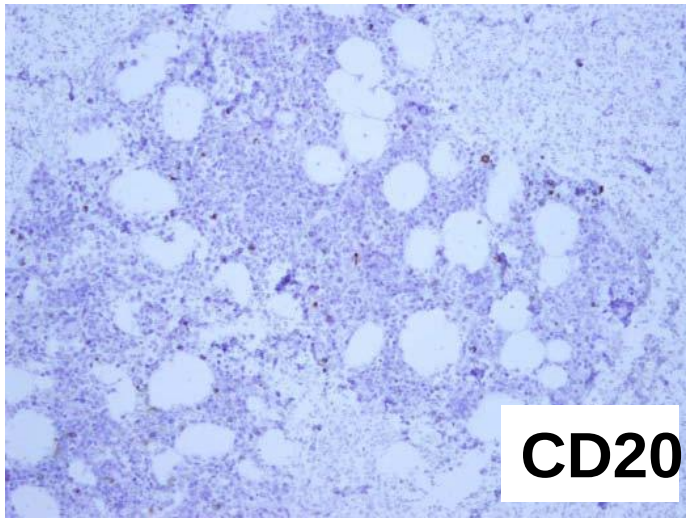
Before treatment



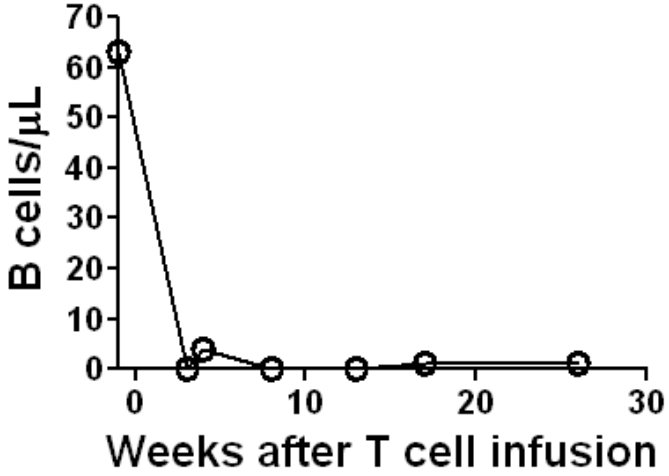
3 months after treatment



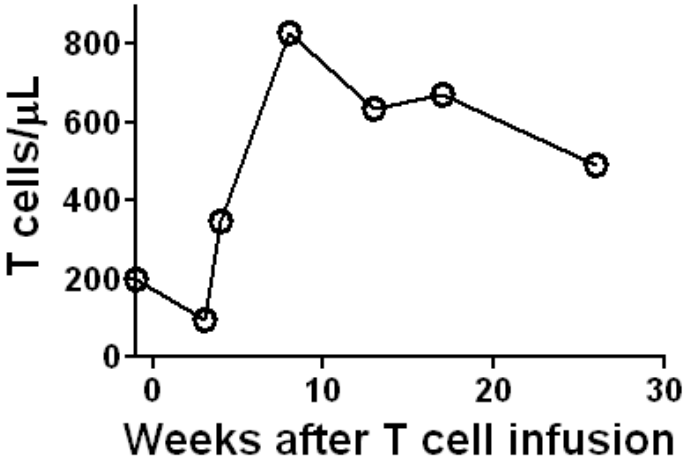


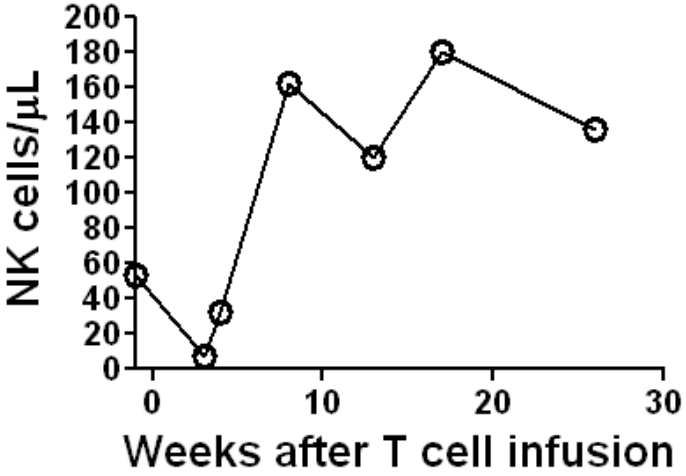


In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion



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





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

---

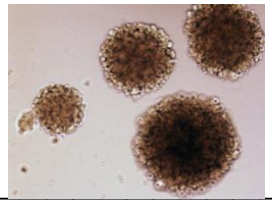
Expressed in 30-50% of glioblastomas

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	<u>4523</u>	<u>3306</u>	<u>3351</u>	<u>4406</u>

***All lines express EGFRvIII**

(R. Morgan, H. Fine et al)

Phase I dose escalation trial in patients with recurrent glioblastoma

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
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)	$3 – 6 x 10^{10}$	3 patients

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Rationale for the anti-VEGFR2 Gene Therapy Protocol

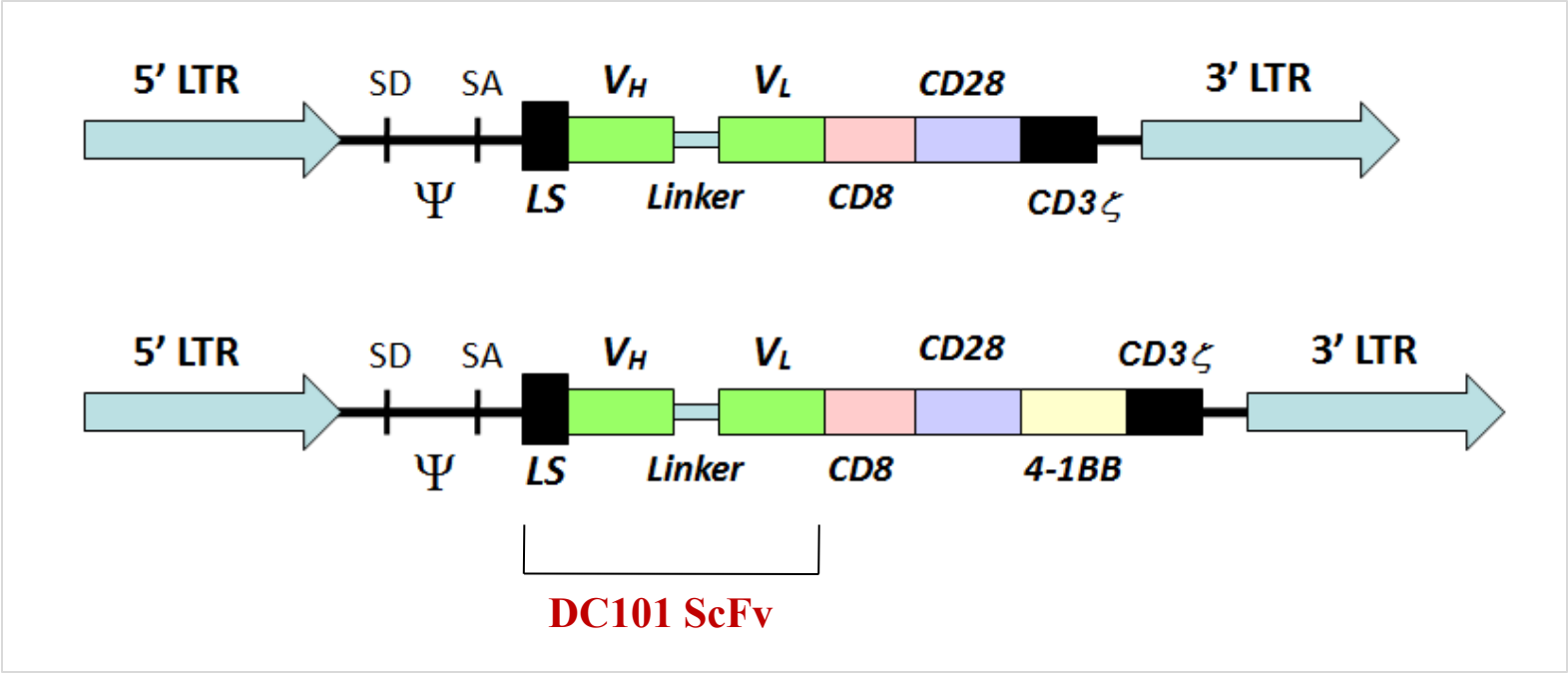
Vascular endothelial growth factor (VEGF) stimulates tumor angiogenesis by binding to its receptor, VEGFR2

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

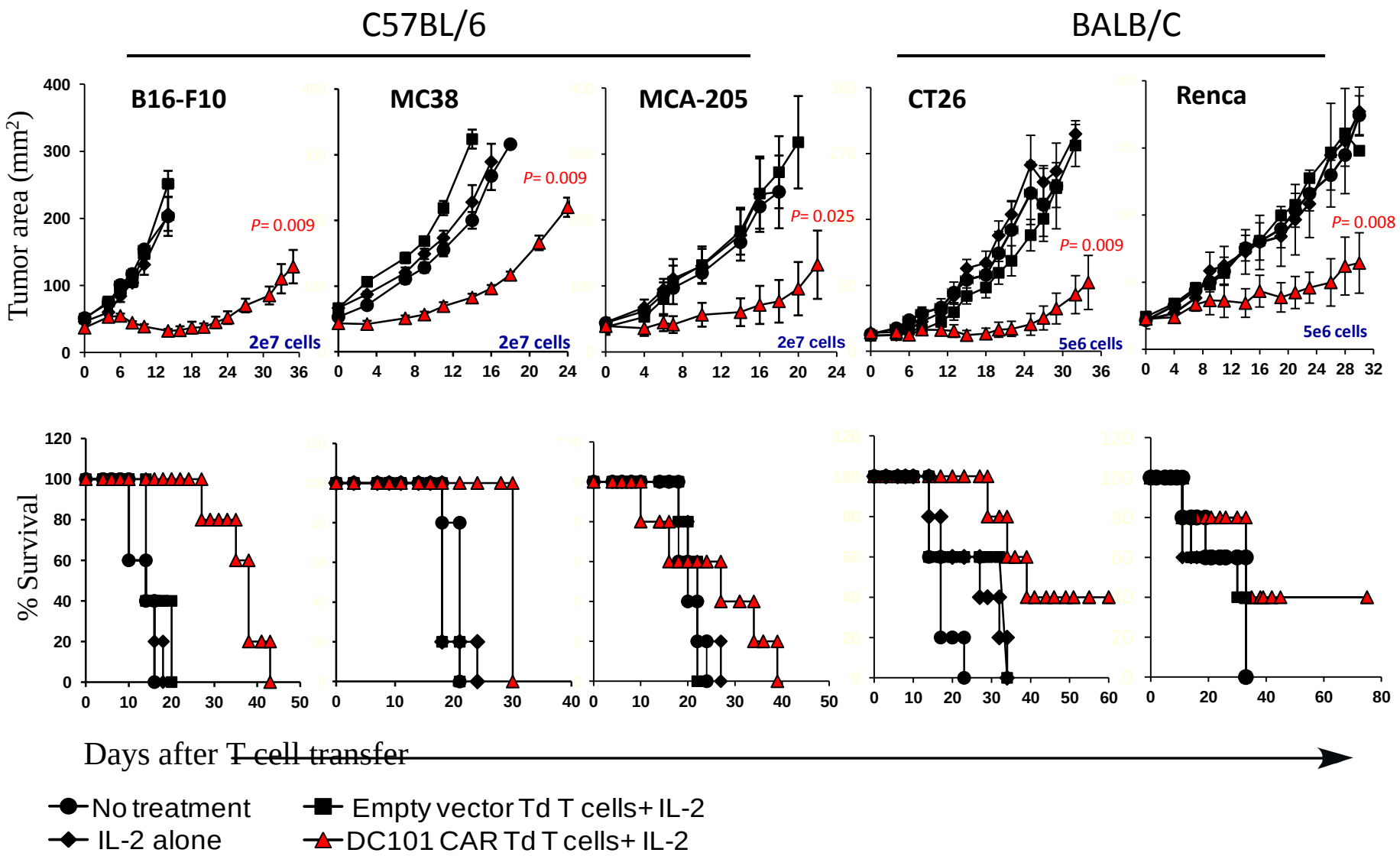
Destruction of cells bearing VEGFR2 may more effectively destroy tumor vasculature and result in effective cancer treatment

Design of DC101 Anti-VEGFR2 CAR Retroviral Vectors



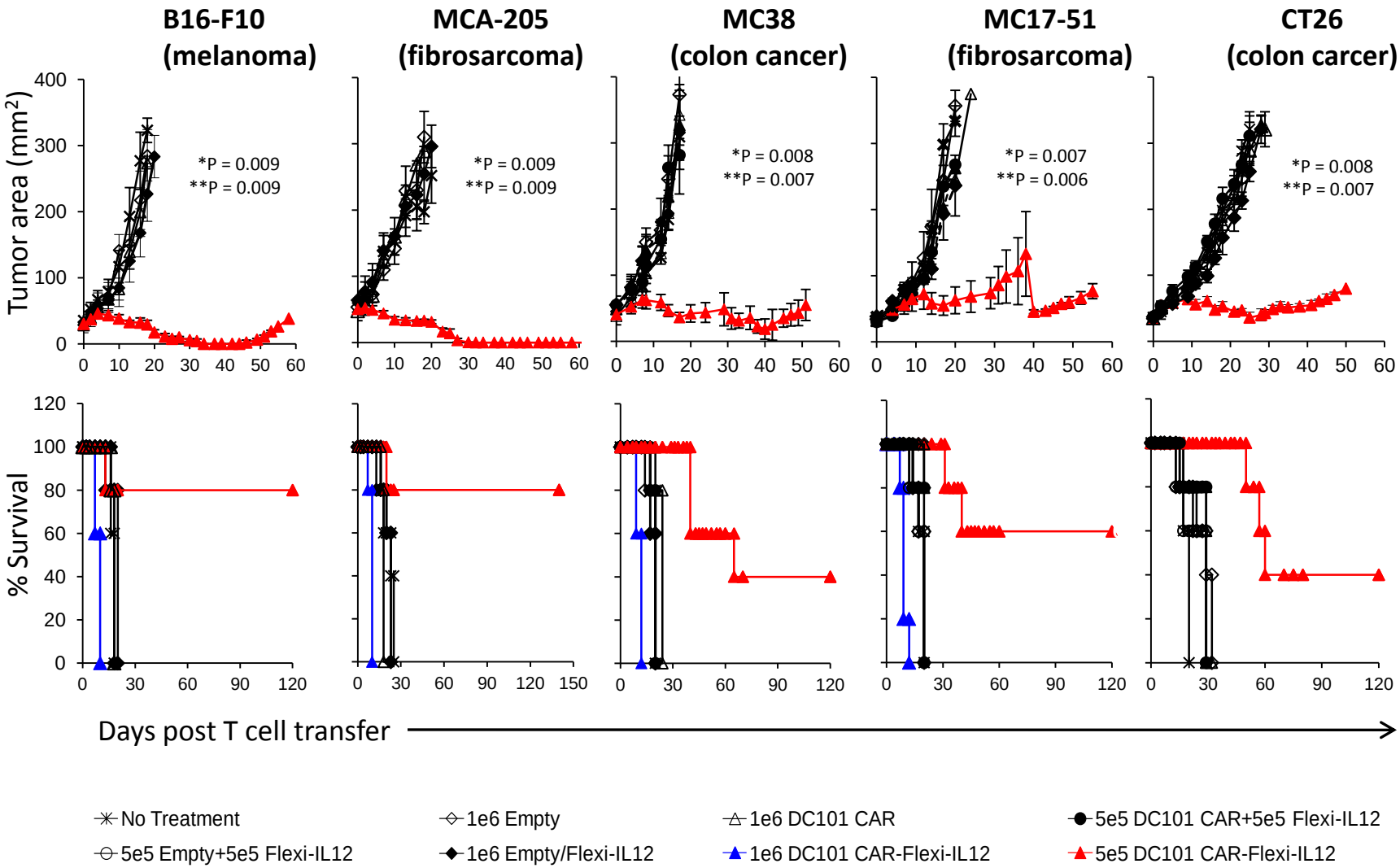
(D. Chinnasamy, J Clin Invest 120:3953, 2010)

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



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

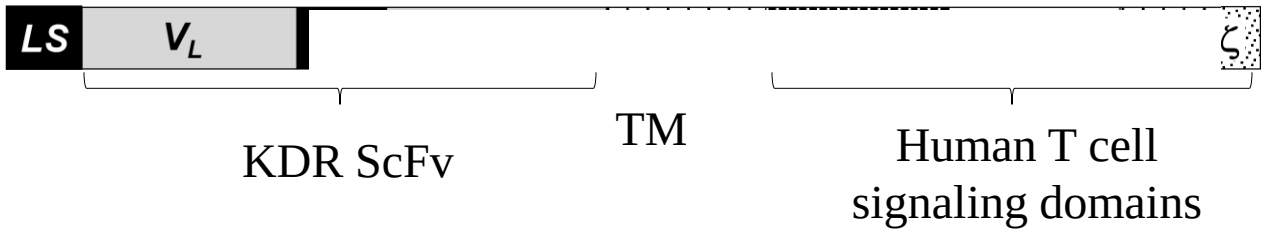
Anti-VEGFR2 CAR and IL-12 cotransduced mouse T cells induced regression of multiple types of vascularized tumors *in mice* without exogenous IL-2 administration



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

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.

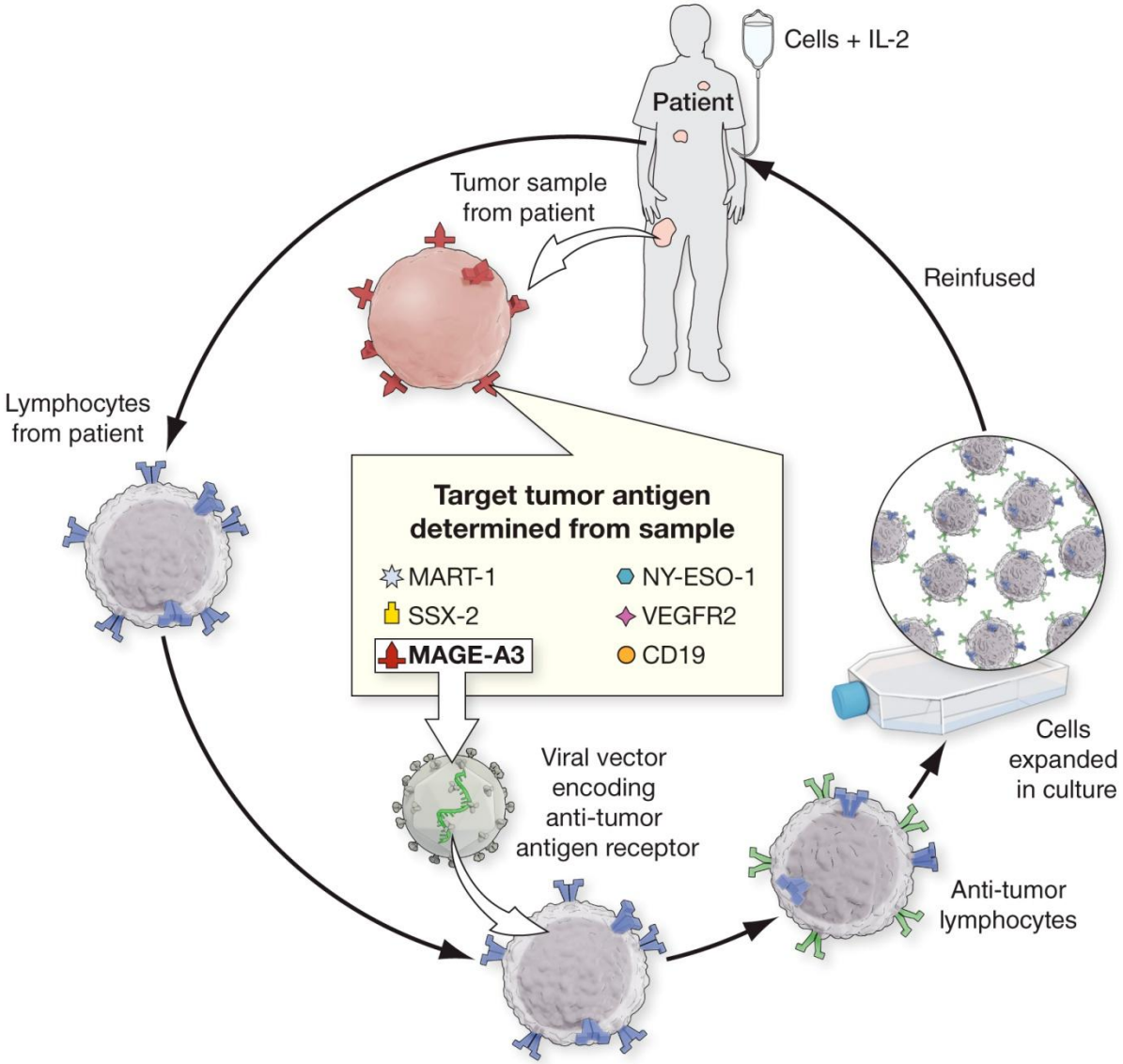


KDR1121-hCD828BBZ) (KDR CAR)

Phase I dose escalation trial ongoing.

Treatment of Metastatic Cancer with Autologous Lymphocytes Expressing anti-VEGFR2 CAR

Patient	Age	Diagnosis	Number cells ($x10^{-9}$)	Doses IL-2	Followup
1. C.B.	61	Colon	0.001	12	NR
2. J.M.	51	Rectal	0.003	6	NR
3. A.R.	40	Melanoma	0.01	8	NR
4. S.W.	54	Melanoma	0.01	8	NR
5. M.J.	64	Melanoma	0.03	9	NR
6. W.E.	65	Renal	0.03	7	NR
7. K.N.	55	Colon	0.1	9	NR
8. R.T.	35	Sarcoma	0.3	4	NR
9. J.L.	43	Colon	1.0	5	NR
10. T.M.	34	Sarcoma	1.0	5	NR*
11. S.V.	61	Melanoma	1.0	2	NR
12. R.J.	44	Colon	1.0	4	TE

*intubated; IL-2 toxicity; recovered

Personalized immunotherapy using anti-tumor receptor gene-modified lymphocytes