

T Cell Immunotherapy - Optimizing Trial Design

Office of Biotechnology Activities

September 10, 2013

Overview

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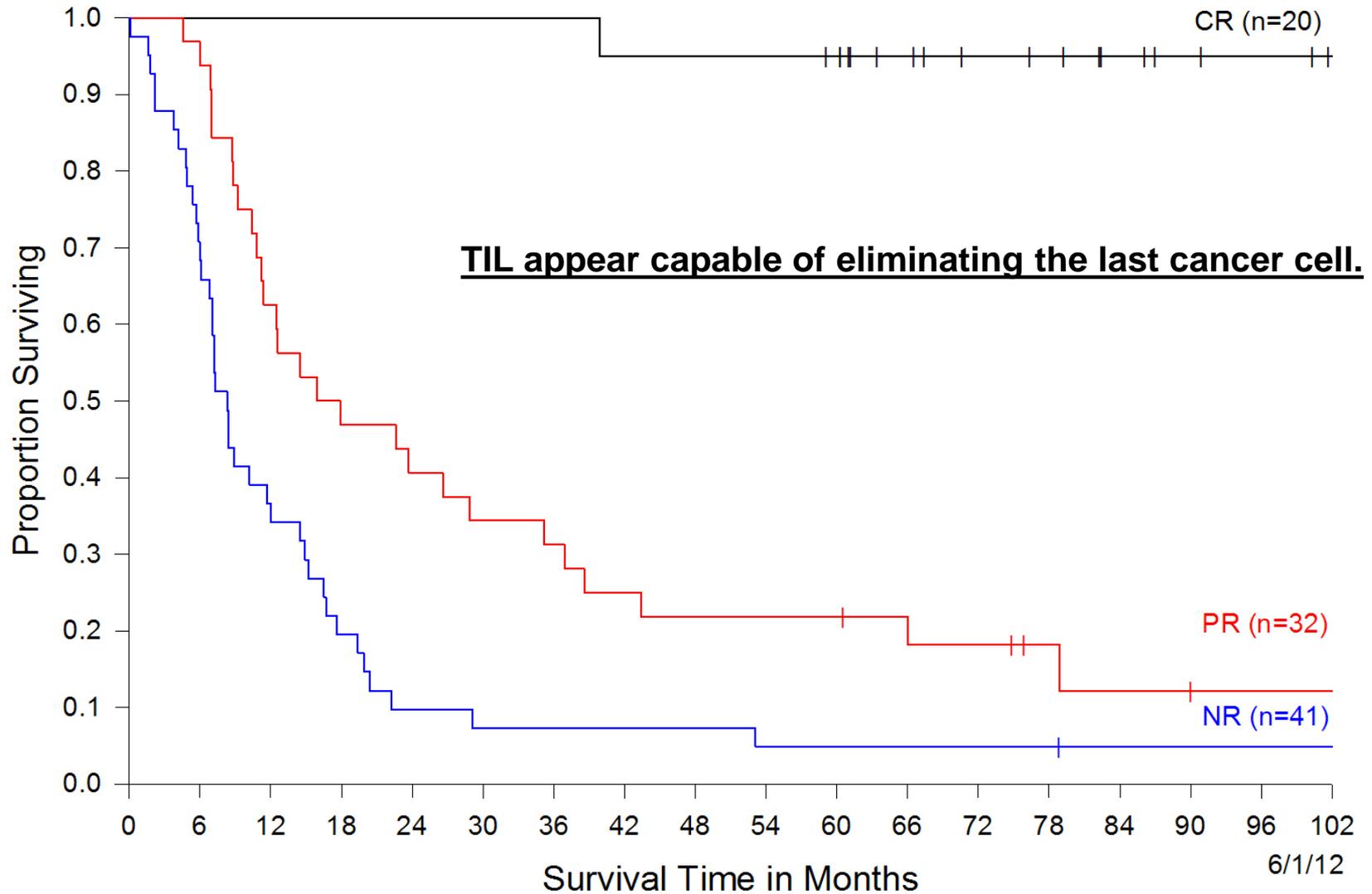
Adoptive Cell Therapy (ACT) is a Powerful Approach to Cancer Immunotherapy

- 1. Large numbers of antitumor cells can be grown in vitro and administered.**
- 2. High avidity anti-tumor cells can be selected using in vitro assays or created in vitro by genetic engineering.**
- 3. Administer cells activated ex-vivo to exhibit anti-tumor effector function (overcome in vivo tolerizing influences)**
- 4. The host can be manipulated to provide a favorable tumor microenvironment prior to administering the cells**

Program for the Application of Cell Transfer Therapy to a Wide Variety of Human Cancers: Tumor Infiltrating Lymphocytes

<u>Cancer</u>	<u>1st patient accrued</u>
Metastatic cutaneous melanoma	1 st 1988 (with lymphodepletion, 2002)
Metastatic ocular melanoma	6/14/13
Metastatic gastrointestinal cancers	9/2/10
Metastatic HPV-induced cancers (cervical, anal, head & neck)	8/16/12

Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2



Program for the Application of Cell Transfer Therapy to a Wide Variety of Human Cancers: Gene Modified Cells

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)

**Assessment of the Safety and Feasibility of Administering T cells
Expressing an anti-CD19 Chimeric Antigen Receptor to Patients with
B-cell Lymphoma or Leukemia**

(OBA: 0809-940; IBC: RD-08-VII-10; CC: 09-C-0082P)

Eligibility: CD19 expressing B cell malignancies

Vector: CAR; CD28,CD3zeta

Total number of patients: 23 (1st patient entered,6/4/09)

Anti-CD19 Protocol** (Plus IL-2)

J. Kochenderfer (Blood 116:4099, 2010)

Type	Total	CR number (duration in months)	PR number (duration in months)	NR
Follicular lymphoma	3	2 (50+*, 15+)	1 (6)	1
Chronic lymphocytic leukemia	4	2 (31+, 20)	1 (6)	1
Splenic marginal zone	1		1 (12)	
Total	8	2 (25%)	4 (50%)	2

75%

*treated twice

**60 mg/kg cyclophosphamide qd x2; 30 mg/m² fludarabine qd x 5

Anti-CD19 Protocol (No IL-2)

J. Kochenderfer

Type	Total	CR	PR	NR
Diffuse large B cell	9	2 (19+, 4+)	4 (6+,2+,2,1+)	3
Chronic lymphocytic leukemia	4	3 (16+,10+,9+)	1 (3+)	
Splenic marginal zone	1		1 (24+)	
Low grade	1		1 (6+)	
Total	15	5 (33%)	7 (47%)	3

80%

(11 patients received 30 mg/kg cyclophosphamide qdx2; 25mg/m² fludarabine qd x5; including 4 CR and 6 PR; 1-5e6 transduced cells/kg)

Anti-CD19 Protocol (No IL-2)

J. Kochenderfer

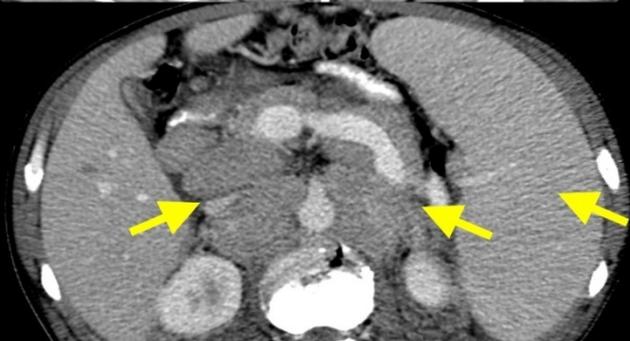
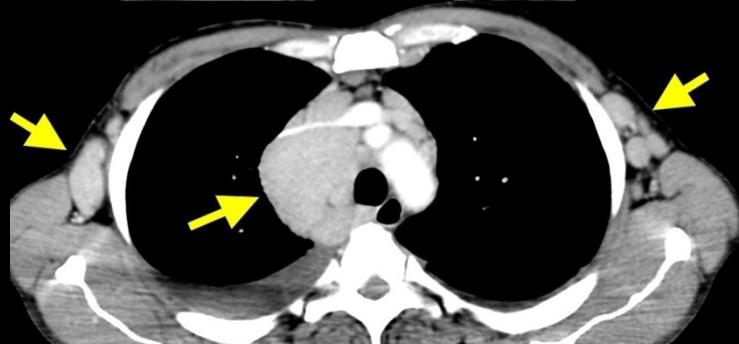
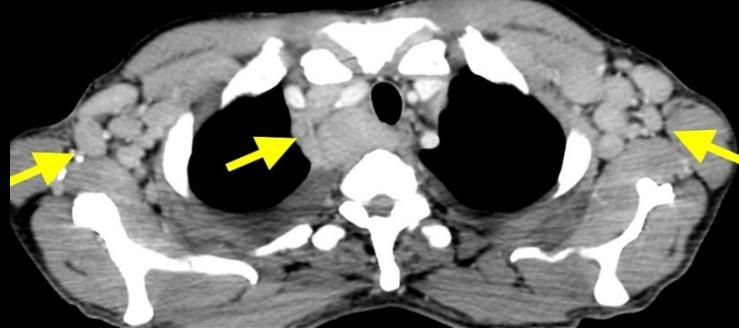
Cell doses 1-5 x 10⁶ CAR + cells/kg

15 patients

- 1 TRM (arrhythmia)
- 3 aphasia (transient, returned to normal)
- 2 obtundation (transient, returned to normal)
- 5 hypotension (transient, returned to normal)

E.K.

Follicular
lymphoma



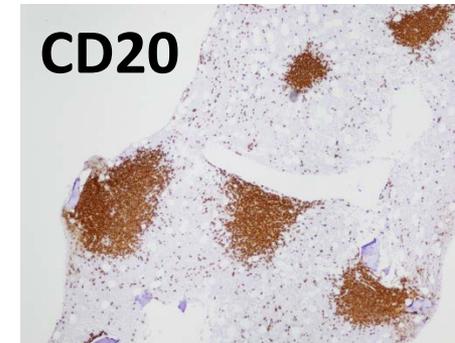
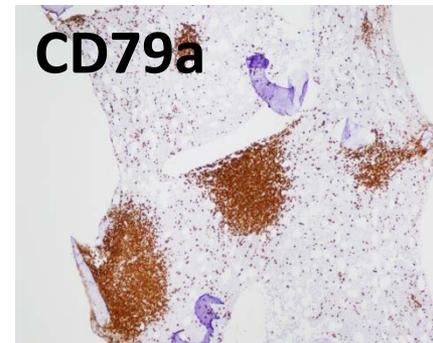
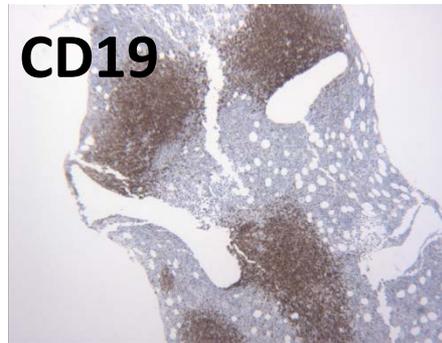
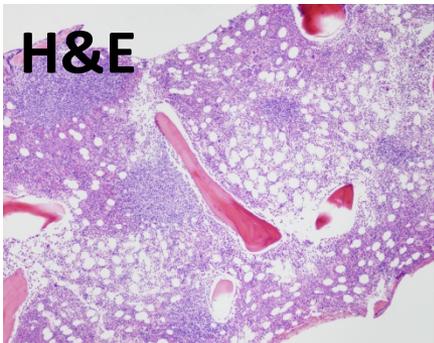
June 2, 2009



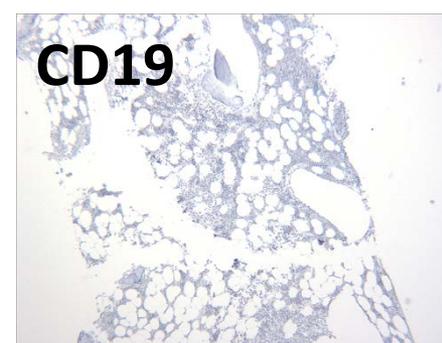
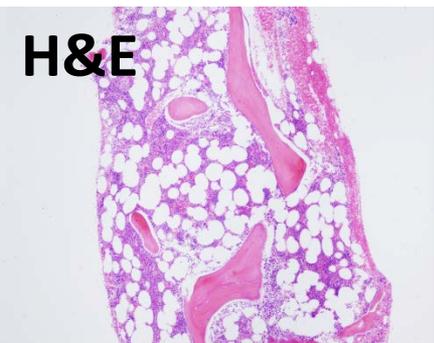
March 14, 2012

Bone marrow biopsies were performed before and 14 weeks after treatment of Patient 1

Before treatment-extensive lymphoma involvement



14 weeks after treatment-absence of all B-lineage cells



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

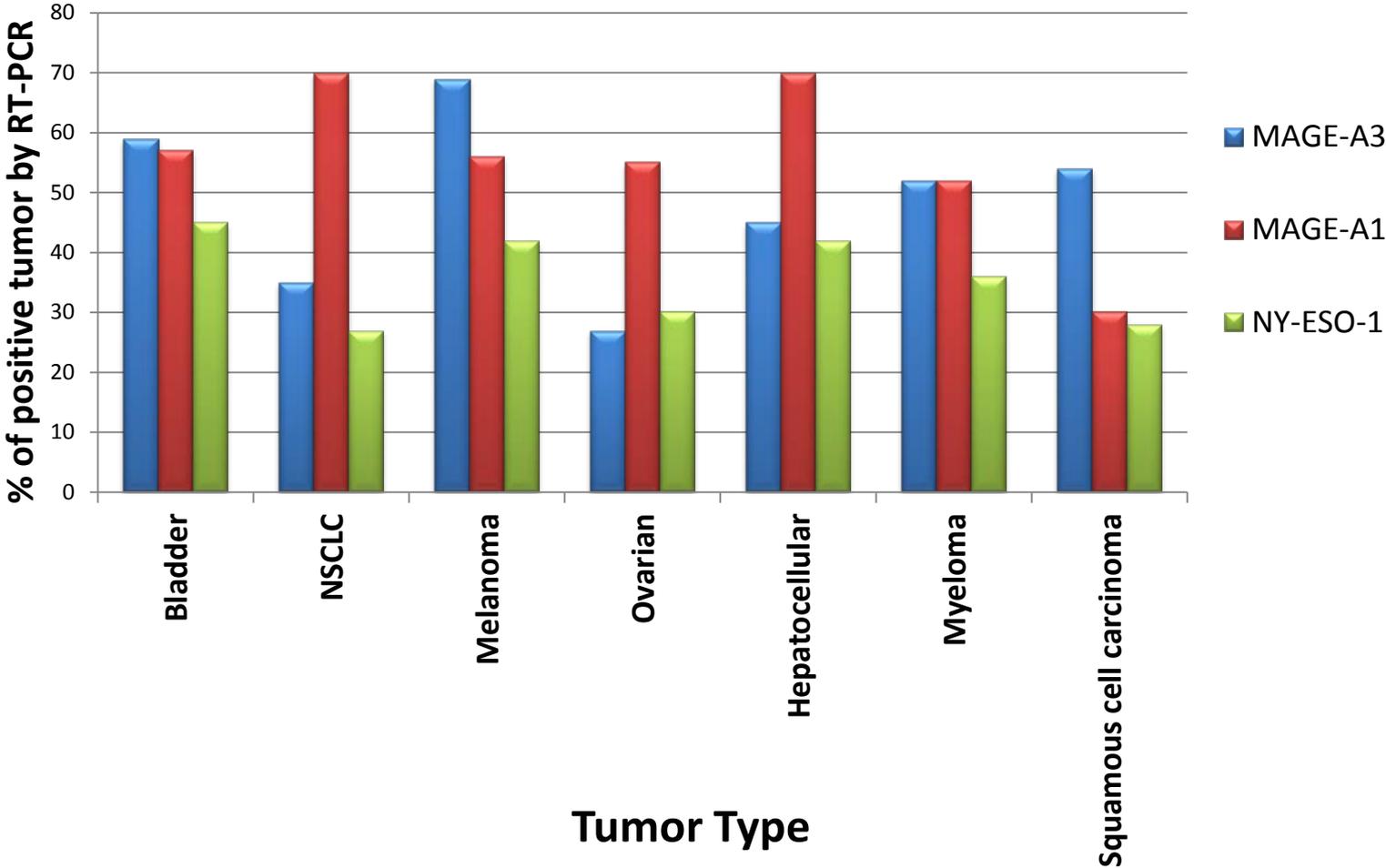
NY-ESO-1 Family

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



**Phase II Study of Metastatic Cancer that Expresses NY-ESO-1 Using
Lymphodepleting Conditioning Followed by Infusion of
Anti-NY-ESO-1 TCR-Gene Engineered Lymphocytes**

(OBA: 0712-886; IBC: RD-07-XII-17; CC: 08-C-0121L)

Eligibility: Metastatic cancer that expresses NY-ESO-1

Vector: TCR gammaretrovirus

Total number of patients: 34 (1st patient entered, 6/5/08)

No off-tumor on-target toxicities.

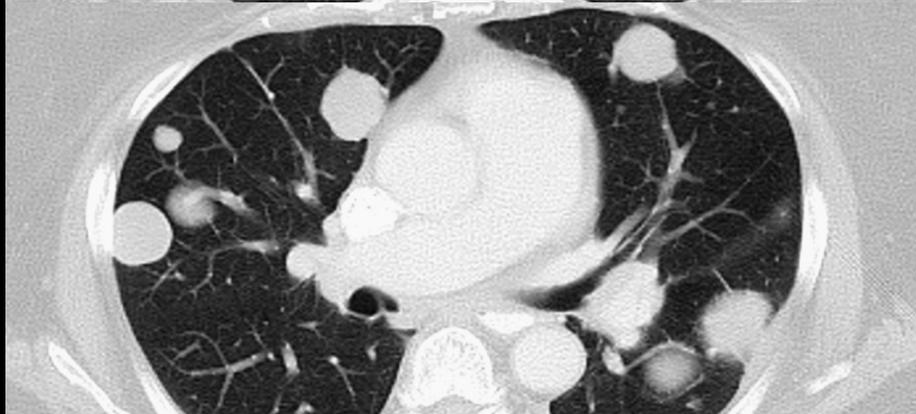
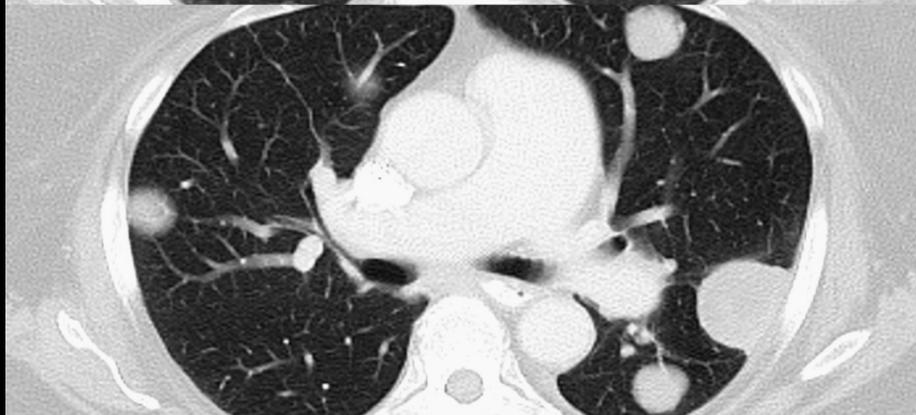
Responses to Therapy with NY-ESO-1 TCR (5/1/13)

	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	19	6 (32%) (10**, 9+, 8, 5, 3, 3)	4 (21%) (50+, 49+, 25, 24+**)	10 (53%)
Synovial Cell Sarcoma	15	9 (60%) (31+**, 18*, 12**, 10, 8, 7, 5, 4, 3**)	1(7%) (5+)	10 (67%)

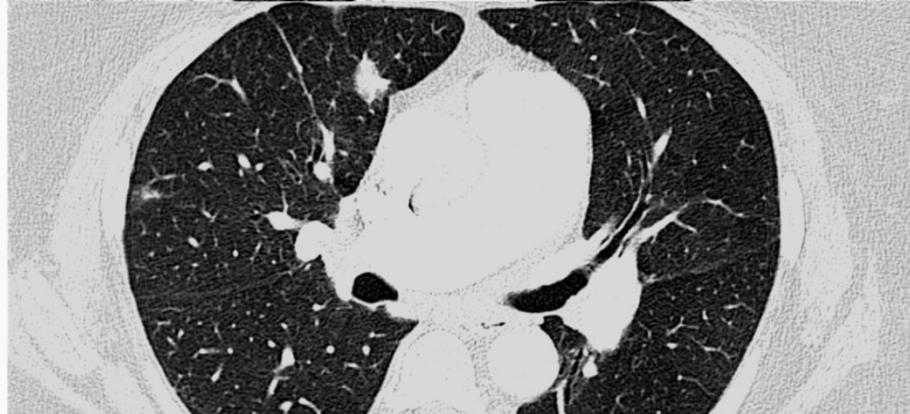
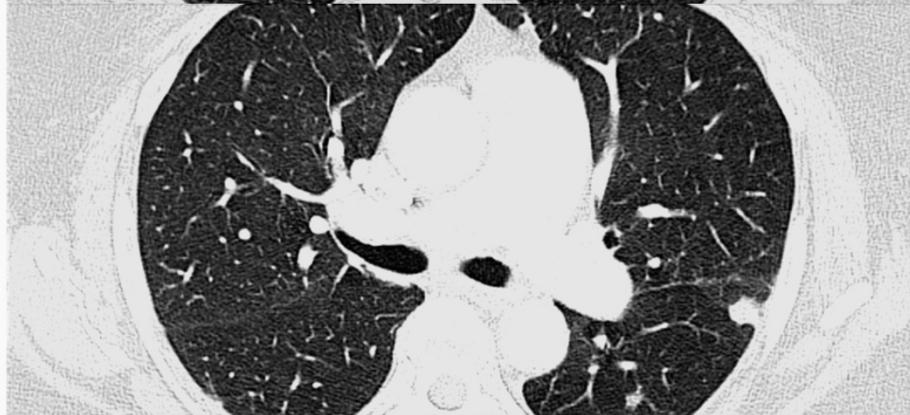
*treated twice

**plus ALVAC vaccine

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



Pre-Treatment



18 Months



Pre-Treatment



18 Months

**Phase I/II Study of Metastatic Cancer that Expresses MAGE-A3/12
Using Lymphodepleting Conditioning Followed by Infusion of
Anti-MAGE-A3/12 TCR-Gene Engineered Lymphocytes**

(OBA: 1009-1065; IBC: RD-10-IX-04; CC: 11-C-0062B)

Eligibility: Metastatic cancer that expresses MAGE-A3

Vector: TCR gammaretrovirus

Total number of patients: 9 (1st patient entered, 2/24/11)

MAGE-A3 TCR Protocol (F/U 3/1/13)

(J. Immunother. 36:133-151, 2013)

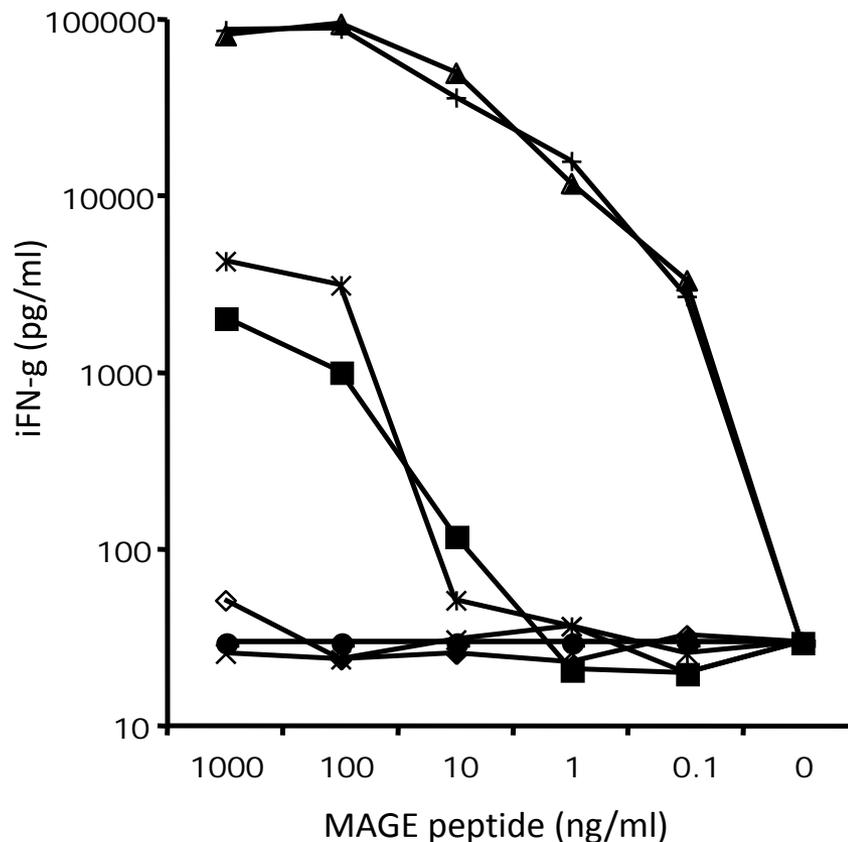
Patient	Diagnosis	Date of cells	# cells (x10 ⁻⁹)	#IL-2 doses	Response	Neurologic
1. L.A.	59 Melanoma	2/24/11	28	6	CR(24+)	None
2. J.P.	38 Melanoma	3/24/11	30	5	NR	None
3. P.M.	56 Melanoma	5/5/11	30	7	PR(4)	None
4. K.H.	21 Synovial Sarc.	6/10/11	41	1	PR(5)	None
5. M.S.	54 Melanoma	7/22/11	79	5	PR(4)	Coma (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(13)	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 KVADLV**G**FL
- MAGE A2 KM**V**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

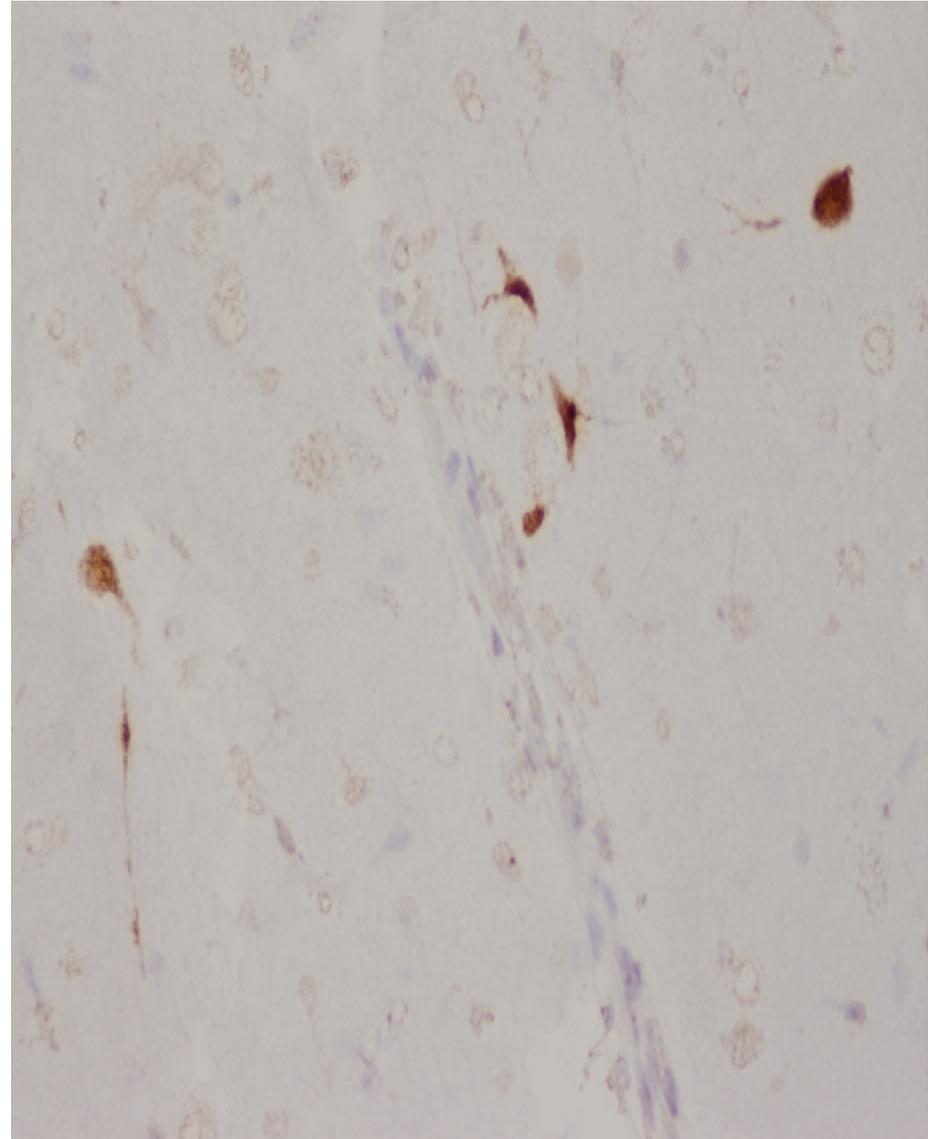
MAGE-TCR patient Bx-IHC staining for MAGE-A

Patient: MS



11-5784; neurons negative for MAGE

Patient: GT



11-5700; few neurons and axons positive for MAGE

Tests for off-target reactivity of MAGE-A3 TCR engineered T cells

1. **MAGE-A3/A9 RNA was not detected by Q-RT-PCR in nine normal brain samples and six biopsy samples from two of the affected patients.**
2. **MAGE-A3/A12 TCR gene engineered T cells do not recognize neural progenitor-derived normal brain cells.**
3. **MAGE-A3/A12 TCR gene engineered T cells do not recognize autologous dendritic cells from affected patients that were pulsed with normal brain cell lysates.**
4. **MAGE-A3, A9, and A12 were not observed in a large SAGE database from two normal human brain samples (>5 million high-quality reads, Parsons, D.W., *et al. Science* 321:1807, 2008).**
5. **Query of a deep sequencing database (containing ~120 million paired end reads that mapped to the reference genome) from 4 patients and 5 normal human brain samples did not find any significant MAGE-A gene expression.**

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

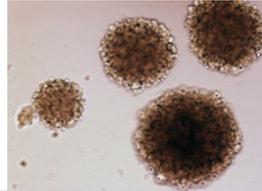
Expressed in 30-50% of glioblastomas

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

Transduction	Media	Targets		Glioblastoma Stem Cell Lines*		
		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)

A Phase I/II Study of the Safety and Feasibility of Administering T cells Expressing Anti-EGFRvIII Chimeric Antigen Receptor to Patients with Malignant Gliomas Expressing EGFRvIII

(OBA: 1103-1095; IBC: RD-11-III-06; CC: 11-C-0266G)

Eligibility: High-grade glioblastomas that express EGFRvIII

Vector: CAR; CD28,41BB,CD3zeta

Phase I/II dose escalation

Total number of patients: 10 (1st patient entered, 5/23/12)

(2 cohorts, with and without steroids)

No off-tumor, on-target toxicities.

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)	3×10^7	1 patient
Cohort 3 (group a & b)	10^8	1 patient
Cohort 4 (group a & b)	3×10^8	3 patients
Cohort 5 (group a & b)	10^9	3 patients
Cohort 6 (group a & b)	3×10^9	3 patients
Cohort 7 (group a & b)	10^{10}	3 patients
Cohort 8 (group a & b)	$3 - 6 \times 10^{10}$	3 patients

**Phase I/II Study of Metastatic Cancer Using Lymphodepleting
Conditioning Followed by Infusion of Anti-VEGFR2 Gene Engineered
CD8+ Lymphocytes**

(OBA: 1004-1036; IBC: RD-10-IV-02; CC: 11-C-0013G)

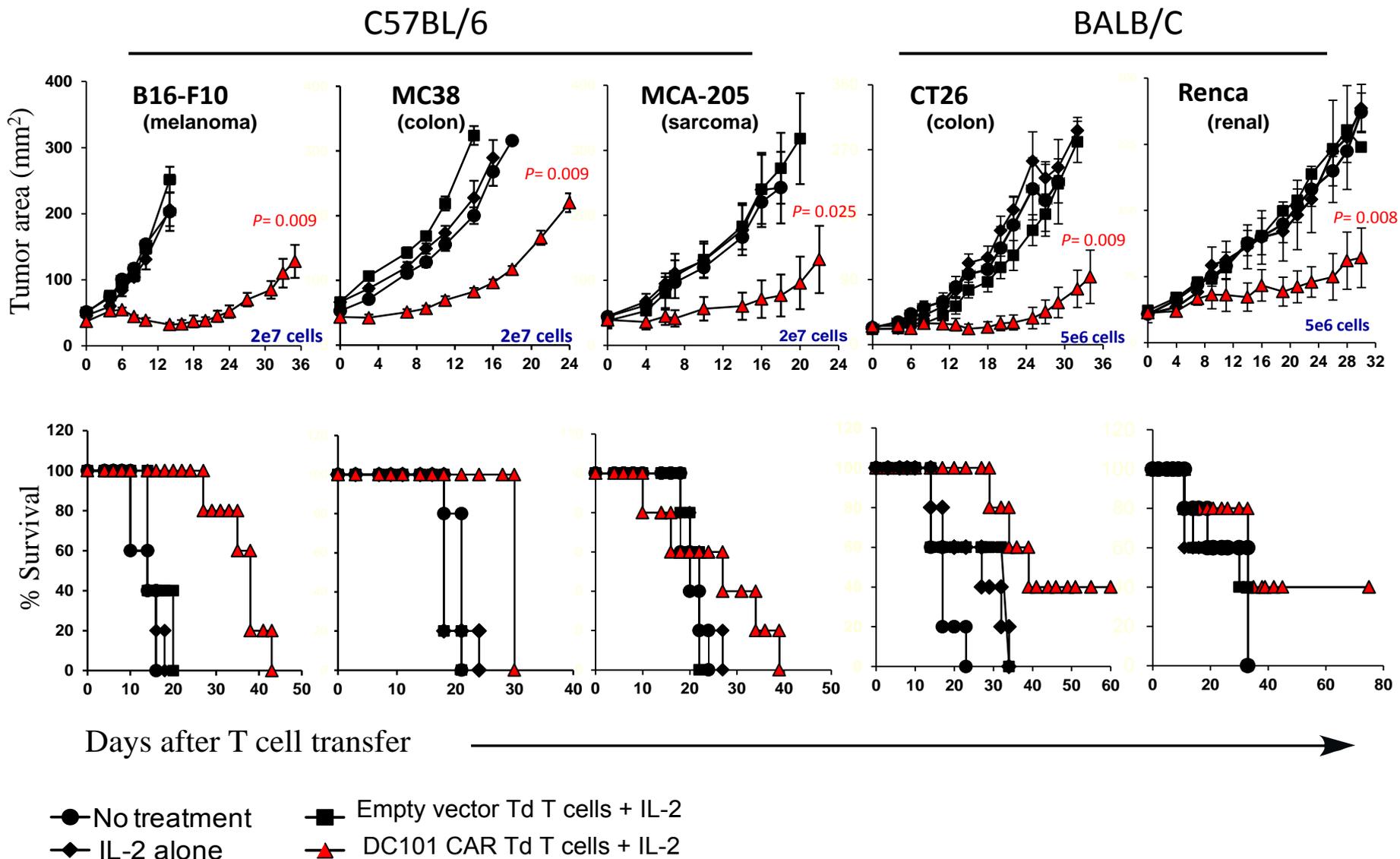
Eligibility: Metastatic cancer refractory to standard treatment

Vector: CAR; CD28,41BB,CD3zeta

Total number of patients: 21 (1st patient entered, 11/18/10)

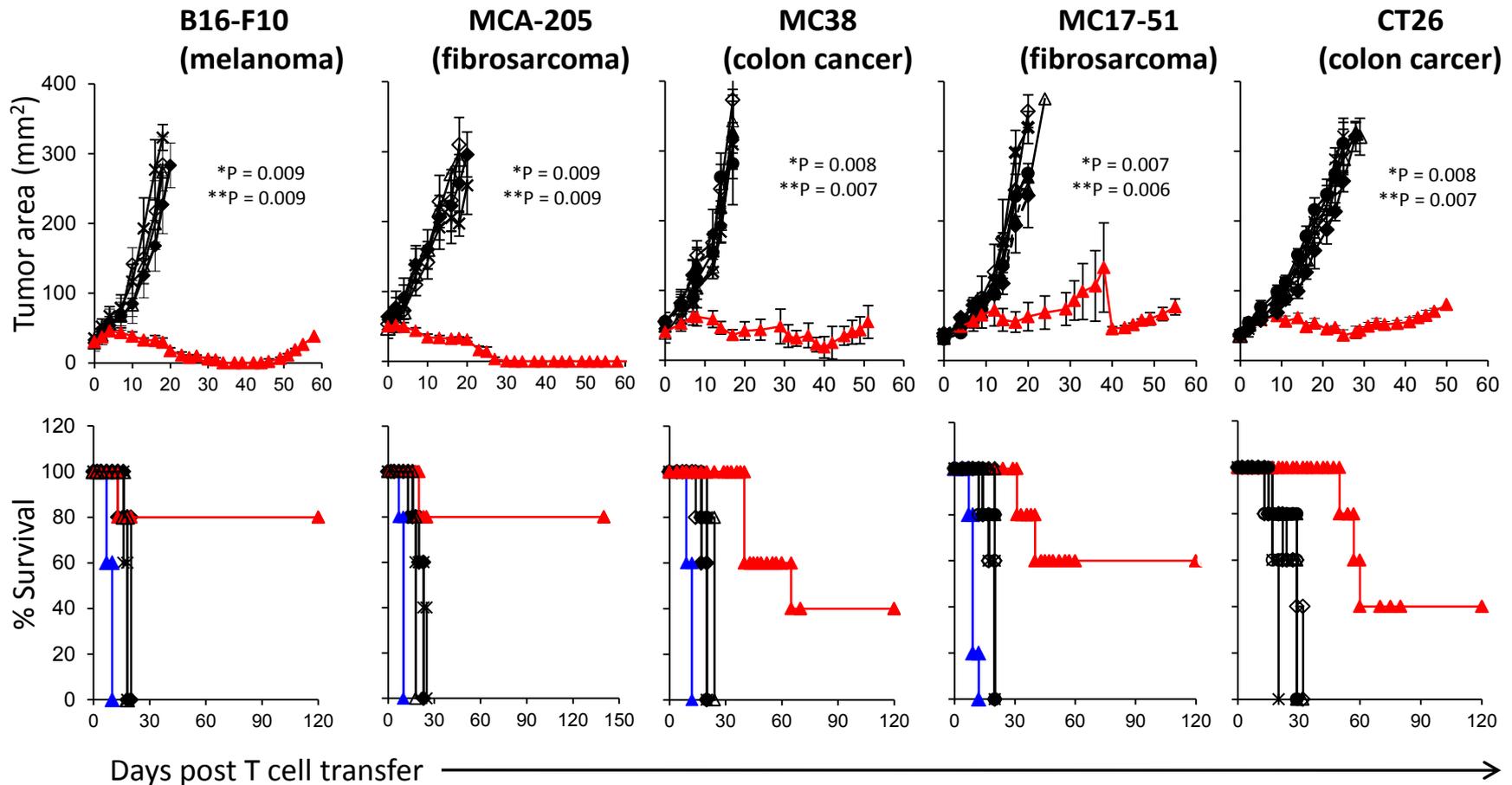
No off-tumor, on target toxicity.

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



* No Treatment ⇐ 1e6 Empty ⇐ 1e6 DC101 CAR ● 5e5 DC101 CAR+5e5 Flexi-IL12
 ○ 5e5 Empty+5e5 Flexi-IL12 ◆ 1e6 Empty/Flexi-IL12 ▲ 1e6 DC101 CAR-Flexi-IL12 ▲ 5e5 DC101 CAR-Flexi-IL12

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

Protocol Design

Phase I/II dose escalation (3 patients per cohort)

Cohort 1	10^6 cells
Cohort 2	3×10^6 cells
Cohort 3	10^7 cells
Cohort 4	3×10^7 cells
Cohort 5	10^8 cells
Cohort 6	3×10^8 cells
Cohort 7	10^9 cells
Cohort 8	3×10^9 cells
Cohort 9	10^{10} cells
Cohort 10	3×10^{10} cells

Phase I/II Study of Metastatic Cancer Using Lymphodepleting Conditioning Followed by Infusion of Anti-mesothelin Gene Engineered Lymphocytes

(OBA: 1112-1139; IBC: RD-12-I-02; CC: 12-C-0111D)

Eligibility: Metastatic cancer that expresses mesothelin

Vector: CAR; CD28,41BB,CD3zeta

Phase I/II dose escalation

Total number of patients: 7 (1st patient entered, 5/11/12)

No off-tumor, on-target toxicity.