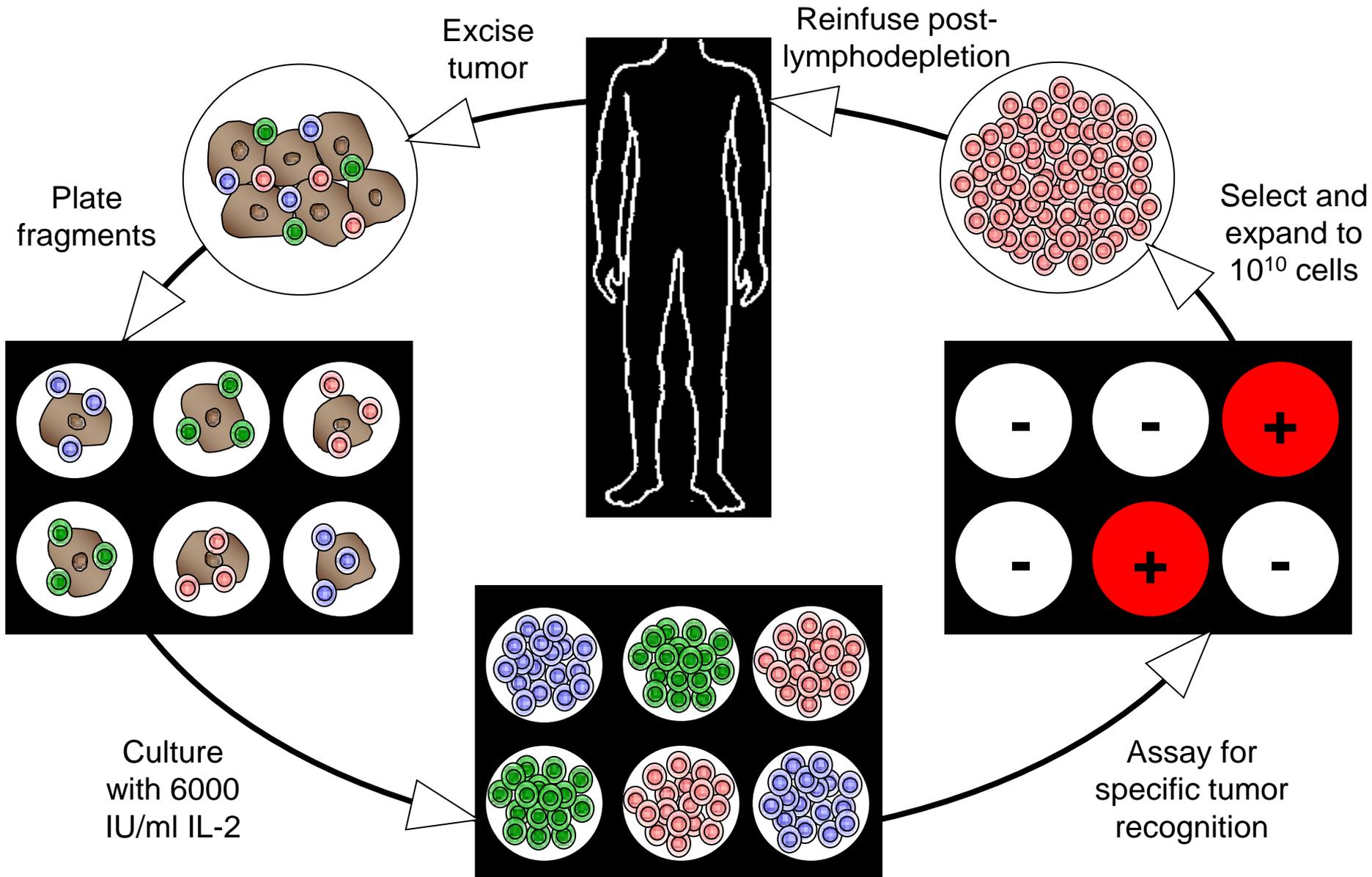


ADVANTAGES OF CELL TRANSFER IMMUNOTHERAPY

- 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**

Adoptive transfer of tumor infiltrating lymphocytes (TIL)



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	
Ablative (200cGy)		Cy Flu	Cy Flu	Flu	Flu	Flu							TBI Cells IL-2	IL-2 IL-2 CD34+
Ablative (1200cGy)	Cy Flu	Cy Flu	Flu	Flu	Flu	Flu TBI	TBI	TBI					Cells IL-2	IL-2 IL-2 IL-2 IL-2 CD34+

Cell Transfer Therapy

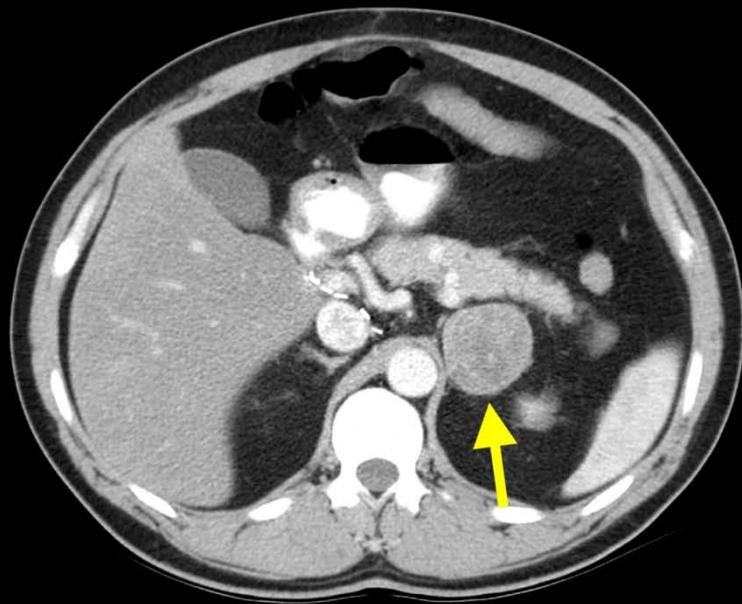
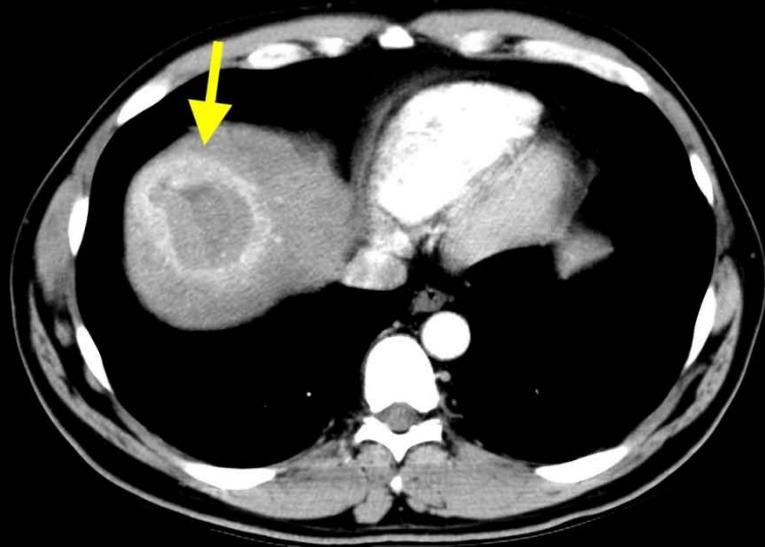
(3/1/11)

Treatment	Total	PR				CR			OR (%)	
		number of patients (duration in months)								
No TBI	43	16				5			21 (49%)	
		(84,	36,	29,	28,	(88+, 86+, 85+,				
		14,	12,	11,	7,	82+, 71+)				
		7,	7,	7,	4,					
		4,	2,	2,	2)					
200 TBI	25	8				5			13 (52%)	
		(14,	9,	6,	6,	(75+, 71+, 67+,				
		5,	4,	3,	3)	64+, 61+)				
1200 TBI	25	8				10			18(72%)	
		(21,	13,	7,	6,	(55+, 52+, 51+,				
		6,	5,	3,	2)	51+, 46+, 45+,				
						45+, 45+, 44+,				
						19)				

(52 responding patients: 42 had prior IL-2; 22 had prior IL-2 + chemotherapy)

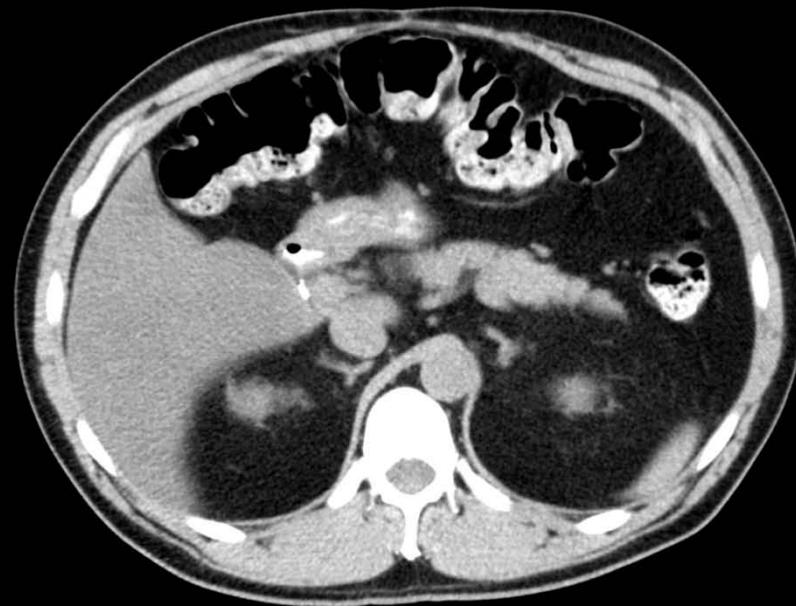
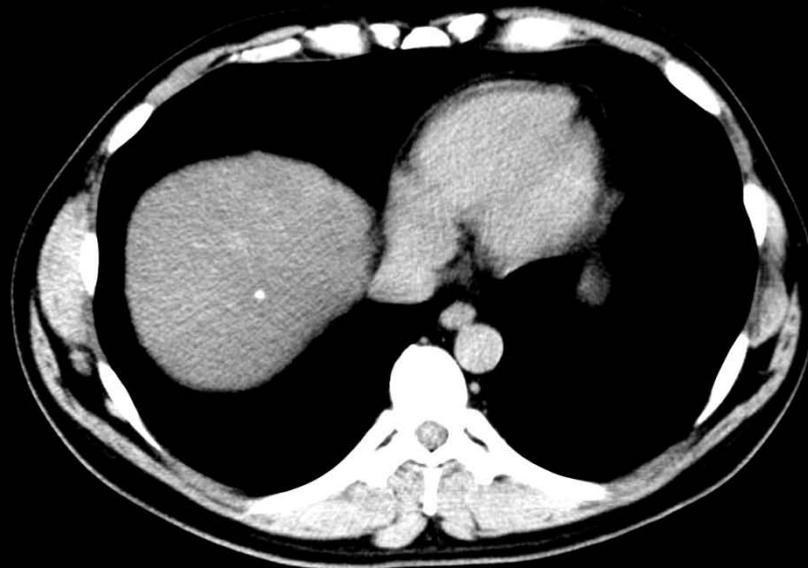
(20 complete responses: 19 ongoing at 44 to 88 months)

Other Sites: L nodes



Nov 7, 2006

CR 39+ mo.



Feb 24, 2010

Pt. M.H.

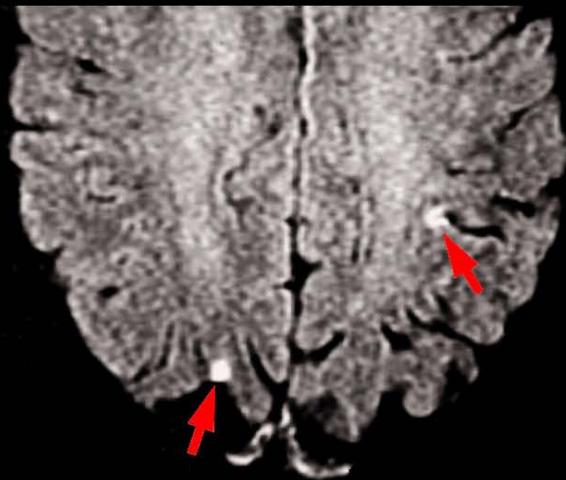
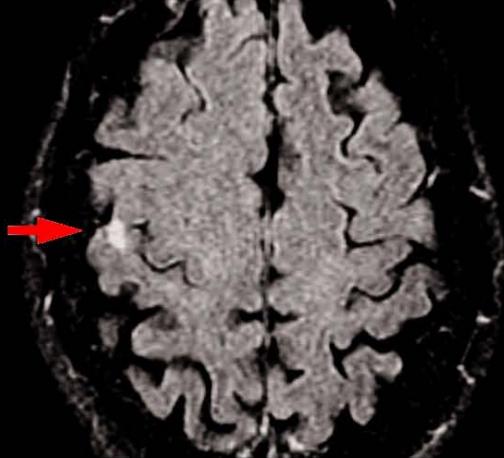


8-27-03

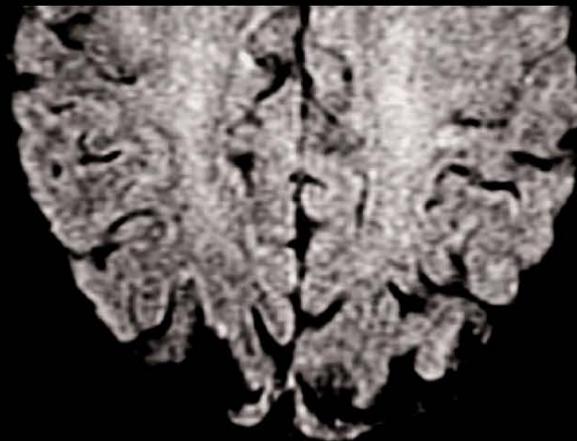
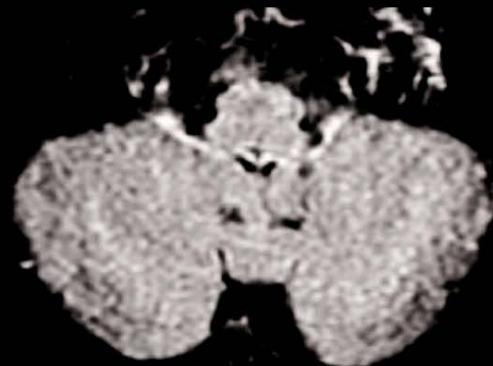
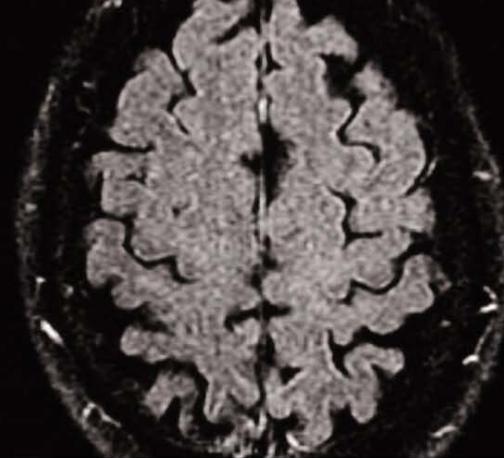
9-22-03

11-7-03

Pt. M.H.



8/03



11/03

Pt.R.B.



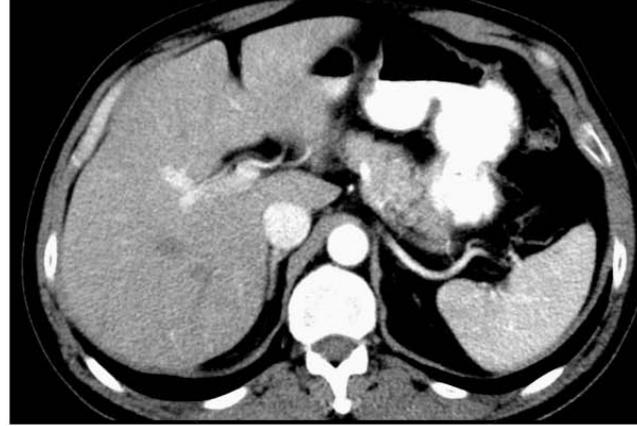
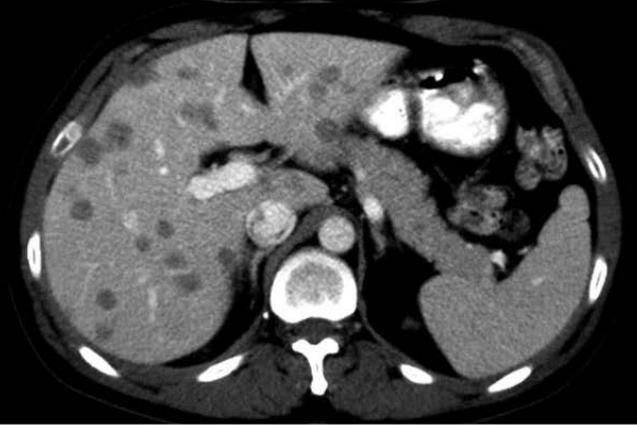
Day -45

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

Hypothesis of Mechanism of Cancer Regression Following Cell Transfer

The lymphopenic 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 the regression of large vascularized, invasive metastatic melanoma in humans.

CHALLENGE

Can we extend this treatment to additional patients with

- 1) melanoma**
- 2) other cancers?**

DMF4 and DMF5 MART1 and gp100(154) TCR retroviral constructs

DMF4 (previous MART1 clinical trial)



DMF5



gp100(154) (high-affinity murine TCR)



Treatment with MART-1 TCR transduced autologous lymphocytes

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

(Science 314:126, 2006)

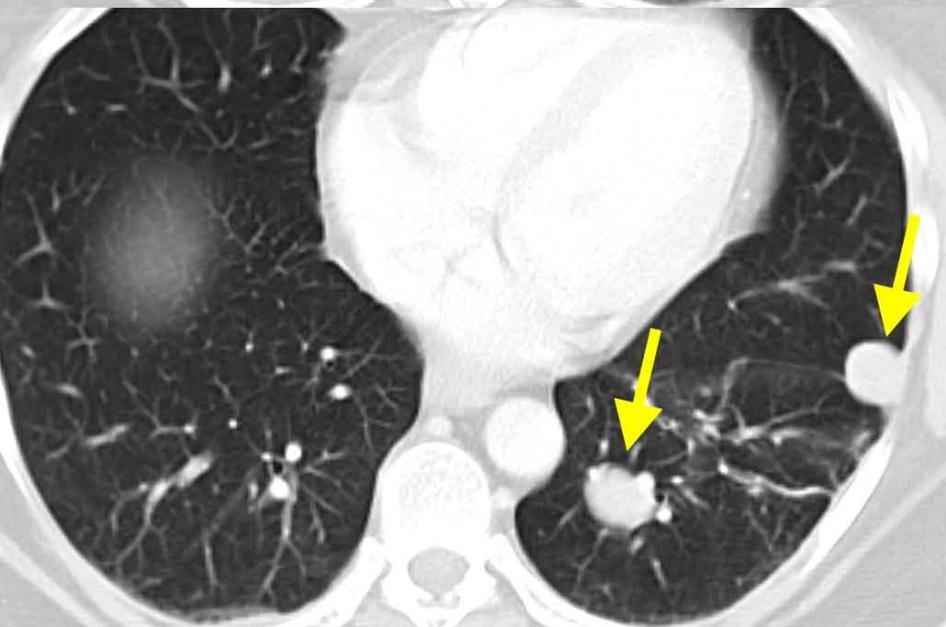
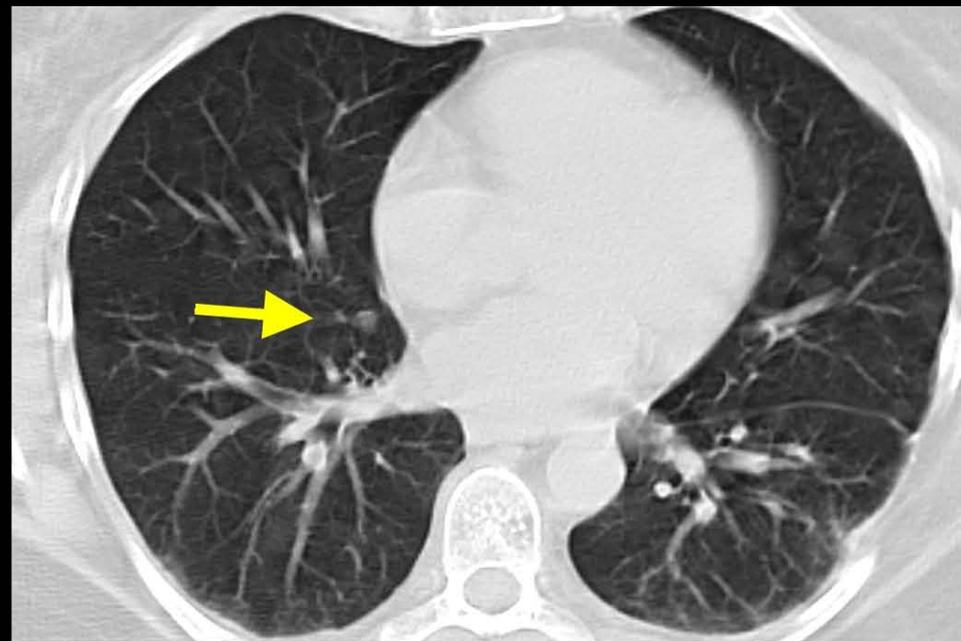
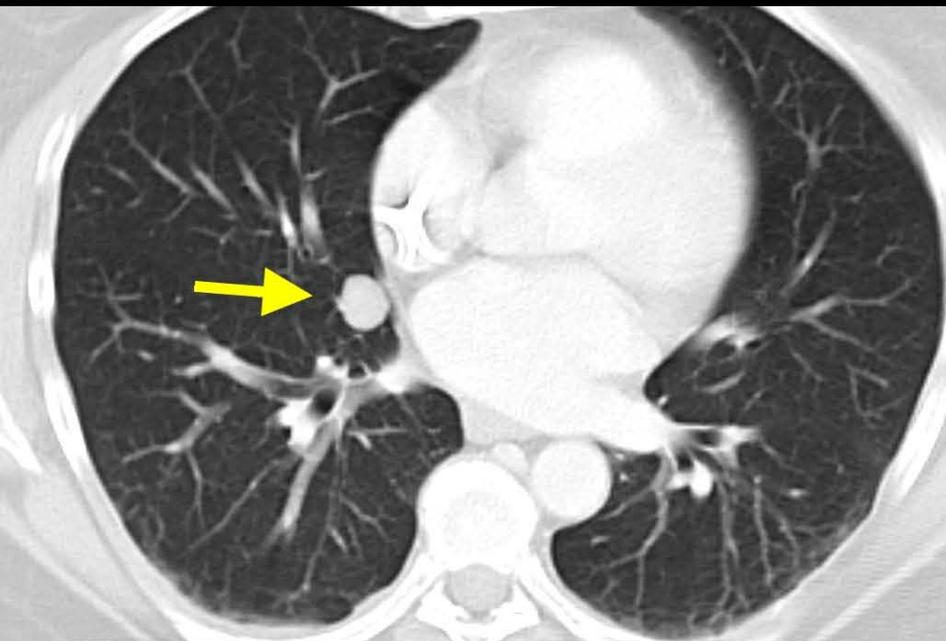
Evaluation of Gene Therapy Using the DMF5 Receptor in Patients with Metastatic Melanoma

Cohort	Cell#	IL-2	Response	
			Total	OR
1	1-3x10 ¹⁰	limited	6	2
2	~3x10 ⁹	to tolerance	6	2
3	1-8x10 ¹⁰	to tolerance	8	2
Total			20	6(30%)

(All patients were refractory to prior treatment with IL-2.)

(Science 314:126-129, 2006; Blood 114:535-546, 2009)

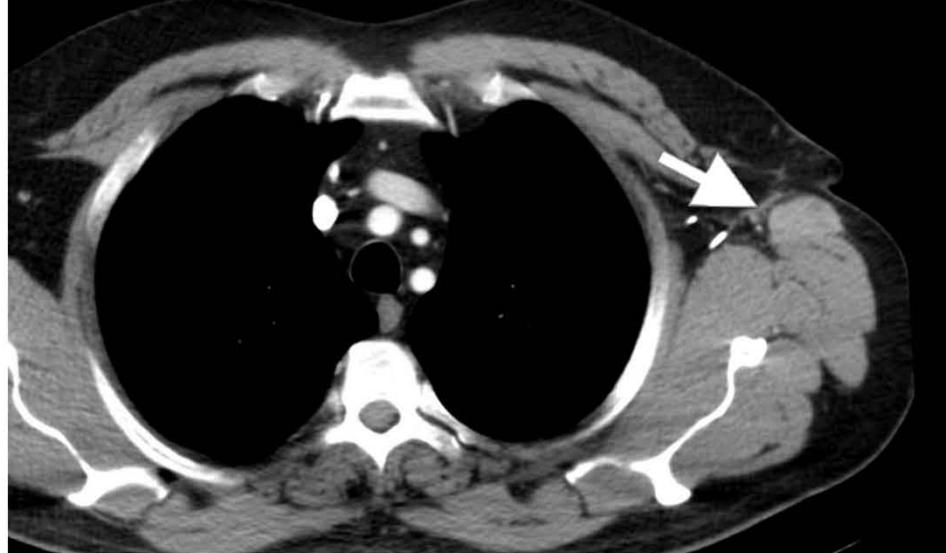
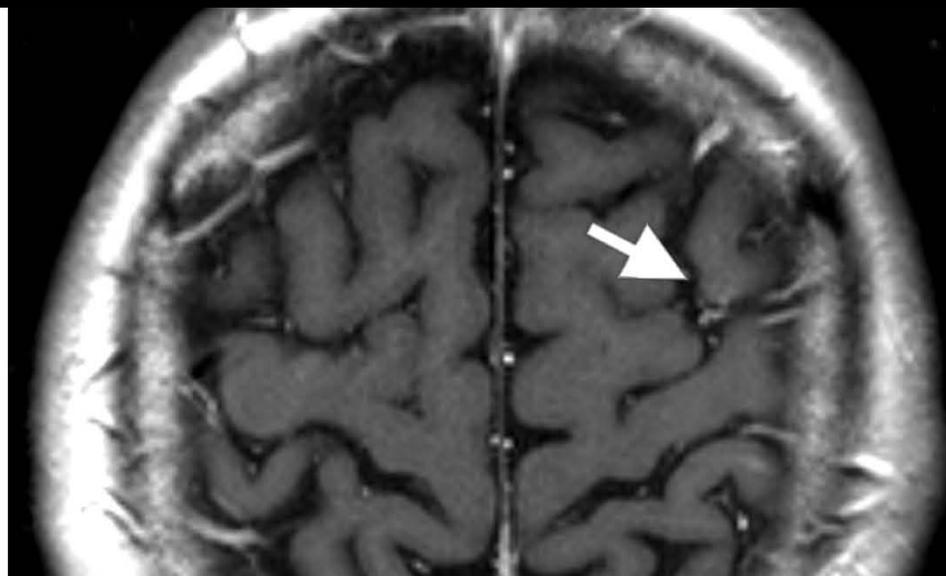
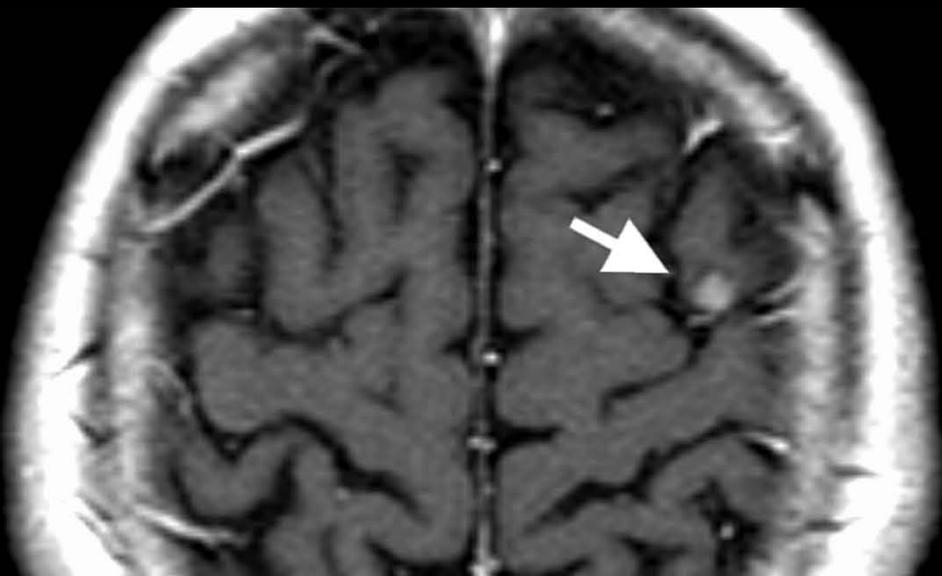
D.T. DMF5 TCR



Pre-Treatment

5+ Months

J.K. F5 TCR



Pre-Treatment

12+ Months

CANCER-TESTES ANTIGENS

No expression on adult human tissues except for testis

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

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

Synovial Cell Sarcoma

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

Chromosomal rearrangement of:

SYT gene on chromosome 18

SSX genes (1 of 5) on chromosome X

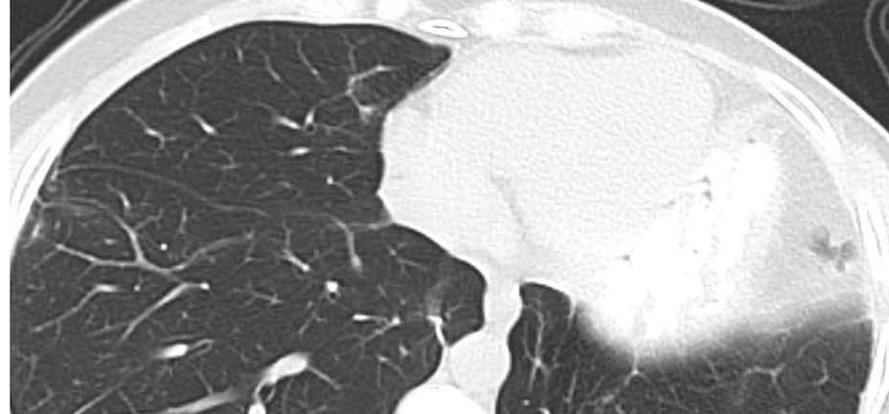
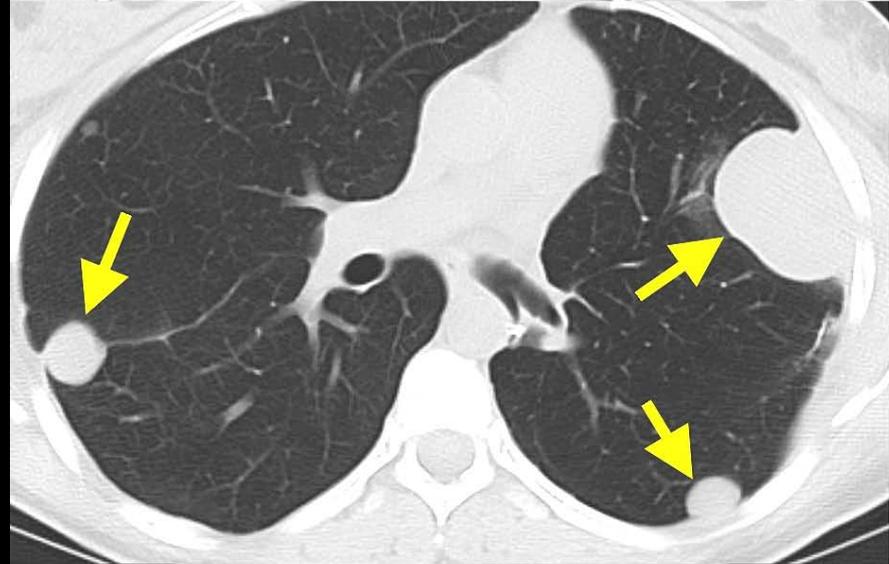
80% of synovial sarcomas express high levels of NY-ESO-1

As of 4/1/11, seven of ten patients (70%) with heavily pretreated synovial cell sarcoma have had objective responses to treatment with autologous TCR gene-engineered T cells.

H.K.

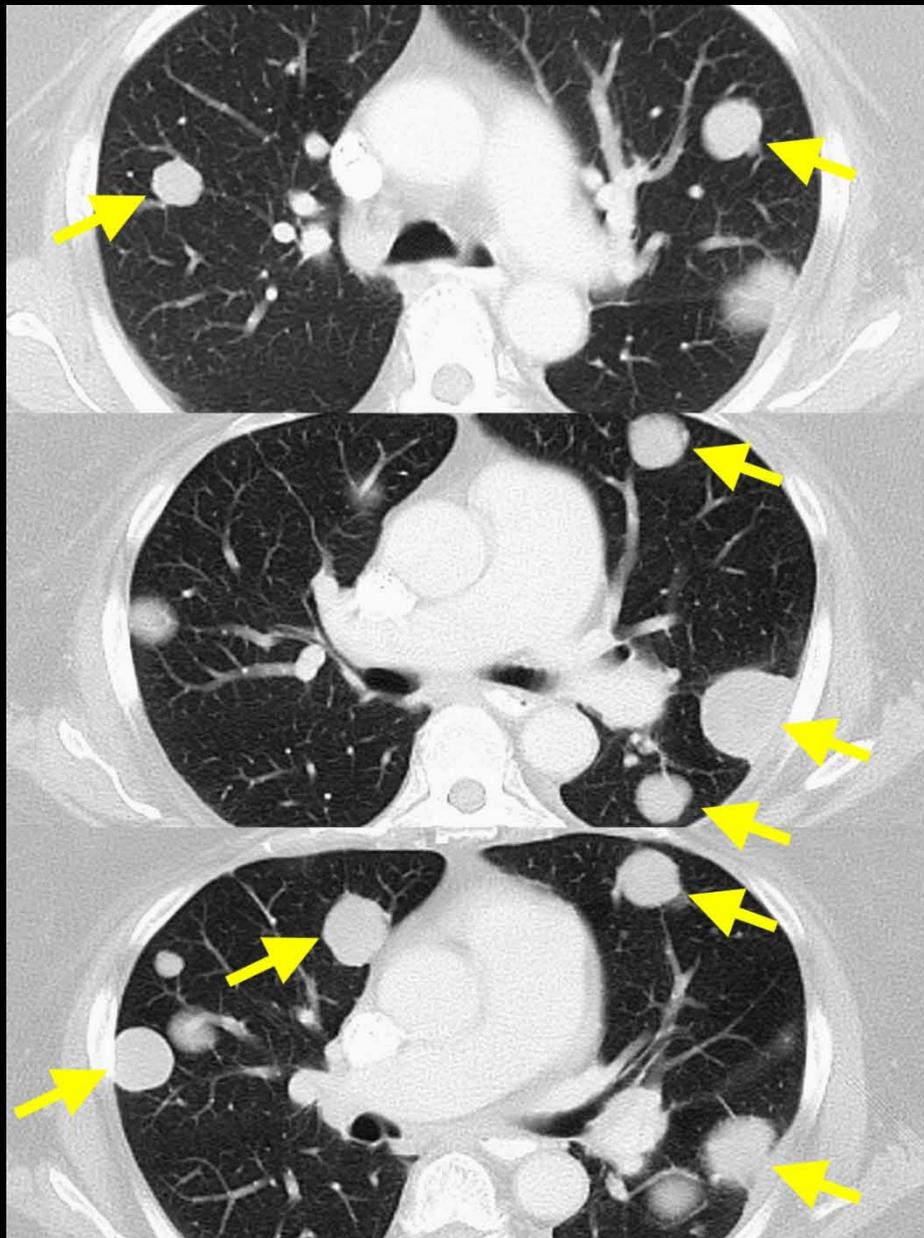
Synovial
Sarcoma

ESO
TCR



Pre-Treatment

14 Months



Pre-Treatment



9 Months

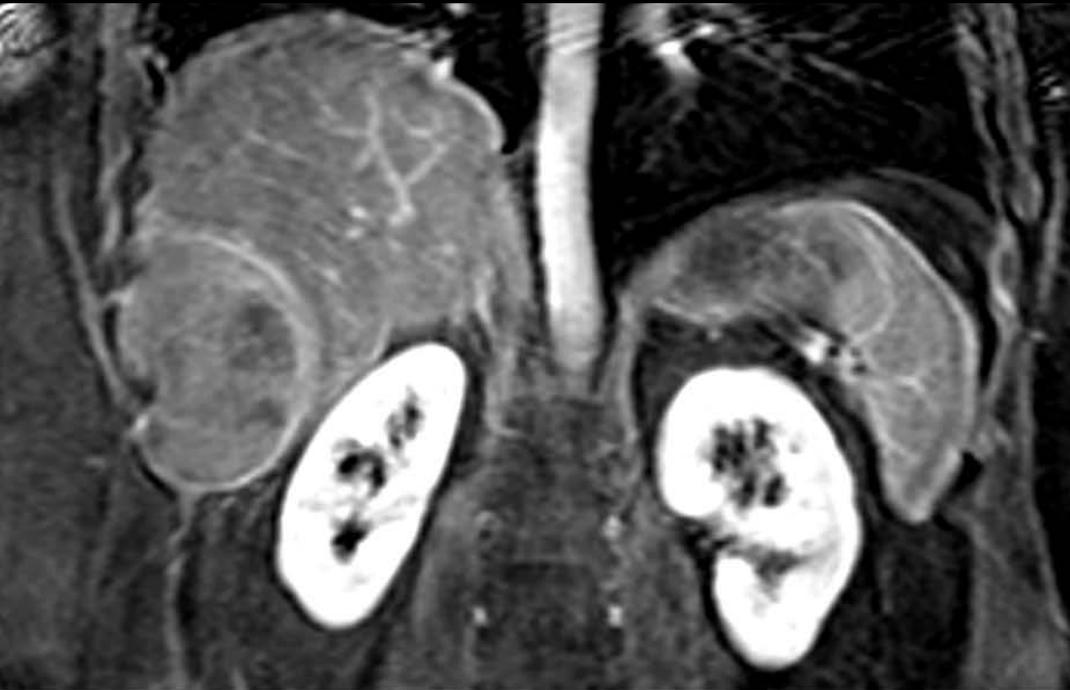
**M.C.
ESO-TCR
Synovial
sarcoma**



Pre



6 months



Pre-Treatment



6 Months

Treatment of Patients with Brain Metastases from Melanoma Using Cell Transfer Therapy

Patients: Metastatic melanoma; all with brain metastases ≤ 1 cm

Treatment:

Preparative regimen: Cyclophosphamide $60\text{mg}/\text{m}^2 \times 2\text{d}$
Fludarabine $25\text{mg}/\text{m}^2 \times 5\text{d}$

Cell Transfer: TIL ($3-10 \times 10^{10}$ cells)
TCR (MART or gp100) transduced cells ($0.4 - 7 \times 10^{10}$ cells)

IL-2: $720,000 \text{ IU}/\text{Kg}$ q 8h

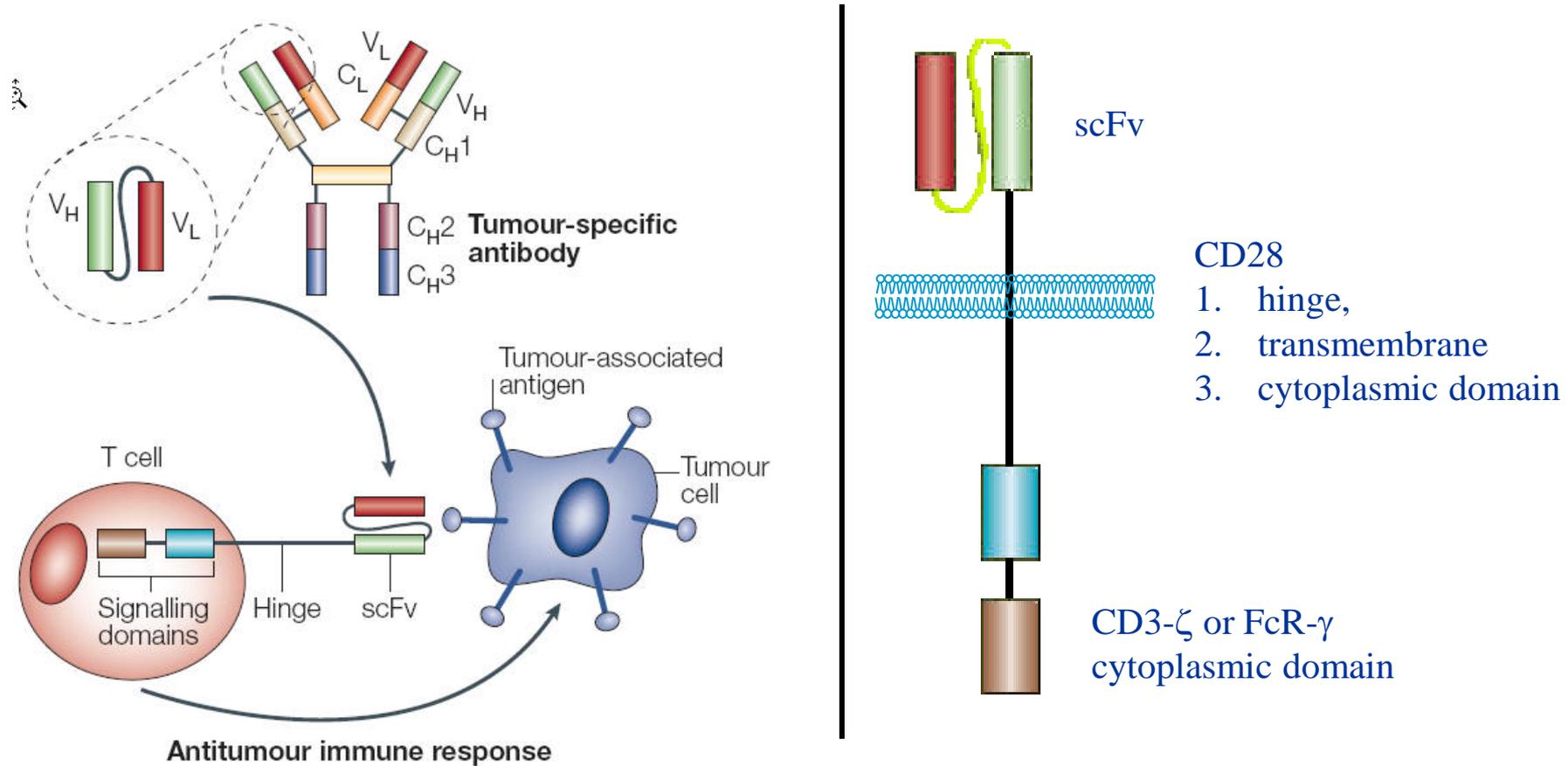
Results: TIL: 7/17 patients (41%) complete regression in brain
(duration: 43+, 26+, 14+, 5+, 4+, 6, 6 months)

TCR: 2/9 patients (22%) complete regression in brain
(duration: 25+, 8 months)

Toxicity: 1/26 patients had a subarachnoid hemorrhage – recovered well after surgery
(Hong et al. Clin Cancer Res. 10:4892, 2010)

Schematic representation of a typical chimeric receptor

Modified from Kershaw MH, Nature Review Immunology. 18 November, 2005



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.

As of 4/1/11 treatment with a chimeric antigen receptor that recognizes CD19 transduced autologous T cells, 5 of 7 (86%) evaluable patients with heavily pretreated NHL or CLL have experienced objective responses.

Patient 1: Pre-infusion INF-gamma ELISA

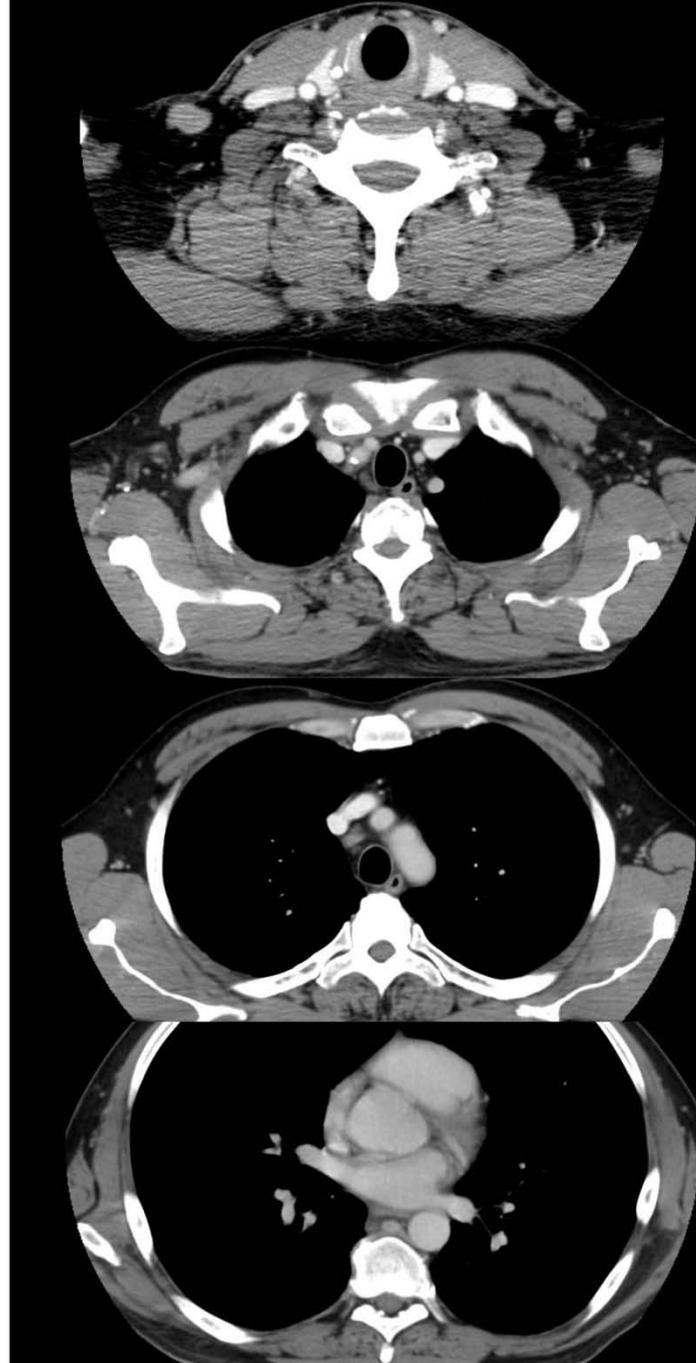
<u>Effector cells</u>	CD19-expressing targets			CD19-negative targets		<u>Effectors alone</u>
	<u>Toledo</u>	<u>Nalm6</u>	<u>CD19-K562</u>	<u>NGFR-K562</u>	<u>CCRL-CEM</u>	
Patient 1 anti-CD19 CAR-transduced	2180	4765	48050	581	193	110
Patient 1 Not transduced	63	70	59	66	66	31

R.K.

CD19 TCR



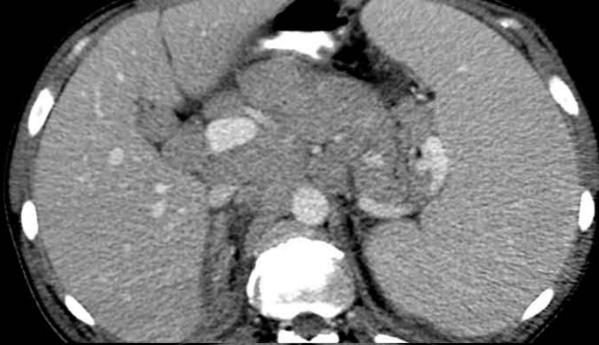
May 26, 2009



Oct 7, 2009

R.K.

**CD19
TCR**

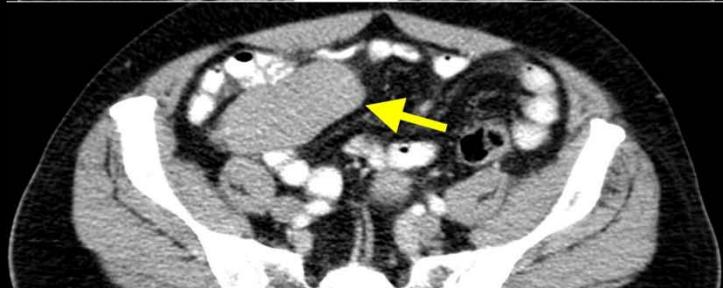
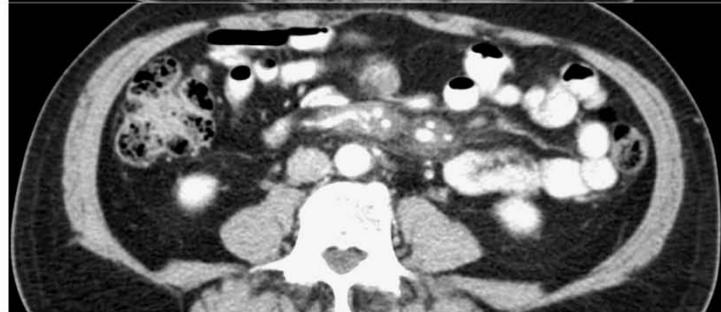
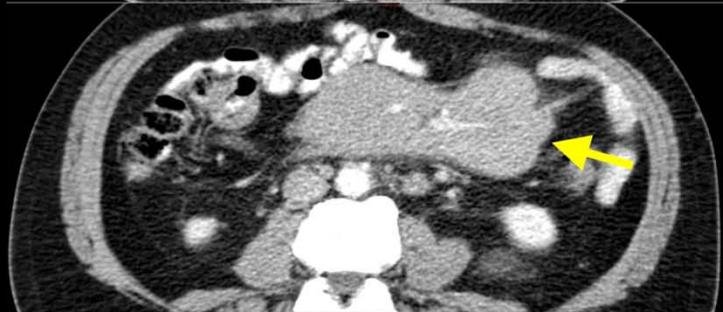
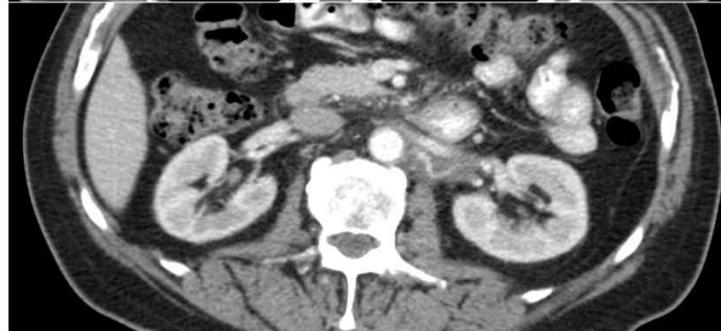
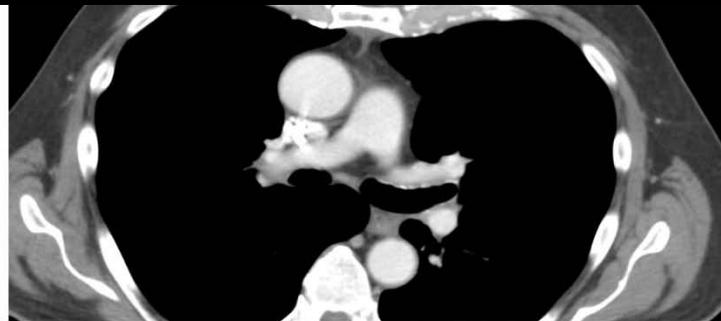
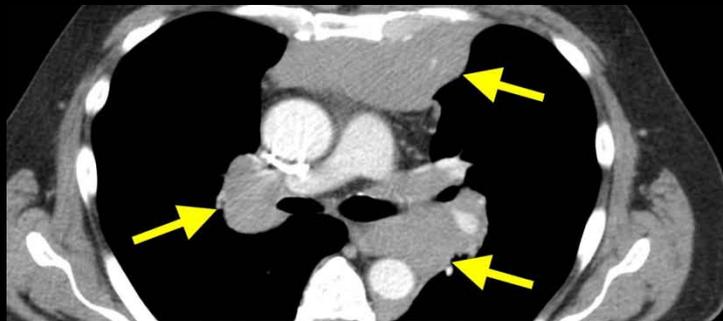


May 26, 2009



Oct 7, 2009

D.G. CD19 TCR Follicular lymphoma



Pre-Treatment

2 Months

Conclusion

Cell transfer immunotherapy can mediate the regression of metastatic cancer (including brain metastases) 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 other cancers.

Glioblastoma

About 10,500 cases/year in U.S.

Most aggressive form of glioma

Current standard of care includes resection, radiotherapy and chemotherapy

14.6 month median overall survival; few survive beyond 18 months

Following recurrence after standard care: 6 month progression-free survival is 7-10%; no cures

New therapeutic approaches needed

EGFRvIII in Glioblastoma

Epidermal growth factor receptor (EGFR)

170 Kda transmembrane tyrosine kinase

Activation of EFGR leads to cell proliferation, invasion, motility

EGFR activating mutation (EGFRvIII)

Expressed in 30-50% of glioblastomas

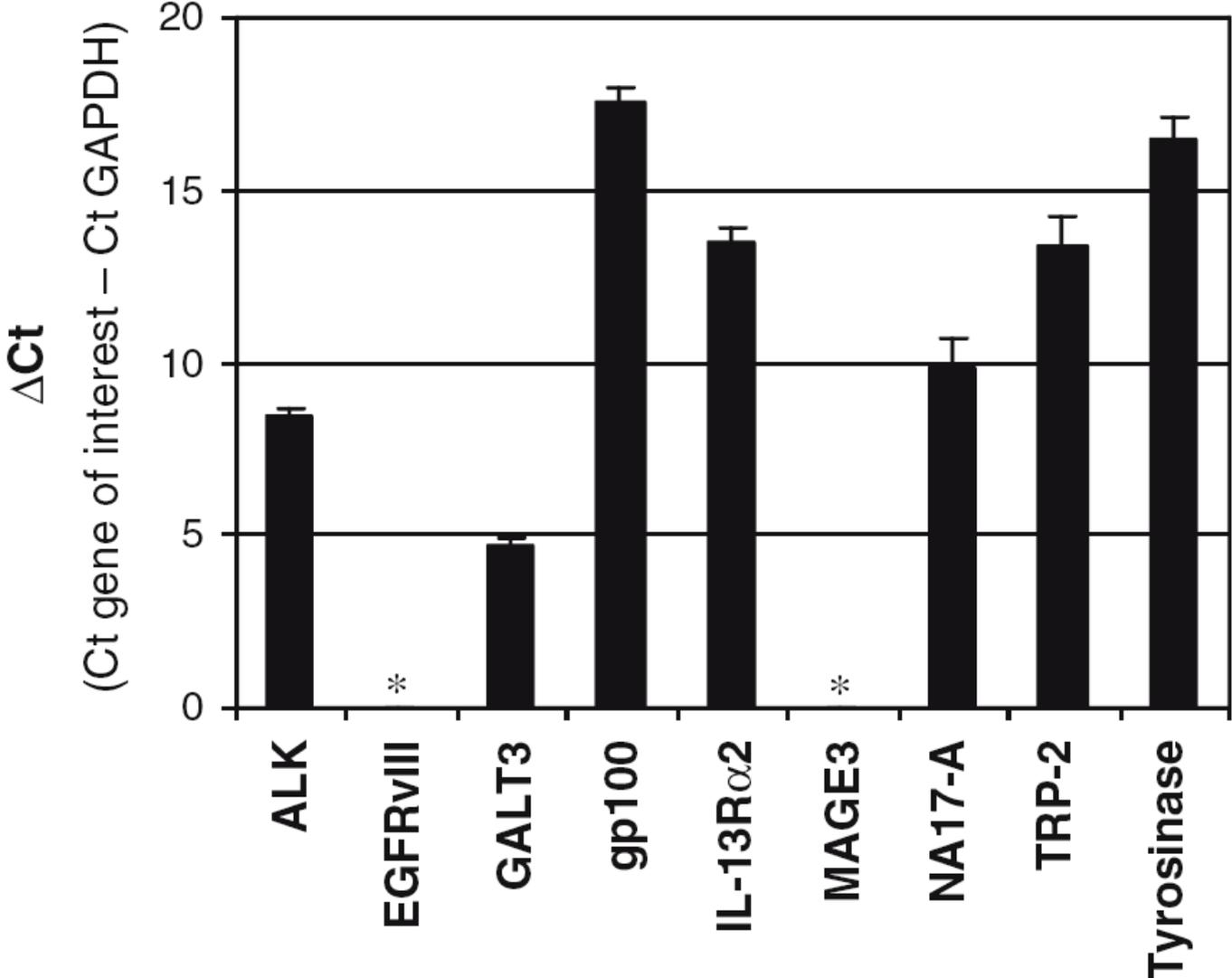
In frame deletion of exons 2-7 results in a truncated extracellular ligand binding domain

Results in constitutive activation of EGFR

Not expressed in any normal tissue

Monoclonal antibodies raised against EFGRvIII (do not have antitumor activity)

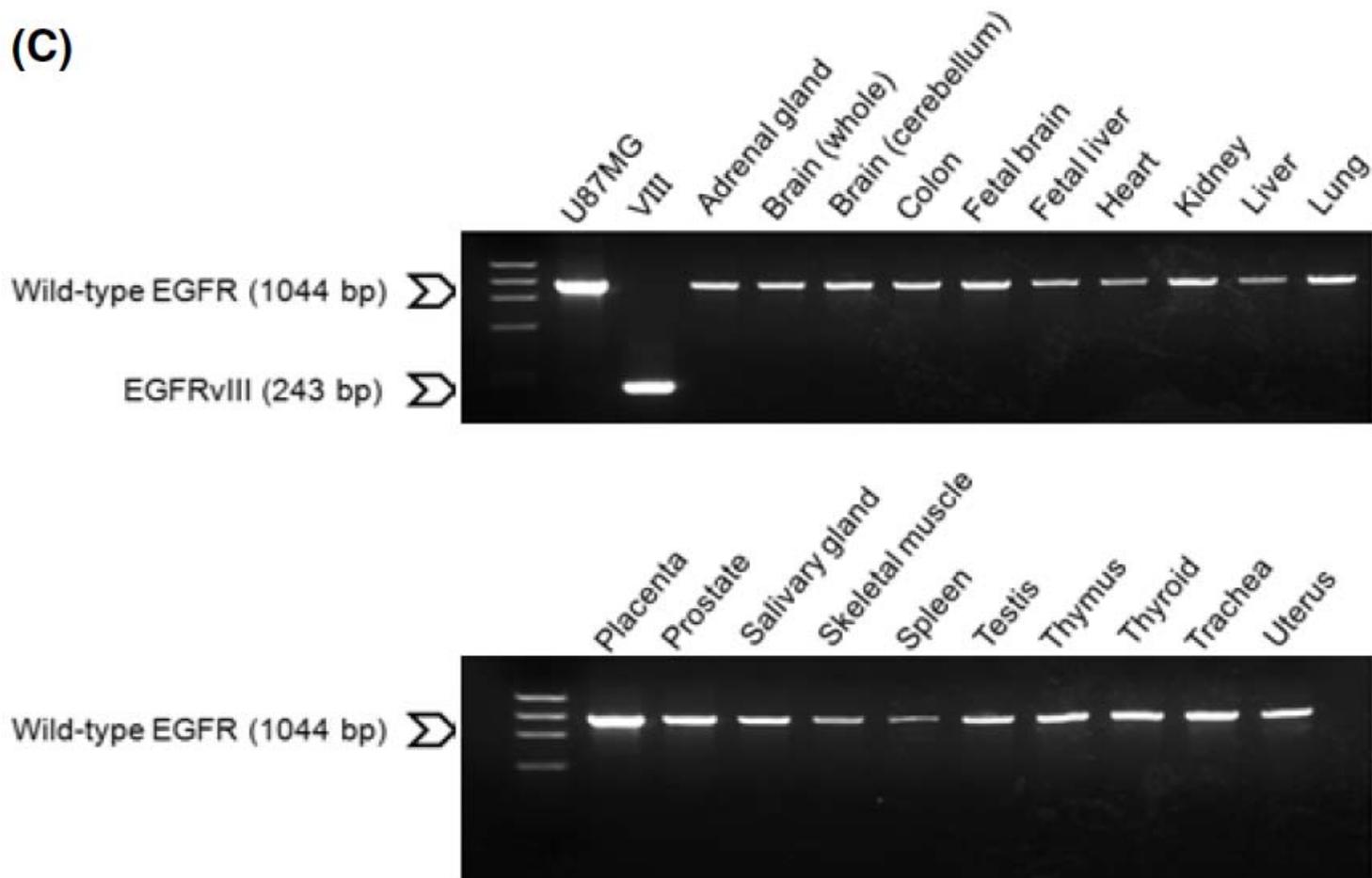
mRNA Expression in Normal Brain



(Saikali et al., J. Neurooncol (2007) 81:139-148.)

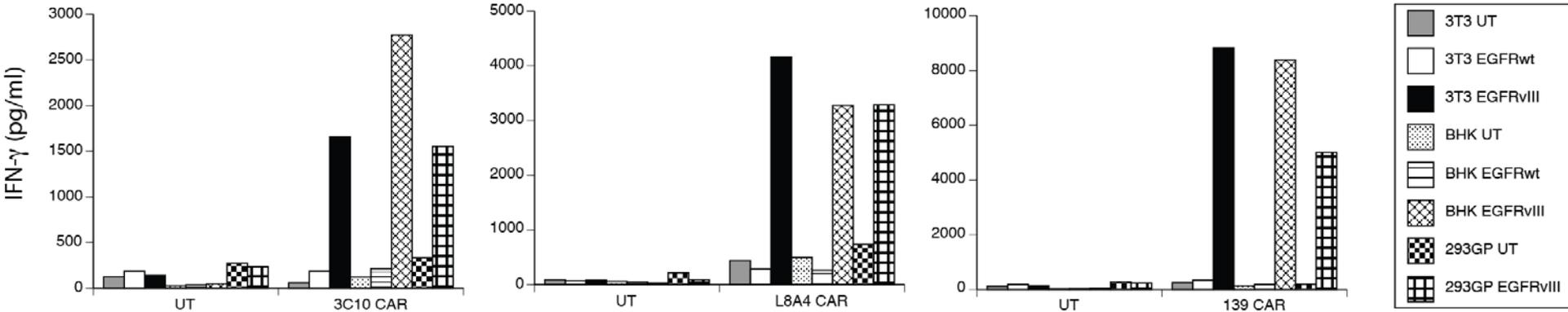
EGFRvIII Expression in Glioblastoma and Normal Tissues

(C)

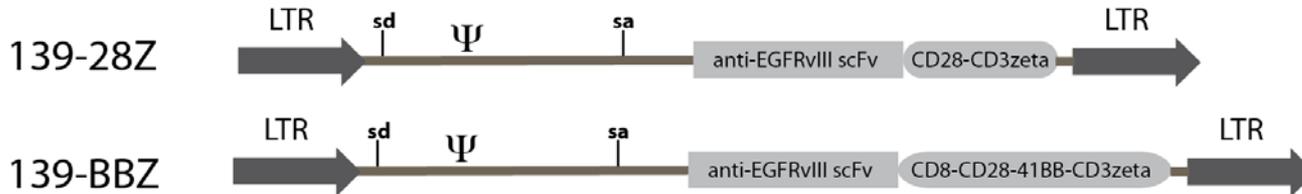


Functional analysis of anti-EGFRvIII CAR vectors

Effector cytokine release:



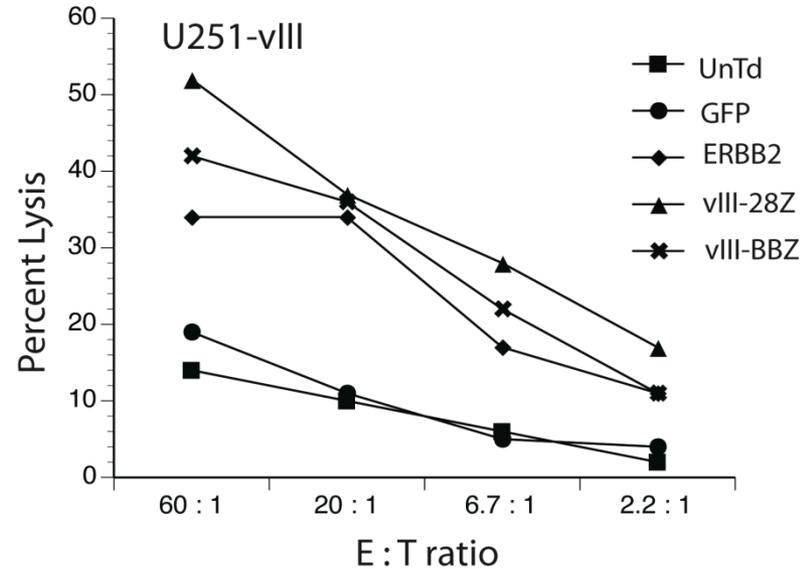
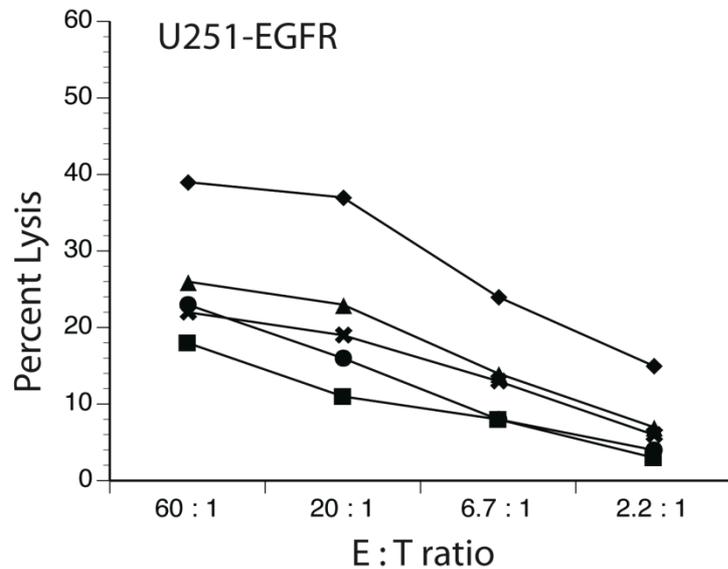
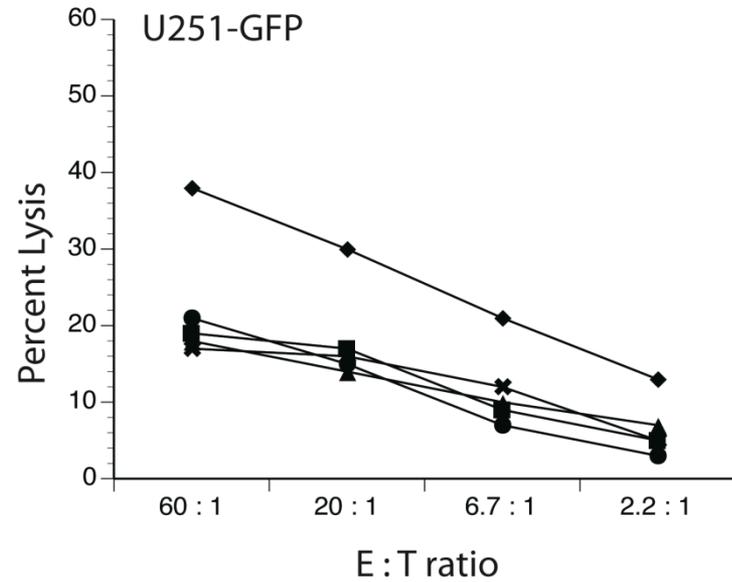
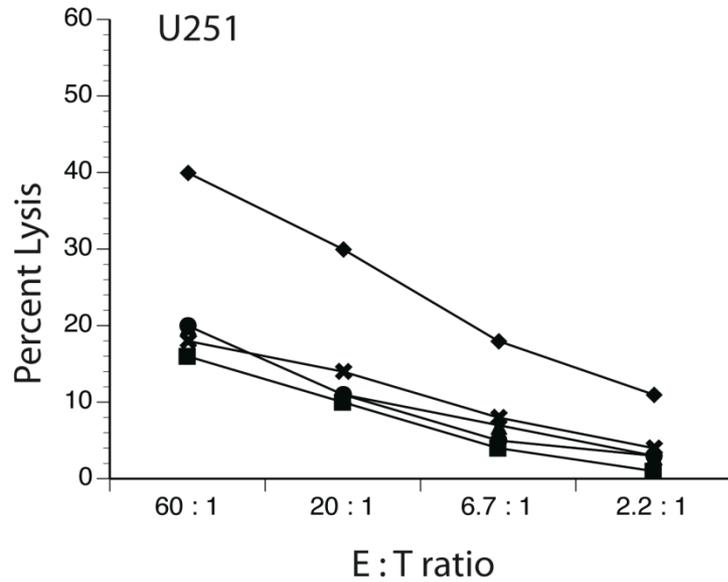
EGFRvIII CAR vectors based on human mAb 139:



Effector T-Cell	U87 cells (IFN-g, pg/ml)		
	GFP	EGFRwt	EGFRvIII
GFP	389	236	339
139-28Z	451	561	1797
139-BBZ	460	499	2117
ERBB2	1061	671	932

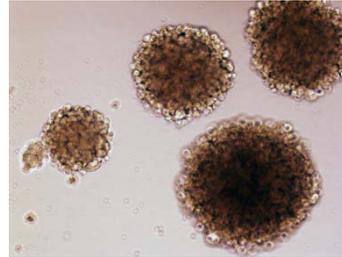
Effector T-Cell	U251 cells (IFN-g, pg/ml)		
	UT	EGFRwt	EGFRvIII
GFP	0	0	0
139-28Z	0	0	2743
139-BBZ	0	0	1820
ERBB2	1195	2201	2692

Specific lysis of U251 EGFRvIII by anti-EGFRvIII CAR transduced T cells

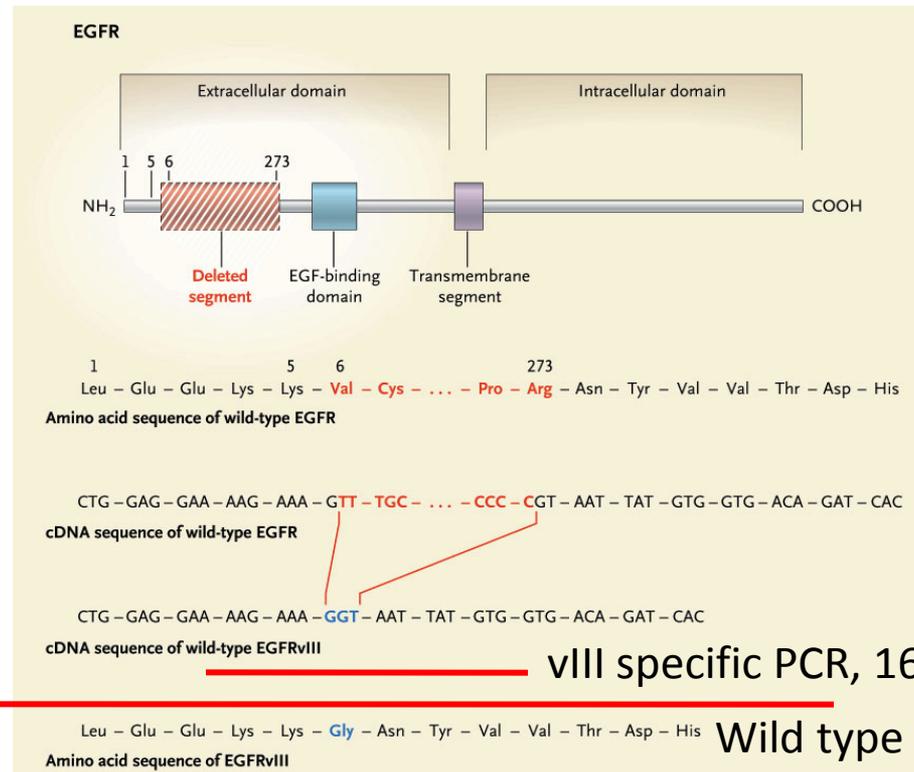


Testing for EGFRvIII expression in GBM-TSC lines

Neuro-Oncology Branch
GBM TSC lines, 308, 822, 1228



Develop PCR primers that span exons 1 and 8 and are vIII deletion specific:

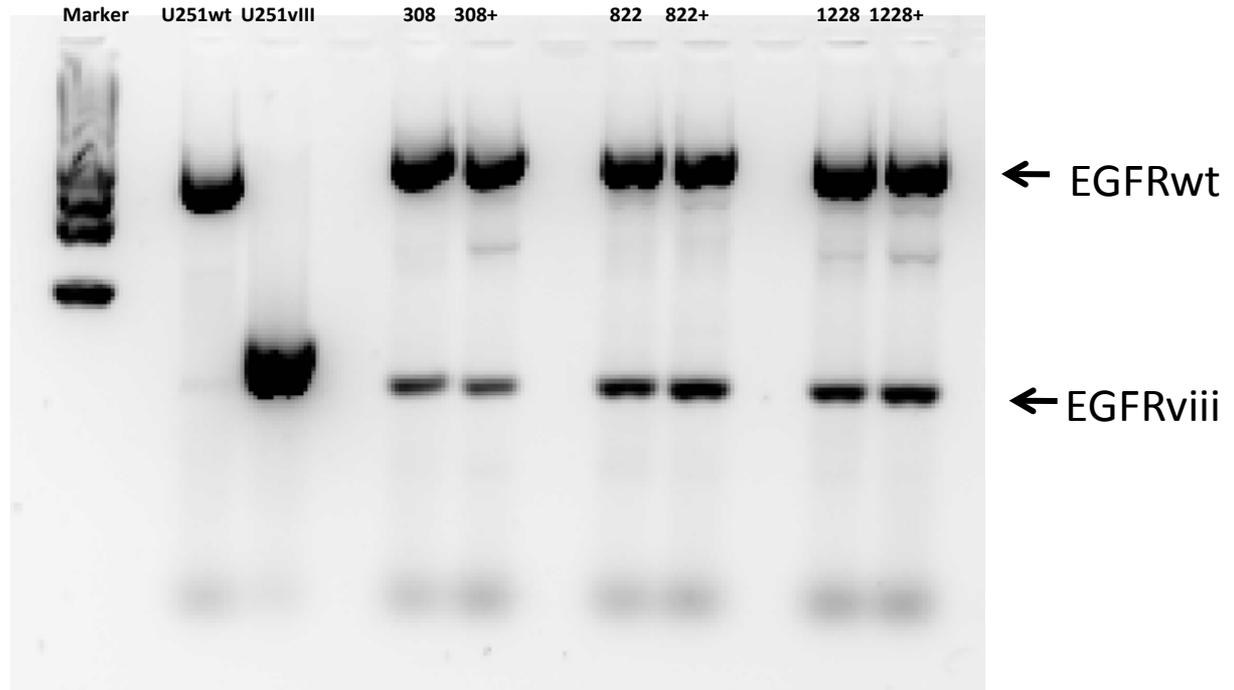


5'-GCTCCTGGCGCTGCTGGCTG-3'

Primers: E1F/E8R

5'-CCTTCGCACTTCTTACACTT-3'

Based on RT-PCR using EGFR wt and EGFRvIII shared primers, EGFRvIII is expressed in GBM-TSC lines.



RT-PCR using vIII specific primers E1F/E8R

Test of EGFRvIII CAR for recognition of GBM-TSC lines

Pt. 1

<u>Effector</u>	<u>media</u>	<u>U251-EGFR</u>	<u>U251-vIII</u>	<u>TSC 1228</u>	<u>TSC 308</u>	<u>308 + serum</u>	<u>TSC 822</u>	<u>822 + serum</u>
UnTd	0	0	0	47	34	0	100	0
GFP	0	0	0	28	34	33	30	2
ERBB2	0	<u>2186</u>	<u>2014</u>	<u>1170</u>	<u>1836</u>	<u>2369</u>	187	42
HMW-MAA	66	48	18	<u>640</u>	<u>470</u>	162	<u>817</u>	<u>1107</u>
EGFRvIII	37	16	<u>1821</u>	<u>729</u>	<u>473</u>	86	<u>918</u>	<u>471</u>

Pt. 2

<u>Effector</u>	<u>media</u>	<u>U251-EGFR</u>	<u>U251-vIII</u>	<u>TSC 1228</u>	<u>TSC 308</u>	<u>308 + serum</u>	<u>TSC 822</u>	<u>822 + serum</u>
UnTd	0	0	0	0	147	0	80	80
GFP	0	0	0	0	0	0	180	0
ERBB2	0	<u>4317</u>	<u>4128</u>	<u>2573</u>	<u>3308</u>	<u>4651</u>	<u>1067</u>	259
HMW-MAA	693	696	720	<u>1950</u>	<u>1807</u>	1385	<u>2786</u>	<u>3162</u>
EGFRvIII	384	331	<u>4523</u>	<u>3306</u>	<u>3351</u>	680	<u>4406</u>	<u>3153</u>

(IFN- γ pg/ml)

Test of 139-vIII-CAR PG13 producer cell clones F10 and H5 for recognition of EGFRvIII

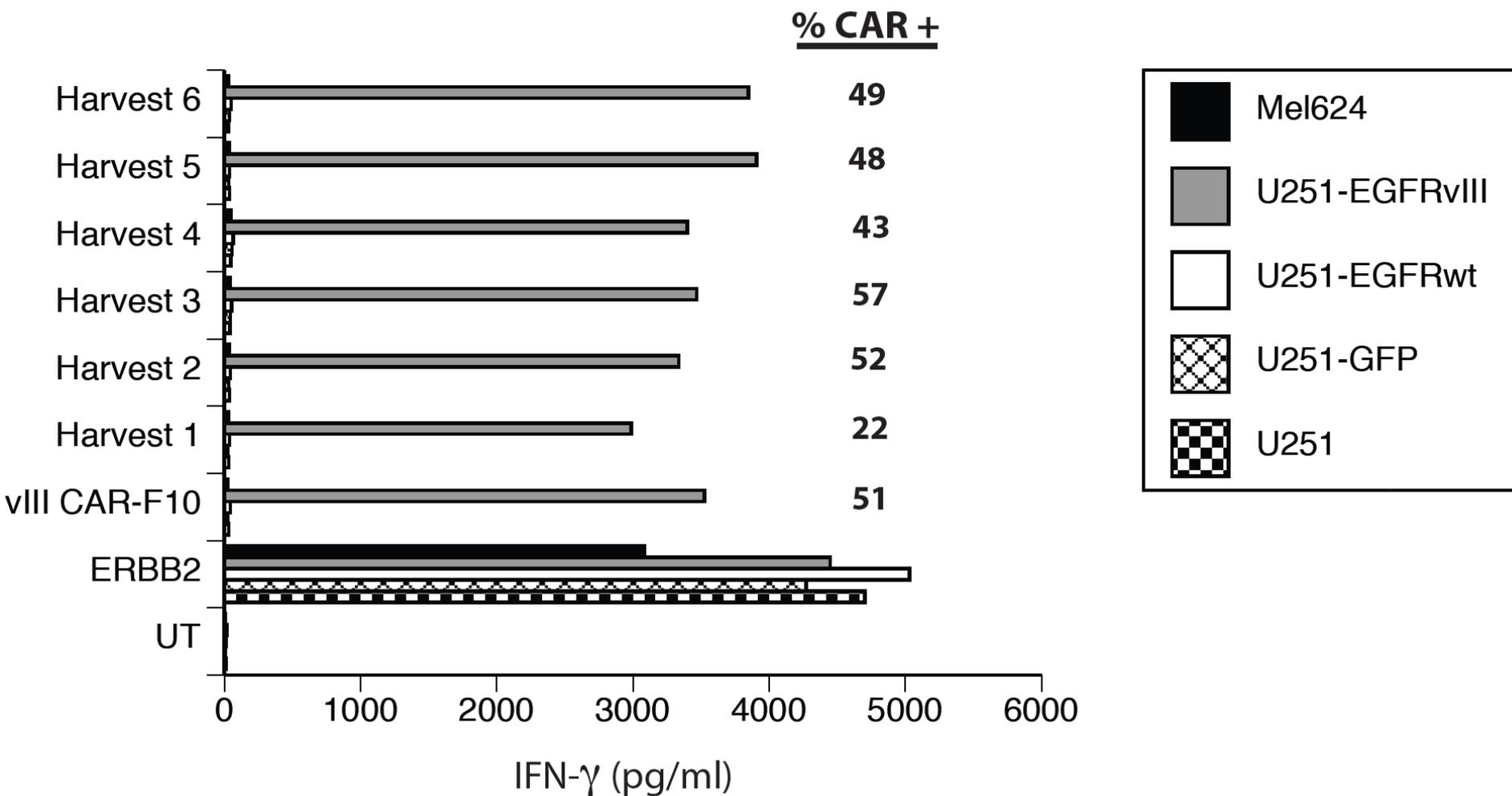
<u>Effector</u>	<u>media</u>	<u>U251-GFP</u>	<u>U251-EGFR</u>	<u>U251-vIII</u>	<u>TSC 308</u>
UnTd	0	21	9	0	200
clone F10	123	103	94	<u>6551</u>	<u>1724</u>
clone H5	6	46	20	<u>6890</u>	<u>921</u>

(IFN- γ pg/ml)

Clone F10 chosen for clinical production, safety testing in progress.

Test of anti-EGFRvIII CAR clinical vector supernatant

Activity of vector harvests:



Test of anti-EGFRvIII CAR clinical vector supernatant

Lack of recognition of normal cells: SAEC, human small airway epithelial cells

HPrEC, human prostate epithelial cells

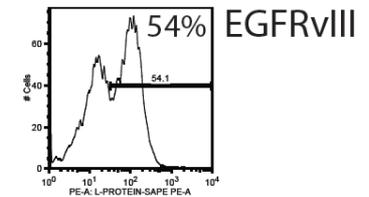
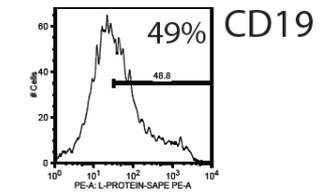
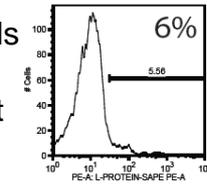
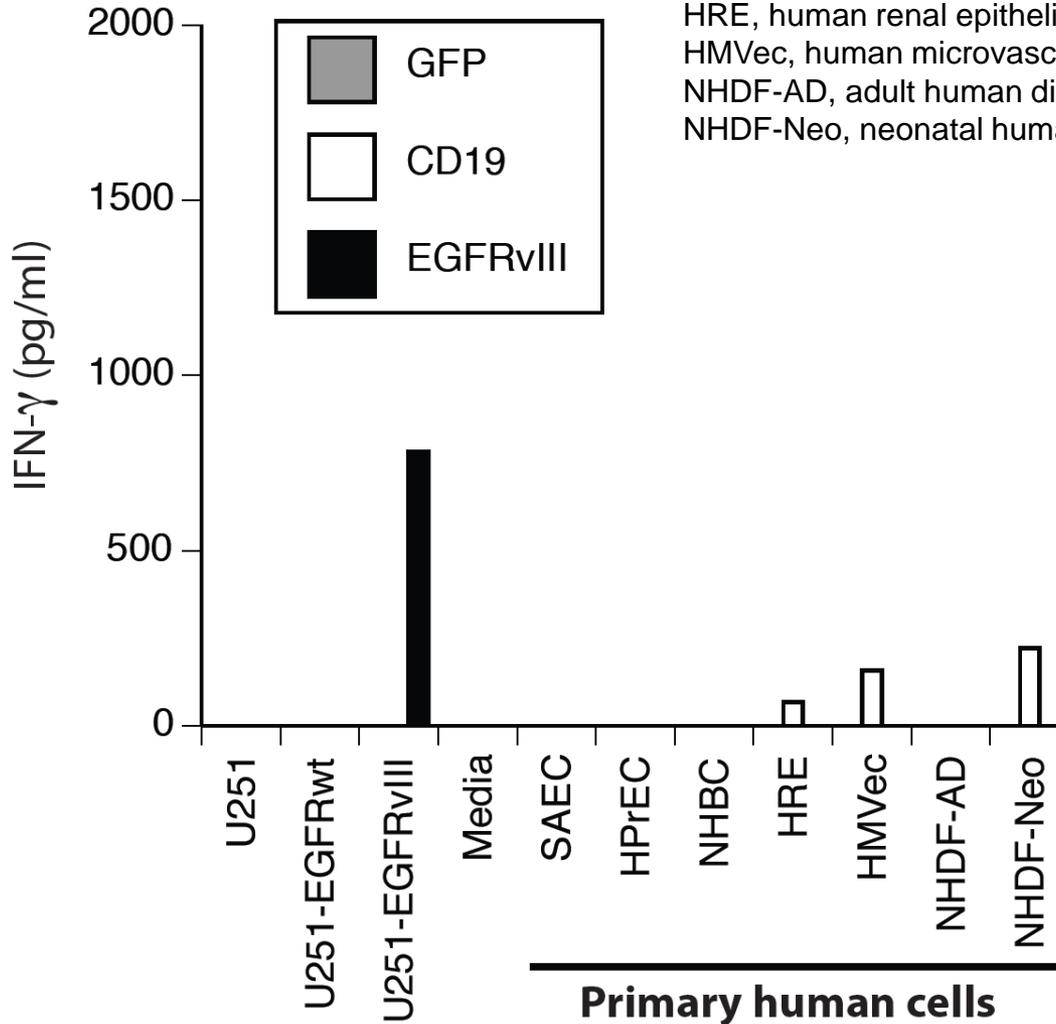
NHBC, normal human bronchial epithelial cells

HRE, human renal epithelial cells

HMVec, human microvascular endothelial cells

NHDF-AD, adult human diploid fibroblast

NHDF-Neo, neonatal human diploid fibroblast



CAR →

EGFRvIII 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

**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**

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

Objectives:

Primary Objectives

To evaluate the safety of the administration of anti-EGFRvIII CAR engineered peripheral blood lymphocytes in patients receiving the non-myeloablative conditioning regimen, and aldesleukin.

Determine the six month progression free survival of patients receiving anti-EGFRvIII CAR-engineered peripheral blood lymphocytes and aldesleukin following a non-myeloablative but lymphoid depleting preparative regimen.

Secondary objectives

Determine the in vivo survival of CAR gene-engineered cells.

Evaluate radiographic changes after treatment

Anti-EGFRvIII CAR to Treat Glioblastomas

Eligibility

Glioblastoma expresses EGFRvIII by RT-PCR or IHC
Progression of disease after radiotherapy
≥ 18 years old
Karnofsky score ≥ 60
Cardiac, pulmonary, laboratory parameters acceptable

Phase I

Two groups: Require steroids at start of treatment
No steroids required
Preparative regimen: Cyclophosphamide 60mg/kg x 2d
Fludarabine 25mg/m² x 5d
Cell dose escalation plus IL-2 (720,000 IU q 8h)

Phase III

At MTD treat patients in 2 groups:
Require steroids
No steroids

Phase I dose escalation

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)	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

(2 week delay after the first and last patient in each cohort before accruing the next patient)

Phase II

At MTD 32 patients will be treated in each of 2 cohorts:

- a) receiving steroids**
- b) no steroids**

Objective: Determine if 30% 6-month PFS can be achieved compared to 10% in historical controls (94% power to detect the difference between 10 and 30% at 0.1 one-sided significance)

MRI brain imaging will also be performed monthly

Persistence of the transferred cells will be determined monthly