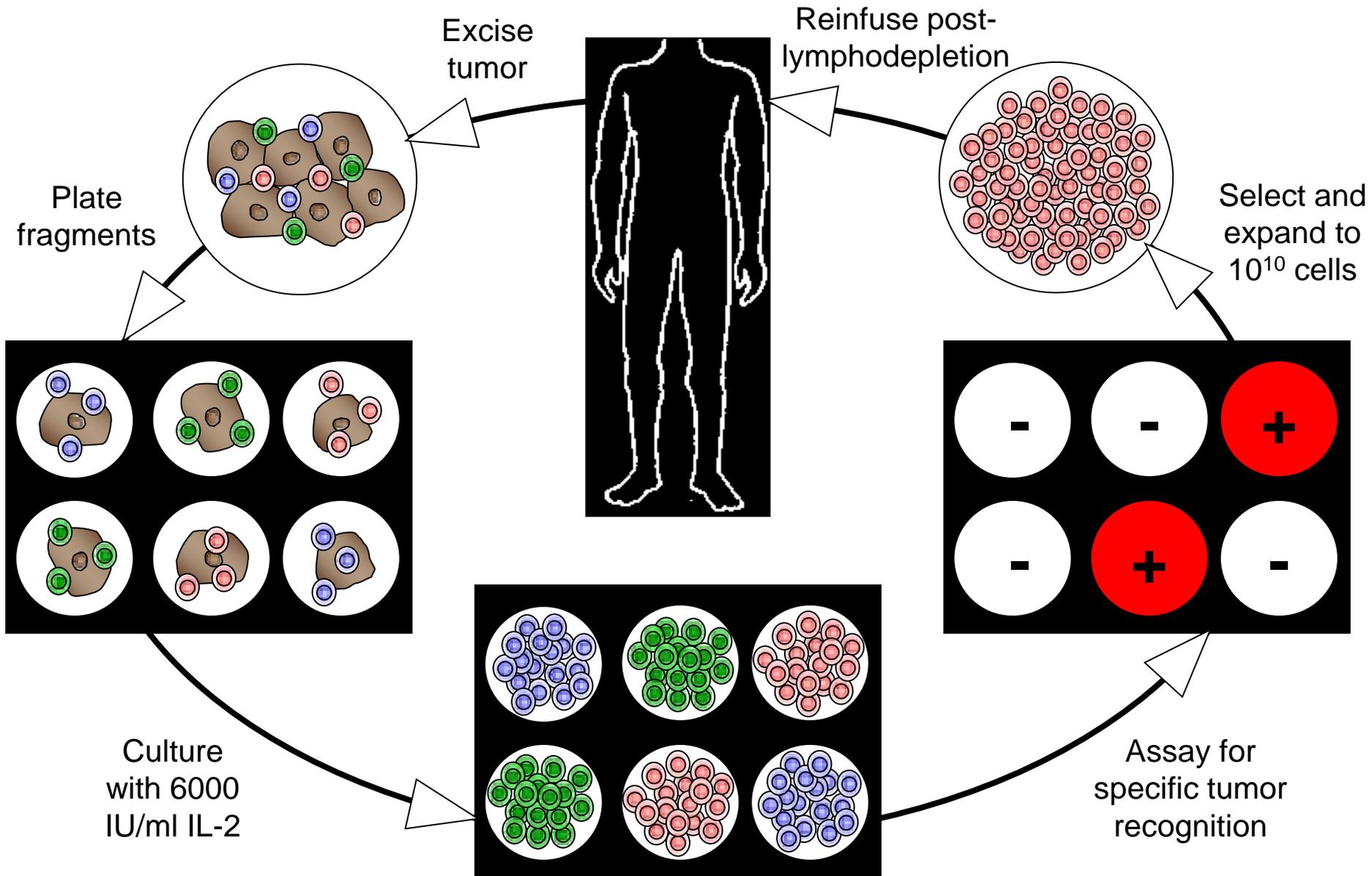


Adoptive transfer of tumor infiltrating lymphocytes (TIL)



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

Cell Transfer Therapy

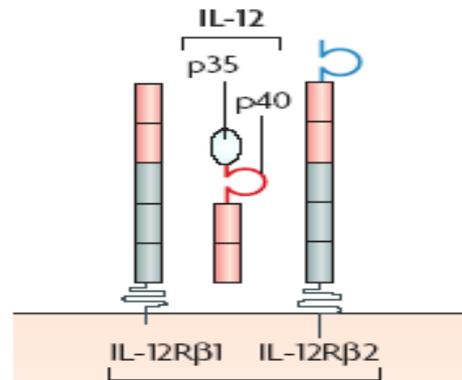
(5/1/10)

Treatment	Total	PR	CR	OR (%)
No TBI	43	16 (84, 36, 29, 28, 14, 13, 11, 8 8, 7, 4, 3, 3, 2, 2, 2)	5 (82+, 78+, 76+, 75+, 61+)	21 (49%)
200 TBI	25	10 (57+, 51+, 14, 9 6, 6, 5, 4 3, 3)	3 (65+, 61+, 54+)	13 (52%)
1200TBI	25	10 (42+, 35+, 21, 13, 7, 6, 6, 5 3, 2)	8 (45+, 41+, 41+, 36+, 35+, 35+, 34+, 19)	18(72%)

(52 responding patients: 42 had prior IL-2, 21 had prior IL-2 + chemotherapy)

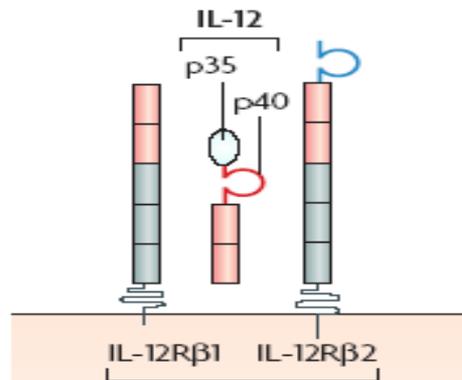
(16 complete responses: 15 ongoing at 34 to 82 months)

Interleukin-12



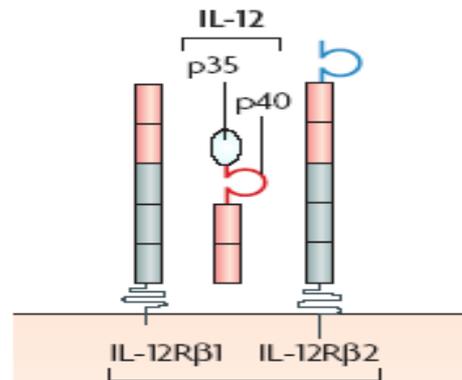
- **Discovered by Dr. Giorgio Trinchieri in 1989:**
 - novel protein/cytokine with multiple biological effects
 - Natural Killer Cell Stimulatory Factor (NKSF)
- **Two sub-units: p35 and p40.**
- **Coordinated production of the two chains lead to the secretion of the biologically active p70**

Interleukin-12



- Central role in both the innate and adaptive immune system
- Produced by dendritic cells, macrophages, and neutrophils
- Activates effector cells: CD4+, CD8+, and NK cells
- Reported to reverse functional anergy of tumor infiltrating lymphocytes
- Implicated in the generation of memory T cells

Interleukin-12

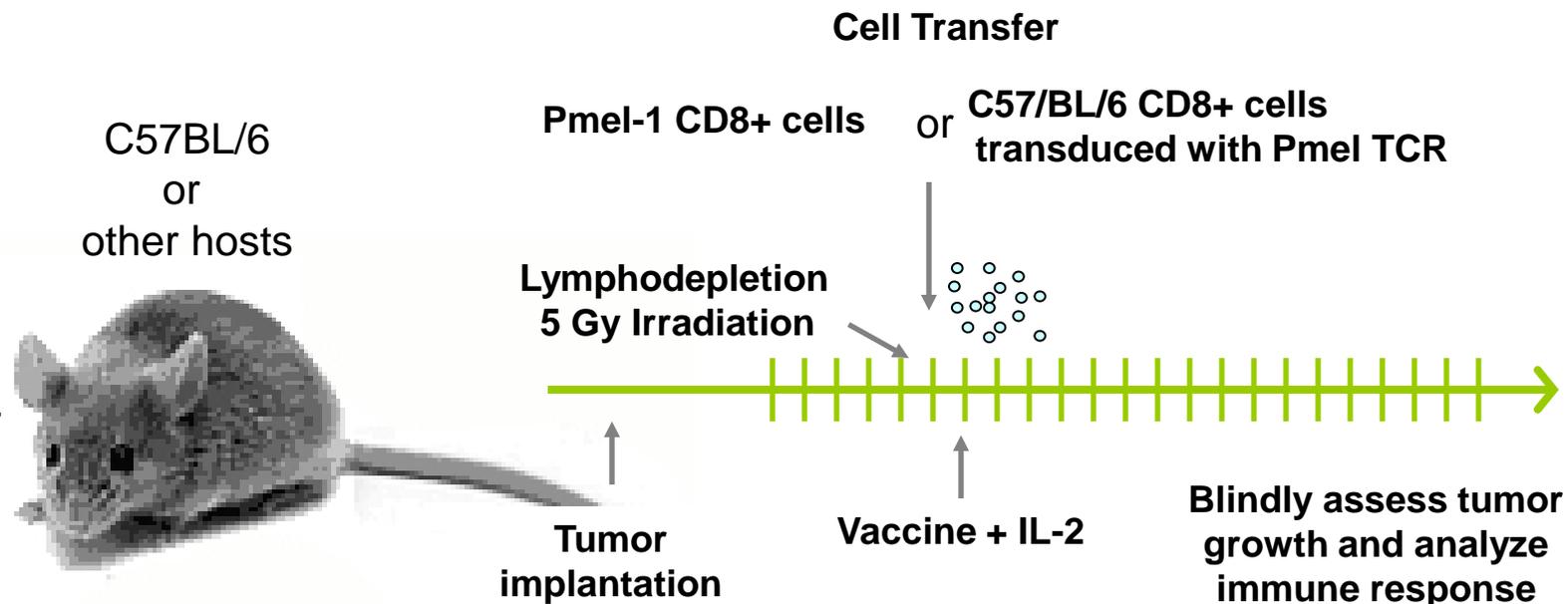


- **Several clinical trials have investigated the use of systemic IL-12 in advanced solid tumors and hematologic malignancies**
- **Objective response rates ranged from 0% - 11%**
 - **except trials treating cutaneous T-cell lymphoma with higher response rates**

Objective of Preclinical Studies

- **To determine whether the local delivery of IL-12 to the tumor site by antigen specific T-cells genetically engineered to produce IL-12 can enhance adoptive cell transfer treatments**

Mouse B16/Melanoma Model of Adoptive Cell Transfer

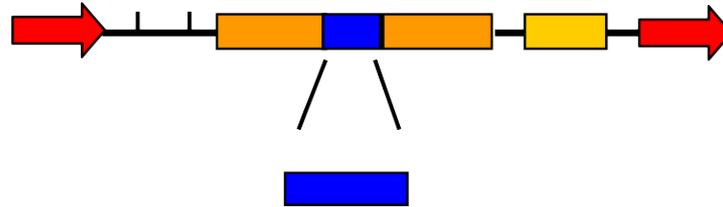


A tripartite regimen is effective:

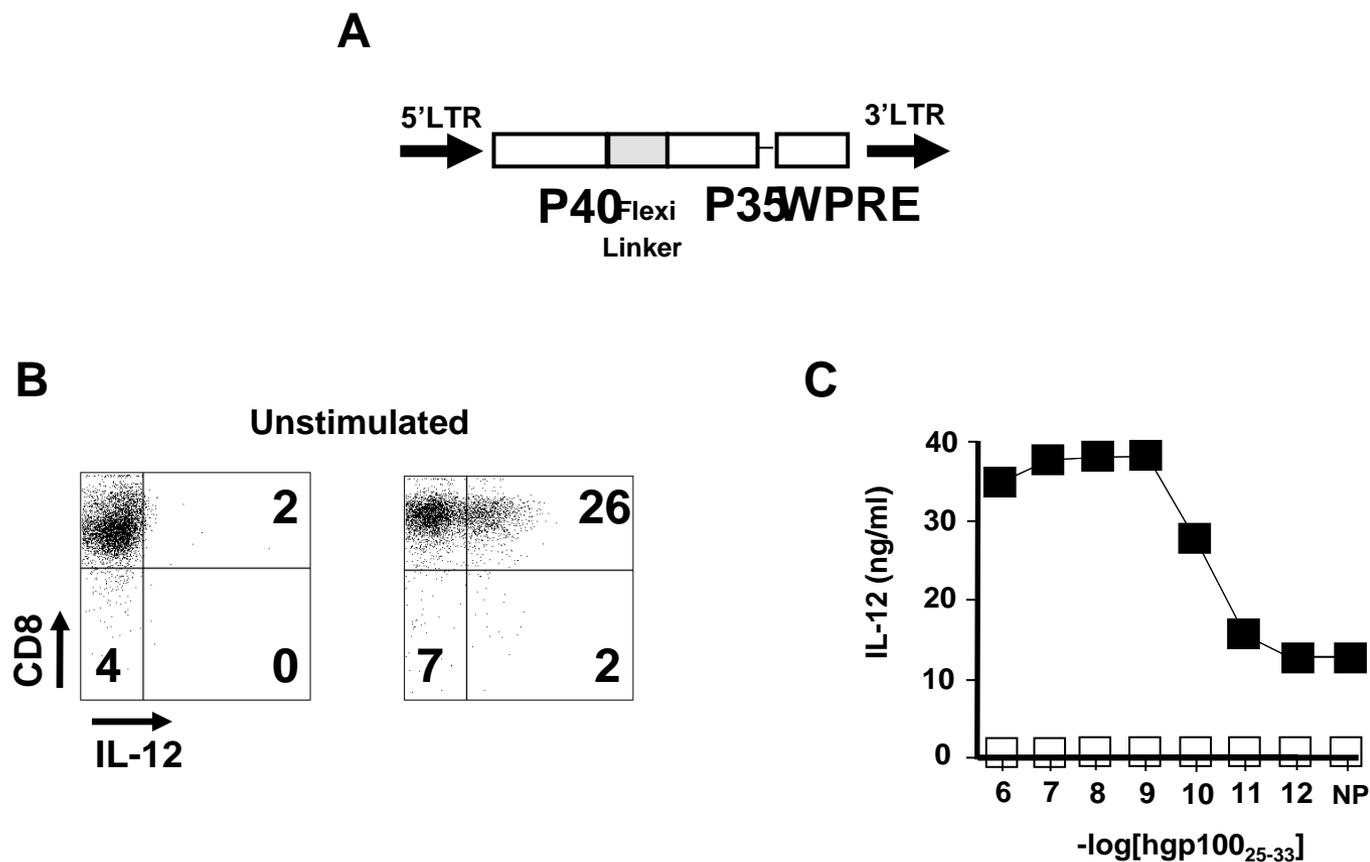
- i) 1 million Pmel-1 CD8+ cells
- ii) 2×10^7 plaque-forming units of rVV encoding hgp100
- iii) administration of high-dose IL-2: 600,000 IU BID for 3 days

(Overwijk, JEM, 2003)

Tumor specific Pmel CD8+ cells can be genetically engineered to secrete IL-12

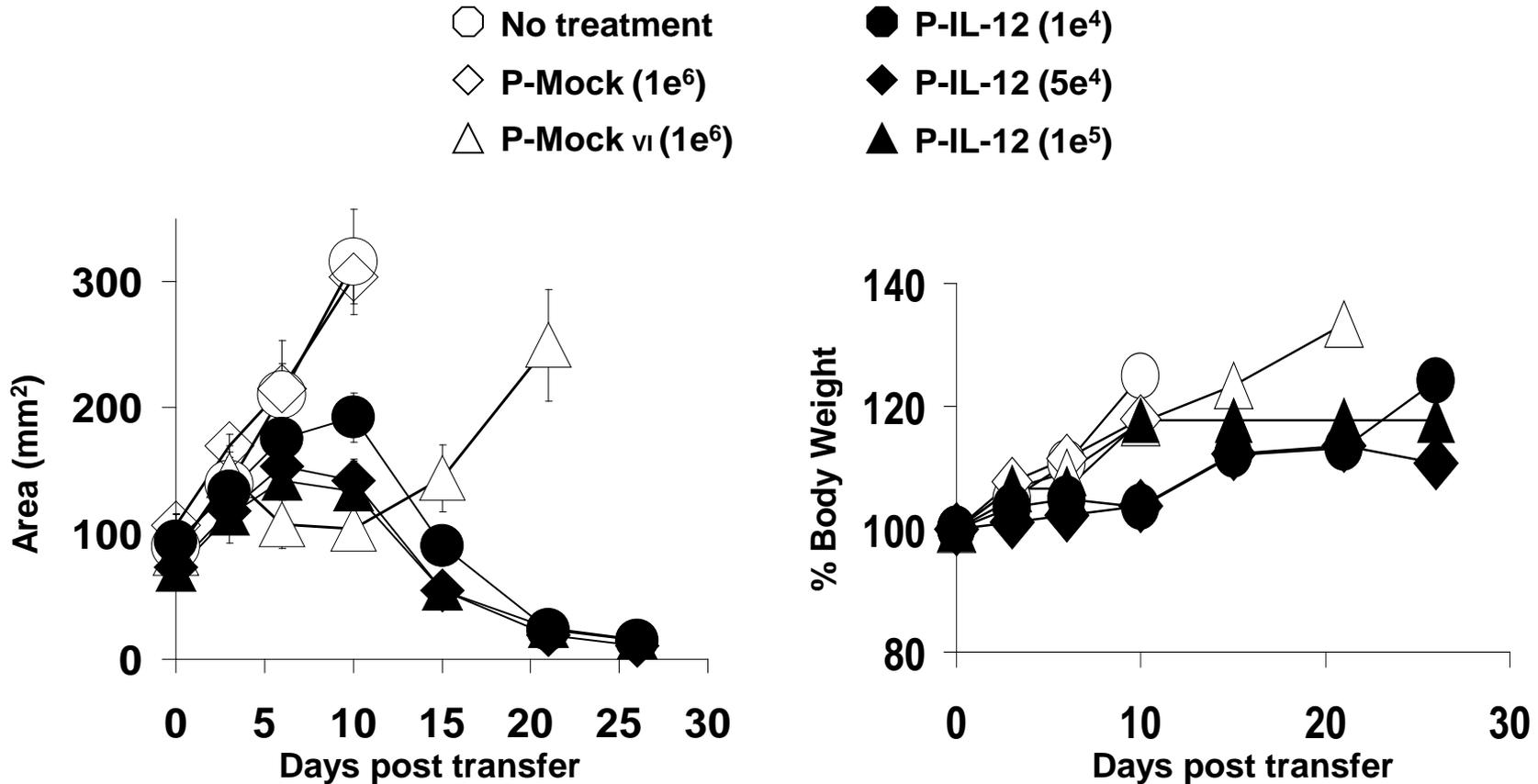


Tumor specific Pmel CD8+ cells can be genetically engineered to secrete IL-12



- The IL-12 vector with the LTR constitutive promoter, leads to high levels of basal IL-12 production

Small Numbers of Pmel-IL-12 cells cause regression of large established tumors



- 10,000 Pmel-IL-12 cells can cause regression of large established B16 melanomas without the need for IL-2 or vaccine
- Transferring few numbers of cells did not lead to any gross toxicity or weight loss

Example of A Complete Response

No Treatment

B16
Melanoma



Day 0

Day 14

Regressing Lesion

100,000 Pmel-IL-12

B16
Melanoma



Day 0

Day 4

Day 9

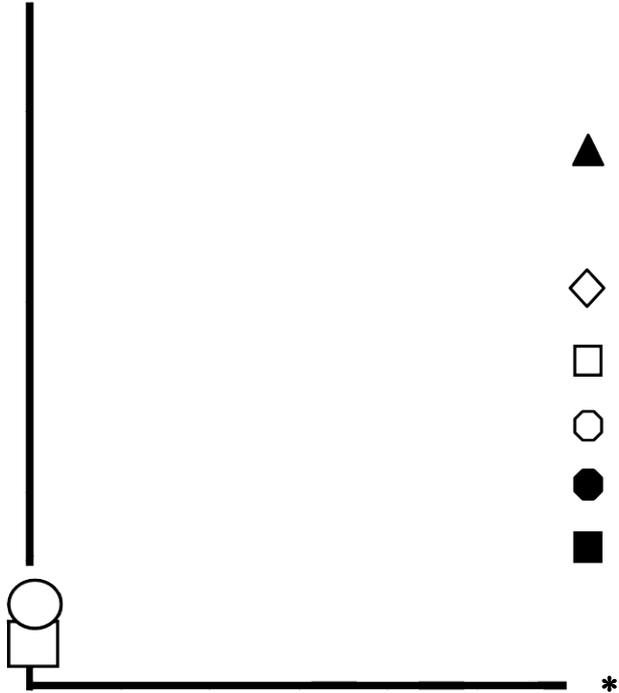
Day 14

Day 18

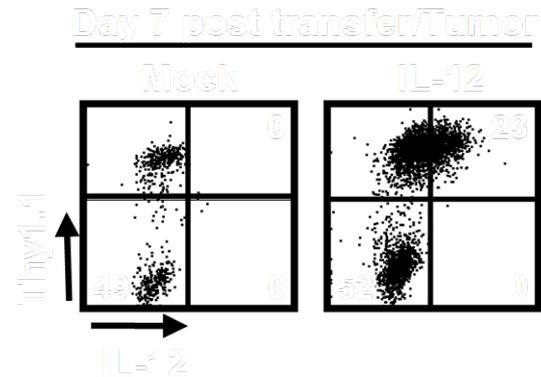
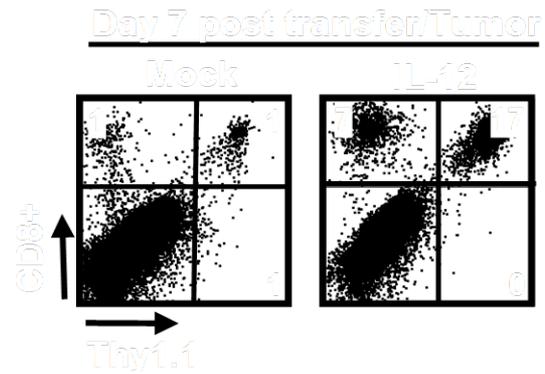
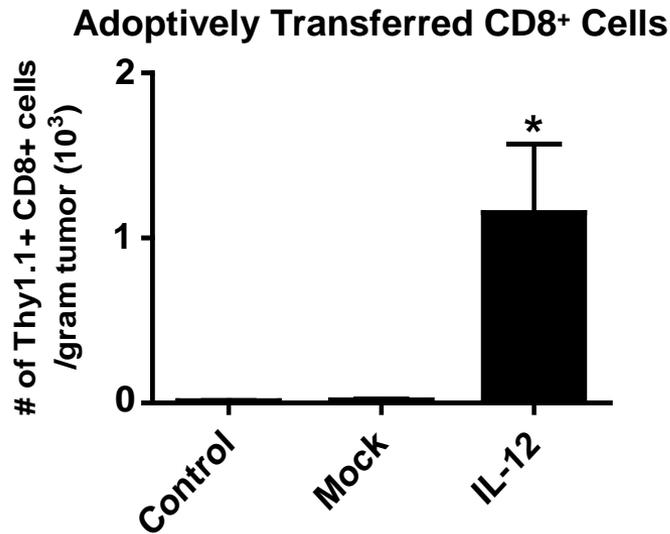
Day 21

Complete
Response

IL-12 engineered pmel-1 CD8+ T cells display enhanced anti-tumor responses compared to rIL-12 provided exogenously



Adoptive transfer of pmel IL-12^{TD} cells leads to increased infiltration of transferred cells that stably produce IL-12 directly at the tumor site

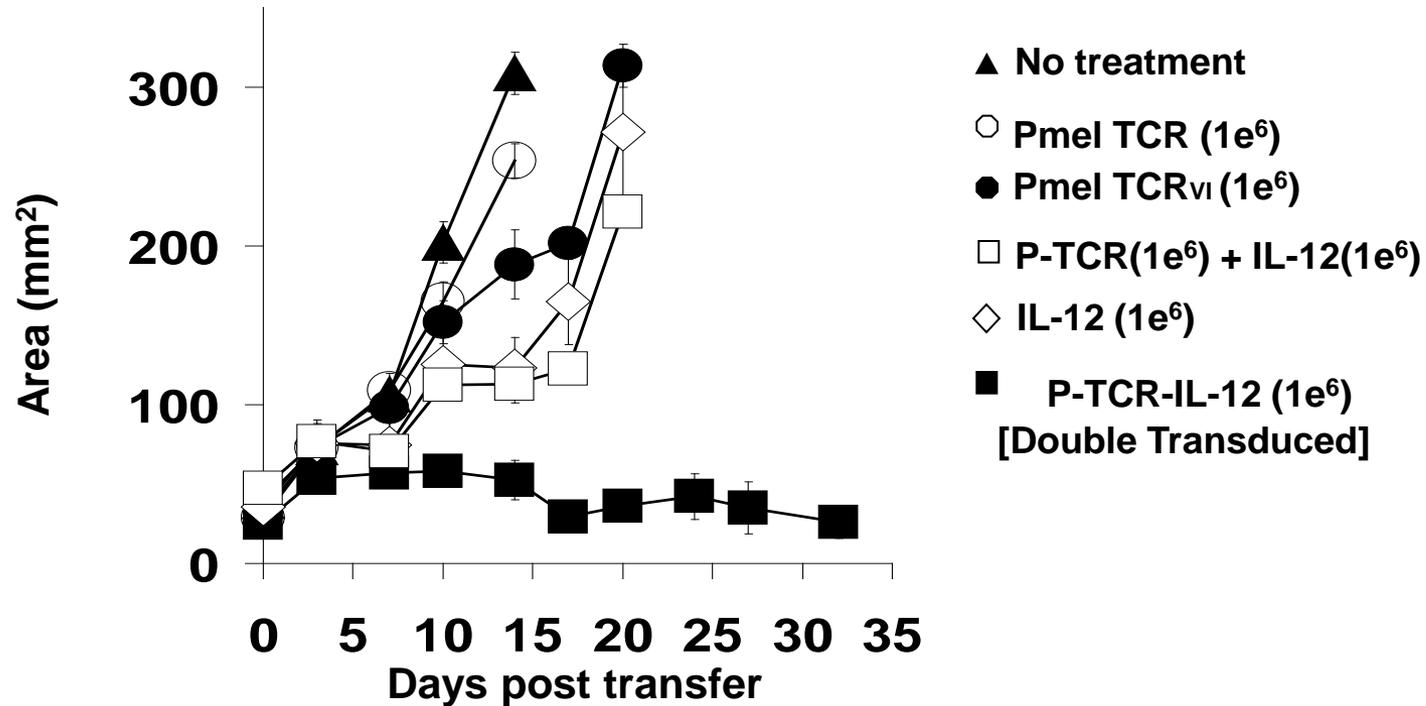


Summary : Cell Transfer Experiments with Pmel-IL-12

- **Small numbers of Pmel-IL-12 cells ($1e^4$) can cause regression of large established B16 melanomas without the need for IL-2 or vaccine**

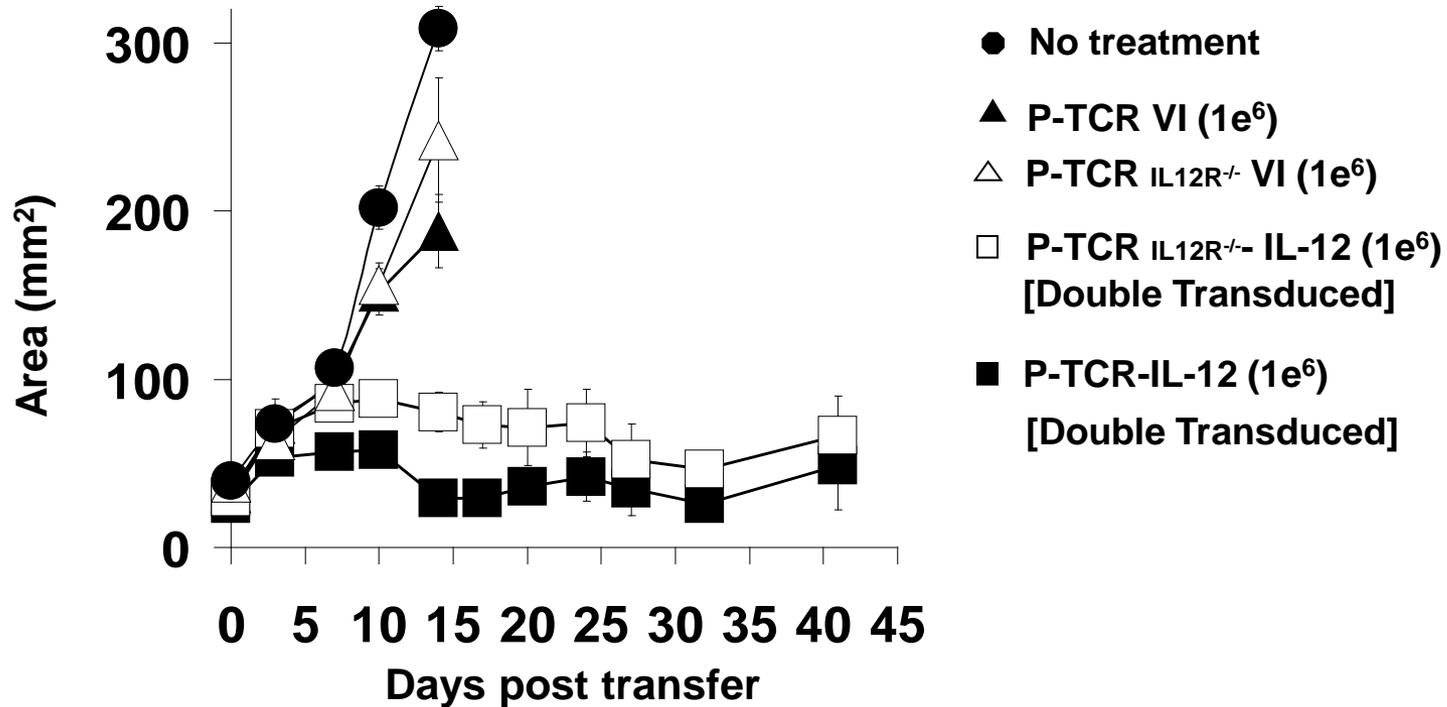
- **Pmel-IL-12 cells treat much more effectively than Pmel cells polarized by IL-12 or Pmel cells with exogenous systemic IL-12 (with no vaccine and no IL-2)**

Pmel TCR-IL-12 Double Transduced Cells Lead to Tumor Response



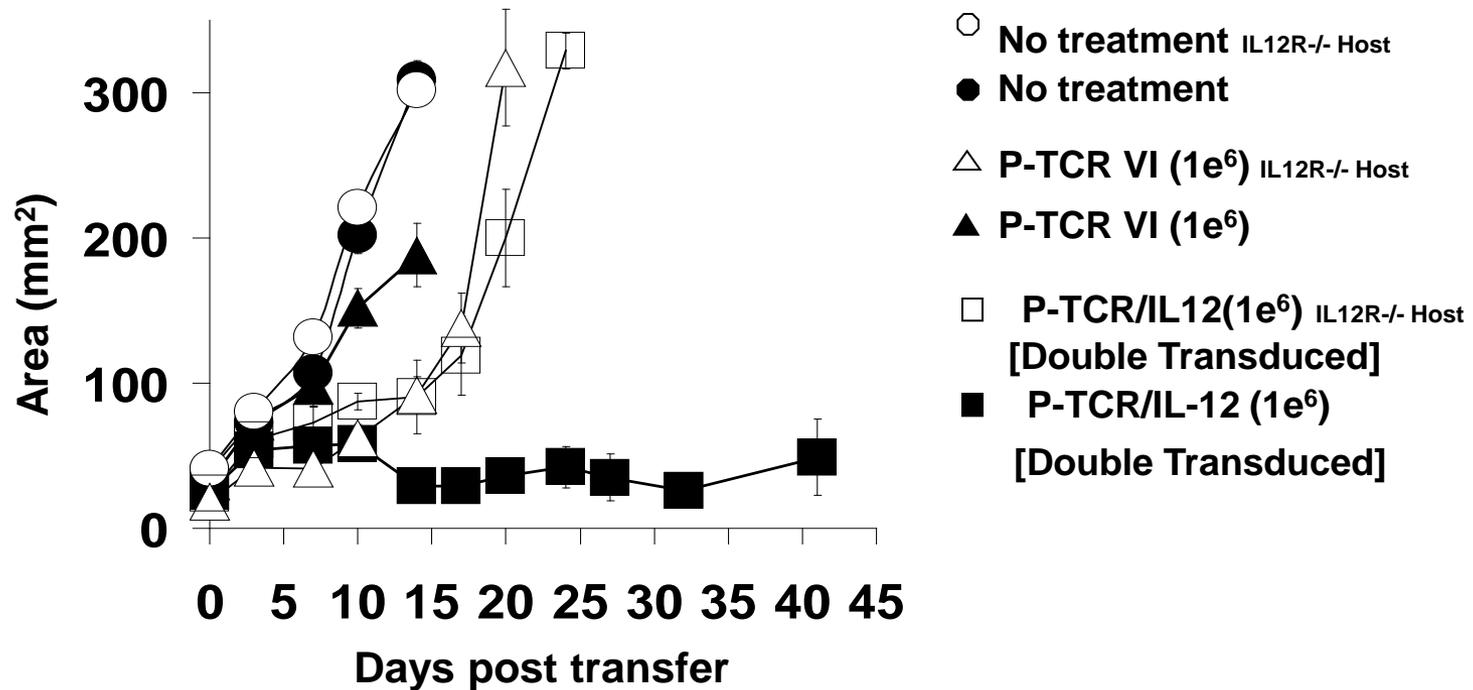
- Open Repertoire C57/B6 CD8⁺ cells double transduced with Pmel TCR and IL-12 can effectively treat B16 melanomas

PmelIL12R^{-/-} TCR-IL-12 Double Transduced Cells Also Leads to Tumor Response



- Open Repertoire IL-12R β 2^{-/-} CD8⁺ cells double transduced with Pmel TCR and IL-12 can also treat B16 melanomas similarly to wild type double transduced cells

Pmel TCR-IL-12 Double Transduced Cells fail to treat tumors on IL-12R β 2^{-/-} Mice



- CD8⁺ cells double transduced with Pmel TCR and IL-12 fail to treat tumors in mice unable to respond to IL-12

Summary: Pmel TCR Experiments

- **Open repertoire murine CD8+ cells can be double transduced to express both the Pmel TCR and IL-12.**
- **Only double transduced CD8+ cells can cause regression of B16 melanomas. The TCR and the IL-12 must be on the same cell.**
- **Double transduced IL-12 Receptor Knockout CD-8+ T-cells can also effectively treat tumors.**
- **Double transduced CD8+ cells can not mediate tumor regression in a host that can not respond to the secreted IL-12.**

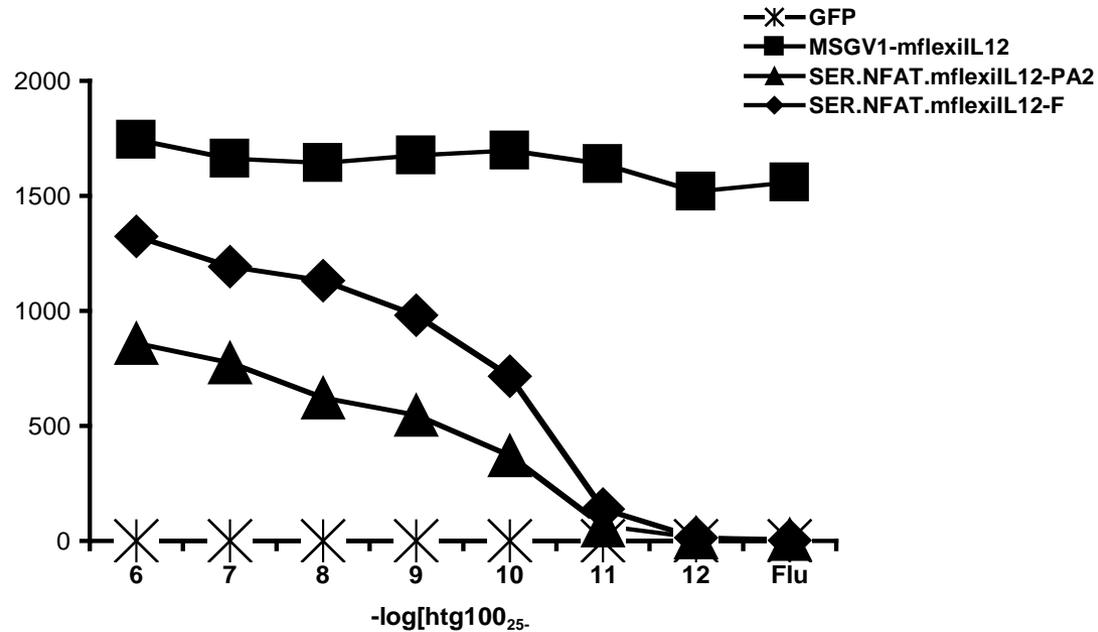
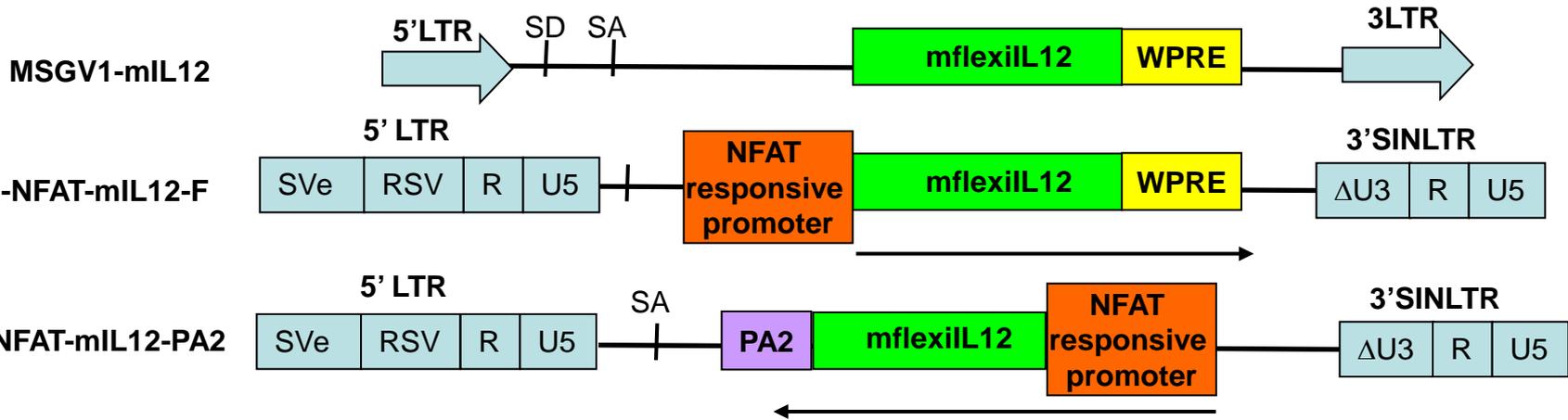
NFAT Inducible Vector to Mediate IL-12 Production in Tumor Microenvironment

Nuclear factor of activated T cells (NFAT) transcription factors

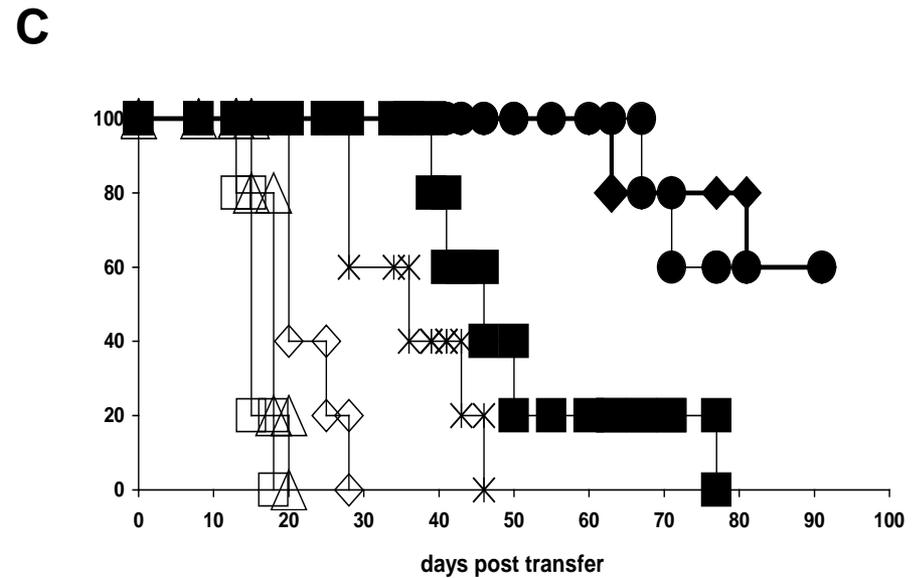
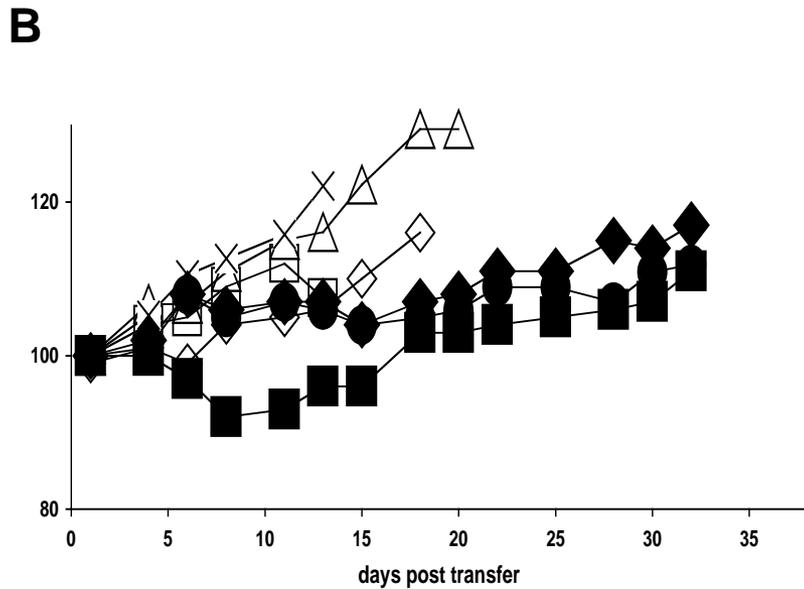
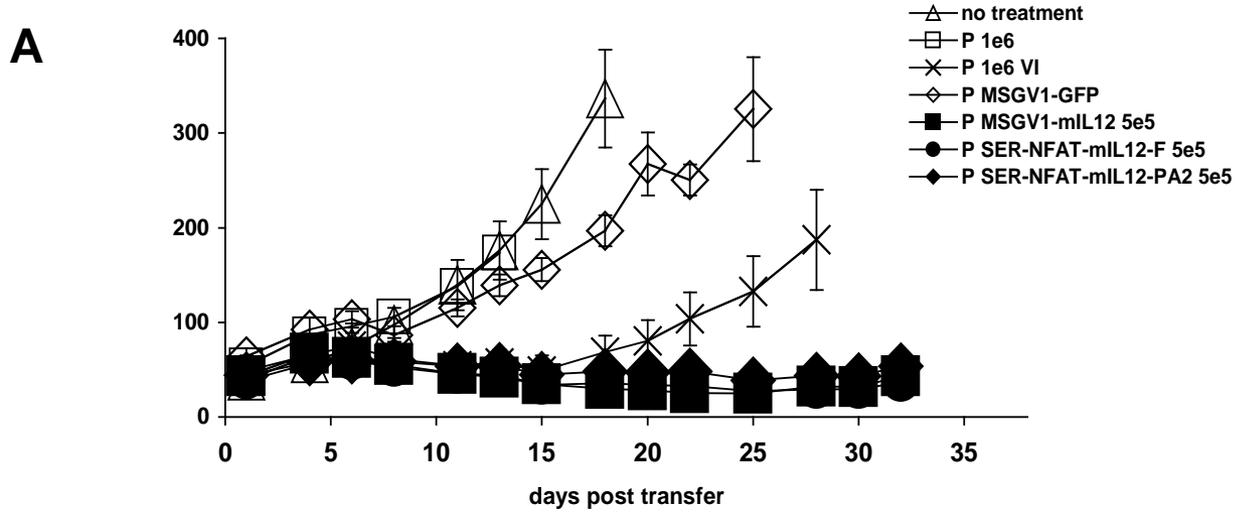
**Discovered as transcription factors bound to IL-12 promoter;
activated by antigen specific triggering of the T cell receptor**

**Thus we used NFAT responsive promoter to drive single chain
IL-12 production by T cells only when encountering specific
antigen stimulation**

Development of Retrovirus NFAT-mflexiIL12 vector for use in murine model

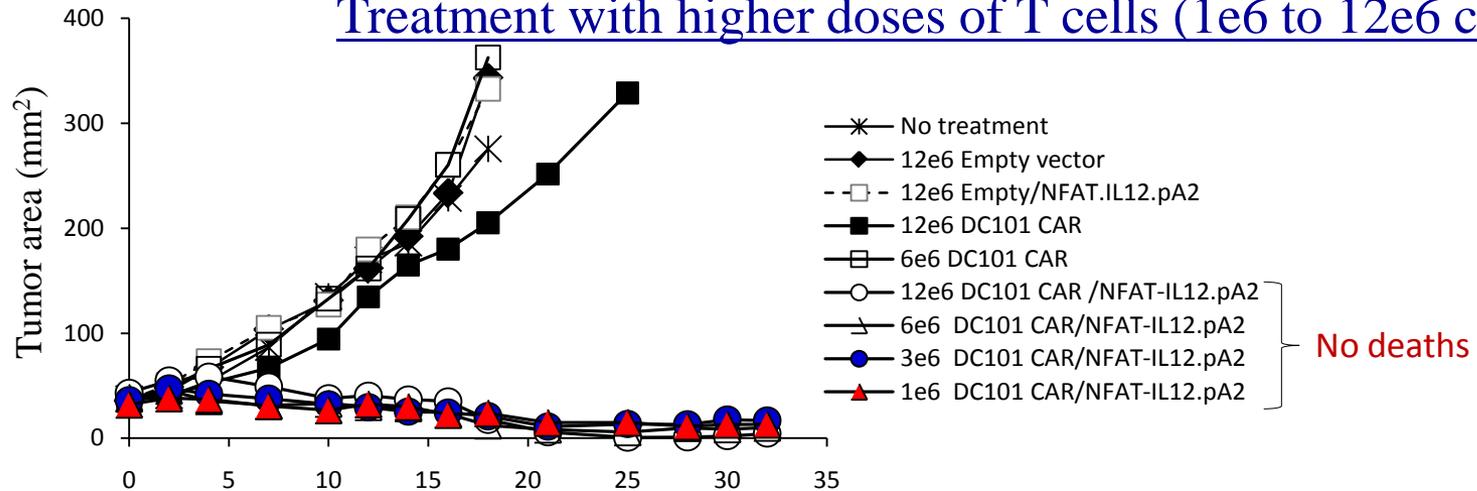


mflexiL12 engineered Pmel-1 T cell improved the efficacy of tumor treatment without need of IL2 and vaccine

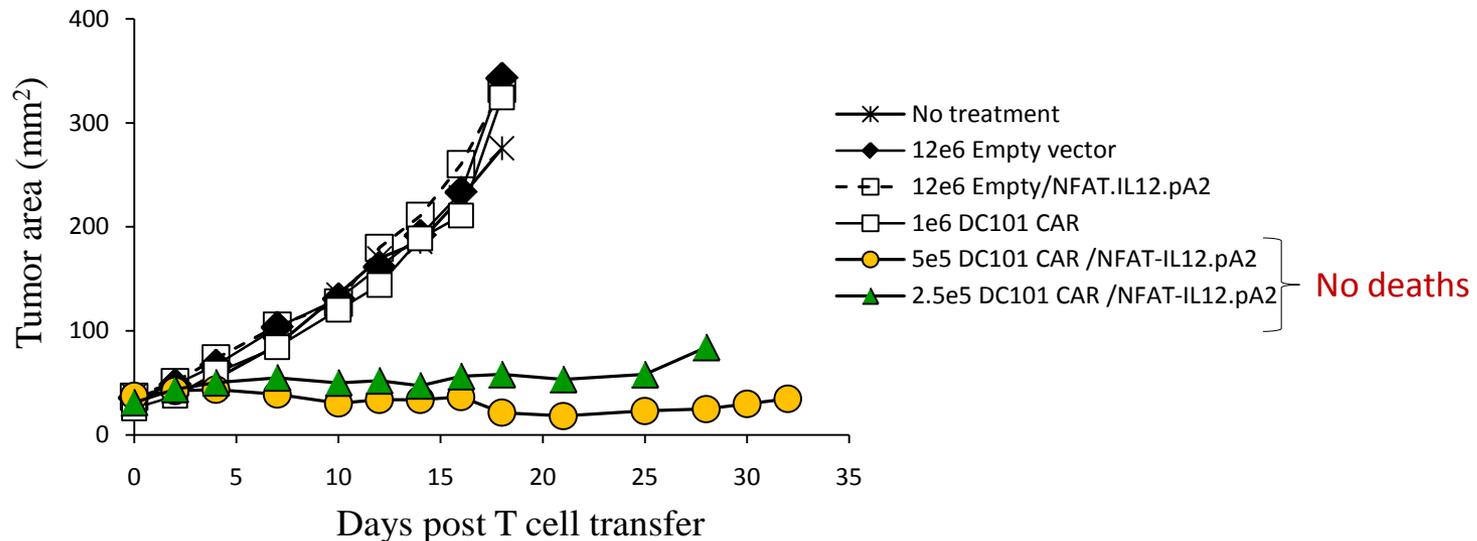


In vivo administration of DC101 (anti-VEGFR2) CAR and NFAT-IL12 cotransduced T cells mediate tumor regression with no IL-2 administration

Treatment with higher doses of T cells (1e6 to 12e6 cells)



Treatment with lower doses of T cells (1e5 to 5e5 cells)

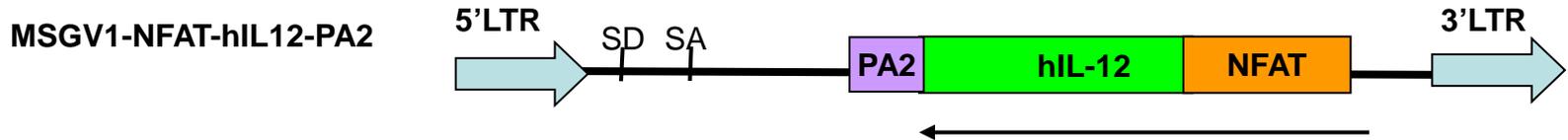


PROTOCOL TITLE

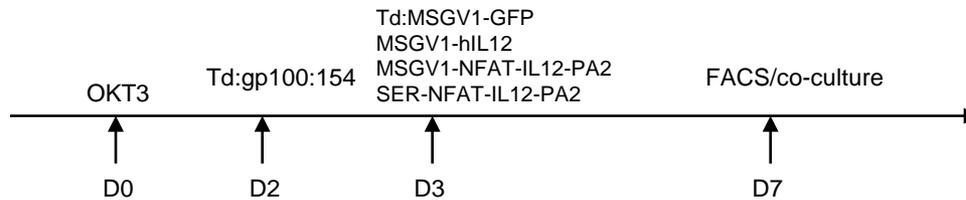
**Phase I/II Study of Metastatic Melanoma
Using Lymphodepleting Conditioning Followed by Infusion of
CD8 Enriched Tumor Infiltrating Lymphocytes Genetically
Engineered to Express IL-12**

Principal Investigator: Steven A. Rosenberg, M.D., Ph.D.

NFAT response promoter induce IL-12 expression in activated PBLs triggered by TCR signaling



Donor: White.E
Stim:5-11-09

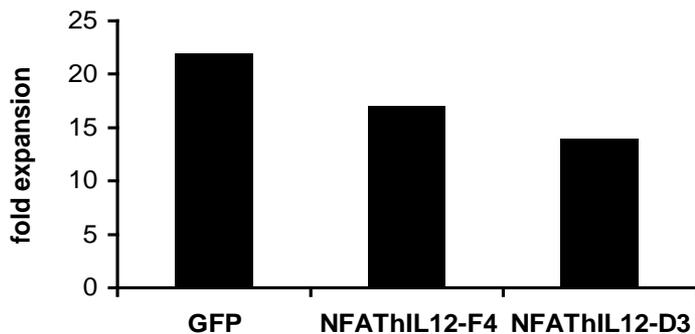
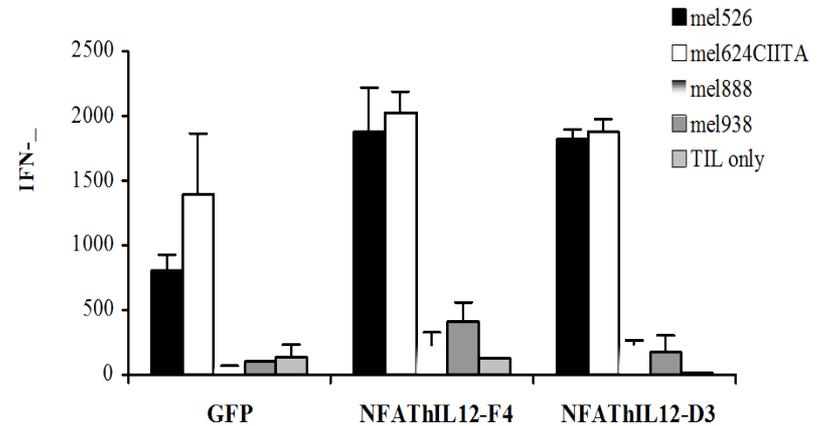
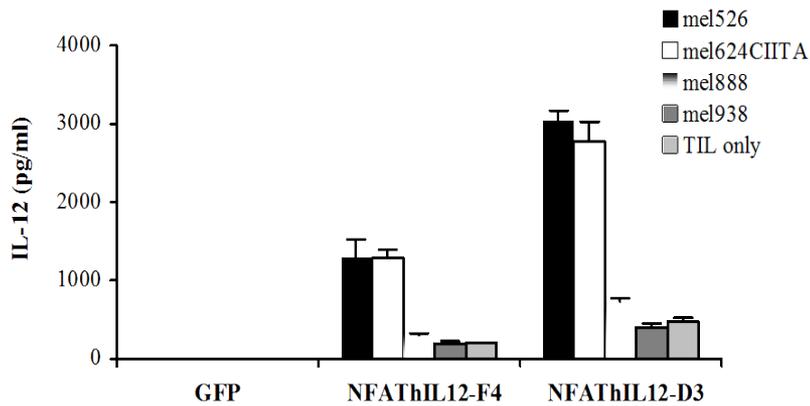
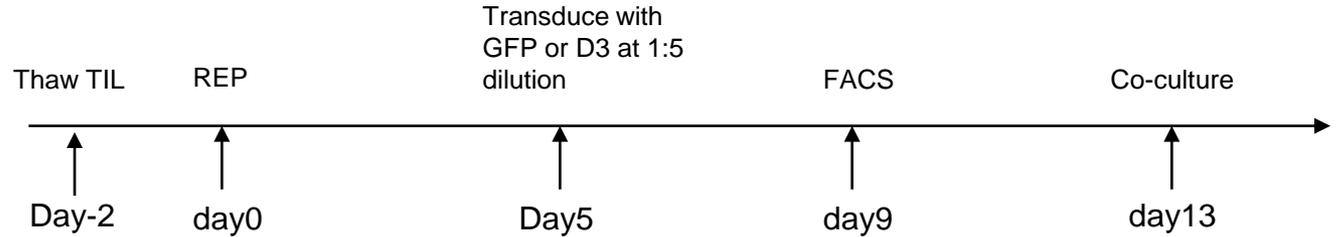


virus sup:	hIL-12 (pg/ml)
MSGV1-GFP	0
MSGV1-hIL12	17756
MSGV1-NFAT-hIL12-PA2	4882

hIL12 (pg/ml)	mel526	mel624	mel888	mel938	PBL alone
gp100(154)+MSGV1-hIL12	>3000	>3000	>3000	>3000	>3000
gp100(154)+MSGV1-NFAT-hIL12-PA2	>3000	>3000	6	0	0

IFN- γ (pg/ml)	mel526	mel624	mel888	mel938	PBL alone
gp100(154) only	9479	4682	0	531	65
gp100(154)+MSGV1-GFP	6896	3670	90	0	0
gp100(154)+MSGV1-hIL12	24720	28640	199	49	271
gp100(154)+MSGV1-NFAT-hIL12-PA2	38492	26858	110	90	80

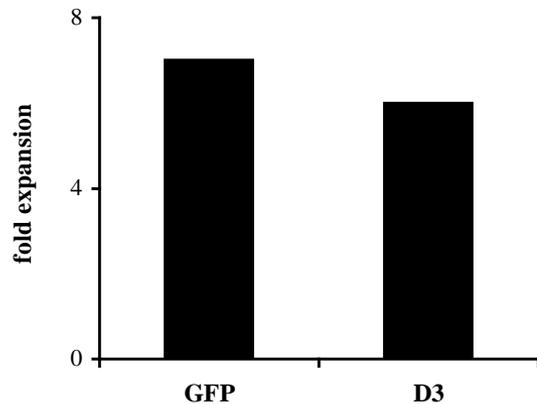
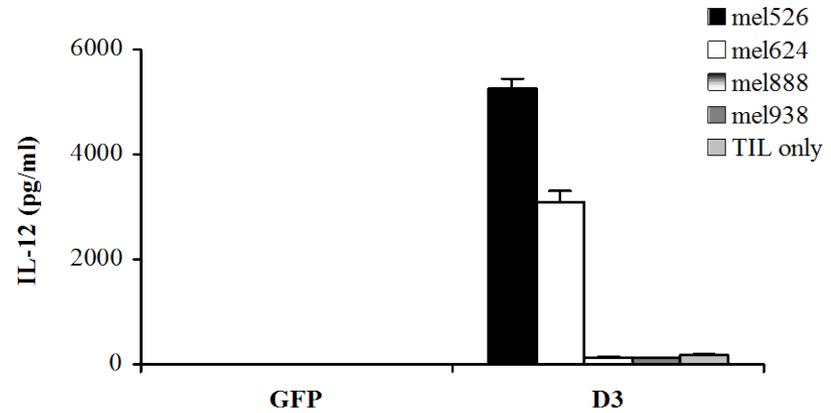
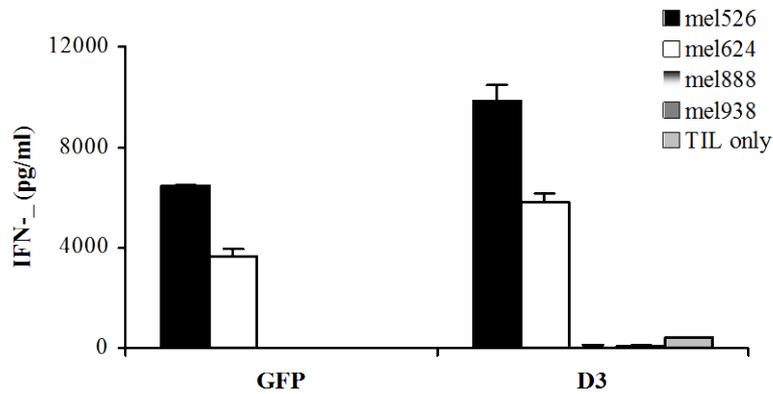
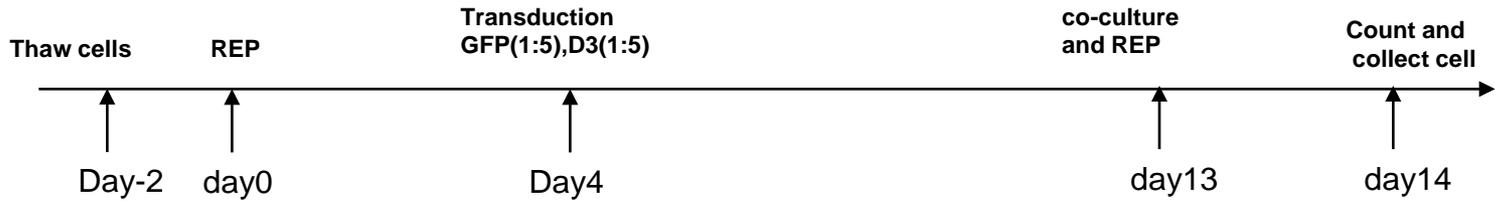
MSGV-1-NFAT-hIL12-PA2 clone: D3; F4 using human tumor reactive TIL



Conclusion: hIL12 could be induced when transduced cells were cocultured with tumor targets and enhance IFN- γ secretion. A modest reduction in cell proliferation was observed in NFAT-IL12 transduced TILs. Clone D3 is chosen for clinical application.

The data is repeated in 5 different donors

Testing NFAT-hIL12-PA2 clone D3 in CD8 enriched TILs



The data is repeated in 3 different donors

Conclusion: CD8 enriched TILs could be efficiently transduced by clone D3. hIL12 was induced when transduced cells were co-cultured with tumor targets.

Gene Therapy: IL-12 in TIL

Objectives:

Primary objectives:

- To evaluate the safety of the administration of IL-12 engineered TIL in patients receiving a non-myeloablative conditioning regimen.
- Determine if the administration of IL-12 engineered TIL to patients following a non-myeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer.

Secondary objective:

- Determine the in vivo survival of IL-12 gene-engineered cells.

IL-12 Gene Therapy: Eligibility

Metastatic melanoma with measurable disease

≥ 18 years old

ECOG 0 or 1

Life expectancy > 3 months

ANC > 1000 mm³

Platelets $> 100,000$ mm³

Hemoglobin > 8 g/dl

Creatinine ≤ 1.6 mg/dl

No active infections or major illnesses of the cardiovascular, respiratory, renal systems

IL-12 Gene Therapy: Treatment Regimen

TIL grown for 2-3 weeks

CD8 enrichment on Miltanyi apparatus

Stimulated with OKT-3, transduced and expanded

Infusion:

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

d0: single infusion (3 patients/cohort)

Cohort 1: 10^6 cells

2: 10^7

3: 10^8

4: 10^9

5: 10^{10}

6: 10^{10} to 5×10^{10}

Mandatory safety assessments:

2 week delay after the first patient in any cohort

2 week delay after last patient in cohort prior to enrollment in next cohort

IL-12 Gene Therapy: Treatment Regimen (cont.)

Dose limiting toxicity (DLT):

All grade 3 and 4 toxicities except:

- (1) myelosuppression due to preparative regimen**
- (2) occurring within 24 hours of cell infusion reversible in < 8 hours by acetaminophen and/or diphenhydramine**

If 2 DLTs develop drop to next lower dose and treat at least 6 patients

MTD is highest dose at which ≤ 1 patient develops DLT

**Phase II protocol at MTD: 21 evaluable patients (if ≥ 2 responses
41 total patients)**

Safety Considerations: IL-12

1994 – 1997: **single test dose**
14 days later daily IL-12
2 responses in 40 patients

When given without test dose: 2 deaths
Thus tachyphylaxis: prior desensitization increased
tolerance to IL-12

2001: **5 published trials, 157 patients**
common well-tolerated regimen:
250 mg/kg i.v. qd for 5d q 21d
5/157 – responder
mild transient transaminase elevations

Safety Considerations: Gene Therapy with IL-12

Human

TIL transduced with gene for IL-12:

1st cohort: 10^6 cells produce 2 ng IL-12 over 24 hours

70 kg person: 0.02 ng/kg

8,000 fold lower than daily dose

40,000 fold lower than 5d dose

Added margin of safety by production at site of antigen

Mouse

25 gram mouse tolerate 10^5 IL-12 transduced cells without toxicity

(4×10^6 cells/kg)

1st cohort: 10^6 cells/70 kg = 0.014×10^6 cells/kg

286 fold lower cell dose than tolerated by mice on a per weight basis

Added margin of safety by production at site of antigen

IL-12 Gene Therapy: Certificate of Analysis

Test	Method	Limits	Result	Initials/ Date
Cell Viability	trypan blue exclusion	> 70%		
Total viable cell number	visual microscopic count	Between 10^6 and 5×10^{10} cells		
Identity	FACS	> 90% CD3+CD8+ after transduction		
Reactivity	IL-12 release when stimulated by PMA/Ionomycin	> 200 pg/ml and > 2 times background		
Microbiological studies	gram stain	no micro-organisms seen		
	aerobic culture	no growth		
	fungus culture	no growth		
	anaerobic culture	no growth		
	mycoplasma test	negative		
Endotoxin	limulus assay	< 5 E.U./kg		
RCR	S+L Assay RCR-PCR	negative		
Presence of tumor cells	Cytopathology	No tumor cells per 200 cells examined		

