Treatment of Patients with Metastatic Melanoma by infusion of anti-MART TCR-Gene Engineered Lymphocytes

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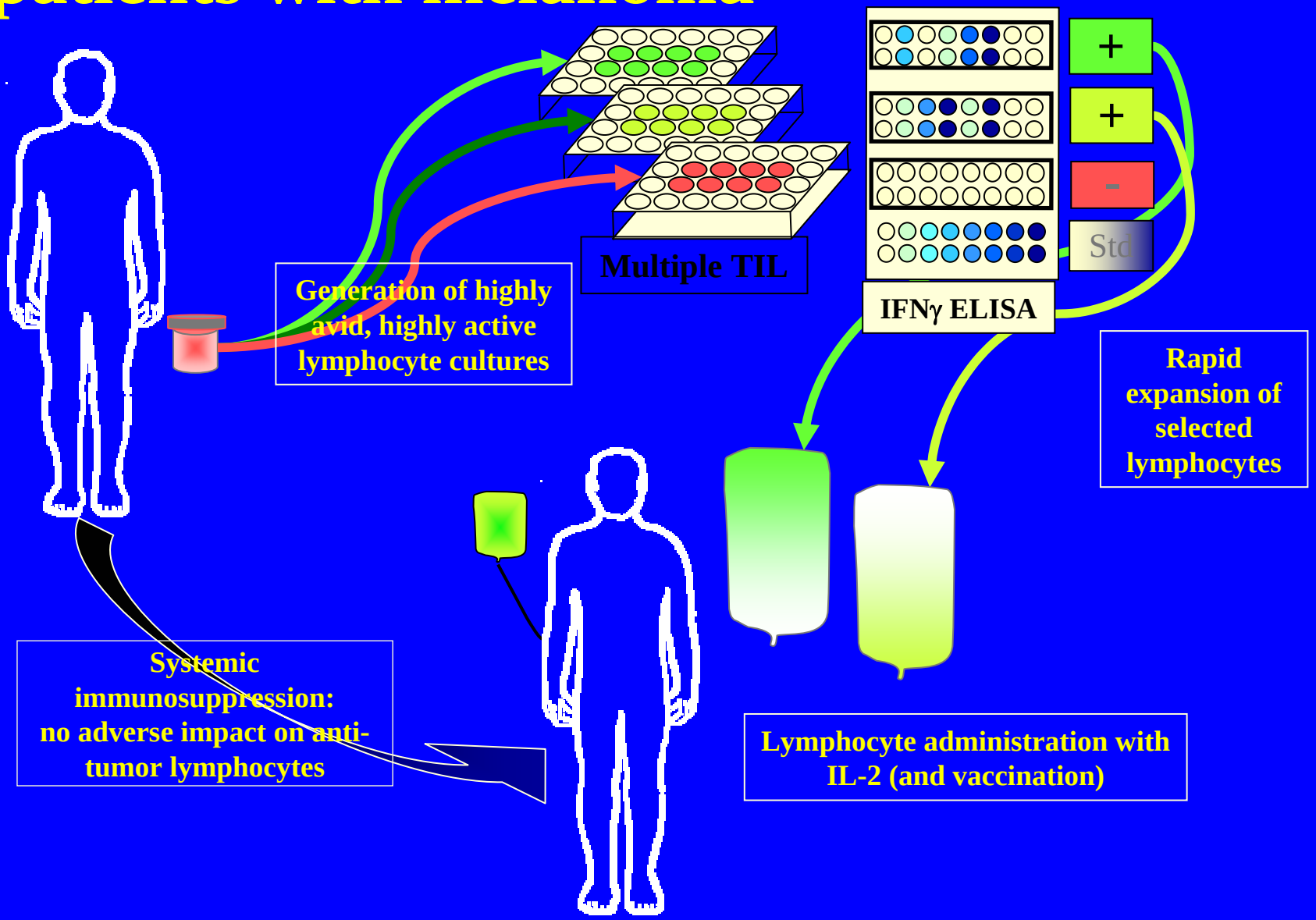
Adoptive Cell Transfer Therapy for patients with melanoma



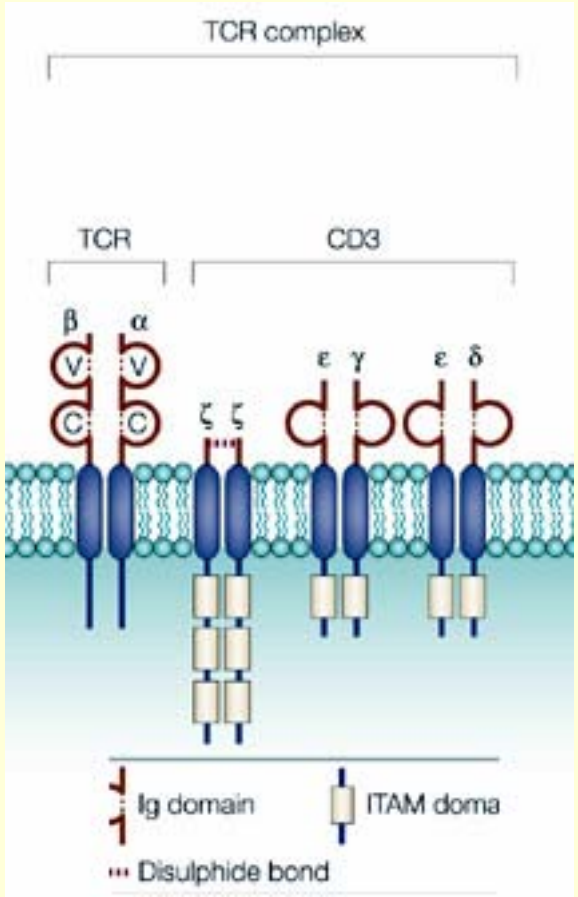
Goal: ***Bring adoptive immunotherapy to patients that lack active TIL and expand technology to common cancers.***

- Adoptive immunotherapy can be thought of as an attempt to circumvent tolerance and induce autoimmunity.

❖ How to break tolerance:

Use *ex vivo* gene transfer to engineer T-cells with new genetic elements that now permit T-cells to recognize self antigens.

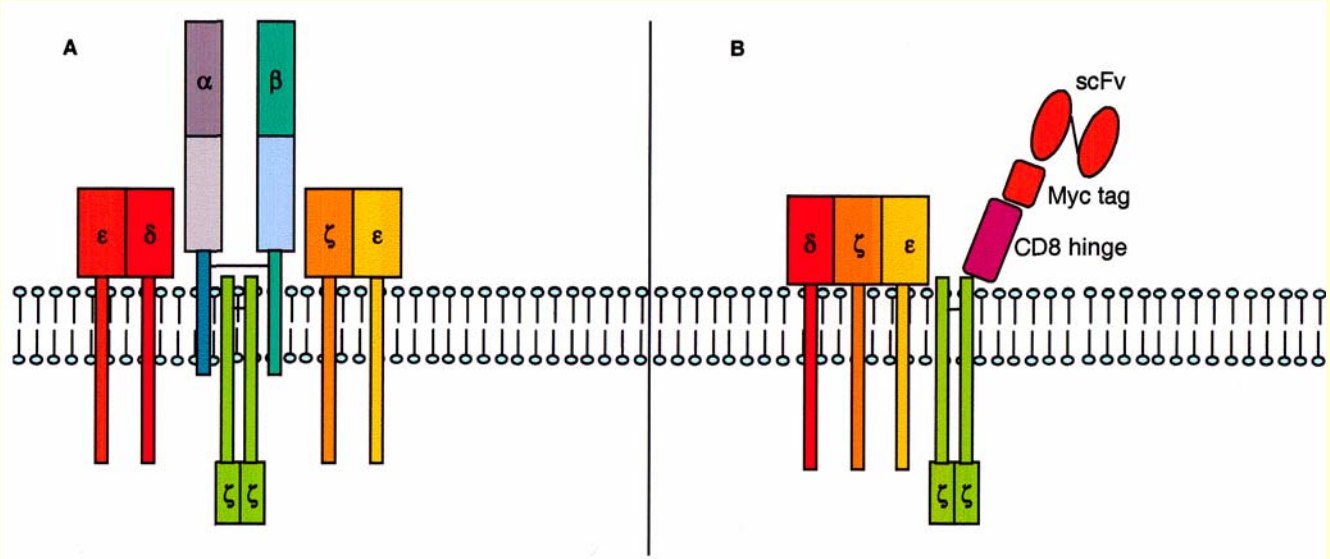
The T-Cell Receptor Complex



Types of TCR Gene Therapy:

Cloned Natural TCR genes

Chimeric TCR-like molecules



Potential advantages of TCR gene therapy:

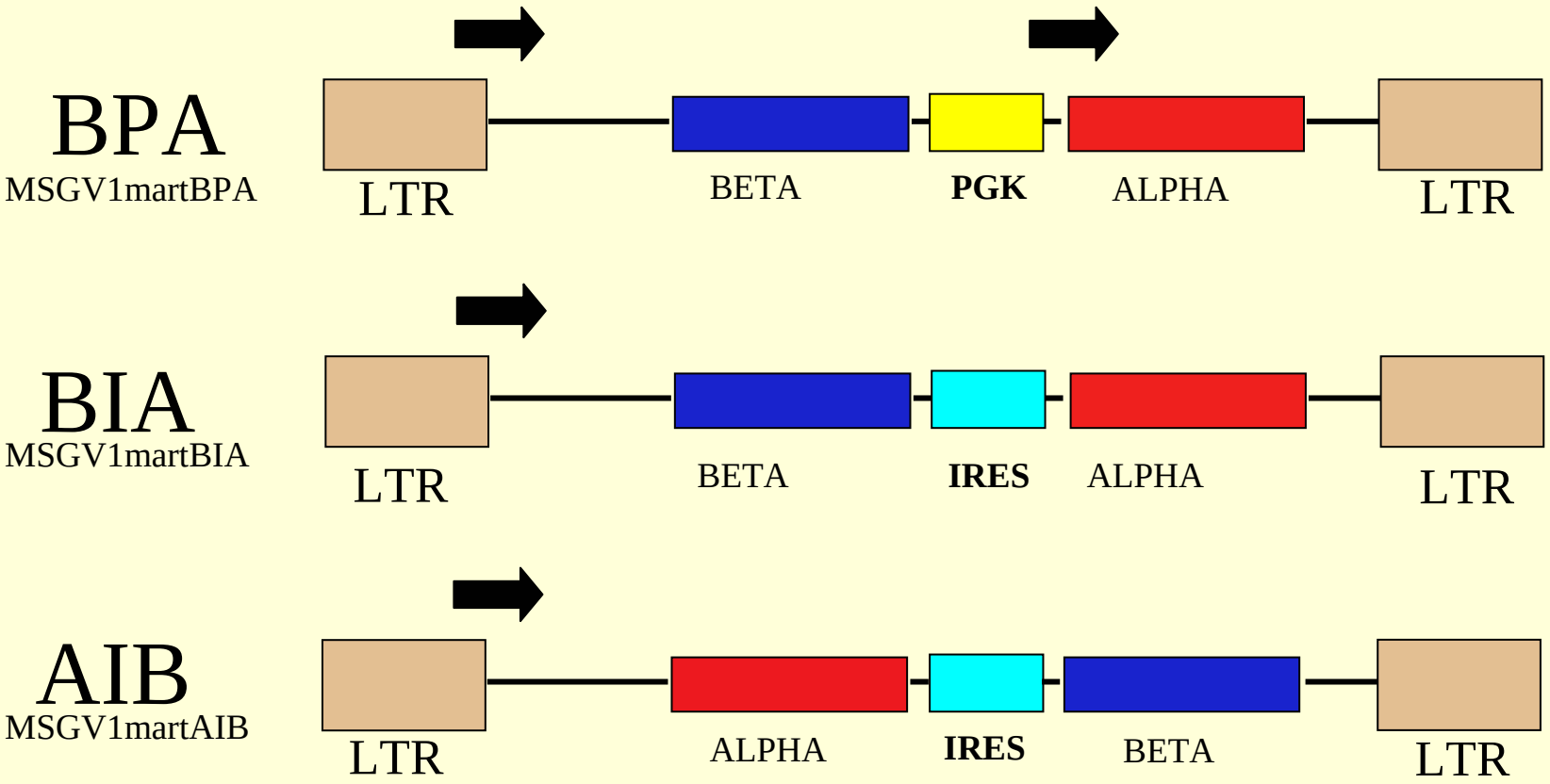
- Immediate, “off-the-shelf” generation of antigen specific T-cells.
- Eliminate need for surgery.
- Introduction of novel antigen specificities not found naturally in patients.
- Potential to “rescue” non-reactive TIL cultures.
- Potential to engineer cells to be more “effective” than natural CTL or TIL.

Isolation of a highly active anti-Mart-1 TCR from a patient demonstrating in vivo clonal expansion and marked tumor regression

Persistence of transferred cells, patient 9:

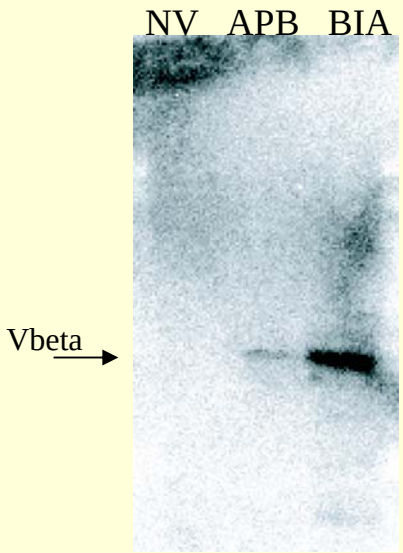
	Percent lymphocytes	
	Vβ12 TCR	MART-1 Tetramer +
Sample		
Pre-treatment PBL	**<1**	**<1**
TIL infusion	**12**	**74**
PBL day 7	**55**	**65**
PBL day 124	**50**	**51**

Anti-MART-1 TCR VECTORS:

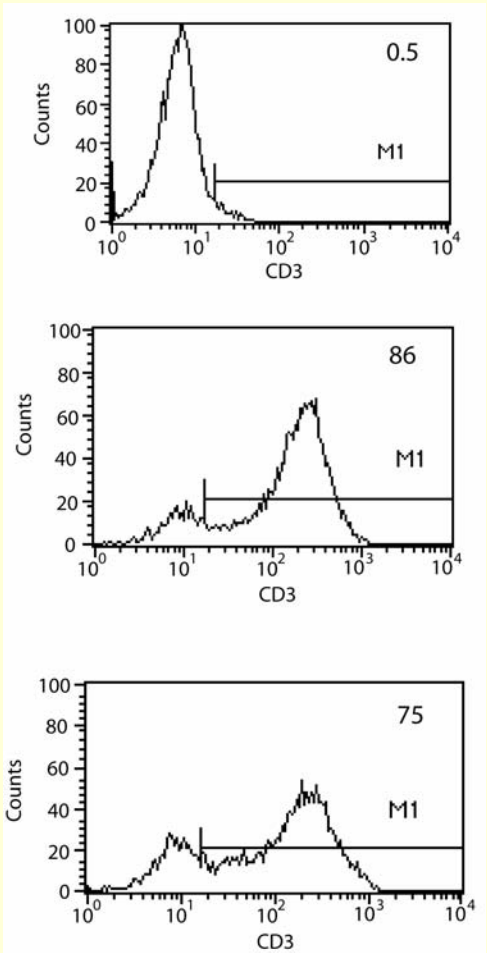


MART-1 TCR vector: TCR derived from the predominant TIL clone of patient Millet cloned into new retroviral vector, MSGV1. The LTR promoter in vector MSGV1 is from the Murine Stem Cell Virus (MSCV) and has been shown to active in lymphocytes and less prone to repression in murine hematopoietic cells.

Test of TCR gene transfer into human SupT1 T-cell line:



Western blot

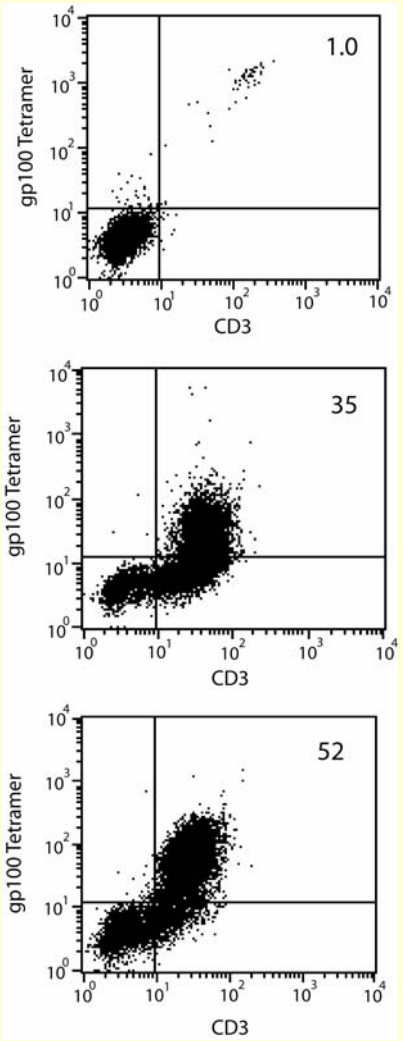


Rescue of CD3 expression

NV

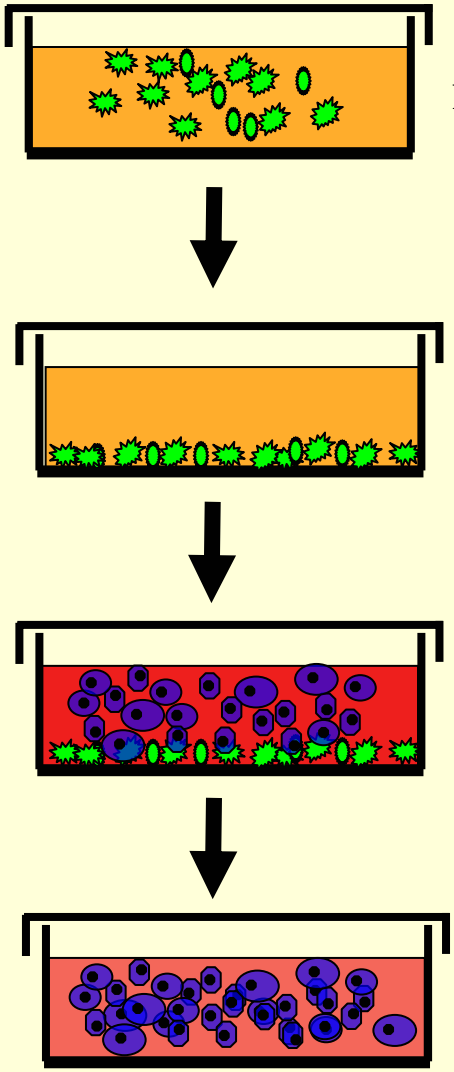
APB

BIA



Tetramer staining

PBL gene transfer procedure

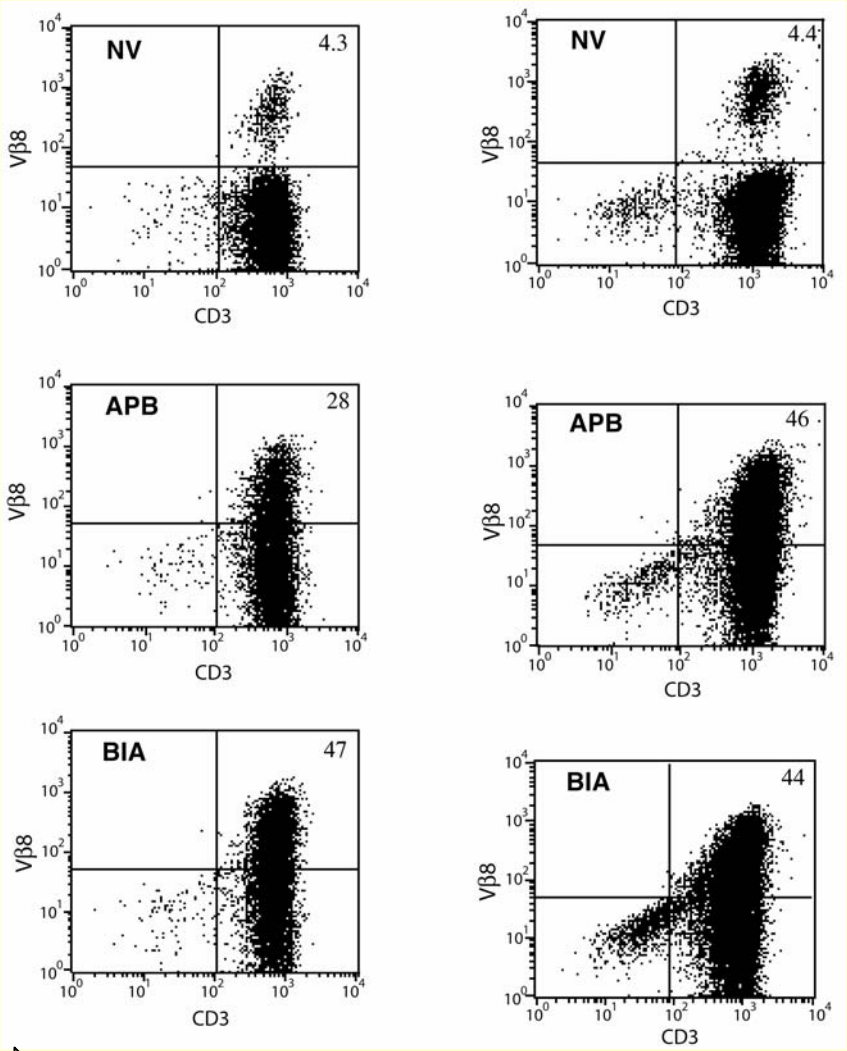


Add vector to Fibronectin plate

Vector binds to plate.

Remove “spent” vector medium add activated T-cells. Repeat X2

Expand T-cells, test for TCR expression



Results: 30-50% TCR gene transfer without selection

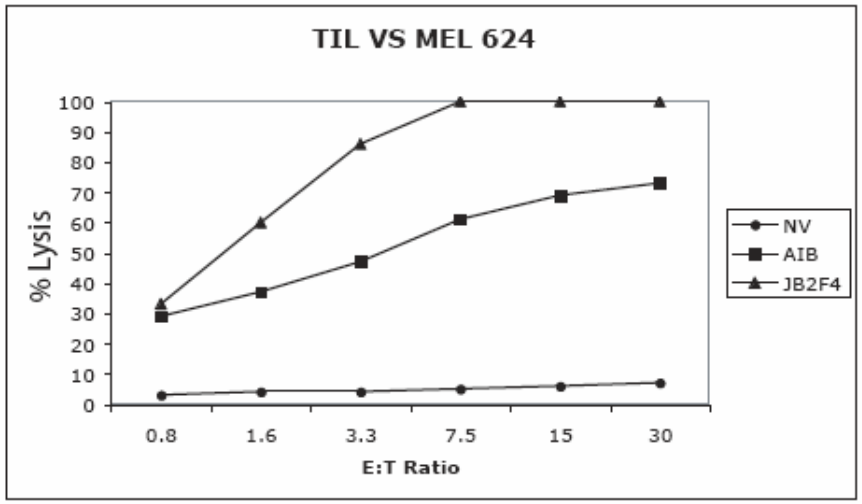
Initial test for TCR engineered PBL reactivity :

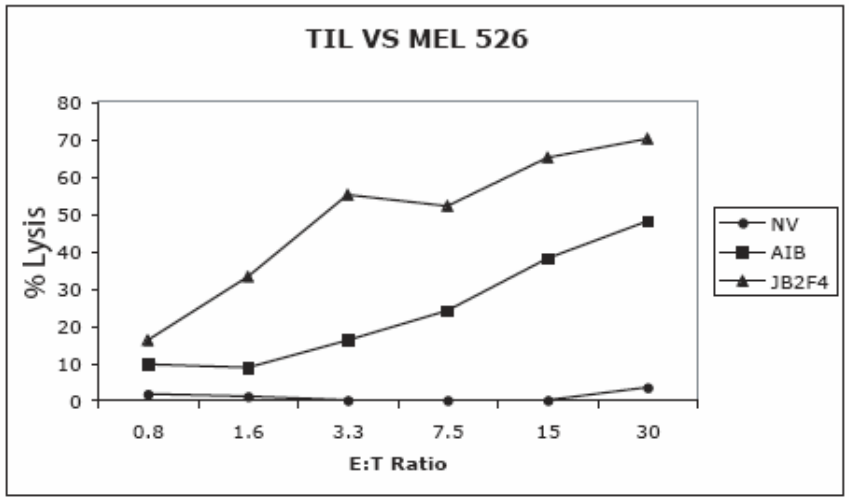
Cytokine release following co-culture with T2 cells pulsed with various peptides.

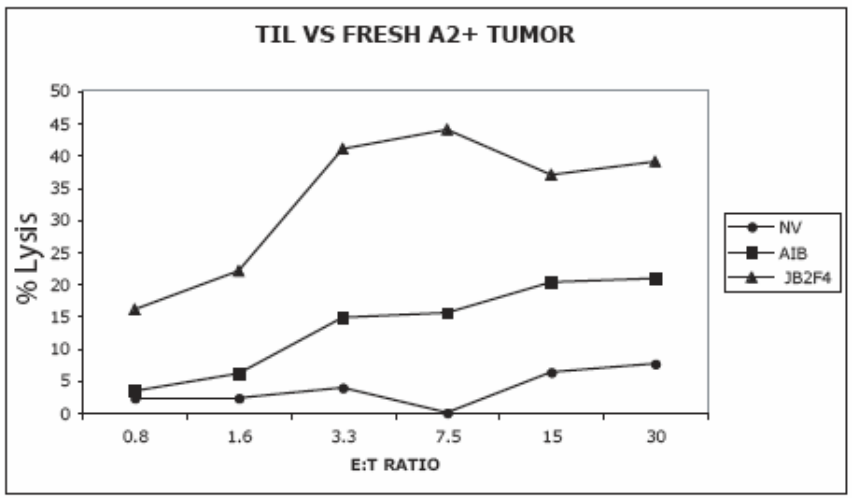
Table 1. Transduced PBL Specificity

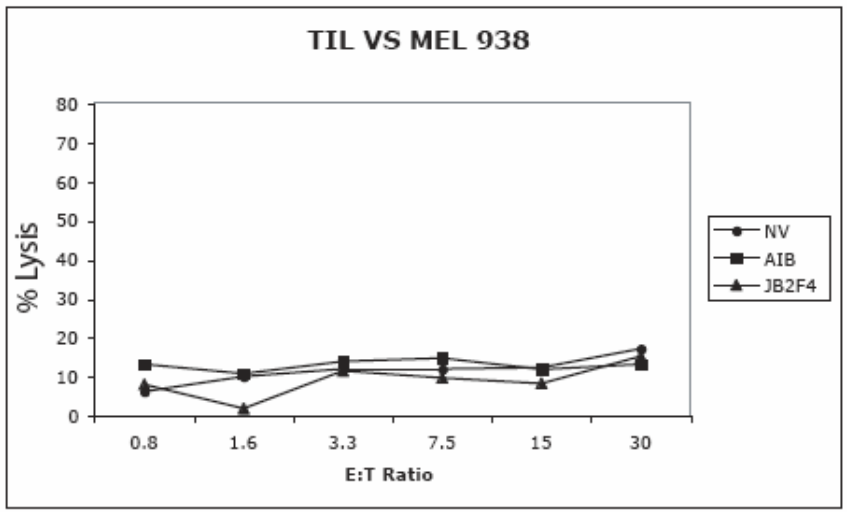
Responder Cells	*Vector*	*Stimulators (pg/ml IFN-γ)*				
		T2 alone	*T2+FLU*	*T2+gp100*	*T2+MART*	
PBL	MSGIN	464	1	10,856	409	
APB 9	gp100	532	659	**44,065**	591	← gp100
AIB 18	MART	209	178	403	**48,375**	MART-1
BPA 34	MART	515	509	687	**52,706**	MART-1
BIA 71	MART	628	519	15,457	**75,546**	MART-1
PBL-MART	NONE	0	0	428	**49,376**	MART-1
		pg/ml GM-CSF				
PBL						
PBL	MSGIN	437	573	722	786	
APB 9	gp100	498	901	1200	715	← gp100
AIB 18	MART	567	702	738	**5,100**	← MART-1
PBL-MART	NONE	1	3	45	**2,200**	
		pg/ml IL-2				
PBL	MSGIN	0	0	0	0	
APB9	gp100	0	0	0	0	← gp100
AIB 18	MART	0	0	0	**72**	MART-1
BPA 34	MART	0	0	0	**78**	MART-1
BIA 71	MART	0	18	0	**189**	MART-1
PBL-MART	NONE	0	0	25	**122**	MART-1
		pg/ml TNF-α				
PBL	NONE	0	0	0	9	
PBL	MSGIN	0	0	0	0	
APB9	gp100	0	0	**287**	0	← gp100
AIB 18	MART	0	0	0	**2,552**	MART-1
AIB 54	MART	0	0	0	**1,941**	MART-1

^{51}Cr lysis assay of TCR transduced T-Cells

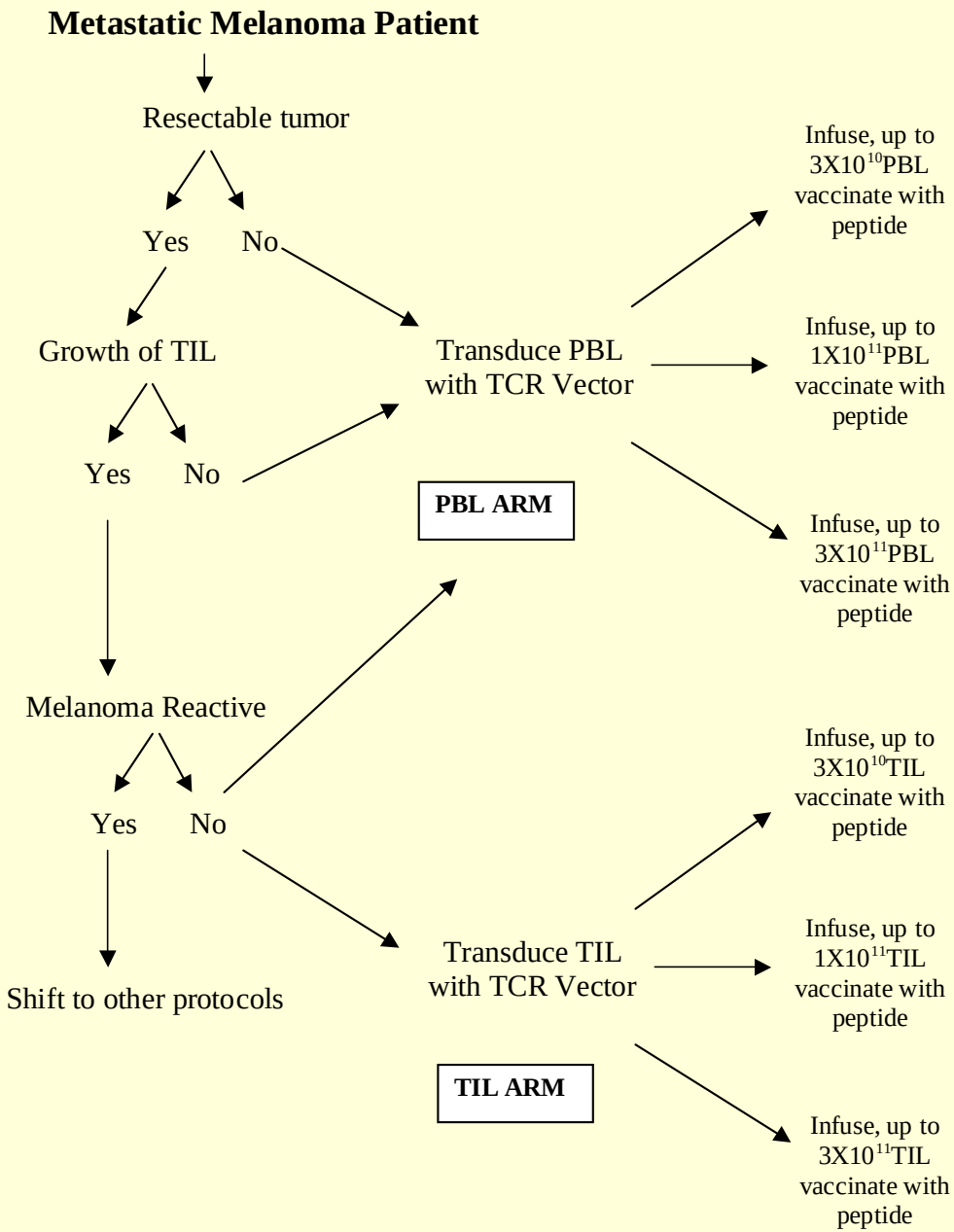








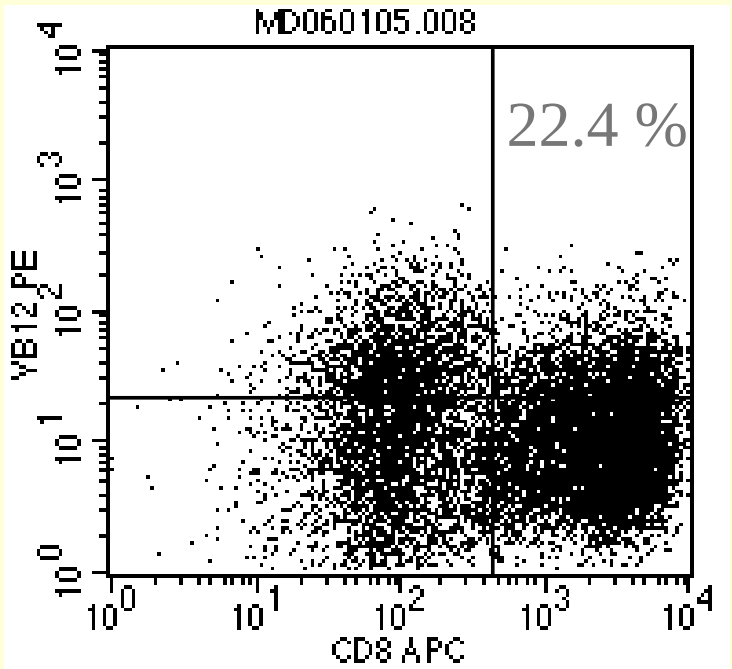
Original Protocol Schema



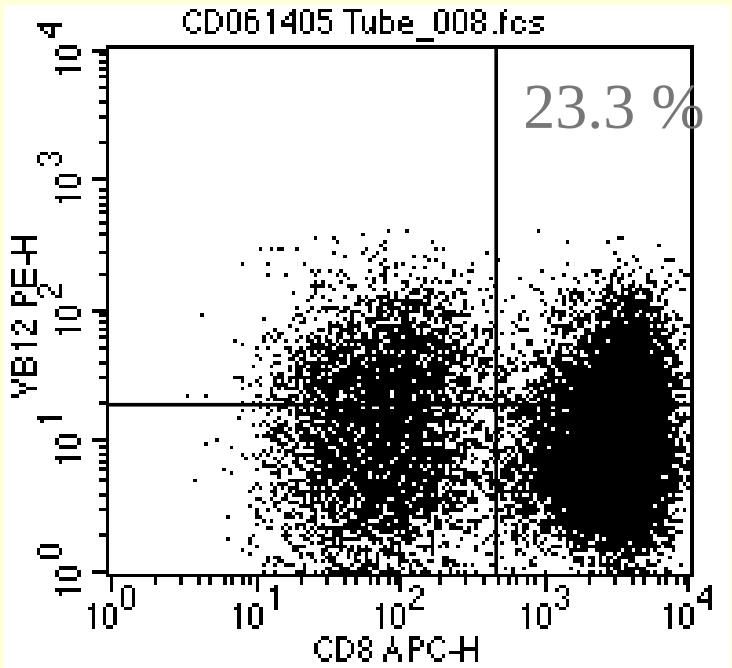
MART TCR infusion samples (n=14)
VB12 expression

CD8: 47% VB12+ (+/- 4.6%)
CD4: 47% VB12+ (+/- 4.0%)

Day 18 cells



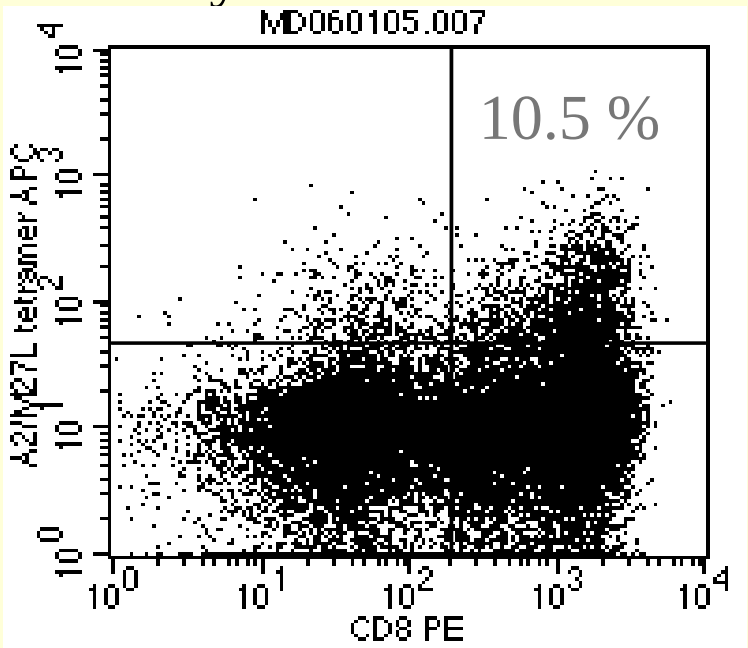
Day 7 cells



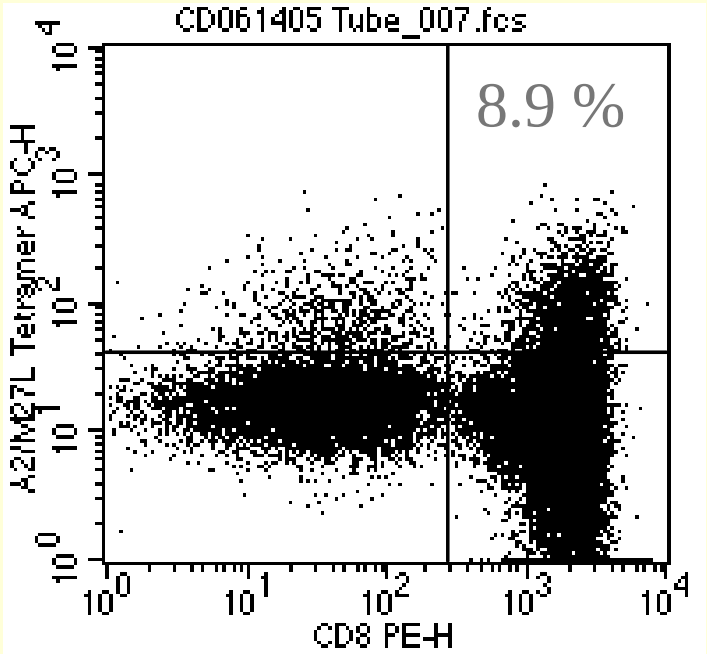
MART TCR infusion samples (n=14)
HLA-A2/MART(27L) tetramer binding

CD8: 15% A2/M27L+ (+/- 2.6%)
CD4: 13% A2/M27L+ (+/- 2.3%)

Day 18 cells



Day 7 cells

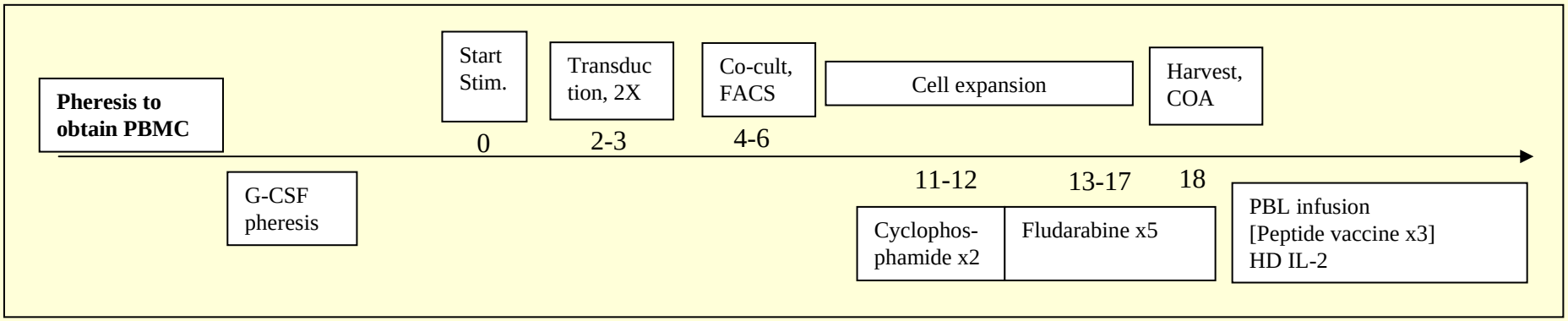


MART TCR transduced PBL – patient demographics, treatments received, and clinical outcomes

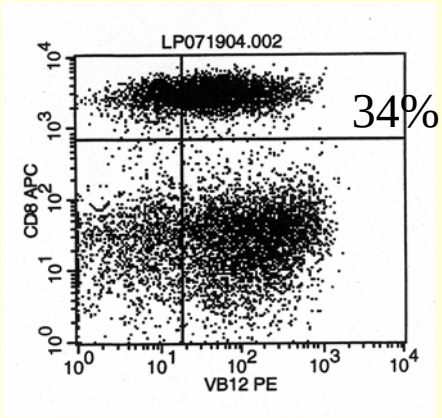
	Age of PBL Cells	Age/ Sex	# (e9)	%VB12	M cells (e9)	Cohort	FNA	Sites of evaluable metastases	Response
3 wk	D19	28/M	11	67	7.4	2	+	LN (Il ,In, Rp), skin, subcutaneous	NR
	D19	44/F	13	64	8.3	2	+	subcutaneous, LN (Il)	NR
	D19	58/M	14	35	4.9	2	+	Cutaneous, subcutaneous	NR
1 wk	D8	50/M	13	17	2.2	1	'+	Lung, pancreas, pelvis, subcutan.	NR
	D7	55/F	7	51	3.6	2	Nd	Pariaortic LN, lung	NR
	D7	56/M	9	40	3.6	2	A2 <25%	LN (Ax), lung	NR
	D7	37/M	6	32	1.9	1	Nd	Lung, LN (In)	NR
	D7	53/M	5	41	2.1	1	Nd	LN (Ax, Ms), renal, adrenal, sebcut	NR
	D6 (admin d +4)	52/M	1.2	42	0.5	1	Nd	Liver, subcutaneous, hilum	PR (10+)
	D6 (admin d +4)	44/F	2	53	1.1	1	+	LN (Il), subcutaneous	NR
	D6 (admin d +4)	45/M	9	34	3.1	2	M+, A2 nd	LN (Ax), subcutaneous	NR
	D6 (admin d +3)	45/M	6	45	2.7	1	+	Pancreas, Ln (hilum), lung	SD
	D6 (admin d +4)	32/F	5	61	3.1	2	M-, A2 nd	Subcutaneous (in trans.), Br	NR
3 + REP	D18 + R1d9	30/M	86	40	22	3	Nd	Hilum, Liver, LN (In)	PR (9+)
	D18 + R1d9	51/M	39	45	14	3	A2 <25%	Lung	NR
	R1d9	25/F	33	11	3.6	2	+	Lung, Liver, Subcutaneous	NR

GROUP I, TCR TRANSDUCTION SOP:

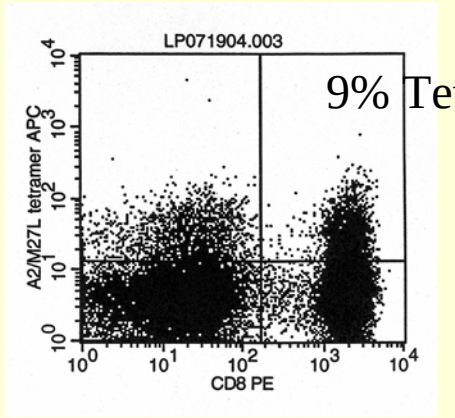
d0: Stimulate cells (OKT3 + 50 CU/ml IL-2)
d2: Transduce on Retronectin coated plates
d3: Transduce on Retronectin coated plates
d4: Harvest, transfer cells flasks
d6: Co-culture and FACS
d7 through17: expand in bags using AIM V + 5% AB serum + 50 CU/ml IL-2
d18: Harvest and infuse (goal > 3 X 10e10 total cells)



Good gene transfer:



34% VB12



9% Tetramer

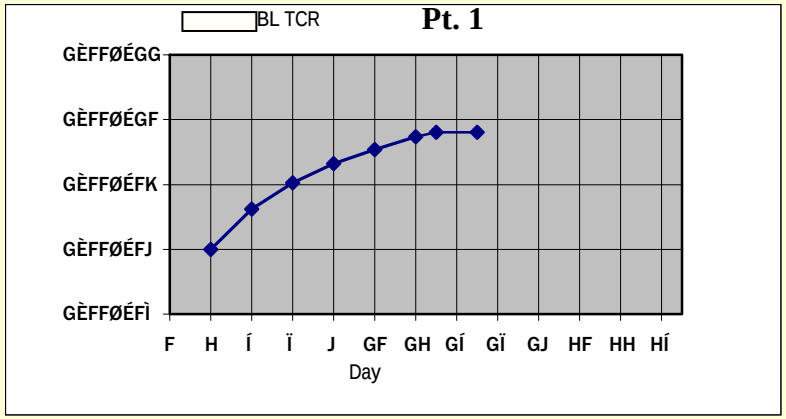
Good reactivity:

INF-γ (pg/ml)

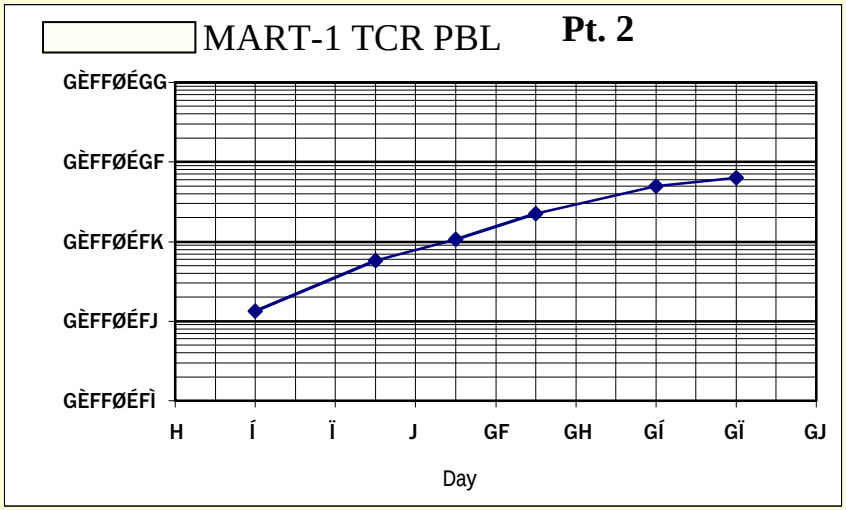
			T2 cells pulsed with peptide			Mel TC lines				
	media	**none**	**M (1.0)**	**M (0.1)**	**M27L**	**209**	**888**	**938**	**526**	**624**
Pt. 1										
JKF6	86	44	**>15960**	**4370**	**>64200**	72	37	25	**>36870**	**>34380**
UT	39	251	337	243	327	206	82	85	47	128
MART TCR	12	46	**5810**	**1820**	**>15580**	150	18	13	**3820**	**4120**
Pt. 2										
JKF6	24	30	**10420**	**2510**	**>43810**	44	26	81	**>45570**	**>45580**
UT	41	48	42	42	60	35	85	28	22	23
MART TCR	29	39	**3370**	**470**	**>20330**	51	81	45	**2220**	**884**
Pt. 3										
JKF6	28	17	**>10930**	**2040**	**>24180**	17	17	18	**5910**	**7080**
UT	21	24	41	29	36	28	52	21	23	14
MART TCR	20	26	**3810**	**680**	**>13240**	24	43	4	**6560**	**9930**

Suboptimal growth

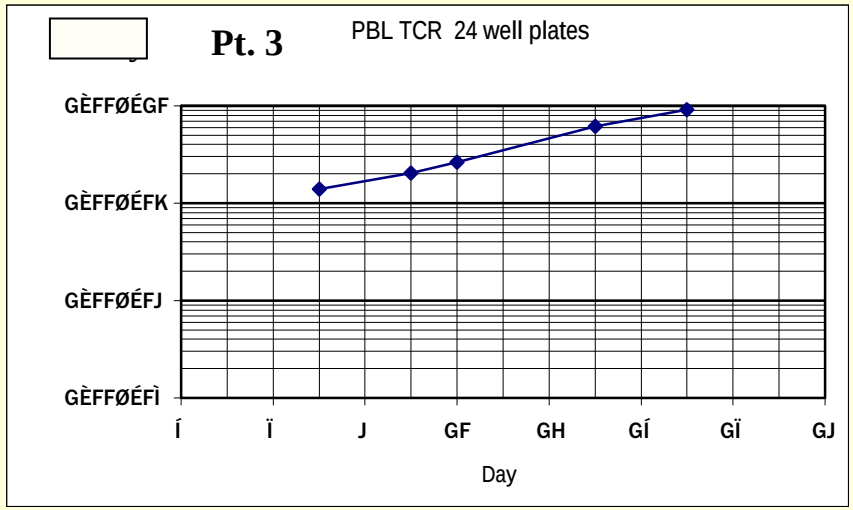
(55-70 fold expansion)



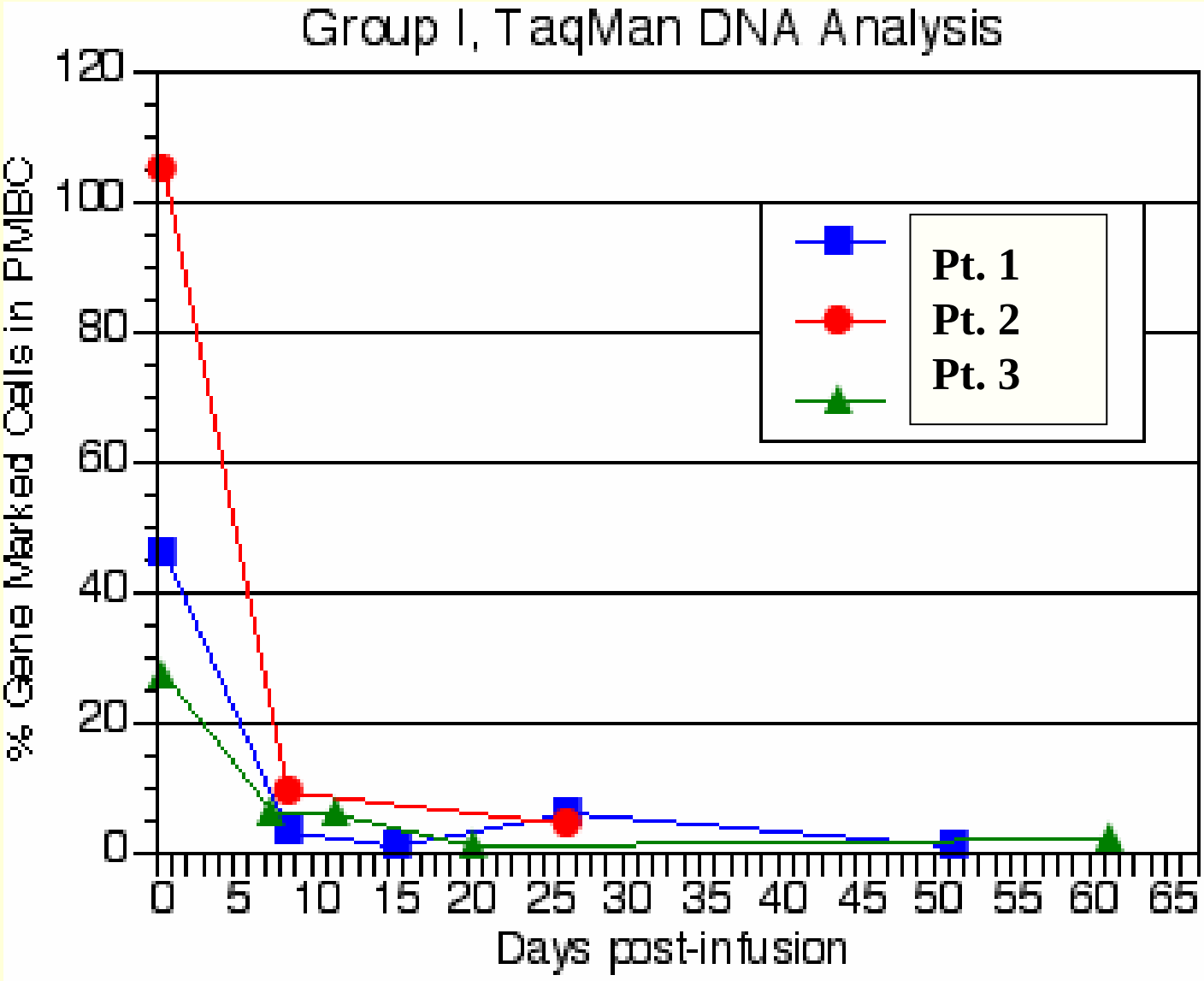
Doubling time days 4-8: 1.7
Doubling time days 15-17: 8.7



Doubling time days 4-7: 1.4
Doubling time days 14-16: 6.2



Doubling time days 2-7: 1.3
Doubling time days 13-15: 3.5


Group I, TaqMan DNA Analysis
% Gene Marked Cells in PMBC
120
100
80
60
40
20
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65
Days post-infusion
Pt. 1
Pt. 2
Pt. 3

MART TCR transduced PBL – patient demographics, treatments received, and clinical outcomes

	Age of PBL Cells	Age/ Sex	# (e9)	%VB12	M cells (e9)	Cohort	FNA	Sites of evaluable metastases	Response
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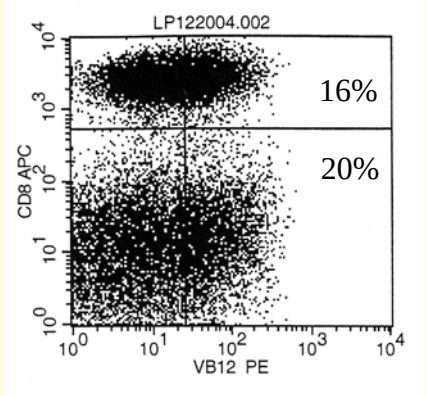
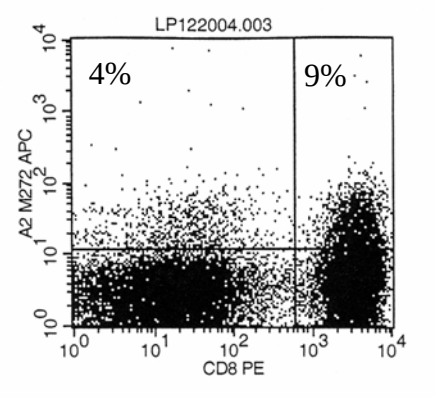
3 + REP	D18 + R1d9	30/M	86	40	22	3	Nd	Hilum, Liver, LN (In)	PR (9+)
	D18 + R1d9	51/M	39	45	14	3	A2 <25%	Lung	NR
	R1d9	25/F	33	11	3.6	2	+	Lung, Liver, Subcutaneous	NR

Short-Term Culture, Single Transduction results:

d0: Stimulate cells (OKT3 + IL-2)
d2: Transduce on Retronectin coated plates
d3: Harvest, transfer cells flasks
d5-6: Co-culture and FACS
d6-7: Harvest cells, administer

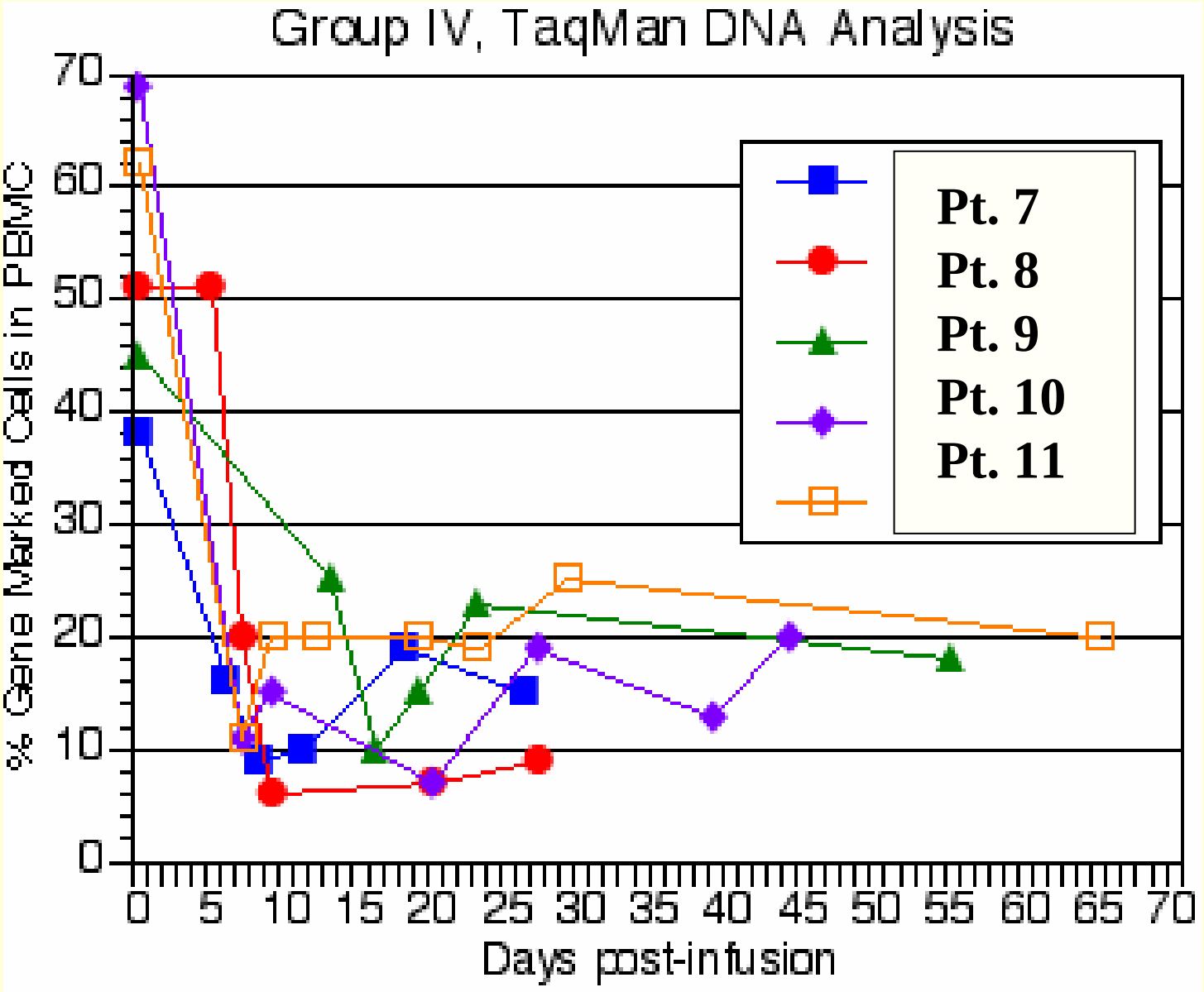


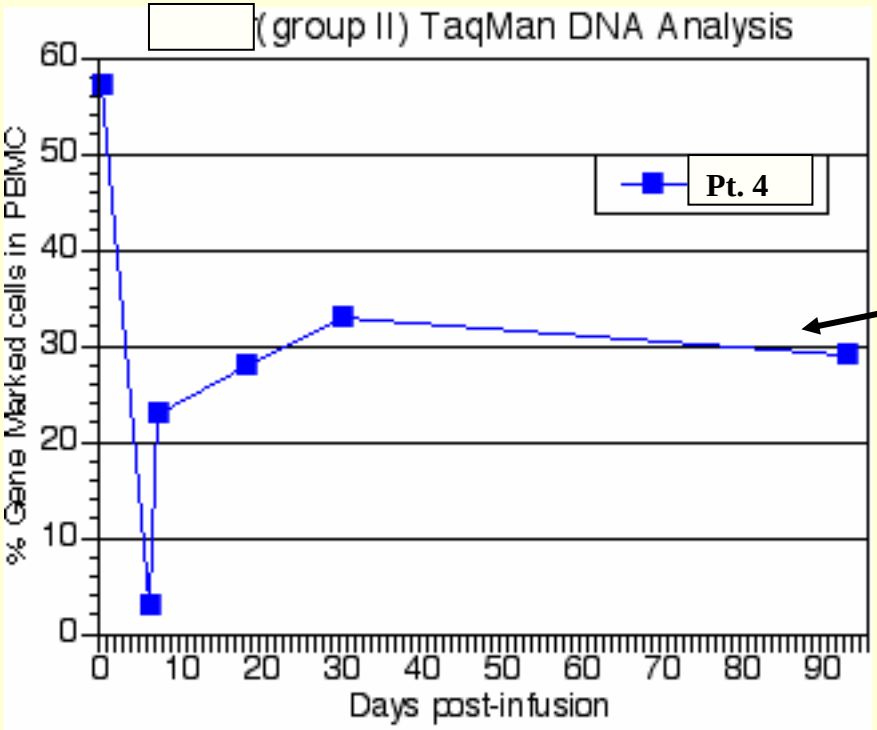
V Beta 12



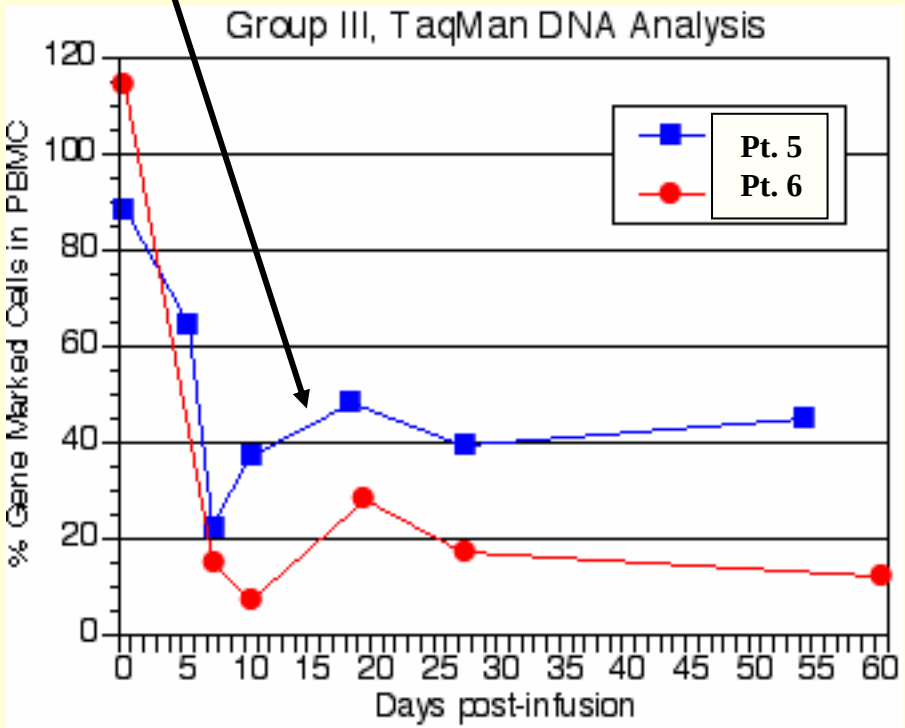
MART-1 Tetramer

		INF-γ (pg/ml)								
		T2 cells pulsed with 1uM peptide					A2-		A2+	
	None	None	g209	MART (1.0)	MART (.01)	M27L	888 A1,24	938 A1,24	526 A2,3	624 A2,3
Controls										
None	29	29	35	26	29	29	38	64	56	167
AK1700-3	247	193	230	162	198	247	32	49	38	157
L2D8	71	84	222	**412**	96	**330**	92	101	**225**	149
JKF6	0	56	10	**7790**	**1870**	**196**	6	35	**164**	140
Pt. 4										
d5 UT	318	492	458	472	330	499	225	162	172	373
Rx1b MART	128	620	555	**5910**	**2700**	186	198	133	**3610**	**2420**
TCR d5	142	555	419	**6720**	**2190**	164	167	137	**2533**	**1980**


Group IV, TaqMan DNA Analysis
% Gene Marked Cells in PBMC
0
10
20
30
40
50
60
70
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
Days post-infusion
Pt. 7
Pt. 8
Pt. 9
Pt. 10
Pt. 11

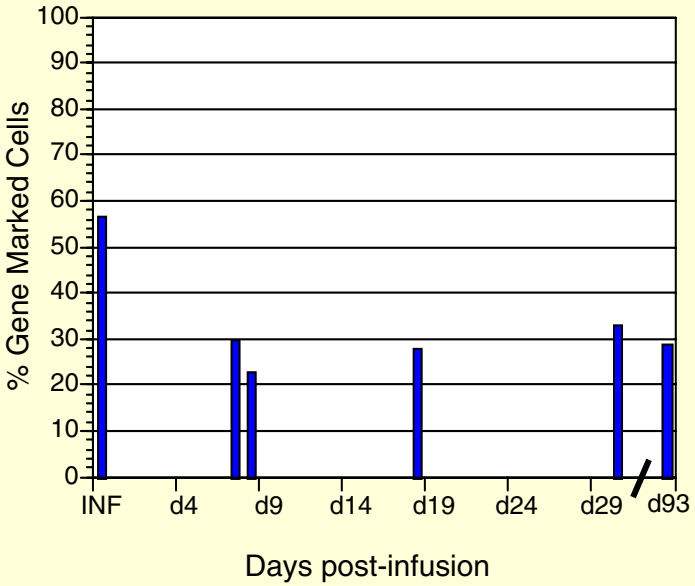


Both protocol responders had >30% marked PBL @ one month

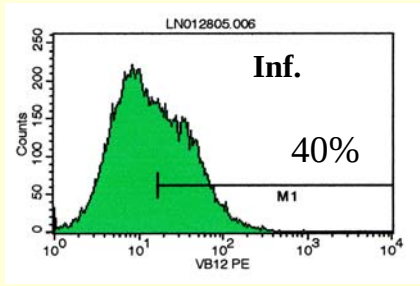


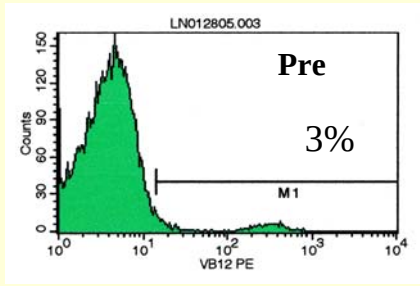
Presence of TCR transduced cells in Patient 4 PBMC

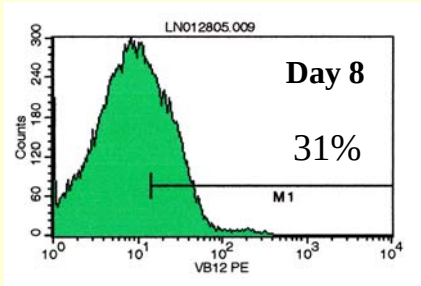
TaqMan DNA PCR:

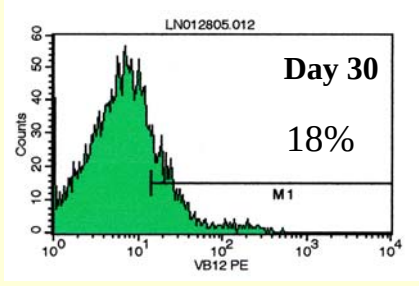


FACS Analysis (gated on CD8+):

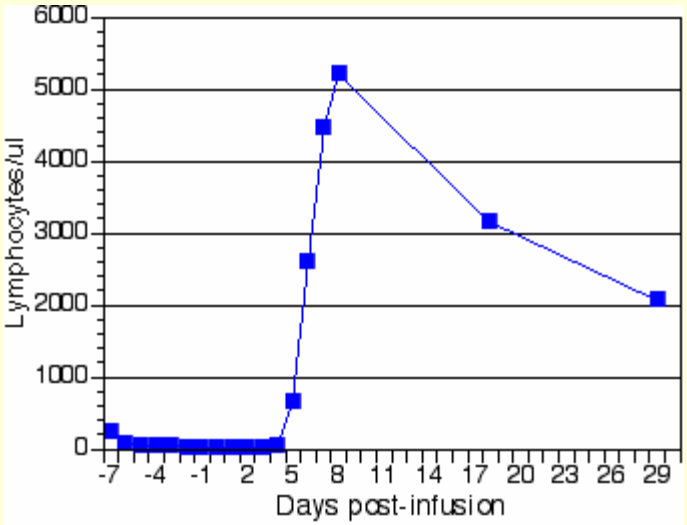








Patient 4, lymphocytosis:



In vivo cell expansion:

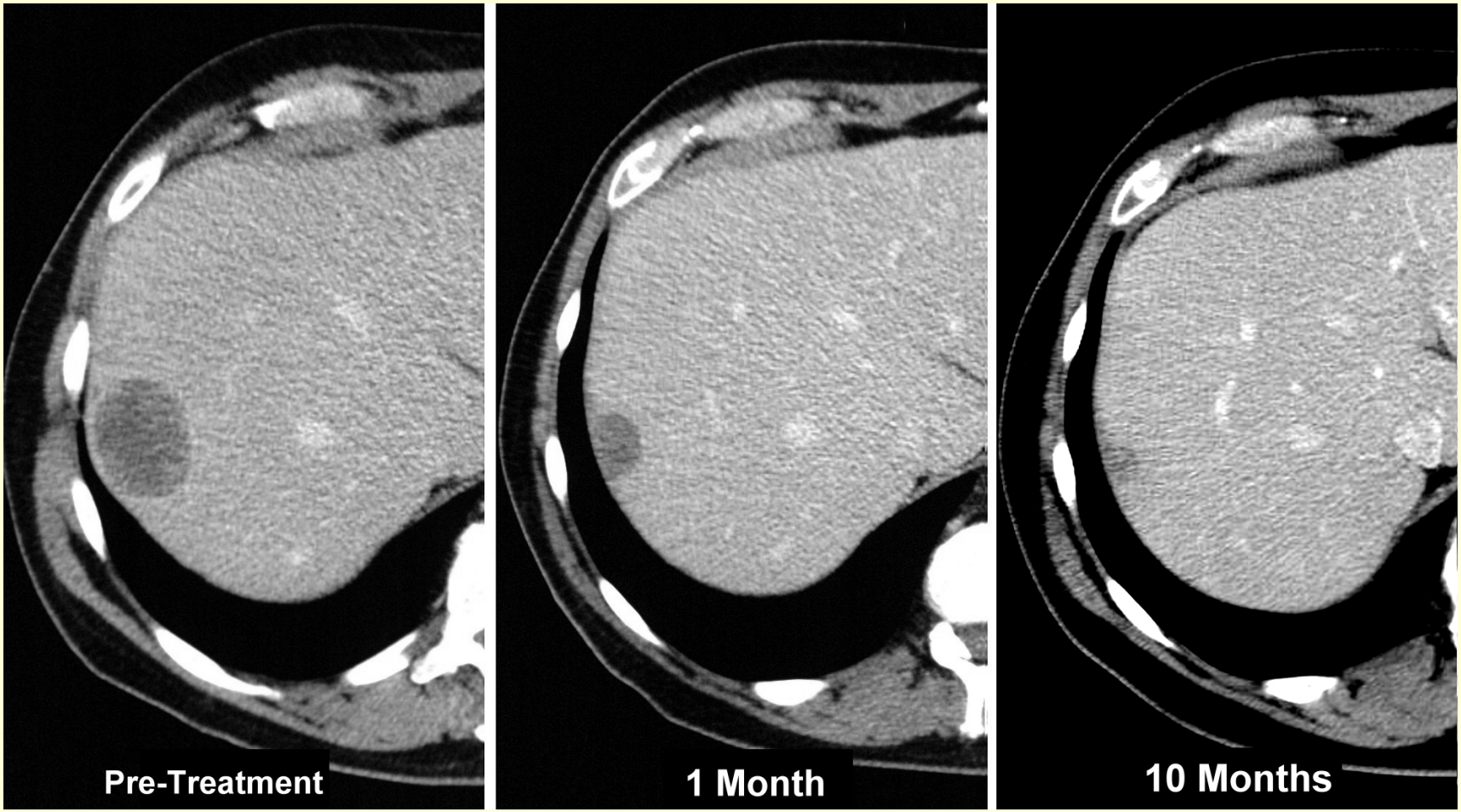
1.1 X 10e9 infused

5.22 X 10e9/L, d8

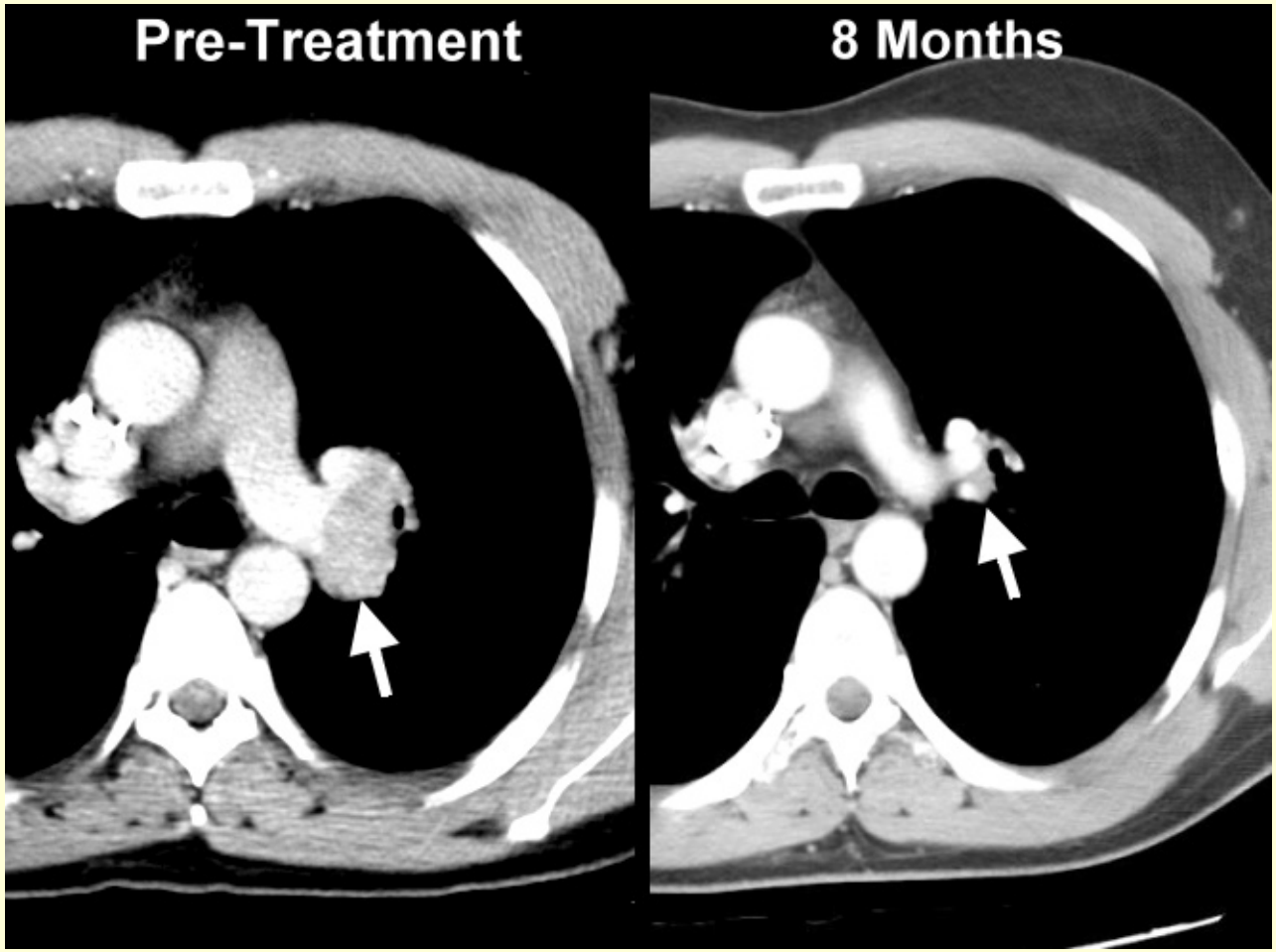
261 X 10e9 total in blood @ d8

Dbl time, < 24 hr

MART-1 TCR Transduced PBL (6 day culture)
Patient 4



MART-1 TCR Transduced PBL (3 wk culture + REP)
Patient 5



Summary:

MART TCR transduced PBL (first 16 patients)
2 on-going PRs, both PRs had highest level of gene transfer (>30%) at one month post-infusion

	Age of PBL Cells	Age/ Sex	# (e9)	%VB12	M cells (e9)	Cohort	FNA	Sites of evaluable metastases	Response
3 wk	D19	28/M	11	67	7.4	2	+	LN (Il ,In, Rp), skin, subcutaneous	NR
	D19	44/F	13	64	8.3	2	+	subcutaneous, LN (Il)	NR
	D19	58/M	14	35	4.9	2	+	Cutaneous, subcutaneous	NR
1 wk	D8	50/M	13	17	2.2	1	'+	Lung, pancreas, pelvis, subcutan.	NR
	D7	55/F	7	51	3.6	2	Nd	Pariaortic LN, lung	NR
	D7	56/M	9	40	3.6	2	A2 <25%	LN (Ax), lung	NR
	D7	37/M	6	32	1.9	1	Nd	Lung, LN (In)	NR
	D7	53/M	5	41	2.1	1	Nd	LN (Ax, Ms), renal, adrenal, sebcut	NR
	D6 (admin d +4)	52/M	1.2	42	0.5	1	Nd	Liver, subcutaneous, hilum	PR (10+)
	D6 (admin d +4)	44/F	2	53	1.1	1	+	LN (Il), subcutaneous	NR
	D6 (admin d +4)	45/M	9	34	3.1	2	M+, A2 nd	LN (Ax), subcutaneous	NR
	D6 (admin d +3)	45/M	6	45	2.7	1	+	Pancreas, Ln (hilum), lung	SD
	D6 (admin d +4)	32/F	5	61	3.1	2	M-, A2 nd	Subcutaneous (in trans.), Br	NR
3 + REP	D18 + R1d9	30/M	86	40	22	3	Nd	Hilum, Liver, LN (In)	PR (9+)
	D18 + R1d9	51/M	39	45	14	3	A2 <25%	Lung	NR
	R1d9	25/F	33	11	3.6	2	+	Lung, Liver, Subcutaneous	NR

Protocol Modification: Selection of PBMC subsets for TCR transduction and adoptive transfer

- Selection of $CD8^+$ cells

  Greater Transduction
- Elimination of T_{REG} ($CD25^+$) cells

CliniMACS (Miltenyi)

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