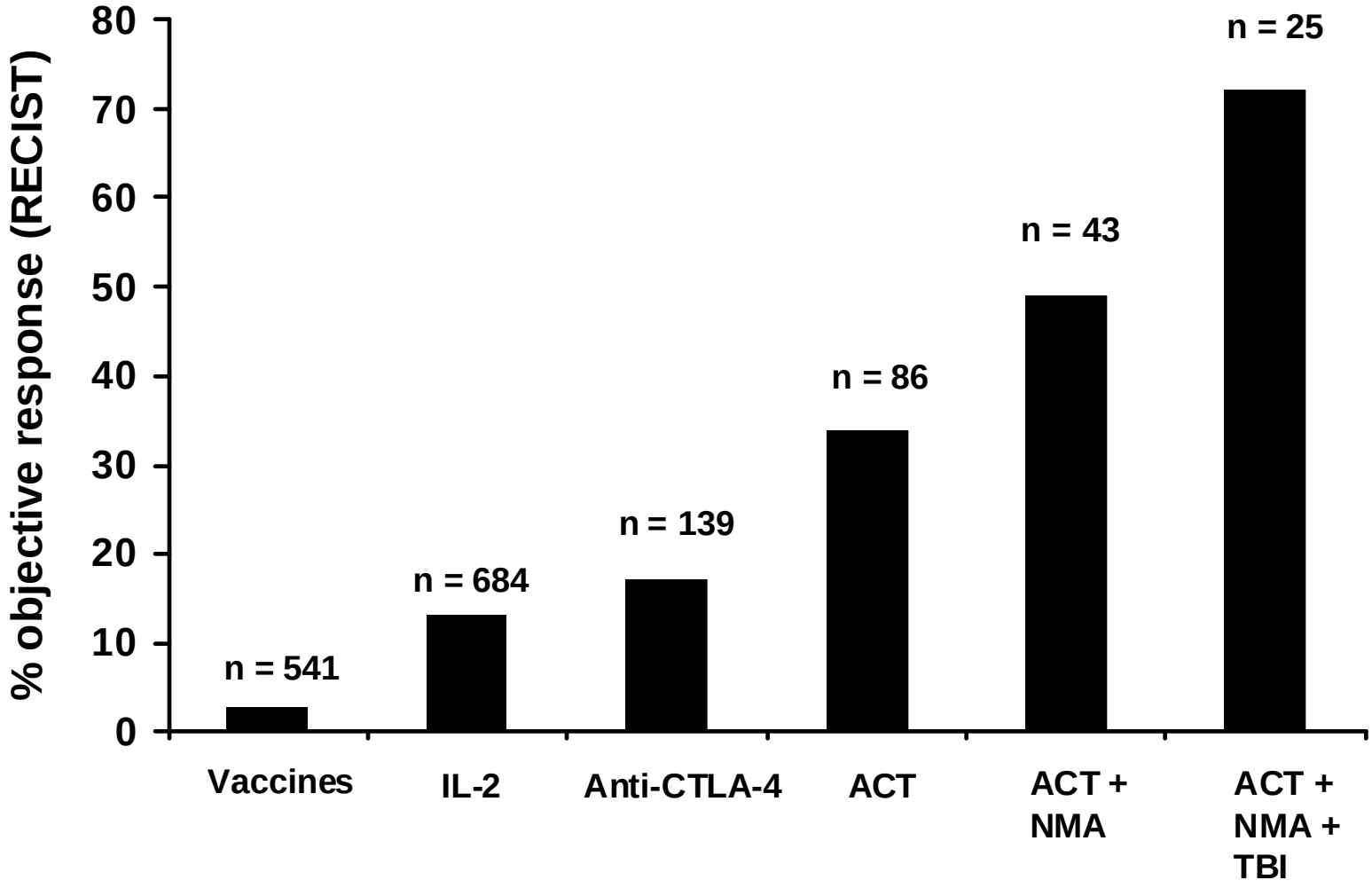
Programming tumor-reactive effector memory CD8$^+$ T cells *in vitro* obviates the requirement for *in vivo* vaccination

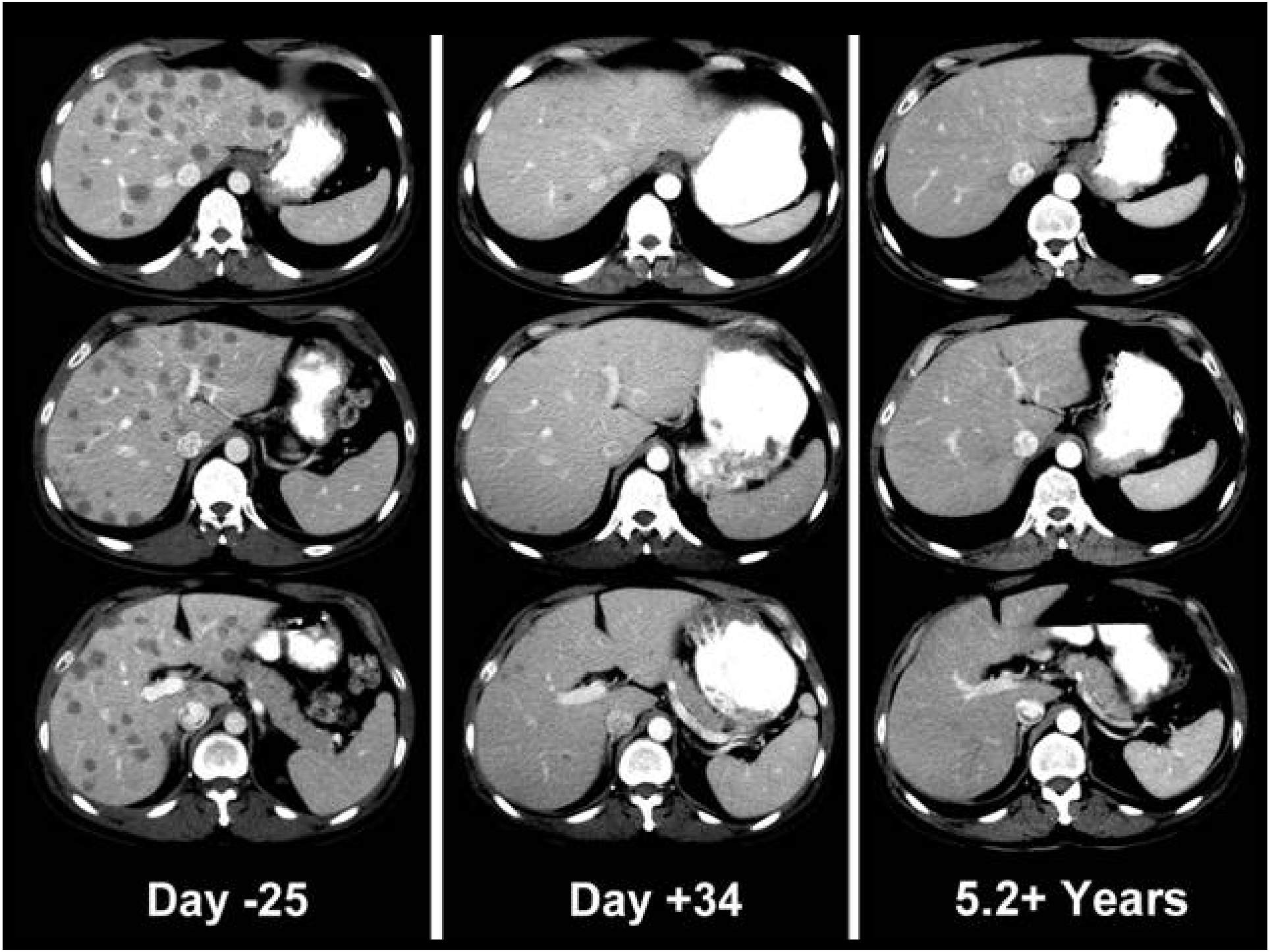
Christopher A. Klebanoff, M.D.

ISBTC 2009 Annual Meeting

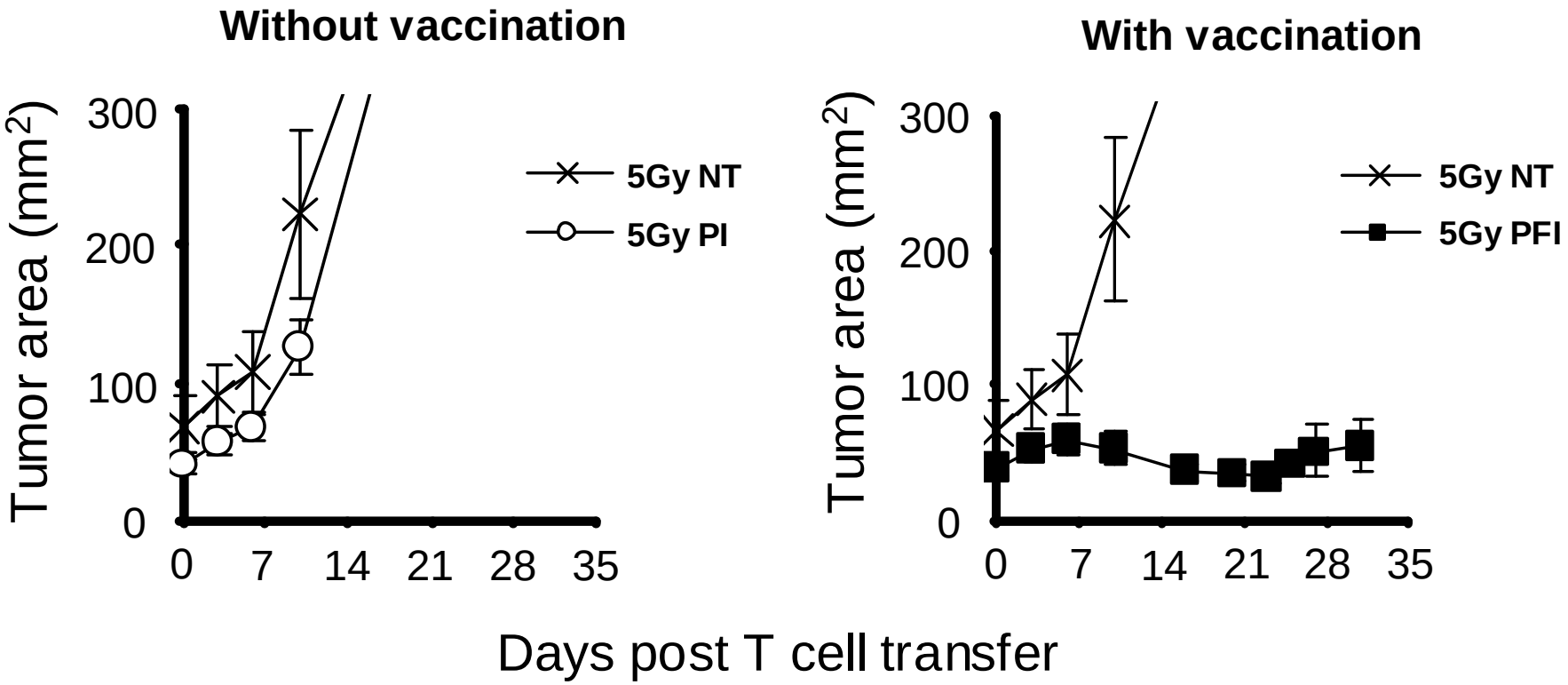
Objective response rates (RECIST) in patients with metastatic melanoma treated at the National Cancer Institute, Surgery Branch



Modified from Rosenberg and Dudley, **COI** 2009.

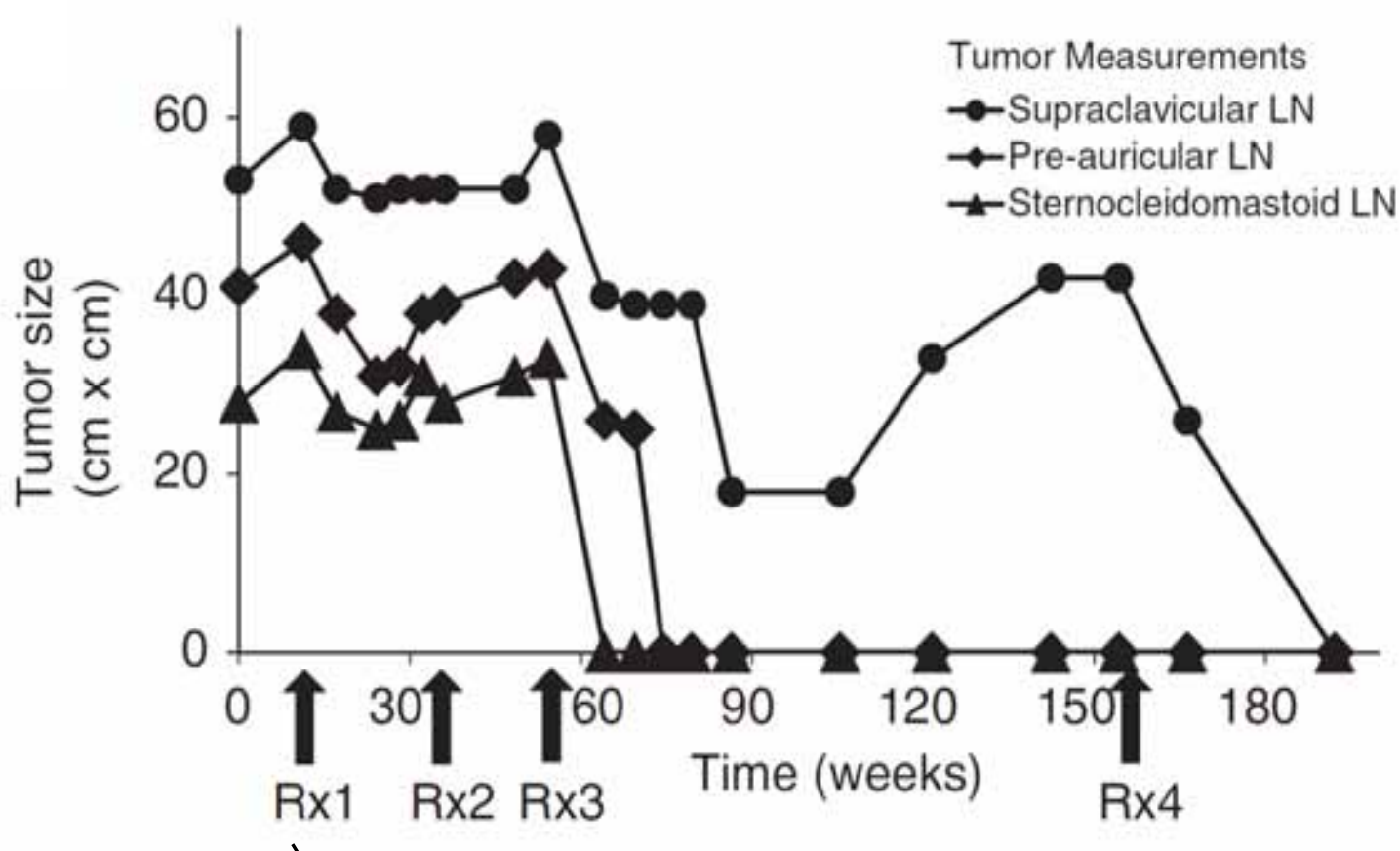

Day -25
Day +34
5.2+ Years

Antigen stimulation can potently enhance the function of adoptively transferred T cells in mouse and man



Modified from: Overwijk WW *et al*, **JEM** 2003.
Wrzesinski C *et al*, **JCI** 2007.

Antigen stimulation can potently enhance the function of adoptively transferred T cells in mouse and man



Cy/Flu + 200 cGY TBI
IV TIL, HD IL-2

IA TIL, HD IL-2

Cy/Flu, IV TIL, HD IL-2,
rFPgp100 2092M

Cy/Flu, IV TIL, HD IL-2,
rFPgp100 2092M

Modified from:
Smith FO *et al*, **JIT** 2009.

Translation of concomitant Ag-stimulation following ACT is technically and practically challenging

Table 3. Treatment Characteristics

Characteristic	No. of Patients	%
Antigens recognized		
Unknown/autologous only	9	26
Gp100 only	2	6
MART-1 only	12	34
Autologous and gp100	2	6
Autologous and MART-1	7	20
Gp100 and MART-1	2	6
Autologous, MART-1 and gp100	1	3

Contains autologous reactivity **55%**

Dudley ME *et al*, **JCO** 2005.

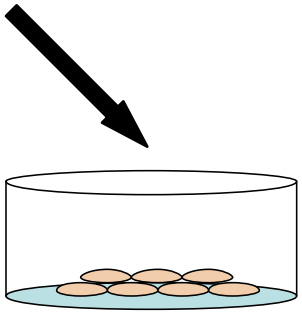
Hypothesis

Antigen-experienced $CD8^+$ T cells may be "programmed" to execute an effector response with a limited duration *in vitro* stimulation, bypassing the requirement for systemic vaccination following ACT for optimal tumor treatment.

Kaech SM and Ahmed R, **Nat. Immunol.** 2001.
Von Stipdonk MJ *et al*, **Nat Immunol.** 2001 & 2003.

Experimental approach

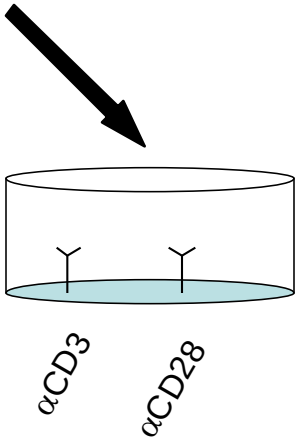
In vitro differentiated Pmel T_{EM}



Autologous hgp100$_{25\text{-}33}$ pulsed irradiated splenocytes

Stimulation x 12hr →72hr

In vitro differentiated Pmel T_{EM}



Stimulation x 12hr →72hr

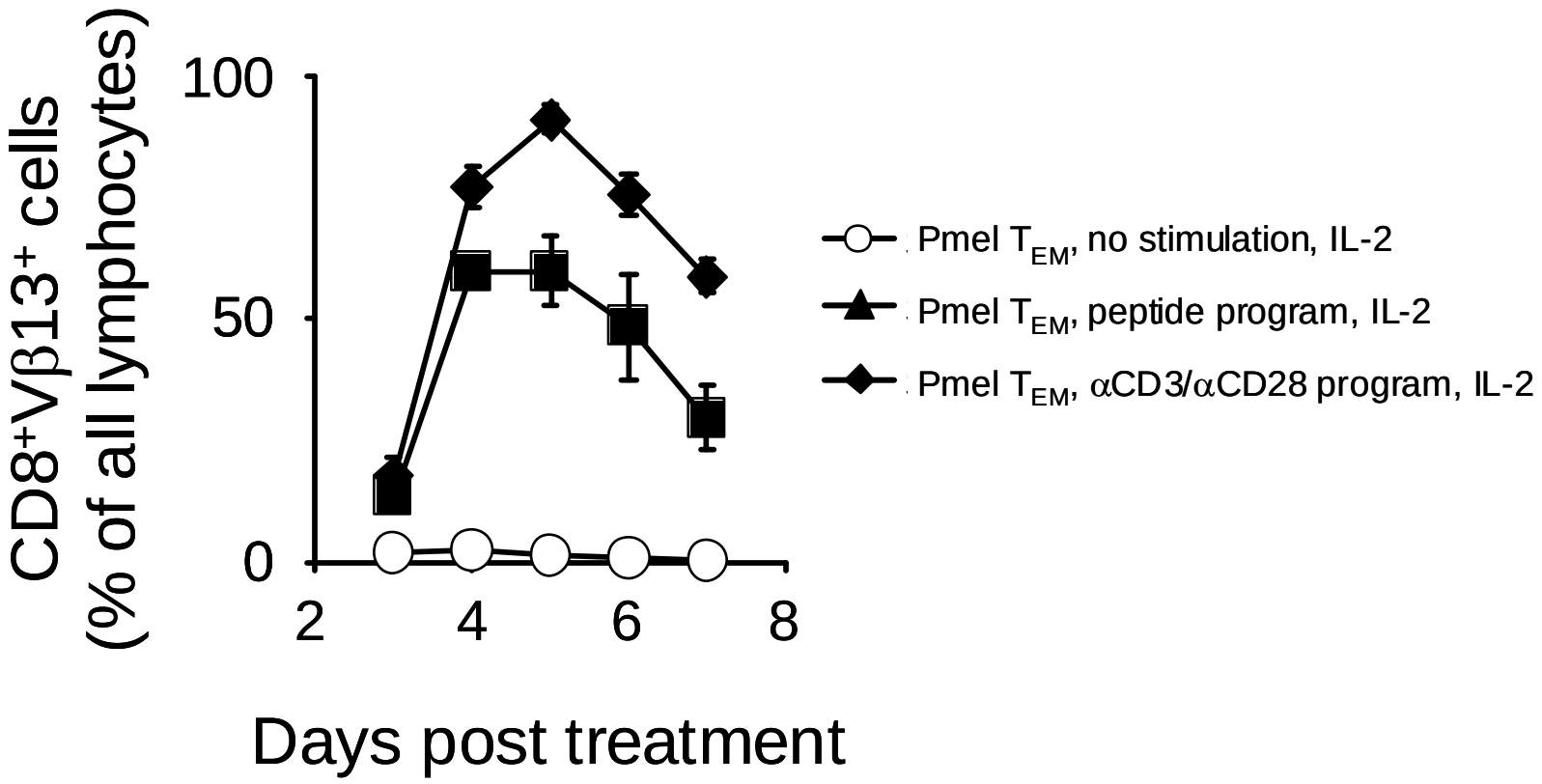
Adoptive transfer into mice bearing established B16 melenoma:

- ✓Lymphodepletion
- ✓Exogenous IL-2 support

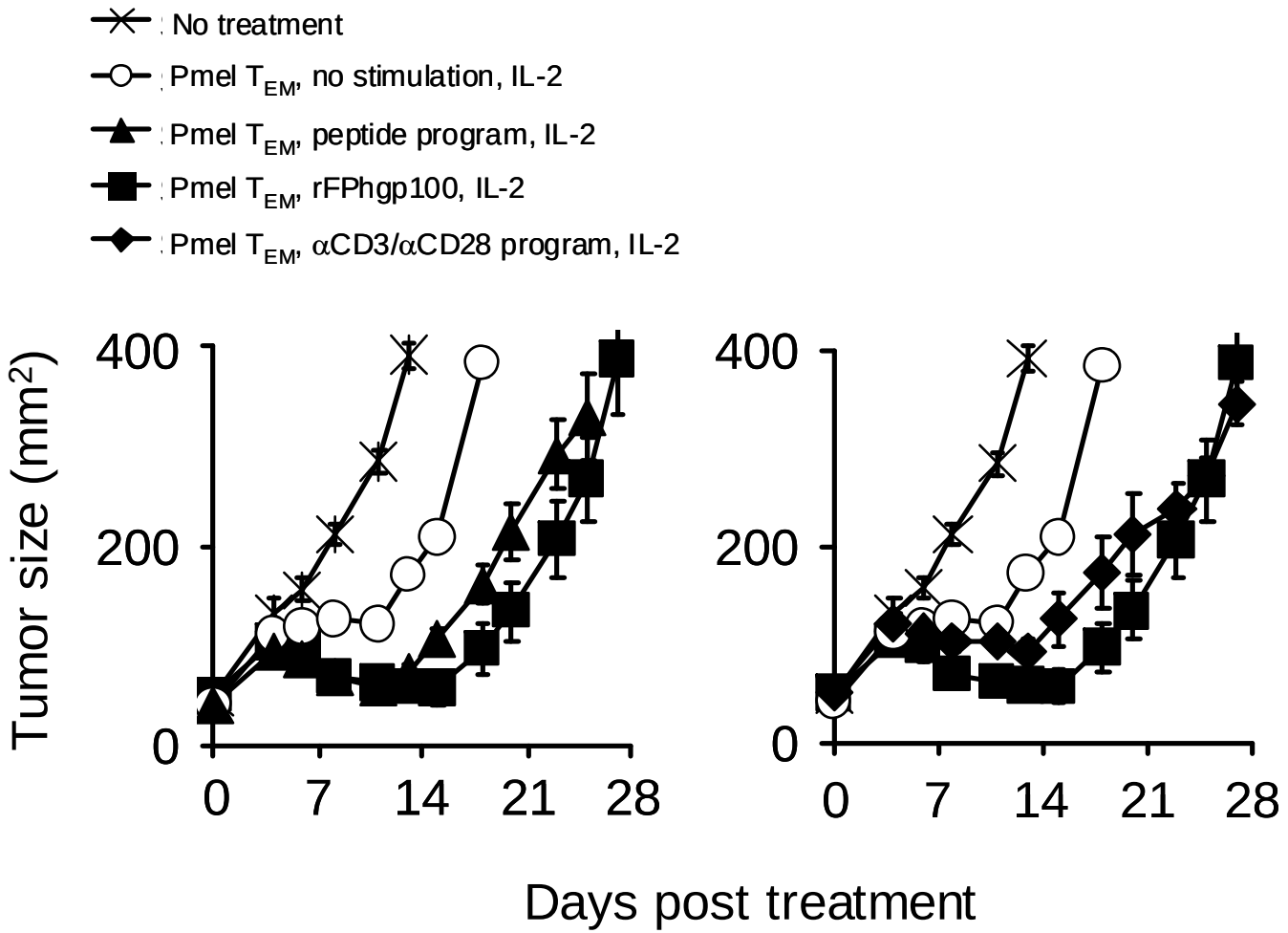
Endpoints:

- ✓In vivo proliferation
- ✓Tumor treatment

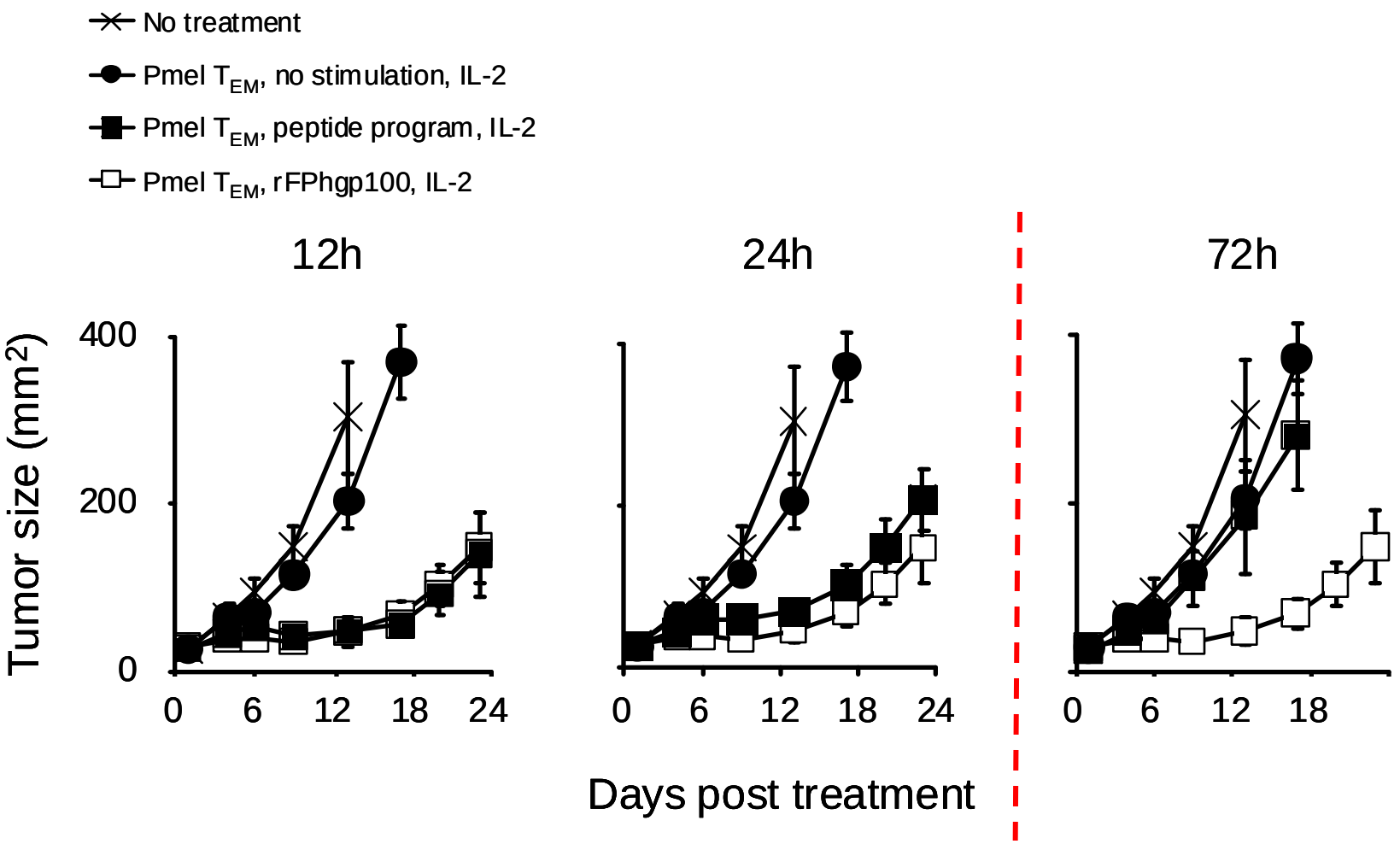
Programmed CD8$^+$ T cells execute a similar *in vivo* proliferative response compared with vaccine stimulated T cells



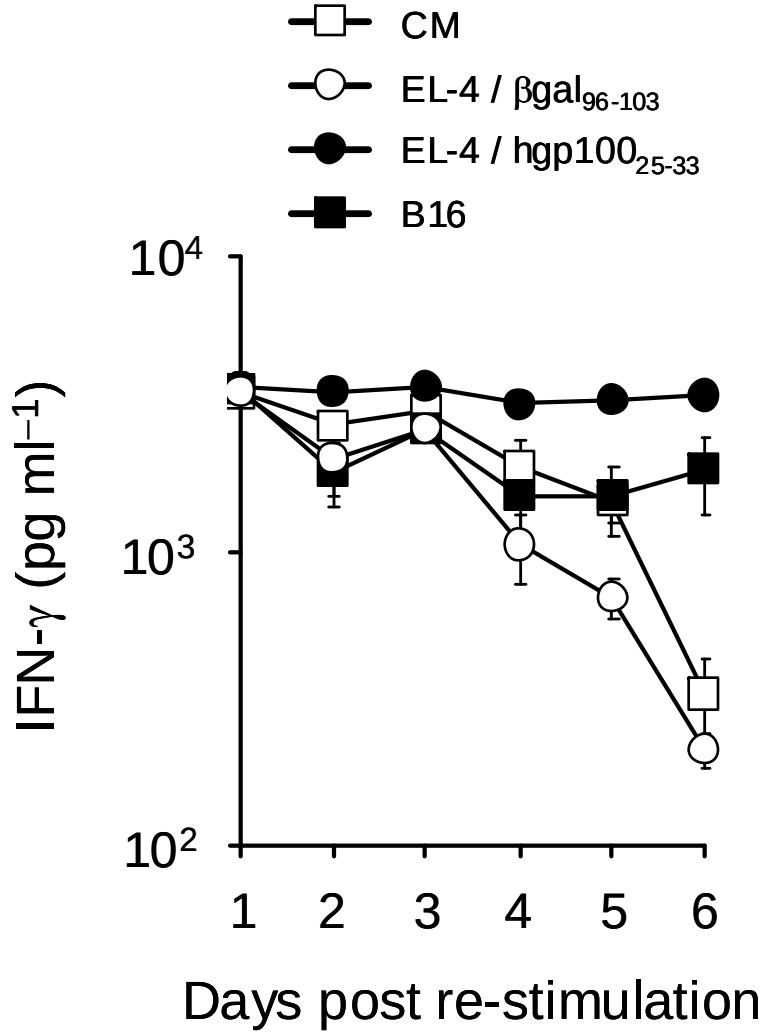
Programming antigen-experienced tumor-reactive T cells prior to ACT replaces the need for vaccination



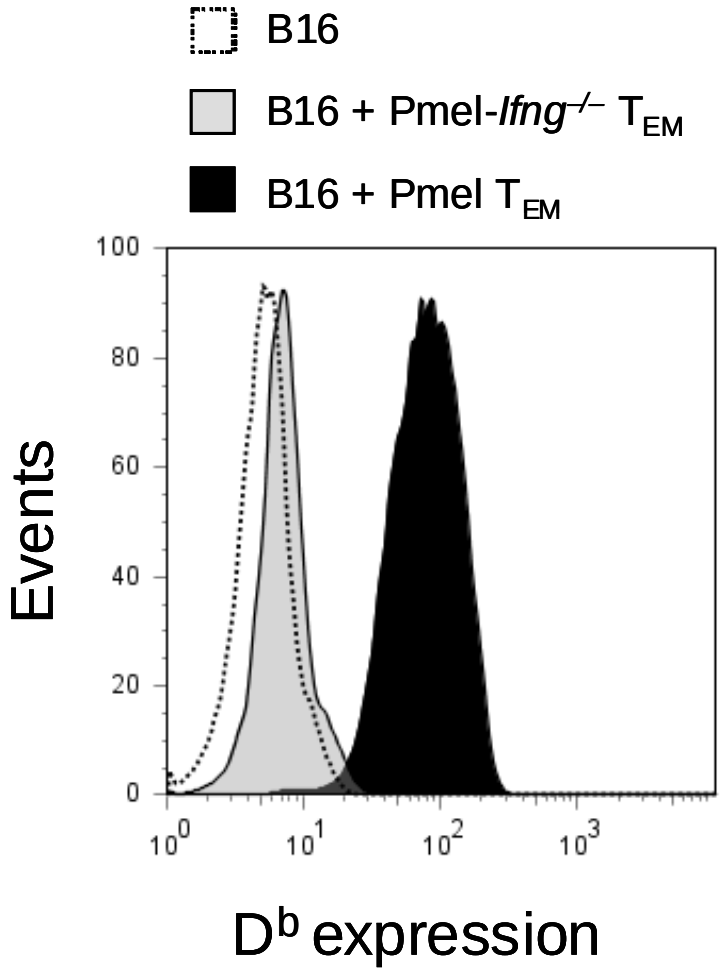
Delayed transfer of programmed CD8$^+$ T cells impairs anti-tumor treatment efficacy

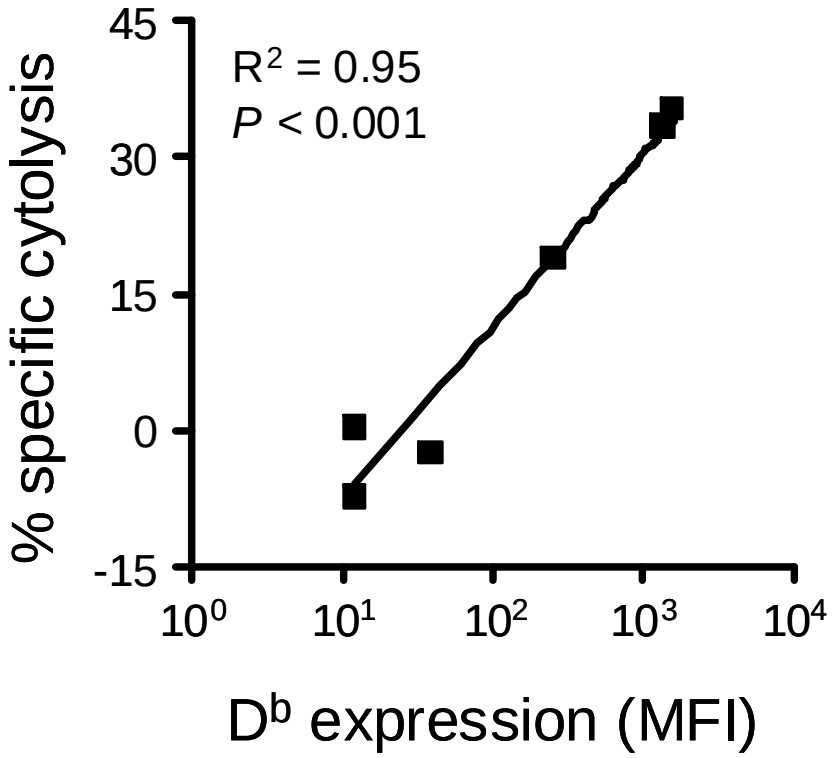


Programming CD8$^+$ T cells incites an interval of antigen independent IFNγ release



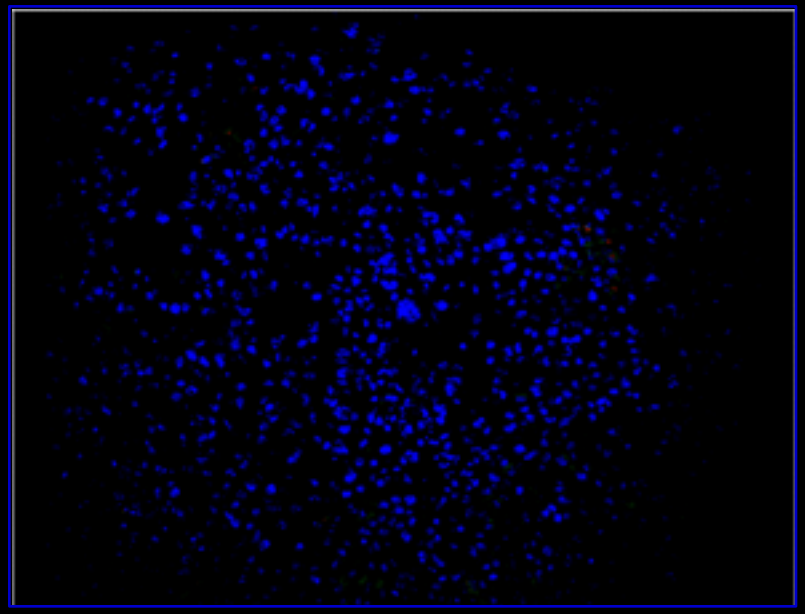
IFNγ production by programmed CD8$^+$ T cells enhances tumor D^b expression and subsequent tumor kill *in vitro*



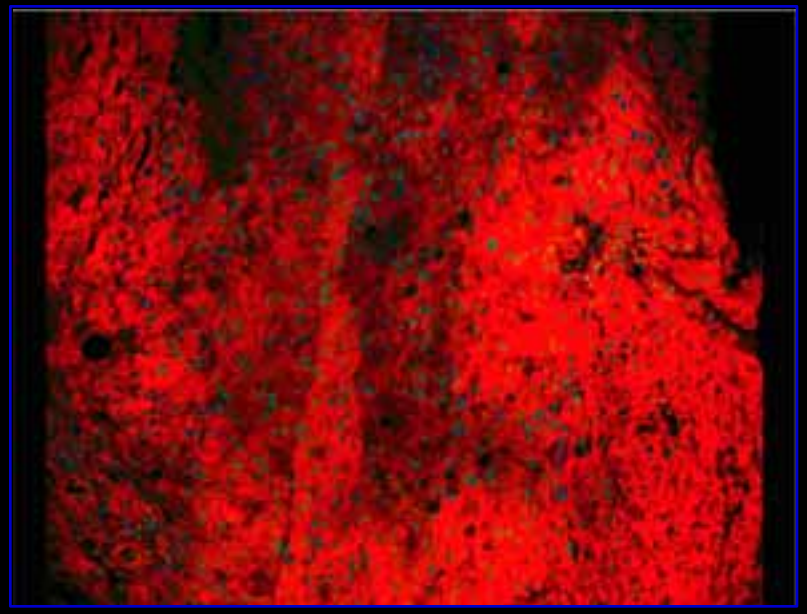


IFNγ production by activated CD8^{+} T cells enhances tumor D^{b} expression *in vivo*

No treatment



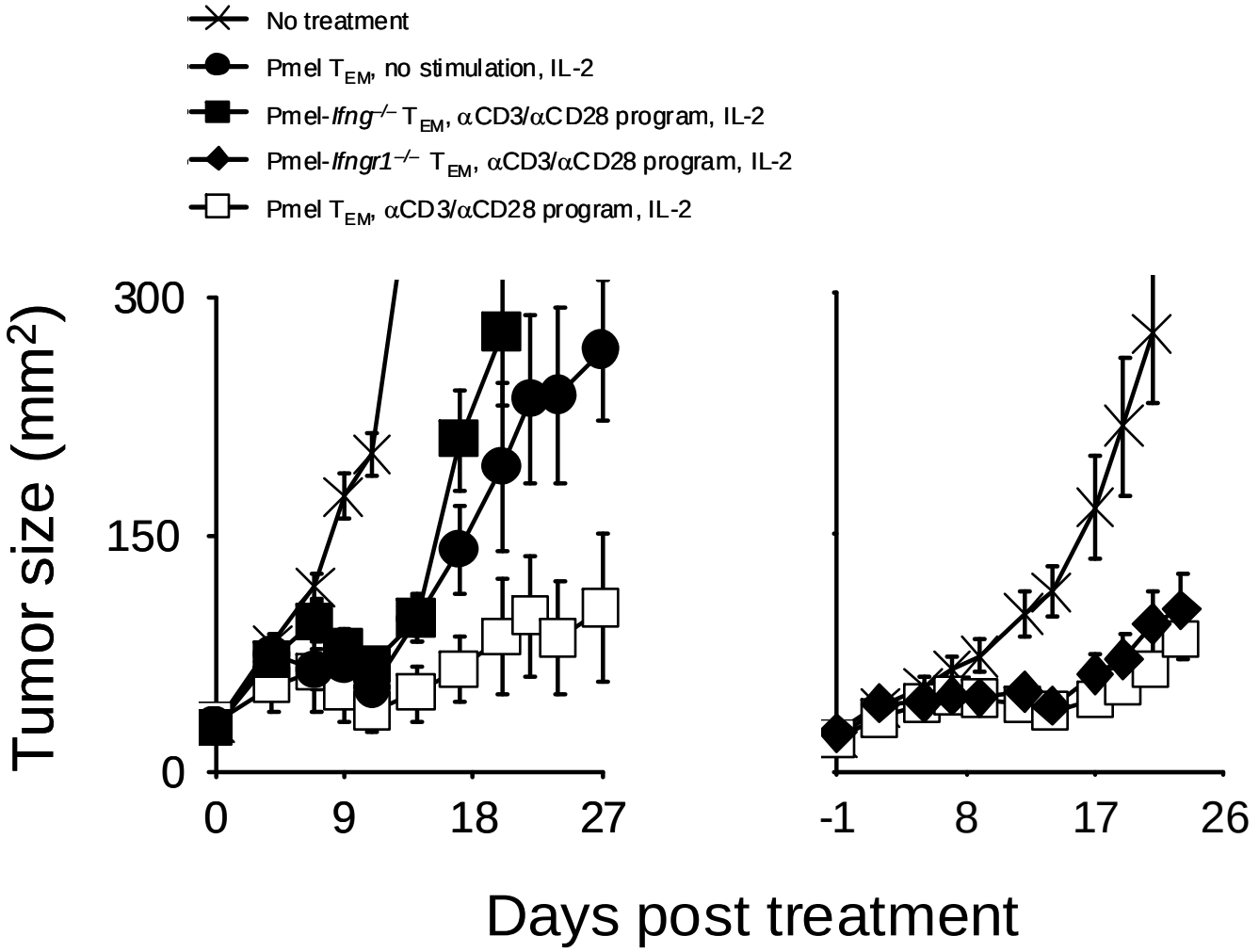
Activated Pmel



DAPI / D^{b}

Modified from Palmer DC *et al*, **PNAS** 2008.

The *in vivo* therapeutic benefit of *in vitro* programmed CD8$^+$ T cells is IFNγ-dependent



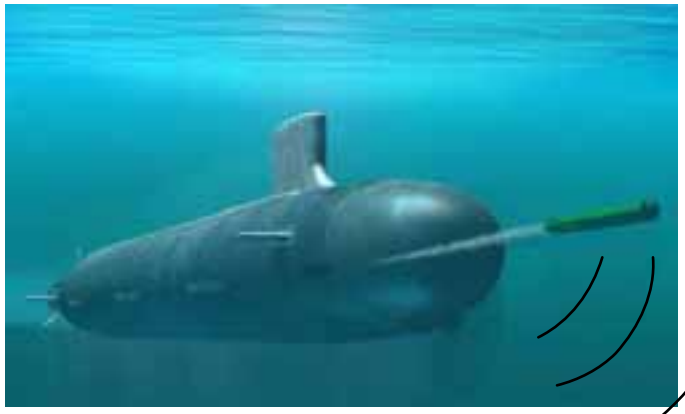
Conclusions

- *In vitro* programming tumor-reactive $CD8^+$ T cells prior to ACT with either peptide-pulsed irradiated feeders or combined αCD3/αCD28 stimulation can bypass the requirement for *in vivo* vaccination.

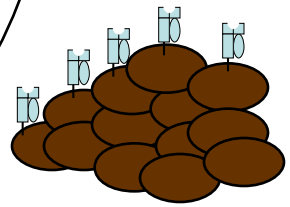
- *In vitro* programming incites an interval of antigen-independent IFNγ release that facilitates tumor recognition both *in vitro* and *in vivo*.

- The benefit of *in vitro* programming $CD8^+$ T cells prior to adoptive transfer is entirely dependent on the ability to release IFNγ.

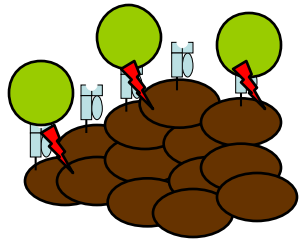
A model for “immunologic sonar”



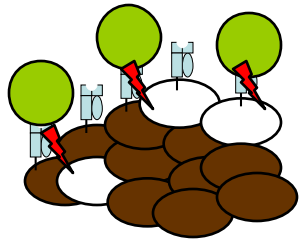
IFNγ



B16 tumor deposit

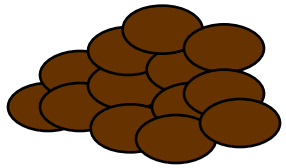


B16 tumor deposit

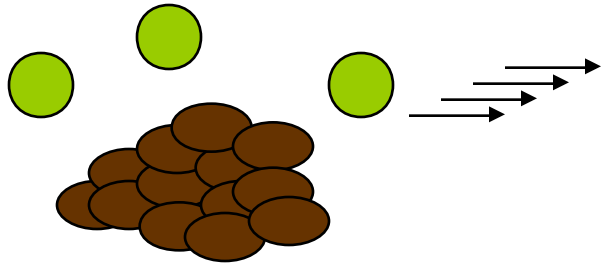


B16 tumor deposit

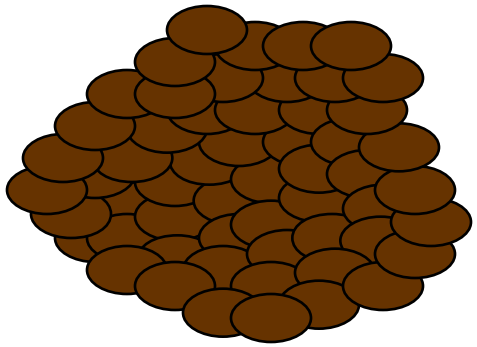
In vitro programmed Pmel **IFNγ$^{-/-}$** T_{EM}



B16 tumor deposit



B16 tumor deposit



B16 tumor deposit

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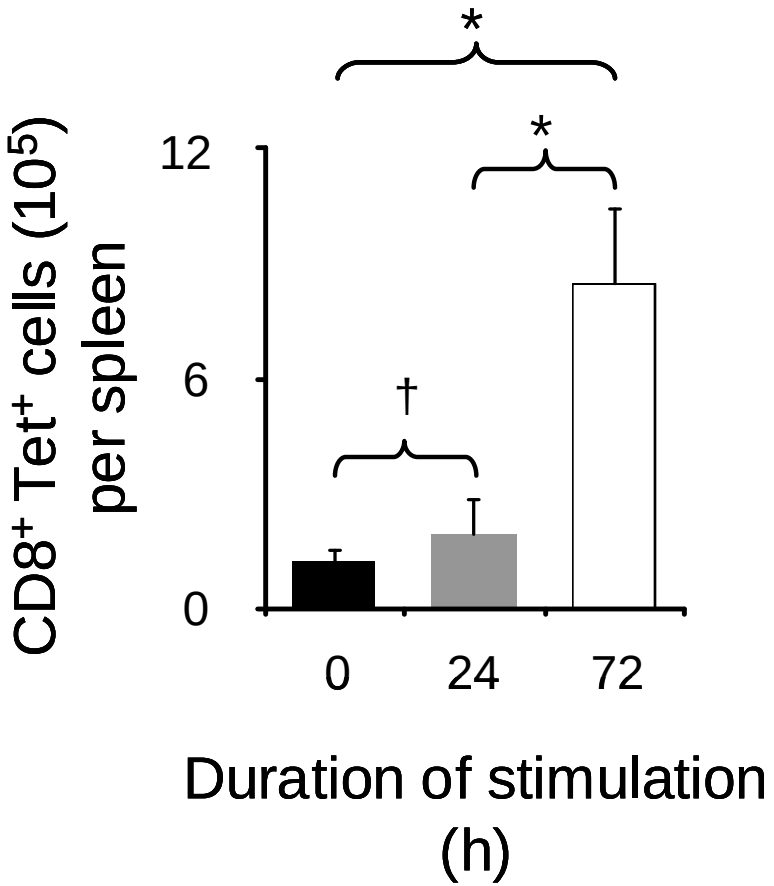
Howard Hughes Medical Institute

Delayed transfer does not impair the relative engraftment efficiency of transferred CD8+ T cells



† = $P > 0.05$; * = $P < 0.05$

Antigen stimulation can potently enhance the function of adoptively transferred T cells in mouse and man

TABLE 1. Schedule of the 4 Treatments Administered on the 3 Protocols of Adoptive Cell Therapy

Day of Treatment	−7	−6	−5	−4	−3	−2	−1	0	1	2	3	//	30	31	32	33
Rx1	Cy	Cy	Flu	Flu	Flu	Flu	Flu TBI	TIL (IV) IL-2	IL-2	IL-2 CD34+						
Rx2								TIL (IA) IL-2	IL-2	IL-2						
Rx3 and Rx4	Cy	Cy	Flu	Flu	Flu	Flu	Flu	Fpgp100 TIL (IV) IL-2	IL-2	IL-2			Fpgp100 IL-2	IL-2	IL-2	

TABLE 2. Characteristics of Treatments*

			Phenotype by FACS (%)				Melanoma Cell Lines				T2 Cells/Peptide (μM)				
					CD8+		HLA-A2−		HLA-A2+		MART	gp100	gp209-2M		
Infusion	Cell Number ($\times 10^9$)	IL-2 (Doses)	CD8+	CD4+	gp209+	MART-1+	888	938	526	624	(1.0)	(1.0)	(1.0)	(0.1)	(0.01)
											Interferon-γ (pg/mL)				
Rx1	31.0	11	65	25	6.3	0.09	5	75	1090	2210	81	8720	2540	12180	1280
Rx2	26.0	10	77	13	2.2	0.92	146	130	7230	13120	49	8500	670	556	482
Rx3	47.0	12, 3	84	6	7.4	0.41	29	110	1760	3260	39	6900	6110	4810	2420
Rx4	22.7	8, 7	85	7	6.5	0.31	57	579	3470	9180	20	17000	10970	8130	5710