Strategies to Enhance Dendritic Cell-Mediated Antitumor Immunity

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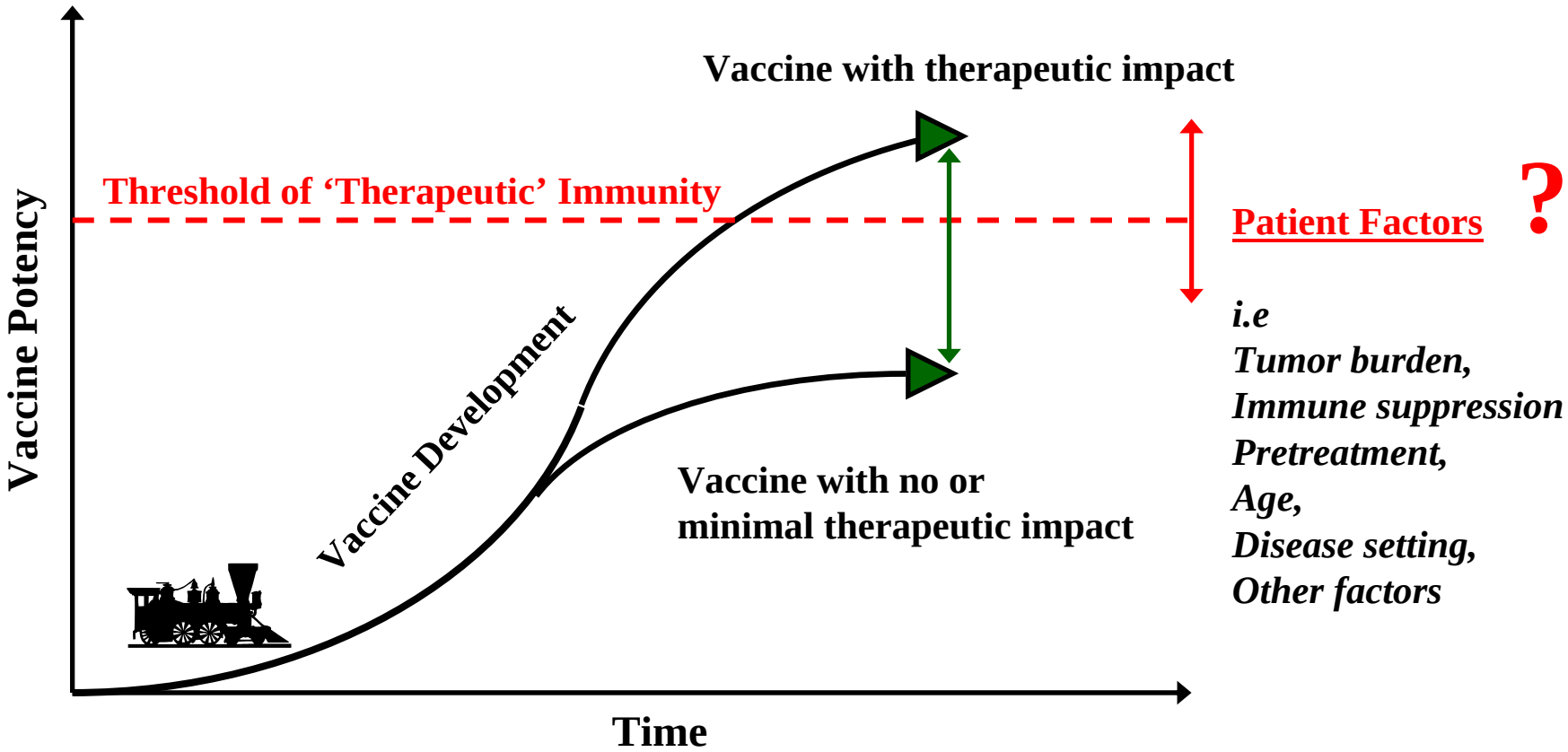
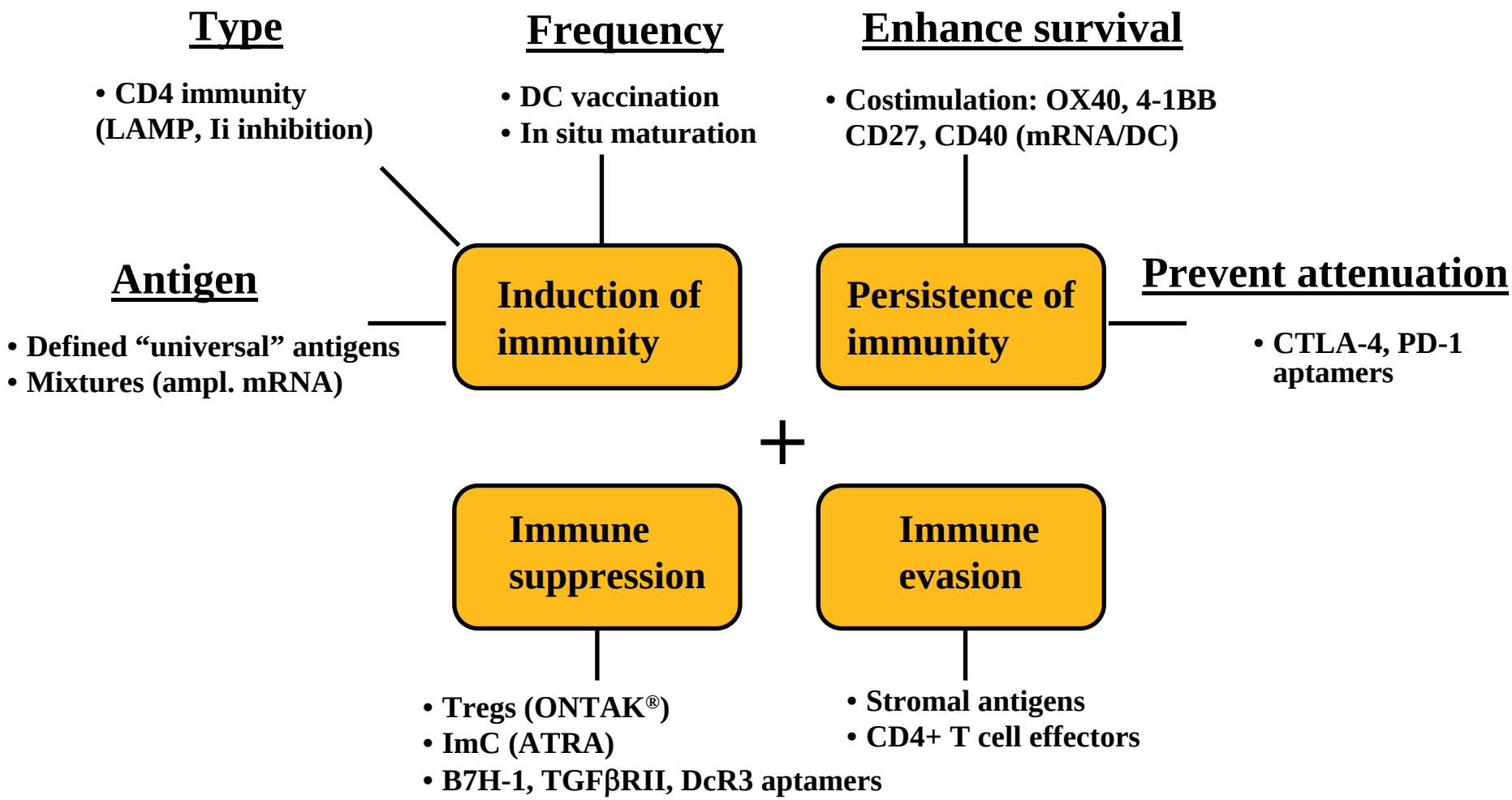
Objective

Therapeutic Immunity

Vaccine Potency = Magnitude x Quality x Persistence



A Multi-Pronged Approach to Cancer Immunotherapy



Activation of T–Cells by APC

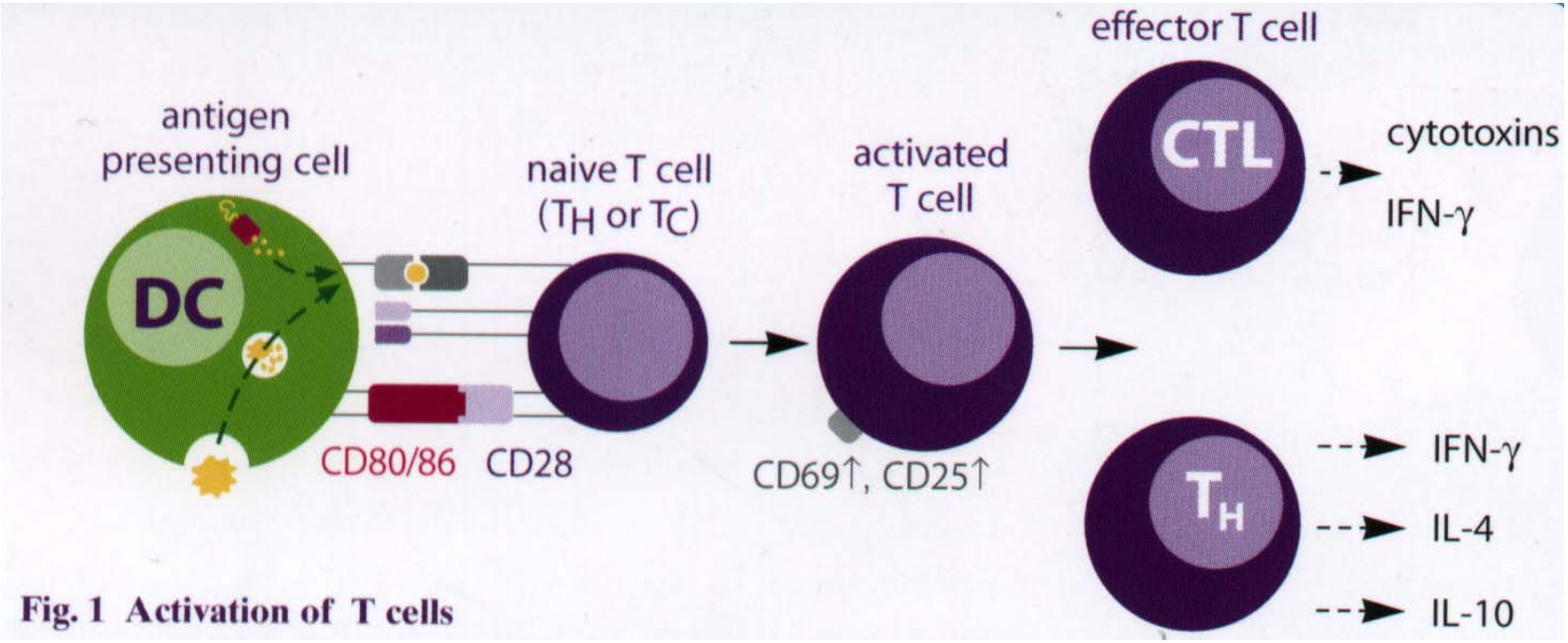


Fig. 1 Activation of T cells

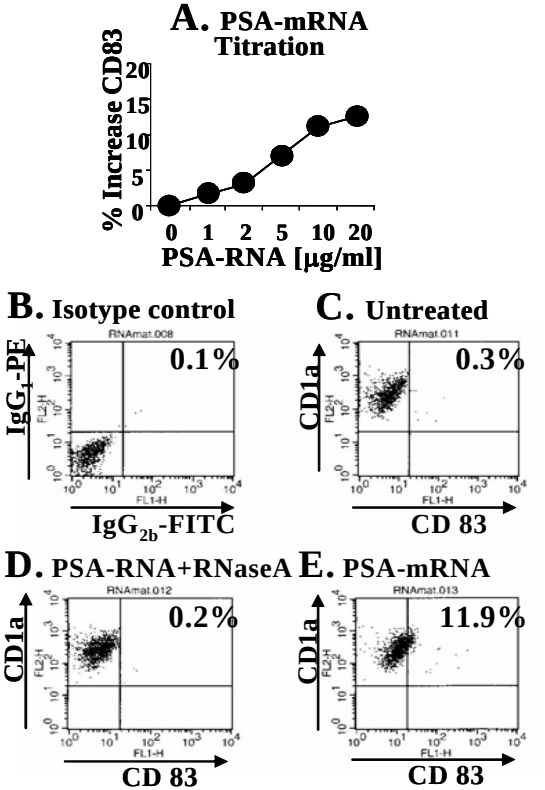
From: Abbas et al. Cancer Immunology

Dendritic cell-based vaccines using tumor antigen in the form of mRNA

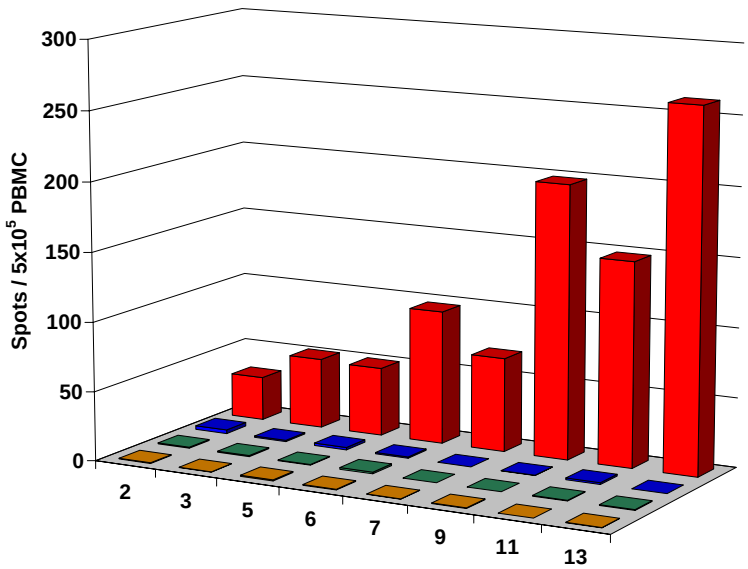
- **Powerful method for stimulating antitumor immunity**
- **Broadly applicable to all cancer types**
- **Solution to the problem of treatment-related emergence of resistant variants**

Background: Phase I Clinical Trial using semi-mature PSA RNA transfected DC

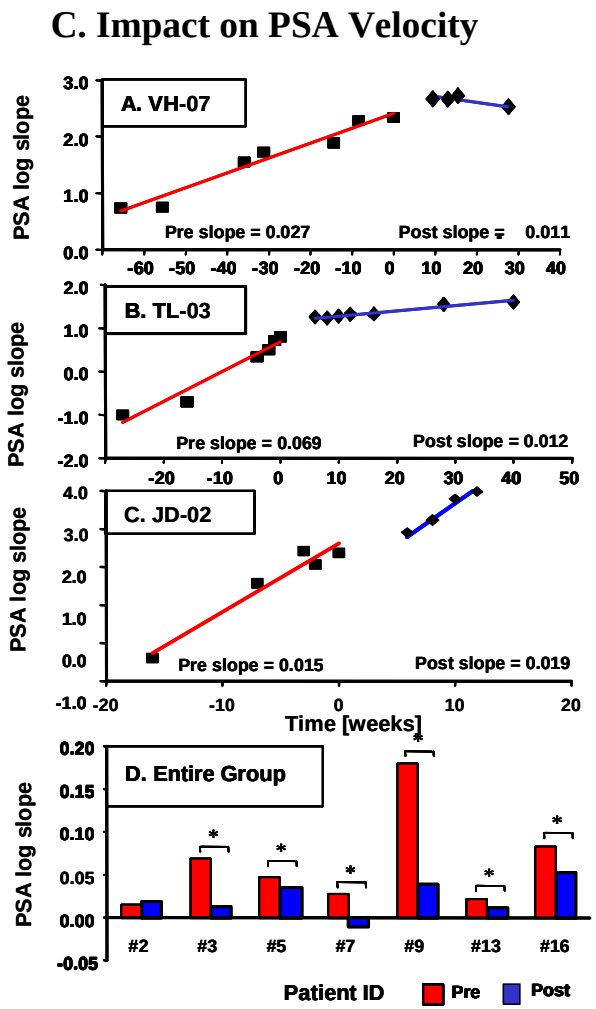
A. Phenotype after RNA Loading

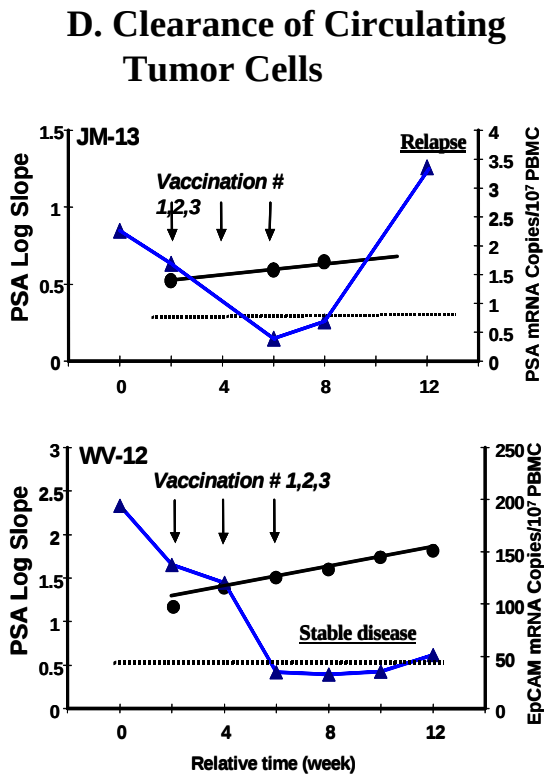


B. Evidence of Immunogenicity

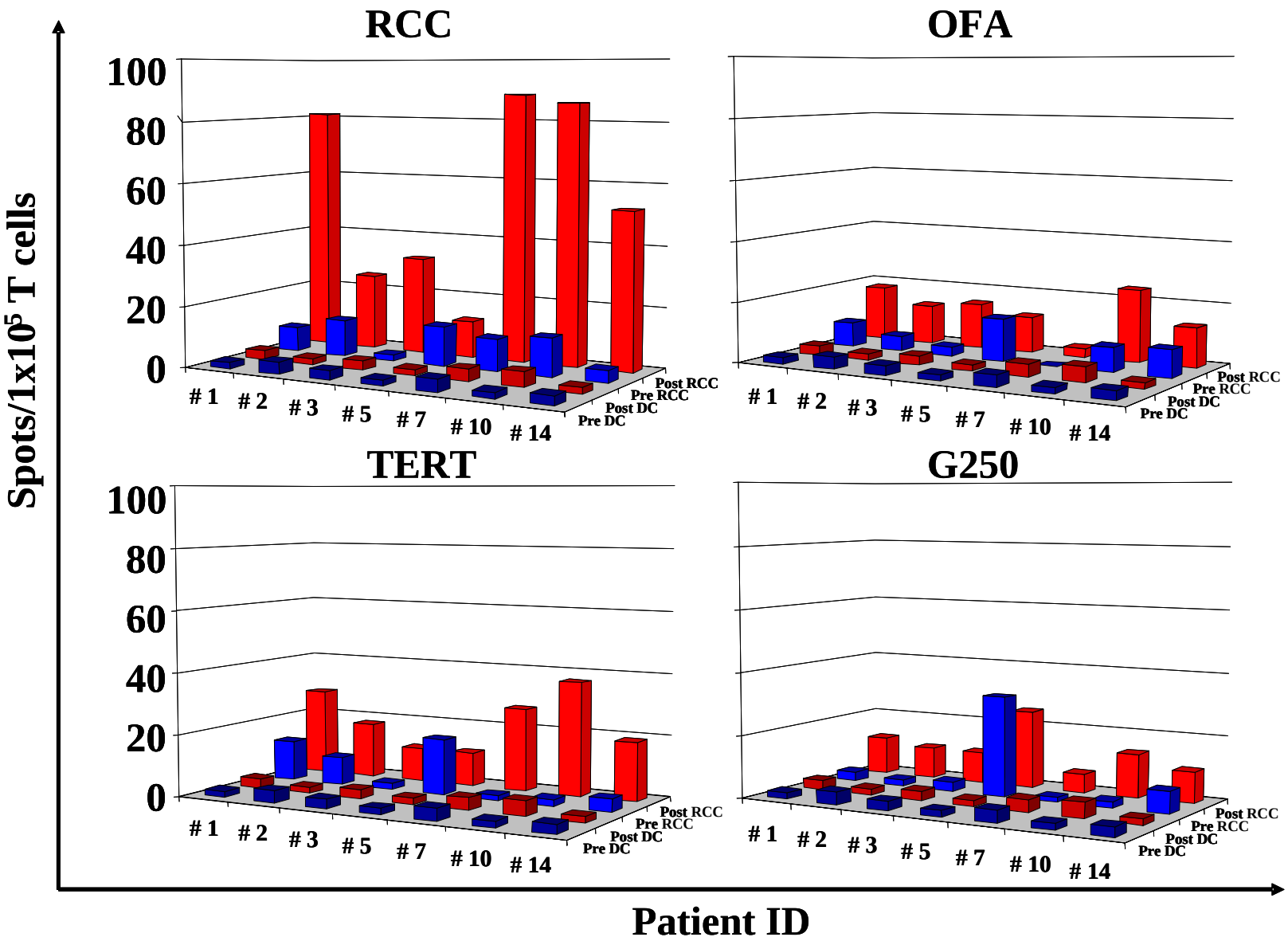


Background: Phase I Clinical Trial using semi-mature PSA RNA transfected DC

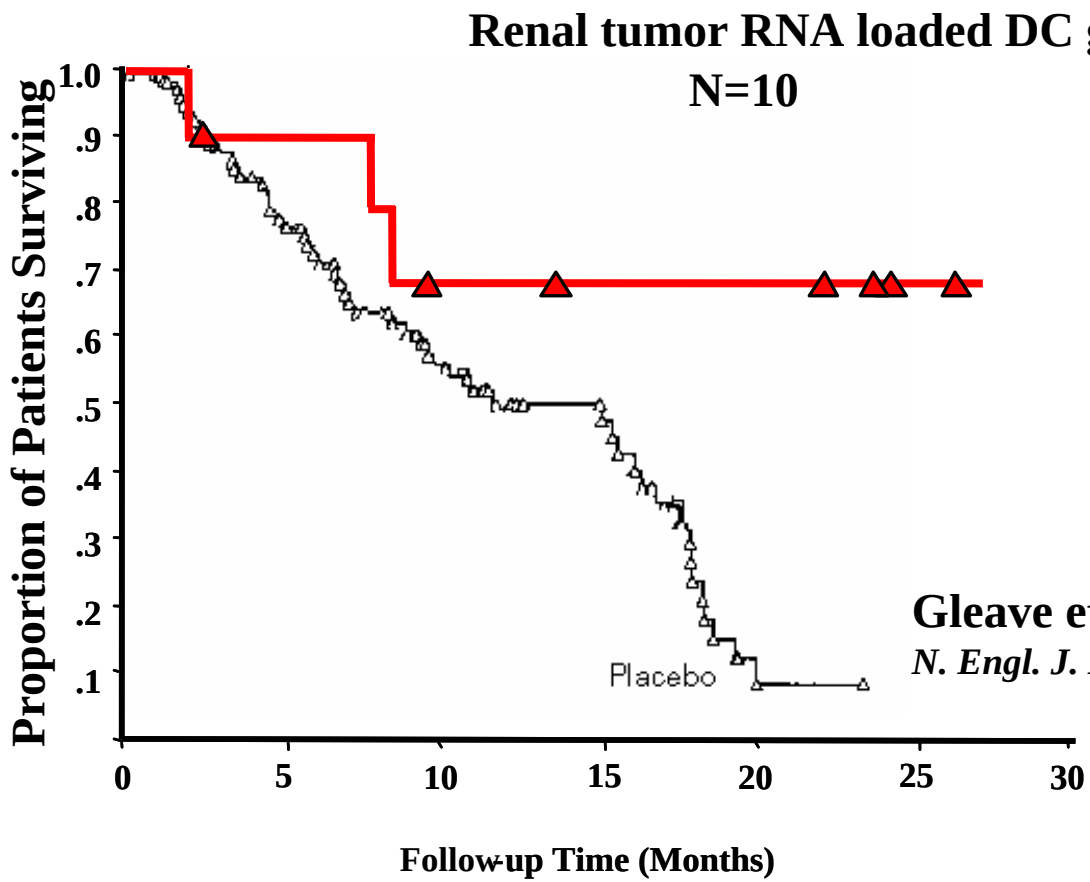




Increase of Tumor-Specific T Cells after Vaccination with semi-mature RCC RNA transfected DC



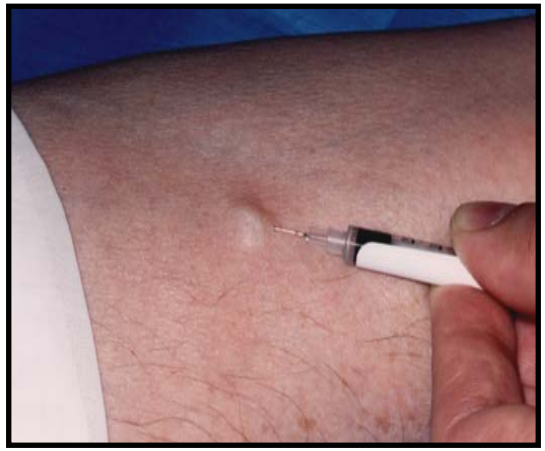
Survival of Subjects immunized with Renal Tumor RNA Loaded DC



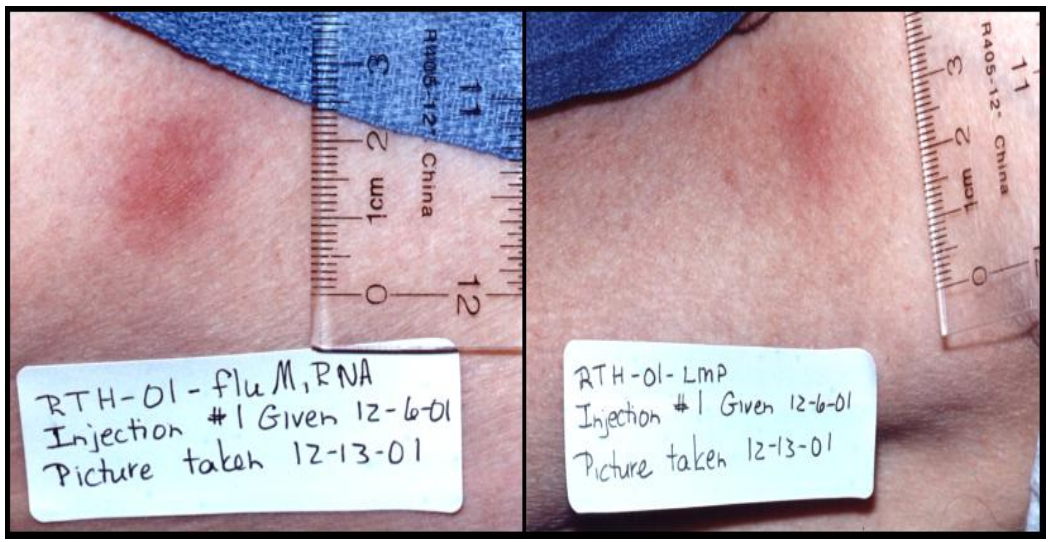
Gleave et al. N=188

N. Engl. J. Med. 338:1265, 1998

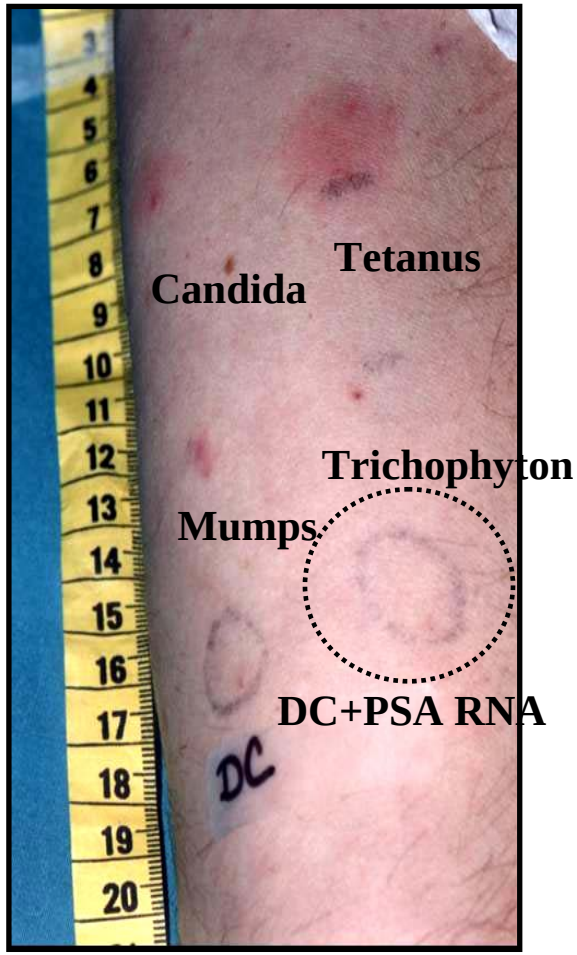
Mature, but not Immature TERT RNA Loaded DC Elicit A Local Inflammatory Response at the Injection Site



Injection Technique

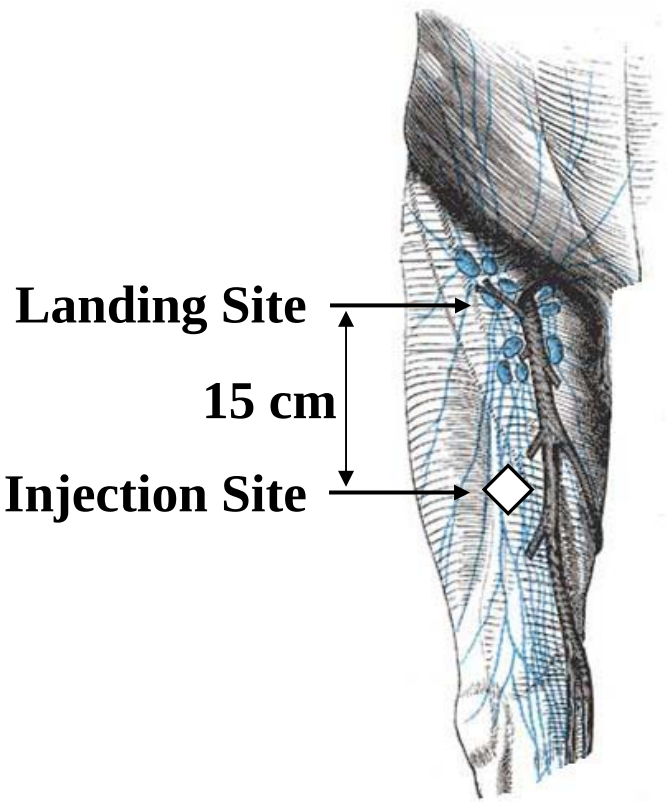


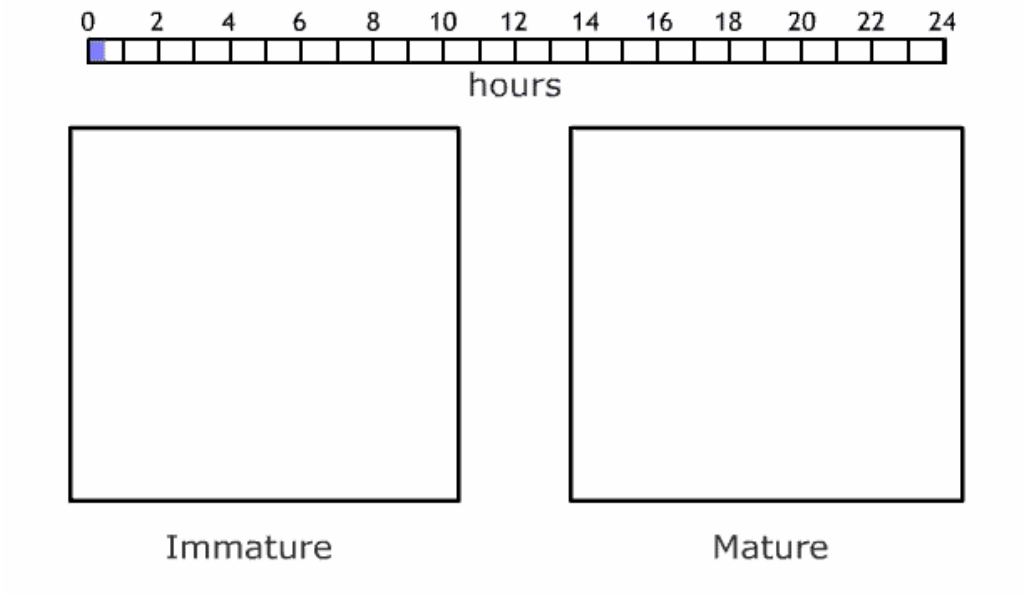
Mature LAMP-TERT RNA Transfected DC



Immature PSA RNA Transfected DC

Mature, but not Immature Dendritic Cells are Capable of Migrating to Draining Lymph Nodes

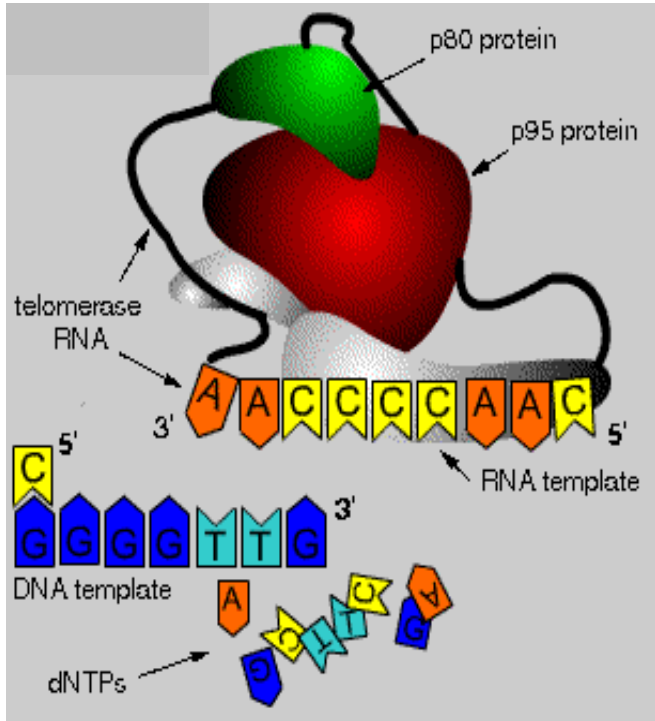




Telomerase (hTERT)

A Broadly Expressed Candidate Tumor Antigen

- **hTERT can be processed for class I presentation in a broad range of human tumors.**
- **Telomerase is an attractive candidate for a broadly expressed tumor rejection antigen**
  - **Silent in most somatic tissues**
  - **Reactivated and over-expressed in the majority of human solid tumors**
- **Reduced risk of antigen-escape tumor cell variants.**



Targeting mRNA-encoded antigens into the endosomal/lysosomal compartment

A.

pGEM4Z/hTERT/A64

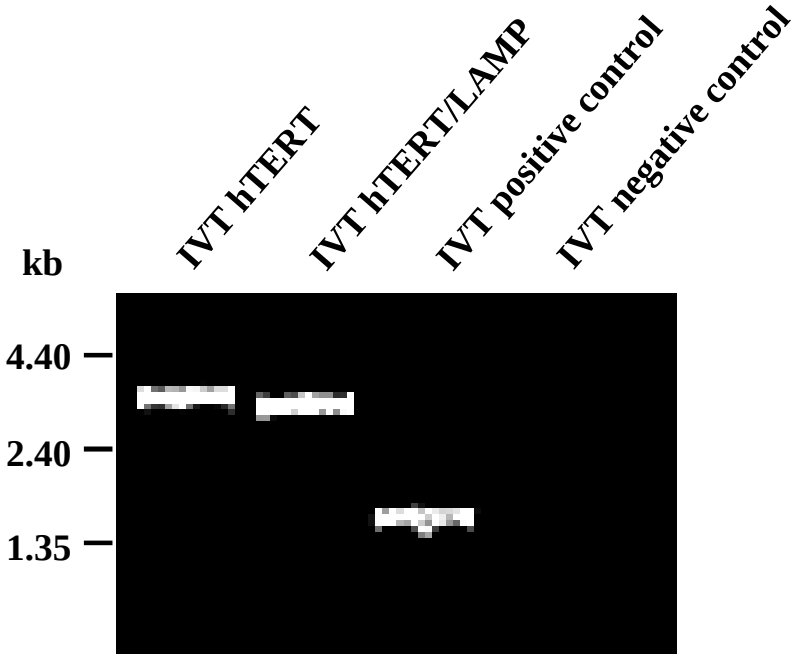
pGEM4Z/hTERT- LAMP/A64

gp96 aa1-27

Leader sequence

hLAMP aa 382-416

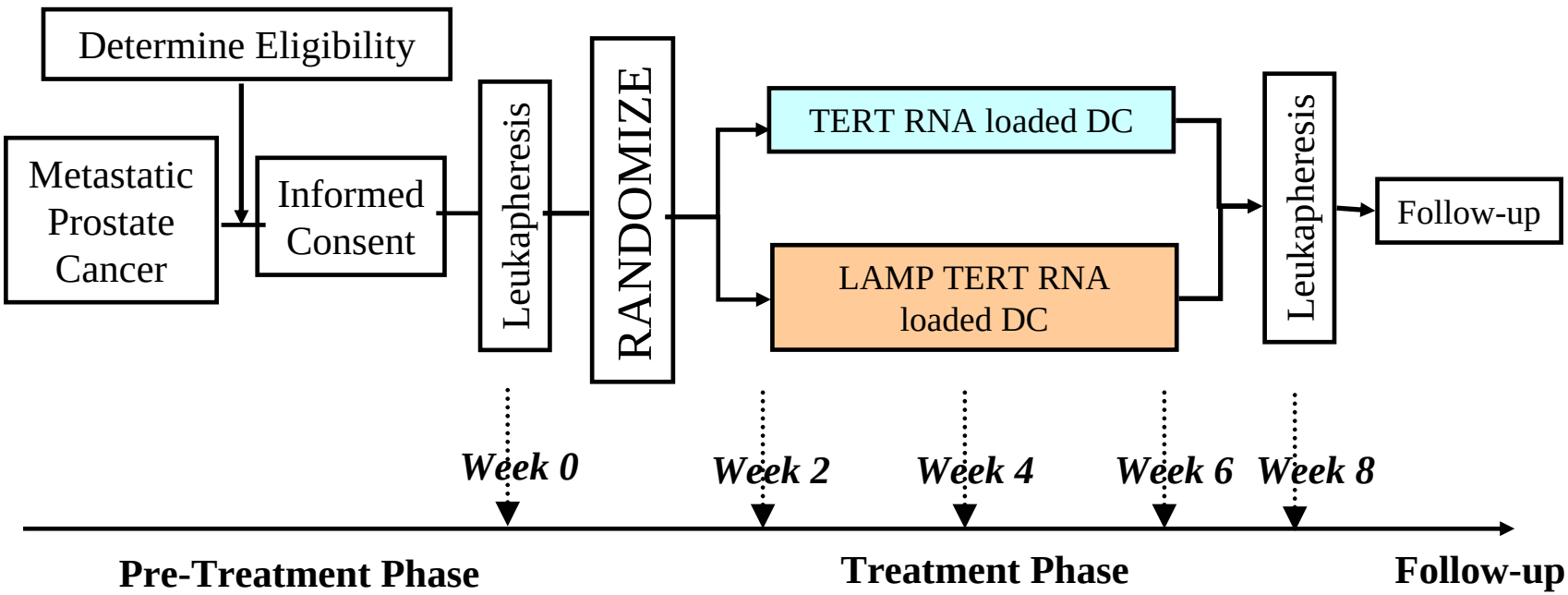
B.



Cancer Research 62 (15): 5322-5328 (2002)

TERT mRNA-Transfected DC
Clinical Trial Design

Dose Schedule A: 3 cycles of $1x10^7$ cells i.d. per cycle
Dose Schedule B: 6 cycles of $1x10^7$ cells i.d. per cycle



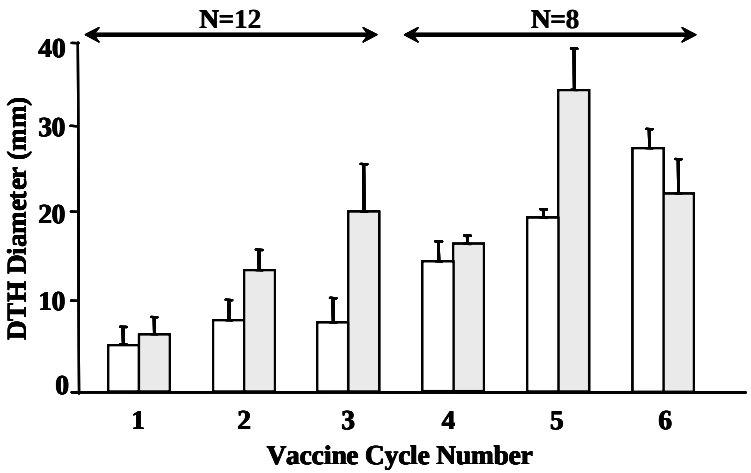
Patient Characteristics

Table 1. Characteristics of subjects enrolled

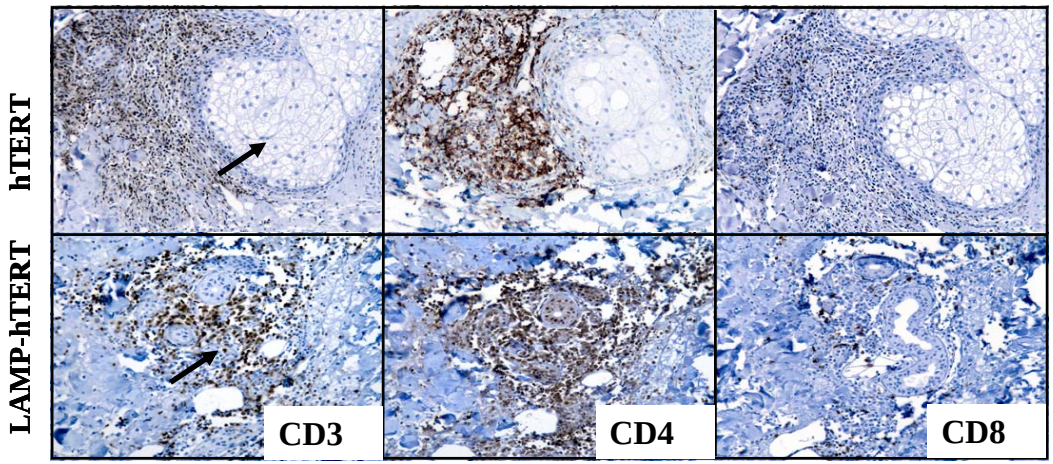
Subject ID No.	Age	Karnofsky Index	Diagnosis of Metastases - Treatment (Months)	Stage (Jewett)	Prior Therapy	Pretreatment PSA (ng/dl)	Metastases (Study Entry)	Assigned Dose Level (Total Dose)	Cell Product (mRNA-Transfected DC)
RTH-01-LMP	68	100	148	D2	RP/XRT[1]/H	4.5	BN	$3x10^7$	LAMP hTERT
RFB-02-TRT	75	90	14	D2	XRT[2]/H	1.6	BN	$3x10^7$	hTERT
DEM-03-LMP	70	100	56	D3	RP/H	54.3	LN/BN	$3x10^7$	LAMP hTERT
RNR-04-TRT	65	90	36	D3	RP/XRT[1]/O	10.7	LN	$3x10^7$	hTERT
BRH-05-TRT	50	100	21	D1	RP	0.3	LN	$3x10^7$	hTERT
DGE-06-LMP	63	100	90	D1	RP/XRT[1]	7.3	LN	$3x10^7$	LAMP hTERT
GWN-08-TRT	58	100	71	D3	RP/XRT[1]/H/C	111.3	LN/BN	$3x10^7$	hTERT
JLA-09-TRT	47	100	26	D3	H	2.9	LN/BN	$3x10^7$	hTERT
TJL-10-LMP	64	90	64	D3	XRT[2]/H/C	60.4	BN/ST	$3x10^7$	LAMP hTERT
JDS-11-TRT	59	100	6	D2	H	0.4	BN	$3x10^7$	hTERT
JLB-12-LMP	62	80	22	D3	RP/XRT[1]/H/C	15.6	BN	$3x10^7$	LAMP hTERT
HTD-13-LMP	59	90	74	D1	RP/XRT[1]/H	4.3	LN	$3x10^7$	LAMP hTERT
JCS-14-LMP	63	80	11	D3	H	287.7	BN	$6x10^7$	LAMP hTERT
JRL-15-TRT	67	90	175	D3	RP/XRT[1]/H/O	11.7	BN	$6x10^7$	hTERT
TMS-16-TRT	59	100	96	D1	RP/XRT[1]	0.1	LN	$6x10^7$	hTERT
JOG-17-TRT	68	90	55	D1	RP/H/C	0.9	LN	$6x10^7$	hTERT
AG-18-LMP	71	90	95	D3	H	38.0	BN	$6x10^7$	LAMP hTERT
FSH-19-LMP	52	100	58	D2	XRT[1]/H	0.4	LN/BN	$6x10^7$	LAMP hTERT
PEZ-20-TRT	72	100	6	D3	RP/H	21.7	BN	$6x10^7$	hTERT
CAH-21-TRT	57	90	9	D2	XRT[1]/XRT[2]/O	0.3	BN	$6x10^7$	hTERT

Pre-treatment: XRT[1], primary irradiation; XRT[2], local (palliative) irradiation for painful bony metastases; RP, radical prostatectomy; H, medical hormonal ablative therapy; C, chemotherapy; O, orchiectomy. Metastases: LN, lymphadenopathy; BN, bony metastases; ST, soft tissue metastases.

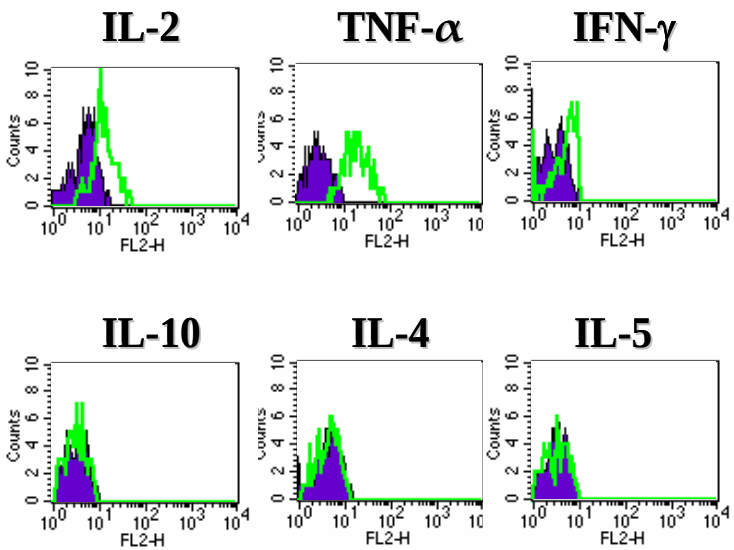
A.


N=12
N=8
DTH Diameter (mm)
40
30
20
10
0
1
2
3
4
5
6
Vaccine Cycle Number

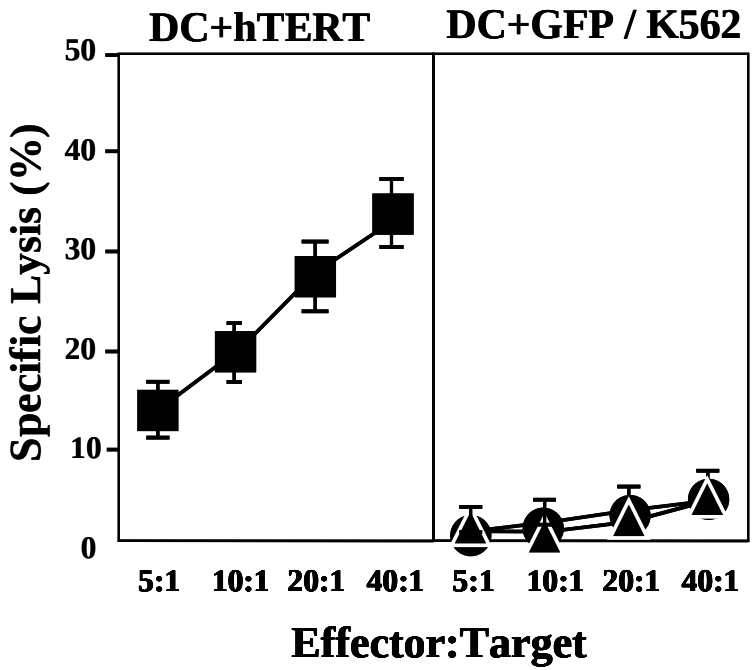
B.


hTERT
LAMP-hTERT
CD3
CD4
CD8

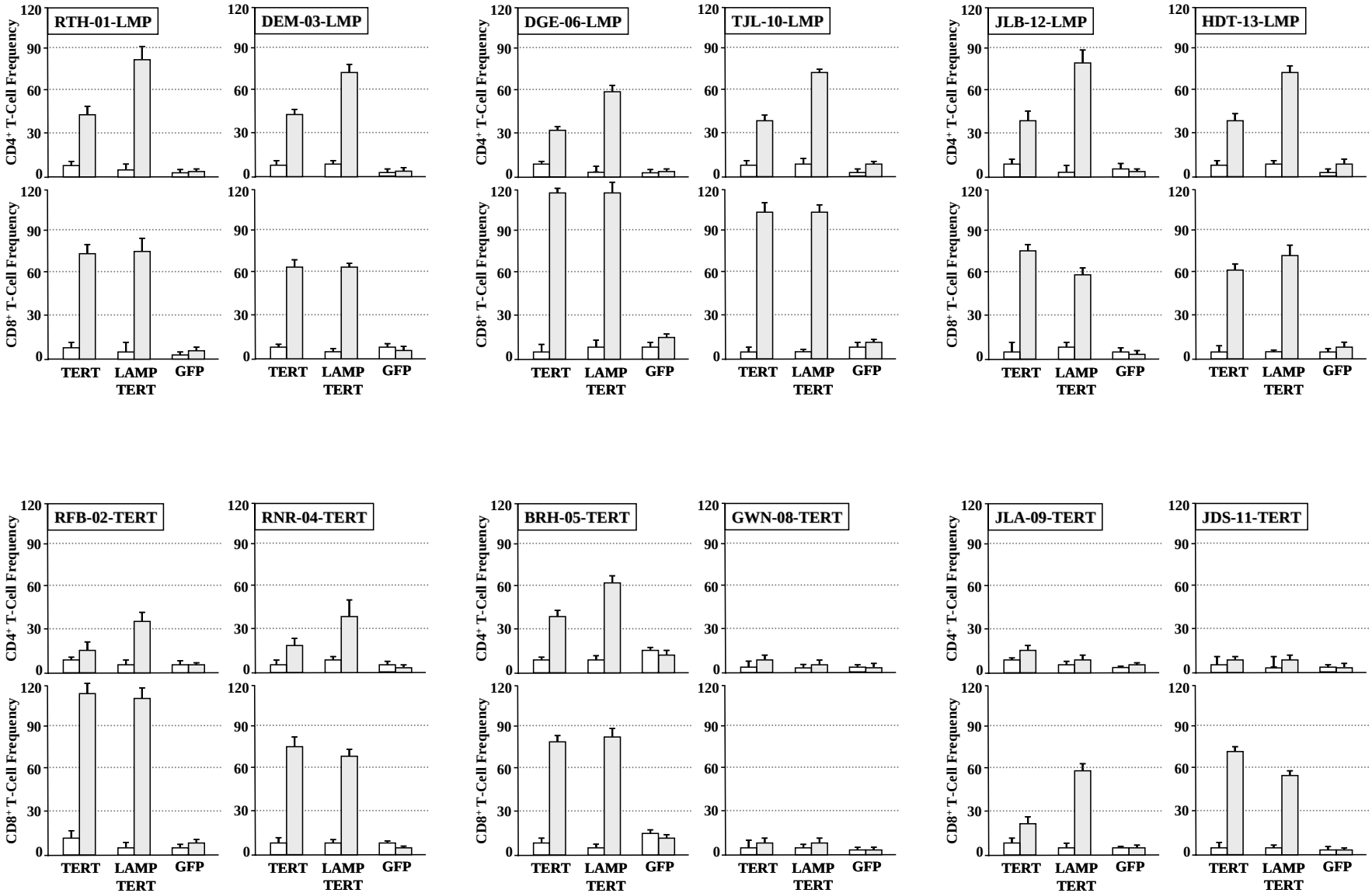
C.


IL-2
TNF-α
IFN-γ
IL-10
IL-4
IL-5
Counts
FL2-H

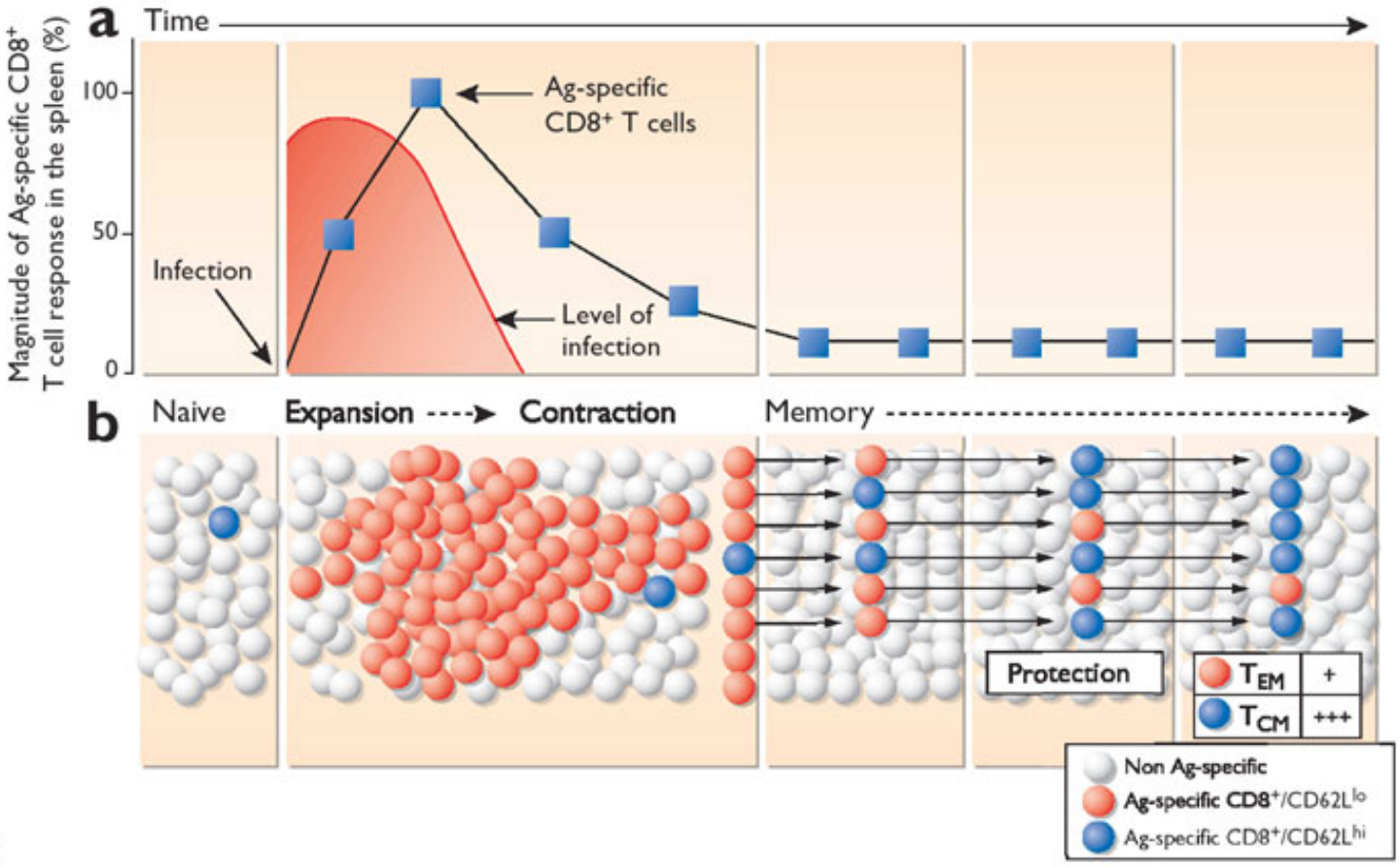
D.


DC+hTERT
DC+GFP / K562
Specific Lysis (%)
50
40
30
20
10
0
5:1
10:1
20:1
40:1
5:1
10:1
20:1
40:1
Effector:Target

Stimulation of hTERT-specific T-cell responses After Vaccination with TERT RNA transfected DC

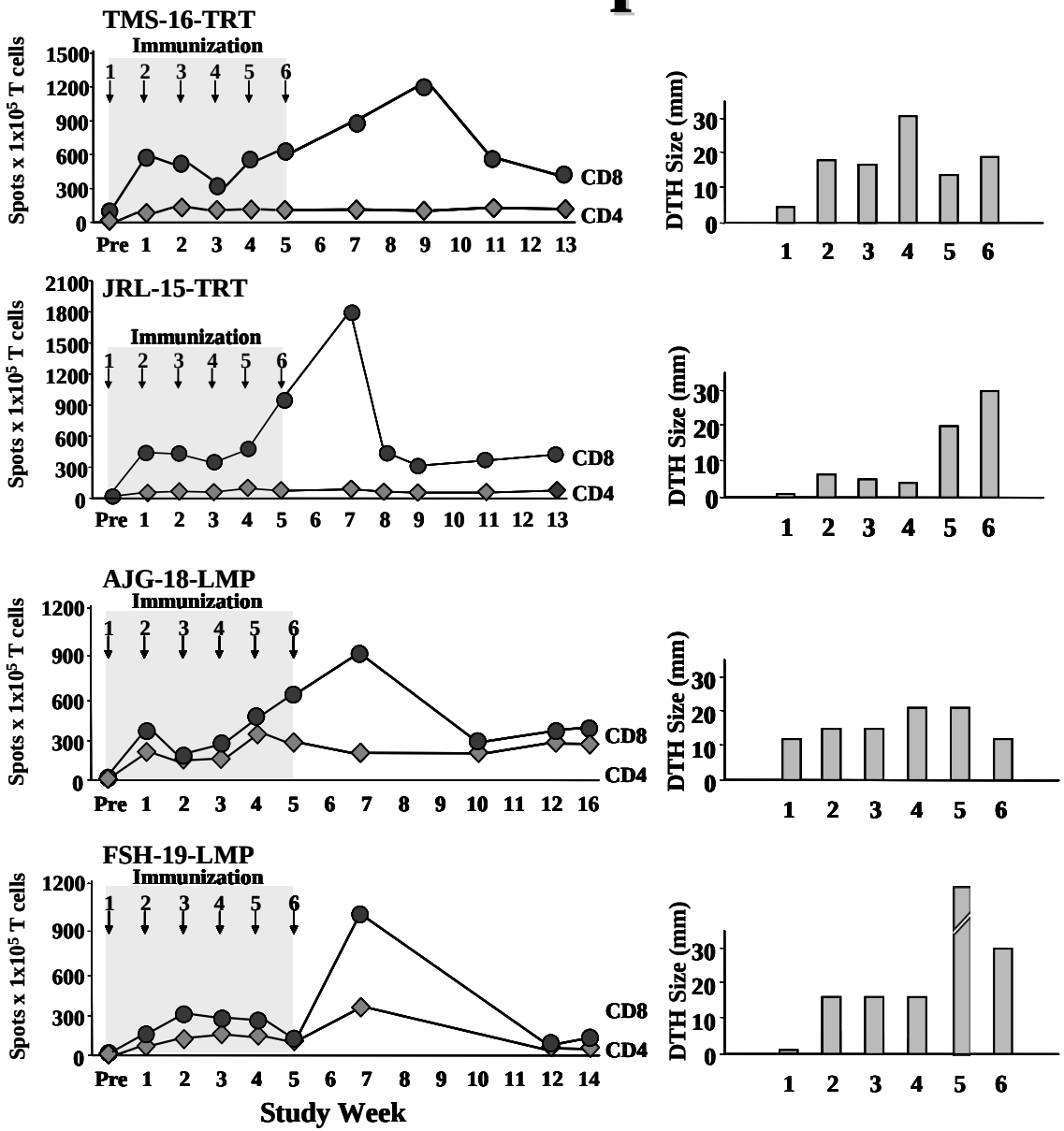


Kinetics of the Antigen-Specific CD8$^+$ T-cell Response



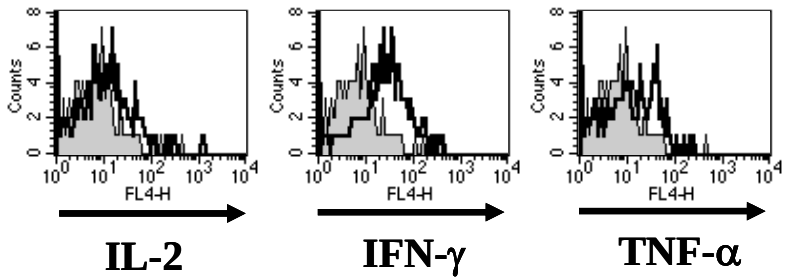
Badovinac et al, Nat Immunol 4:212, 2003

Longitudinal Evolution of $CD8^+$ and $CD4^+$ T cell Responses

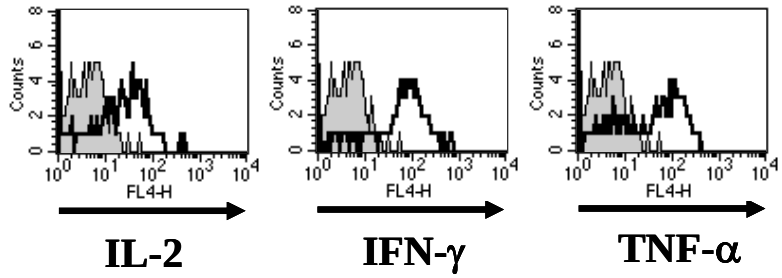


Characterization of Vaccine-induced $CD8^+$ T cells

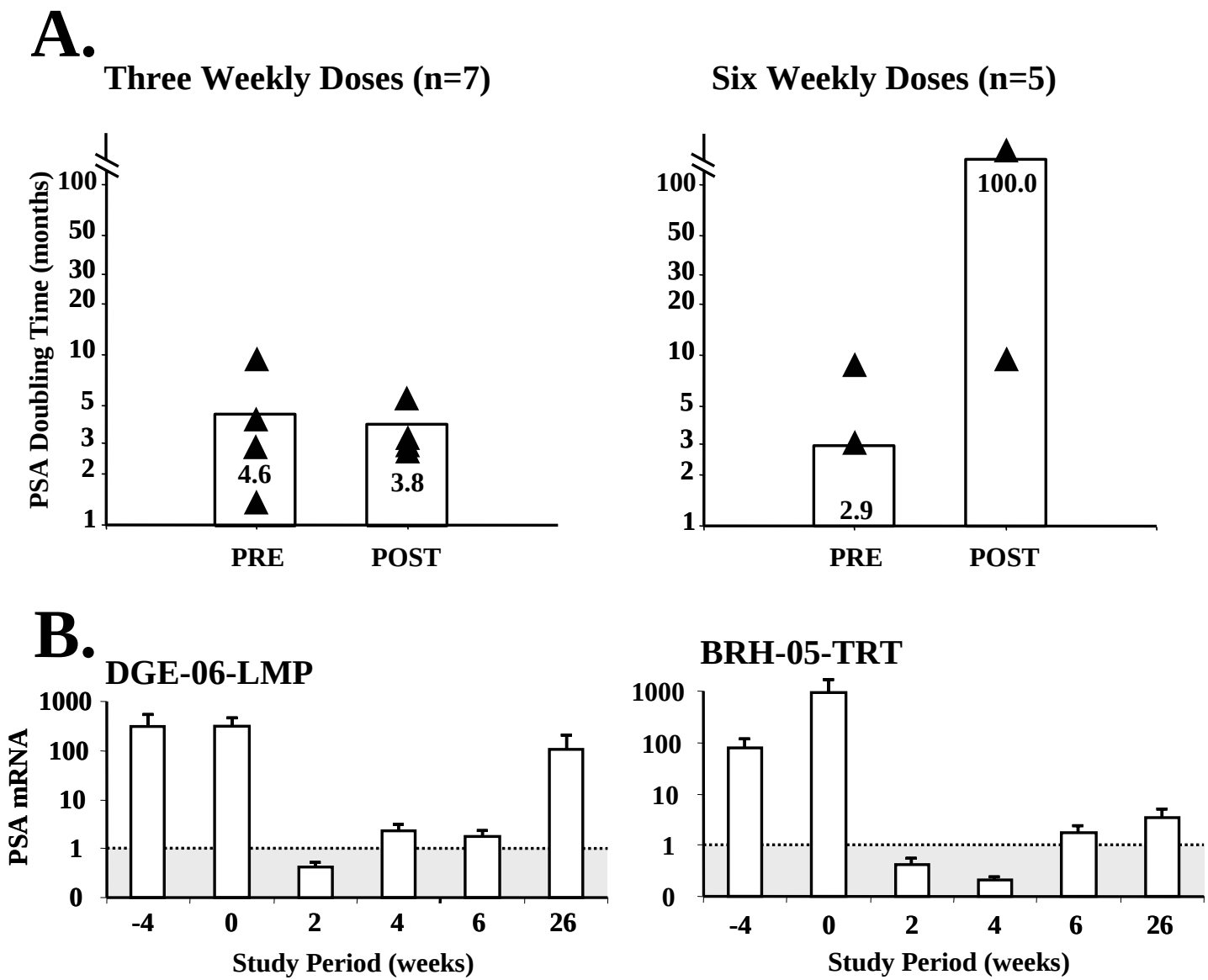
$CD8^+$ hTERT



$CD8^+$ LAMP hTERT



Impact on PSA Doubling Time and Circulating Tumor Cells



Conclusions

- **Powerful method of stimulating hTERT-specific $CD4^+$ and $CD8^+$ T cell responses in cancer patients.**
- **Evidence that LAMP-hTERT RNA transfected DC are capable of stimulating higher frequencies of hTERT – specific $CD4^+$ T cells**
  - **DTH reactions/ELISPOT/cytolytic assays**
  - **Induction of central T cell memory**
- **Lack of tolerance with increasing numbers of vaccinations.**
- **Impact on PSA doubling time and clearance of circulating tumor cells.**

Elimination of Regulatory T cells

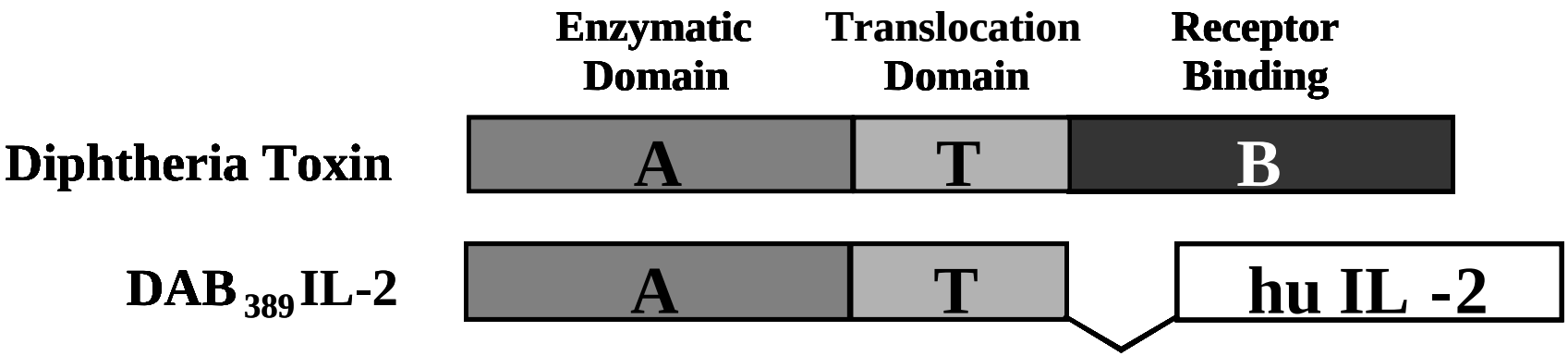
Rationale

- **Existence of $CD4^+/CD25^+$ regulatory T cells in humans (Treg) that suppress immune responses to self- and tumor antigens.**
- **Some studies suggest increased levels of Treg in cancer patients.**
- **Antibody-mediated elimination of Treg has shown to elicit antitumor immunity in tumor-bearing mice.**
- **Anti-CD25 *m*AB therapy was capable of enhancing the therapeutic effects of tumor vaccines.**

Elimination of Regulatory T cells

Approach

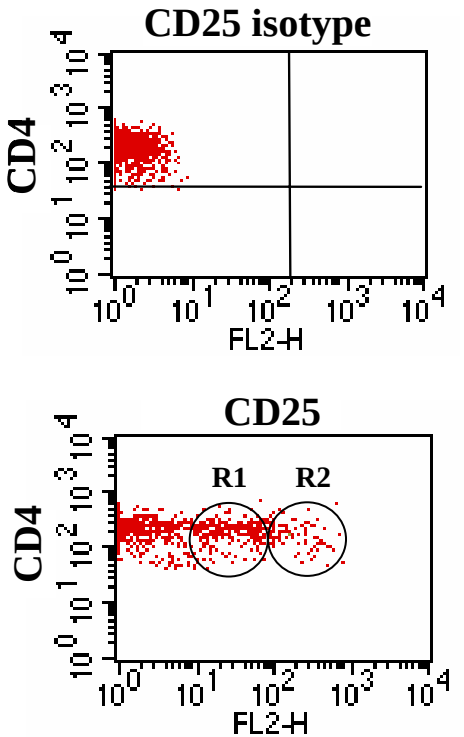
DAB_{389}IL-2 is a recombinant fusion protein that contains the catalytical- and membrane translocation domain of diphtheria toxin fused to human IL-2, allowing targeting of $CD25^+$ cells.



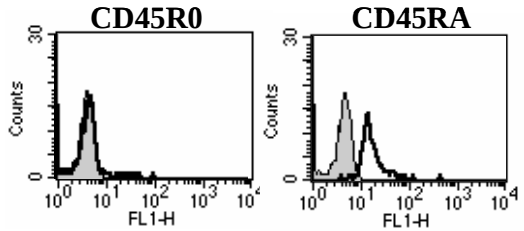
Human $CD4^+CD25^+$ Regulatory T cells

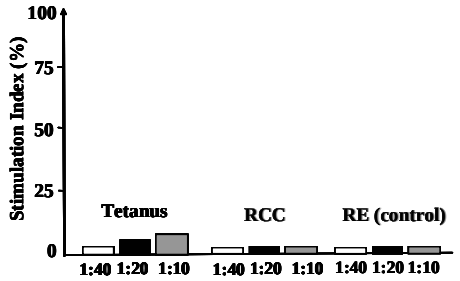
Definition

A. $CD4^+/CD25^+$

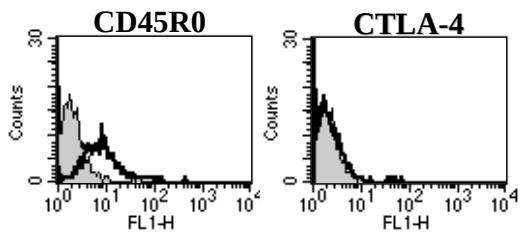


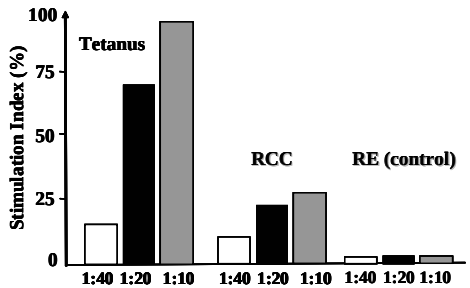
B. $CD4^+/CD25^{neg}$



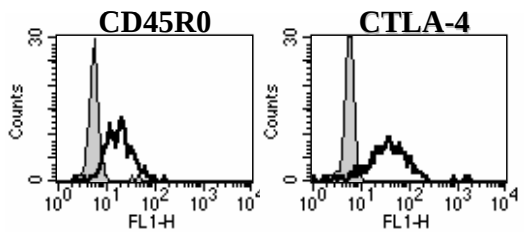


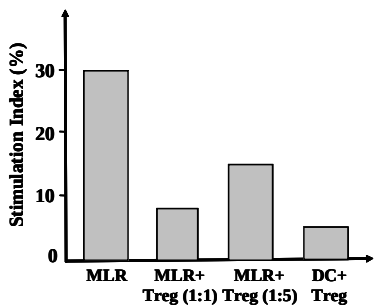
C. $CD4^+/CD25^{int}$



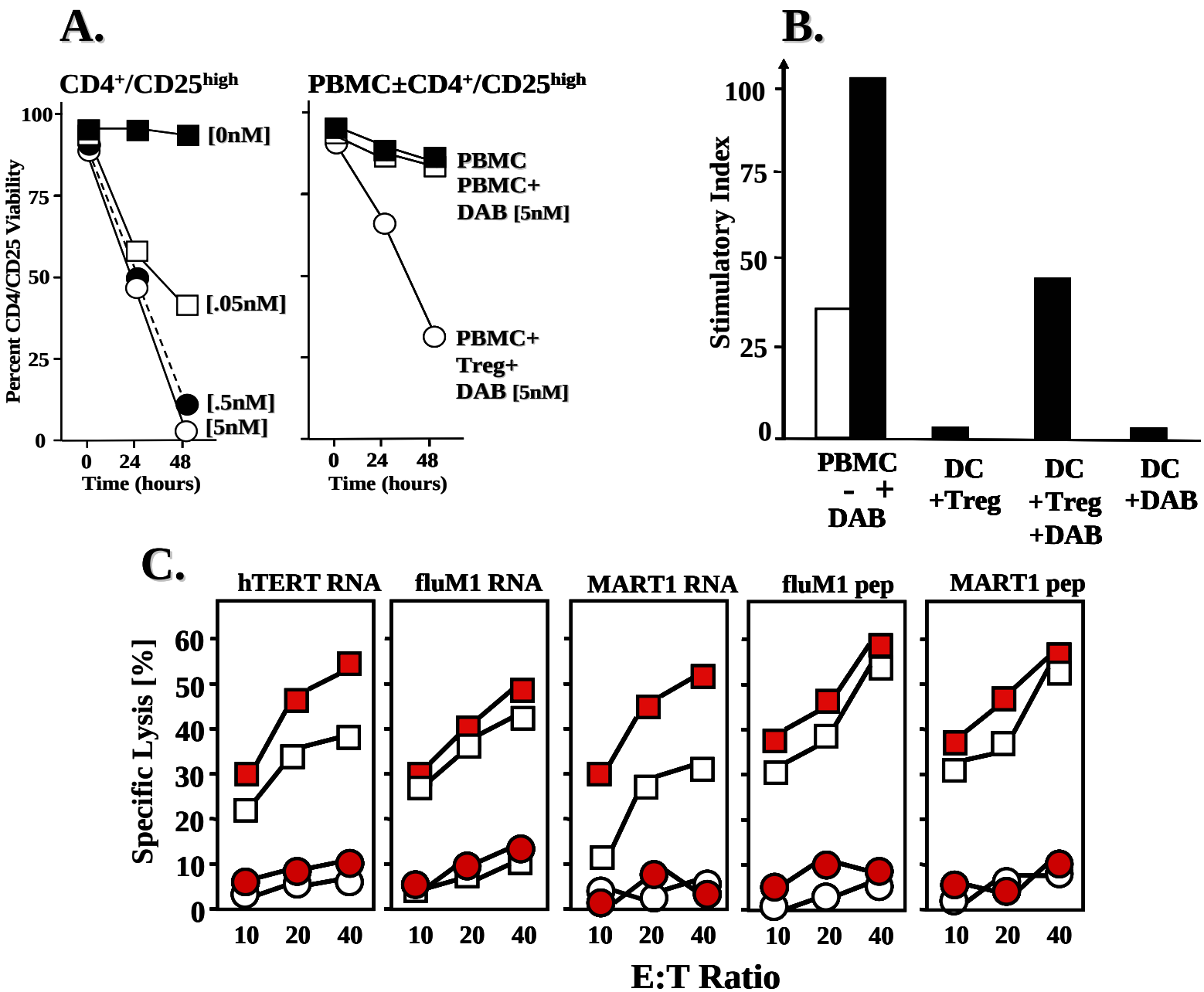


D. $CD4^+/CD25^{high}$

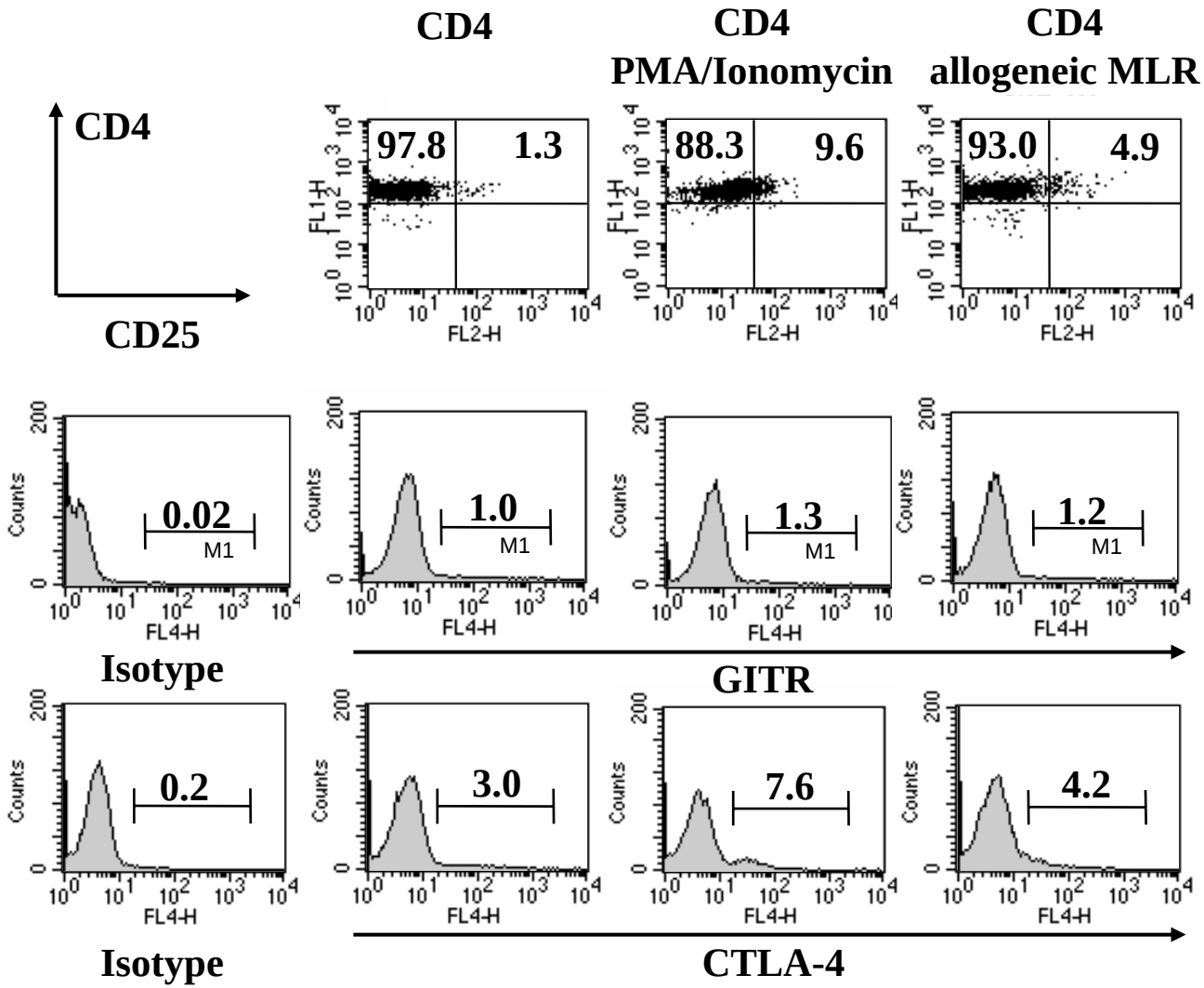




Enhancement of T-cell Immunity after T_{reg} Depletion

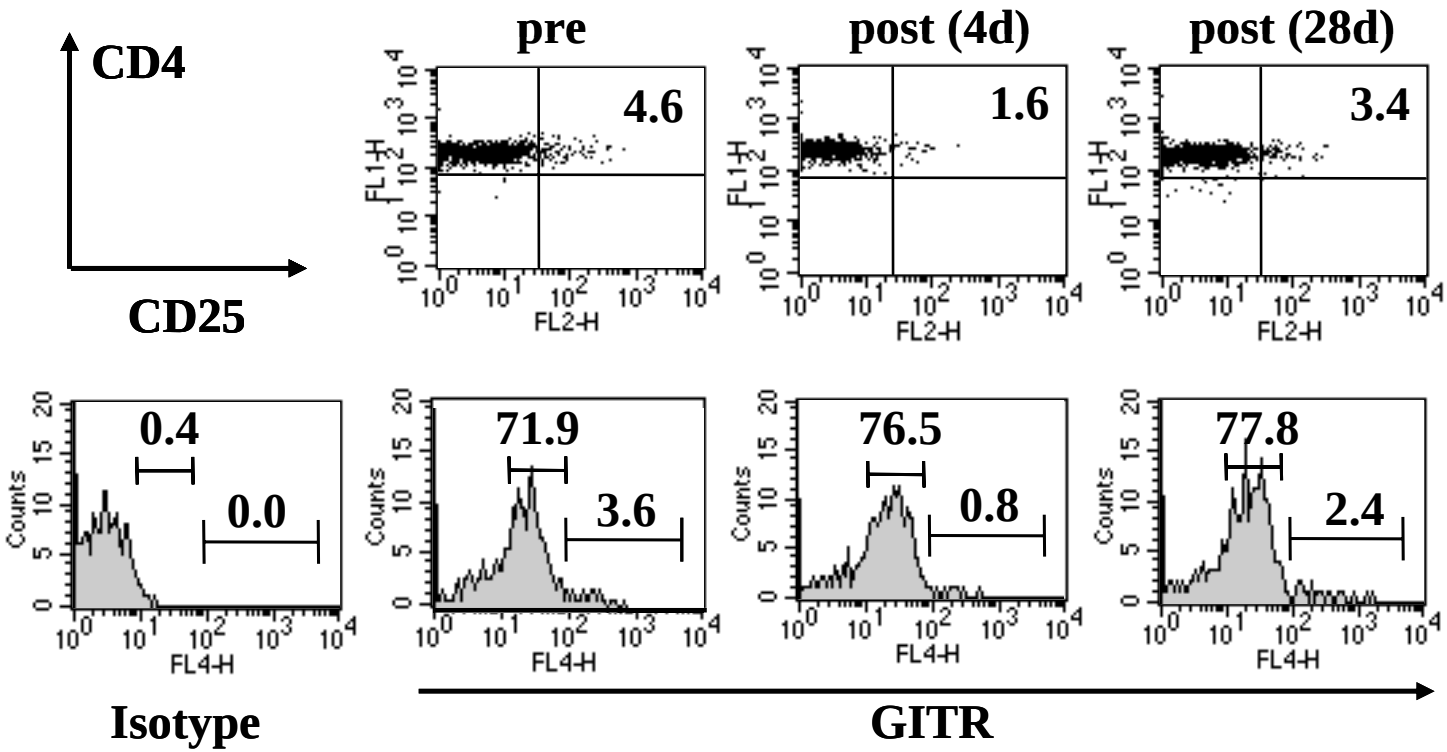


Monitoring for Regulatory T cells in a Vaccination Setting



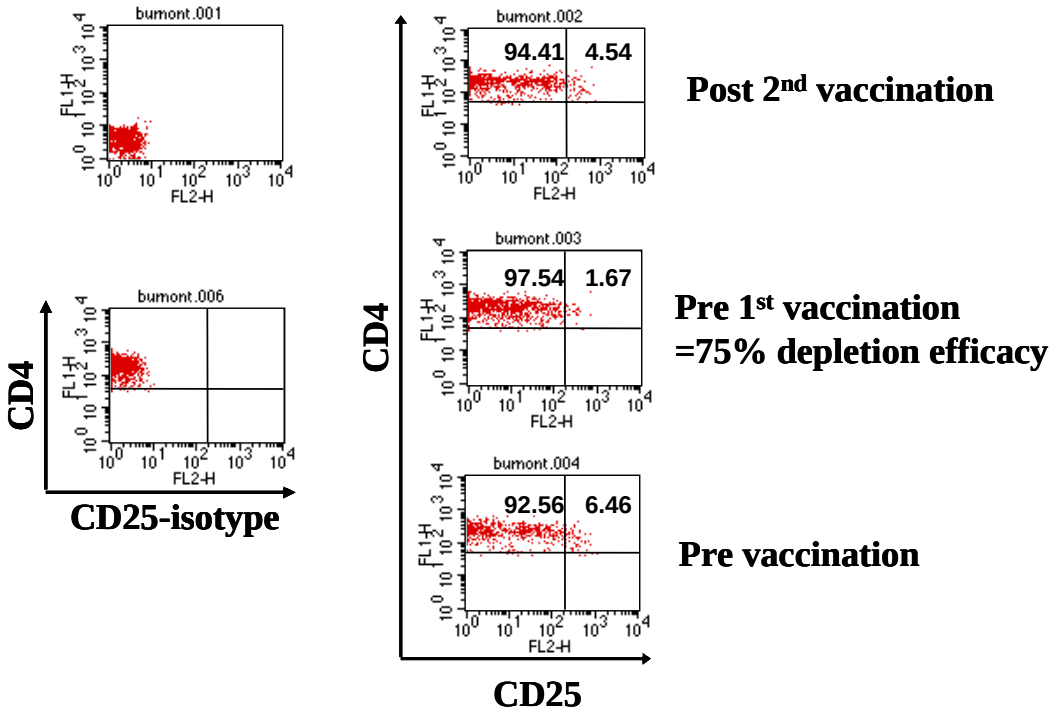
Depletion of $CD4^{+}/CD25^{high}$ Regulatory T cells After $DAB_{386}IL\text{-}2$ Administration

Patient: HMT-04-DAB



Depletion of $CD4^+/CD25^{high}$ Regulatory T cells After $DAB_{386}IL\text{-}2$ Administration

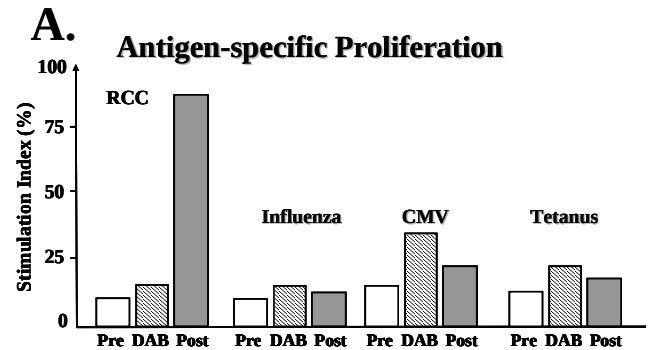
Patient: JB01-RCC ONTAK 18μg/kg + RCC RNA DC (2 doses)

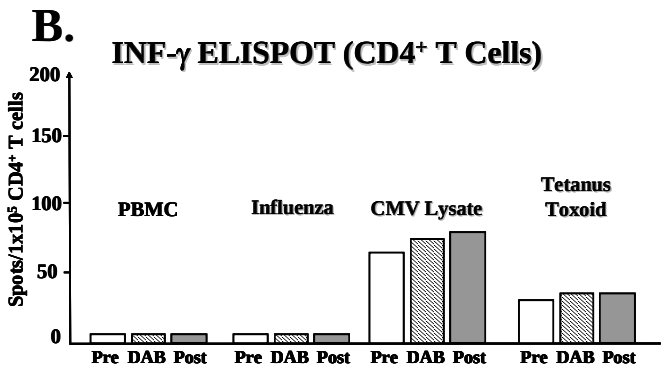


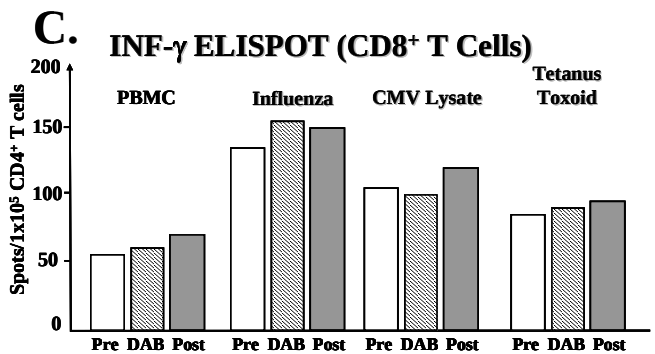
Post 2nd vaccination

Pre 1st vaccination
=75% depletion efficacy

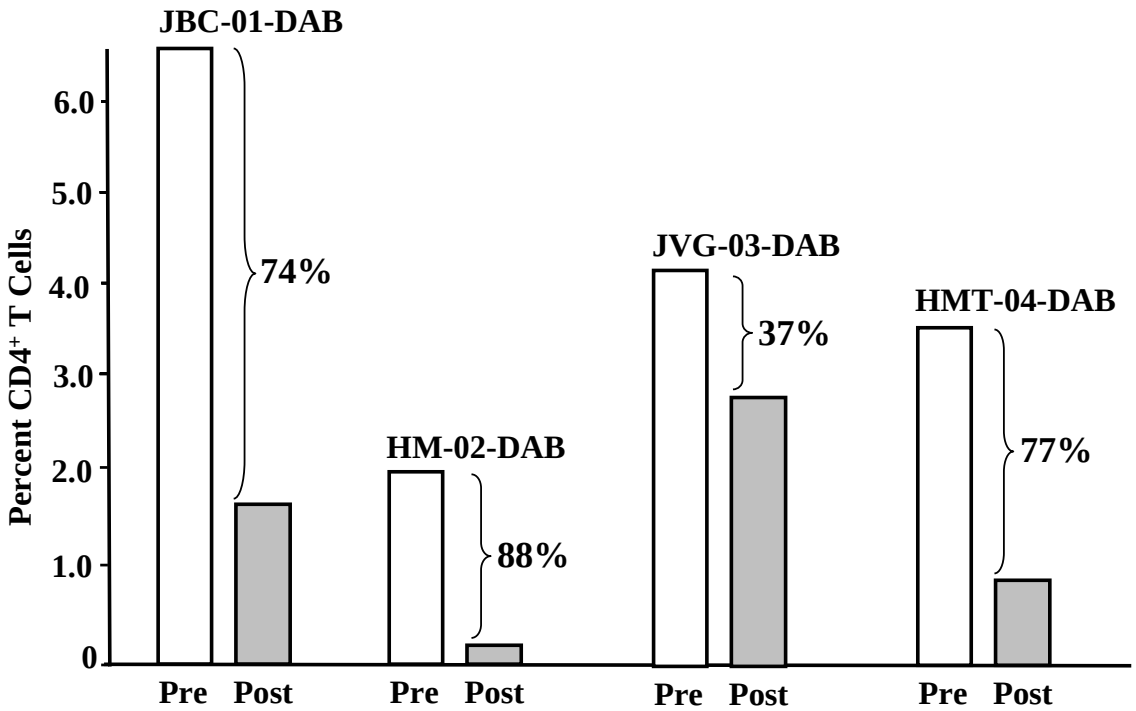
Pre vaccination





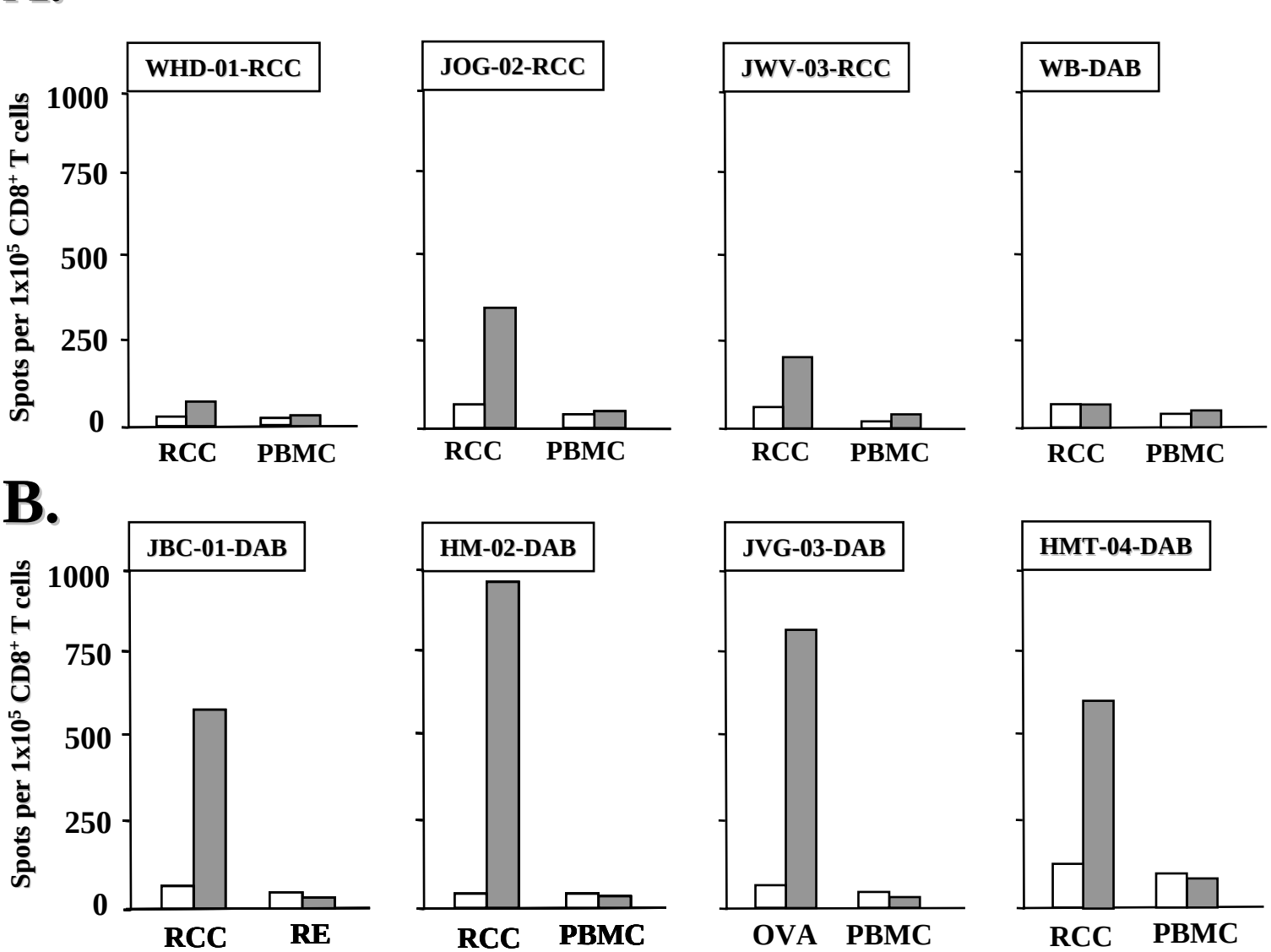


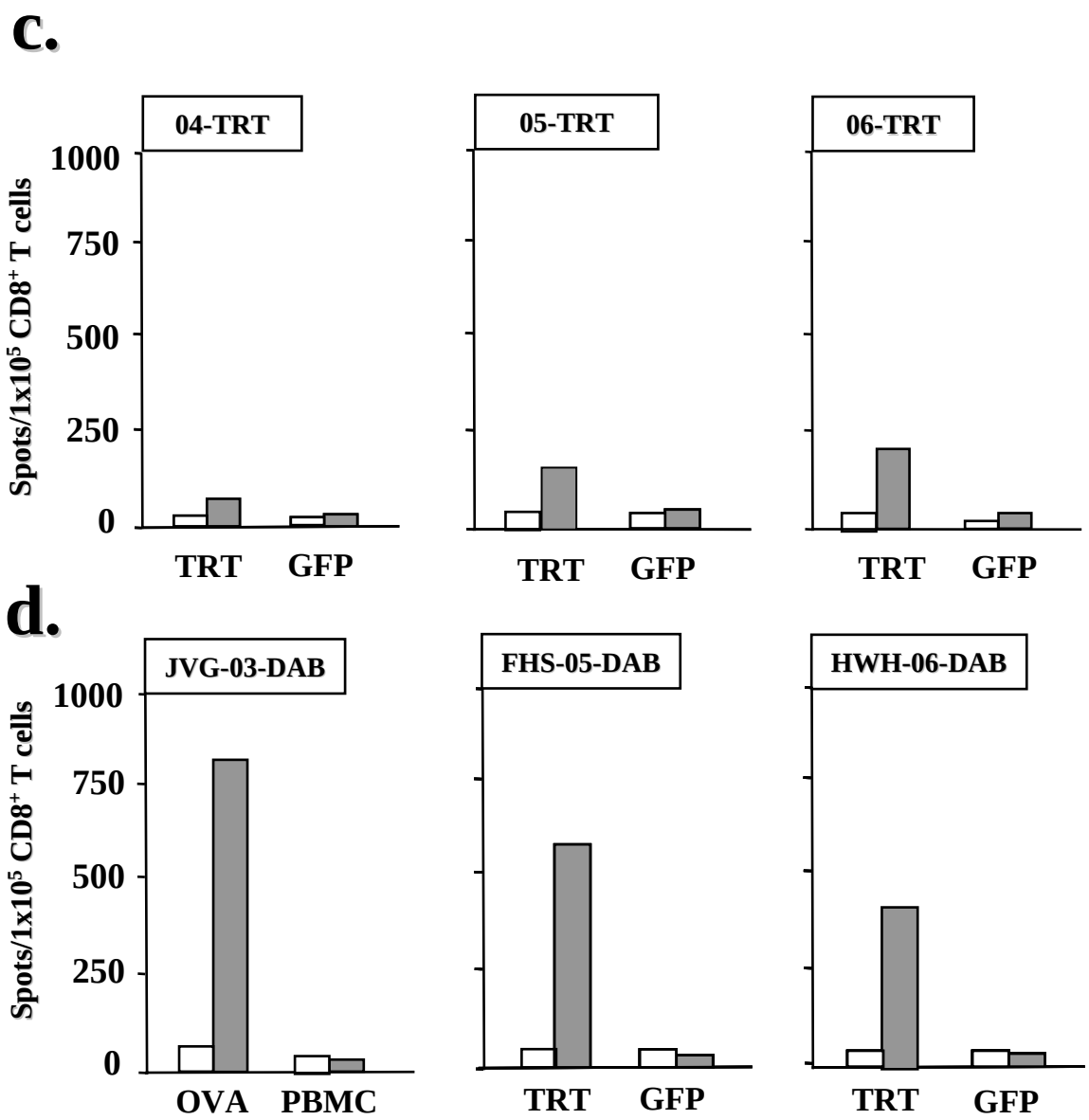
Efficacy of Depleting Regulatory T cells in Metastatic Cancer Patients



Marker	Cell Type	Single Positive (% positive of PBMC)		CD25 Double Positive (% of Single Positive)	
		Pre	Post	Pre	Post
CD4	T cells	28.0	25.0	42.8	40.0
CD8	Macrophages	17.0	19.0	16.5	22.1
CD14	Monocytes	13.9	15.4	10.0	10.7
CD19	B cells	27.3	24.6	15.4	13.4
CD56	NK cells	18.9	21.3	-	-
CD69	NK/effector cells	21.2	19.6	7.8	9.2

Elimination of T_{reg} is Capable of Enhancing Vaccine-mediated T-cell Responses




c.
04-TRT
05-TRT
06-TRT
Spots/1x10^5 CD8+ T cells
1000
750
500
250
0
TRT
GFP
d.
JVG-03-DAB
FHS-05-DAB
HWH-06-DAB
OVA
PBMC

Elimination of Regulatory T cells
Conclusions I

- NIH and FDA-approved clinical trial.
- Demonstration of selective Treg depletion following single dose of $DAB_{389}IL\text{-}2$.
- Enhancement of T cell responses *in vitro*, predominantly against 'naturally processed' self-antigens.
- Safety, no clinical signs of autoimmunity in 10 patients treated thus far.

Elimination of Regulatory T cells

Conclusions II

- No interference with $CD4^{+}/CD25^{int}$ memory T cell pool.
- Stimulation of high frequencies of RCC-specific T cells *in vivo* after combined therapy.
- Polarization of RCC-specific $CD4^{+}$ T cells towards Th-1, but not Th-2.
- This strategy could have broad implication for the design of active and passive immune-based protocols.