Molecular Manipulation of the Anti-tumor Immune Response

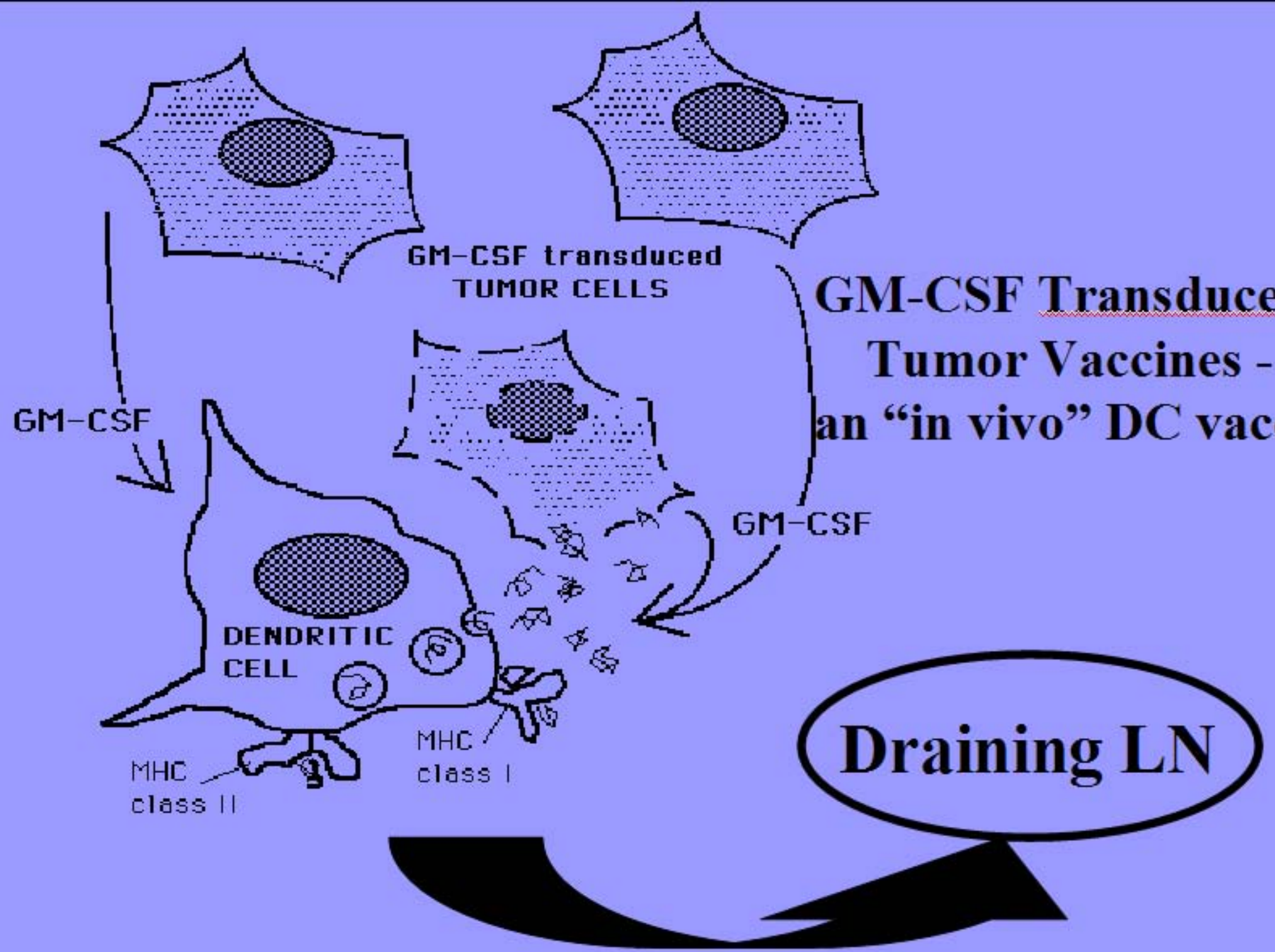
Drew Pardoll, SKCCC,JHMI

How can we finally develop a successful therapeutic vaccine approach?!?!

1) Use decent vaccines (Multivalent, indispensable Ag, good adjuvants, costim.,DC targeting)

2) Use vaccines in the low tumor burden, MRD setting (50-75% of cancer deaths due to recurrence after MRD)

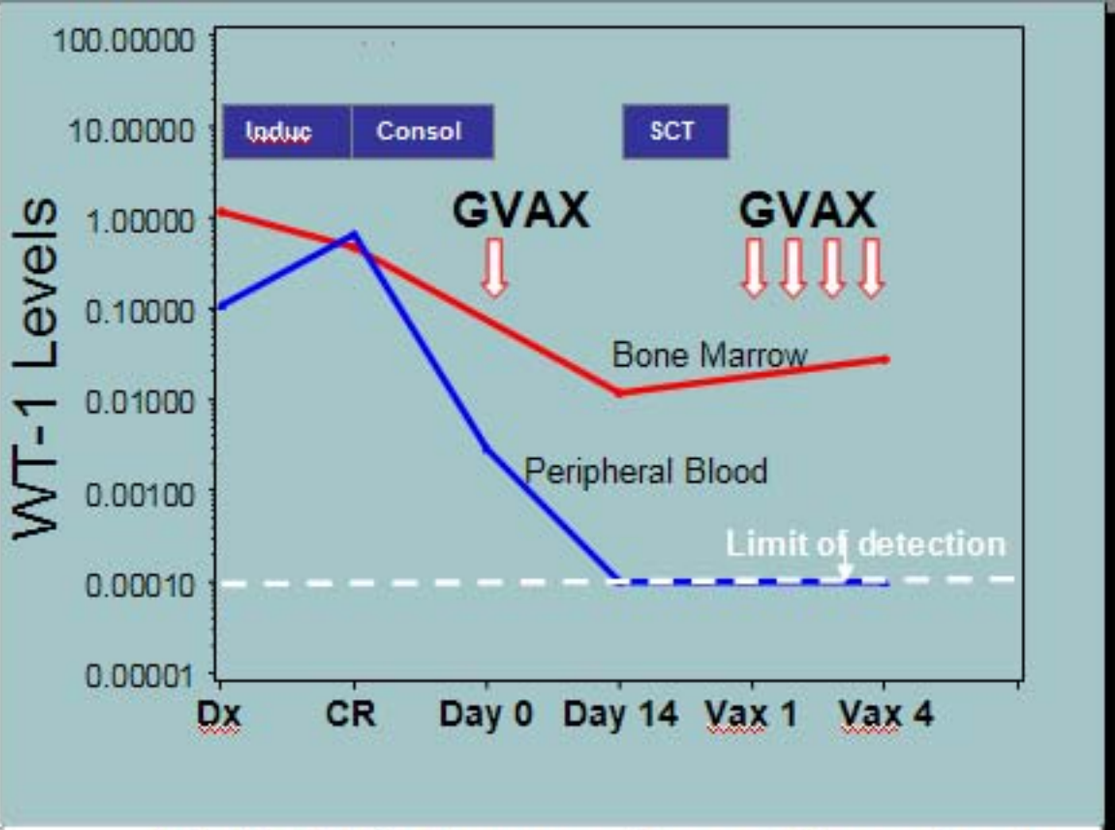
3) Combine vaccines with agents that break tumor-induced tolerance and inhibition of effector T cell function

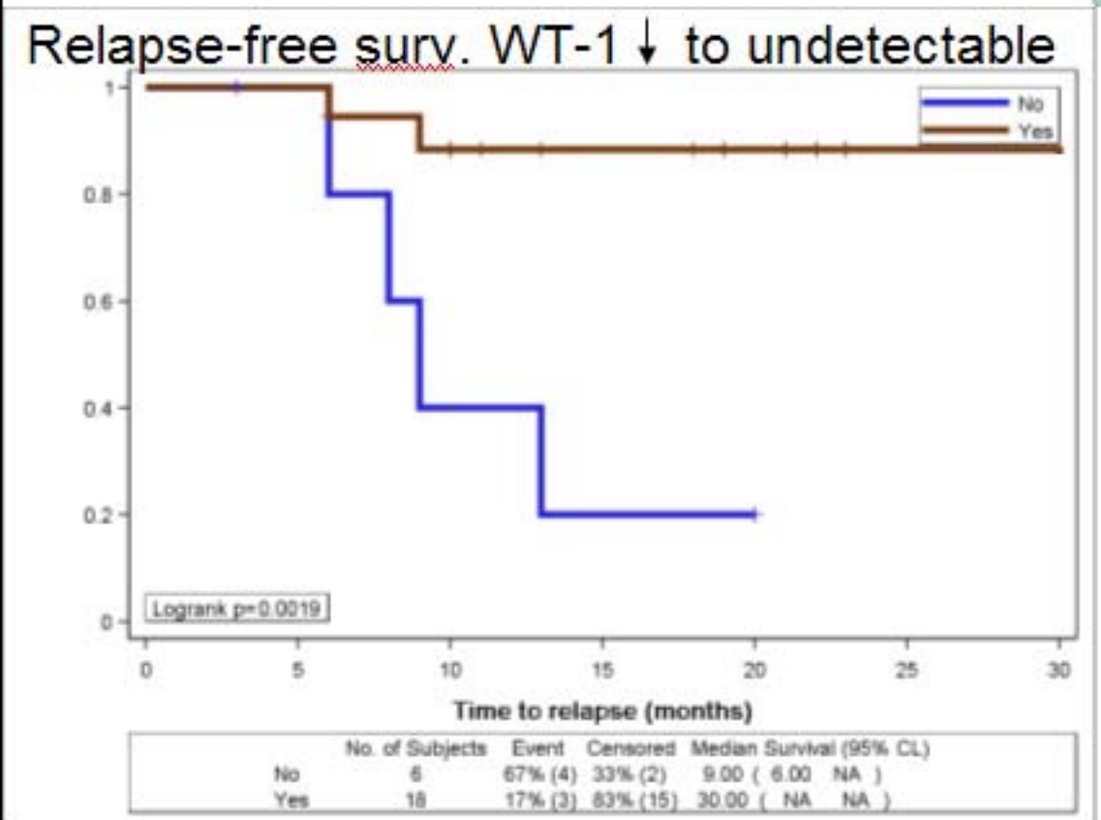


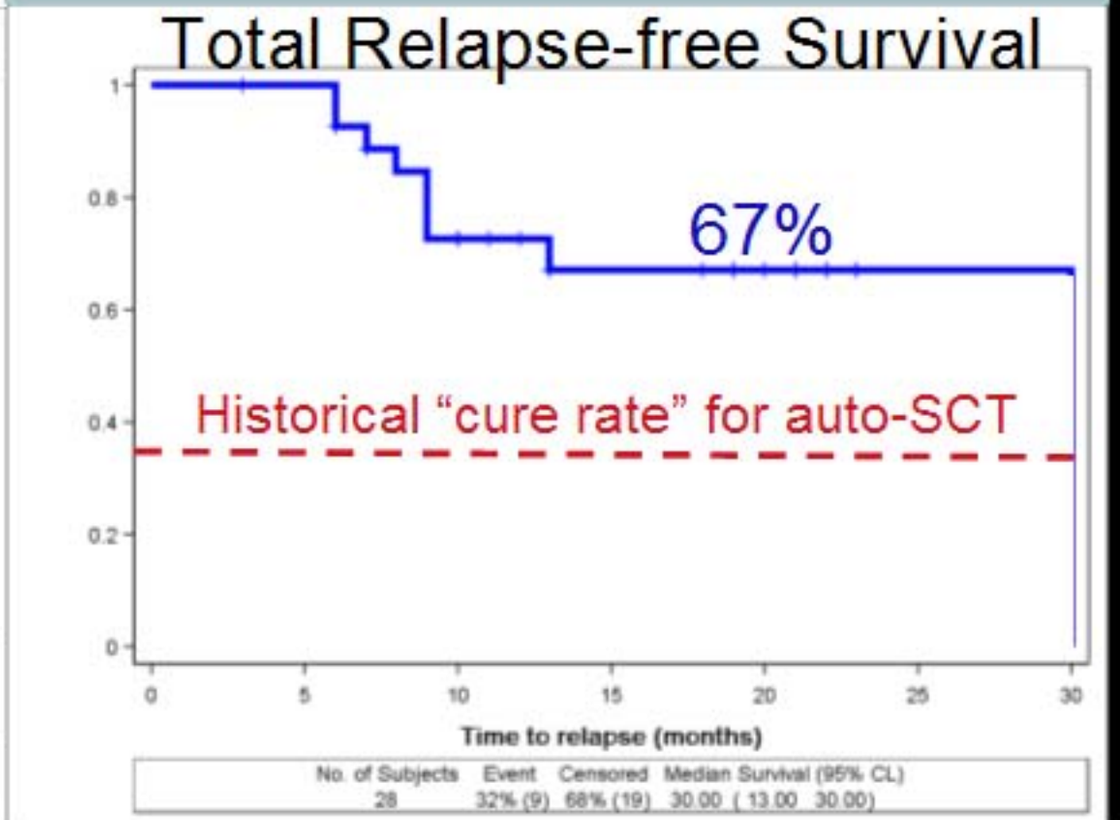
GM-CSF Transduced Tumor Vaccines - an "in vivo" DC vaccine

GVAX + auto-SCT for Refractory AML

Hy Levitsky/Ivan Borrello
together with CellGenesys



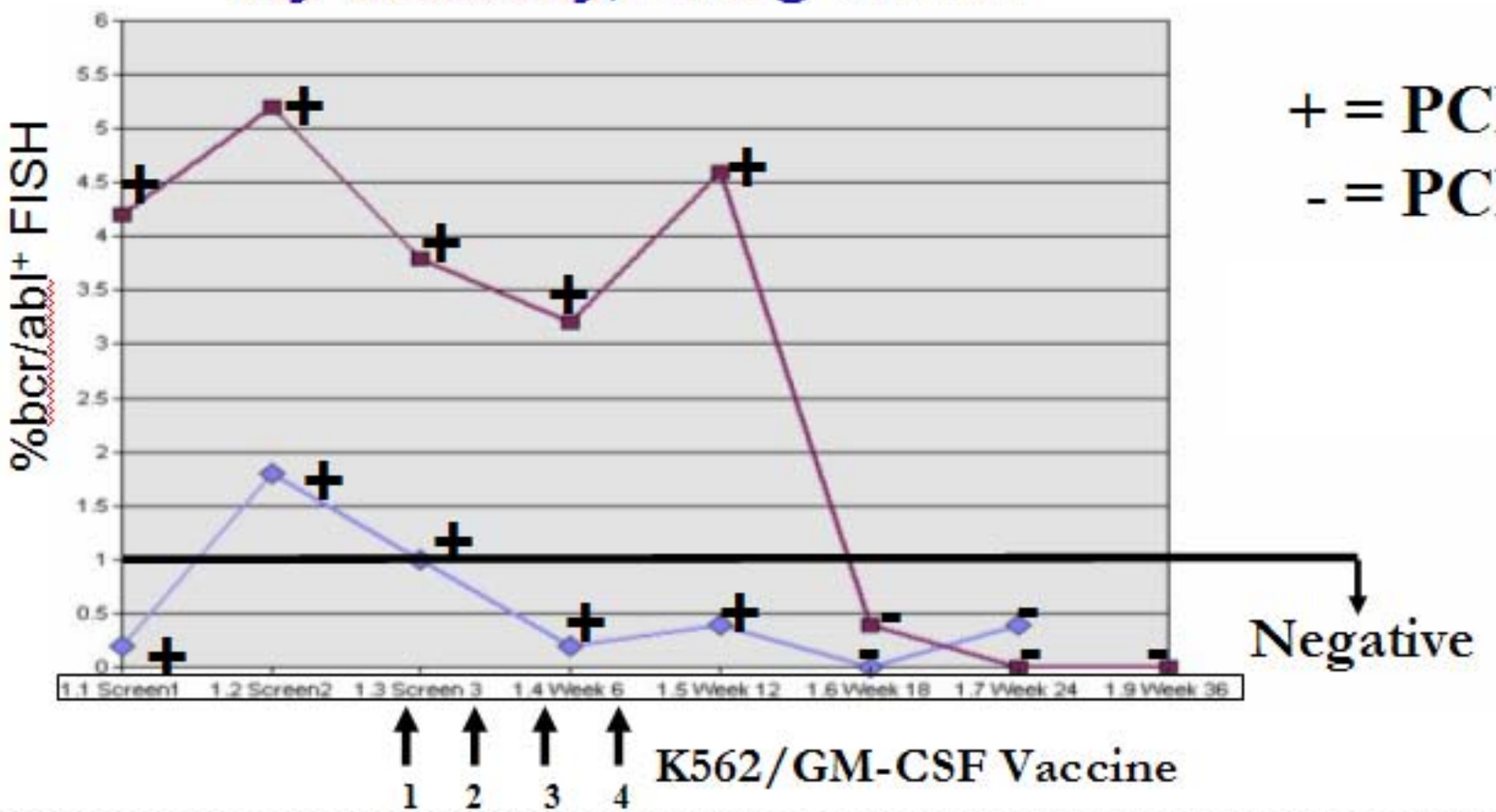




K562/GM-CSF Vaccine for residual CML on Imatinib

Hy Levitsky, Doug Smith

Example of 2 pts

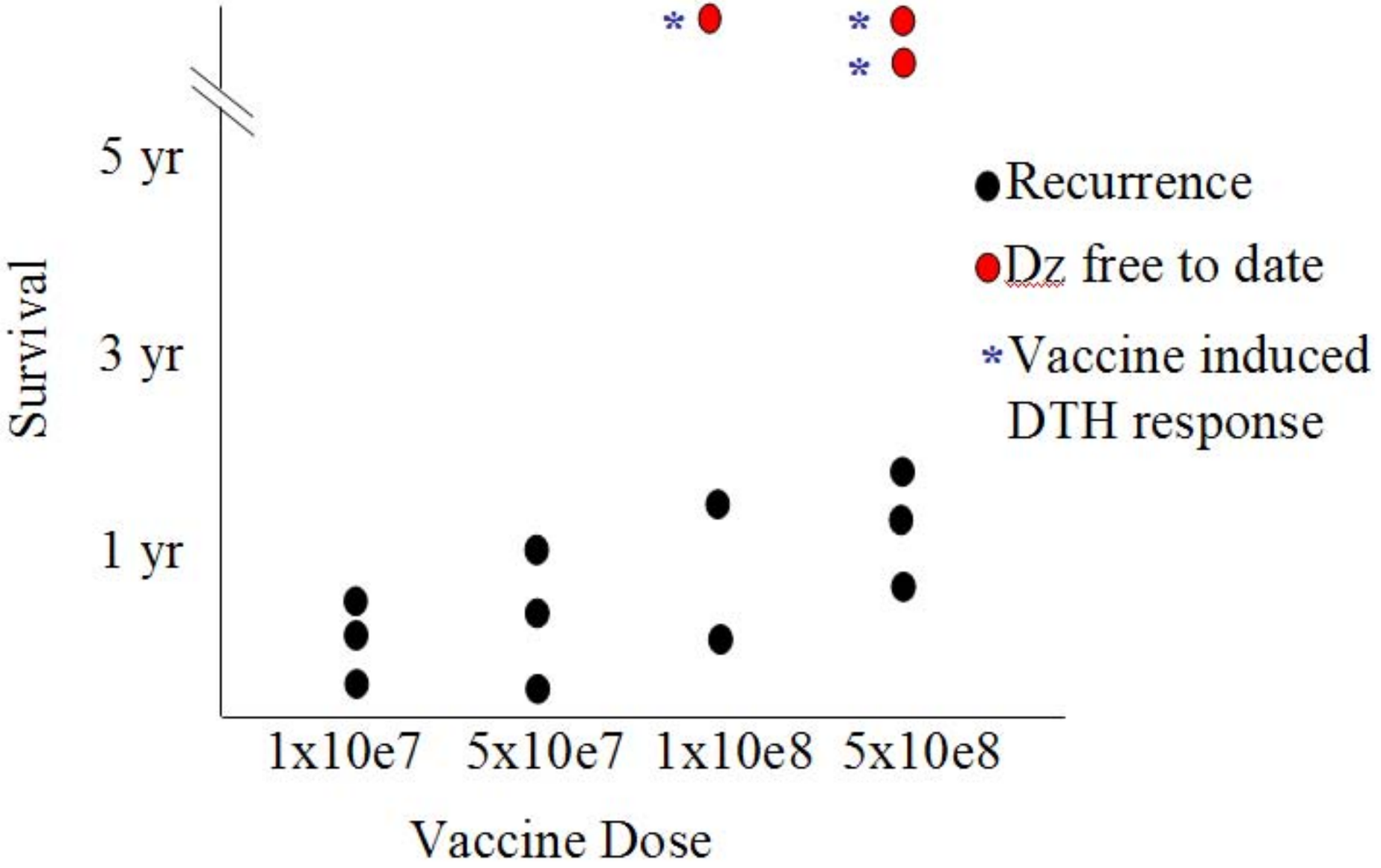


Compiled results of 17 pts

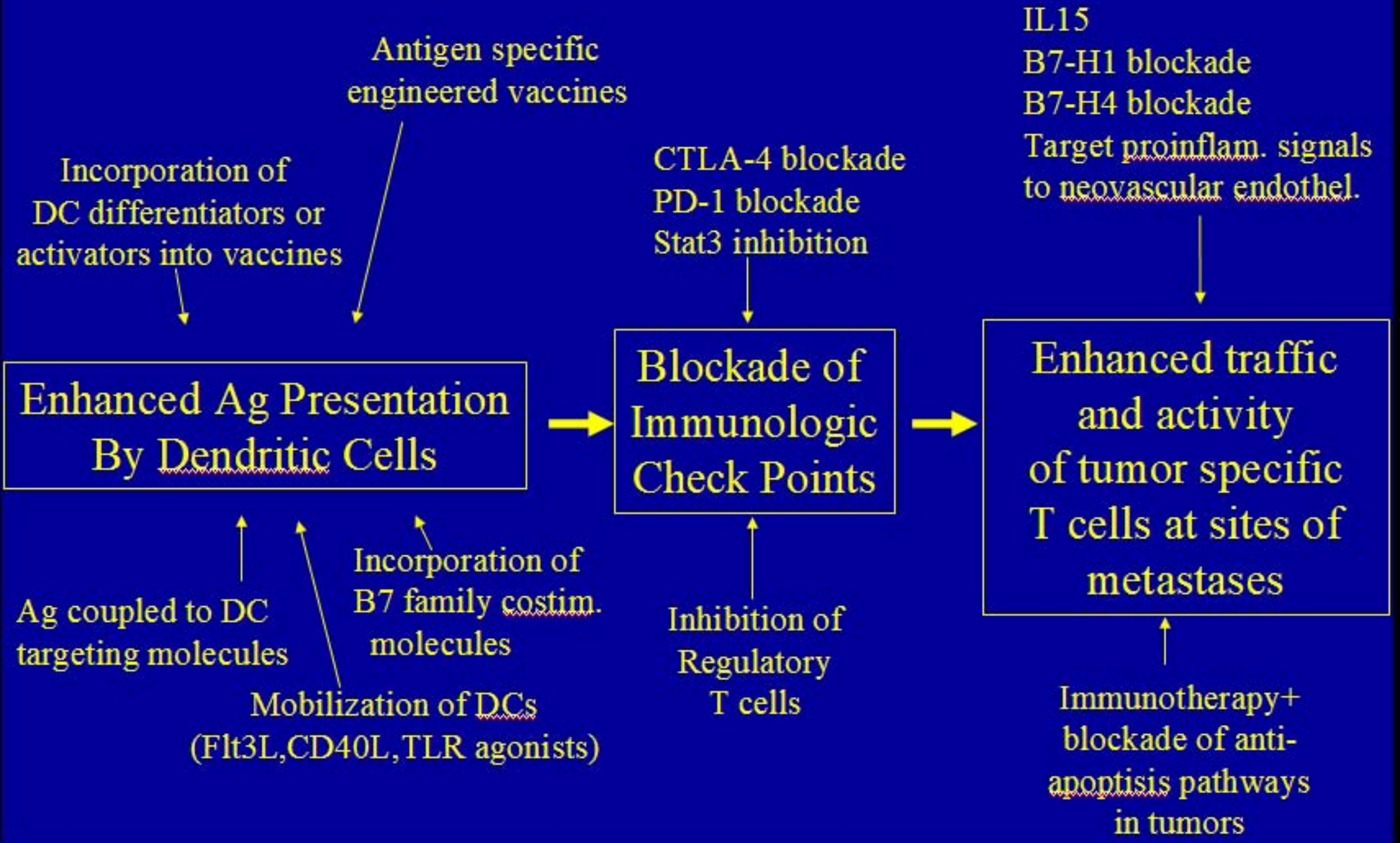
Best previous response	Post vaccine response FISH-/PCR+	FISH-/PCR-
FISH+/PCR+ (5)	2/5	1/5
FISH-/PCR+ (12)	-	4/12

Phase I Study of GM-CSF transduced allogeneic vaccine post resection for stage II/III pancreatic cancer

Liz Jaffee, Dan Laheru, Johns Cameron, Charles Yeo



Three levels at which the anti-tumor immune response must be manipulated => combination rx



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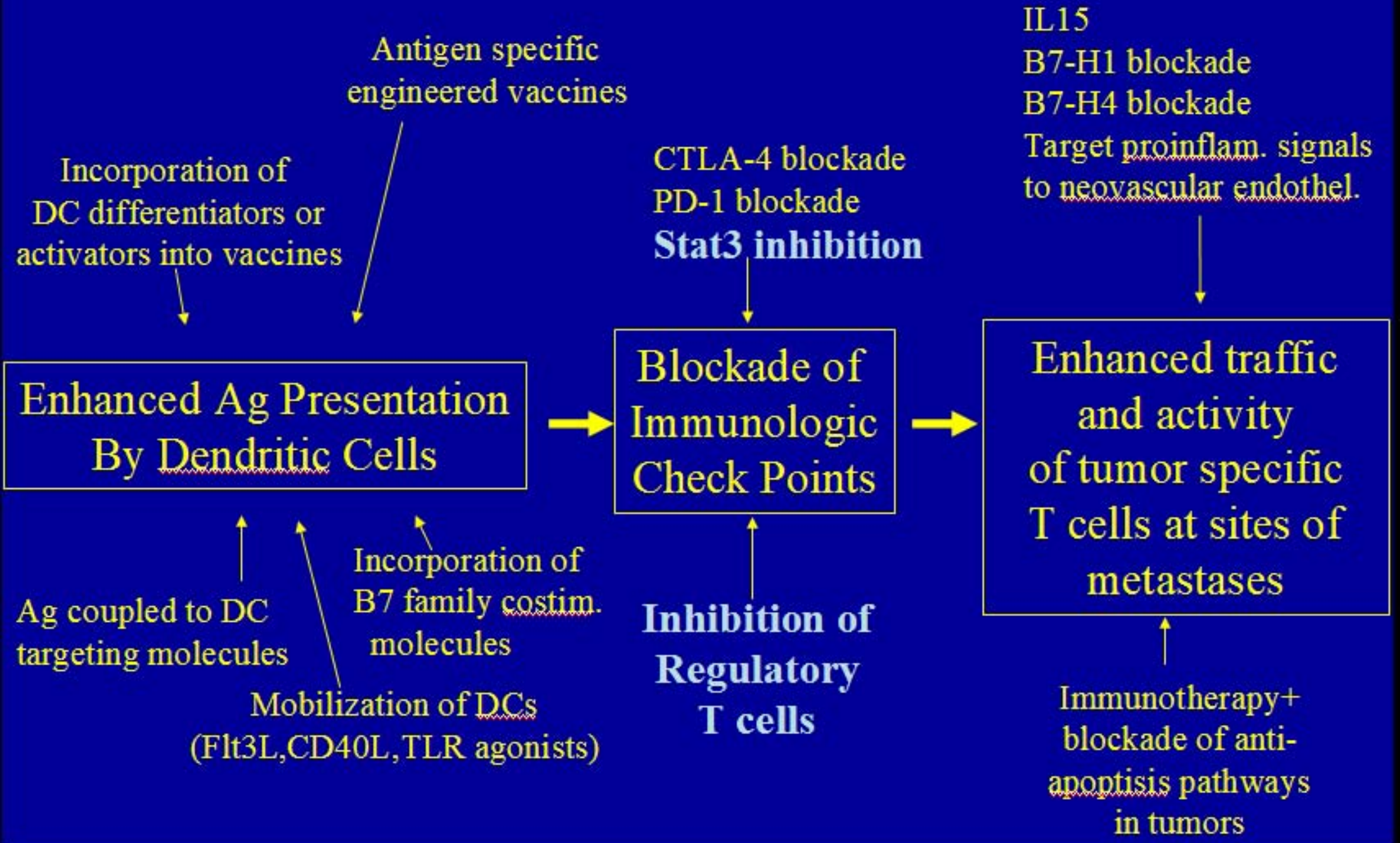
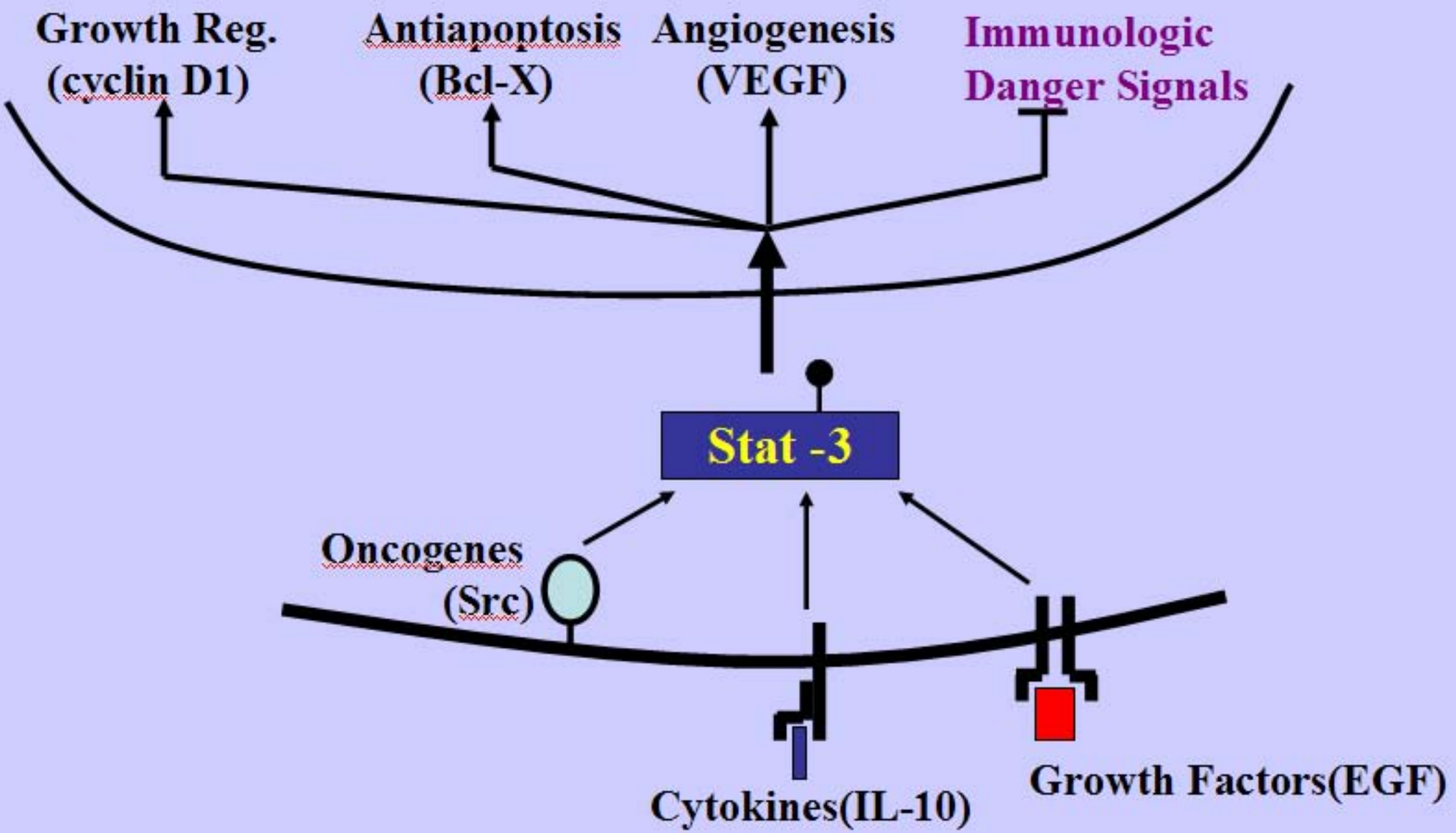
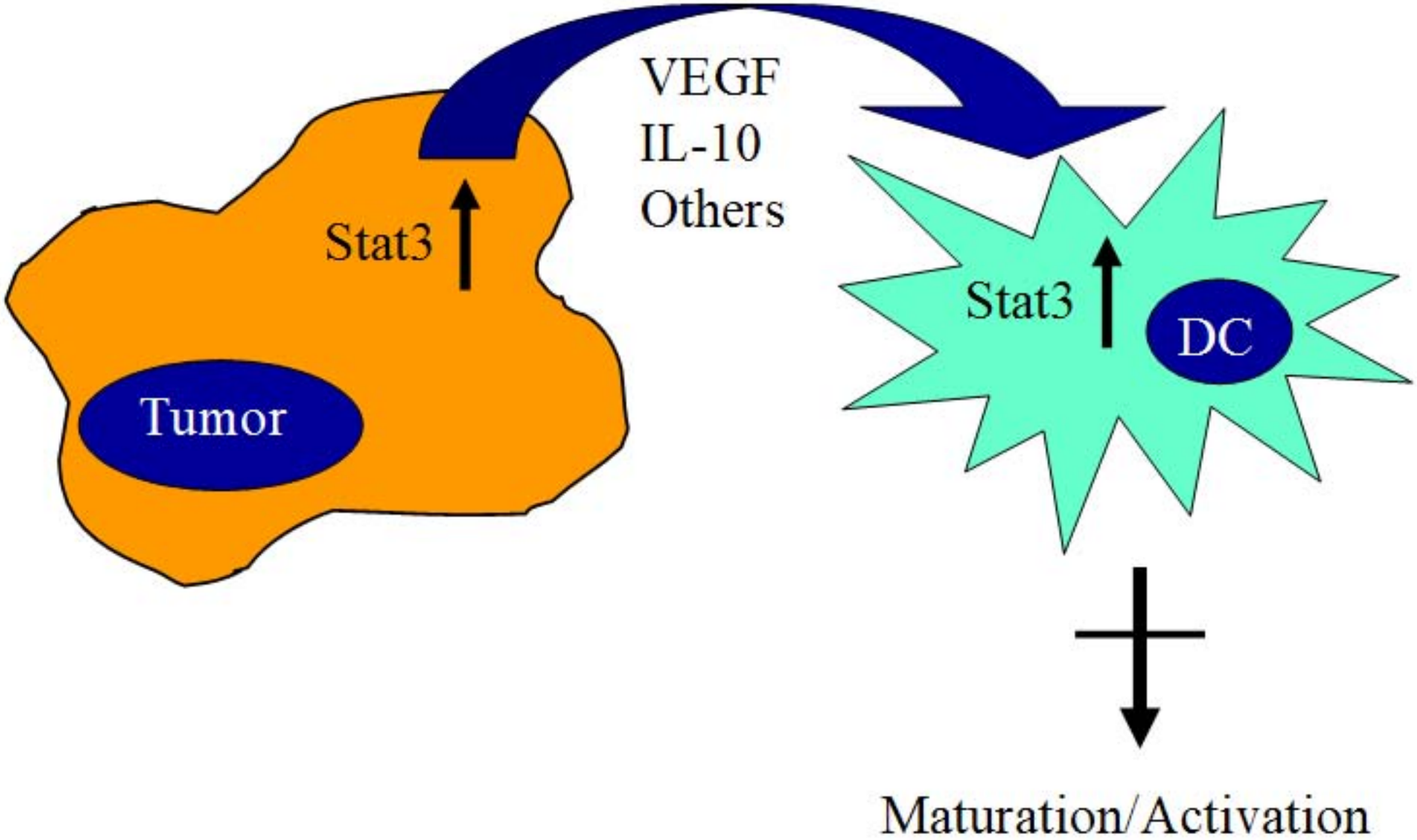


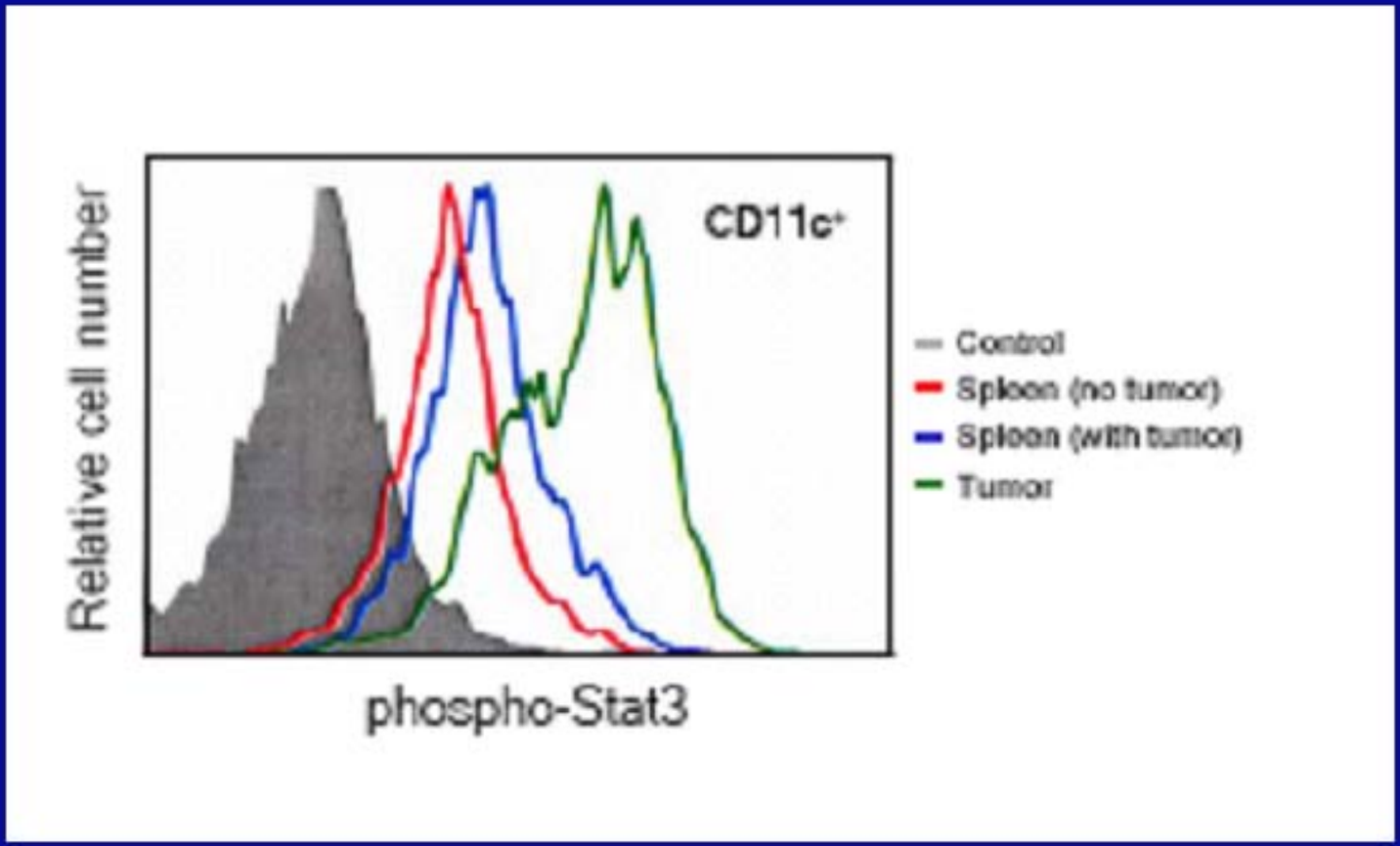
Stat 3 as a Major Signal Transducer in Tumor Cells



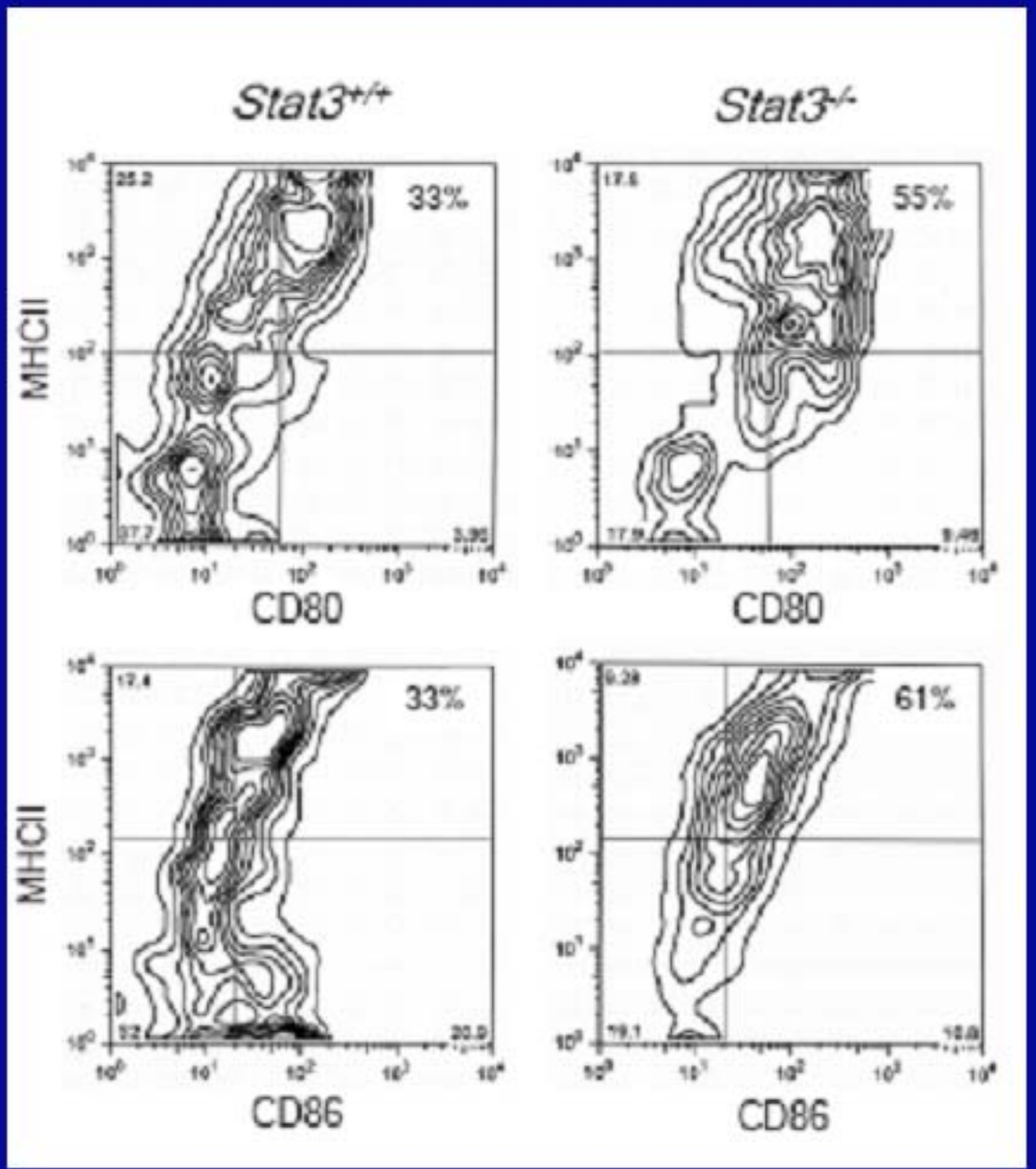
Tumors Inactivate DCs via a Stat3 Activation Cascade



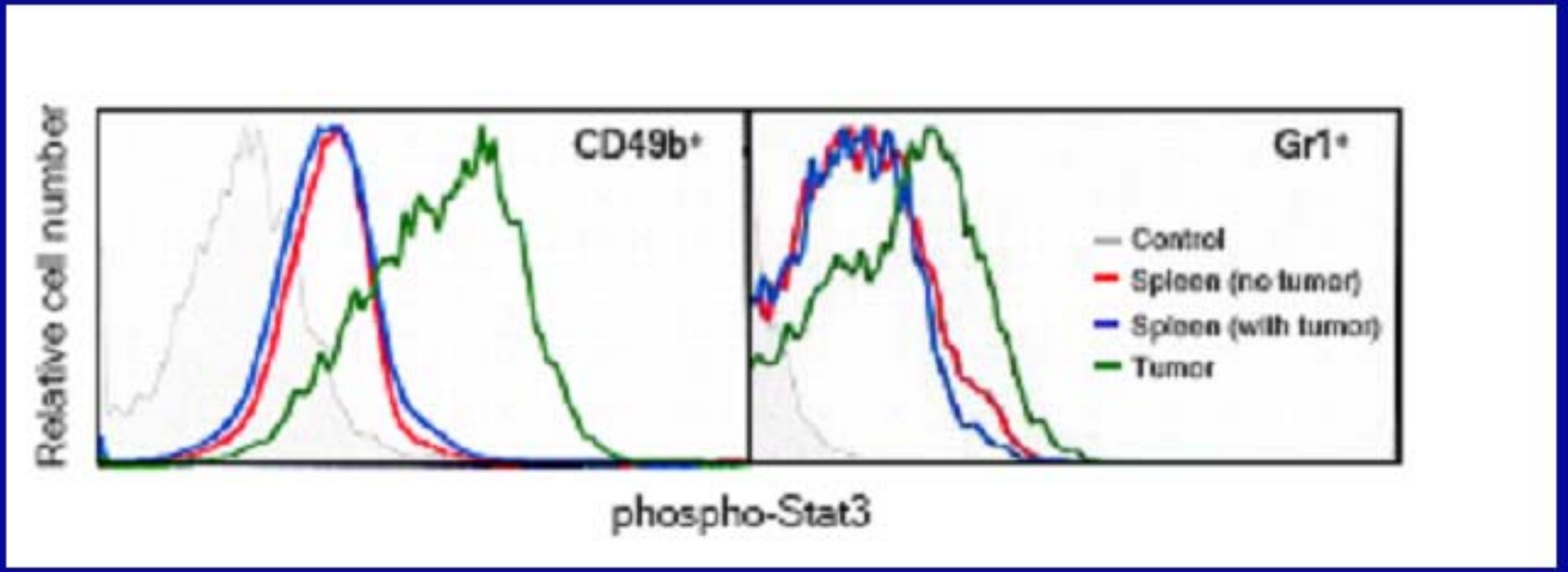
Stat3 is constitutively activated in tumor infiltrating DCs



Tumor infiltrating Stat3−/− DCs express more MHC II, CD80 and CD86

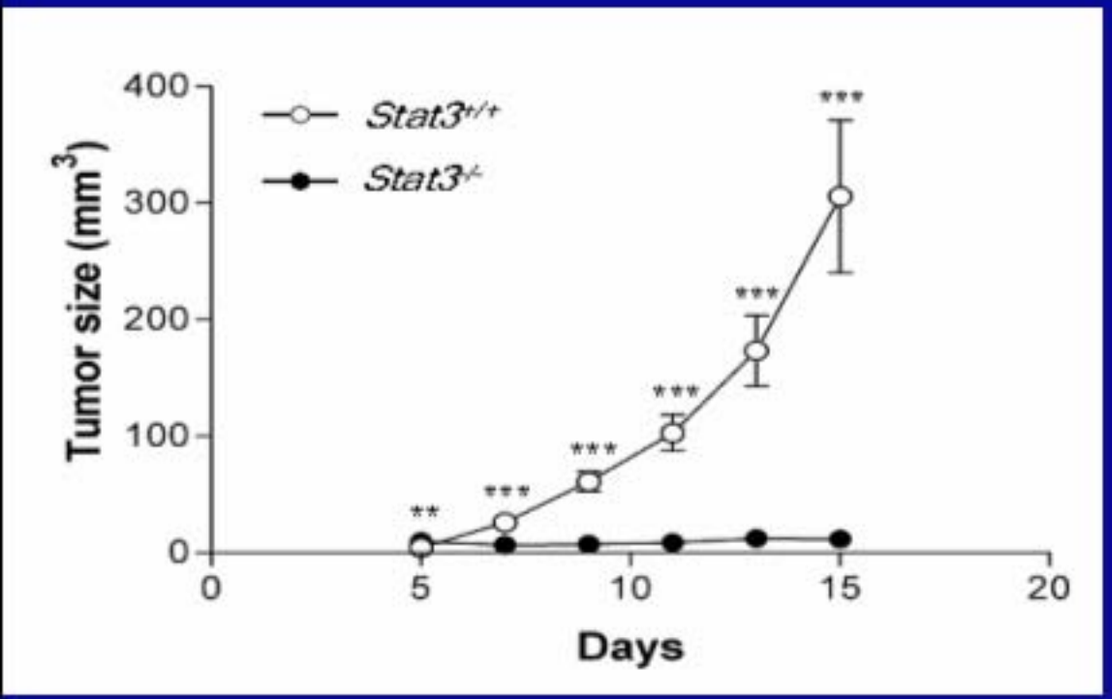


Stat3 is constitutively activated in tumor infiltrating NK cells and neutrophils

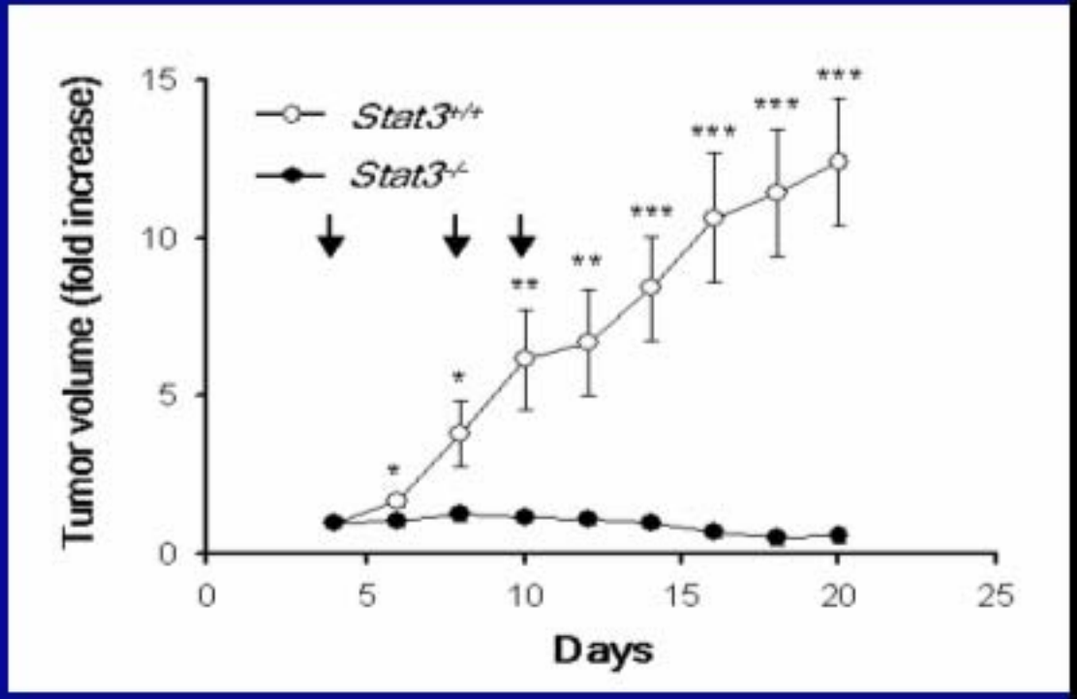


Inhibition of Stat3 in bone marrow cells leads to T-cell mediated anti-tumor effects

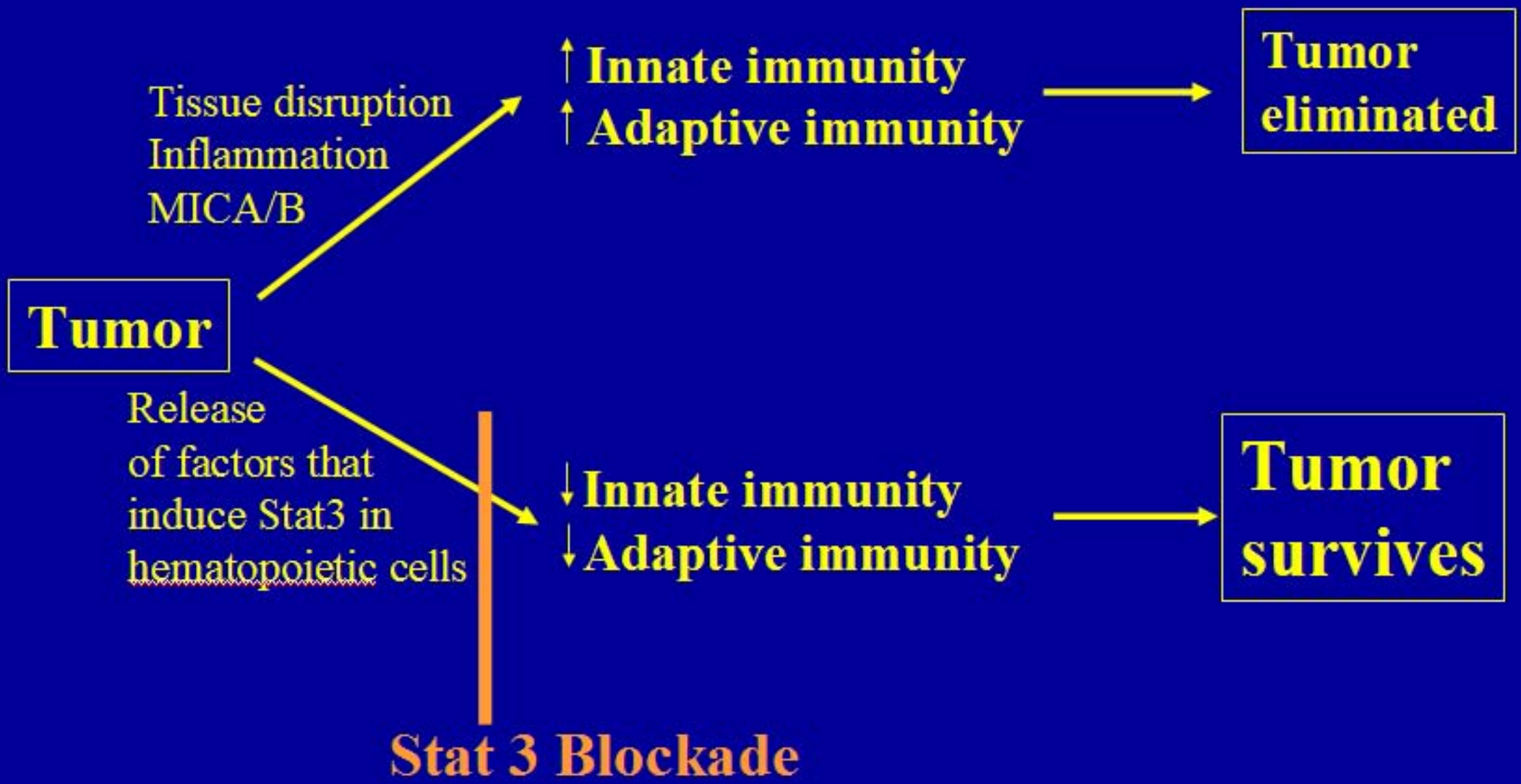
Stat3 ablation prior to introduction of tumor



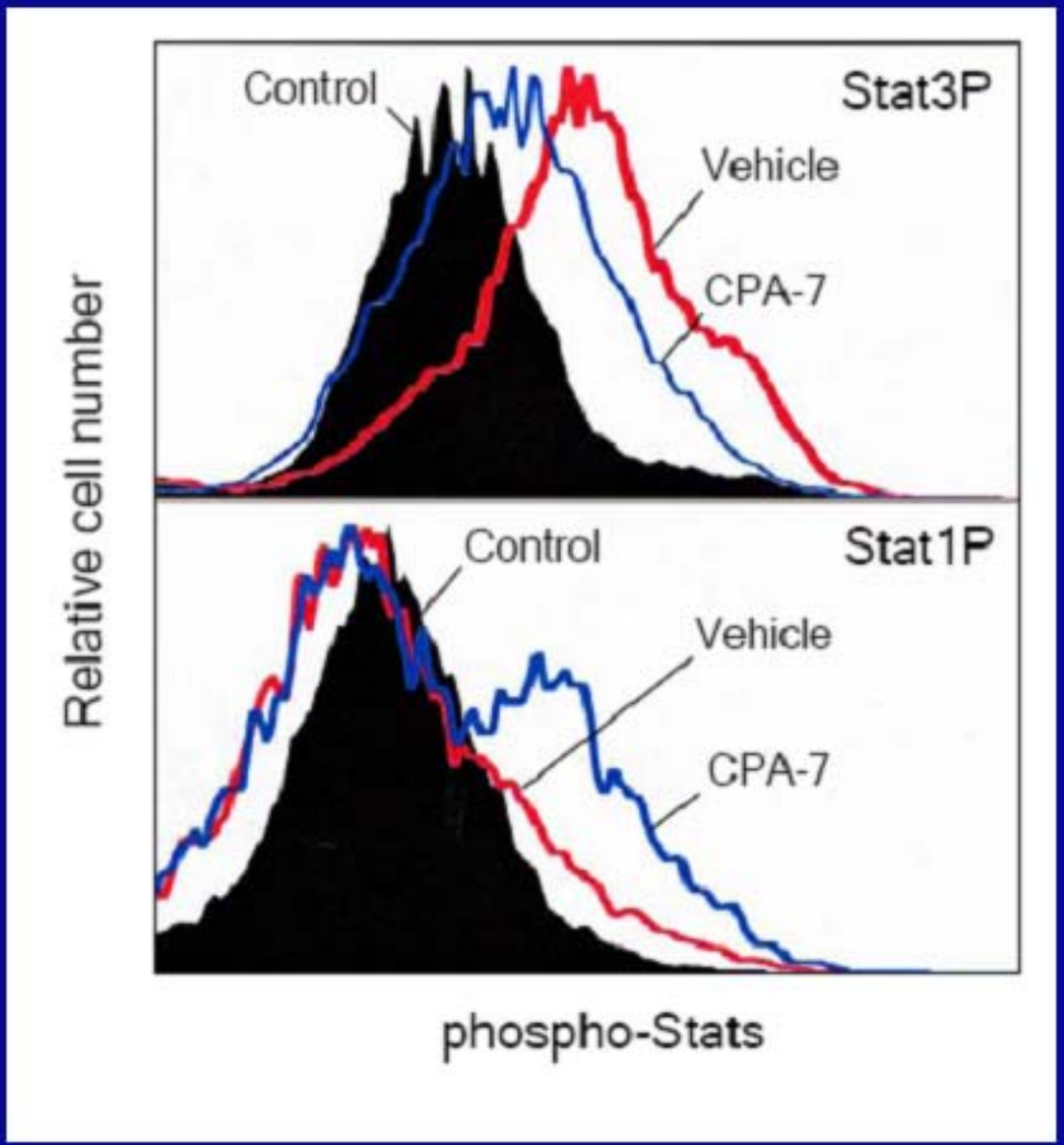
Stat3 ablation 1 wk after introduction of tumor



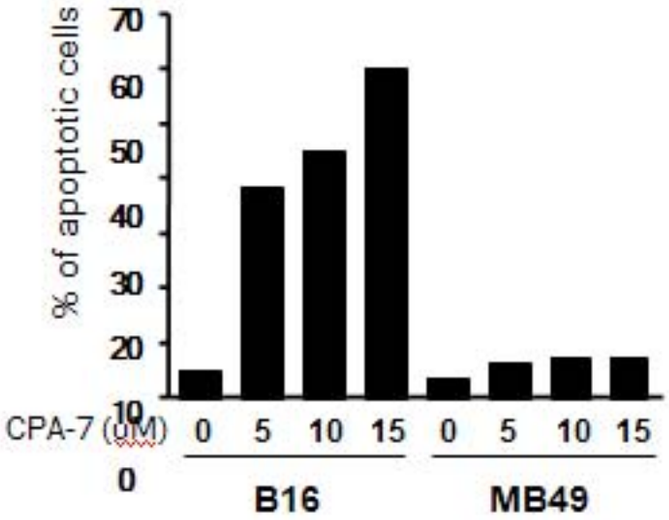
Stat3 restrains tumor immune surveillance



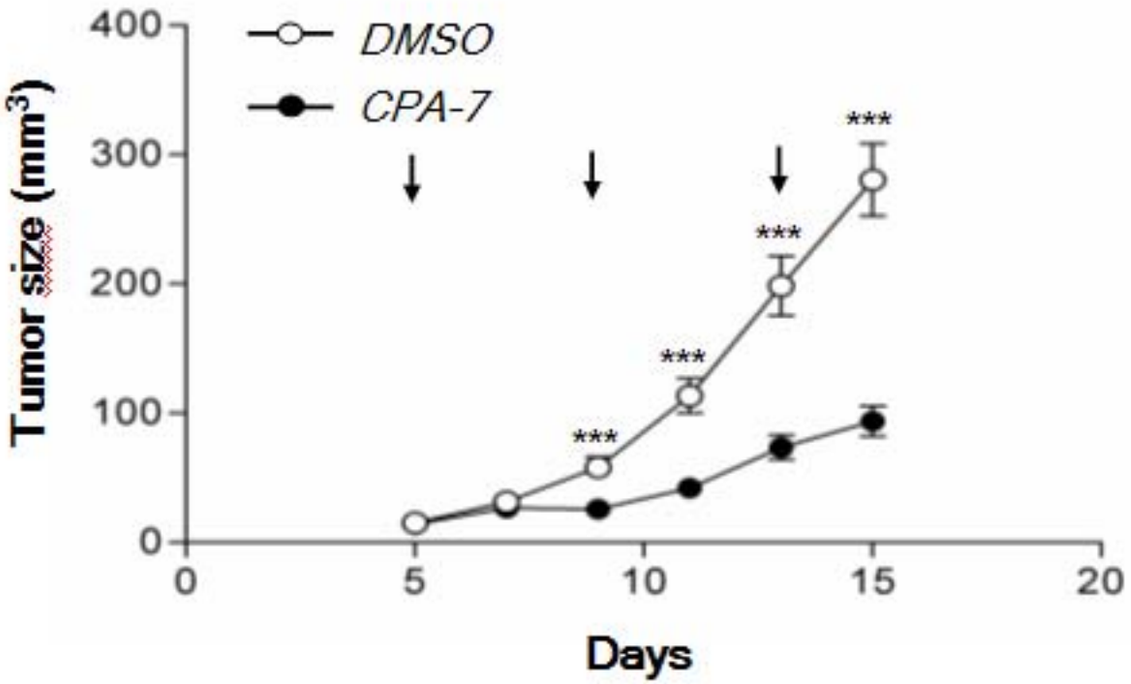
Targeting Stat3 with an inhibitor reduces Stat3 in tumor infiltrating DCs



In vitro sensitivity to CPA-7

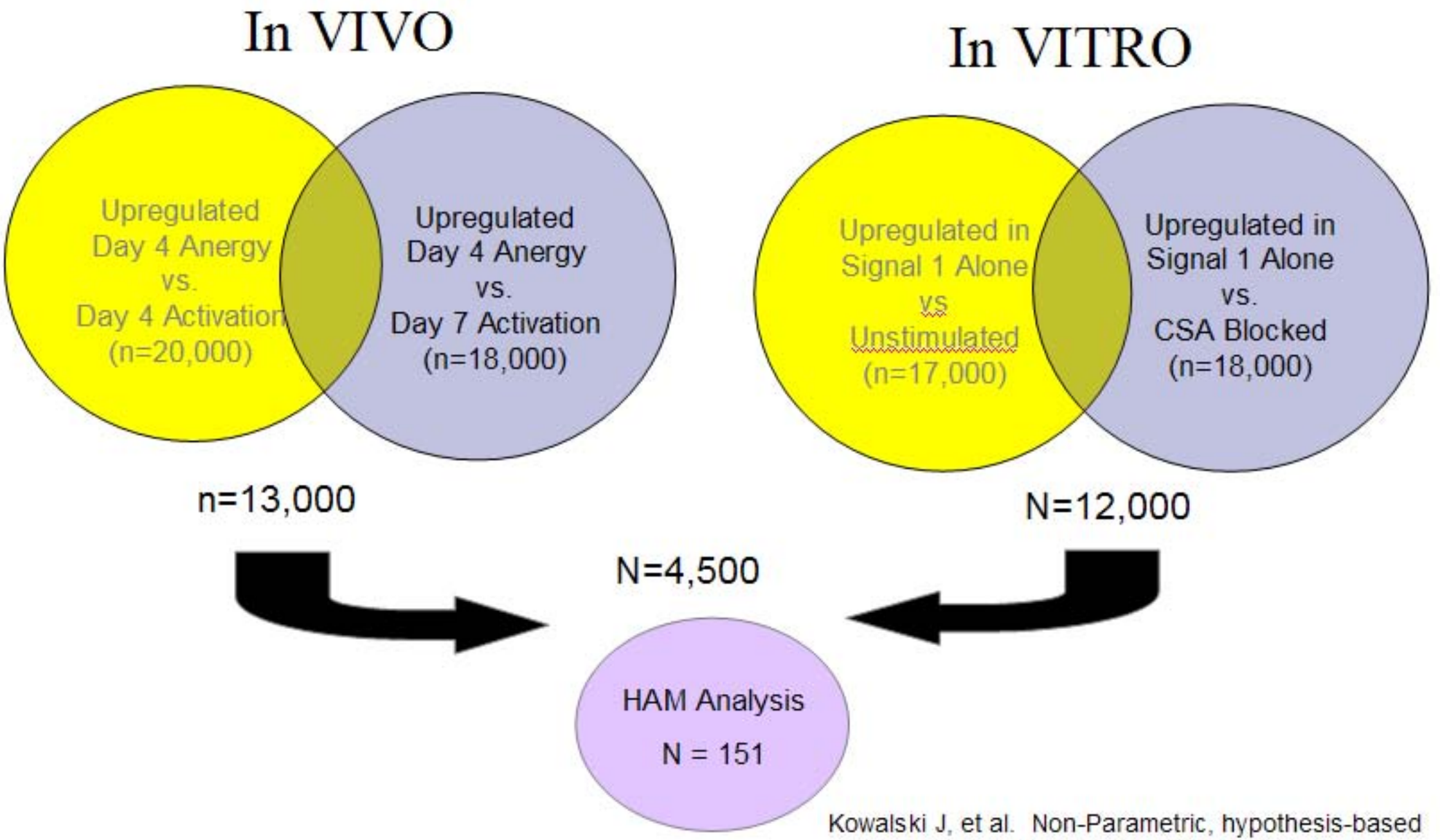


In vivo sensitivity to CPA-7 of MB49



Defining regulatory T cell specific molecules as targets for therapeutic manipulation

Genes Involved in Expression of the Tolerant Phenotype in T Cells

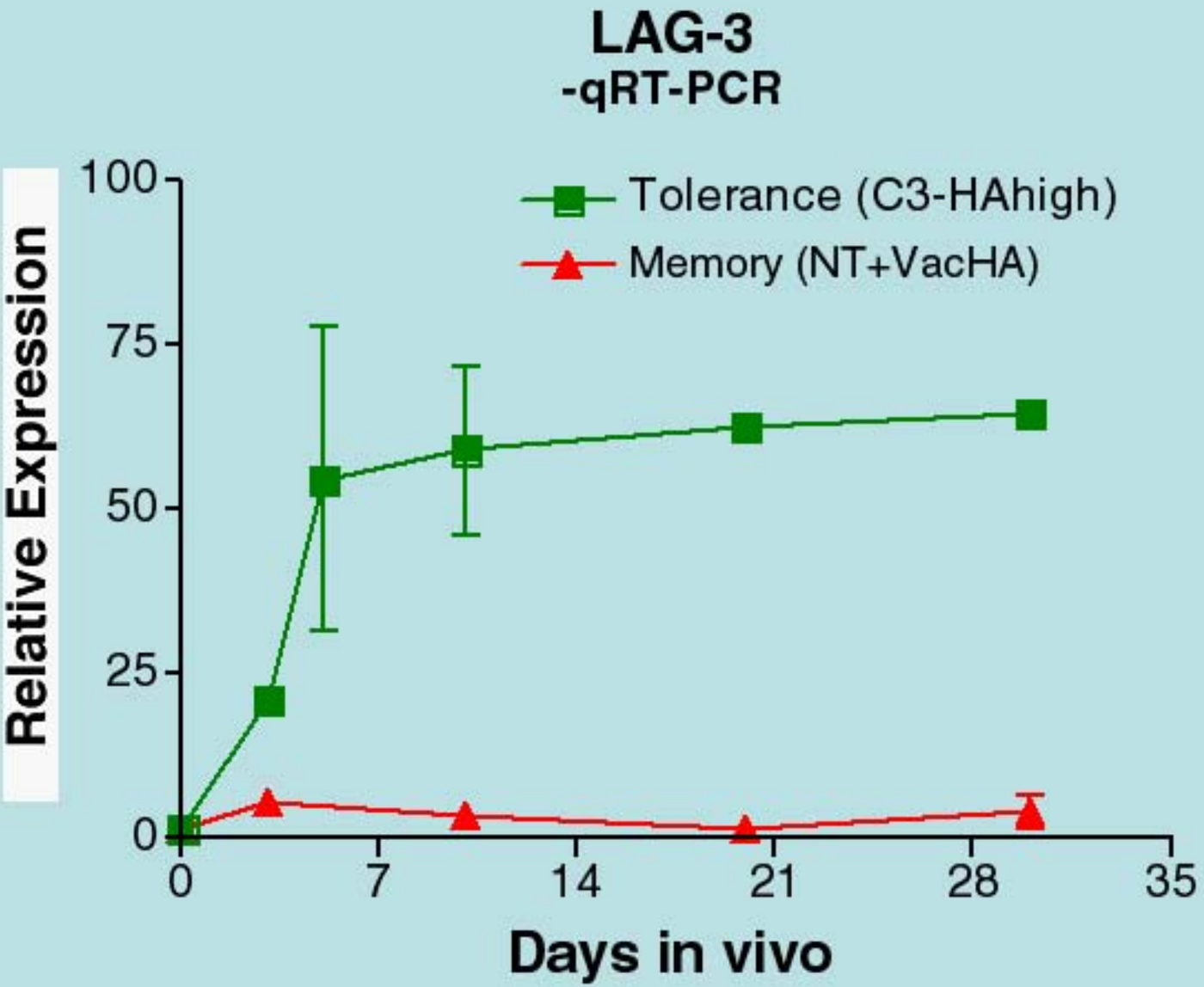


Kowalski J, et al. Non-Parametric, hypothesis-based analysis of microarrays for comparison of several phenotypes. *Bioinformatics* 20: 364-373, 2004

HAM Analysis of Tolerance Specific Genes

Symbol	IN VIVO Ratio Anergy / Memory	IN VITRO Ratio Anergy / Resting	NAME - FROM GO Ontogeny Search
Lag3	3.6	8.7	Lymphocyte-activation gene 3
Bcl3	3.5	14.6	B-cell leukemia/lymphoma 3
Tnfrsf4	2.9	5.7	tumor necrosis factor receptor superfamily member 4
Nfatc1	2.7	14.3	NF-ATc isoform a (NF-ATca)
Il5	2.7	10.7	Interleukin 5
Bcat1	2.6	34.0	branched chain amino acid transferase 1, cytosolic
Ptprs	2.5	4.5	Protein tyrosine phosphatase, receptor type, S
Mapkapk2	2.4	10.5	MAP kinase-activated protein kinase 2
Tubb5	1.8	4.5	beta-tubulin (isotype Mbeta 5)
Bcap37	1.8	4.1	B-cell receptor-associated protein 37
Fhl2	1.7	8.3	Four and a half LIM domains
Il1r2	1.5	3.9	Interleukin 1 receptor, type II
Cish	1.4	5.9	Cytokine inducible SH2-containing protein
Ndrg1	1.4	8.0	N-myc downstream regulated gene 1
Etf1	1.4	4.6	Eucaryotic translation termination factor 1
Prkch	1.4	6.0	Protein kinase C, eta
Cnil	1.3	24.7	cornichon-like protein
Tnfsf11	1.2	26.0	Tumor necrosis factor (ligand) superfamily, member 11
Il13	1.2	95.7	Interleukin 13
Kcnn4	1.2	5.2	calcium -activated potassium channel, small conductance
Ccl1	1.1	159.9	chemokine (C-C) motif ligand 1
Egr2	1.1	75.9	Early growth response 2
Ier3	1.1	8.7	immediate early response 3
Gch	1.1	30.5	GTP cyclohydrolase 1
Rgs16	1.1	13.4	regulator of G protein signalling 16
Csf1	1.1	3.7	colony-stimulating factor 1 (macrophage)
Fkbp8	1.0	8.2	FK506 binding protein 8
Nr4a1	1.0	46.9	nuclear receptor subfamily 4, group A member 1
Lcp2	1.0	4.4	lymphocyte cytosolic protein 2
Dnajc5	1.0	16.1	DnaJ (Hsp40) homolog

LAG-3 is differentially expressed between Teffector/memory & anergic/Treg cells



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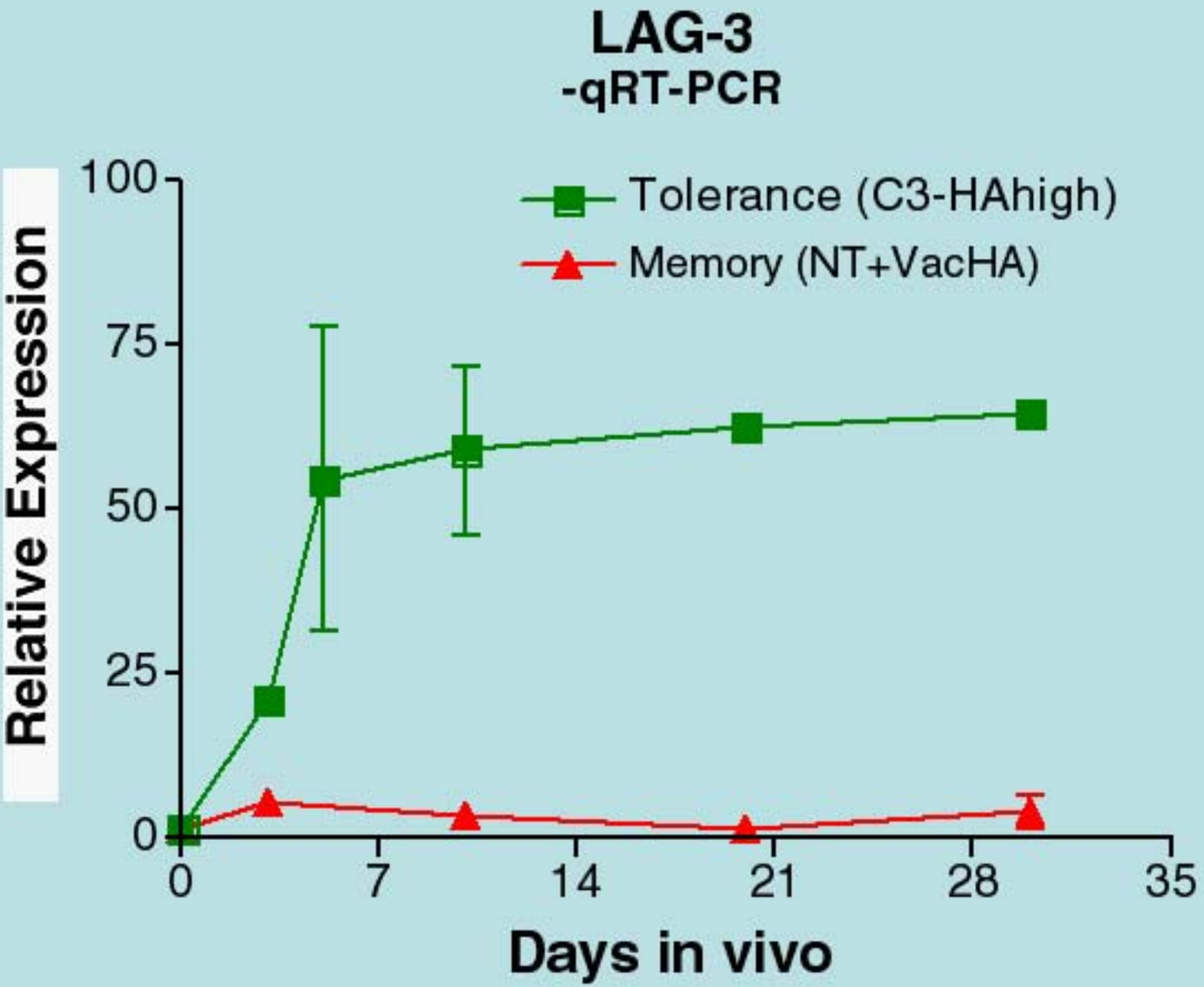
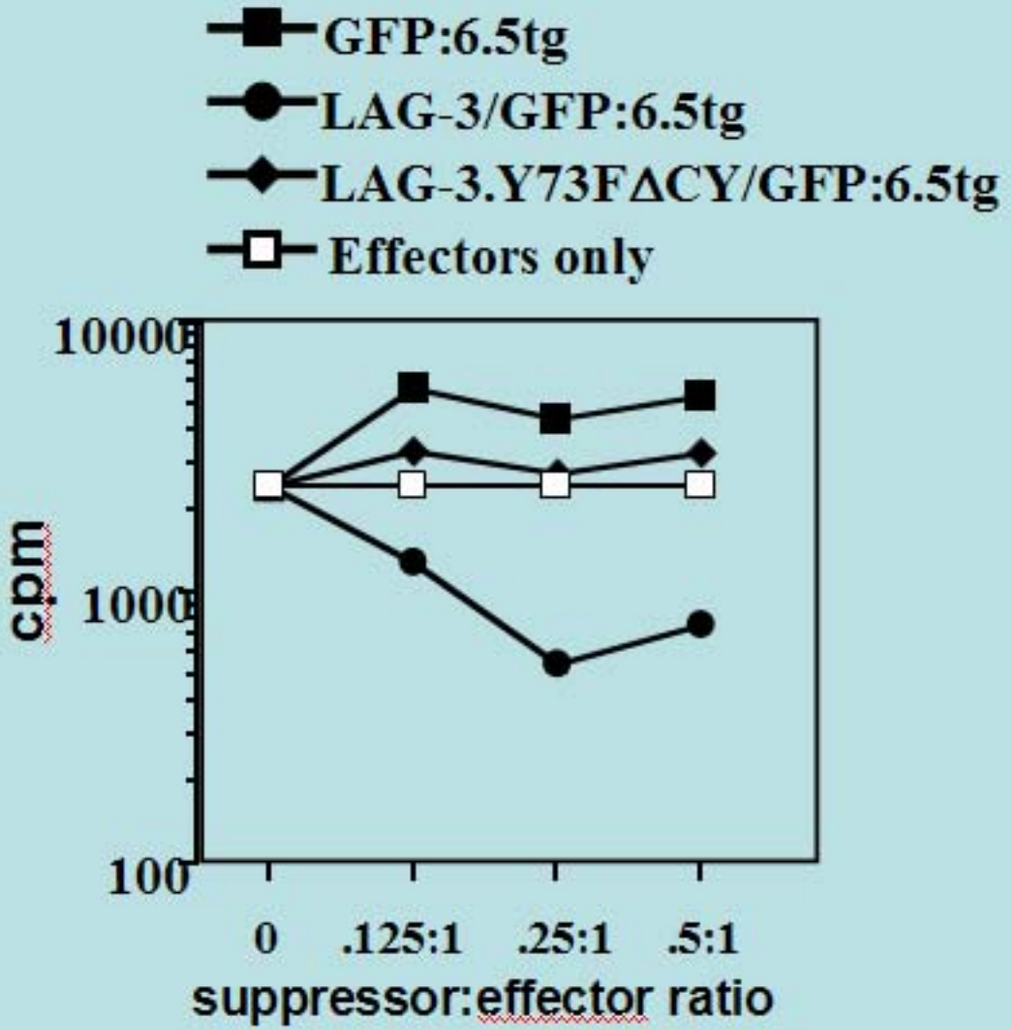
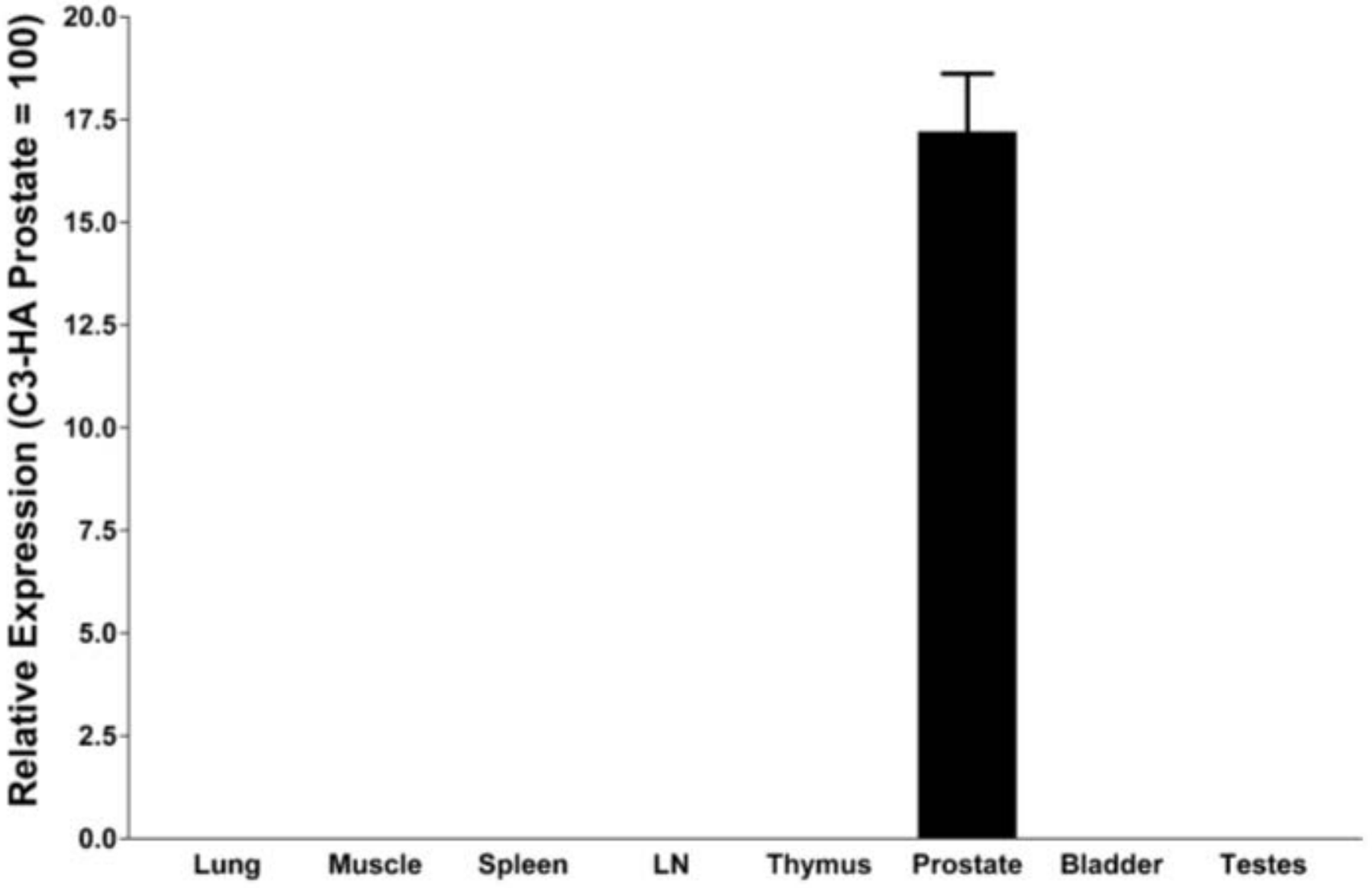


Transduction of CD4+CD25- T cells with wild type LAG-3 confers regulatory capacity



Do Anti-LAG-3 antibodies break tumor tolerance ?

Selective Expression of HA in the Prostate of Probasin-HA Transgenic Mice



The TRAMP Mouse (Pro-SV40Tag)

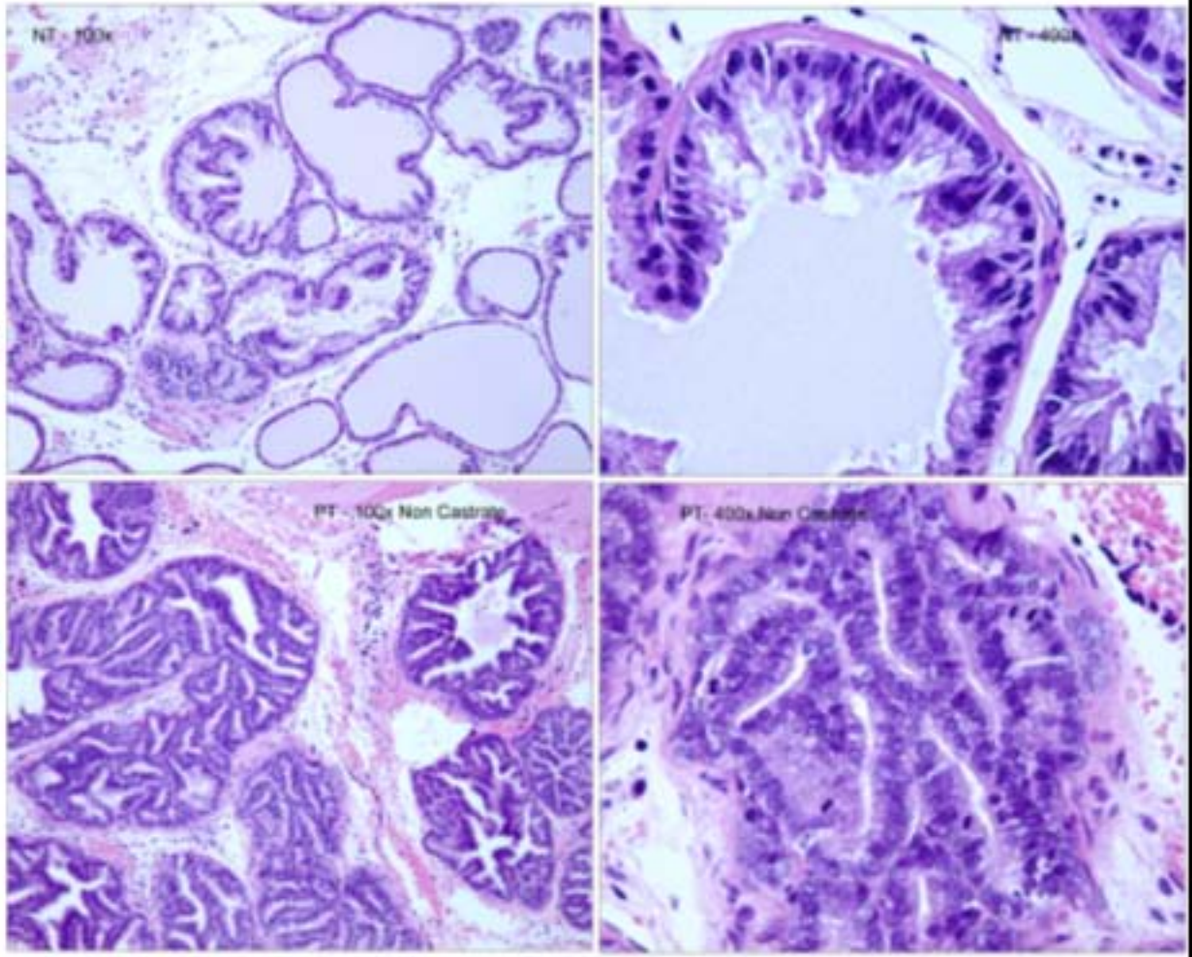
- TRansgenic Adenocarcinoma of the Mouse Prostate
- T Antigen Downregulates Both p53 and Rb pathways
- An "AUTOCHTHONOUS" model
- PIN at 8-12 Weeks
- Moderately Differentiated Adenocarcimoma at 18 weeks
- Poorly Differentiated Adenocarcinoma at 24-30 weeks
- Metastases to Lung, Lymph Nodes (Occasionally Bone)
- Death Before 33 Weeks

The ProHA x TRAMP Mouse (ProTRAMP)

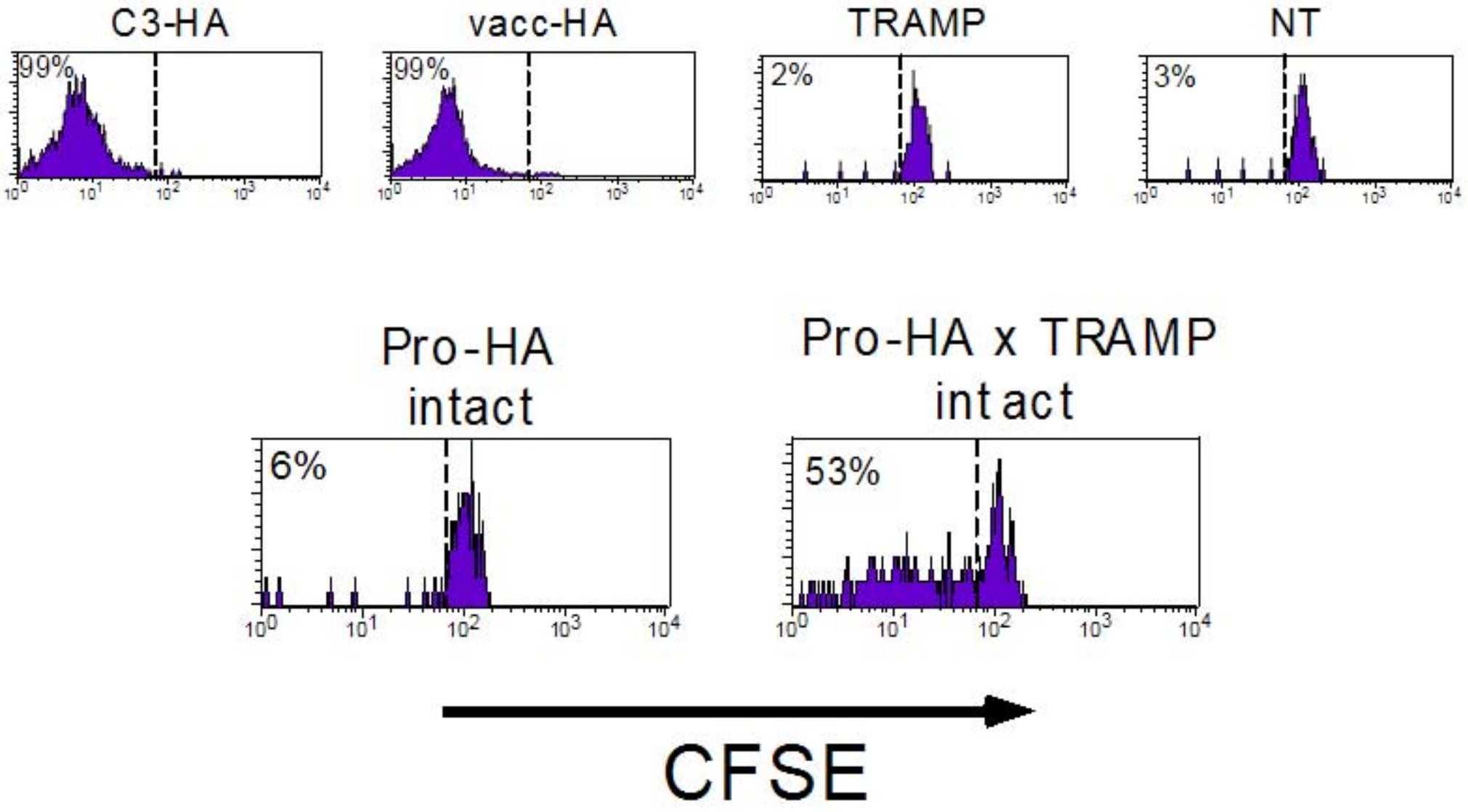
- ProSV40 – Oncogenic
- ProHA – A Tumor and Tissue Specific Antigen
- Disease grossly identical to TRAMP

Normal prostate

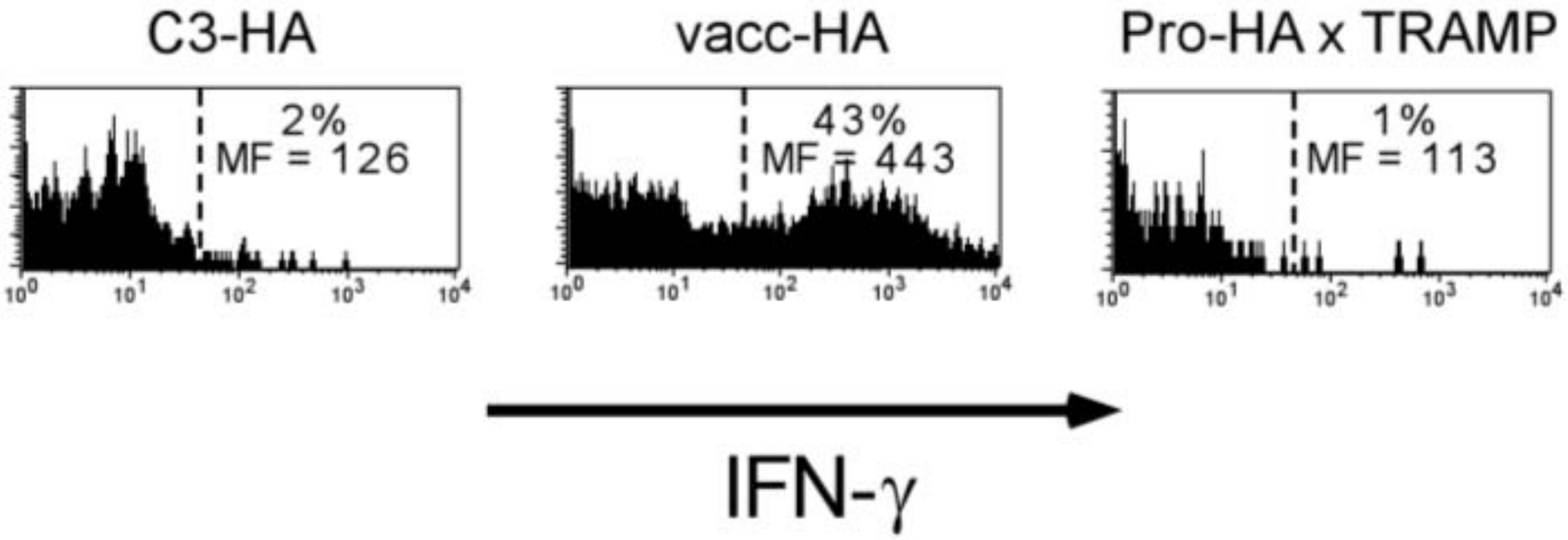
ProTRAMP prostate



Tumorigenesis Increases Prostatic Antigen Recognition



This Increased Recognition is Tolerogenic



Can LAG-3 blockade alter endogenous T cell function?

Day 0: α-LAG-3 (0.2mg)

Day 1: VAC-HA

Day 3: α-LAG-3 (0.2mg)

Day 6: Adoptive transfer targets

NT
ProTramp
C3-HA

Mix 1:1; inject i.v.

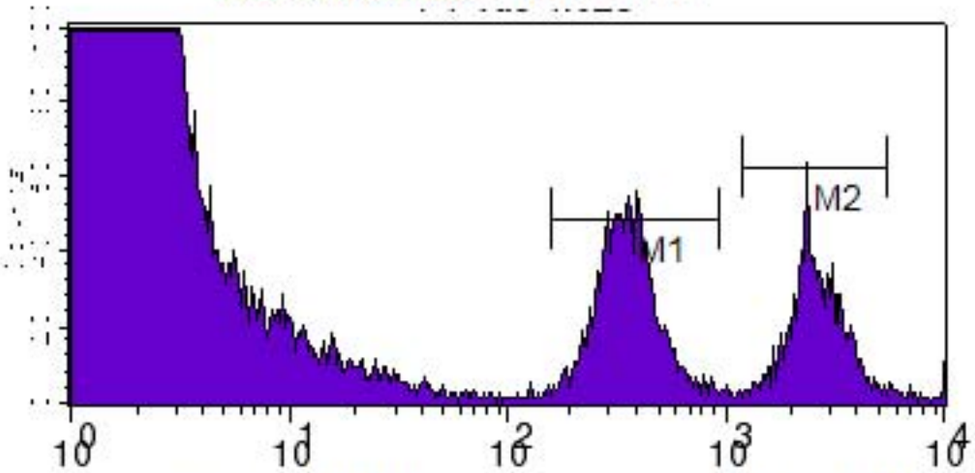
Unstimulated
CFSE^{lo}

HA peptide-pulsed
CFSE^{hi}

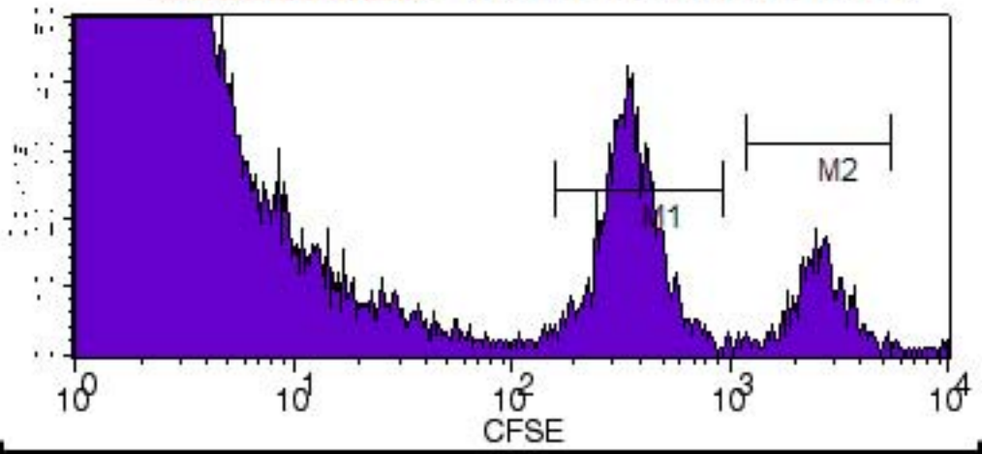
B10.D2 splenocytes

Day 7: Harvest spleens; Flow

ProTRAMP+vaccine

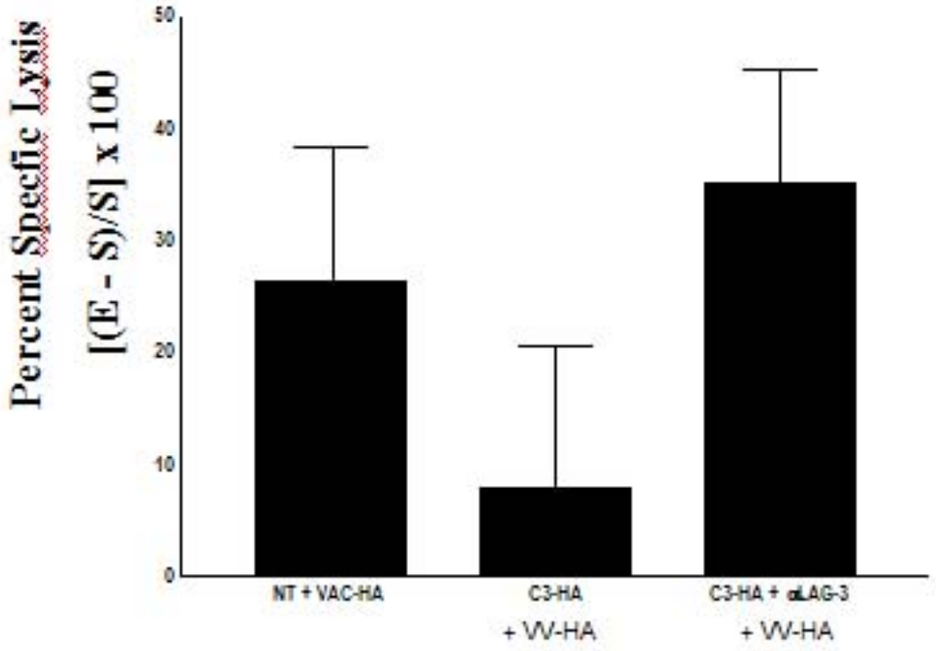


ProTRAMP+vaccine+anti-LAG3

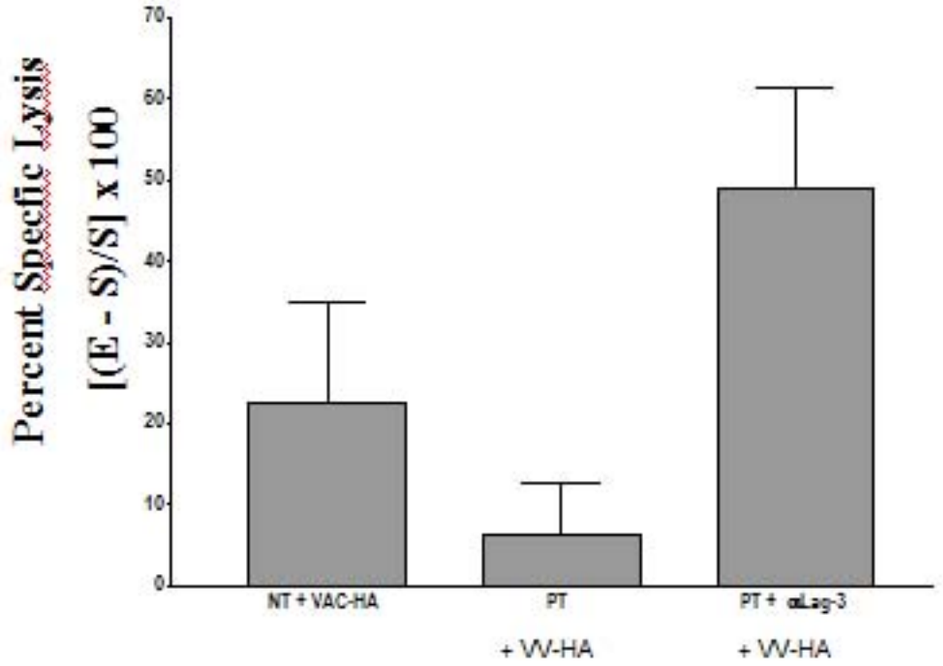


Self- and tumor-tolerized endogenous CTL regain effector function in vivo after LAG-3 blockade

C3-HA

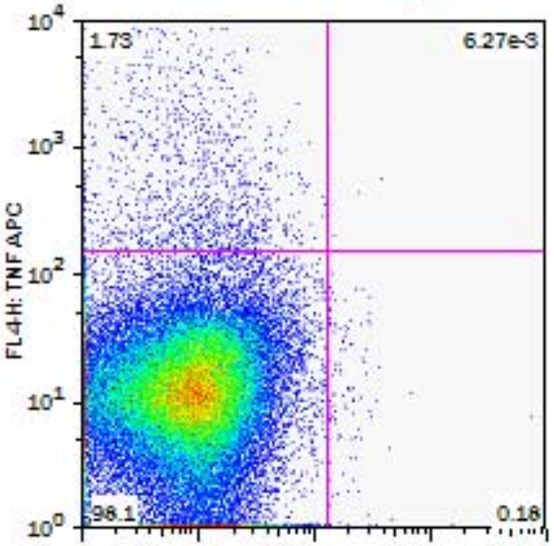


ProTramp (14-16 wk old)

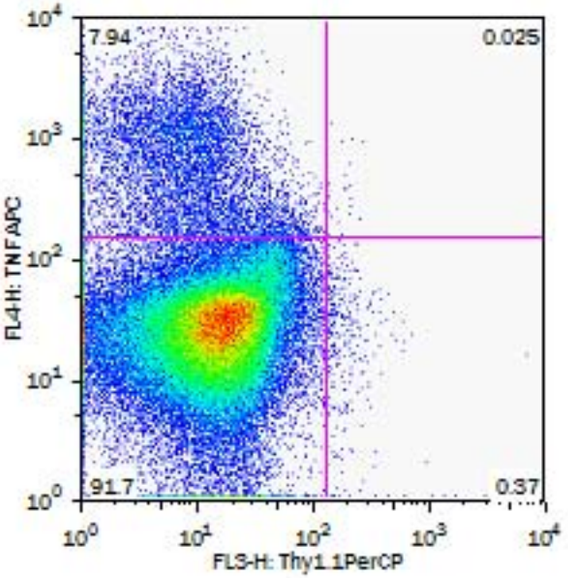


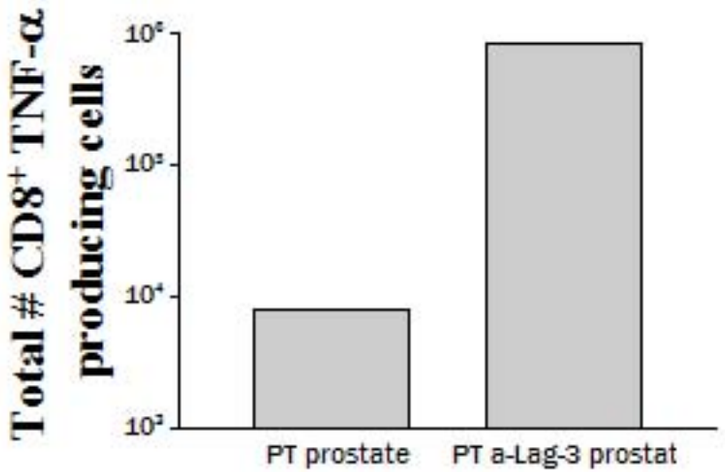
α-LAG-3 leads to endogenous CD8 migration and TNF-α production within prostates of ProTRAMP mice

ProTramp



ProTramp + α-LAG-3

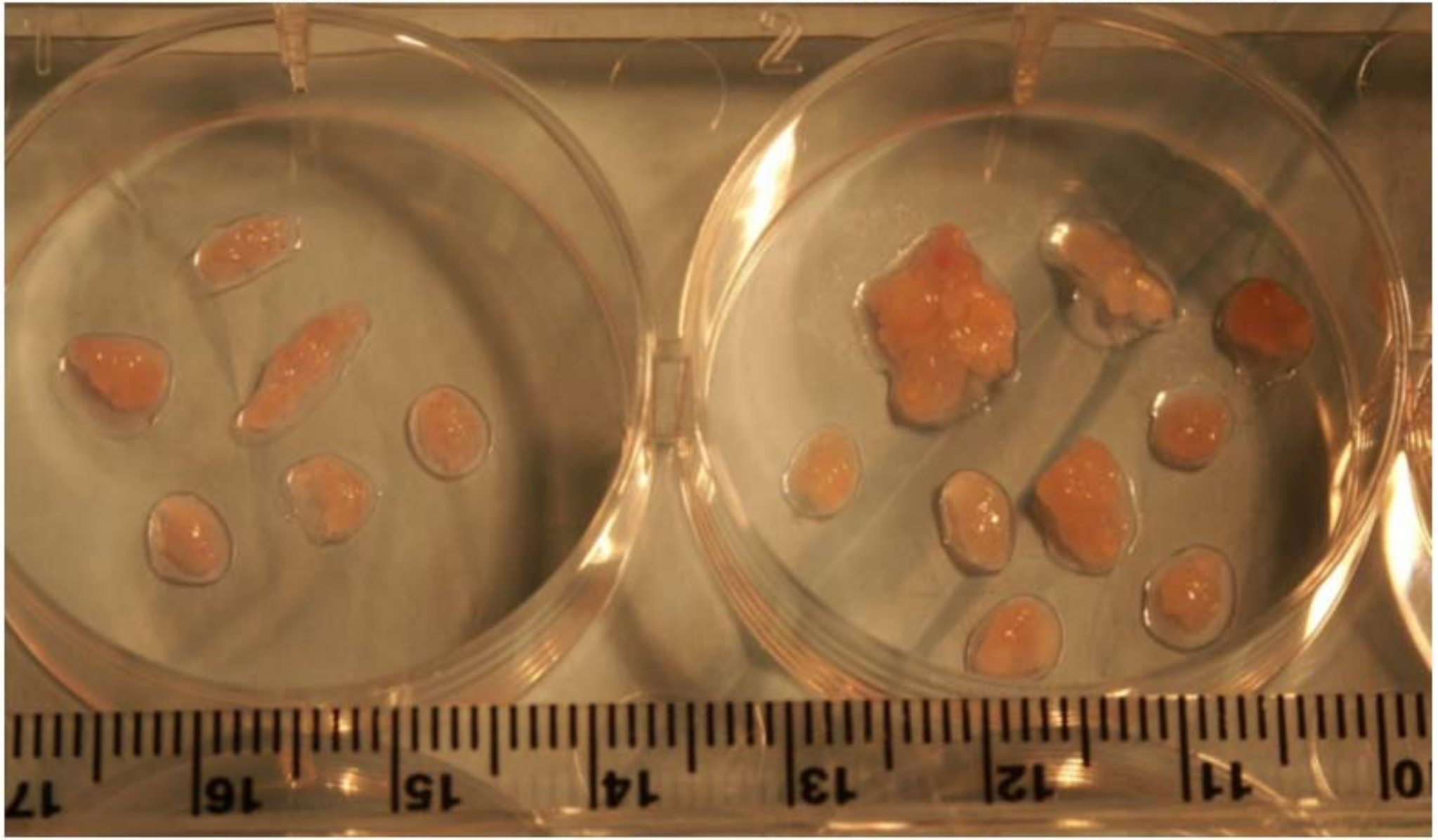




Prostate tissue from Pro-Tramp mice 7 days post-AT

Clone 4 Adoptive Transfer

Clone 4 Adoptive Transfer + anti-LAG-3



Johns Hopkins

Chuck Drake

Joe Grasso

St Jude

Dario Vignali

Craig Workman

Moffitt/City of Hope

Hua Yu

Richard Jove

Marcin Kortelewski