Considerations to overcome downstream resistance to melanoma antigen-specific effector T cells

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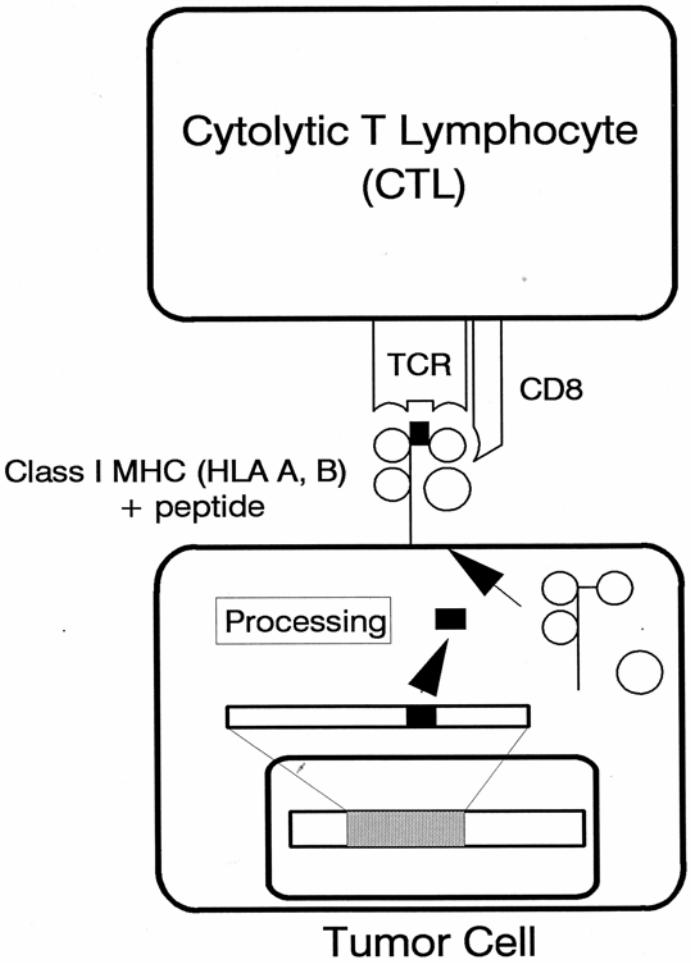
Recognition of class I MHC-restricted tumor antigen peptides by CD8$^+$ CTL



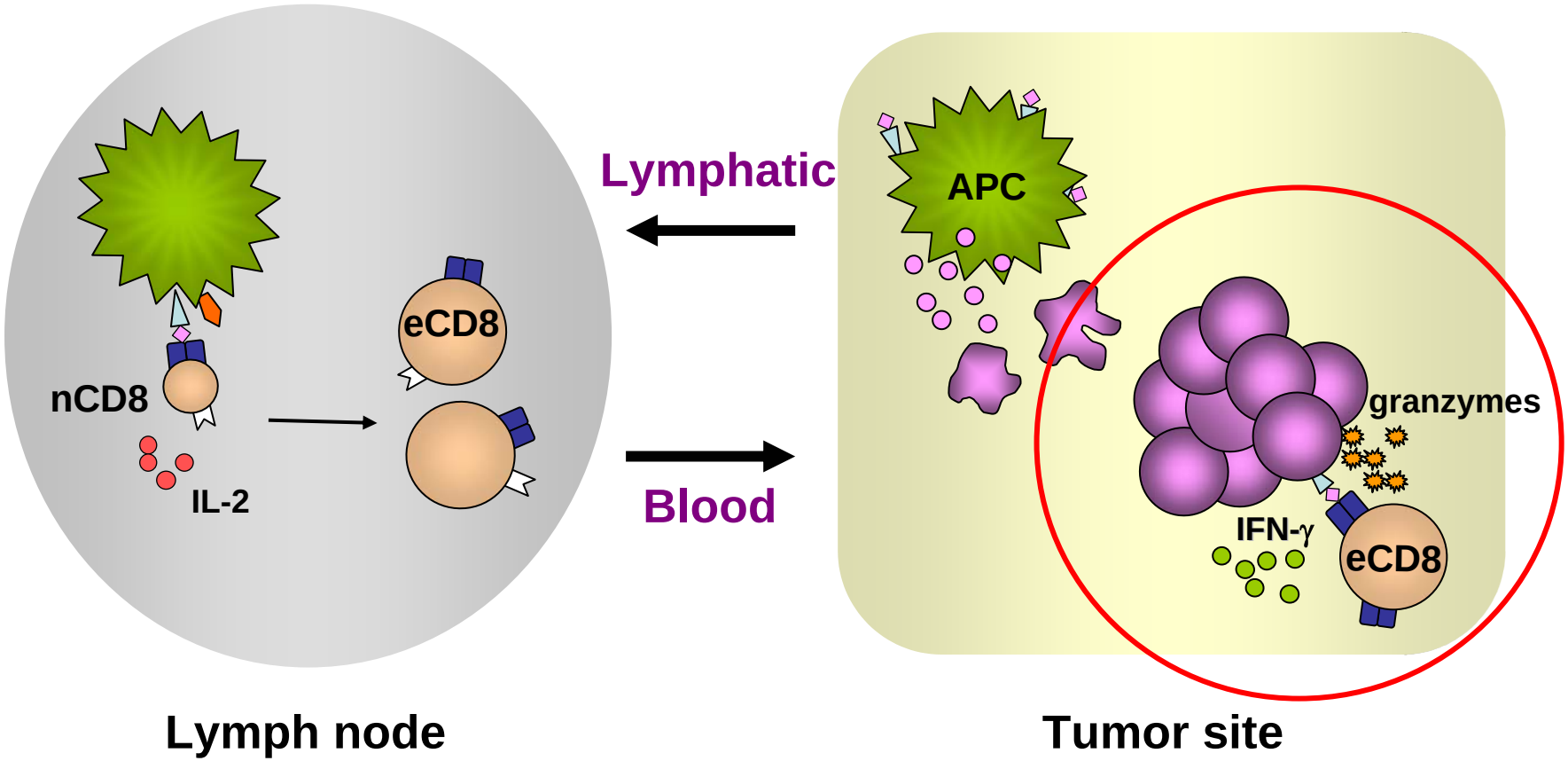
Antigen discovery:

- Quickly led to vaccine clinical trials
- Based on notion that fundamental defect in patients is failed T cell priming
- Results: Vaccines often increase specific CD8$^+$ T cells in blood
- Nonetheless, tumor regressions are rare

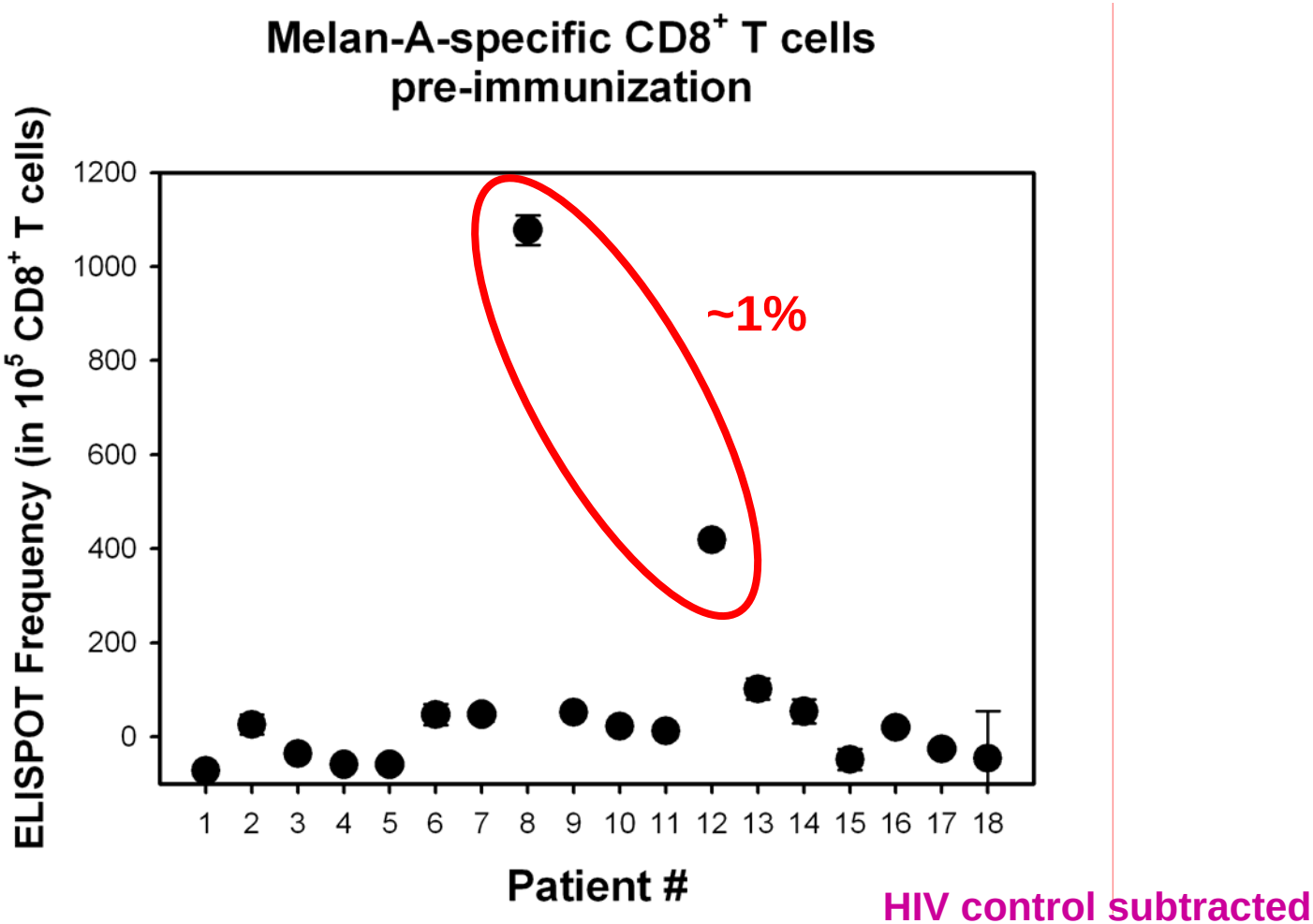
Present conundrum:

- Was that the right hypothesis?
- Spontaneously activated melanoma antigen-specific T cells can be found in patients
- Detected in blood and within tumors
- e.g. this is starting point for TIL therapy
- ***Points to downstream resistance as dominant defect in many patients***

Tumor escape from the effector phase of an anti-tumor immune response may be a major obstacle

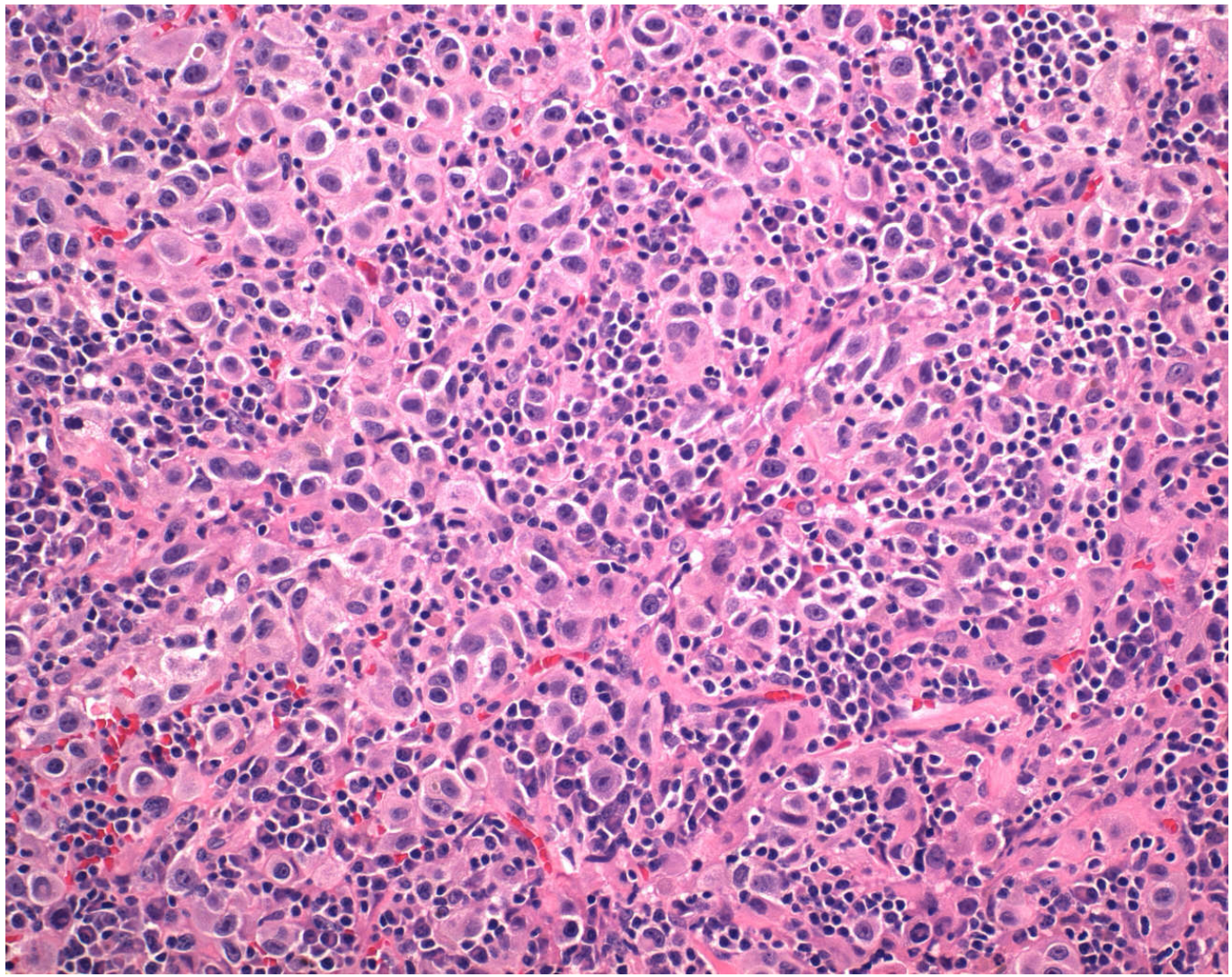


Melanoma patients can exhibit very high frequencies of circulating Melan-A-specific IFN-γ-producing CD8$^+$ T cells

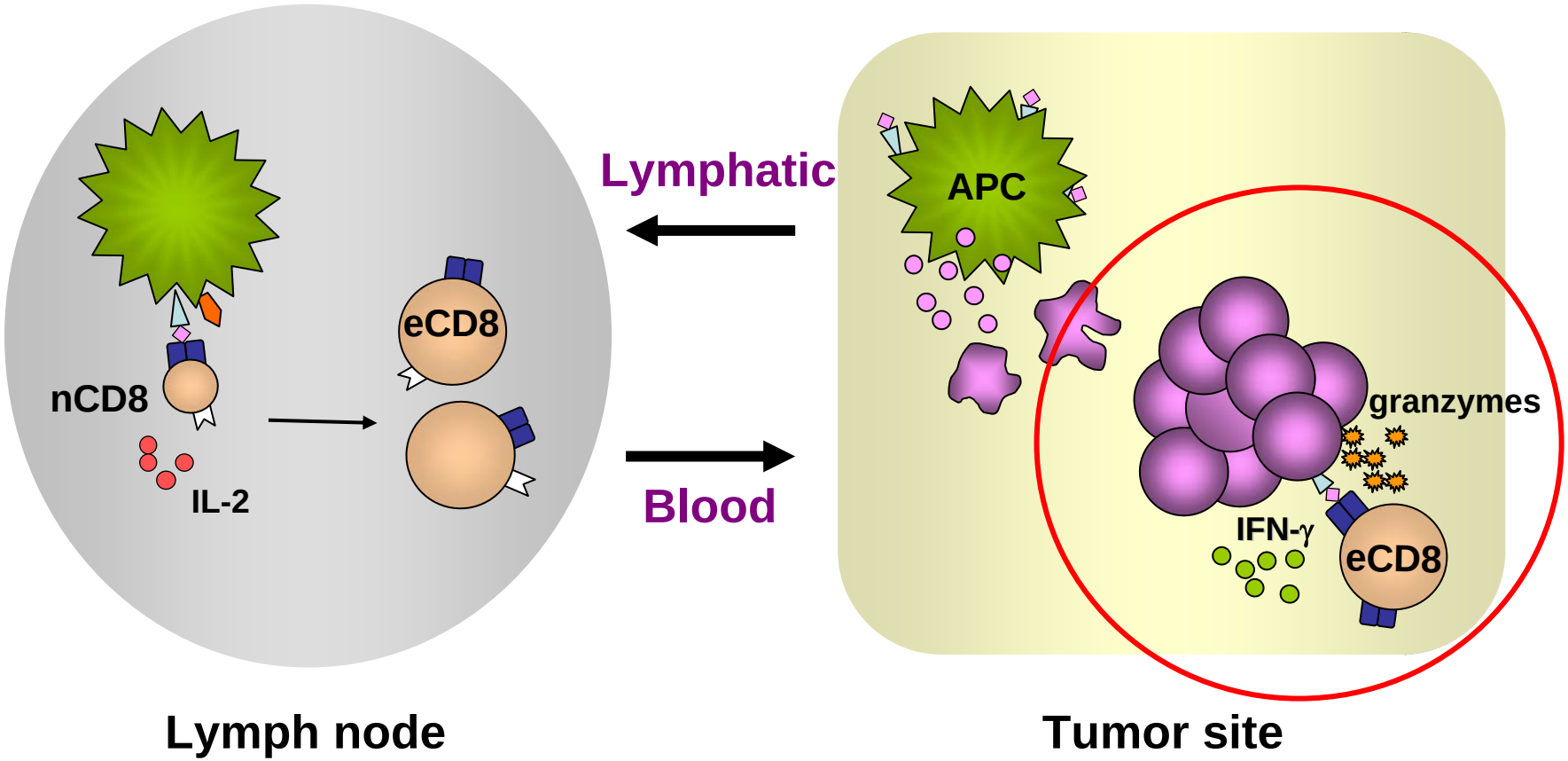


HIV control subtracted

Some melanoma metastases are replete with lymphocytes



Focus on defect in effector phase of immune response in tumor microenvironment



Understanding mechanisms of negative regulation of T cell function in tumor microenvironment

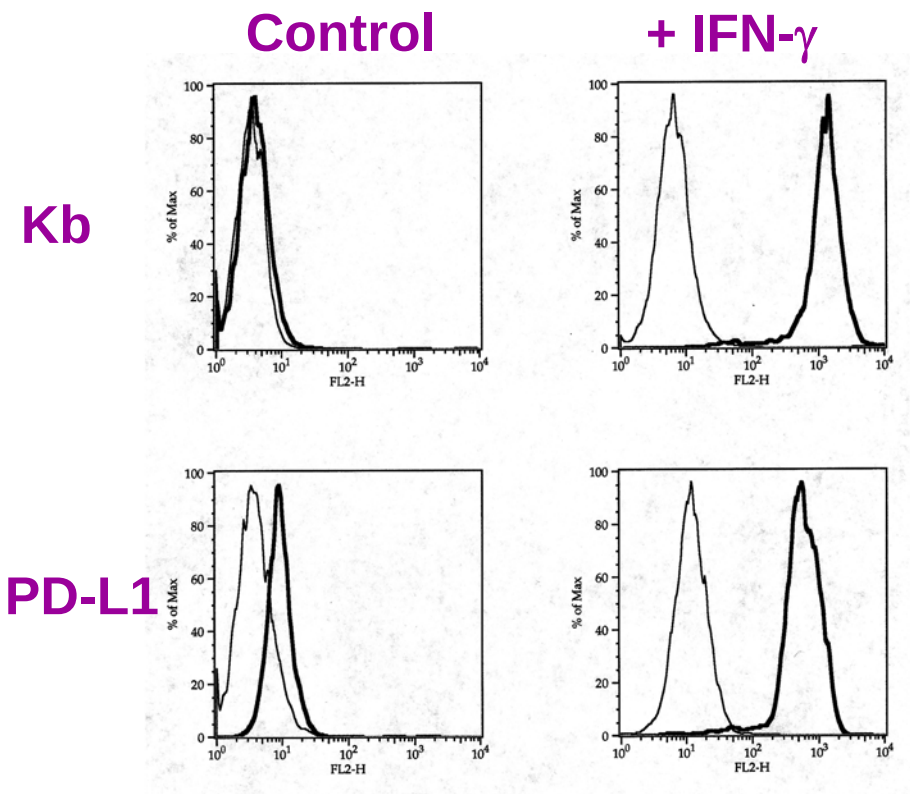
- Candidate processes
  - Inhibitory receptors (e.g. PD-L1/PD-1)
  - Inhibitory cell populations (e.g. Tregs)
  - T cell intrinsic dysfunction (e.g. anergy)
- Analyze tumor microenvironment from metastatic melanoma tumors
  - TIL function, phenotype, and molecular profile
  - Real-time RT-PCR candidates and validation
  - Gene array analysis of stromal elements

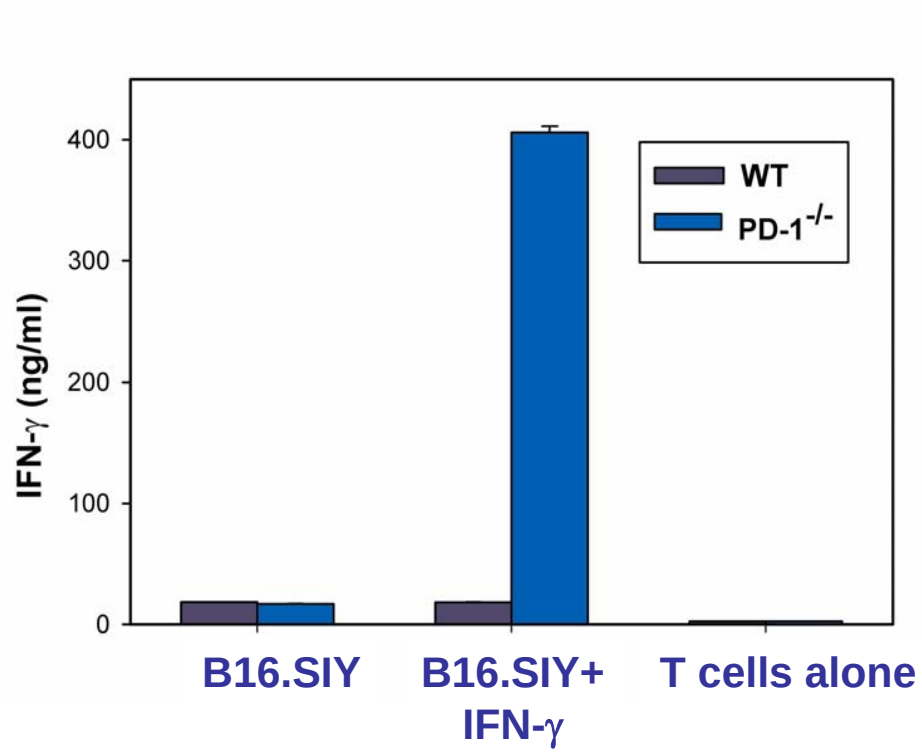
Are there drugable targets?

1. PD-1/PD-L1

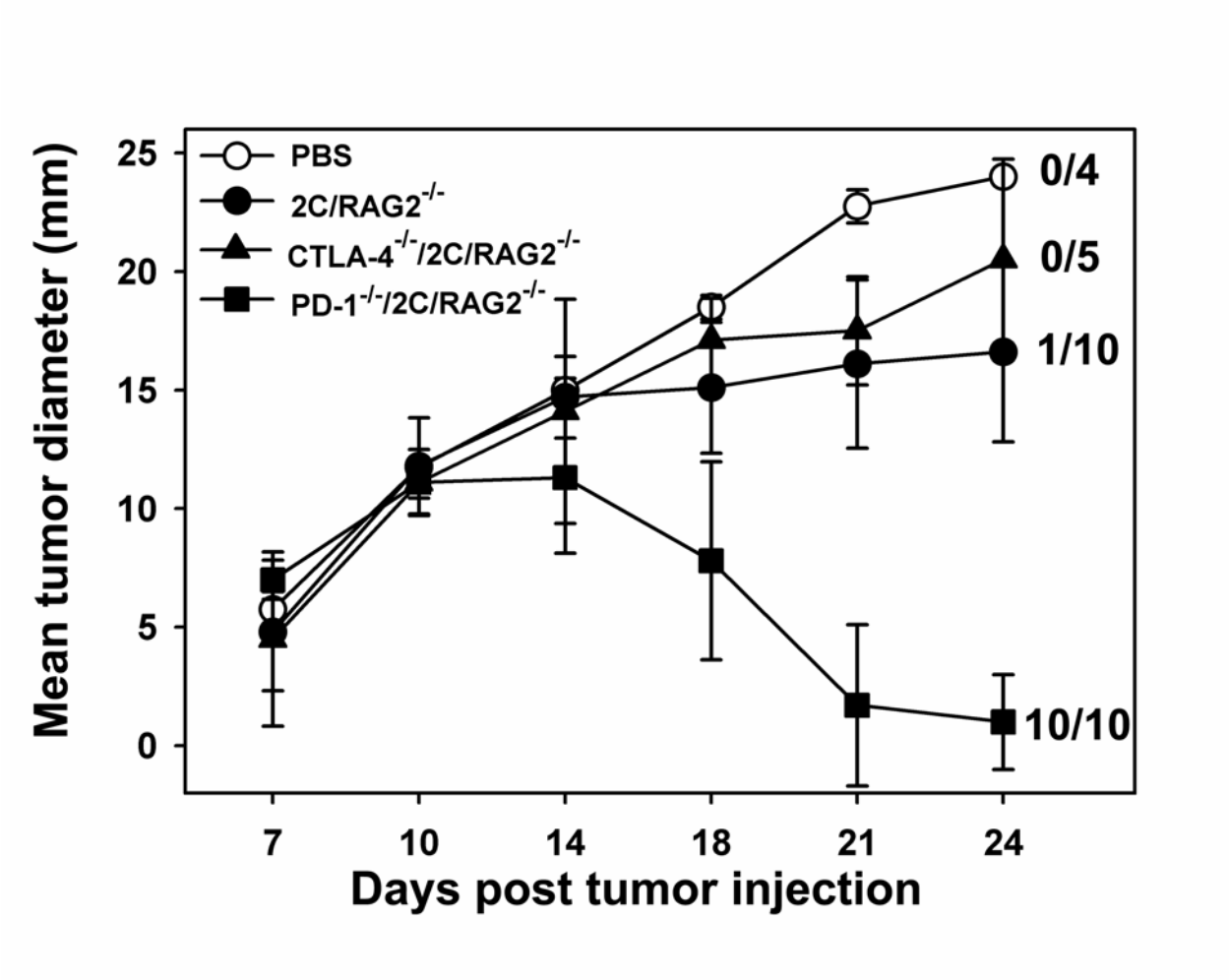
- PD-1: receptor induced on activated T cells
- Contains ITIM and ITSM domains that can recruit SHP2
- PD-1-deficient mice develop autoimmune syndromes => dominant role is negative
- Two defined ligands: PD-L1/B7-H1 and PD-L2/B7-DC
- PD-L1 can be expressed in non-hematopoietic tissues, including tumor cells

IFN-γ-treated B16.SIY-GFP melanoma stimulates PD-1$^{-/-}$ but not PD1$^{+/+}$ 2C TCR Tg T cells in vitro

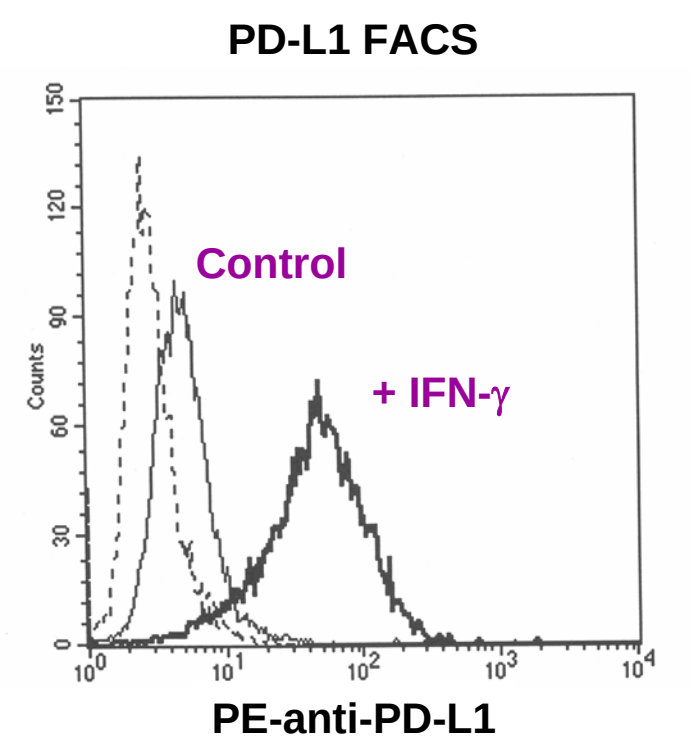


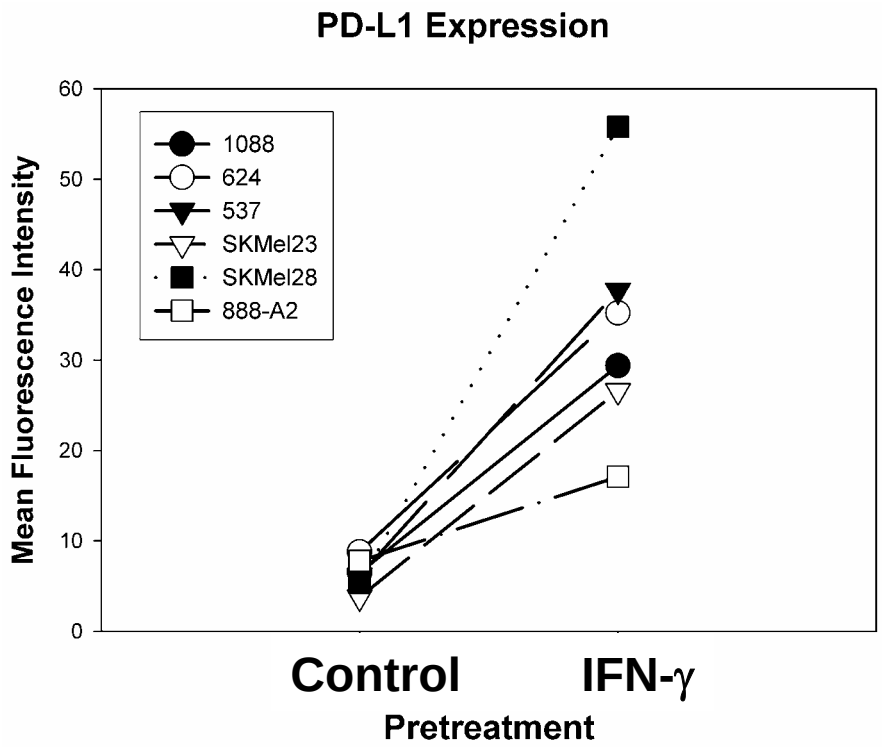


PD-1$^{-/-}$ 2C T cells reject tumors in vivo under conditions in which CTLA-4$^{-/-}$ 2C cells do not

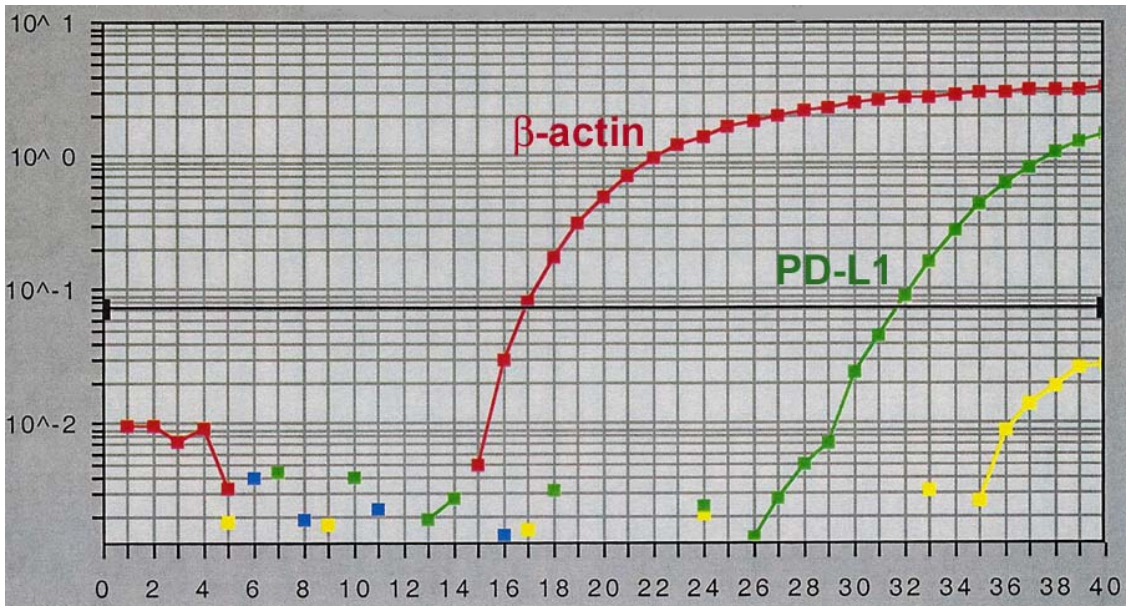


IFN-γ upregulates PD-L1 on all human melanoma cell lines tested





PD-L1 mRNA is expressed in fresh melanoma tumor biopsies



Tumor cells also positive by IHC

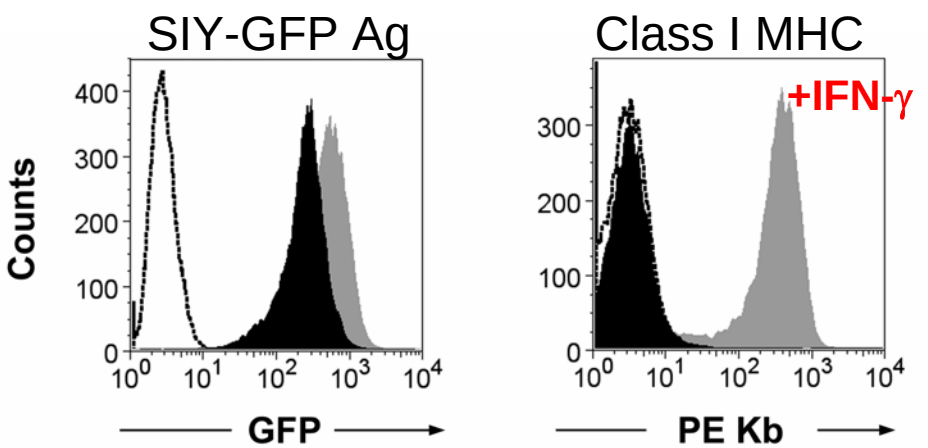
Therefore, the PD-1/PD-L1 interaction is an important candidate negative regulator of anti-tumor immunity in human melanoma

2. Regulatory T cells

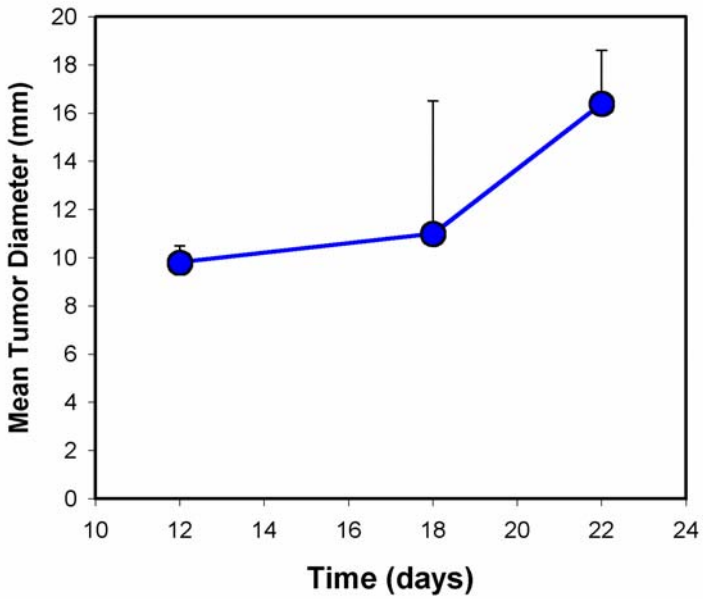
- Defined by $CD4^+/CD25^+$ phenotype
- Selectively express the transcription factor FoxP3, and preferentially express the TNFR family member GITR
- Functionally suppress activation of $CD4^+$ and $CD8^+$ effector T cells in vitro and in vivo
- Observed to be present in increased numbers in cancer patients and within tumors

B16 melanoma cells expressing the model antigen SIY-GFP grow progressively in vivo

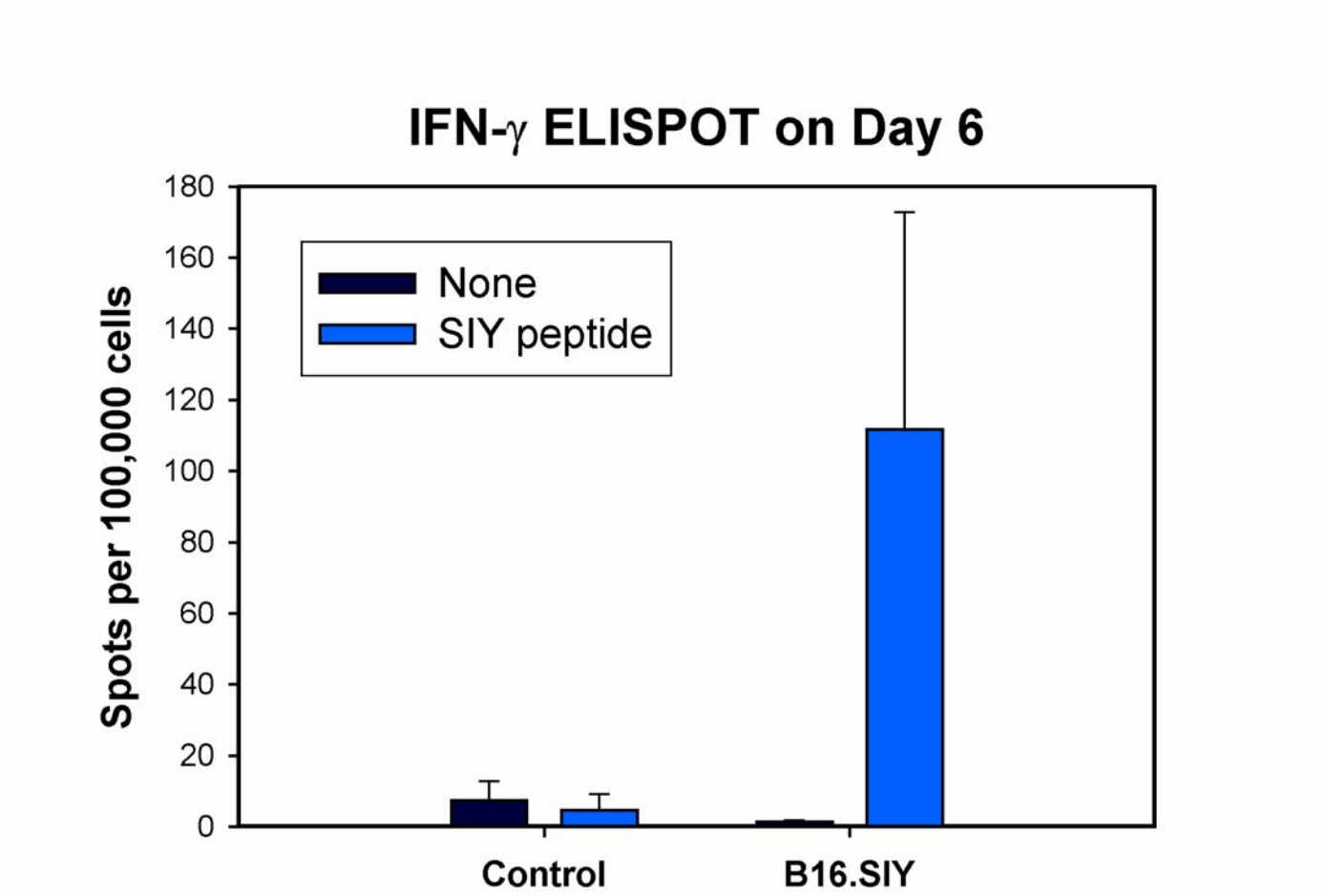
FACS analysis



Tumor growth (B6 mice)

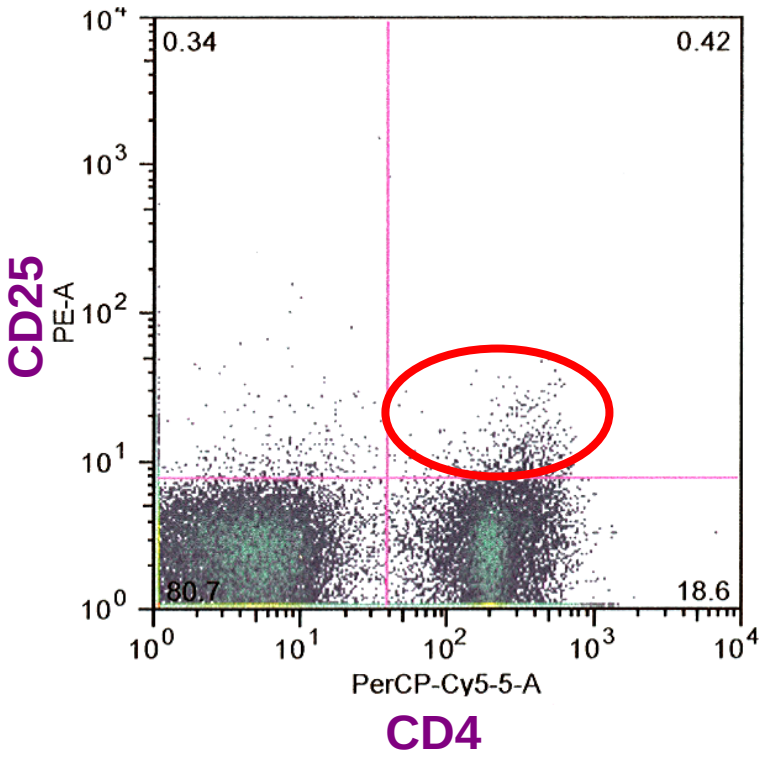


Spontaneous induction of anti-SIY $CD8^+$ T cells on day 6 in vivo despite lack of tumor rejection



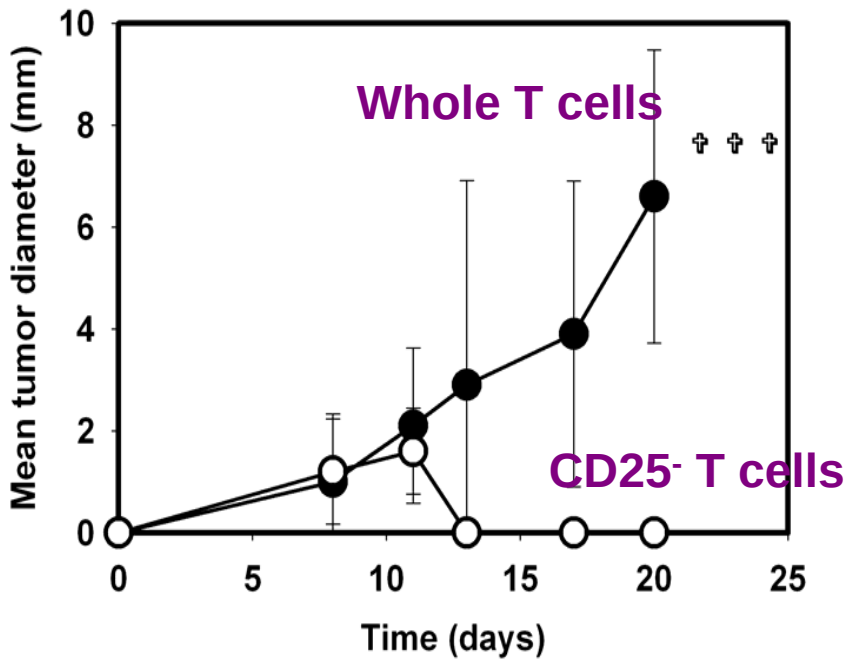
Involvement of CD25^{+} Tregs in preventing spontaneous rejection of B16.SIY melanoma

TIL FACS analysis

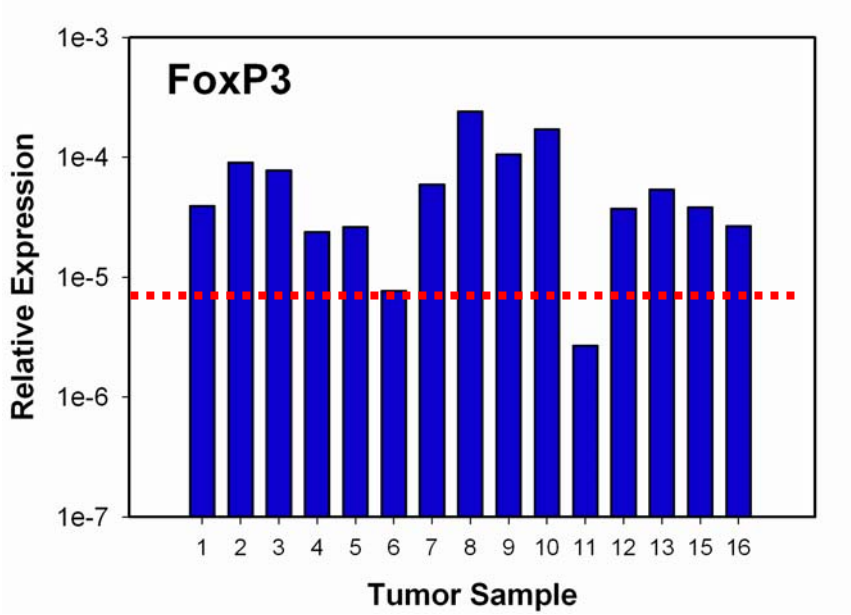


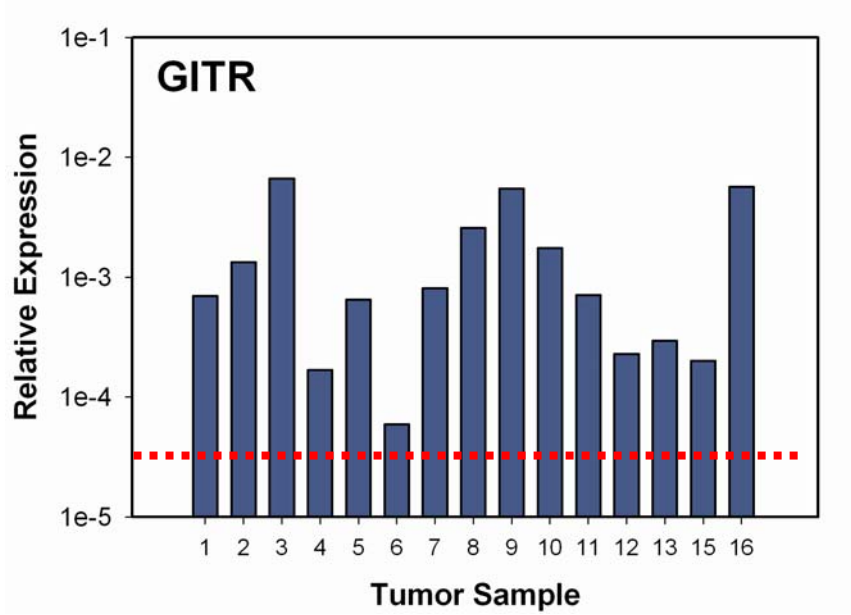
Tumor day +28

Tumor rejection

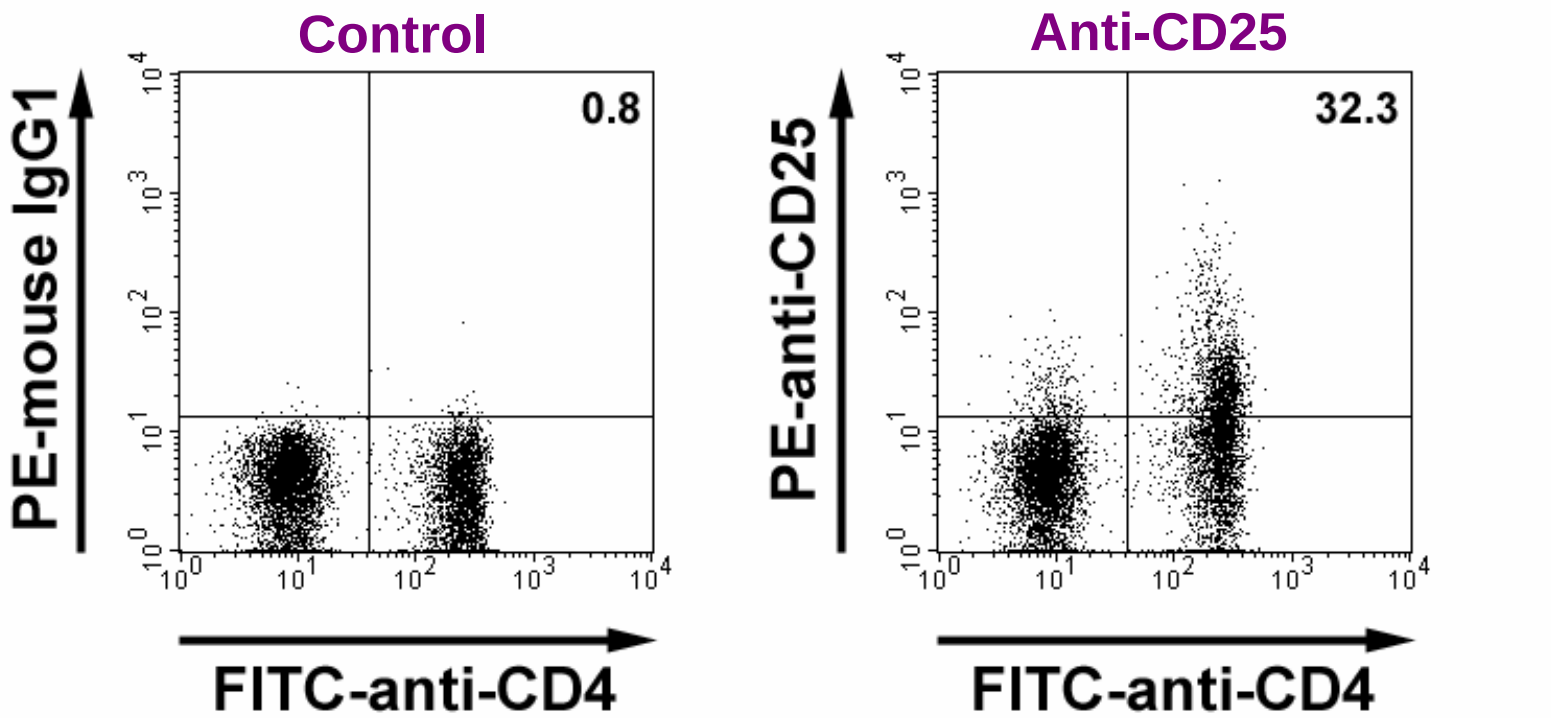


Human metastatic melanoma biopsies contain FoxP3 and GITR transcripts





$CD4^+CD25^+$ cells are present among human melanoma TILs

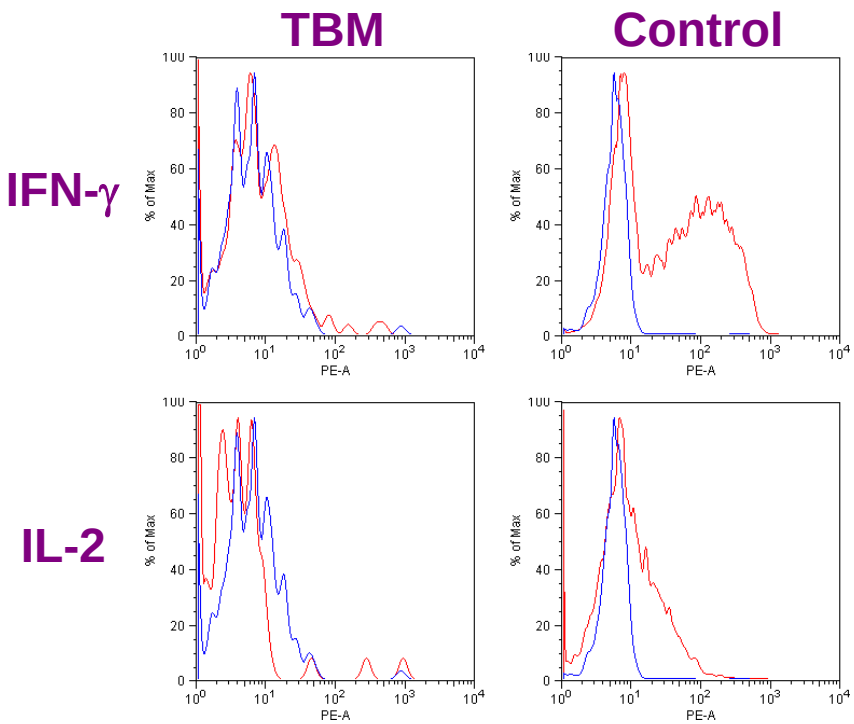


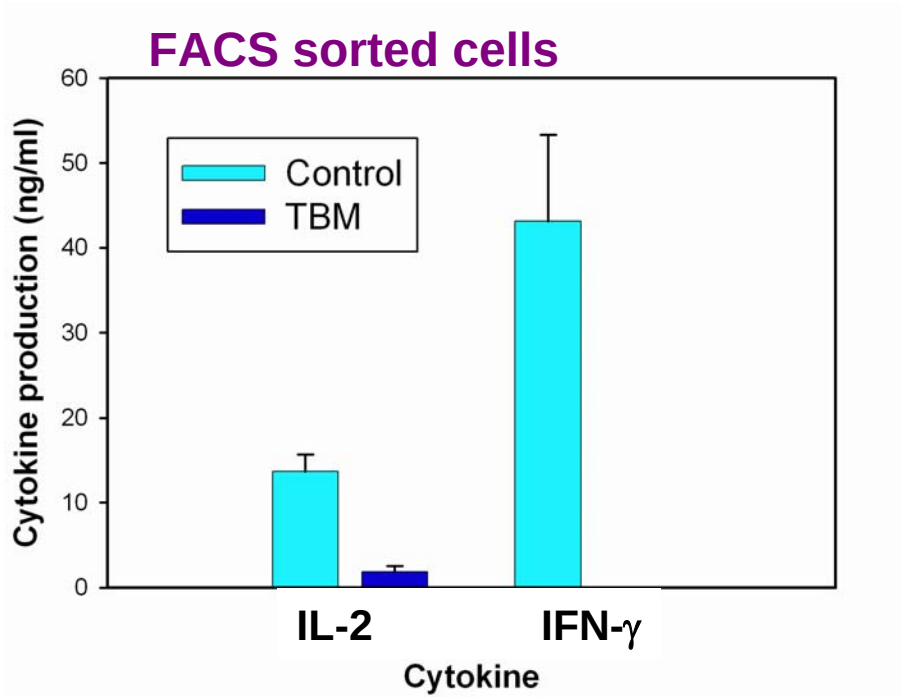
Therefore, regulatory T cells represent an important candidate negative regulator of anti-tumor immunity in human melanoma

3. T cell anergy

- Can result from TCR ligation in the absence of CD28 costimulation by B7-1/B7-2
- Characterized by defective TCR-induced cytokine production and proliferation
- Hypothesized to represent one mechanism of tolerance to tumor antigens
- Reversible by proliferation via cytokines (IL-2, IL-7, IL-15)

Hyporesponsiveness of 2C TCR Tg T cells isolated from P1.HTR tumor-bearing P14/RAG2$^{-/-}$ mice (day 28)

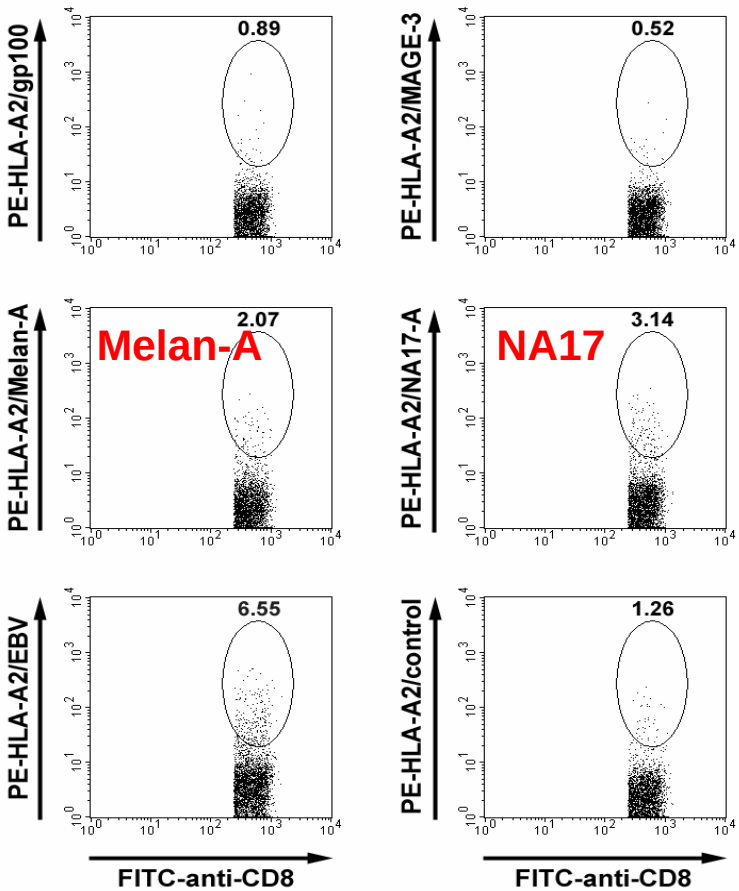




- Restimulated 16 hrs with antigen in vitro
- Cytokine production to PMA+Ionomycin intact

Malignant melanoma ascites fluid contains melanoma antigen-specific CD8$^+$ T cells bearing an activated phenotype

Tetramer staining



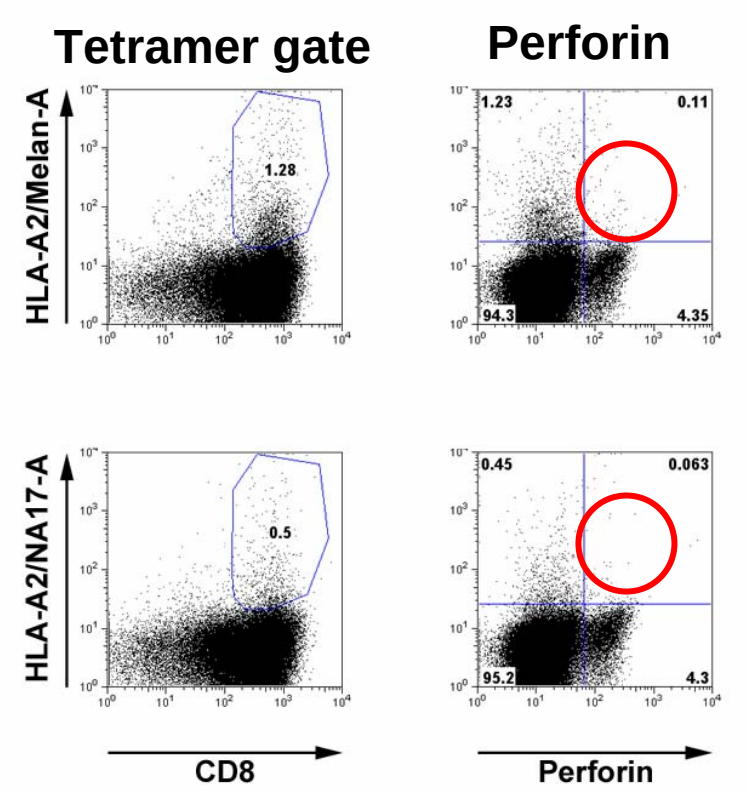
Additional phenotyping

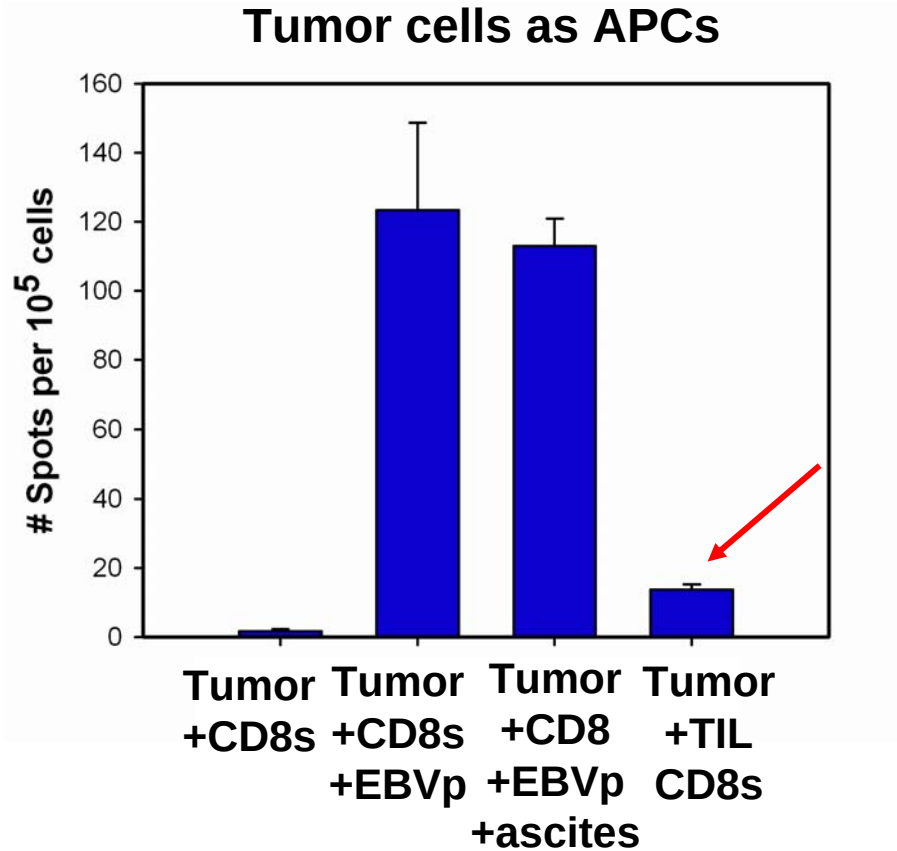
	Overall CD8$^+$	EBV tetramer$^+$	Melan-A tetramer$^+$	NA17-A tetramer$^+$
CD45RA$^+$/CD62L^{hi}	12.9	1.2	6.2	4.4
CD45RA$^+$/CD62L^{lo}	26.2	13.0	20.5	10.2
CD45RA$^-$/CD62L^{hi}	4.6	1.2	1.9	1.7
CD45RA$^-$/CD62L^{lo}	56.2	84.6	71.4	83.8

Majority CD45RA^{lo}, CD62L^{lo}, CD28$^+$

	Overall CD8$^+$	EBV tetramer$^+$	Melan-A tetramer$^+$	NA17-A tetramer$^+$
CD45RA$^+$/CD28$^+$	20.1	8.2	14.0	18.3
CD45RA$^+$/CD28$^-$	4.7	2.0	2.5	3.3
CD45RA$^-$/CD28$^+$	44.4	49.9	47.2	46.3
CD45RA$^-$/CD28$^-$	11.9	25.9	18.5	8.1

Ascites CD8$^+$ T cells lack perforin and fail to respond to autologous tumor cell line





Therefore, effector T cell dysfunction represents an important candidate negative regulatory process in human melanoma

New interventions aiming to potentiate effector phase of anti-tumor T cells in clinical development

1. Interfere with PD-1/PD-L1 interactions
   - Neutralizing anti-human PD-1 mAbs
2. Remove regulatory T cells
   - Deplete in vivo, alone or prior to vaccination
   - Adoptively transfer $CD25^-$ T cells
3. Prevent/reverse T cell anergy
   - Transfer into lymphopenic recipients (IL-7-dependent homeostatic proliferation)
   - Intratumoral B7-1 (Fowlpox virus vector)

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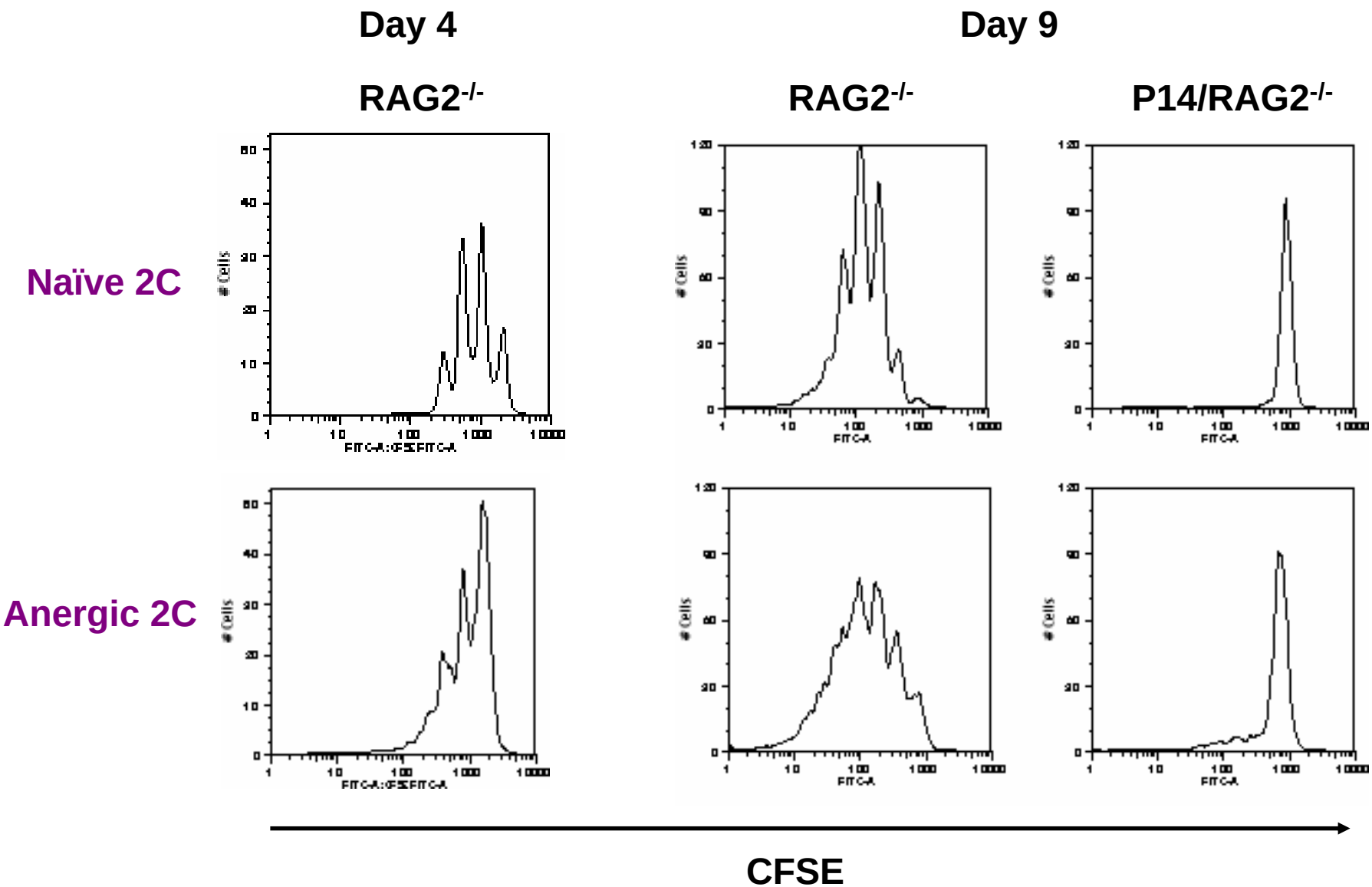
Homeostasis-driven T cell proliferation

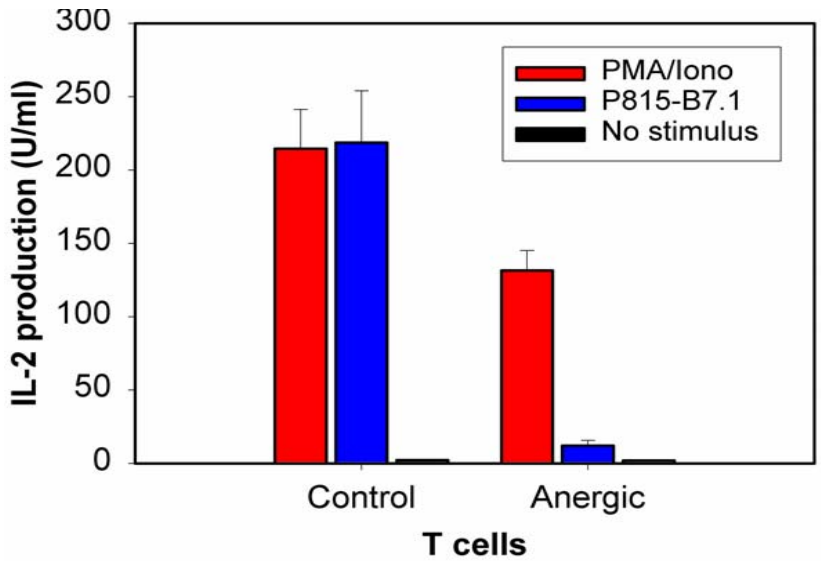
- Occurs when T cells are transferred into lymphopenic recipients
- Driven by excess available IL-7
- Results in partial activation and differentiation of transferred cells (pseudo-memory phenotype)
- We hypothesized that homeostatic proliferation would restore function and tumor rejection by anergic $CD8^+$ T cells

Peptide-anergized 2C T cells undergo homeostatic proliferation in RAG2$^{-/-}$ mice

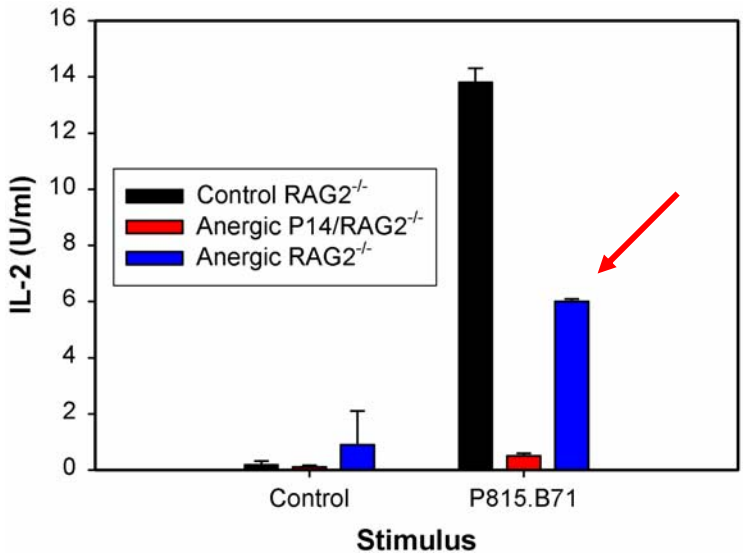


Anergic 2C T cells recover cytokine production following homeostatic proliferation in RAG2$^{-/-}$ mice

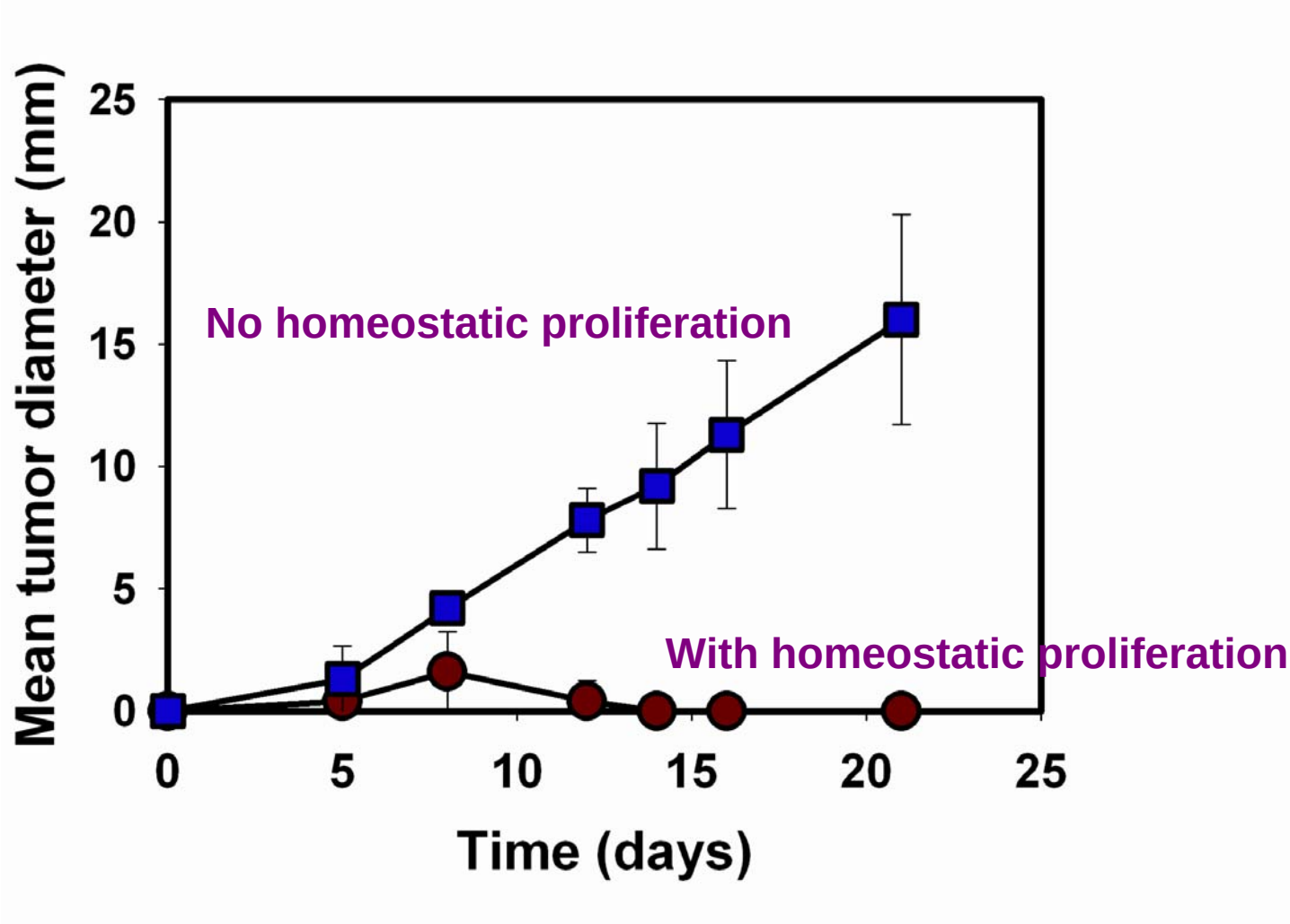
Pre-transfer



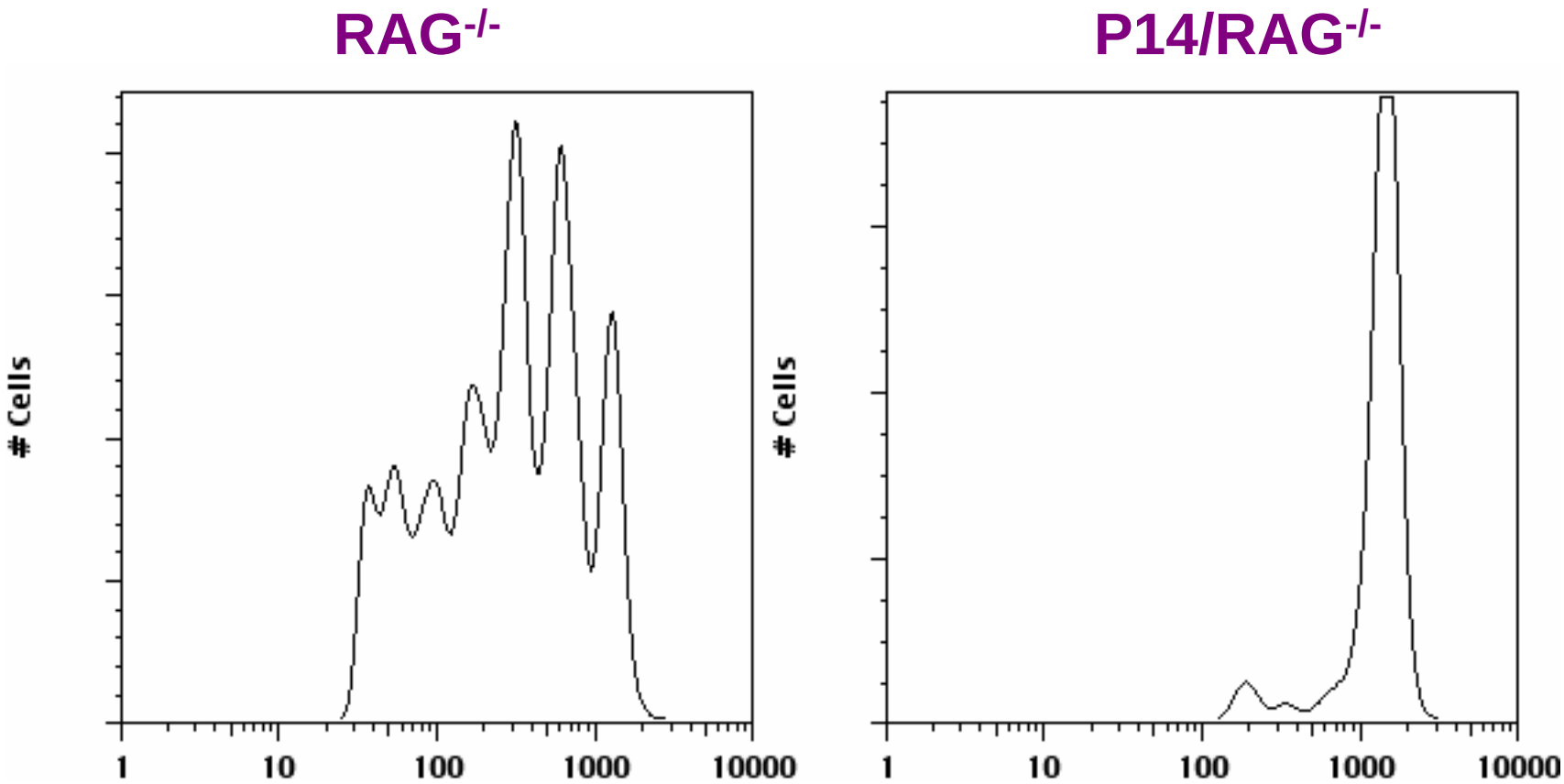
Post-transfer



Anergic 2C T cells reject tumors after homeostatic proliferation in RAG2$^{-/-}$ hosts

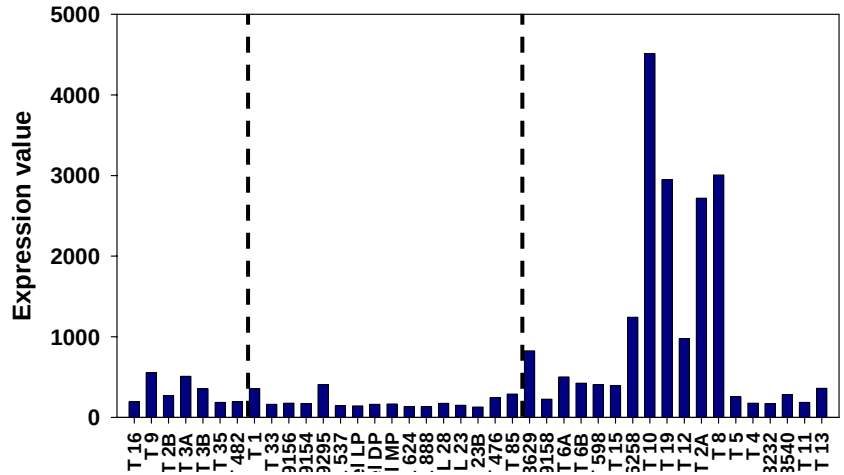


Wildtype B6 CD8^{+} T cells dilute CFSE on transfer to RAG$^{-/-}$ but not P14/RAG$^{-/-}$ recipients

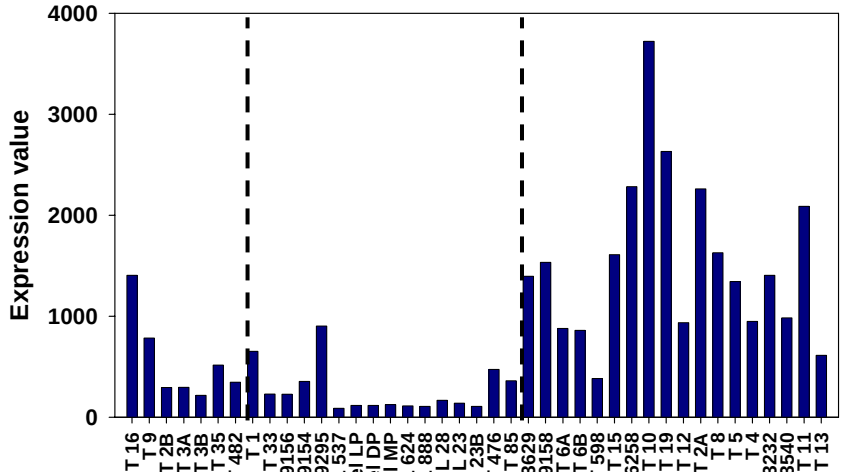


B7-1 transcripts are minimally expressed in metastatic melanoma tumors

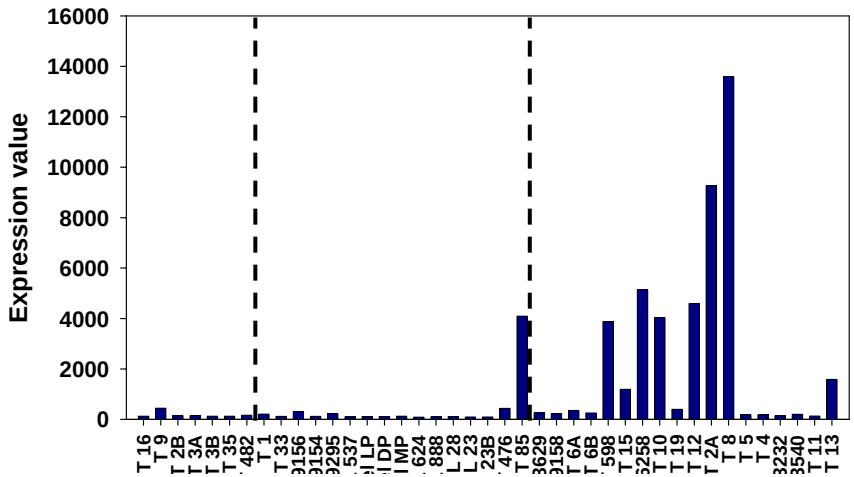
TCRβ



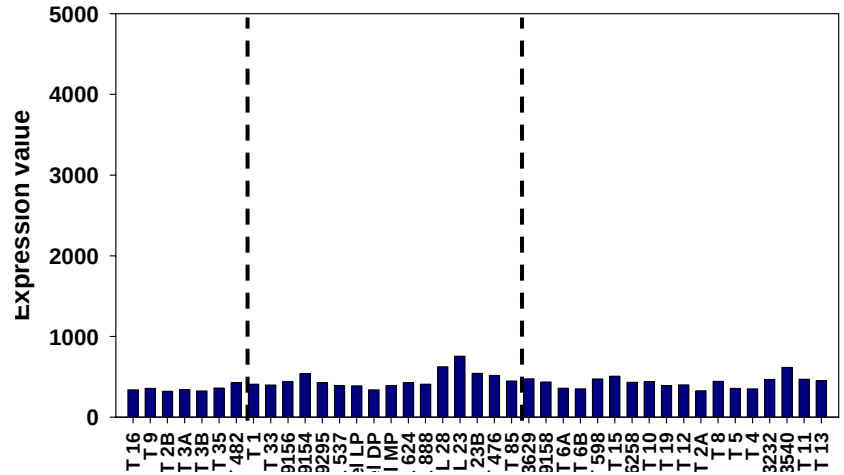
CD14



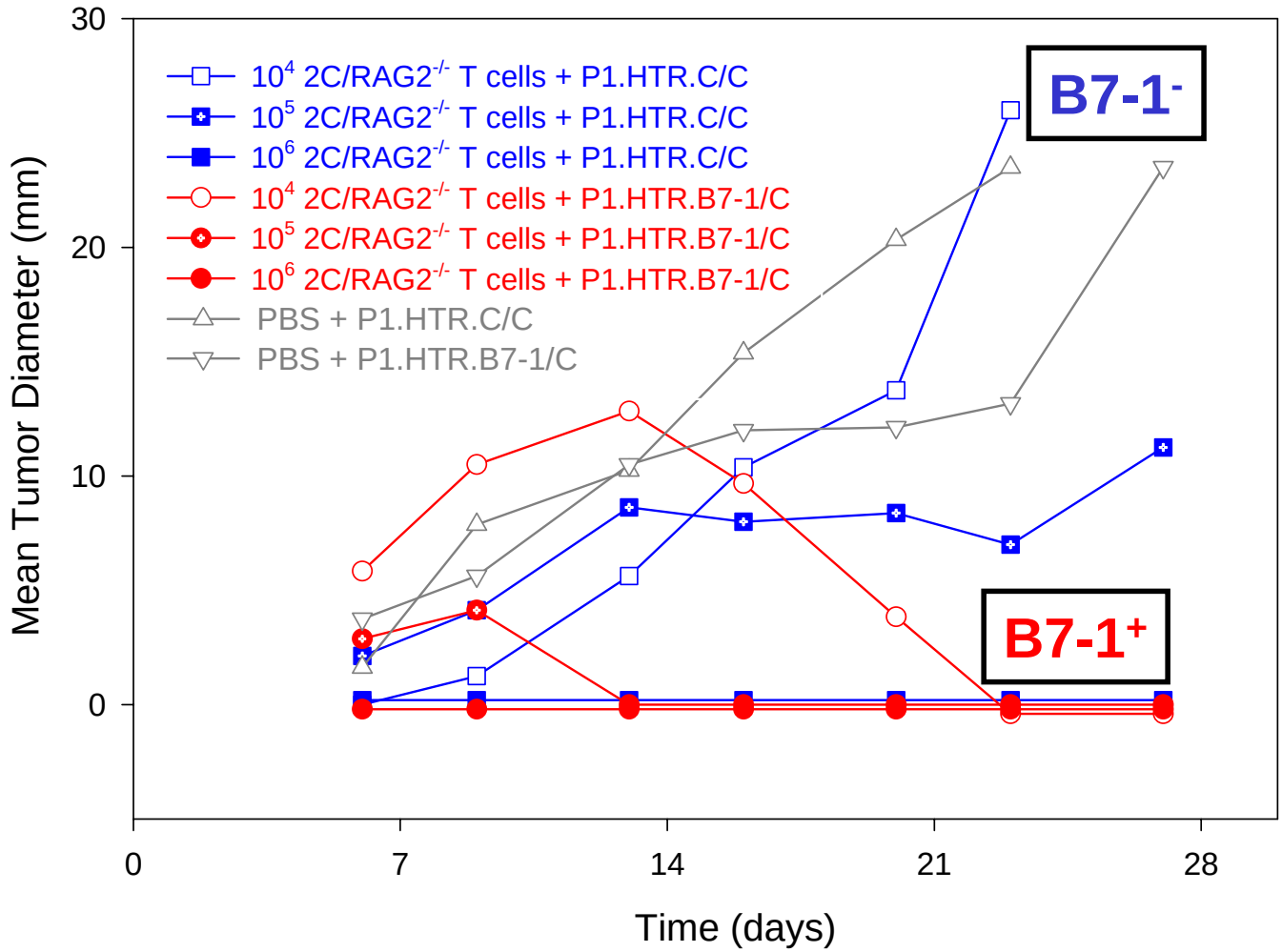
Ig kappa



B7-1



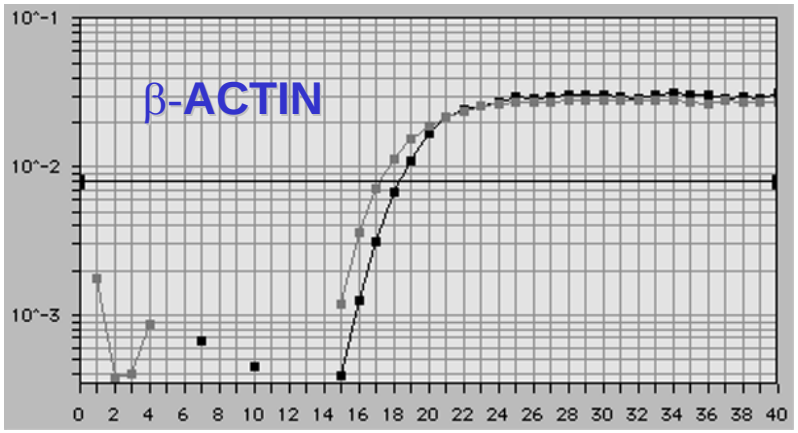
B7-1 expression in tumor allows rejection with at least 10X fewer primed CD8+ effector cells

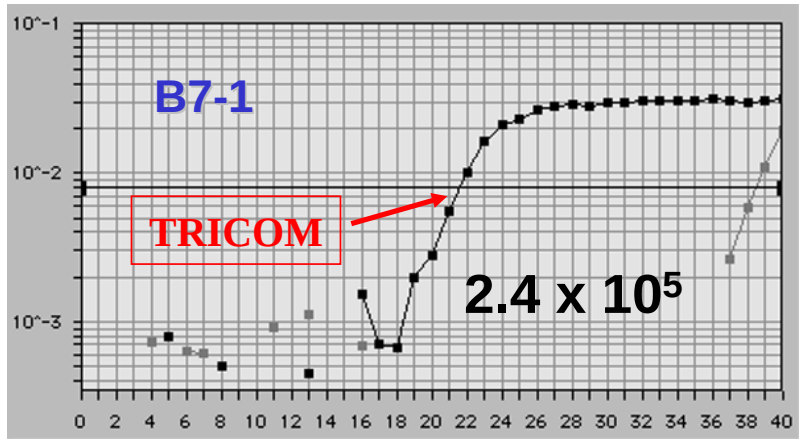


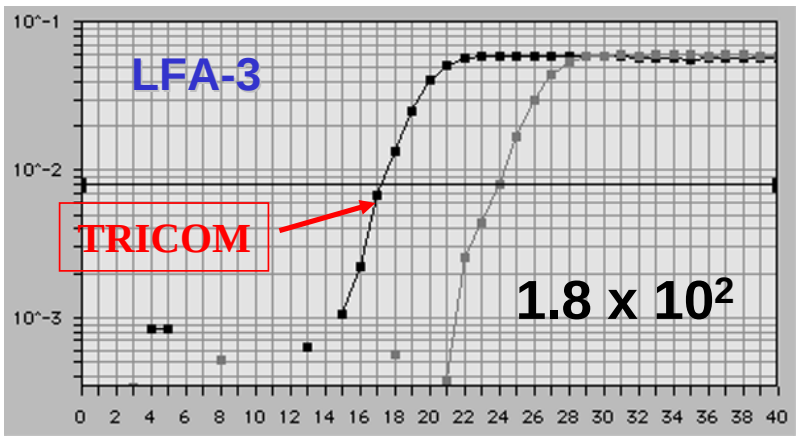
Pilot clinical trial of intratumoral rfTRICOM in melanoma patients with detectable peptide-specific T cells

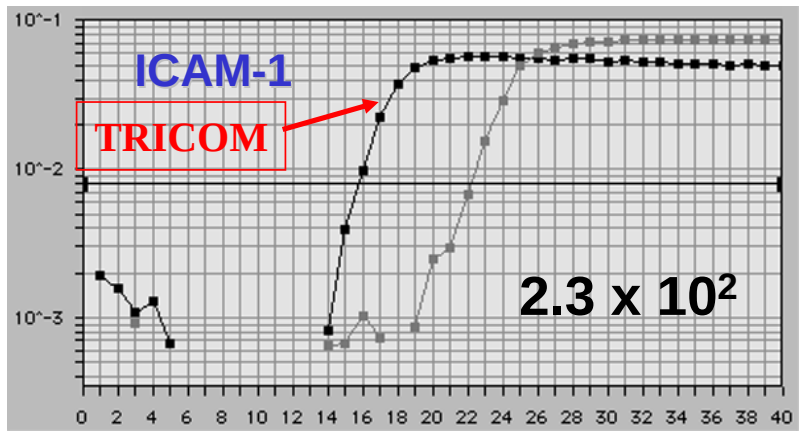
- HLA-A2^{+} patients with detectable circulating CD8^{+} T cells specific for defined melanoma epitopes
- Palpable lesions amenable to injection and biopsy
- Direct intratumoral injection of rfTRICOM (fowlpox virus encoding B7-1, ICAM-1, and LFA-3)
- Core biopsy pre- and post- to assess B7-1, ICAM-1, and LFA-3 expression by real-time RT-PCR
- Clinical response of injected and non-injected lesions assessed
- ELISPOT analysis pre- and post- to measure secondary changes in T cell frequency

rF-TRICOM efficiently transduces human melanoma cell lines in vitro





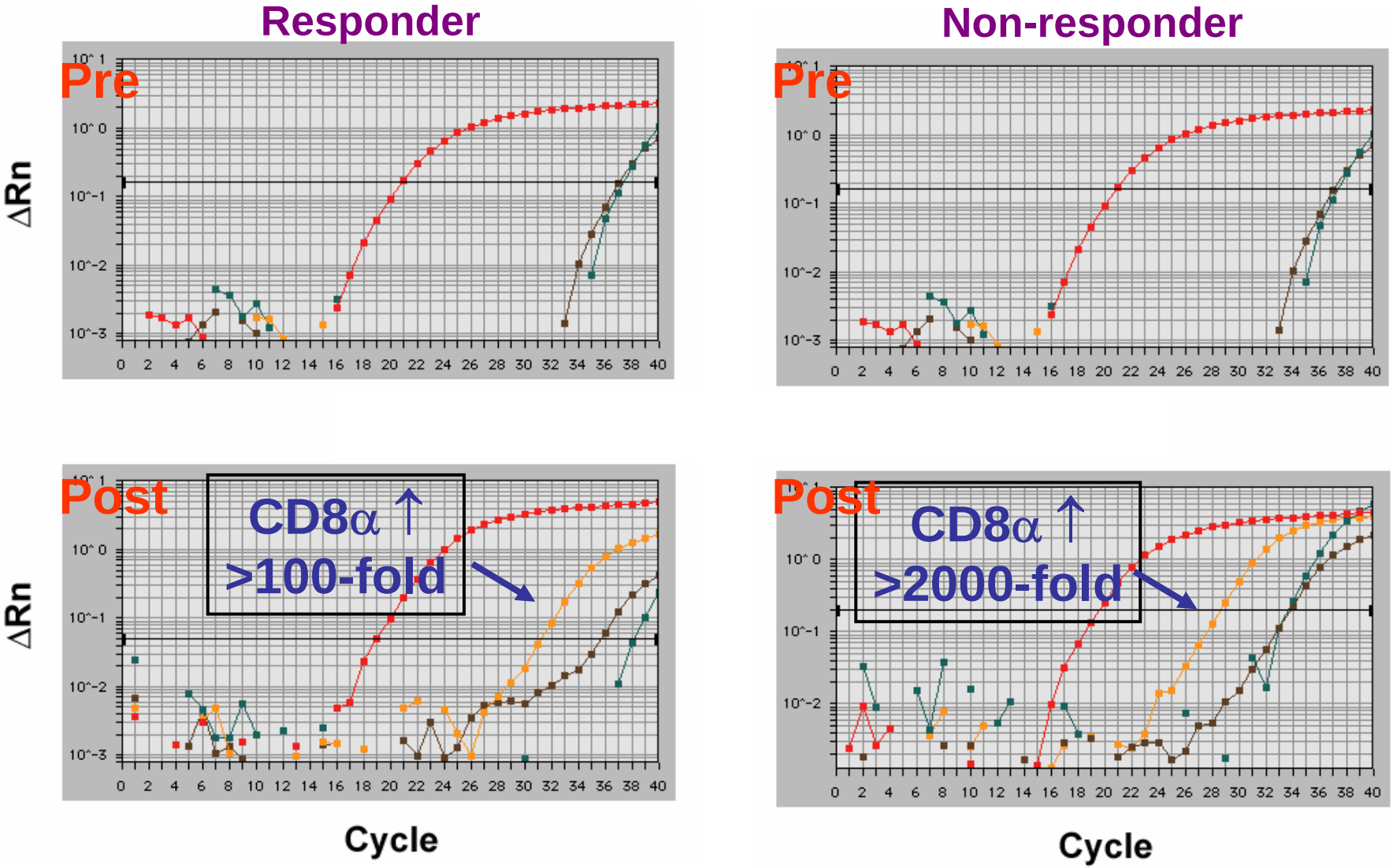




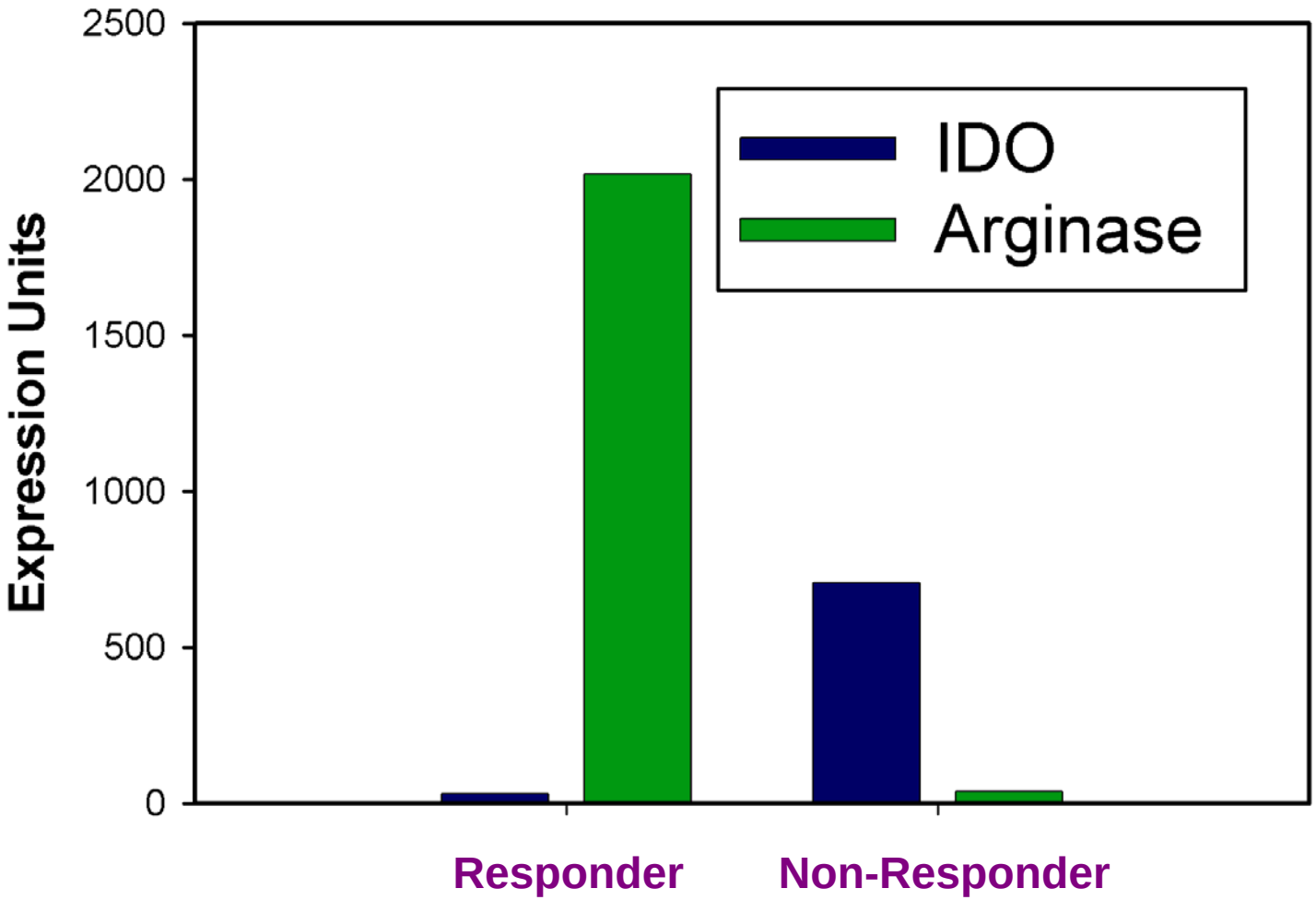
Additional insights gained by molecular analysis of metastatic melanoma tumors undergoing rejection or progressing

- Real-time RT-PCR for candidate genes and to follow effector phase dynamically
- Affymetrix gene array analysis
  - Aim to find stromal elements that correlate with regression versus progression

Real-time RT-PCR: Increased CD8 transcripts in tumors post-vaccination



Affymetrix gene array: expression of IDO by non-responder and arginase by responder



IDO and Arginase

- Indoleamine 2,3-dioxygenase
  - Catabolizes tryptophan, an essential amino acid
  - Expressed in placenta, but also in cells in tumor microenvironment
  - Induced by IFN-γ
  - Leads to T cell hyporesponsiveness and apoptosis
  - Inhibitor, 1-methyl-L-tryptophan, can potentiate anti-tumor immunity in mice
- Arginase I
  - Catabolizes arginine
  - Induced by IL-4/IL-13
  - Expressed by myeloid cells in tumor microenvironment
  - Leads to diminished CD3-ζ expression in T cells, thus blunting TCR signaling

Conclusions

- Sufficient evidence exists to suggest that barriers to immune-mediated tumor regression downstream from T cell priming can be dominant
- New candidates for intervention: PD-1 blockade, depleting Tregs, reversing T cell anergy, and antagonism of IDO or arginase
- Ongoing studies analyzing gene expression profiles of tumor antigen-specific T cells and of cells in the tumor microenvironment from patients should identify major mechanisms that are clinically relevant
- Uncoupling the negative regulation of the effector phase of the anti-tumor immune response should allow an appropriately activated T cell population to mediate effective tumor regression

Acknowledgments

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Amy Peterson
Ian Brown
Sujit Janardhan
Chiayi Kao

Metabolism
Candace Cham

Homeostatic proliferation
Ian Brown
Christian Blank
Harald Wouters

Melanoma gene array
Helena Harlin
Amy Peterson
Functional genomics core

Vaccine patient #13 (non-responder): immune markers