

# Cancer Immunotherapy with Cytokines

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# Outline

- Cytokines in use and in development
- Host vs. Tumor
- One dose, many responses
- Less is more
- No receptor means no signal
- Accentuate the negative

# **Cytokines in Clinical Use**

- **Interleukin-2 for metastatic renal cell carcinoma and malignant melanoma**
- **Interferon-alpha-2b as adjuvant therapy in melanoma**
- **Toxic**

# Cytokines in Development

- Interleukin-12 (recently halted)
- Interleukin-18 (inducer of IFN-gamma)
- Interleukin-21 (IL-2 family)
- Interleukin-28 (interferon family)

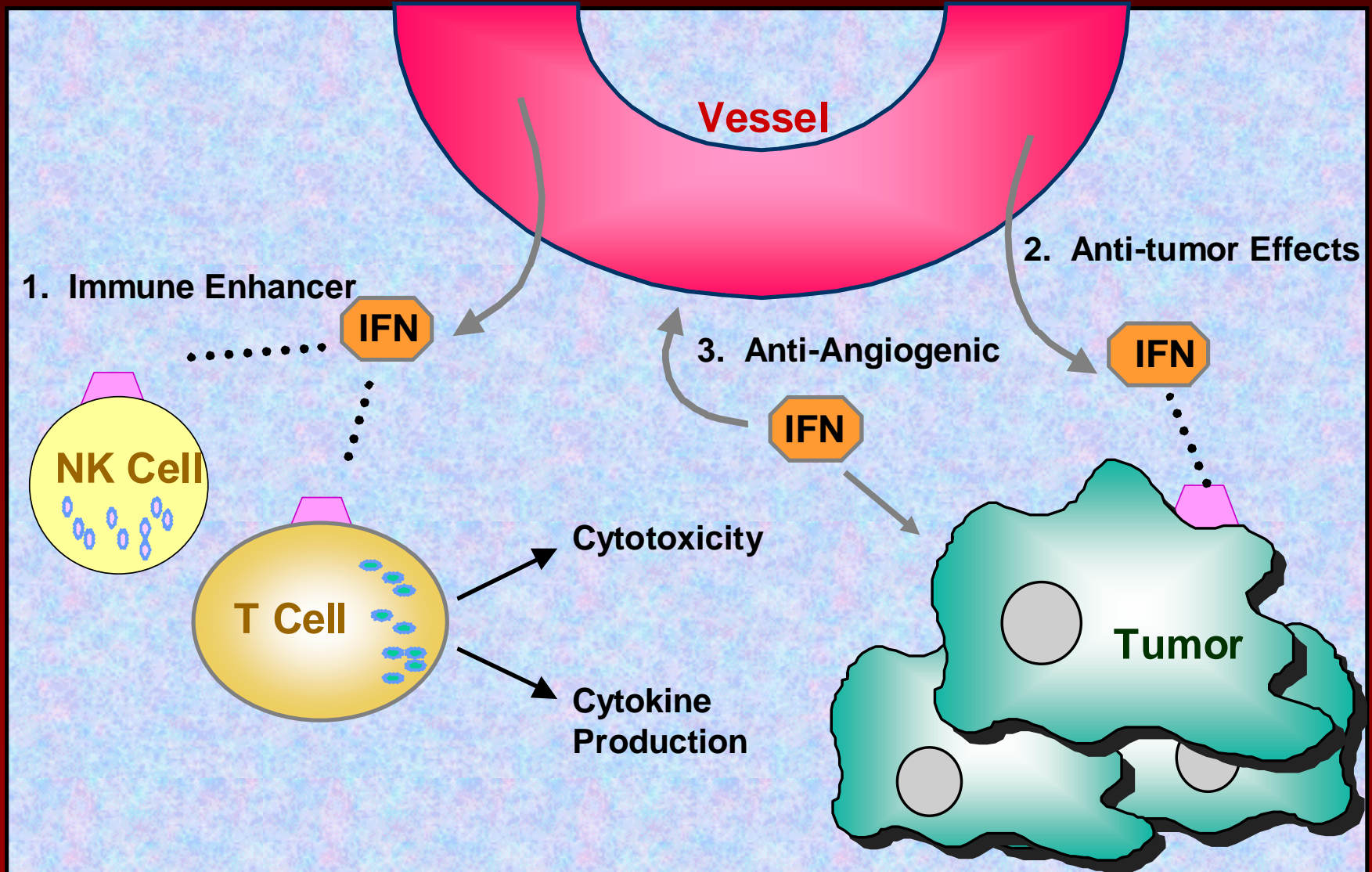
**→ A cytokine may work via effects on the host, the tumor, or a combination.**

# **Clinical Uses of IFN-alpha**

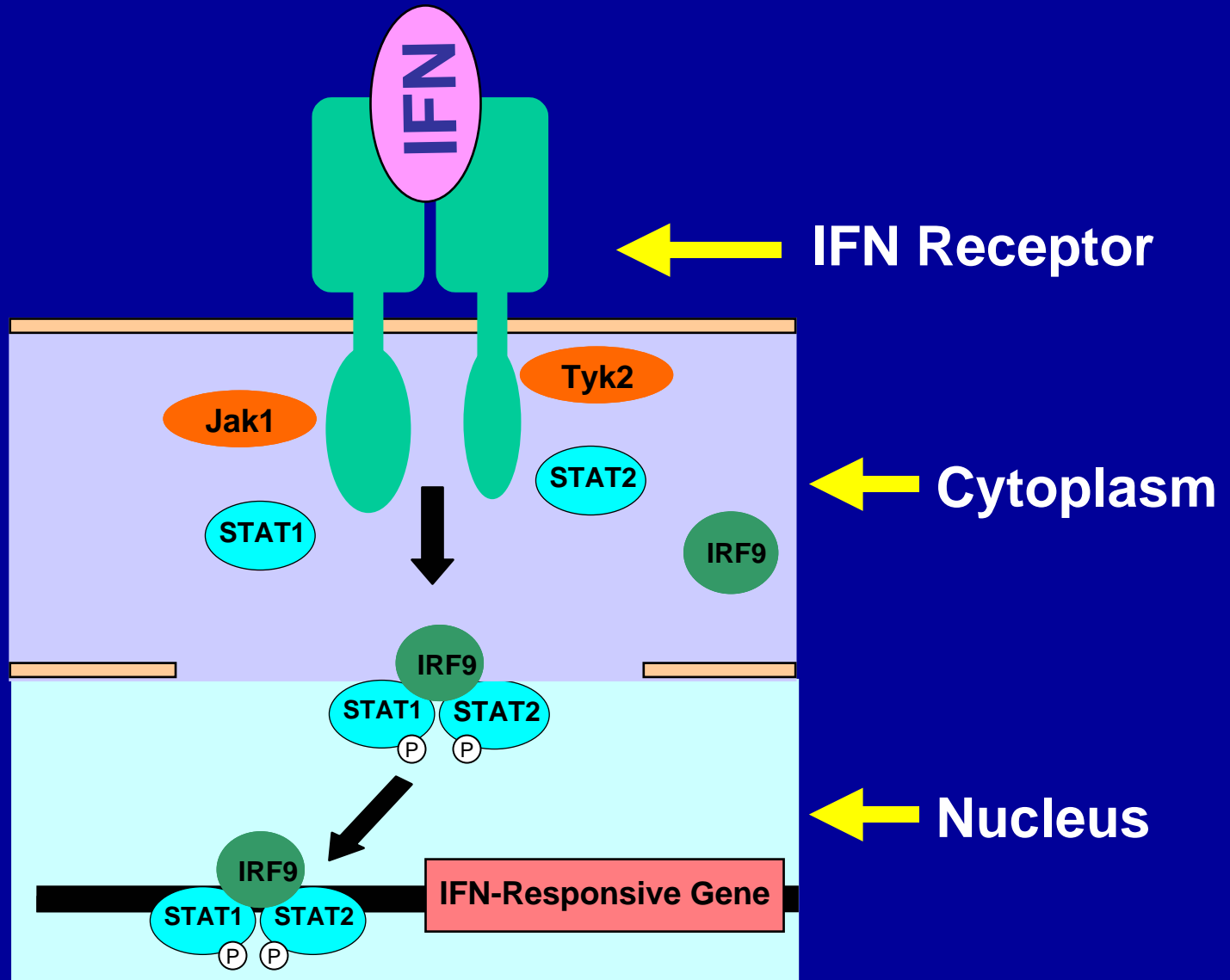
- **Metastatic Renal Cell Carcinoma**
- **Metastatic Malignant Melanoma**
- **Adjuvant therapy in melanoma**
- **Kaposi's sarcoma**
- **Cutaneous T cell lymphoma**

# How Does IFN- $\alpha$ Therapy Work?

## Some Potential Mechanisms ....



# Jak-STAT Pathway

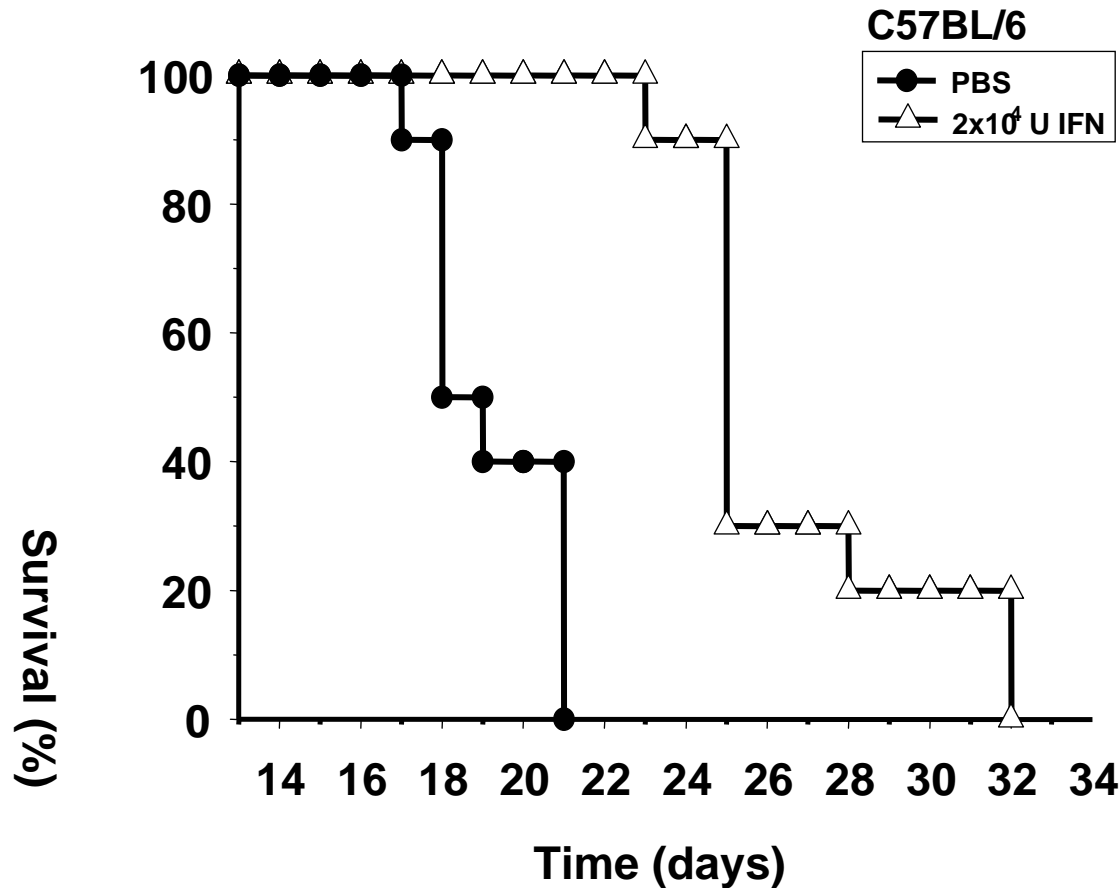




# The IFN-alpha Receptor

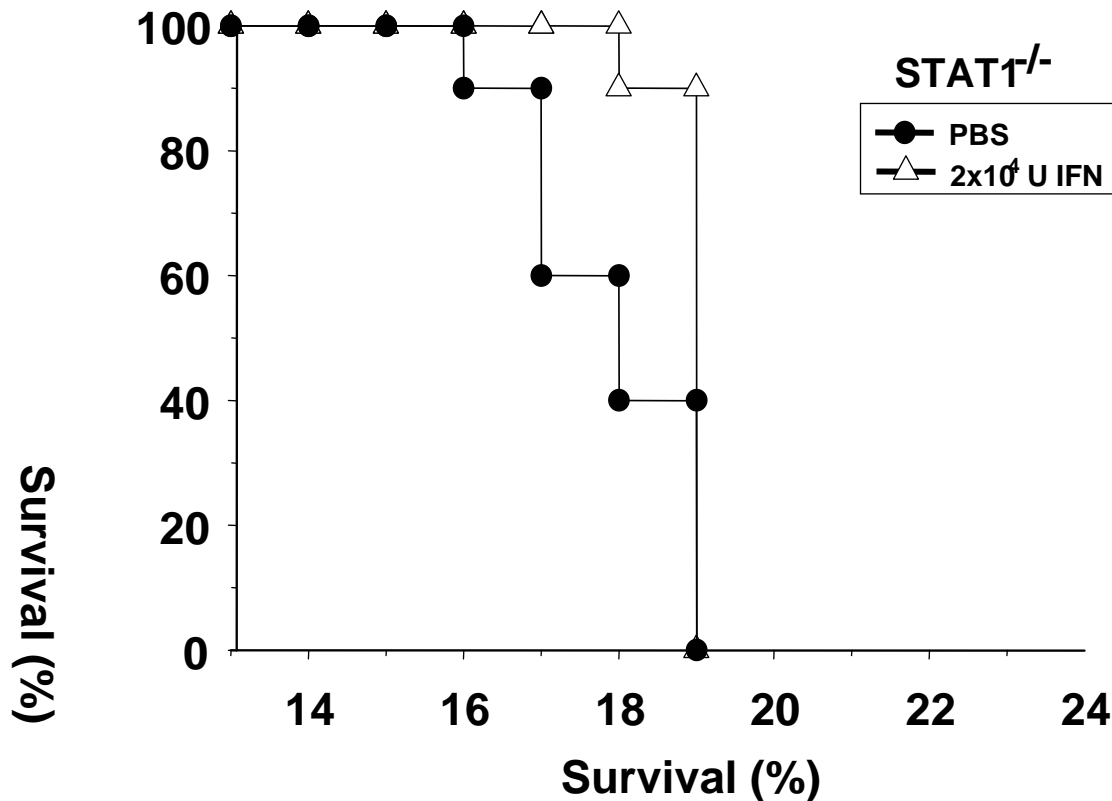
- Two subunits
- Widely expressed on immune cells
- Expressed on most tumor cells

# Murine Model of Malignant Melanoma



Host: STAT1+  
Tumor: STAT1+

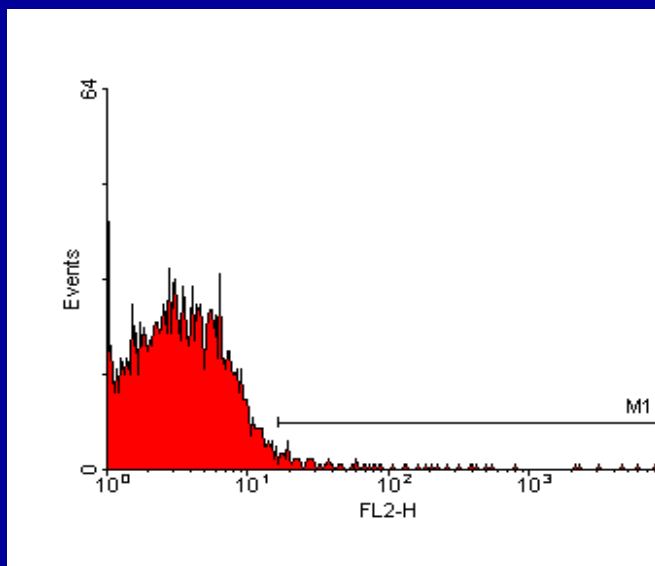
# Effects of IFN-alpha in a STAT1<sup>-/-</sup> Host



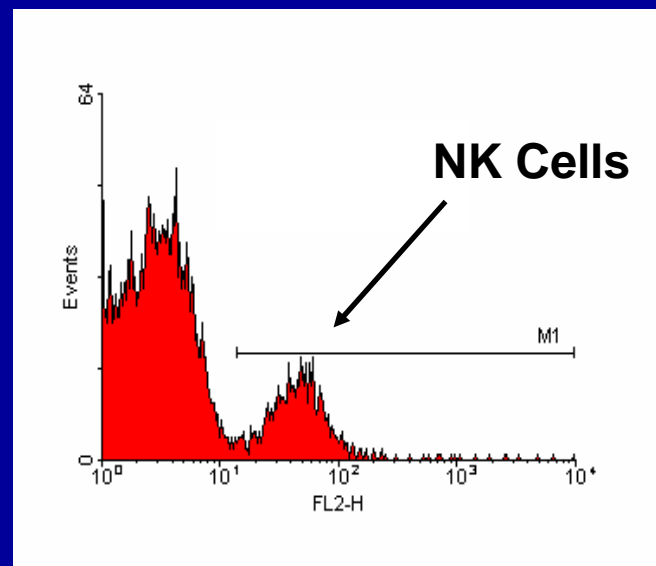
Host: STAT1-neg  
Tumor: STAT1+

# Peritoneal Washings

PBS

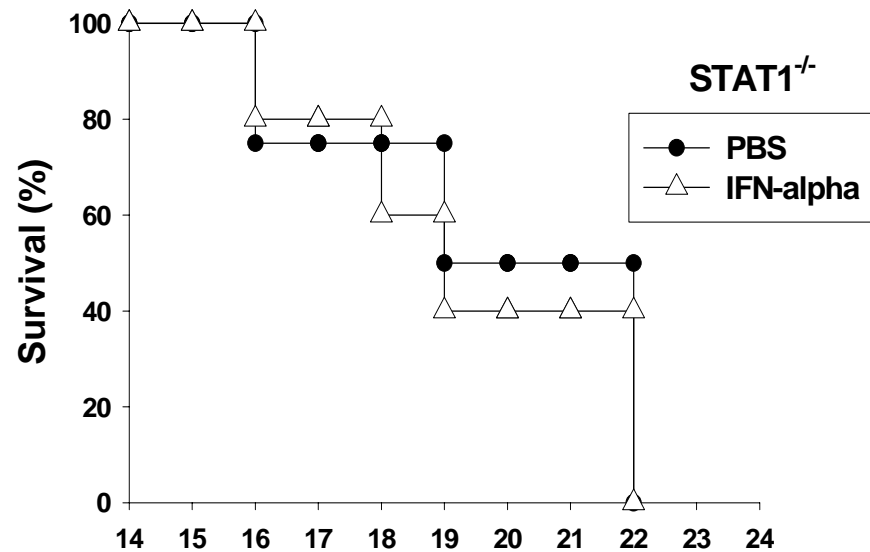
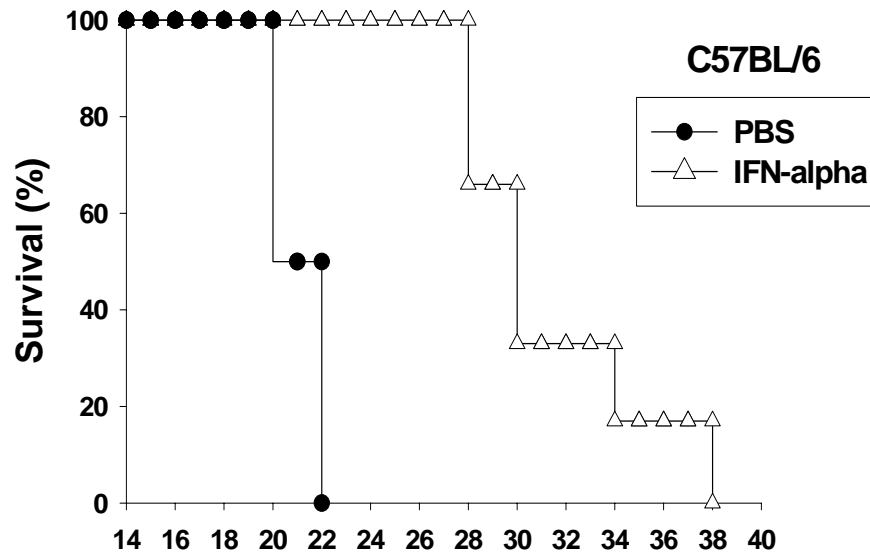


IFN



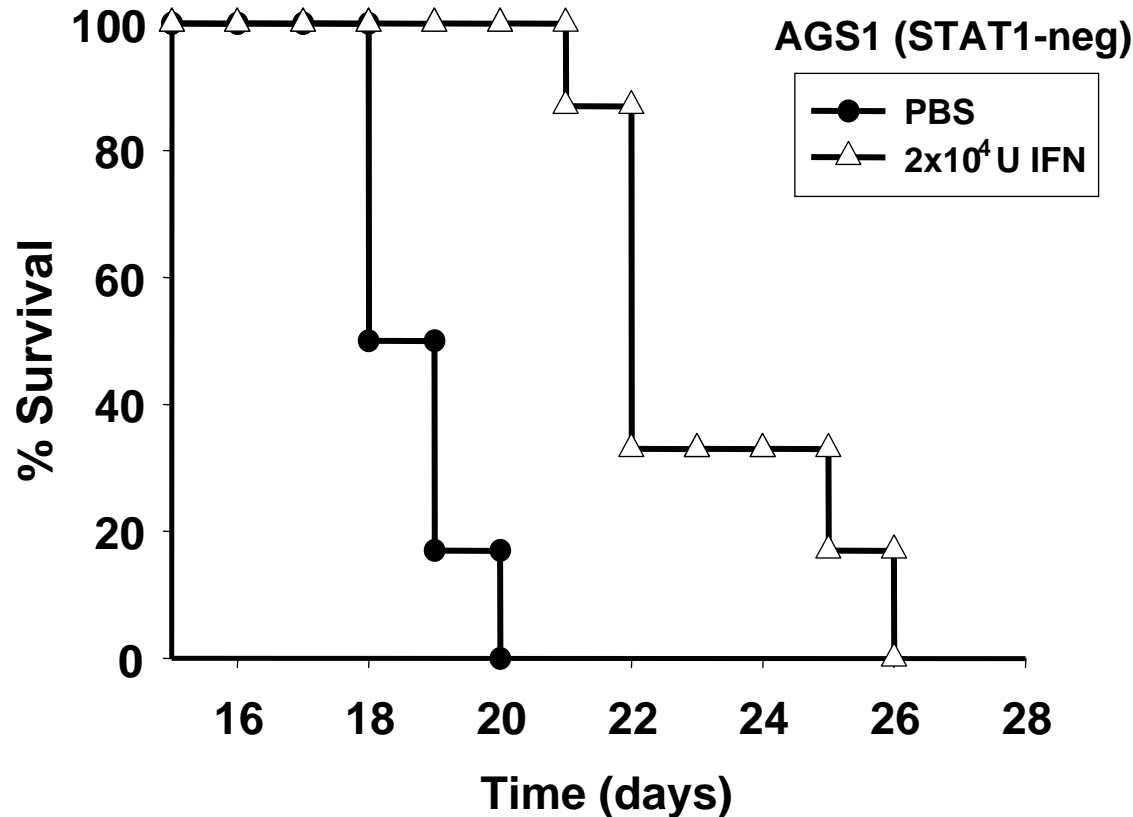
← DX5-PE →

# Adjuvant Model



Host: STAT1-neg  
Tumor: STAT1+

# A STAT1-negative Murine Melanoma



Host: STAT1+  
Tm: STAT1-neg

# Summary

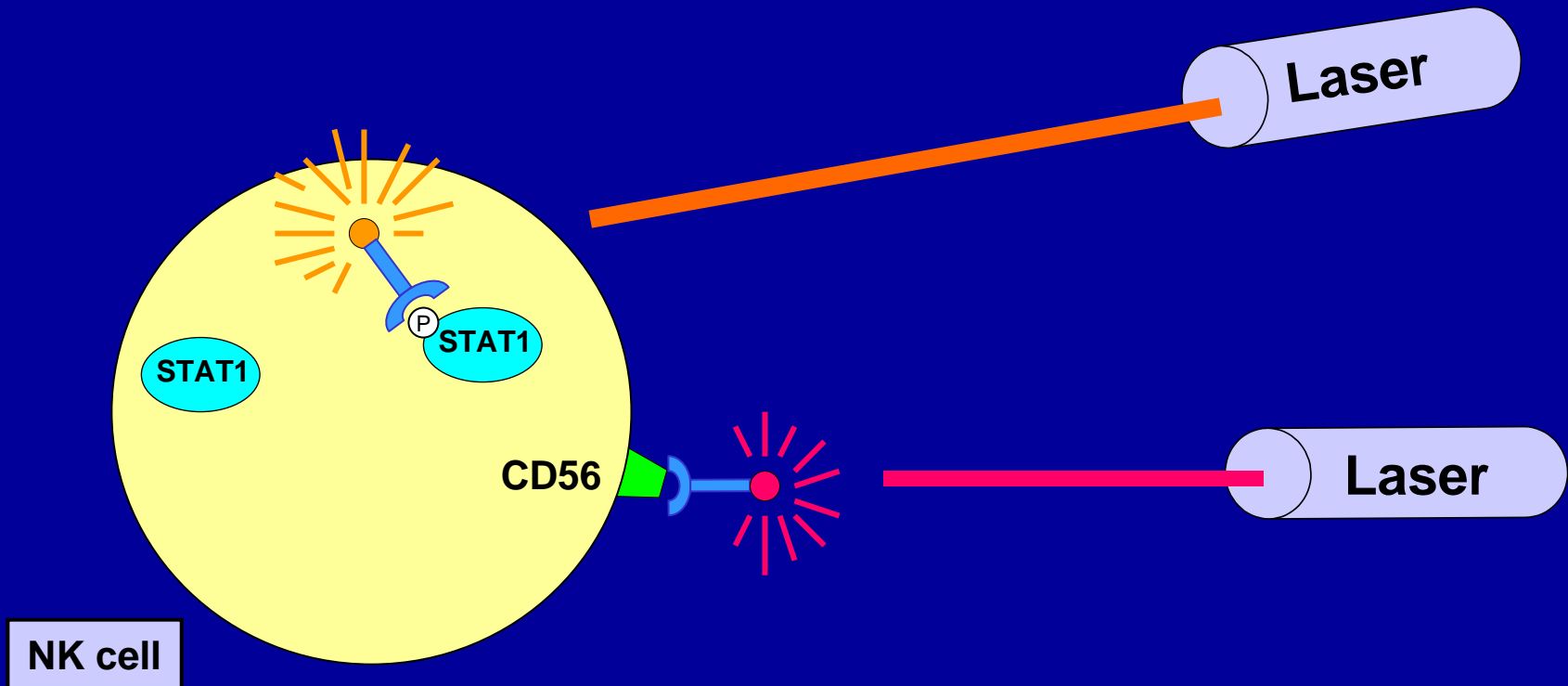
- **STAT1 in the host is critical for the anti-tumor actions of IFN-alpha**
- **STAT1 in the tumor not important**
- **NK cells = effector arm**

# **Analysis of Jak-STAT Signal Transduction in Immune Subsets**



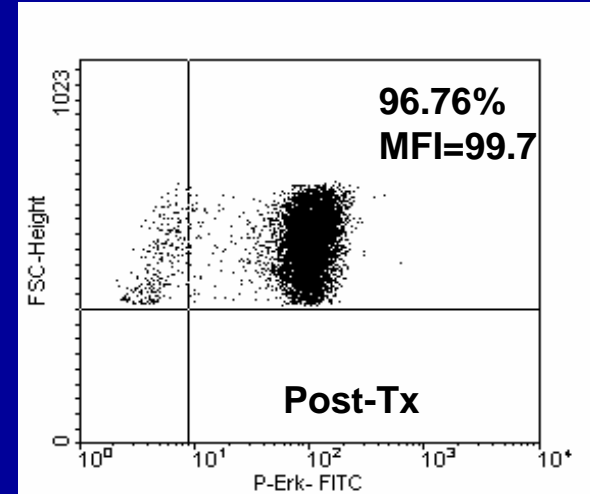
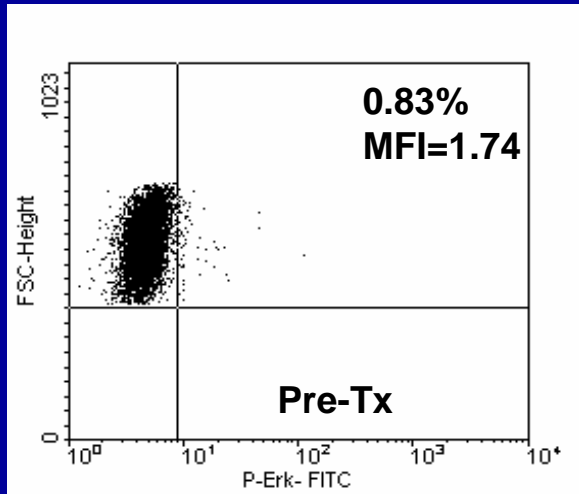
# Monitoring STAT1 Activation in Immune Cells

- Use an anti-phospho-STAT1 Ab 
- Sensitive, quantitative, fast, uses few cells



# The Dot Plot

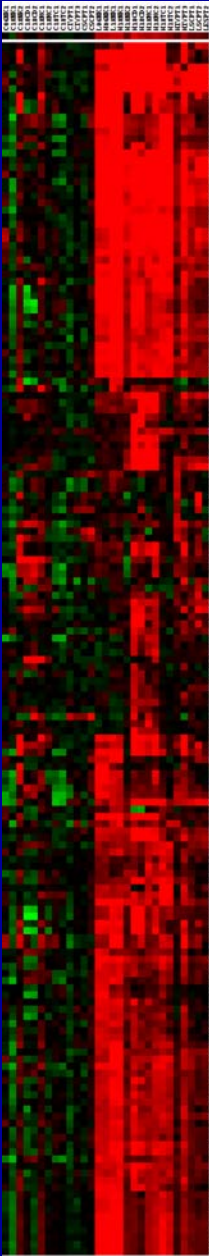
CD3 →



Phospho-Protein →

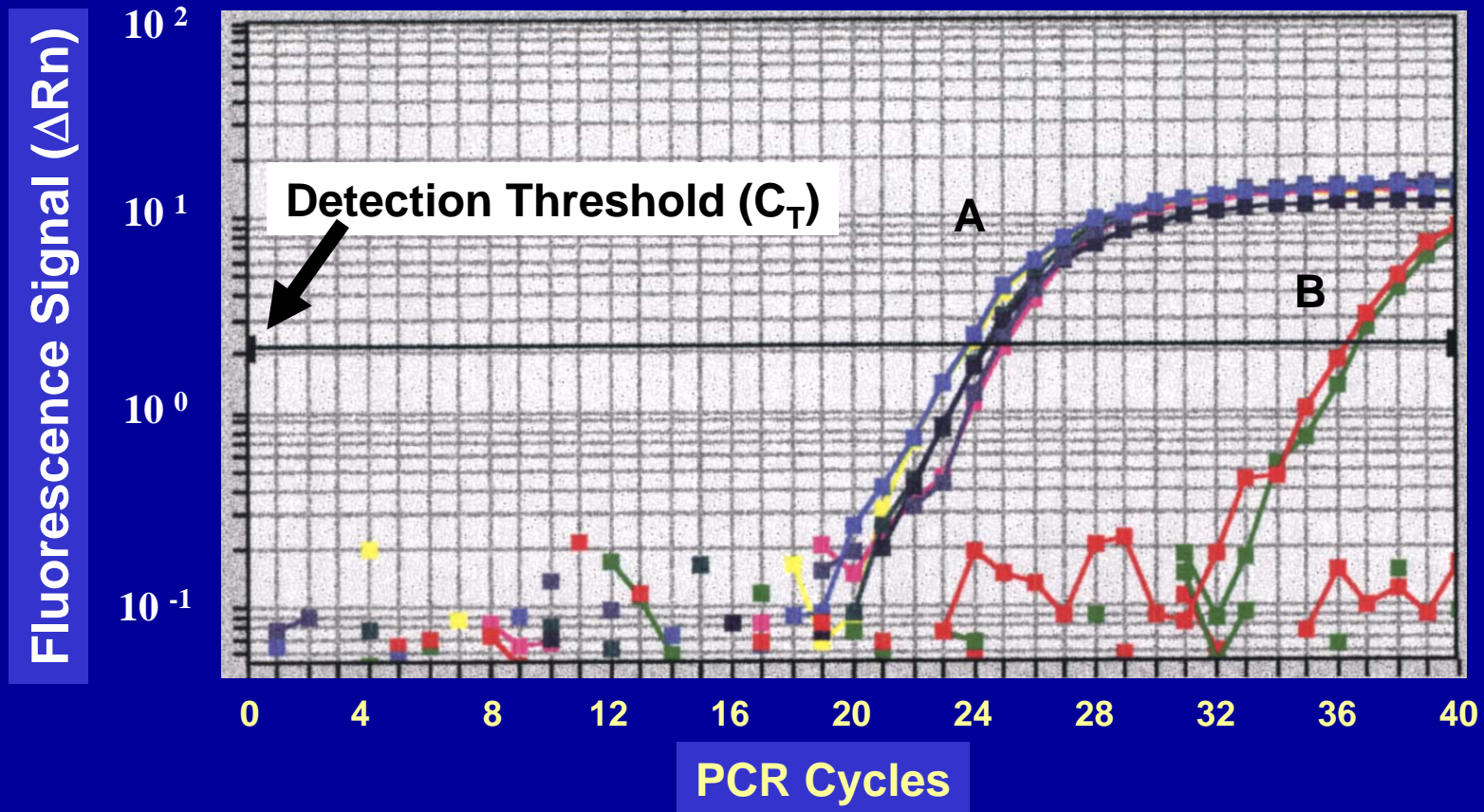
# Other Tools of the Trade

- **Microarray analysis**
- **Quantitative RT-PCR**
- **Proteomics**



# Microarray Analysis of Patient Immune Cells Post-Cytokine Therapy

- The PCR cycle at which the fluorescence is first detected (detection threshold =  $C_T$ ) inversely correlates with the amount of target transcript present in a given sample.



# Proteomic Analysis

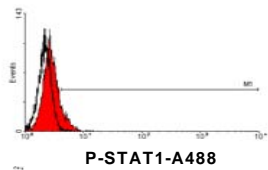
- MALDI-MS
- SELDI-TOF mass spectrometer

**→ Maximal cytokine signaling occurs  
at relatively low doses**

**IFN- $\alpha$  IU/ml**

**Fsp**

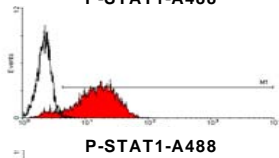
**PBS**



**0.53**

P-STAT1-A488

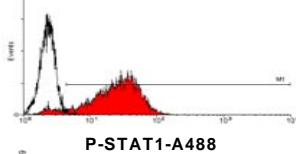
**1**



**13.84**

P-STAT1-A488

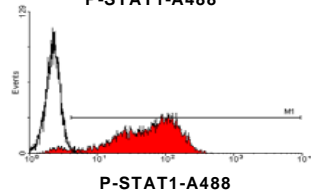
**10**



**24.09**

P-STAT1-A488

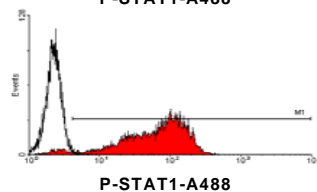
**100**



**61.53**

P-STAT1-A488

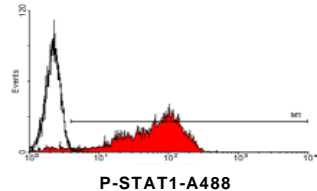
**10<sup>3</sup>**



**72.72**

P-STAT1-A488

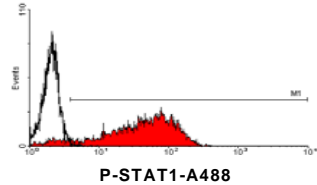
**10<sup>4</sup>**



**66.33**

P-STAT1-A488

**10<sup>5</sup>**



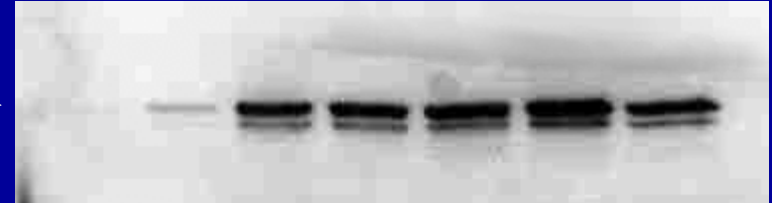
**44.51**

P-STAT1-A488

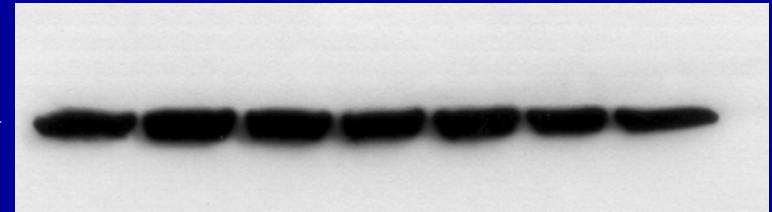
# Dose Response

IFN- $\alpha$  (IU/ml): 0 1 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup>

P-STAT1 →

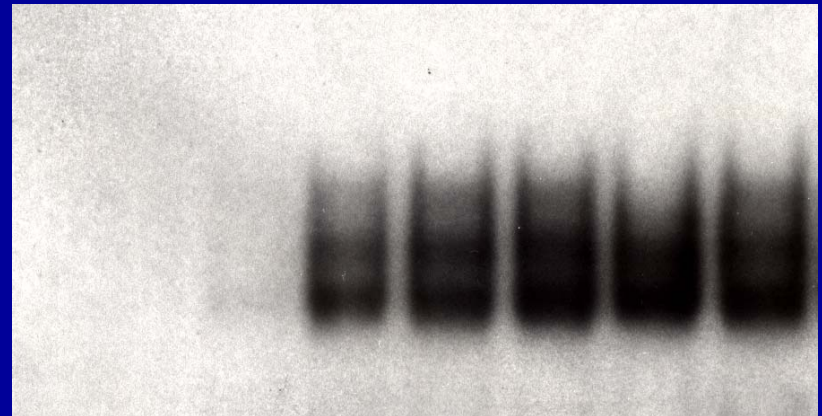


B-Actin →



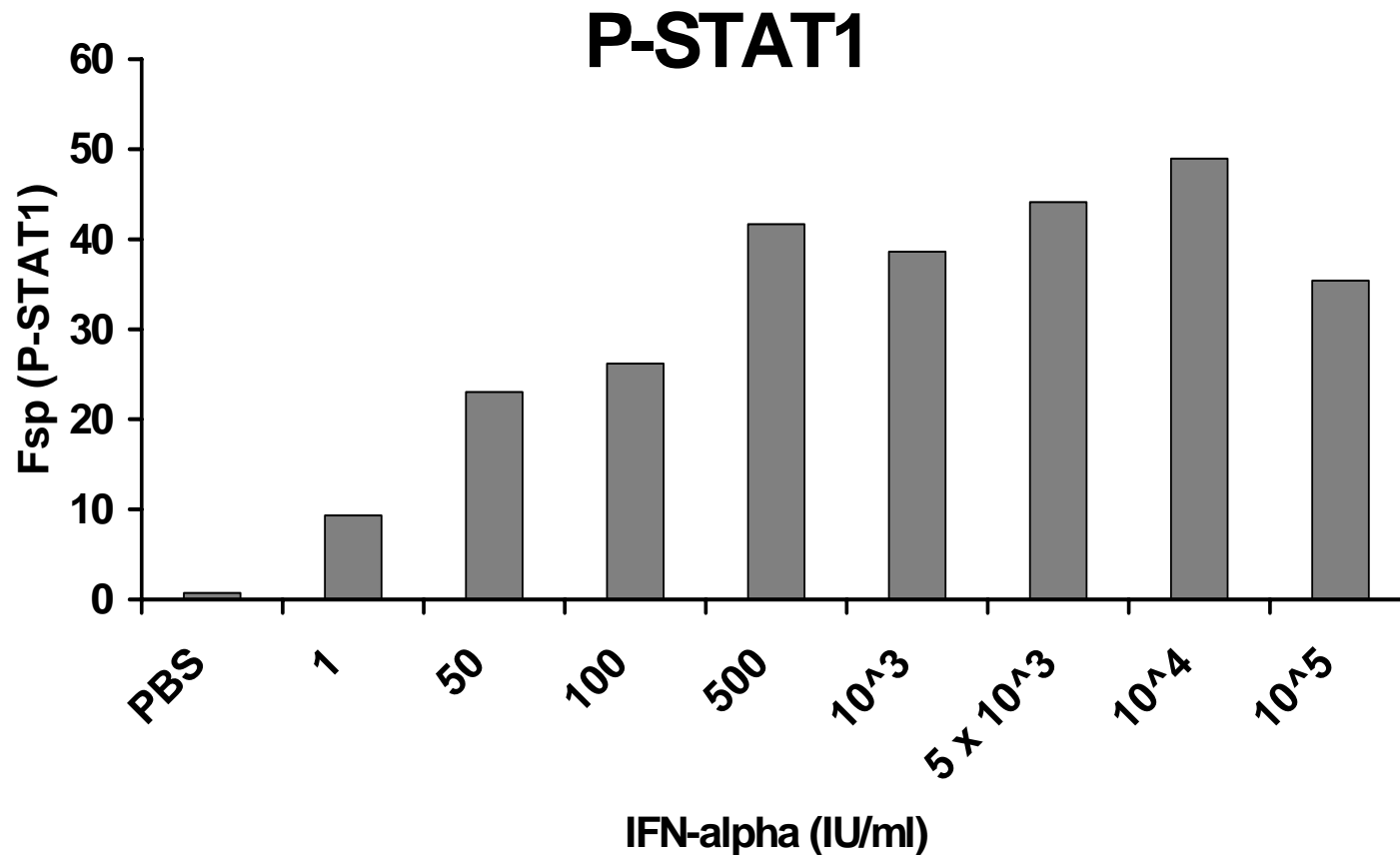
IFN- $\alpha$  (U/ml): 0 1 10 100 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup>

SIE →



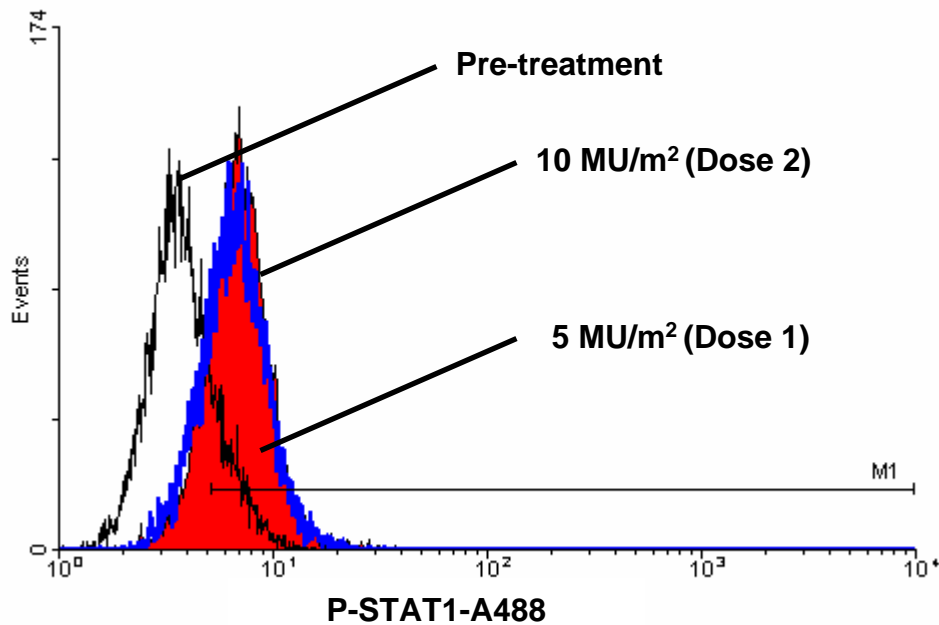


# P-STAT1 in PBMCs: Activation at Low Doses



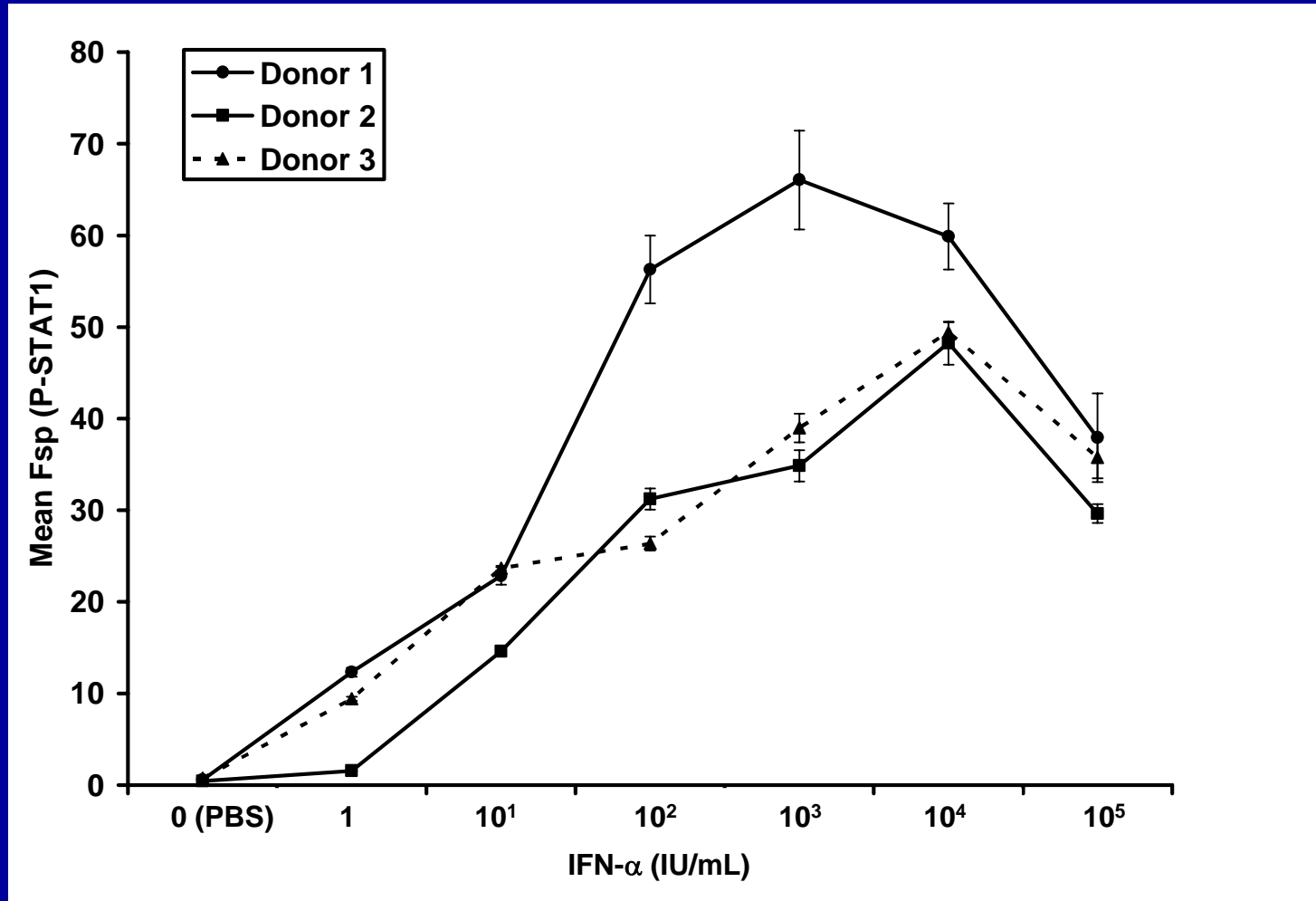
# What Is an Optimal Dose?

Patient C



**→ There is significant inter-patient variation in response to cytokines**

# P-STAT1 Levels In Human PBMCs: Interpatient Variation



Lesinski *et al.* J. Natl. Cancer Inst.. 96:1331-42, 2004.

# Multi-Parametric Analysis of STAT1 Activation

Treatment:

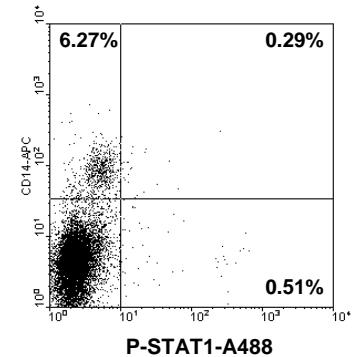
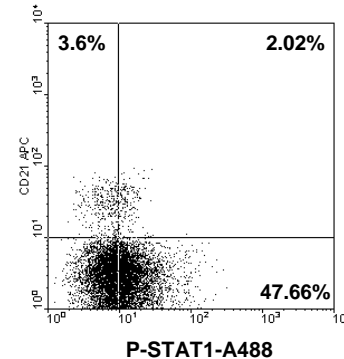
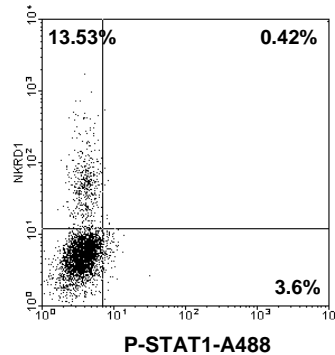
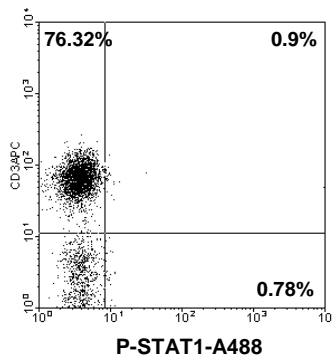
T lymphocytes

NK cells

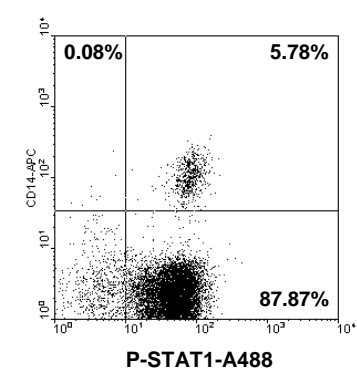
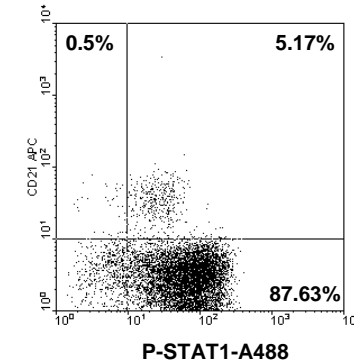
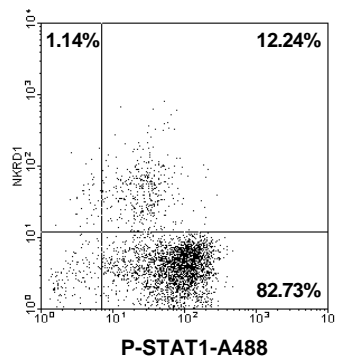
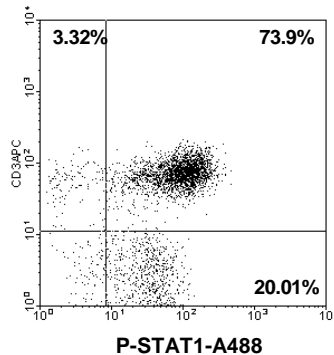
B lymphocytes

Monocytes

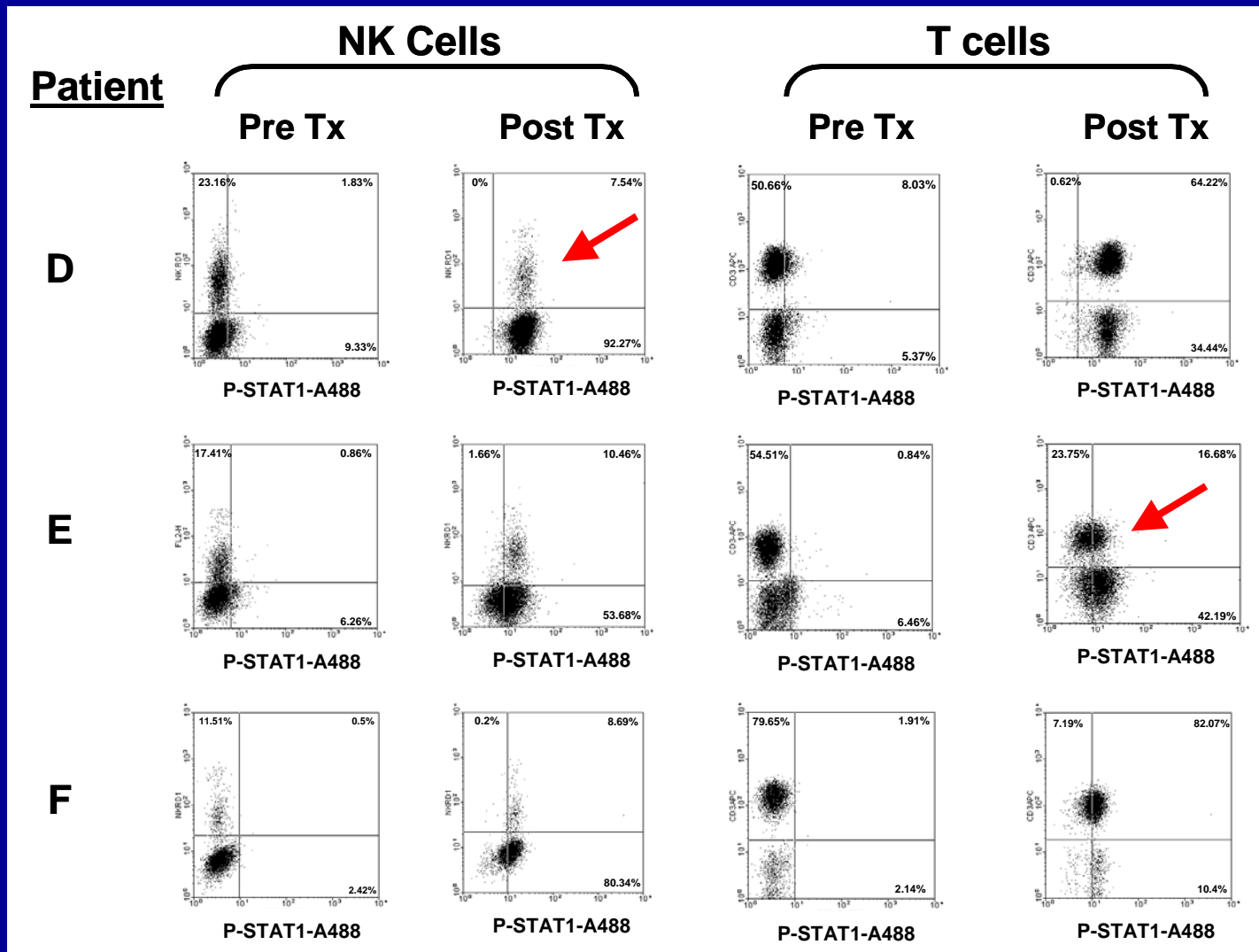
PBS



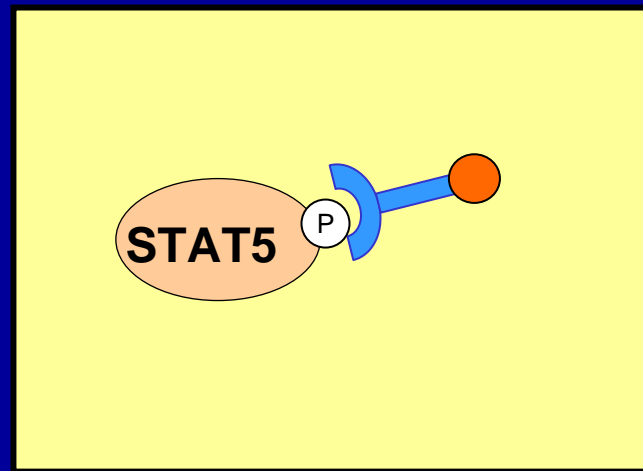
IFN- $\alpha$



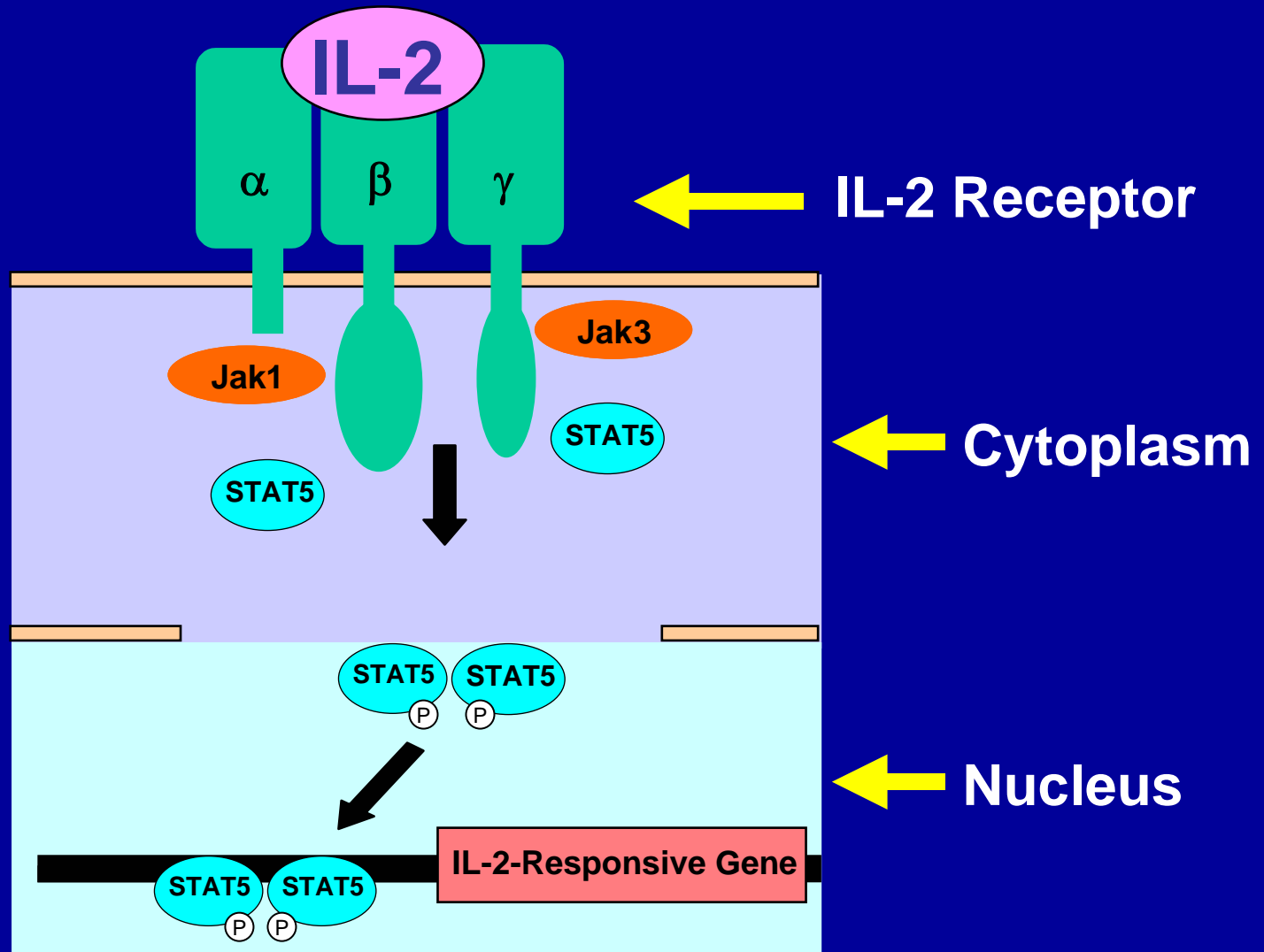
# STAT1 Activation in Patient Lymphocytes During IFN- $\alpha$ Immunotherapy



# Other Phospho-Specific Antibodies?



# IL-2 Signal Transduction

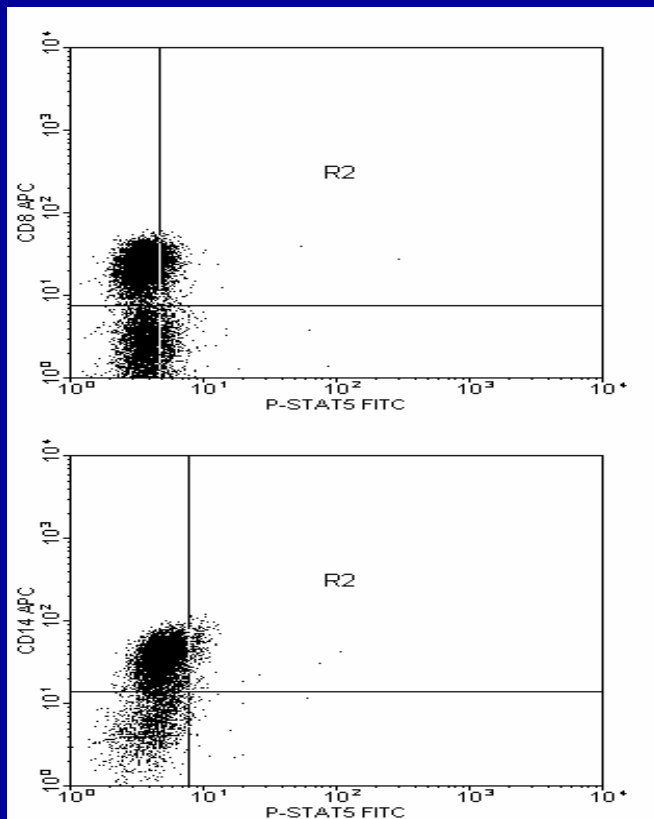




**→ Cytokines will not activate a cell that lacks the corresponding receptor**

# IL-2 Patient No. 5

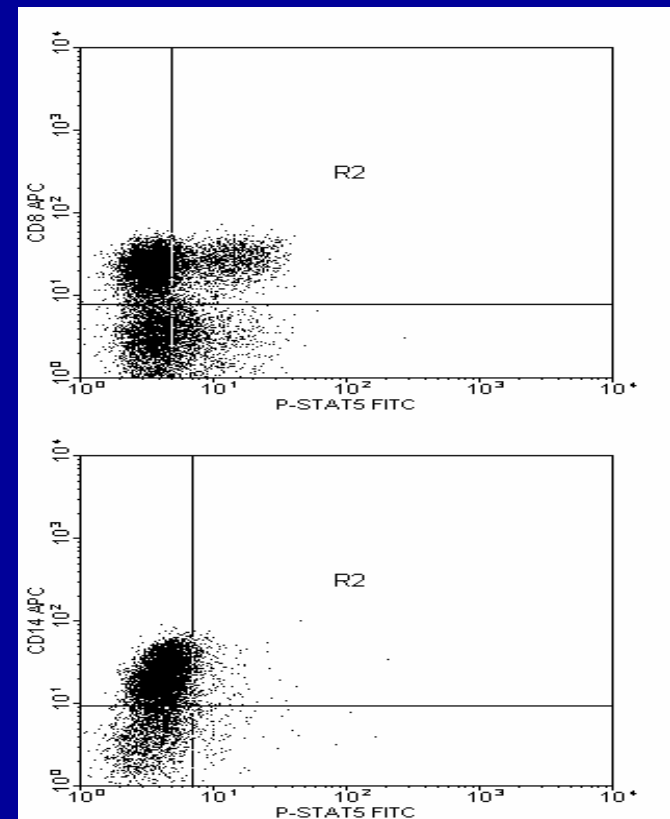
**Pre-Tx**



**CD8**

**CD14**

**Post-Tx**

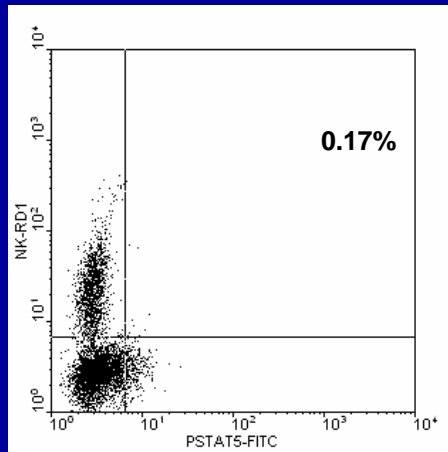


**→ Cytokines may exert prolonged effects**

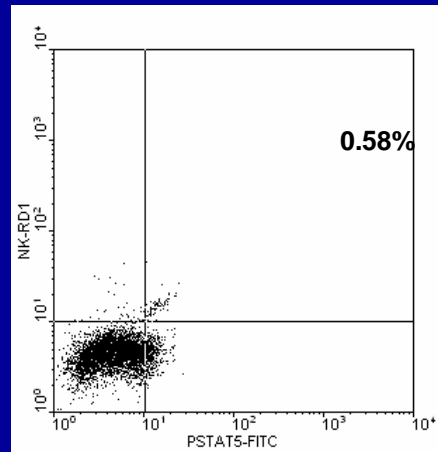
# P-STAT5 in NK Cells

NK

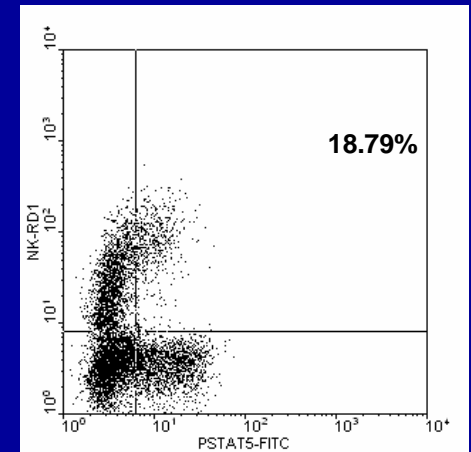
Pre-IL-2



1 hr Post-IL-2



1 wk post-IL-2



P-STAT5

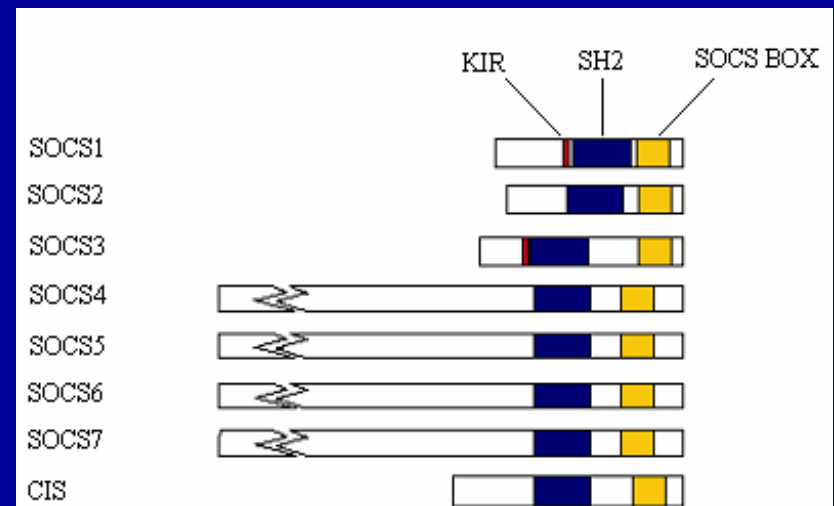
**→ Every signaling system  
has a set of brakes**

# **Regulation of IFN- $\alpha$ Signal Transduction**

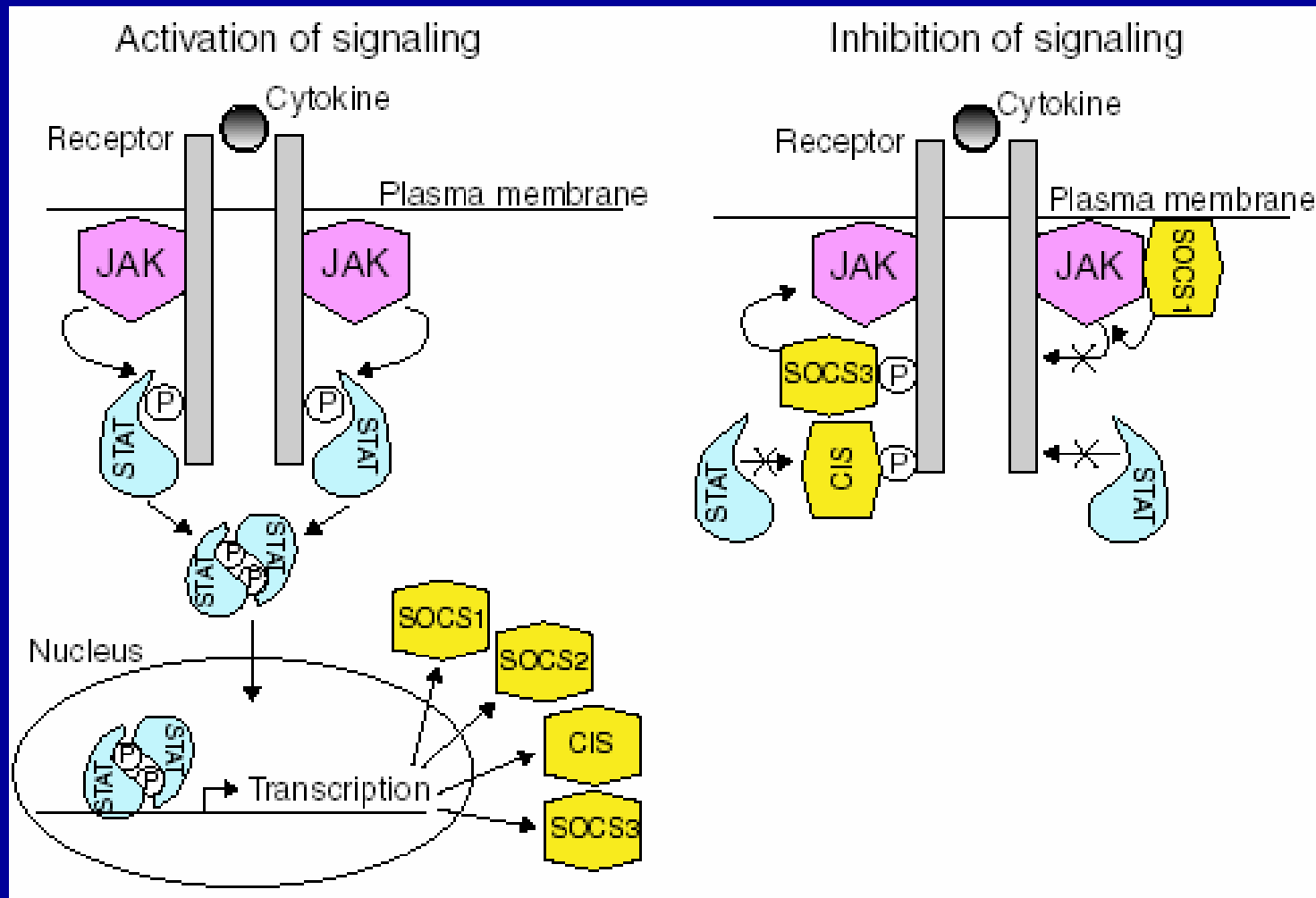
- **Dose-response curve is not linear**
- **Signaling is inhibited at higher concentrations**
- **Role of negative regulators?**

# SOCS

- **Suppressors of Cytokine Signaling Family (negative feedback loop)**
- **SOCS Family = SOCS1-7 and CIS**
- **Src-homology 2 domain (binding to JAKs)**
- **SOCS1 KO mice die @ 3 weeks of uninhibited IFN-gamma signaling.**

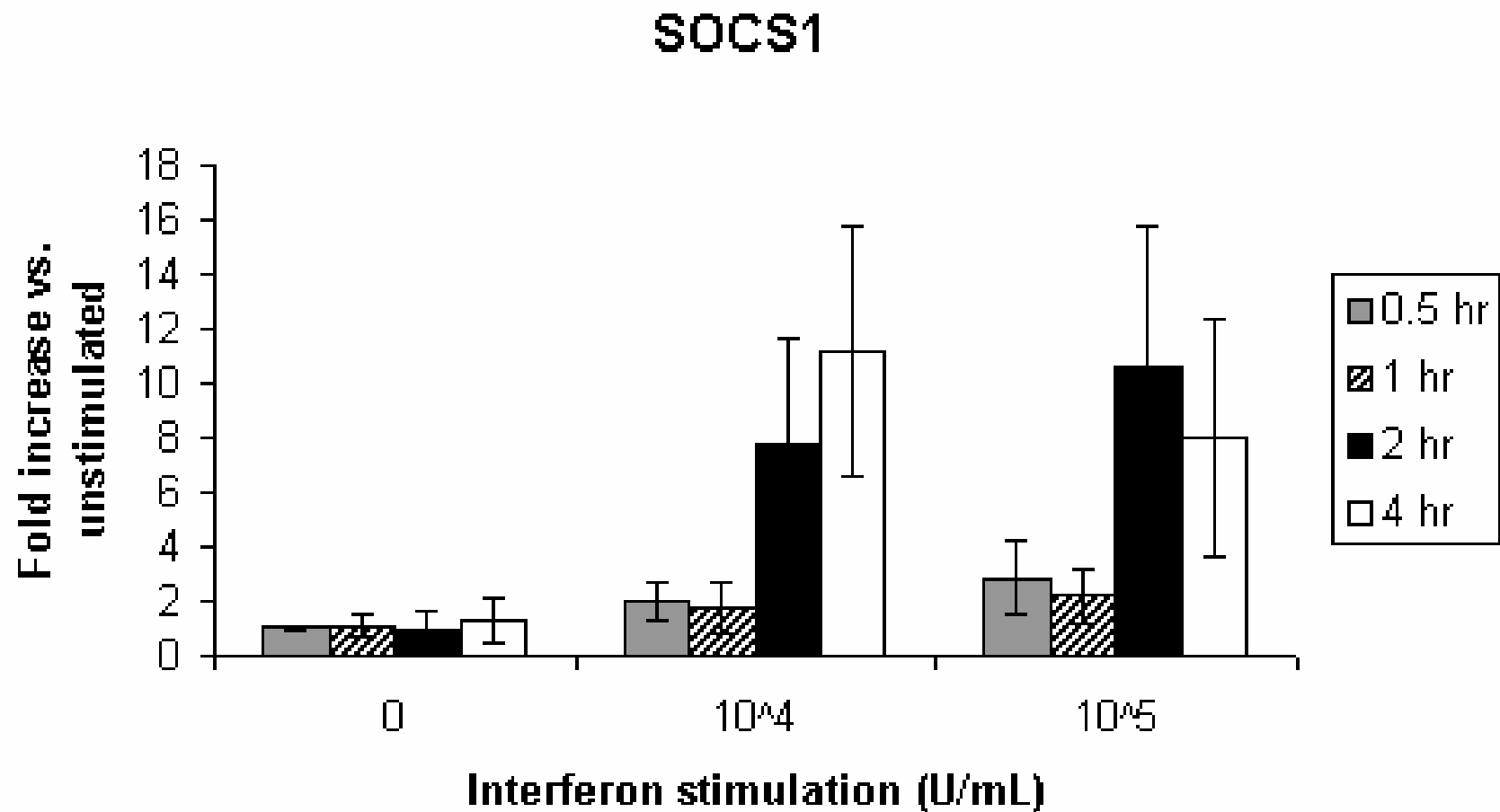


# Negative Feedback Inhibition

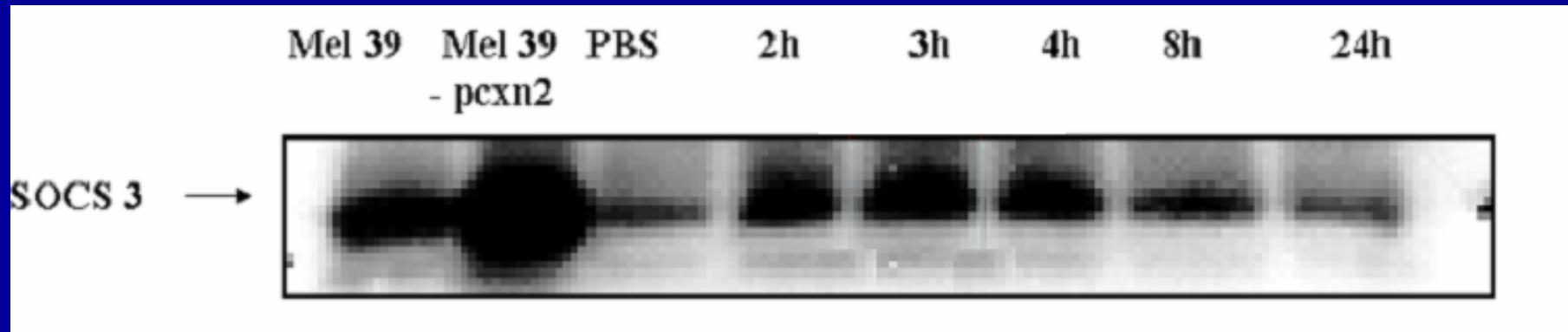




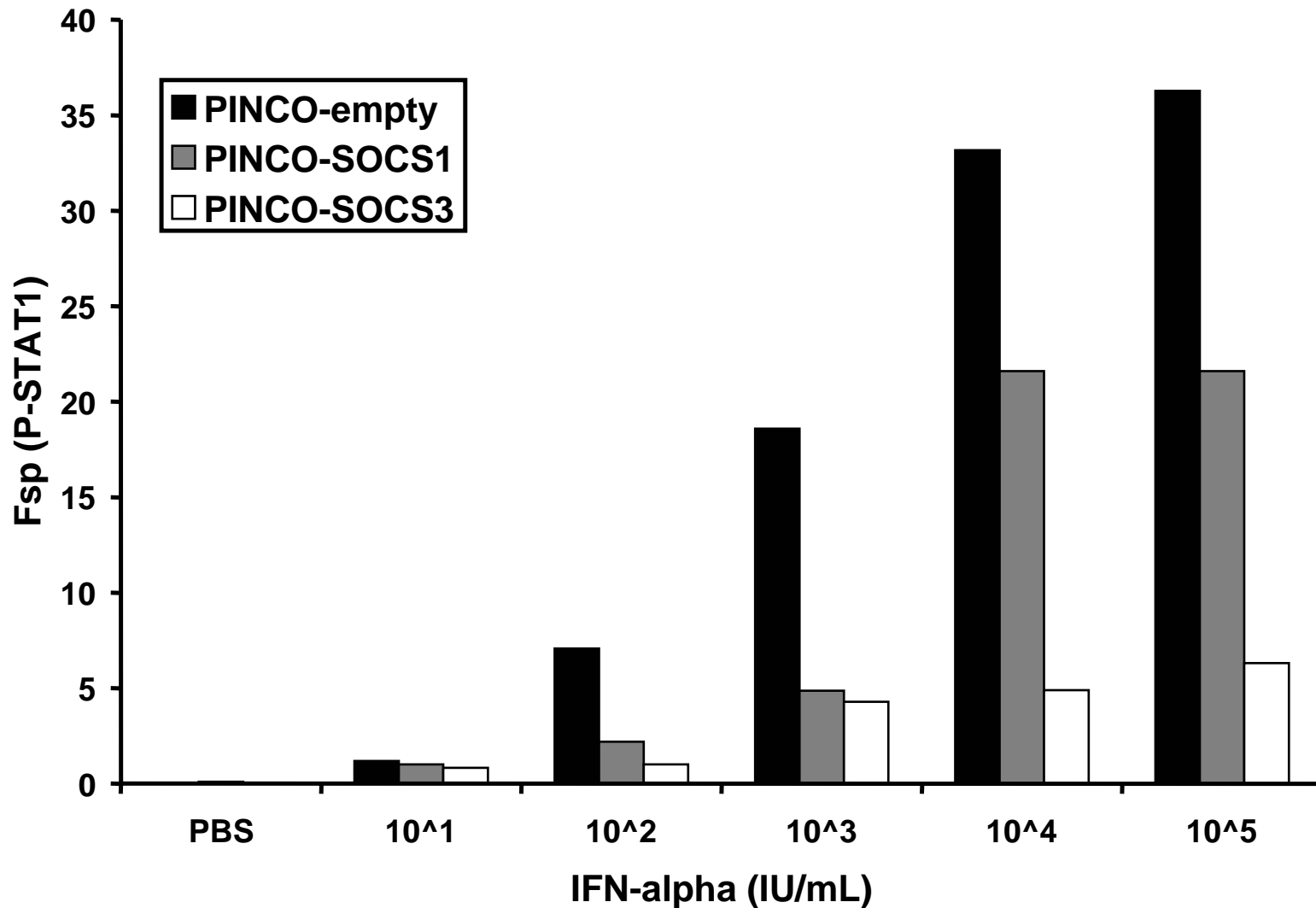
# IFN- $\alpha$ induces SOCS1 mRNA in PBMCs



# SOCS3 Protein Expression



# Jurkat Cells (15 min. IFN- $\alpha$ stimulation)



# Summary

- Cytokines can act via host or on the tumor
- Patients vary in their response to cytokines
- Higher doses of cytokines may be less effective
- If there is not a receptor, then there will not be a response
- Negative regulatory pathways may have dominant effects

# Acknowledgments

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- Jason Zimmerer, B.S.
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- Michael Walker, M.D.