

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

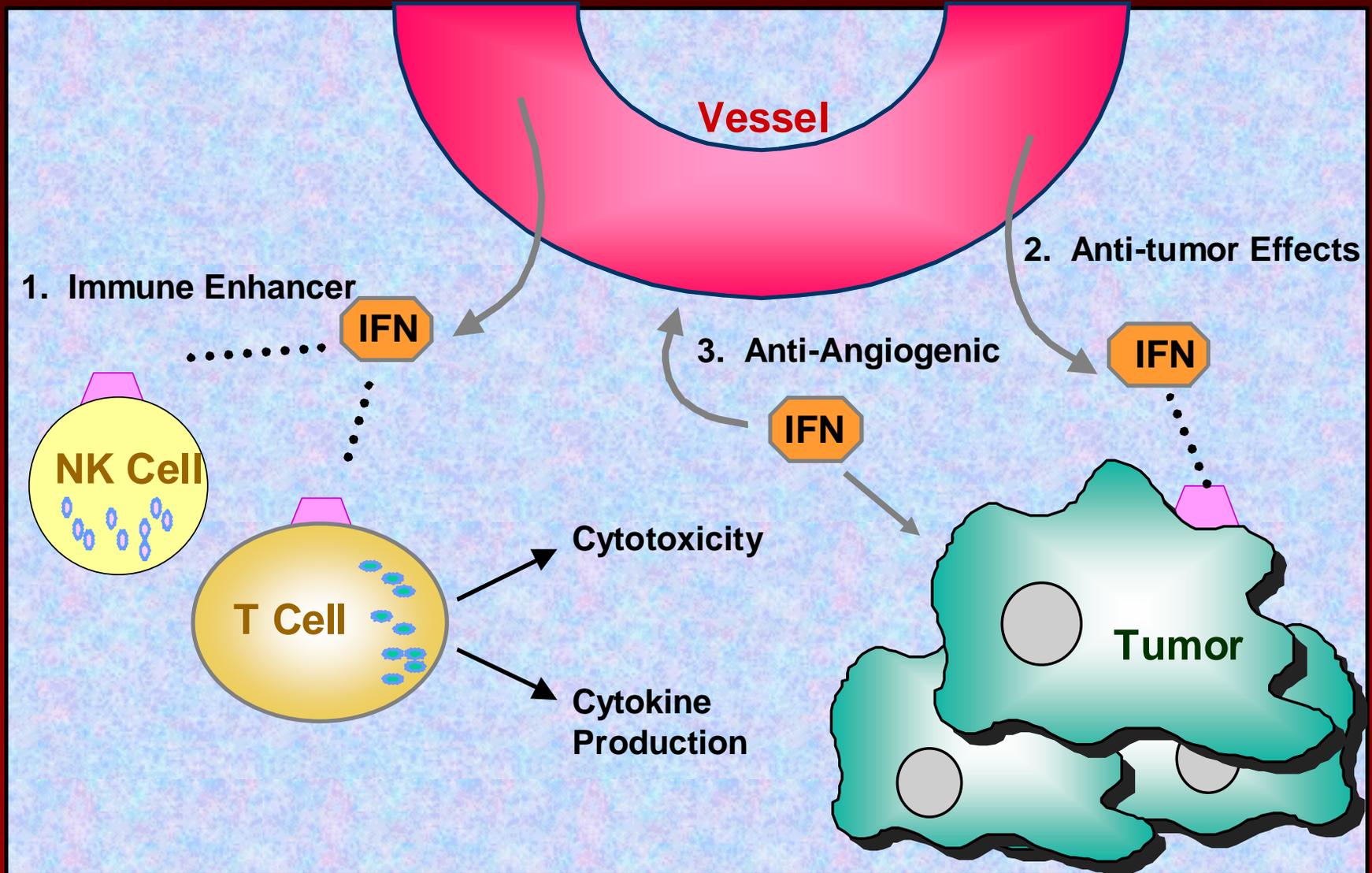
- **Interleukin-12 (recently halted)**
- **Interleukin-18 (inducer of IFN-gamma)**
- **Interleukin-21 (IL-2 family)**
- **Interleukin-28 (interferon family)**

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

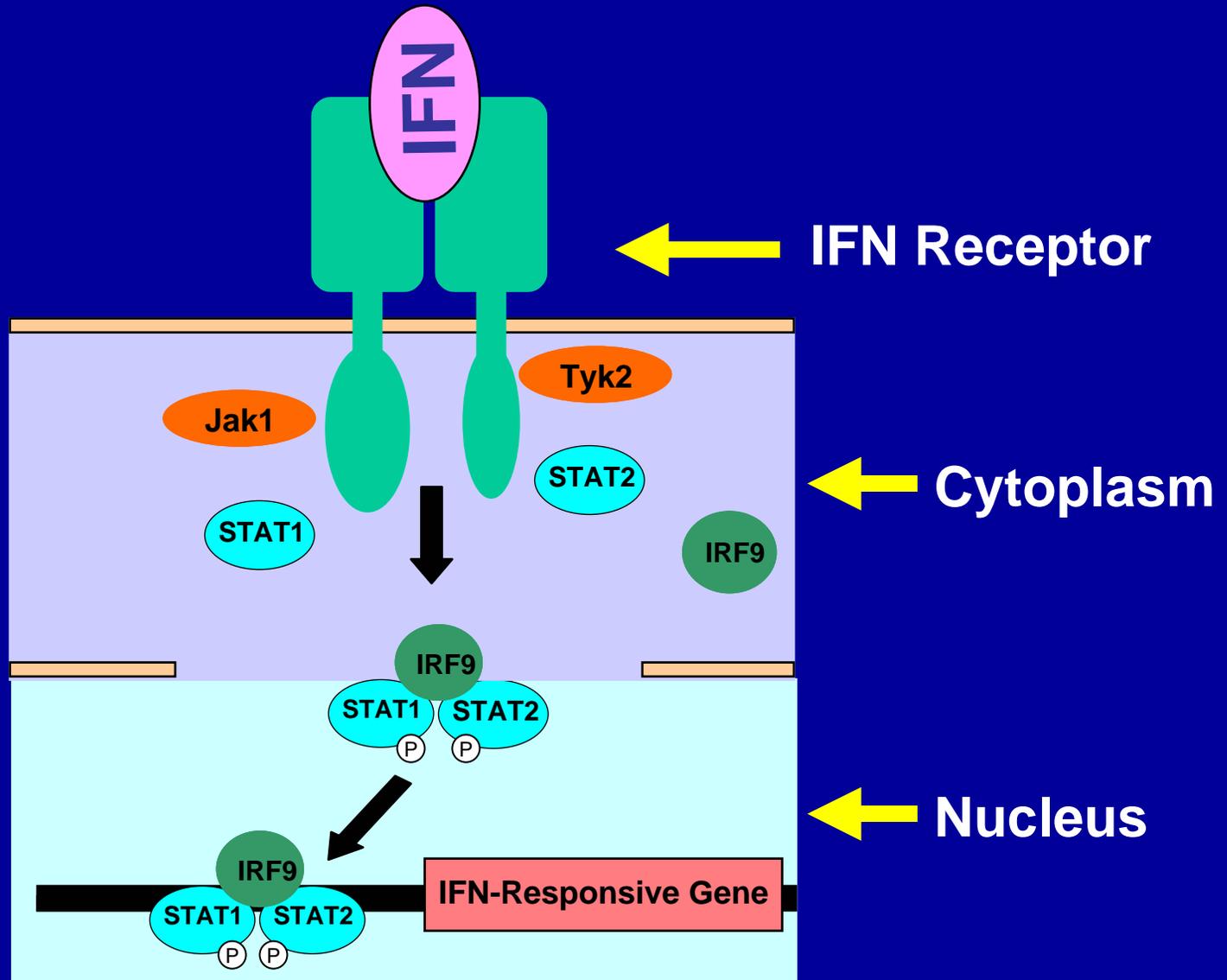
Clinical Uses of IFN-alpha

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

How Does IFN- α Therapy Work? Some Potential Mechanisms



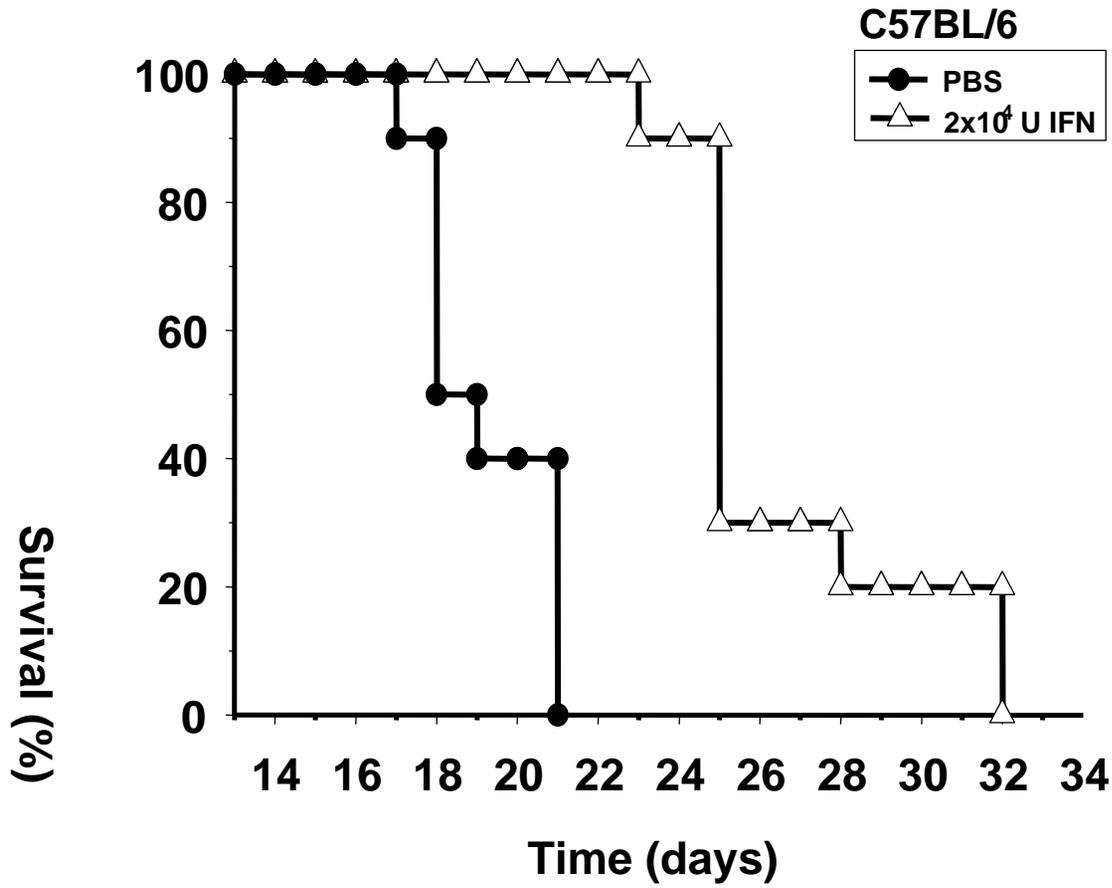
Jak-STAT Pathway



The IFN-alpha Receptor

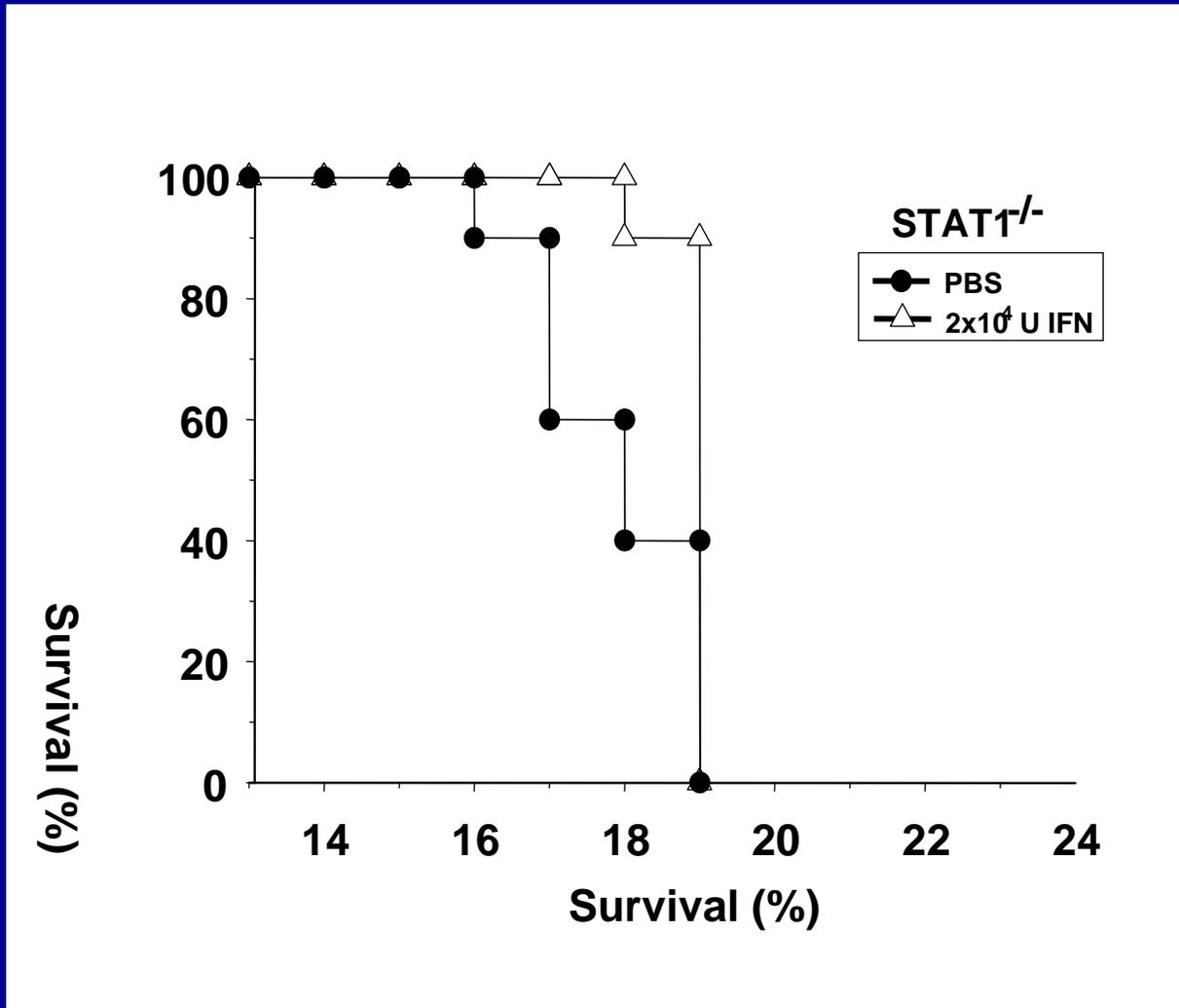
- **Two subunits**
- **Widely expressed on immune cells**
- **Expressed on most tumor cells**

Murine Model of Malignant Melanoma



Host: STAT1+
Tumor: STAT1+

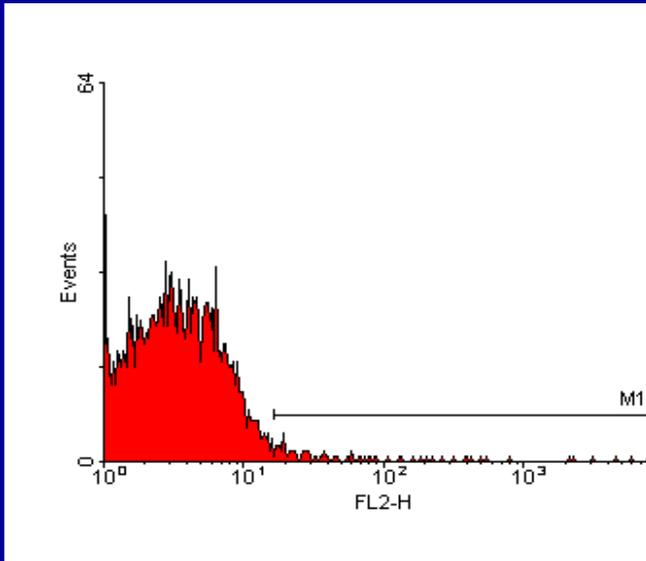
Effects of IFN-alpha in a STAT1^{-/-} Host



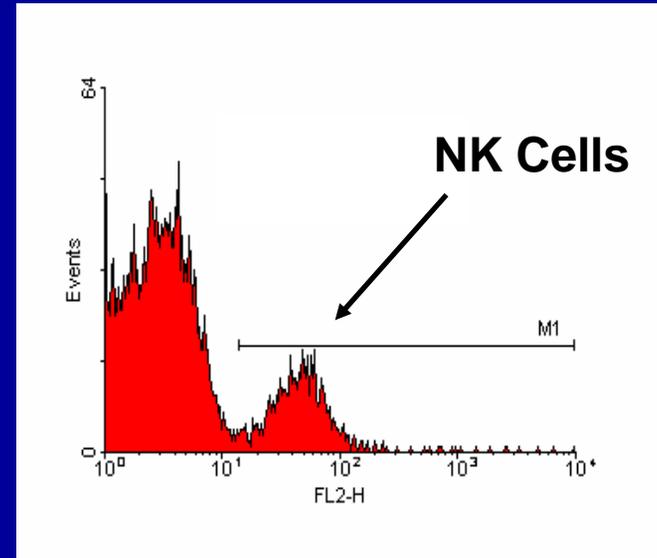
Host: STAT1-neg
Tumor: STAT1+

Peritoneal Washings

PBS

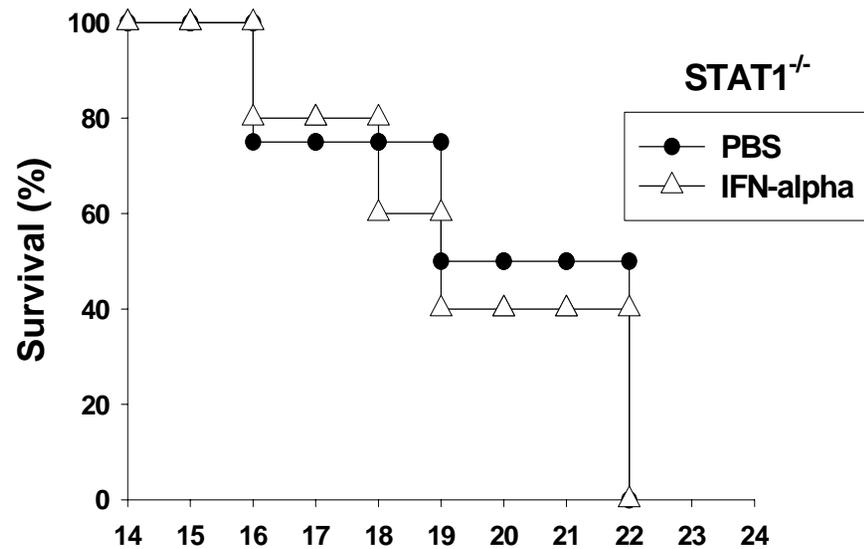
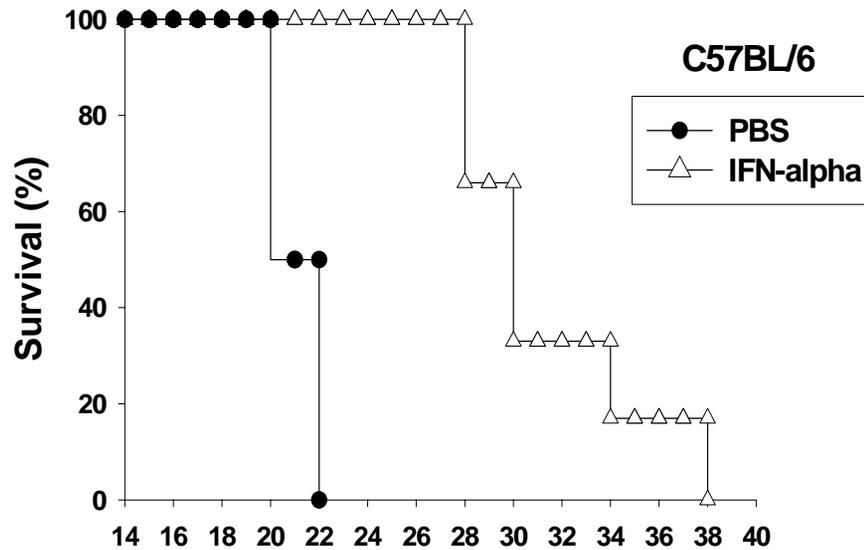


IFN



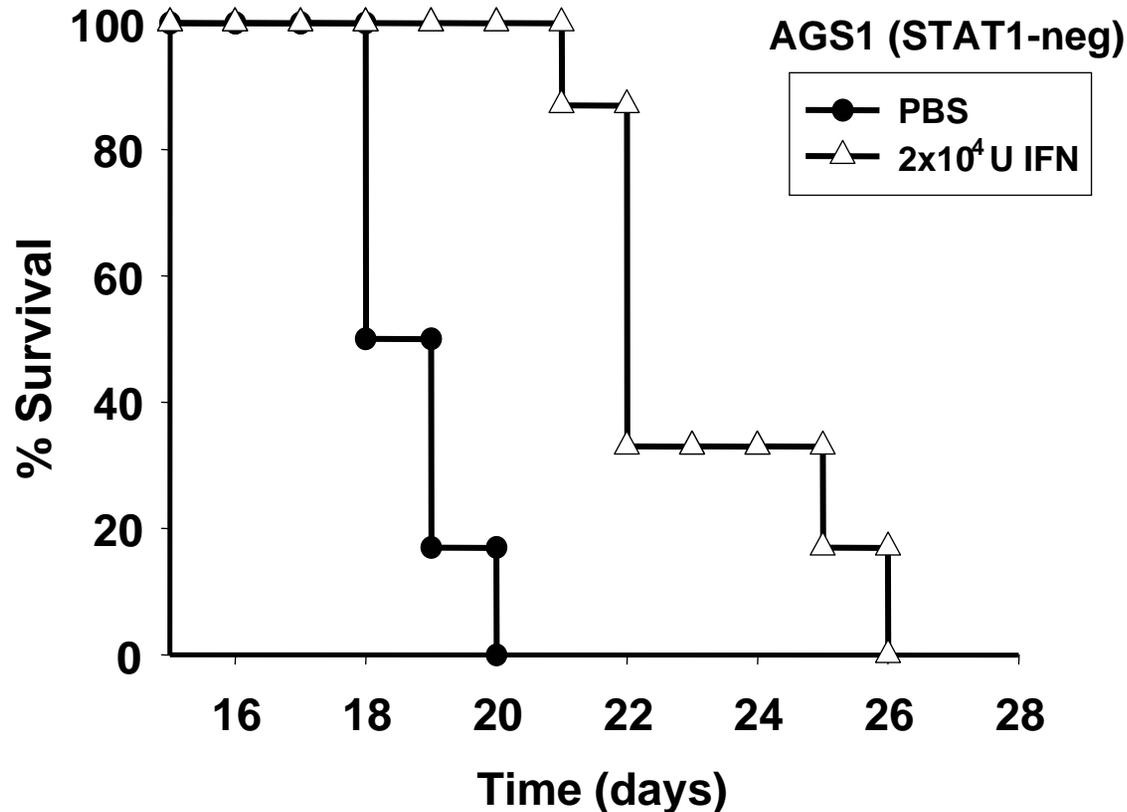
← DX5-PE →

Adjuvant Model



Host: STAT1-neg
Tumor: STAT1+

A STAT1-negative Murine Melanoma



Host: STAT1+
Tm: STAT1-neg

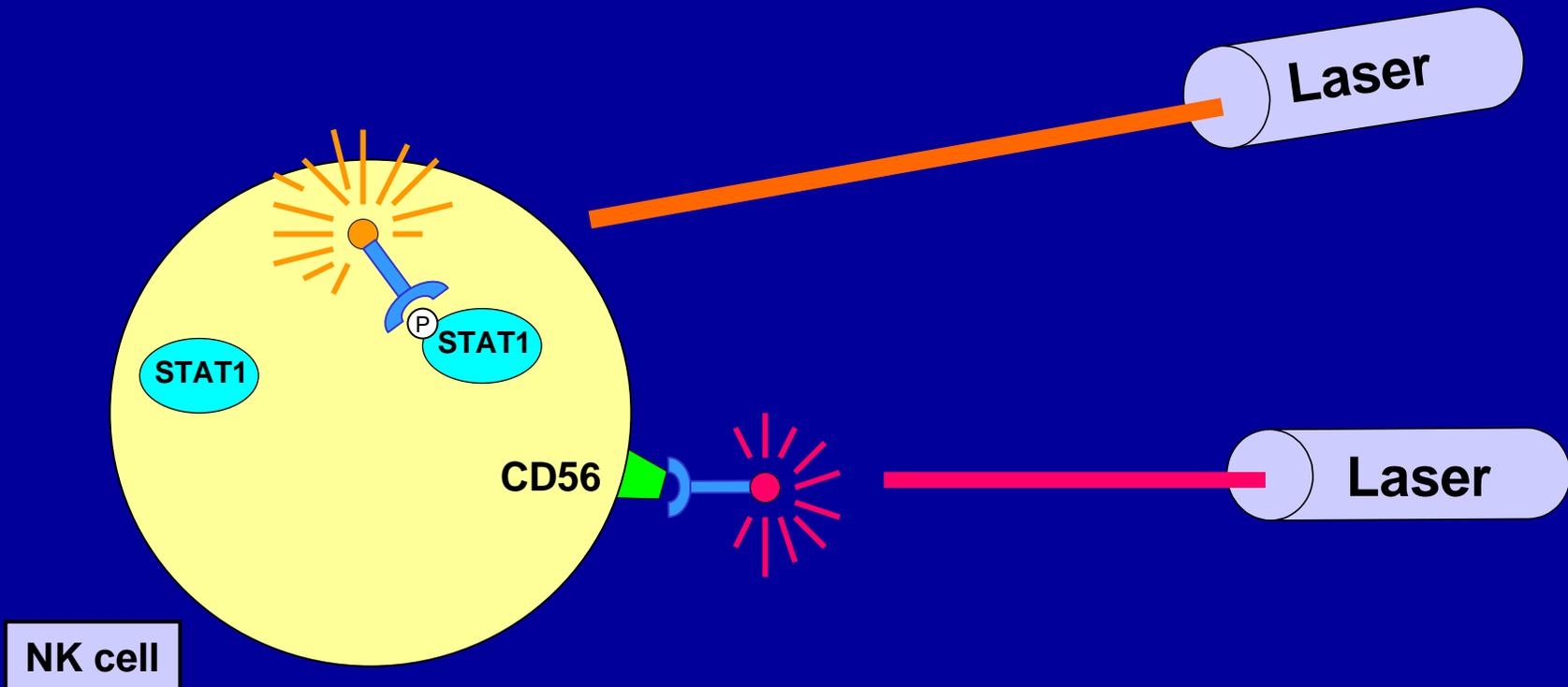
Summary

- **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

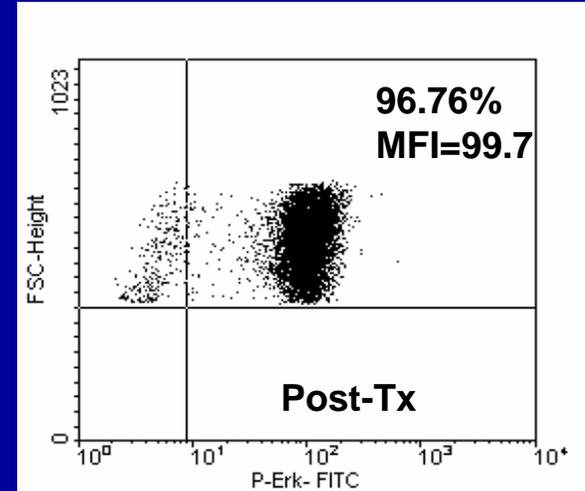
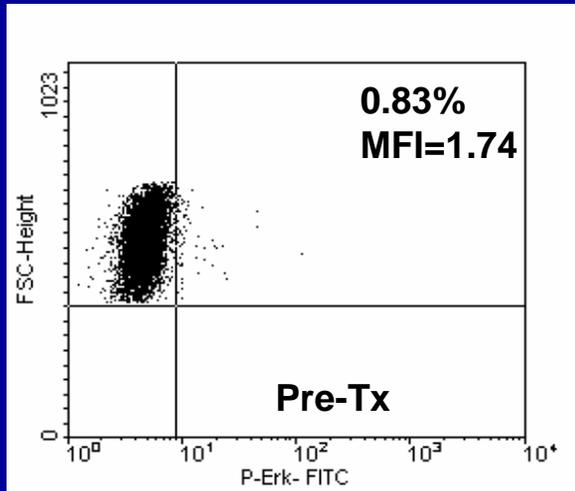
Monitoring STAT1 Activation in Immune Cells

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



The Dot Plot

CD3 →

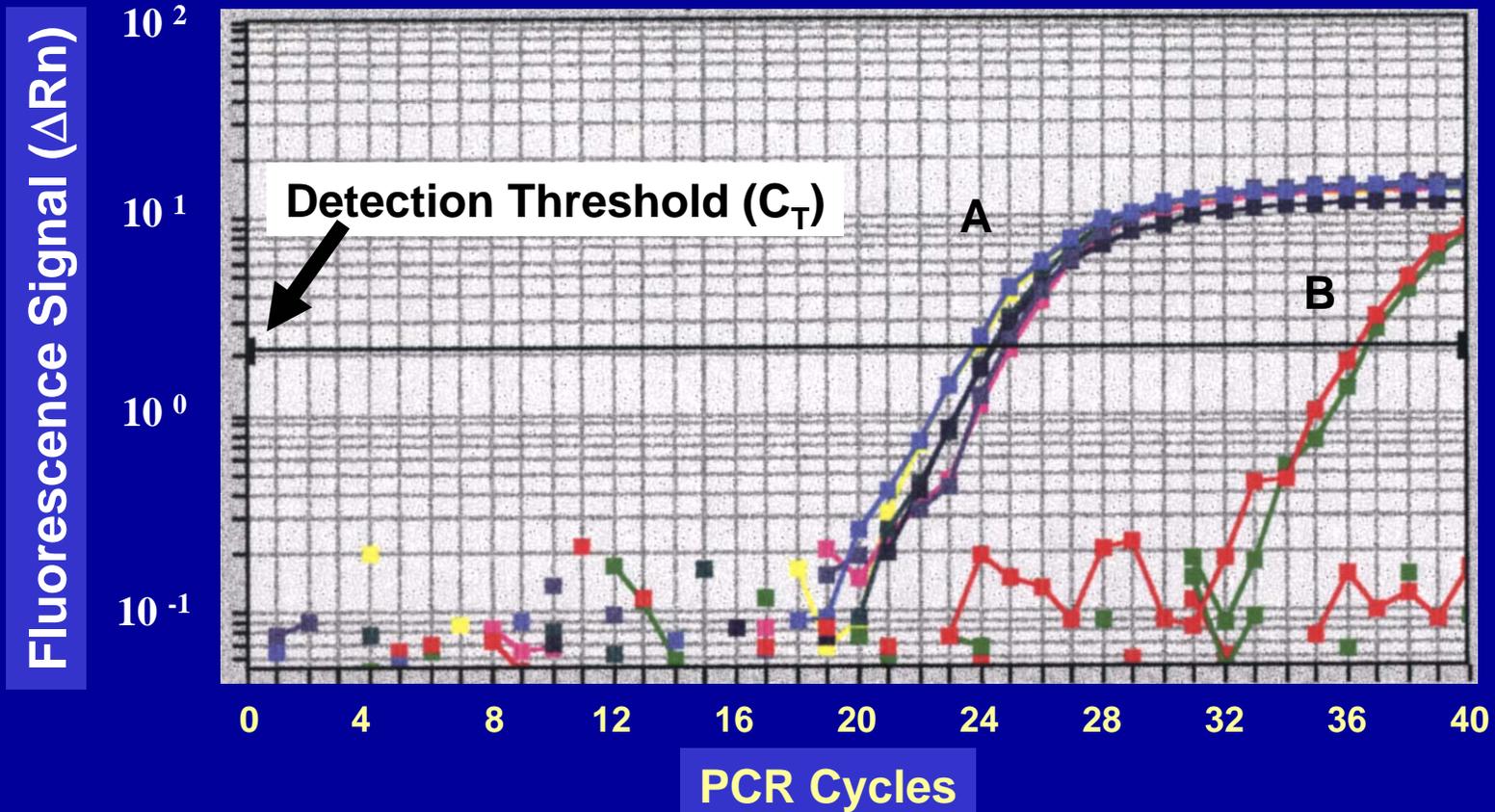


Phospho-Protein →

Other Tools of the Trade

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

- 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

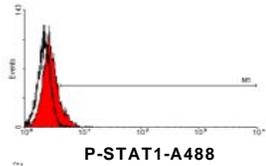
- MALDI-MS
- SELDI-TOF mass spectrometer

→ Maximal cytokine signaling occurs at relatively low doses

IFN- α IU/ml

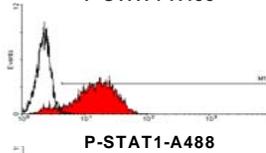
Fsp

PBS



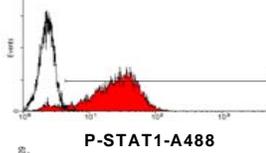
0.53

1



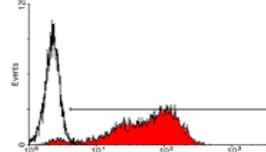
13.84

10



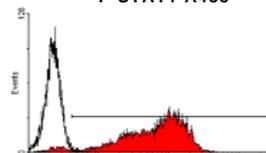
24.09

100



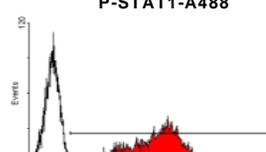
61.53

10³



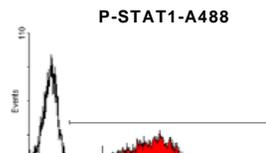
72.72

10⁴



66.33

10⁵

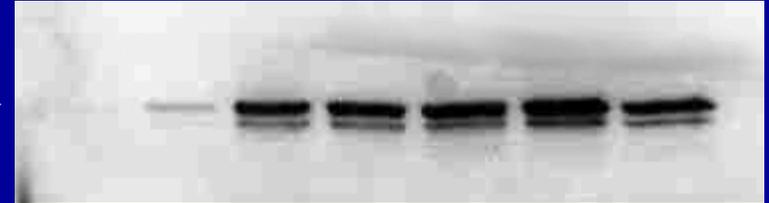


44.51

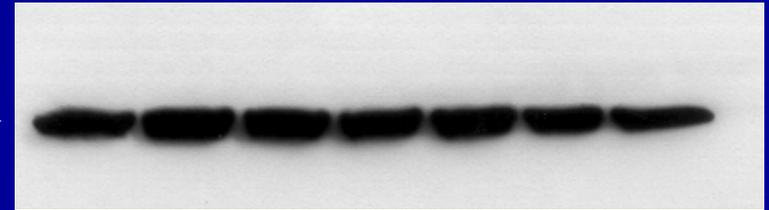
Dose Response

IFN- α (IU/ml): 0 1 10¹ 10² 10³ 10⁴ 10⁵

P-STAT1 →

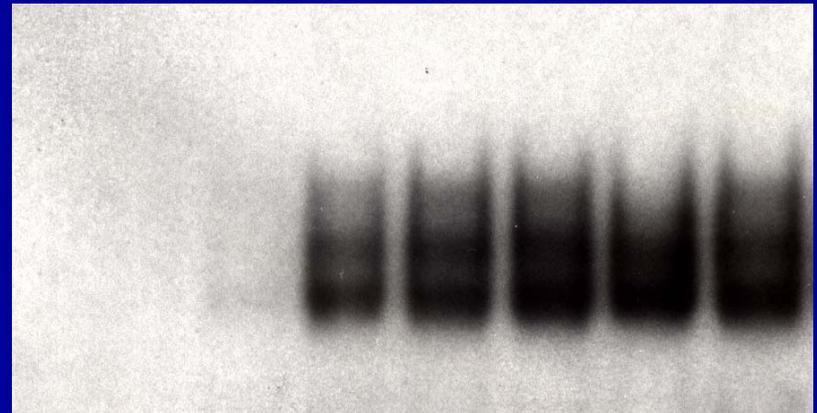


B-Actin →

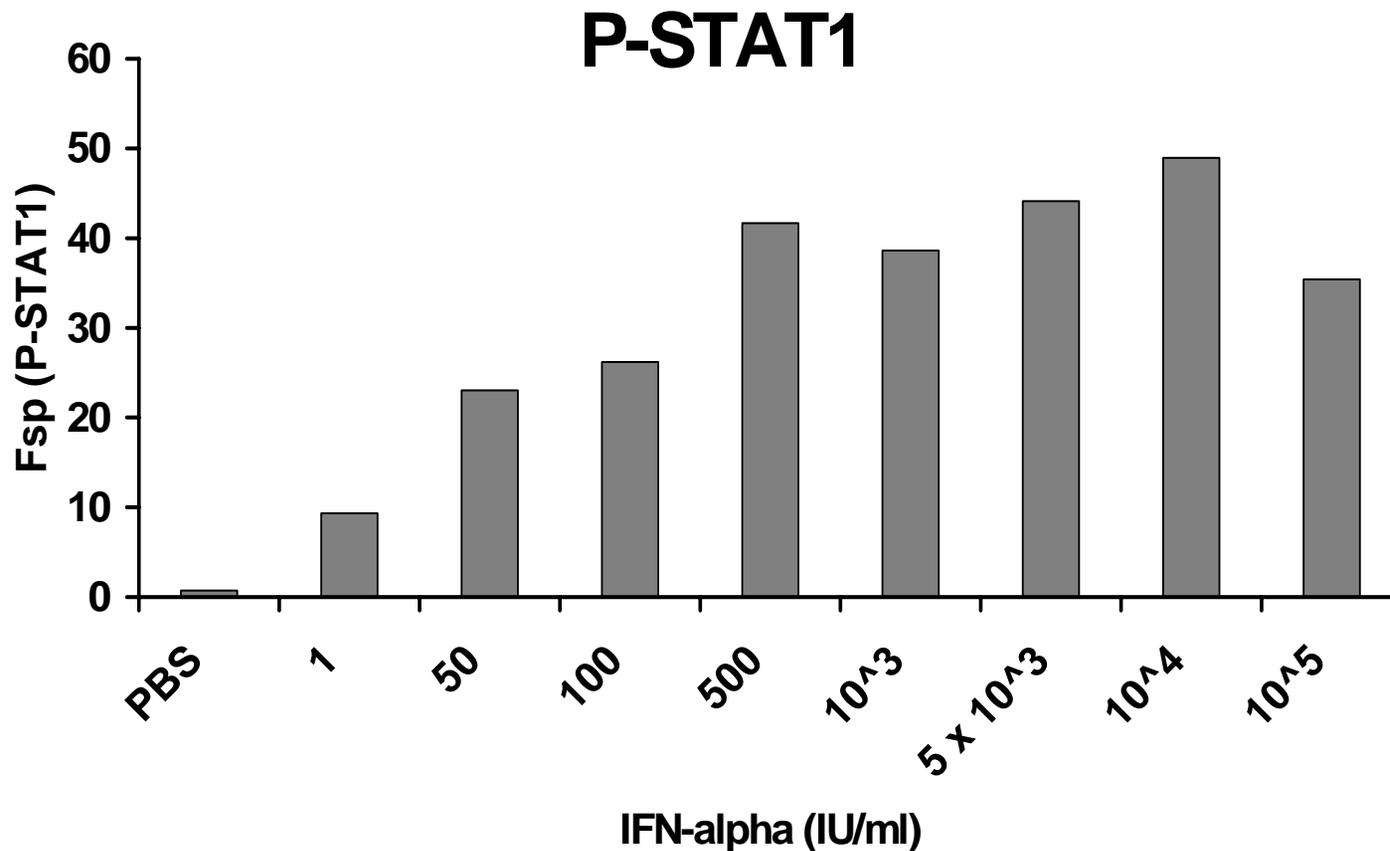


IFN- α (U/ml): 0 1 10 100 10³ 10⁴ 10⁵

SIE →

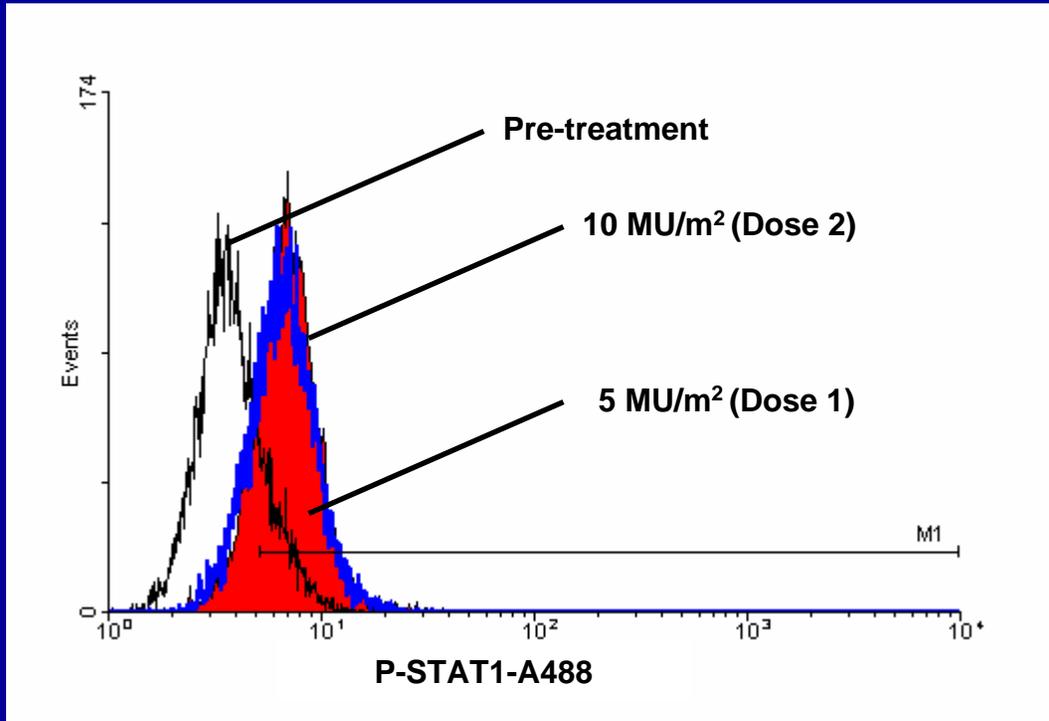


P-STAT1 in PBMCs: Activation at Low Doses



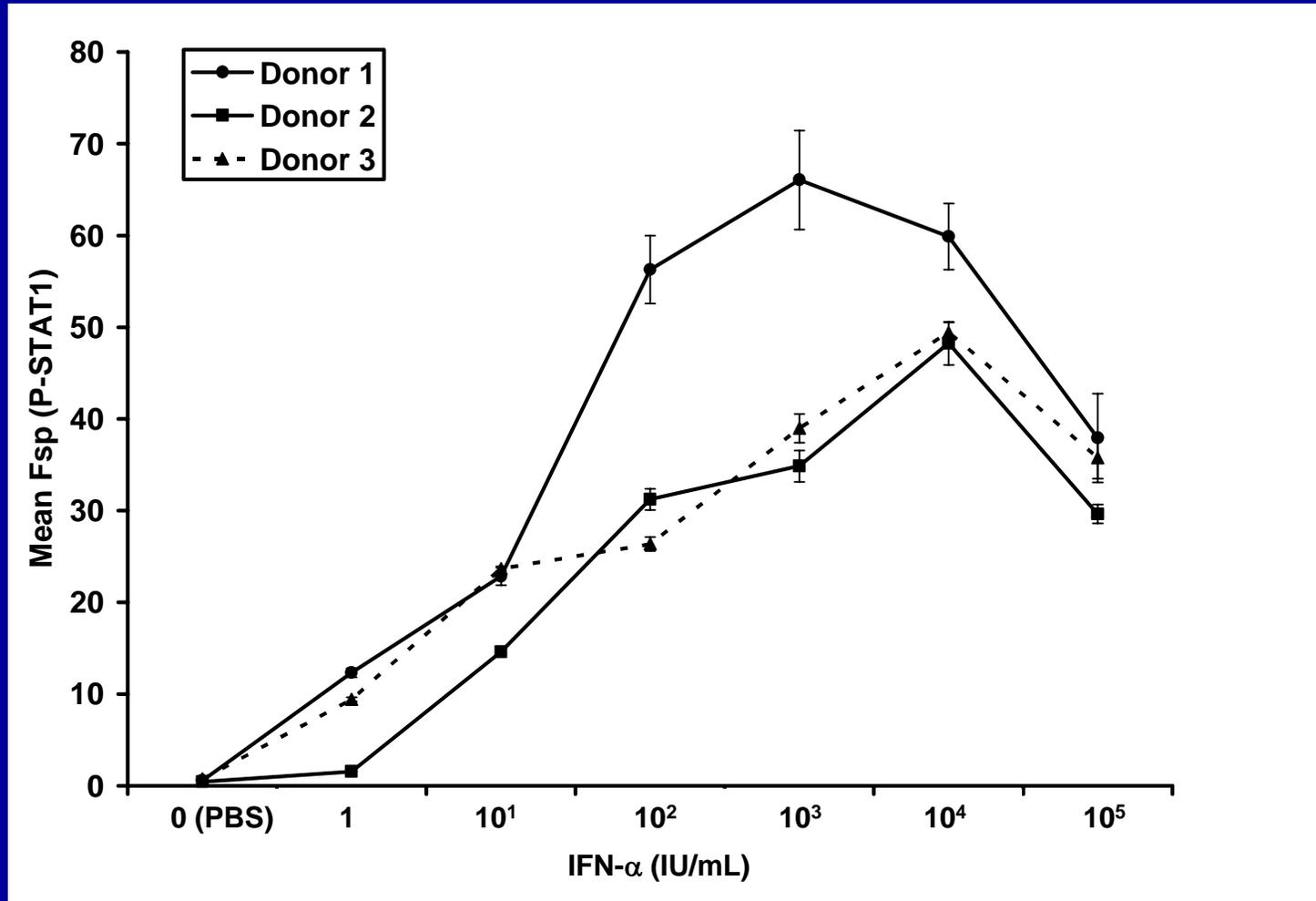
What Is an Optimal Dose?

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:

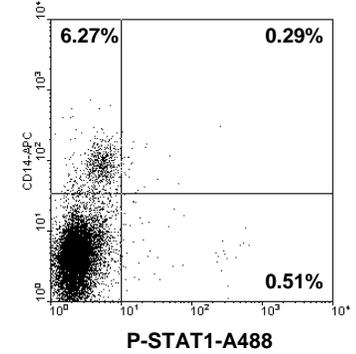
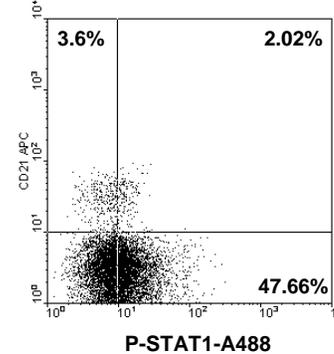
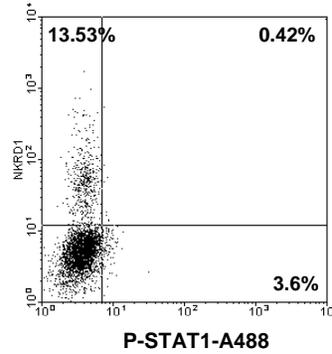
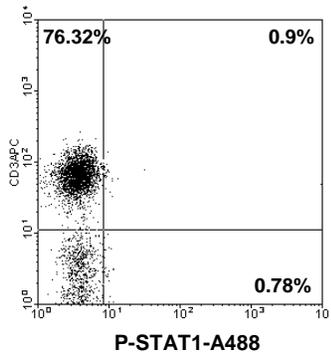
T lymphocytes

NK cells

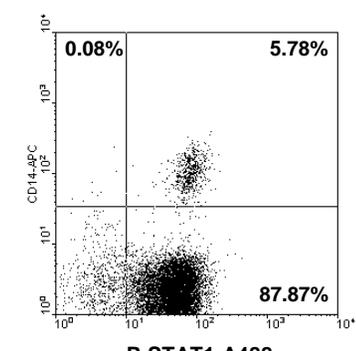
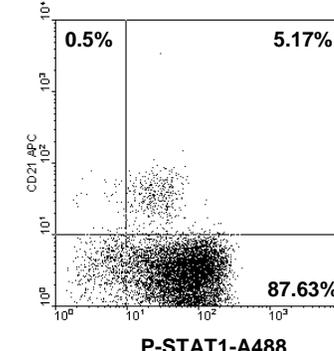
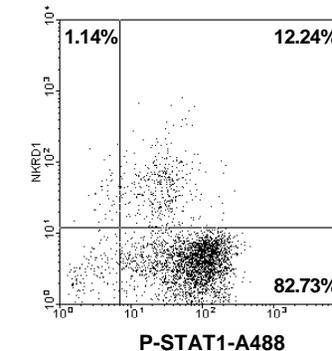
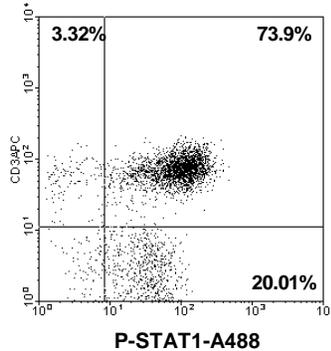
B lymphocytes

Monocytes

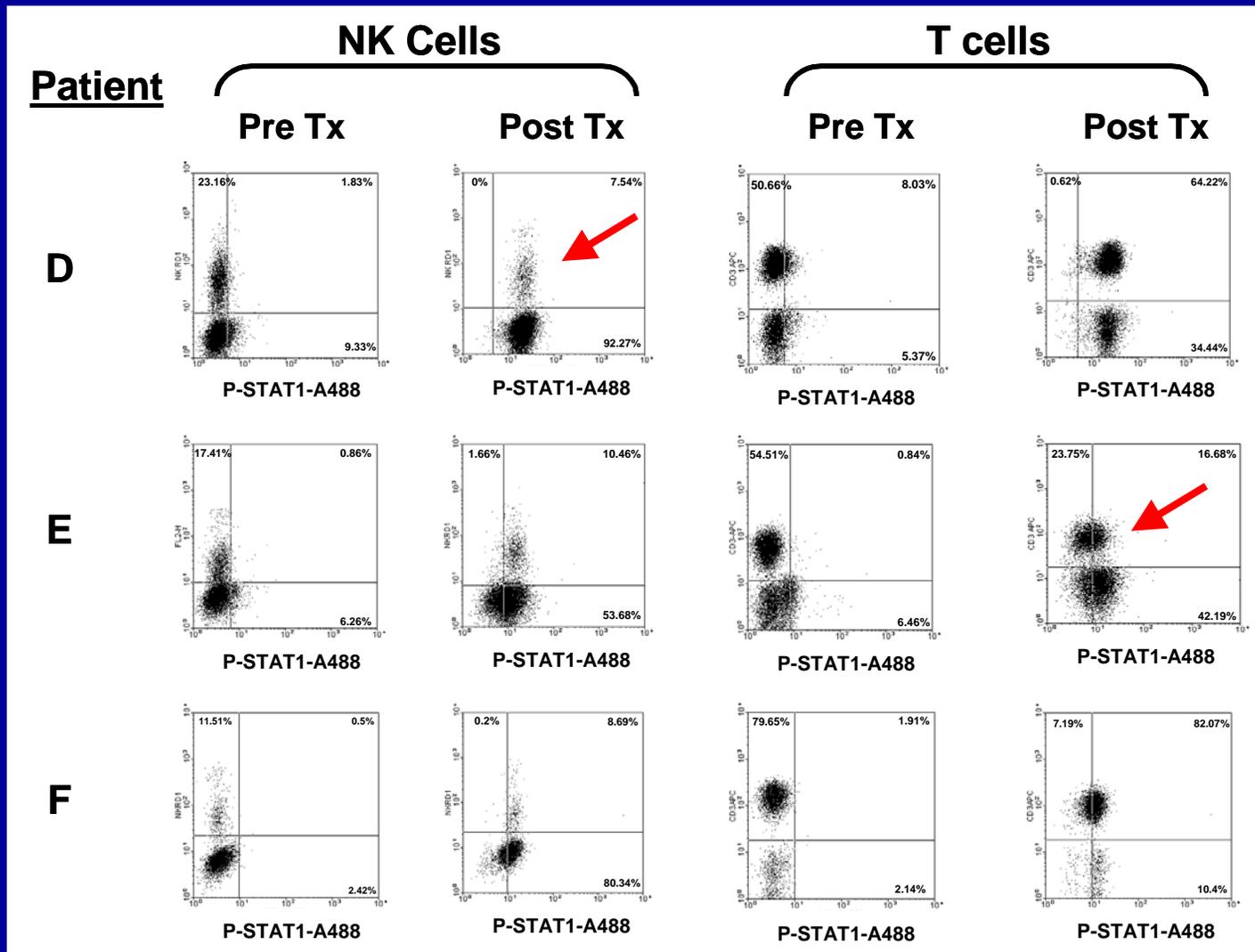
PBS



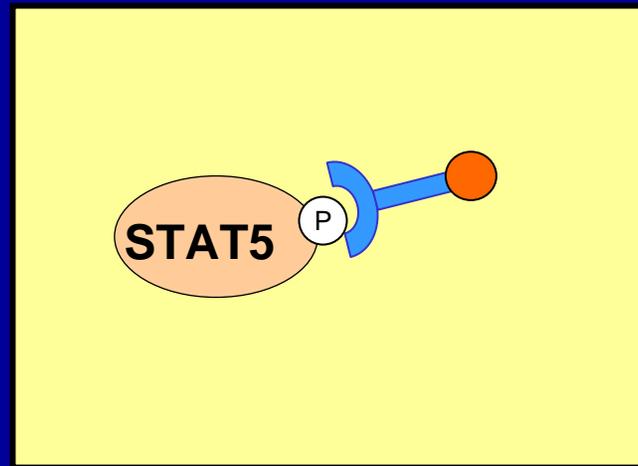
IFN- α



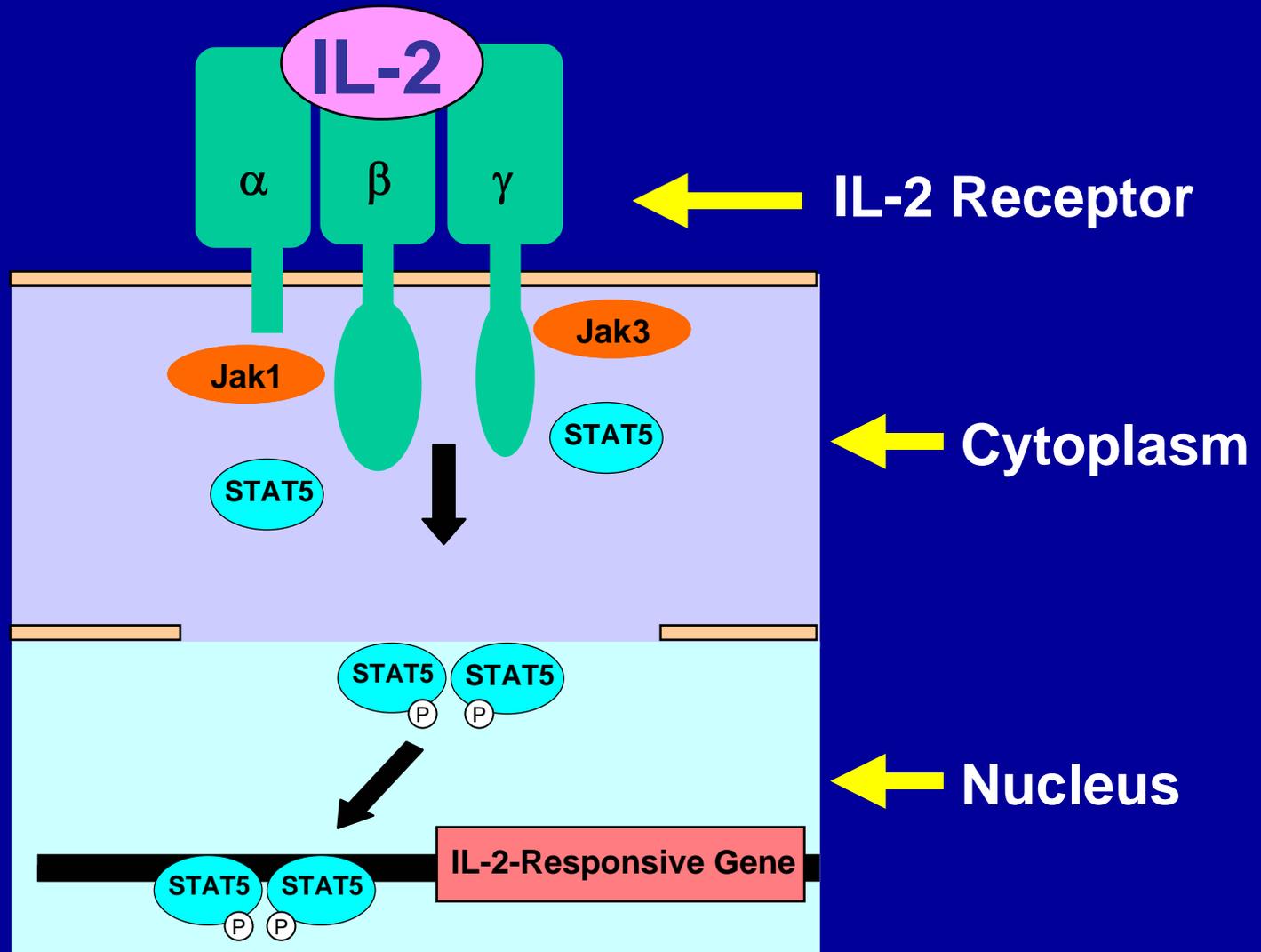
STAT1 Activation in Patient Lymphocytes During IFN- α Immunotherapy



Other Phospho-Specific Antibodies?



IL-2 Signal Transduction

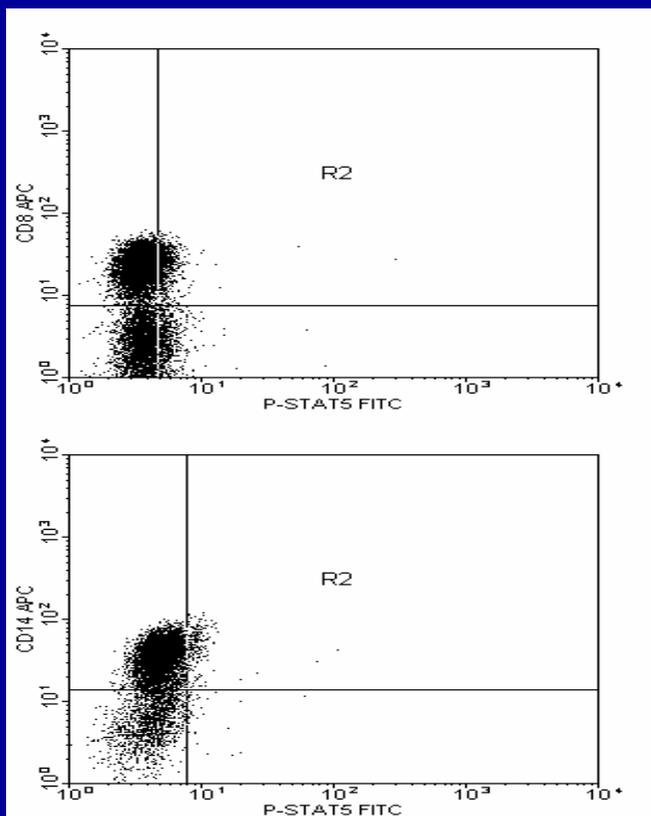


→ Cytokines will not activate a cell that lacks the corresponding receptor

IL-2 Patient No. 5

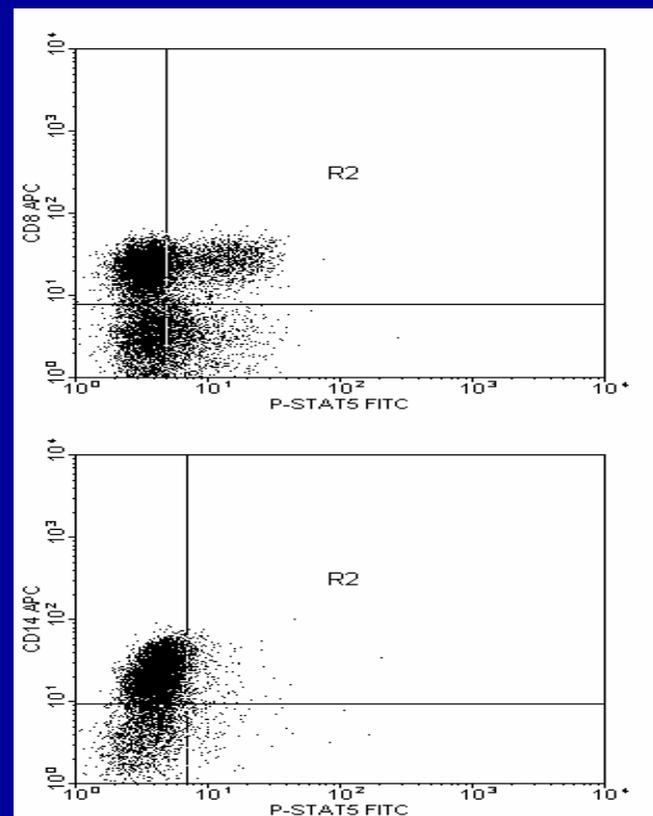
Pre-Tx

Post-Tx



CD8

CD14

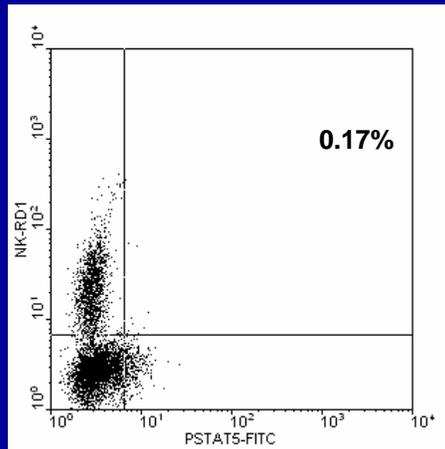


→ Cytokines may exert prolonged effects

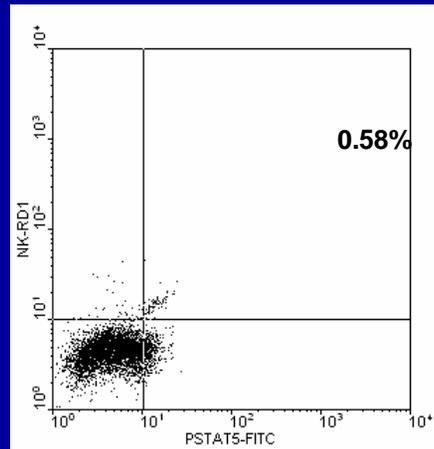
P-STAT5 in NK Cells

NK

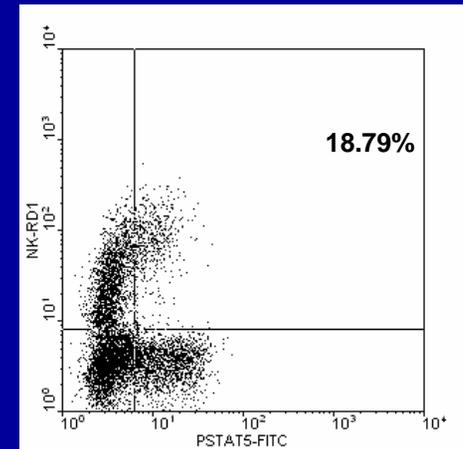
Pre-IL-2



1 hr Post-IL-2



1 wk post-IL-2



P-STAT5

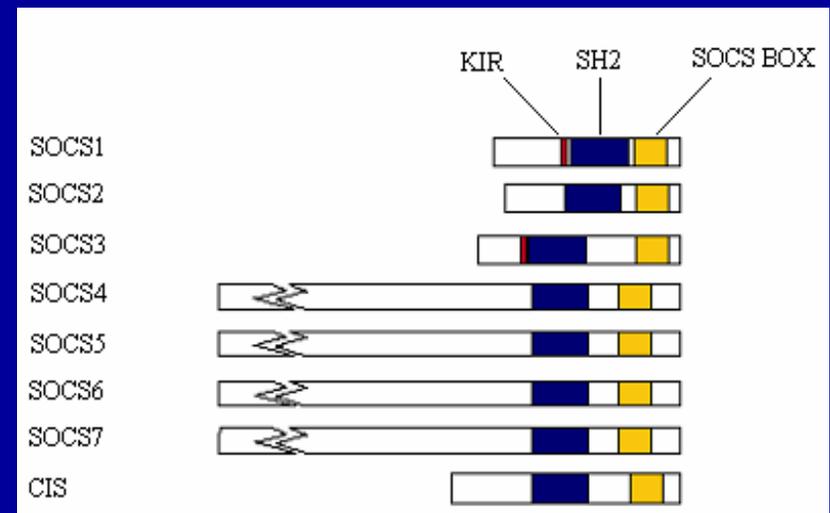
**→ Every signaling system
has a set of brakes**

Regulation of IFN- α Signal Transduction

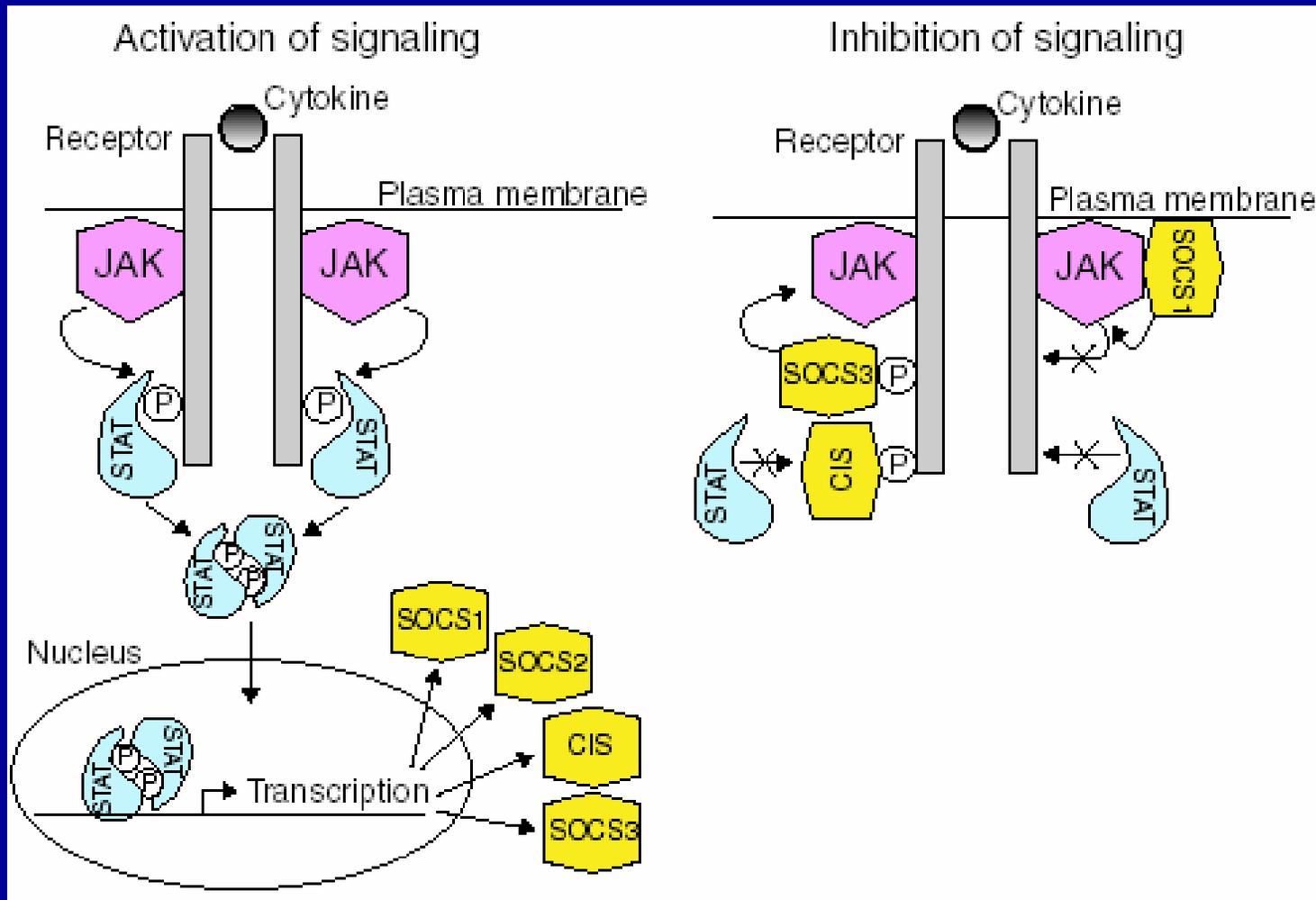
- Dose-response curve is not linear
- Signaling is inhibited at higher concentrations
- Role of negative regulators?

SOCS

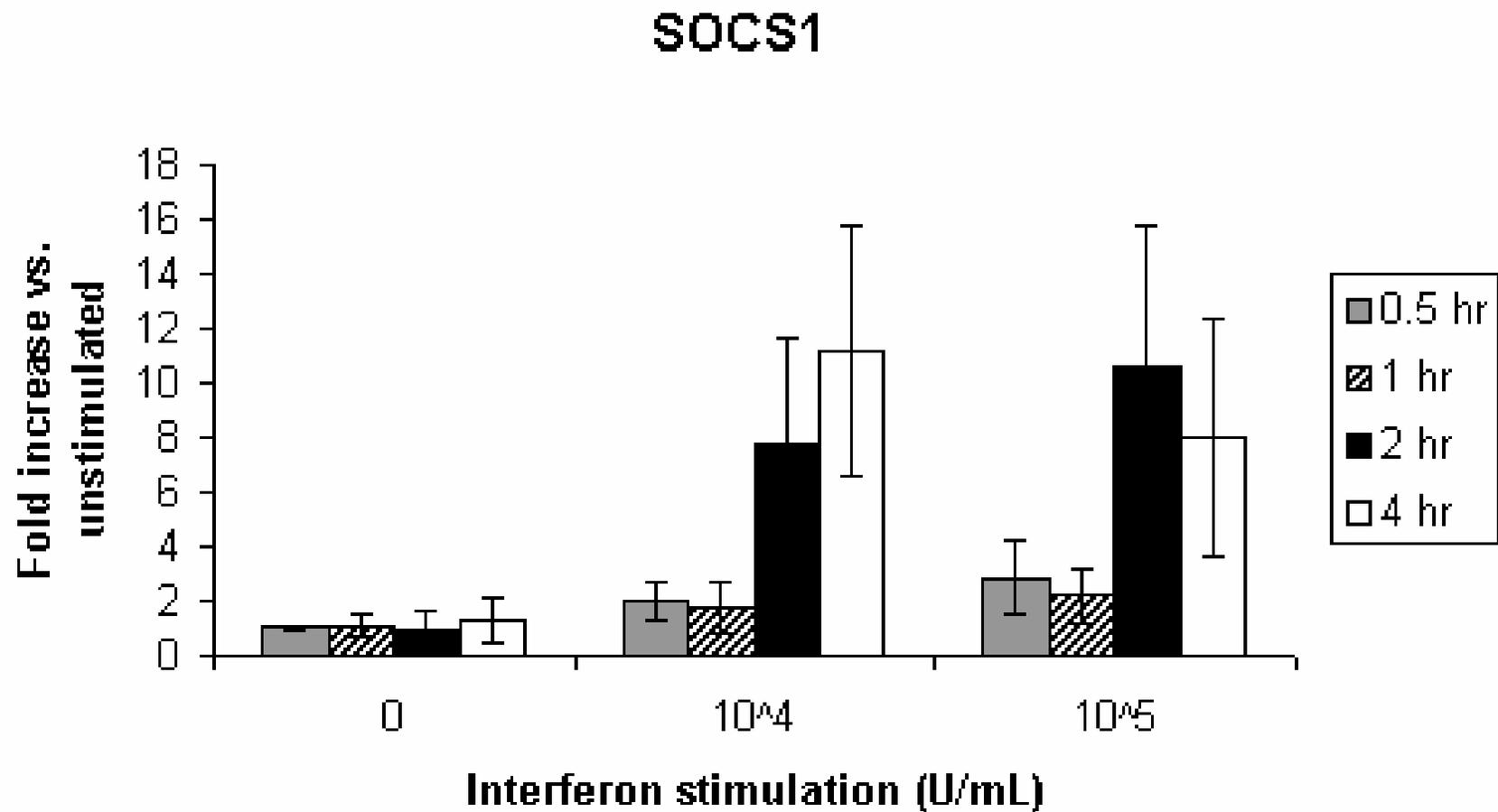
- **Suppressors of Cytokine Signaling Family (negative feedback loop)**
- **SOCS Family = SOCS1-7 and CIS**
- **Src-homology 2 domain (binding to JAKs)**
- **SOCS1 KO mice die @ 3 weeks of uninhibited IFN-gamma signaling.**



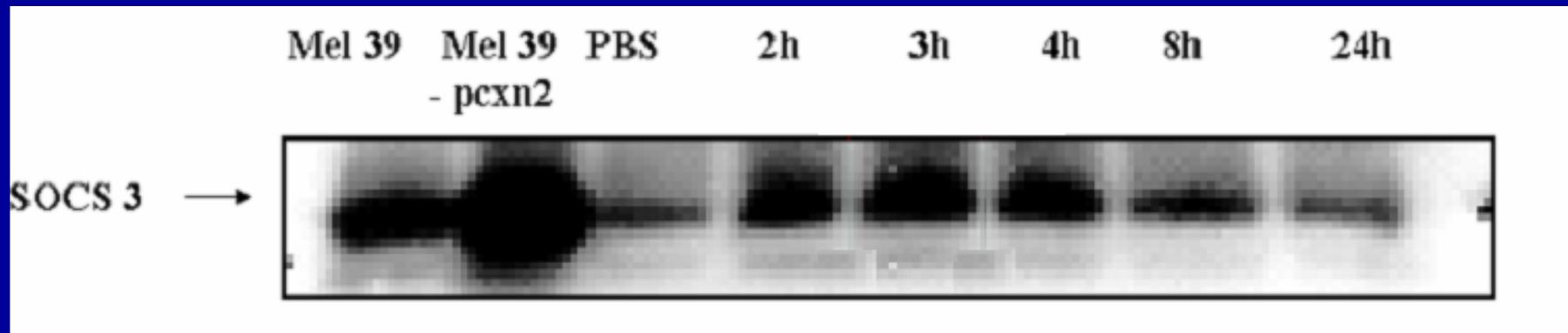
Negative Feedback Inhibition



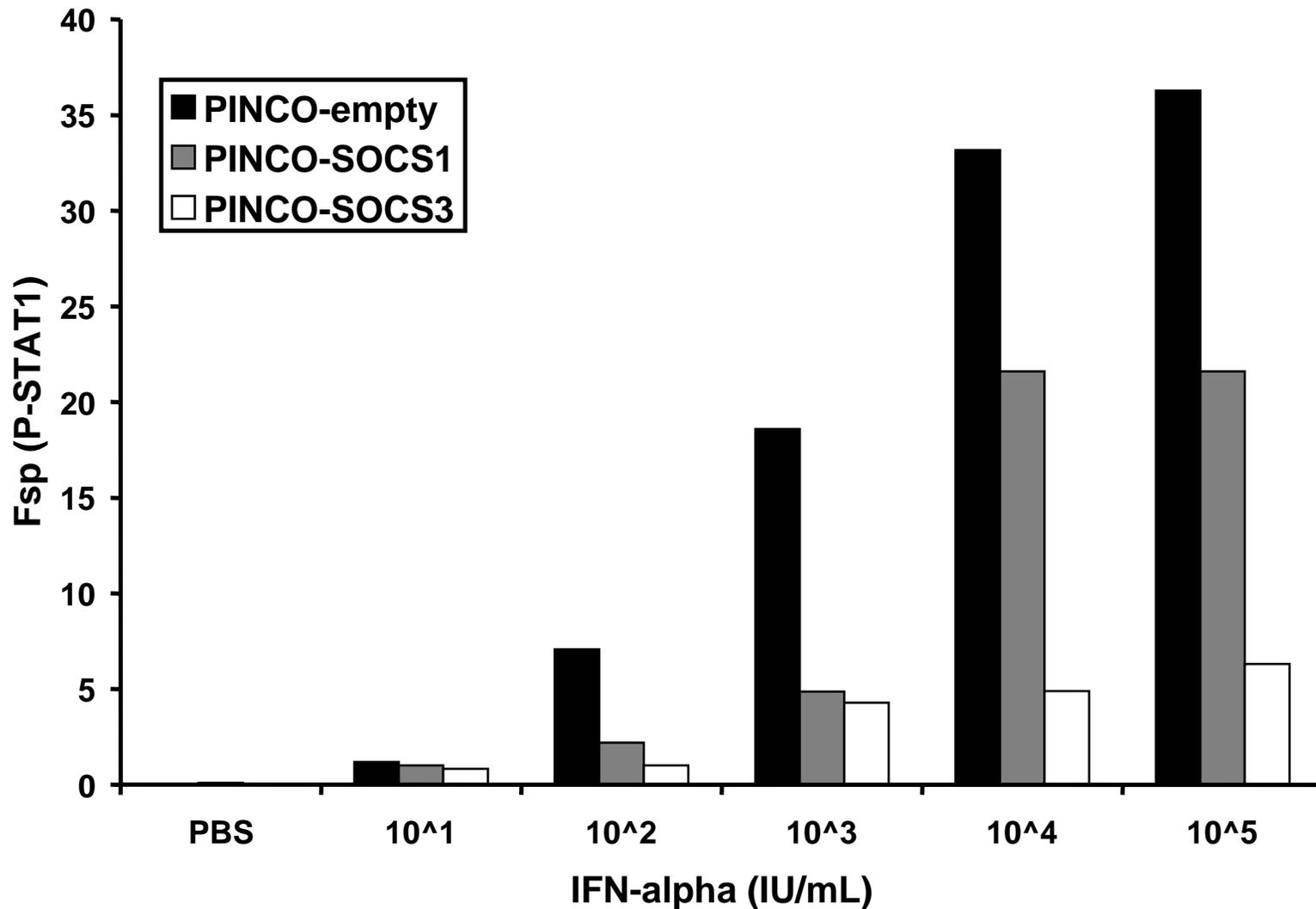
IFN- α induces SOCS1 mRNA in PBMCs



SOCS3 Protein Expression



Jurkat Cells (15 min. IFN- α 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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