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 ….

1. Immune Enhancer
2. Anti-tumor Effects
3. Anti-Angiogenic

- IFN
- NK Cell
- T Cell
- Tumor
- Vessel
- Cytotoxicity
- Cytokine Production
Jak-STAT Pathway

IFN Receptor

Cytoplasm

Nucleus

IFN-Responsive Gene

Jak1

Tyk2

STAT1

STAT2

IRF9

P

P

P

Jak-STAT Pathway

STAT1

STAT2

IRF9

P

P

P
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

Survival (%) vs. Survival (%) graph showing the effects of IFN-alpha in a STAT1^-/- host. The graph compares survival rates under different conditions:

- **Host:** STAT1-neg
- **Tumor:** STAT1+

Lines represent:
- PBS (filled circles)
- 2x10^6 U IFN (open triangles)
Peritoneal Washings

PBS

IFN

DX5-PE

NK Cells
Adjuvant Model

Host: STAT1-neg
Tumor: STAT1+

Survival (%)

C57BL/6

STAT1^-/

PBS

IFN-alpha

PBS

IFN-alpha
A STAT1-negative Murine Melanoma

% Survival

Time (days)

PBS

2x10^4 U IFN

AGS1 (STAT1-neg)

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

Pre-Tx

Phospho-Protein

Post-Tx
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
Dose Response

IFN-α (IU/ml): 0 1 10^1 10^2 10^3 10^4 10^5

P-STAT1

B-Actin

IFN-α (U/ml): 0 1 10 100 10^3 10^4 10^5

SIE
P-STAT1 in PBMCs: 
Activation at Low Doses

![Graph showing P-STAT1 activation at different doses of IFN-alpha.](image-url)
What Is an Optimal Dose?

Pre-treatment

10 MU/m² (Dose 2)

5 MU/m² (Dose 1)

Patient C

P-STAT1-A488
There is significant inter-patient variation in response to cytokines.
P-STAT1 Levels In Human PBMCs: Interpatient Variation

Multi-Parametric Analysis of STAT1 Activation

<table>
<thead>
<tr>
<th>Treatment:</th>
<th>T lymphocytes</th>
<th>NK cells</th>
<th>B lymphocytes</th>
<th>Monocytes</th>
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<tbody>
<tr>
<td>PBS</td>
<td>76.32%</td>
<td>13.53%</td>
<td>3.6%</td>
<td>6.27%</td>
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<td></td>
<td>0.9%</td>
<td>0.42%</td>
<td>2.02%</td>
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<td>0.70%</td>
<td>3.6%</td>
<td>47.66%</td>
<td>0.51%</td>
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<tr>
<td>IFN-α</td>
<td>3.32%</td>
<td>1.14%</td>
<td>0.5%</td>
<td>0.08%</td>
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<tr>
<td></td>
<td>73.9%</td>
<td>12.24%</td>
<td>5.17%</td>
<td>5.78%</td>
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<tr>
<td></td>
<td>20.01%</td>
<td>82.73%</td>
<td>87.63%</td>
<td>87.87%</td>
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</table>
STAT1 Activation in Patient Lymphocytes During IFN-α Immunotherapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>NK Cells</th>
<th>T cells</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre Tx</td>
<td>Post Tx</td>
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<td><img src="image" alt="Graph" /></td>
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</table>
Other Phospho-Specific Antibodies?
IL-2 Signal Transduction

IL-2 binds to the IL-2 receptor, which consists of α, β, and γ chains. This binding leads to the activation of Jak kinases (Jak1 and Jak3). Upon phosphorylation, STAT5 is activated and translocated to the nucleus, where it binds to IL-2-responsive genes.
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

Pre-IL-2

1 hr Post-IL-2

1 wk post-IL-2

P-STAT5

NK

0.17%

0.58%

18.79%
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

Diagram showing the activation and inhibition of signaling pathways involving JAK, STAT, SOCS1, SOCS2, SOCS3, and CIS proteins in the nucleus and plasma membrane.
IFN-α induces SOCS1 mRNA in PBMCs
SOCS3 Protein Expression
Jurkat Cells (15 min. IFN-\(\alpha\) stimulation)

![Bar graph showing Fsp (P-STAT1) levels in different conditions of IFN-alpha (IU/mL) for PINCO-empty, PINCO-SOCS1, and PINCO-SOCS3.]

- **IFN-alpha (IU/mL)**: PBS, 10^1, 10^2, 10^3, 10^4, 10^5
- **Fsp (P-STAT1)**: PINCO-empty, PINCO-SOCS1, PINCO-SOCS3
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
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