Cytokines in Cancer Immunotherapy

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  – GSK
  – Lilly
  – Pfizer
  – Roche

• DMC
  – Cougar/J and J
  – Medimmune
Immunobiology of Cytokines

For discussion
- Structure/function relationships
- Cellular source/stimuli for synthesis/secretion
- Target cell(s)/receptor structure
- Signaling induced by cytokine binding
- Preclinical applications
- Cytokine gene transfer/vaccine
- Clinical application/status

Not for discussion
- Cytokines/polymorphisms in pathogenesis
  - Malignancy
  - Autoimmune disease
- Cytokine-directed therapies for nonmalignant conditions
- Complex interactive cytokine networks, innate
- Alternative structures (immunotoxins, immunocytokines)
- Intratumoral delivery
Generic cytokine signaling

Cytokine

Receptor

Cytoplasm

Gene expression

Nucleus
Cytokine receptor families

Receptor for:

- IL-2
- IL-15
- IL-7
- IL-9
- IL-4
- IL-13

- IL6 Rγ
- IL11 Rγ
- LIR Rγ
- GMCSF Rα

- IL-6
- IL-11
- OSM
- LIF
- CNTF
- IL-3
- IL-5
- GMCSF
GM-CSF as immunotherapy

- Cells of origin
  - Th1, Th2
  - Others include epithelial, fibroblast, tumor
- Target cell: immature DC (& myeloid progenitor)
- Biological functions
  - Stimulation of T cell immunity via effect on APC
  - Myeloid cell proliferation, differentiation
- Clinical development
  - Hematopoietic support
  - Not a potent stand-alone cytokine in cancer
  - Adjuvant for melanoma: (-) results+/- peptide vaccine
  - Immunocytokine in prostate cancer DC product
  - Transgenic expression (GVAX) [and other cytokines]
GM-CSF transgenic tumor “GVAX”
Interferons

- **Type I**
  - α: from neutrophils, Mφ
  - β: from fibroblasts, epithelial cells
- **Type II γ-IFN, type 1, from T, NK cells**
- **Immunomodulatory effects**
  - MHC class I/II upregulation
  - Modulation of T/NK cell cytolytic activity
  - Modulation of macrophage/DC function
  - Decreased Treg/increased Th1
Interferons (cont.)

- Direct effects on tumor cells
  - MHC upregulation
  - Antiproliferative/pro-apoptotic effects
- Anti-angiogenic effects
  - IP-10
  - Thrombospondin
IFN-α Signaling
IFN-γ Signaling

- IFN-γ binding to γR1 and γR2
- Activation of Jak1 and Jak2
- Phosphorylation of Stat1α
- Recruitment and dimerization of Stat1α
- Nuclear translocation
IFN autoimmunity/adjuvant benefit in melanoma

IFN in melanoma, other malignancies remains a work in progress

Relapse-free survival

Overall survival

Gogas et al NEJM 2006
Interleukin-2

- Short chain type I cytokine
- Four $\alpha$-helical bundles
- Produced by activated T cells
- TCR/CD3 engagement plus CD28 ligation required
- Main targets are T, NK cells
- Stimulates immune responses and prevents tolerance
- Also downregulates immune response: role in $T_{\text{reg}}$ development/activity
Interleukin-2

“T cell growth factor”
Produced by Th1 cells
Many cell types express IL-2R
- B, NK/NKT, monocytes
- Affinity, functions depend on subunit $\alpha\beta\gamma$ expression

Signaling
- JAK-STAT
- MAPK
- PI3K

Proliferation, cytotoxicity
IL-2 Signaling
**In Vivo Effects of IL-2**

- Induction of multiple cytokines
  - TNF, interferon-gamma, GM-CSF, M-CSF, G-CSF, IL-4, IL-5, IL-6, IL-8, IL-10
- Increase in soluble IL-2r
- Lymphopenia followed by rebound
- Increased NK activity (during rebound)
- Increased CD25, HLA-DR expression on T-cells
- Decreased PBMC proliferative responses
- Tissue infiltration by lymphocytes
- Eosinophilia
- Neutrophil chemotaxis defect
Pioneering NCI studies

**Biology/source**
- T cell growth factor
- Jurkat source
- Recombinant E. coli

**Preclinical models**
- Toxicities from capillary leak
- Toxicities vary by species
- Dose-dependent activity

**Early clinical studies + LAK**
- Supportive role in adoptive cell-Rx strategies

Extramural IL-2 studies

**In solid tumors**
- With LAK cells
- Single agent high/low doses
- With α-IFN
- With other cytokines
- With chemotherapy
- Toxicity modulation
- Biological predictors of benefit

**In heme malignancies**
- Preclinical activity
- Clinical benefits not achieved
Responses in melanoma

- **CR**
- **CR + PR**
- **PR**

<table>
<thead>
<tr>
<th>Months</th>
<th>CR</th>
<th>CR + PR</th>
<th>PR</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>12</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>24</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>48</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>72</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>96</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>12</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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</tbody>
</table>
Responses in renal cancer

## IL-2 Grade 3-4 Toxicity

<table>
<thead>
<tr>
<th></th>
<th>NCI-SB HD IL-2</th>
<th>CWG HD IL-2</th>
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<tbody>
<tr>
<td>Median Doses per Course</td>
<td>12 (28)</td>
<td>68% (19 doses)</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>1%</td>
</tr>
<tr>
<td>Hypotension</td>
<td>36.4%</td>
<td>56.8%</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>4.2%</td>
<td>13.7%</td>
</tr>
<tr>
<td>CNS orientation</td>
<td>10.2%</td>
<td>14.7%</td>
</tr>
<tr>
<td>CNS consciousness</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>2.8%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>13.4%</td>
<td>9.5%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9.2%</td>
<td></td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>3.2%</td>
<td>11.6%</td>
</tr>
<tr>
<td>ALT</td>
<td>3.2%</td>
<td></td>
</tr>
<tr>
<td>Creatinine &gt; 8.0 mg/dL</td>
<td>1.1%</td>
<td>13.7% (gr 3-4)</td>
</tr>
<tr>
<td>Oliguria (&lt; 80 ml/8h)</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Atrial Arrhythmia</td>
<td>4.2%</td>
<td>8.4% (all cardiac)</td>
</tr>
<tr>
<td>Malaise</td>
<td>20.5%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>
## Severe toxicities of high-dose IL-2

<table>
<thead>
<tr>
<th>Grade 3 or 4 Toxicity</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 255 (% of patients)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>74</td>
</tr>
<tr>
<td>Pulmonary (dyspnea)</td>
<td>17</td>
</tr>
<tr>
<td>Renal (creatinine elevation)</td>
<td>14</td>
</tr>
<tr>
<td>Hepatic (hyperbilirubinemia)</td>
<td>21</td>
</tr>
<tr>
<td>CNS</td>
<td>32</td>
</tr>
<tr>
<td>Myocardial injury (ischemia, infarction, myocarditis)</td>
<td>6</td>
</tr>
<tr>
<td>Arrhythmias (all grades)</td>
<td>14</td>
</tr>
<tr>
<td>Infection</td>
<td>6</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>21</td>
</tr>
<tr>
<td>Death</td>
<td>4</td>
</tr>
</tbody>
</table>
How does high-dose IL-2 work?

Multiple hypotheses from animal data, but rigorous human data lacking.

Gene expression profiling on FNA of tumor, serial PBMC in melanoma patients on HD-IL-2

**HD IL-2**

**Tumor-site inflammation**

- **Activation of Monocytes**
- **Chemokine production; Immune cell recruitment**
- **Activation of NK cells**
- **Adaptive immune response**

Panelli MC et al. Genome Biology 2002
Phase III Trial of High-Dose IL-2 ± Peptide Vaccine in Patients With Metastatic Melanoma

Eligibility Criteria

- Pts with stage IV or locally advanced stage III cutaneous melanoma
- HLA-A*0201 positive (N=185)

High-dose IL-2 720K IU/kg/dose IV every 8 hours x 12 Repeat every 3 weeks (N=94)

Vaccine ([gp100:209-217(210M)] subcutaneous d1 q3w) + High-dose IL-2 (as above) (N=91)

- Primary endpoint: RR
- Secondary endpoints: toxicity, PFS, quality of life, and immunologic monitoring
- Central HLA typing, pathology review, and blinded response assessment performed at the National Institutes of Health

Schwartzentruber. ASCO. 2009 (abstr CRA9011);
High-Dose IL-2 ± Peptide Vaccine: Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RR* (%)</th>
<th>PFS (mos)</th>
<th>OS (mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-dose IL-2</td>
<td>9.7</td>
<td>1.6</td>
<td>12.8</td>
</tr>
<tr>
<td>High-dose IL-2 + vaccine</td>
<td>22.1</td>
<td>2.9</td>
<td>17.6</td>
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</tbody>
</table>

*Investigator assessment.

Reproduced with permission from Schwartzentruber. ASCO. 2009 (abstr CRA9011).
Adoptive T-Cell Therapy and Intensive Myeloablative Chemoradiation: Results

<table>
<thead>
<tr>
<th>Regimen</th>
<th>RR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBI 12 Gy</td>
<td>72</td>
</tr>
<tr>
<td>TBI 2 Gy</td>
<td>52</td>
</tr>
<tr>
<td>NMA</td>
<td>48.8</td>
</tr>
</tbody>
</table>
IL-2: Current and future

• Structural alterations
• Toxicity modulation without loss of activity
• Combinations
  – Anti-angio/cytotoxics/STIs/other cytokines
  – Vaccines (melanoma peptides enhanced IL-2)
• Greater insight into mechanisms
  – Renal “Select” trial to validate prior observations re: histology, hypoxia genes
  – Melanoma “Selection” trial to study immune polymorphisms, tumor gene expression
  – Autophagy? (Lotze)
Interleukin-4

- Pleomorphic Th2 cytokine
- Net effects depend on milieu
  - Mainly a B cell-stimulator
  - Inhibits non-specific NK activity
  - Enhances other adaptive immune functions
    - Growth factor for Th2
    - Promotes proliferation, cytotoxicity of CTL
    - Stimulates MHC class II expression
    - Contributes to DC maturation
    - Enhances mΦ tumoricidal activity
IL-4 Signalling
Interleukin-4

- Promising preclinical data, especially transgenic secretion by tumor
- Clinical experience limited
  - Studied like IL-2 at MTD
  - Unfavorable therapeutic index
- Used routinely to elicit i-moDC from PBMC
  - Used *ex vivo* w/GM-CSF
  - Shares some structure, function with IL-13
IL-4 and IL-13

**Similarities**
- Predominantly anti-inflammatory effects
- Favor Th$_2$ responses
- Partially common receptor
- Promotes Ig class switch
- Used w/ GM-CSF → moDCs

**Differences**
- IL-13 activity on monocyte/mΦ cells
- IL-13 lacks B, T cell effects

**IL-13 receptors on tumor cells, especially glioma**
- Immunotoxins
- Chimeric T cell Ag receptor

Assortment of receptor subunits depend on cell type
IL-13 and IL-4 receptor combinations and binding

- IL-4
- IL-13

- \( \gamma_c \)
- IL-4R\( \alpha \)
- IL-4R\( \alpha \)
- IL-4R\( \alpha \)
- IL-4R\( \alpha \)
- IL-13R\( \alpha 1 \)
- IL-13R\( \alpha 2 \)
- IL-13R\( \alpha 1 \)
- IL-13R\( \alpha 2 \)
IL-6 is pleiotropic

IL-6

- **Tumor source**
  - Unfavorable prognostic for renal cancer
  - Important growth factor for myeloma
  - Mediates paraneoplastic thrombocytosis

- **Adaptive system**
  - B cell growth/differentiation
  - CTL differentiation, Type 2 responses

- **Preclinical data suggested antitumor activity**

- **Clinical data**
  - Too toxic
  - ?tumor promotion → Blockade may be therapeutic
IL-6 signaling
Examples of cytokine-mediated counterregulation
\( \gamma_c \) cytokines
IL-7

Signaling/gene expression
JAK 1,3 → STAT 5
PI3K → mTOR activation

Regulation contrasts with IL-2, IL-15
Unique to IL-7 is receptor downregulation
IL-7 accumulates during lymphopenia due to ↓ utilization

Mediates homeostatic expansion of naïve cells during lymphopenia (greatest clinical potential, possibly with IL-15, IL-21)

Kaech, Nat Imm 2003
IL-15

- Unique $\gamma_c$ cytokine—complexes with receptor from cell of origin, then signals target cell

- With IL-2 and IL-7 in $\gamma_c$ cytokine family promoting T cell growth, differentiation
IL-2 and IL-15 compared

**IL-2**
- Activated T, B express high-affinity $\alpha\beta\gamma$ receptor
- Prolif/differentiation NK, T, B
- Promotes activation-induced cell death
- Maintenance of Treg
- +/- KO develops autoimmunity

**IL-15**
- Produced by DC, monos
- Surface-bound on DC/mono ↔ receptors on NK, CD8a1 cells
- Promotes proliferation NK, T, B, memory CD8
- Inhibits AICD
- Does not support Treg
- +/- KO is lymphopenic
Activated monocyte or dendritic cell

Endocytic vesicle

IL-15–IL-15Rα recycling

IL-2/15Rβ

Trans-presentation of IL-15

CD8+ T cell or NK cell
**IL-12**

- Link between innate, adaptive immune response
  - Receptors on variety of immune cells
  - Induces IFN-\(\gamma\), a prototypical type I cytokine

- Potent inducer of counterregulatory type 2 cytokines
  - Emerged in clinical trials for advanced malignancy
  - Schedules and doses may be manipulated

- Clinical potential
  - Vaccine adjuvant
  - Induction of anti-angiogenesis
  - In combinations e.g. w/\(\alpha\)-IFN, IL-2?
IL-12 and innate/adaptive immune system

IL-12 family
Jak/Stat Signaling: IL-12 versus IL-2

IL-12

- \(\beta_1\)
- \(\beta_2\)
- Tyk2
- Jak2
- Stat4
- Stat3
- Stat1
- Stat5

IL-2

- \(\alpha\)
- \(\beta\)
- \(\gamma_c\)
- Jak1
- Jak3
- Stat5
- Stat1
- Stat3

\(p\) and \(s\) indicate phosphorylation and sumoylation states, respectively.
IL-21 is a pleiotropic cytokine
Interleukin-21 and its receptor
Clinical activity of IL-21

1 CR, 11 SD among 24 melanoma patients

4 PR, 13 SD among 19 renal cancer patients

Other Clinical Trials of IL-21

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Trial Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>¹Phase I: Alternative schedules 5+9 schedule x 3 and 3x/week</td>
</tr>
<tr>
<td></td>
<td>²Phase IIa: 5+9 schedule x 3</td>
</tr>
<tr>
<td></td>
<td>³NCIC PHASE II: 5+9 schedule x 3, q 8 weeks  Treatment-naive; bulky disease excluded</td>
</tr>
<tr>
<td>Renal cell Cancer</td>
<td>IL-21 plus Sunitinib</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>⁴IL-21 and Rituximab in Relapsed/Refractory Indolent Lymphoma</td>
</tr>
</tbody>
</table>

IL-10: a Th2 counter-regulatory cytokine
Thank you

Any questions?