Immune Potentiating Cytokines

Howard L. Kaufman, MD
An Introduction to Cytokines

• General mechanisms of action
• FDA-Approved Cytokines for Cancer Immunotherapy
  – Interferon-α2b (Intron-A)
  – Pegylated interferon-α2b (Sylatron)
  – Interleukin-2 (Proleukin)
• Other Cytokines in Development
General mechanism of cytokine signaling
Interferons

- Type I
  - $\alpha$: from neutrophils, m
  - $\beta$: from fibroblasts, epithelial cells

- Type II
  - $\gamma$: 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
  - Inhibition of angiogenesis
Interferon Signaling
Interferon Administration

• Induction Phase
  – 20 Million Units/m² IV Monday through Friday for 4 weeks

• Maintenance Phase
  – 10 Million Units/m² SQ M-W-F for 11 months

• Dose reductions or discontinuation for toxicity
E1684: Estimated Relapse-Free Survival

Probability of relapse-free survival

Arm | Median RFS
---|---
IFN-2b (n=143) | 1.72 yr
Observation (n=137) | 0.98 yr


P=0.0023
E1684: Estimated Overall Survival

<table>
<thead>
<tr>
<th>Arm</th>
<th>Median OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN 2b</td>
<td>3.82 yr</td>
</tr>
<tr>
<td>Obs</td>
<td>2.78 yr</td>
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Probability of survival

Arm IFN 2b (n=143)
Arm Observation (n=137)

P=0.0237

Meta-analysis of IFN effect on DFS

<table>
<thead>
<tr>
<th>Study</th>
<th>HR</th>
<th>LL</th>
<th>UL</th>
<th>SE</th>
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<td>NCCTG (Creagan, 1995)</td>
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Morcellin et al. JNCI 2010
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Morcellin et al. JNCI 2010
Common Interferon Toxicity

- Flu-like syndrome: Acetaminophen/benadryl
- Fatigue: Dose reduction, if severe
- Cytopenias: Dose reduction, if severe
- Increased LFTs: Dose reduction, if severe
- Weight loss: Dose reduction, if severe
- Alopecia
Less Common Interferon Toxicity

- Nausea/vomiting: Anti-emetics
- Diarrhea: Anti-diarrheals
- Hypotension: Fluids, dose reduce
- Depression: Anti-depressants
- Cough: Symptomatic
- Dry mouth: Fluids
- Skin rash: Moisturizers
- Irritability: Dose reduce
Pegylated Interferon

Drug with Low Solubility
Short Biological Half Life
Unstable in Biological Fluid

+ PEG
(Polyethylene glycol)

Solubility Enhancement
Prolonged Biological Half Life
Increased Stability
PEG-Interferon Administration

• Induction phase
  – 6 mcg/kg SQ weekly for 8 weeks
• Maintenance Phase
  – 3 mcg/kg SQ weekly for up to 5 years
• Dose reduction or discontinuation for toxicity
Pegylated IFN vs. Observation in Resected Stage III Melanoma

Recurrence-free Survival

Overall Survival

Eggermont et al. Lancet 2008
PEGylated IFN in N1a Disease

Recurrence-free Survival

Overall Survival

Eggermont et al. Lancet 2008
PEGylated IFN in N1b Disease

Recurrence-free Survival

Overall Survival

Eggermont et al. Lancet 2008
PEGylated IFN in patients with ulcerated primary melanomas

Eggermont et al. JCO 2012
Induction of autoimmunity correlates with survival in IFN-α treated patients

Relapse-free Survival

Overall Survival

Gogas et al. NEJM 2006
Interleukin-2 (IL-2)

- Natural biologic immunomodulatory agent
- T cell growth factor
- Proliferation of T cells and NK cells
- Promotes the killing activity of these cells
- Powerful anti-tumor effects in animal studies
- Extensively evaluated in patients with cancer
- FDA approved for metastatic renal cell in 1992
- FDA approved for metastatic melanoma in 1998
Interleukins and Their Receptors

Cytokine produced by:
- IL-2: T cells and DCs
- IL-4: T cells, NKT cells, eosinophils and mast cells
- IL-7: stromal cells, epithelial cells and fibroblasts
- IL-9: T cells
- IL-15: monocytes, DCs and epithelial cells
- IL-21: CD4+ T cells and NK T cells
- TSLP: stromal cells, epithelial cells, fibroblasts, mast cells and basophils

Receptor expressed by:
- IL-2Rα
- IL-1Rα
- IL-4Rα
- IL-7Rα
- IL-9Rα
- IL-15Rα

Margolin, Lazarus and Kaufman 2013
IL-2 Receptor

- Binds chain
- Forms heterotrimeric complex
- Signals through and c chains
- Induces T cell growth and promotes survival

Malek and Bayer, Nature Immunol Rev, 2004
Mechanism(s) of IL-2 anti-tumor activity

Malek and Bayer, Nature Rev Immunol 2004
Tregs exhibit a paradoxical response to IL-2 treatment

Cesana et al. JCO 2006
High-dose Bolus rIL-2 Regimen

- IL-2 600,000 IU/kg every 8 hours by 15-minute IV infusion for a maximum of 14 doses
- 9-16 day rest period
- Repeat schedule for another 14 doses
- Maximum 28 doses per course of therapy
- No dose reductions are performed during high-dose IL-2 therapy
- Excessive toxicity treated by withholding dose or discontinuing treatment for that cycle
Patient Selection for IL-2 Treatment

- ECOG performance status 0 or 1
- Adequate pulmonary function
  - FEV1 and FVC $\geq 75\%$ of predicted
  - No evidence of symptomatic pulmonary disease
- Normal cardiac function
  - For patients $>50$ years of age or with ischemic symptoms, consider stress thallium or other stress tests
- Adequate renal function
  - Creatinine levels should be $\leq 1.6$ mg/dL

Patient Selection (cont)

- Adequate hepatic function
  - Bilirubin $\leq$ 2.0 g/dL
  - SGOT $< 3 \cdot$ ULN, unless due to liver metastases
- Adequate hematologic function
  - ANC $\geq$ 1500/mm$^3$
  - Platelets $> 100,000$
  - Hemoglobin $\geq$ 9.0 gm/100 mL
- No CNS metastases (unless adequately treated)
  - MRI brain within one month
- No corticosteroids
IL-2 Toxicity

- Most side effects are preventable
- Nearly all side effects are reversible
- All side effects can be managed by qualified physicians and nurses

- Management begins with pre-treatment screening
Vascular Leak Syndrome

- Increased capillary permeability
  - Decreased vascular resistance
    - Breakdown of blood-brain barrier
    - Neuropsychiatric toxicity
    - Hypovolemia
      - Hypotension
      - Fluid retention/weight gain
        - Rales/SOB
        - Diarrhea
        - Edema/ascites
        - Pleural effusion
    - Hypoperfusion
      - Sinus tachycardia
      - Myocardial ischemia
      - Decreased renal perfusion
        - Prerenal azotemia
        - Oliguria and anuria

Used with permission from Lori Stover, RN.
# Pivotal High Dose IL-2 Trials: The NCI Experience

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>No. of Patients (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CR</td>
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<tr>
<td>Melanoma</td>
<td>182</td>
<td>12(6.6)</td>
</tr>
<tr>
<td>Renal Cell</td>
<td>227</td>
<td>21(9.3)</td>
</tr>
<tr>
<td>Total</td>
<td>409</td>
<td>33(8.1)</td>
</tr>
</tbody>
</table>

High-dose IL-2 for Renal Cell Carcinoma

- 115 patients
- Renal cell carcinoma
- 720,000 IU/kg (147)
- Median follow-up 52 months
- No mortality

Yang, Cancer J, 1997
High-dose IL-2 for Melanoma

- 270 patients, 1985-93
- Melanoma
- 600,000 IU/kg (118)
- 720,000 IU/kg (147)
- 6 (2.2%) mortality
  - 5/6 had ECOG PS 1
  - Bacterial sepsis in all 6
  - No prophylactic antibiotics
  - No deaths after 1990

Atkins et al, J Clin Oncol, 1999
High-dose IL-2 induces durable objective clinical responses in 15-20%
Case Example of Melanoma Patient Treated With High-Dose IL-2

Provided by D Schwartzentruber MD.
Case Study: 8 year old girl with melanoma spread to the liver

Before Treatment

After Treatment

Soni et al. J Pediatr Hematol Oncol 2002
High-dose IL-2 after No Response on Biochemotherapy
High-dose IL-2 promotes durable disease free survival in responders

Klapper et al. Cancer 2008
IL-2 and radiation therapy: Abscopal effect?

66% objective response rate

Seung et al. Sci Transl Med 2012
What correlates with response to IL-2 treatment?

- Performance status
- Development of autoimmunity
  - Autoimmune thyroiditis
  - Vitiligo
- Amount of IL-2 given during first course
- Height of the rebound lymhocytosis
- CA IX (in renal cell carcinoma)?
- Pre-treatment VEGF/fibronectin levels?

Biomarkers of IL-2 response: Proteomic analysis

A Validation set
B VEGF and Fibronectin map
C Training and Validation set

Sabatino et al. JCO 2009
VEGF predicts survival following IL-2 treatment

Survival, by VEGF group

Survival, by VEGF group

\[ P = 0.0031 \]

Sabatino et al. JCO 2009
IL-15 Signaling

- Unique cytokine that complexes with receptor from cell of origin, then signals target cell

- With IL-2 and IL-7 in cytokine family promoting T cell growth and differentiation but may not expand Tregs

- Clinical trials starting

Margolin, Lazarus and Kaufman 2013
Comparing IL-2 and IL-15

**IL-2**
- Activated T, B express high-affinity receptor
- Prolif/differentiation of NK, T, and B cells
- 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 T cells
- Promotes proliferation NK, T, B, and memory CD8 T cells
- Inhibits AICD
- Does not support Treg
- +/- KO is lymphopenic
Interleukin-21
IL-21

- Dendritic cells
  - Antigen uptake
  - Antigen presentation
  - Maturation

- Activated CD4+ T cells
  - Proliferation
  - Tc1/Tc7 differentiation
  - Resistance to T_{reg} inhibition

- NKT cells
  - Survival
  - Proliferation
  - Granular morphology
  - Cytokine production

- B cells
  - Isotype switch
  - Immunoglobulin production
  - Plasma cell differentiation
  - Anti-CD40 mAb-induced proliferation
  - TLR-induced proliferation
  - Apoptosis

- Macrophages
  - TCXCL8/IL8 production
  - Activation

- T_{reg} cells
  - No proliferative effect.

- Cytotoxic CD8+ T cells
  - Proliferation
  - Perforin, INFγ, granzymes
  - Cytotoxicity

Nature Reviews | Drug Discovery
Phase I IL-21 clinical trial

Melanoma

Renal cell carcinoma

Thompson et al. JCO 2008
IL-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 tumorcidal activity
IL-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 \textit{ex vivo} w/GM-CSF
  - Shares some structure, function with IL-13
IL-4 and IL-13

- **Similarities**
  - Predominantly anti-inflammatory effects
  - Favor Th₂ 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
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
May have clinical potential, possibly with IL-15, IL-21)
IL-12 Cytokine Family

Interleukin-12
- p35
- p40
- β2
- β1
- Production of T-helper 1 cells
- Interferon-γ production
- Adaptive immunity

Interleukin-23
- p19
- p40
- β1
- Induction of interferon-γ and other cytokines by memory T cells, macrophages and dendritic cells

Interleukin-27
- p28
- EBI3
- WSX-1/CCR9
- Production of T-helper 1 cells
- Interferon-γ production by naive T cells in synergy with interleukin-12
- Differentiation and proliferation of naive T cells

p35-EBI3
- ?
- Inside cell
- Unknown
IL-2 vs. IL-12 Signaling
IL-12 links innate and adaptive immune responses
IL-12

• Link between innate, adaptive immune response
  – Receptors on variety of immune cells
  – Induces IFN-γ, 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/ -IFN, IL-2?
GM-CSF

- 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]
Mechanism of GM-CSF anti-tumor activity?
Non-immune potentiating cytokines

- IL-6
- IL-10
- IL-17??
- TGF-β
- VEGF
Conclusions

• Immune-potentiating cytokines have shown clinical benefit in patients with cancer
  • IL-2
  • Interferon-α
• The mechanism of cytokine-mediated tumor regression is unclear
• Several cytokines are in clinical development
• Combination studies are in progress
  • Cytokines and immunotherapy (e.g. anti-CTLA-4, PD-1)
  • Cytokines and targeted therapy (e.g. BRAF inhibitors)
  • Cytokines and radiation (e.g. absocopal effect)
• Predictive biomarkers are in development
  • Autoimmunity
  • VEGF