Cytokines in the (Immuno)Therapy of Malignancy

Kim Margolin, M.D.
City of Hope Comprehensive Cancer Center
Cytokines—according to Google
Biology of the cytokines

- Details to be discussed for IL-2>>other cytokines
  - Cytokine structure
  - Stimuli leading to induction of cytokine synthesis
  - Cell(s) responsible for cytokine production
  - Cytokine-responsive cell(s)/receptor structure
  - Signaling induced by cytokine binding
  - Overall result of cytokine function

- NOT for detailed discussion
  - Transgenic cytokine expression
  - Cytokines in adoptive cell therapies
  - Cytokines in tumor vaccine investigation
Clinical development

- **Early trials**
  - Dose-seeking, proof of principle
  - Toxicity, schedule considerations

- **Disease-directed studies**
  - Pilots
  - Phase II
  - Phase III

- **Modulation of activity, toxicity**

- **Current status and future possibilities**
  - Combination cytokines
  - With other modalities and classes of Rx, esp. “targeted”
  - Fusion molecules for focussed therapies
The players

For discussion
- GM-CSF
- Interleukin-2
- Interleukin-4/13
- Interleukin-6
- Interleukin-7/15
- Interleukin-12
- Interleukin-18
- Interleukin-21

Not for detailed discussion
- Interferons
- Hematopoietic: 3, 11, flt-3L
- Complex innate: TNF, IL-1
- “Suppressive”: IL-10, TGFβ
- Miscellaneous: TRAIL etc.
T cells are mobilized
when they encounter a cell such as a macrophage or a B cell that has digested an antigen
and is displaying antigen fragments bound to its marker molecules

Lymphokines help the T cell to mature.

The T cell, alerted and activated, secretes lymphokines

Some lymphokines spur the growth of more T cells.

Some lymphokines attract immune cells — fresh macrophages, granulocytes, and other lymphocytes — to the site of infection. Yet other lymphokines direct the recruits once they arrive on the scene.

Some T cells become killer cells and track down body cells infected by viruses.
Cytokine and Cell Interactions

GM-CSF → thymocytes

IL-2

IL-4 → Immature APC

GM-CSF

IL-10

IL-7 → thymocytes

IL-12(+) → NK

IL-15 → NK

IL-18

IL-21

IL-6

APCs (including macrophages and dendritic cells) are recruited into the tumour site by MIP-1α.
Cells of origin for some therapeutic cytokines: Type I and Type II

Antigen

Ag-MHCII

TCR

IL-1

IL-12

THO

TH1

TH2

IL-10

IFN-γ

IL-2

IFN-γ

IL-12

IL-4

IL-5

IL-10

IL-13

GM-CSF
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
  - **Transgenic expression (GVAX)**
  - **Adjunct to immunotherapy**
GM-CSF function in immunity

Th1

GM-CSF
IL-4

Phagocytosis

CD154 (CD40L)
or LPS

MonopDC1

iDC1

Adaptive immunity

DC1

Th1

Cell-mediated immunity

Bendzen 1999
Interleukin-2

- The mother of all therapeutic cytokines
- Produced by Th1 cells for T cells but...
- Many other cells express IL-2R
  - B, NK/NKT, monocytes
  - Variable affinity depending on subunit expression
  - Response to IL-2 depends on cell type, receptor, milieu

- Signaling
  - JAK-STAT
  - MAPK
  - PI3K

- Proliferation, cytotoxicity
IL-2 Signalling
Induction of an immune response

Recognition  Processing  Presentation  Activation

Erkennung  Prozessierung  Präsentation  Aktivierung

Antigen  APZ  B7-1 (CD80)  CD28  CD4  TCR  MHC II  Peptid  T_H

IL-2R  IL-2

Recognition  Processing  Presentation  Activation
Where do cytokines come from?
Will cancer come to an end?
Which came first, the T cell or NK cell?

A BRIEF HISTORY OF IL-2

High Dose Interleukin-2
Kidney Cancer

Partial Response (n=26)
Complete Response (n=17)
Overall Response (n=43)

Probability of Continuing Response

Duration (months)

RCC

0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00
0 12 24 36 48 60 72 84 96 108 120 132

(10 Years)
### Pioneering NCI studies

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

- **Preclinical models**
  - DLTs due to CLS
  - Toxicities vary by species
  - Dose-dependent activity

- **Early clinical studies + LAK**

- **Role of IL-2 in adoptive cell-Rx strategies**

### Extramural IL-2 studies

- **In solid tumors**
  - With LAK cells
  - Single agent
  - With α-IFN
  - With other cytokines
  - With chemotherapy
  - Toxicity modulation

- **In heme malignancies**
  - Trial methodology challenging
  - Phase II data promising
  - Phase III data disappointing
IL-2 in hematologic malignancy

- **Preclinical:** IL-2 exposure of BM/PB induced cytotoxic lymphocytes vs. chemo-S/R leukemia, NHL, cancer

- **Early clinical**
  - Ex vivo Rx of HCT product w/IL-2 feasible, may promote h’poiesis
  - IL-2/LAK cell Rx had slight activity in HD, NHL
  - Autologous GvHD endpoint promising

- **Post-transplant IL-2 had dose-dependent toxicities**
  - Less technically demanding than treating cells w/IL-2
  - Encouraging pilot data from Seattle, other centers
  - Auto-HCT feasible, allo-HCT too complex

- **Phase III designs included HCT and non-HCT regimens**
  - Acute leukemia: feasibility problems, better alternatives
  - NHL: Negative result of post-aHCT IL-2 (J Thompson/SWOGI)
Overall conclusions: Clinical IL-2 studies ~1985-2000

- 15-20% pts w/RCC, Mel benefit
- Rx ratio not improved by
  - IL-1 receptor agonist (decoy)
  - TNF blockade (Ab or decoy)
  - Lysophylline (lipid mediators)
  - Histamine (inhibit mϕ ROS)
  - iNOS blockade (inhibit CLS)
- Dose-response inconclusive
- Not effective in biochemo
  - RCC w/pyrimidines, vincas
  - Melanoma w/DTIC, CDDP

- Novel strategies did not improve therapeutic index
  - With IFN α or γ
  - With tumor-directed Ab
  - With agonistic OKT3 Ab
  - Structure-function alterations
    - PEG-IL-2
    - Liposomal IL-2
    - IL-2 “specific agonist”
    - Albuleukin

Worth pursuing in RCC, melanoma, ?heme
IL-2 2001-2006: General considerations

Investigations continuing on structural alterations to reduce capillary leak

Toxicity modulation approaches have been overtaken by investigation of mechanisms and patient selection factors: different paths for different diseases

Rational combinations hold promise for improving therapeutic ratio
RCC: Ag-specific strategies elusive

Defining correlates of benefit

- Target organs
  - Lymphocytes, ?other cells
  - Blood count changes
  - Cytokines
  - Autoimmune events
- Prognostic
  - Sites of mets
  - Pace of mets
  - Nephrectomy state
- Predictive
  - Histology
  - Hypoxia-related, other cell pathways
New directions for IL-2-based Rx in RCC

- “Select” trial to validate predictors, correlates
  - CA-IX
  - Histology
    - Favorable: 😊 clear, alveolar, <50% granular,
    - Unfavorable: 😞 papillary, >50% granular
  - New exploratory endpoints
    - VHL gene mutations
    - Other pathways: glut-1, PTEN/AKT, CXCR4
    - Immuno”suppressive” factors-preRx analyses
      - CD11b immature myeloid cells
      - arginase, ξ-chain function

- Rational new combos
  - With targeted agents
  - With angiogenesis inhibitors

} ongoing
Melanoma: Ag-specific strategies remain at forefront

- **Multicomponent Rx**
  - Lymphodepletion/reconstitution
  - Vaccine (many options)
  - Regulatory blockade (IL-2 effects on Treg need further elucidation)
    - CTLA-4Ab
    - Ontak
    - Other resistance modulators
  - Passive/active
    - Cloned/expanded Ag-specific effector lymphocytes
    - Chimeric receptor-expressing T cells
    - Immunocytokine to redirect effector cells
  - Methylation inhibitors to ↑ expression of immune response genes

- **Immunological insights plentiful, but useful predictors remain under investigation**
Interleukin-4

- Pleomorphic cytokine signals through STAT 6
- Th$_2$ cytokine mediates T-B, other interactions
- Net effects depend on cytokine and cell milieu
  - Mainly a B cell-stimulatory cytokine
  - Inhibits non-specific NK activity but
  - Enhances other adaptive immune functions
    - Growth factor for Th2
    - Promotes proliferation and cytotoxicity of CTL
    - Stimulates MHC class II expression
    - Contributes to DC maturation
    - Enhances mΦ tumorcidal activity
IL-4 Signalling
Interleukin-4

- Promising original data
  - One of the first transgenically expressed cytokines
  - Tumor-associated immune infiltrate was prototype
- Clinical experience limited
  - Studied like IL-2
  - Minimal activity, much toxicity (mucocutaneous, cardiac)
- Most promise as Rx to “elicit” moDC from PBMC
  - Phase I was directed at in vivo DC expansion (Gitlitz JIT ’03)
  - How does this compare w/flt-3L?
  - IL-13 may be superior in vitro to IL-4
  - What is the current status of in vivo DC work?
IL-4 and GM-CSF in DC

Th1 → GM-CSF

IL-4

Phagocytosis

GM-CSF

Mon pDC1

iDC1

CD154 (CD40L) or LPS

Adaptive immunity

DC1 → Th1

Cell-mediated immunity

Bendtzen 1999
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 to elicit moDCs

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

**Most important: IL-13 receptors on tumor cells, especially glioma**
- Immunotoxins under evaluation
- Chimeric T cell Ag receptor in clinical trials

Shared receptor subunits depend on cell type
IL4R and IL13R subunit interactions
Sinks, suppressors and antigen presenters: how lympho-depletion enhances T cell-mediated tumor immunoRx

IL-6: A very pleiotropic cytokine

- Induction of T-Cell Differentiation
  - T Cell
  - Cytotoxic T Lymphocyte

- Induction of Acute-Phase Reactants
  - Hepatocytes
  - C-Reactive Protein

- Induction of Nerve Cell Differentiation
  - Astrocytes
  - NGF-Like Activity

- Induction of B-Cell Differentiation
  - B Cell
  - Plasma Cell
  - Myeloma Cell
  - Malignant Transformation

- Induction of Myeloma-Plasmacytoma Growth
  - Megakaryocyte

- Induction of Leukemic Cell Differentiation
  - M1
  - Macrophages

IL-6

- Tumor source
  - Associated with unfavorable outcome renal CA, melanoma
  - An important growth factor for myeloma
  - Major effector of paraneoplastic thrombocytosis

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

- Preclinical data showed activity in selected tumor models

- Phase I and II clinical data
  - Hematologic (thrombocytosis, anemia), arrhythmias, neurotox
  - Insufficient clinical activity
  - Concern about potential tumor-promoting effects

- Paradox: IL-6 Ab now in trials alone or w/IL-2 based on preclinical, clinical leads
IL-12

- Prototypical type I cytokine, induces IFN-γ
- Link between innate, adaptive immune response
- DC production triggered by variety of stimuli
- Receptors mainly on activated T and NK cells
- Anti-angiogenic activity via IFN induction
- In animals, ↑ antitumor effects in combo w/other type I cytokine (IL-2)
- Probable role in vaccine development, ? tumor vaccine
IL-12 and its receptor

- IL-12 and IL-10 work to inhibit each other.
- IL-12 stimulates Th1 Development.
- INF-γ release stimulates Monocytes leading to further IL-12 release.
- INF-γ stimulates Th1 Cell.

IL-12 and IL-10 act on the immune system, with IL-12 promoting Th1 cell development and INF-γ release, while IL-10 inhibits this process.

Diagram illustrates the complex interactions involving IL-12, IL-10, Th1 cells, and INF-γ, showing how these cytokines and cells interact within the immune system.
IL-12 Clinical trial experience

- Subcutaneous dosing in initial trials
  - Lymphopenia, hepatoxicity dose-limiting
  - Fever, headache, fatigue, nausea common
  - Attenuation of toxicities with re-exposure

- i.v. dosing featured test dose
  - Markedly reduced toxicity at similar doses
  - Type II cytokines increased, especially IL-10
  - Type I cytokines decreased, especially IFN-γ

- Phase II i.v. IL-12 without test dose had excess toxicity

- b.i.w. schedule for i.v. IL-12
  - Less attenuation of IFN-γ
  - Clinical activity associated with maintenance of IFN-γ induction

- Combinations w/IFN-α
  - Feasible
  - Therapeutic ratio not favorable
IL-18

- Member of IL-1 family of cytokines
- Activates NK cells and induces type I cytokines
- Promotes Th1 and memory CD8 T cells
- Upregulates FasL on effector lymphocytes
- Cytotoxicity MOA “complementary” to that of IL-12
  - Not IFN-γ-dependent
  - 18 uses Fas/FasL while 12 uses perforin/granzyme

- Antitumor activity in animals
  - Alone
  - W/IL-2, IL-12

- Phase I DLTs
  - Leukopenia
  - Fever/chills
  - Hepatotoxicity

Phase II in melanoma ongoing [caveat: like IL-6, may be GF for selected CAs]
IL-21: another pleiotropic cytokine

Durable anti-tumour activity
- Clonal expansion/proliferation
- Increased effector function
  - cytotoxicity
- cytokine production (IFN-γ, TNF-α, IL-10)
  - increased granzyme A/B, perforin
  - Inhibition of IL-15 induced proliferation

Humoral immunity
- B cell proliferation (CD40)
  - Increased IgG production
  - Decreased IgE production (IL-4)
  - B cell growth inhibition (IgM)

Anti-tumour activity
- Increased IFN-γ, IL-10, TNF-α production
- Increased granzyme A/B, perforin
- Increased cytotoxicity
  - Decreased growth
Phase I i.v. outpatient IL-21

J. Thompson et al, ASCO 2006

IL-21 Treatment Schedule
(outpatient administration of two 5-day cycles)

- Tolerable outpatient regimen identified
- Multiple dosing cycles feasible
- IL-21 pharmacodynamic activity
  - Direct effect on lymphocyte count
  - Increase in sCD25
- Four responses observed at different dose levels
  - One patient with Complete Response
  - Three patients with Partial Response

Phase II studies planned
RCC w/TKI (Phase I/II); Melanoma as SA
The Corrections: Some of the Lessons Learned

Biological insights → potential new targets

- Activation-induced death of effector T, NK cells
- Intrinsic, acquired tumor resistance mechanisms
- Counterregulatory cytokines, other substances in tumor-effector cell milieu
- CD4+CD25+FoxP3+ regulatory T cells
- Enhanced understanding of effector cell gene expression, polymorphisms
Other strategies based on cytokine structure and biology

- **Immunocytokines**: Ab chain fused to cytokine
- **Immunotoxins**
  - Localized toxin delivery similar to Ab-based RxS
  - Cytokine-receptor targeting e.g. Ontak (IL-2-diptheria)
- **Novel T cell-receptor engineering**
  - Xgenic TCR→IL-13 recognizing IL13R on glioma
  - Immunofusions: Ag-specific TCR fused to cytokine
**Immunocytokine structure**

*well, sort of…*

![Diagram of immunocytokine structure]

**Immunofusion TCR-IL-2**

**Single chain TCR (264scTCR)**

![Diagram of single chain TCR (264scTCR)]
If only it were so easy...
Cytokine Therapy report card

- Points taken off for
  - Empiric approach
  - “Me-too” research and development designs

- Extra credit given for
  - Incorporation of cytokine biology into novel structure, strategies
  - Recent exploration of mechanisms of resistance, predictive factors and selection strategies

Overall grade B+
Thank you

Any questions?