Cytokines: Lessons From Double Digit Cytokines
IL-10, IL12, IL-15, IL18 and Counting [IL-35]

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## Cancer Vaccines 1990-2000

<table>
<thead>
<tr>
<th>Study</th>
<th># Patients</th>
<th>Objective Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muc1+ BCG</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>Mel Peptide+Adj</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Mel Peptide+IL-12</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Mel Peptide+iDC</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>ANK+IL-2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>TIL/IL-2/IL-4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>IL-12 protein</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>IL-4 Gene Rx</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>IL-12 Gene Rx</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>259</strong></td>
<td><strong>13 [5%] 1 Long Term Survivor</strong></td>
</tr>
</tbody>
</table>
Cytokines: Lessons From Double Digit Cytokines

IL-10, IL12, IL-15, IL18 and Counting [IL-35]

• During the early days of biologic therapy, novel purified recombinant cytokines were seen as suitable agents for exploration in clinical trials –”BRMs”.

• Their major perceived role was to promote inflammation and to expand hematopoietic cells more generally and more specifically to enhance tumor mediated killing by NK cells and T-cells; similar to what we expected from ChemoRx and RØTx

• Many of these earnest early attempts at cytokine or cytokine gene therapies were based on an erroneous sense that there was defective recognition of tumors by immune effectors

• Most adult tumors arise in the setting of chronic inflammation, have an established T-cell response and simple cytokine therapies will unlikely be effective for most patients with cancer.
Cytokines: Lessons From Double Digit Cytokines

IL-10, IL12, IL-15, IL18 and Counting [IL-35]

• Key Strategic Decisions Almost Always Made Based on Incomplete Information; Mouse Models Biased and Can be Misleading

• Impact of Regulatory Interactions – Fear of Adverse Outcomes and Decreased Tolerance of Risk for Patients with Limited Longevity and Options

• Financial Considerations: Projected Costs vs. Reality; Cost of Goods; Competition in the Marketplace; Corporate History

• Lessons Learned Not Used in Future Trials
Melanoma Patient Response Before and After High Dose IL-2
Vitiligo in Patients Receiving IL-2
A Crack in the Fabric of the Universe - IL-2

• Roche after substantial investment bowed to Cetus PEG-IL2
• Only one approved therapy for melanoma in Aug 1990, DTIC
• Cetus Application 8/90 - Sentiment that spontaneous regressions in the disease more common than currently believed
• ‘Toxicities’ including death had been realized by this time
• Risk/benefit ratio not thought to be suitable
• Safety pattern comparable to antibiotics was thought to be critical
• 100’s of patients Rx with well tolerated regimen; 2% death
• ODAC did not recommend for approval

• TNF Gene Therapy approved by the RAC for clinical testing with no data in murine models
Other IL-2 Family [IL-9, IL-15, IL-21]

• **Interleukin 9.** Originally described as a TH2 cytokine, it has not been given to patients. Its inhibition is suggested by intriguing studies in allogeneic skin transplants in which it appears to be critical for maintenance of Tregs through a mast cell dependent process; Ab MedImmune.

• **Interleukin 15.** IL-15 has yet to enter clinical trials; Shares β and γ chains of IL-2; increases T-cells specific for tumor without impacting on Tregs and to be required for NK expansion; might be useful in the treatment of patients with human T cell lymphotropic virus I-associated myelopathy/tropical spastic paraparesis, rheumatoid arthritis, multiple sclerosis or refractory celiac disease, **mucosal protection.**

• **Interleukin 21.** Shares a common γ chain receptor with other members of the extended IL-2 family; has recently entered clinical trials in patients with renal cell carcinoma and melanoma and is associated with cytokine like effects. Anecdotal responses have been observed in patients with these diseases.
ROLE OF FDA IN NEW DRUG DEVELOPMENT

PRE-ClinICAL RESEARCH

Synthesis & Purification

IL-19, 22-35

Animal Testing

Short-Term

Long-Term

Avg 18 Months

IND

DISCOVERY/SCREENING

CLINICAL STUDIES

Phase 1

Phase 11

IL-12

IL-12

IL-18

IL-4

IL-7

IL-10

IL-1

IL-21

NDA REVIEW

Accelerated Approval

Parallel Track

Avg 5 Years

NDA

POST-MARKETING

IL-2

Adverse Reaction Surveillance Product Defect Reporting

Phase IV

Surveys Sampling Testing Post-Approval Inspections

Avg 24 Months

APPROVAL

Sponsor/FDA Meeting Encouraged

Avg 18 Months

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Interleukin 10 [IL-10, -19, -20, -22,-24]

• The IL10 family members are closely related to the interferons. Promotes NK and T cell cytotoxicity; retention
• IL-10 also exerts anti-inflammatory actions by counteracting many biological effects of interferon gamma (IFN-γ)
• IL-10 has never been tested in patients with cancer; has been given to patients with inflammatory bowel disease, with minimal improvement in patients treated
• Treated patients had significant increases in serum neopterin and PHA induced IFN-γ production
• The newer IL-10 family members, IL-19, IL-20, IL-22, and IL-24 have yet to be tested in the clinic or to have demonstrable antitumor activity.
**Interleukin 10 [IL-10, -19, -20, -22, -24]**


Virtually every gene therapy with IL-10 profound antitumor activity [vIL-10 promotes]

Being developed in a Small Drug Big Pharma
IL-2+IL-10=Interleukin 12

- First possible utility observed in murine gene therapy models
- Toxicity observed at much lower dose than expected [long half life of IL-12]
- Major collaboration [Genetics Institute and Roche] made impossible by financial considerations
- Corporate histories [Genetics Institute – Erythropoietin; Roche – Interleukin 2] complicated development
- Drugs developed by Oncology teams experienced with small molecule development; inexperienced with biologics
- Findings of early toxicity with two deaths likely unrelated to direct cytokine effects limited development
- Tachyphylaxis suggested alternative drug development strategies but these were not promoted.
Interleukin 12


IL-10
### Interleukin 12

<table>
<thead>
<tr>
<th><strong>aTumors</strong></th>
<th><strong>Route</strong></th>
<th><strong>Pt</strong></th>
<th><strong>O.R</strong></th>
<th><strong>cImmune modulation</strong></th>
<th><strong>cAngiogenesis-related effects</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Different solid tumors*</td>
<td>i.v.</td>
<td>40</td>
<td>5%</td>
<td>-Dose-dependent ↑ sIFN-γ; peak at 24-48 hrs ↓ CD4+/CD8+ and CD16+ cells; nadir at 24 hrs ↑ of NK cell adhesion molecules (CD2, LFA-1).</td>
<td>N.D.</td>
</tr>
<tr>
<td>Melanoma *</td>
<td>s.c.</td>
<td>10</td>
<td>0%</td>
<td>- ↑ sIFN-γ within 24 hrs - ↑ IL-10 during the second cycle;</td>
<td>- ↓ urine bFGF in 2/3 pts with MR</td>
</tr>
<tr>
<td>Renal cell carcinoma *</td>
<td>s.c.</td>
<td>51</td>
<td>2%</td>
<td>- ↑ sIFN-γ with peak level at 24 hrs after the first maintenance dose</td>
<td>N.D.</td>
</tr>
<tr>
<td>CTCL</td>
<td>s.c. or i.t.</td>
<td>10</td>
<td>56%</td>
<td>- ↑ CD8+ and/or TIA-1+ T cells in skin biopsy from regressing lesions.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Melanoma, renal cell carcinoma</td>
<td>i.v.</td>
<td>28</td>
<td>3%</td>
<td>- Induction of IFN-γ, IL-15 and IL-18, maintained in pts. with tumor regression or prolonged disease stabilization</td>
<td>N.D.</td>
</tr>
<tr>
<td>Renal cell carcinoma ♦</td>
<td>s.c.</td>
<td>30</td>
<td>7%</td>
<td>- ↑ sIFN-γ, IL-10 and neopterin, maintained in cycle 2.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Abdominal tumors*</td>
<td>i.p.</td>
<td>29</td>
<td>7%</td>
<td>- ↑ peritoneal CD3+ and ↓ CD14+ cells</td>
<td>- ↓ bFGF and VEGF in tumor;</td>
</tr>
</tbody>
</table>
# Interleukin 12

<table>
<thead>
<tr>
<th>Condition</th>
<th>Route</th>
<th>Dose</th>
<th>RR</th>
<th>Effect</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer*</td>
<td>Intra-vesical</td>
<td>15 pts</td>
<td>0% RR</td>
<td>No urine/serum IFN-γ induction</td>
<td>N.D.</td>
</tr>
</tbody>
</table>
| Renal cell carcinoma*                         | s.c.  | 26 NA |      | -dose-dependent ↑ sIFN-γ, TNF-α, IL-10, IL-6 and IL-8 at first injection.  
                              |       |      |     | -Lymphopenia; -Further ↑ IL-10 during treatment                      | N.D.                                       |
| Cervical carcinoma ♦                          | i.v.  | 34 3% |      | -↑ lymphoproliferative responses to HPV 16 E4, E6 and E7 peptides. | N.D.                                       |
| Head-neck carcinoma ♦                         | intratumoral | 10 ND |      | -↑ CD56+ NK cells in the primary tumor;  
                              |       |      |     | -high IFN-γ mRNA expression at lymph node level                        | N.D.                                       |
| AIDS-related Kaposi Sarcoma*                  | s.c.  | 34 50% |      | -↑ sIFN-γ after 1st dose, persisting after week 4                      | ↑ sIP-10 after the 1st dose, persisting after week 4 |
| Mycosis fungoides ♦                           | s.c.  | 23 43% |      | N.D.                                                                    | N.D.                                       |
IL-2 Receptor-\(\alpha\) (CD25) Expression Increases With DC Maturation

**INTERLEUKIN 2 RECEPTOR ALPHA CHAIN**

*Autophagy*

IMMATURE DC  MATURE DC/MAC SUPE  IL1,IL6,IFN,IL6
MM1 Anti-gp100 280-288 CD8+ PBMC-T Cell Response: IFN-g ELISPOT Analysis Pre-/Post- Vaccination
Interleukin 12 Gene Therapy

- **Skin Biopsy**
- **Fibroblasts in Culture**
- **Peritumoral Injection of Graded Doses of IL-12, Weekly x4**
- **Transduction with Retroviral Vector (TFG-hIL12)**
- **Selection Using G418**
- **Measure IL12 Expression Level and Harvest**
- **Myco/Bact., RCR Test**
- **Radiation**

iSBTc Oncology Biologics Development Primer
Responses to IL12 Gene Therapy in Patients with Melanoma/H&N Cancer
Repeated Administration of IL-12-DC Is Associated with Profound Antitumor Effects

![Graph showing mean tumor area (mm²) over days after tumor inoculation for different groups: HBSS, Fibro.-IL-12, and DC-IL-12. The graph illustrates that repeated administration of IL-12-DC is associated with profound antitumor effects.](image-url)
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon, Anne Cester, Fatima Sanchez-Cabo, Amos Kirilovsky, Bernhard Mlecik, Christine L'agarde-Page, Marie Tosolini, Matthieu Camus, Anne Berger, Philippe Wind, Franck Zinzindohoué, Patrick Bruneval, Paul-Henri Cugnenc, Zlatko Trajanoski, Wolf-Heinrich Friedmann, Franck Pages

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor sample) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.
IL-23 promotes tumour incidence and growth

John L. Langowski¹*, Xueqing Zhang¹*, Lingling Wu¹, Jeanine D. Mattson¹, Taiying Chen¹, Kathy Smith¹, Beth Basham¹, Terrill McClanahan¹, Robert A. Kastelein¹ & Martin Oft¹
Interleukin 35

IL-35 suppressed disease development in CIA in DBA/1 mice.

In our article (this issue), the two sentences “We have constructed a heterodimeric protein covalently linking EBI3 and p35, to form a novel cytokine which we now call IL-35” and “We propose to call the novel cytokine IL-35” imply that we were the first to propose this nomenclature; however, Dario Vignali first proposed the name IL-35 for the EBI3/p35 heterodimer at the 13th International Congress of Immunology, Rio de Janeiro, Brazil, as part of his presentation (Collison LW, Workman CJ, Kuo TK, Boyd K, Wang Y, Vignali K, Cross R, Sehy D, Blumberg RS and Vignali DAA. The inhibitory cytokine IL-35 contributes to regulatory T cell function. Nature, in press). Dario Vignali also received confirmation of his proposed nomenclature from the International Union of Immunological Societies (IUIS) Subcommittee on Interleukin Nomenclature and by the HUGO Gene Nomenclature Committee on 25 June 2007. We would, however, like to point out that the construct of EBI3/p35-Fc and the biological functions described in Figures 1, 2, 5 and 6 of this manuscript were first published by FY Liew and Xq Wei in a patent application in 2005 (PCT/GB2005/001037, priority date 4/5/3).
Ebi3–/– and Il12a–/– Treg cells fail to cure IBD.
IL-35 suppresses Teff cell proliferation.
Interleukin 18 [IL-1F1-F10, IL-33]

• Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H. Differential antitumor effects of administration of recombinant interleukin 18 (rIL-18) or rIL-12 are mediated by Fas-Fas ligand and perforin-induced tumor apoptosis, respectively. *Journal of Immunology* 1999; 163:583-589.


Interleukin 18 [IL-1F1-F10, IL-33]

• My plan, rapid combination with IL2 and/or IL-12
• IL-18 and IL-18BP circulate in normal and cancer patients.
• Phase I/II trials of IL-18 have been carried out and recently reported.
• Patients given rhIL-18 ranging from 3 to 1,000 μg/kg had chills, fever, nausea, headache, and hypotension along with neutropenia, thrombocytopenia, anemia, hypoalbuminemia, hyponatremia, and elevations in liver transaminases but with limited, unconfirmed responses.
• Ongoing trials in combination with therapeutic monoclonal antibodies are ongoing (Z. Jonak, personal communication).
Cancer Necrosis Correlates with Poor Prognosis

- Mesothelioma \((Edwards, 2003)\ p=0.008\)
- Renal-clear cell carcinoma \((Cheville 2003; Tollefson 2007)\ p<.001\)
- Colon carcinoma \((Hunter, 1983)\)
- NSCLC \((Swinson, 2003)\ p=0.0016\)
- Breast \((Gilchrist, 2003)\ p=0.0003; Kato, 2002)\ p=0.0068\)
- Mucosal melanoma \((Prasod, 2002)\ p=0.007\)
- Melanoma \((Balch, 2001)\)
- Sarcoma \((Miyajima 2002; Gustafson 2003)\)
Biomarkers and Surrogates - DAMPS

- LDH
- S100b, S100p, HMGB1, HSPs
- DNA
- Uric acid, other purine metabolites
# Damage-Associated Molecular Pattern Molecules (DAMPs)

<table>
<thead>
<tr>
<th>Cell Constituents:</th>
<th>HMGB1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heat shock proteins</td>
</tr>
<tr>
<td></td>
<td>Uric Acid, ATP, Adenosine</td>
</tr>
<tr>
<td></td>
<td>s100 proteins</td>
</tr>
<tr>
<td></td>
<td>Hepatoma derived growth factor</td>
</tr>
<tr>
<td></td>
<td>Cardiolipin</td>
</tr>
<tr>
<td></td>
<td>Interleukin 1 Family</td>
</tr>
<tr>
<td></td>
<td>DNA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secreted molecules:</th>
<th>Fibrinogen domain A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surfactant protein A</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Matrix elements:</th>
<th>Heparan sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble hyluranan</td>
</tr>
<tr>
<td></td>
<td>Fibronectin</td>
</tr>
</tbody>
</table>
Rubartelli A, Lotze MT. Inside, outside, upside down: Damage associated molecular pattern molecules and Redox. Trends in Immunology [2007].
# Eosinophils

## Wound Healing
- <1% circulating leukocytes
- Do not re-circulate
- IL-2, IL-4, GM-CSF, CTLA-4
- Associated with chronic inflammation including asthma, allergy, cancer, and transplant rejection
- Remove debris [opsonization]

## Eosinophils

### Adhesion molecules
- CD11a, CD44
- CD11b, CD49d
- CD15, CD62L
- CD15s, CD162
- CD18, CD174
- CD29 (αβ integrin)

### Chemokine, complement and other chemotactic factor receptors
- CD35, CCR1
- CD88, CCR3
- C3aR, CCR6
- PAFR, CXCR1
- LTβR, CXCR3
- LTD4R, CXCR4
- fMLP-R, CRTH2

### Histamine
- (H4 receptor)

### Immunoglobulin receptors and other members of the immunoglobulin superfamily
- CD4
- CD16
- CD31
- CD32
- CD33
- CD47
- CD48
- CD50
- CD54

### Enzymes
- CD13
- CD45
- CD45RB
- CD48RO
- CD46
- CD55
- CD59
- CD87
- PAR-2

### Apoptosis, signaling and others
- CD9, CD97
- CD17, CD98
- CD24, CD99
- CD28, CD137
- CD37, CD139
- CD39, CD148
- CD43, CD149
- CD52, CD151
- CD53, CD161
- CD69, CD165
- CD65, Siglec-8
- CD69, Siglec-10
- CD76, LIR1
- CD81, LIR2
- CD82, LIR3
- CD86, LIR7
- CD92
- CD95

### Cytokines
- CD25, CD124
- CD116, CD125
- CD117, CD131
- CD119, IL-9R
- CD120, IL-13R
- CD123, TGFβR

### MBP, EPO, EDN

---

Eos within necrotic tissue and capsule

Day 10  Necrotic  Day 16

Viable

Capsule

iSBTc Or
Case Studies: Lessons and Issues

• What were the most important strategic decisions the team made that you would recommend to others facing similar issues? Follow the data and make decisions after MTD reached, not before

• Regulatory authorities
  – What feedback did you get from regulatory authorities that was helpful to the strategic development plan? Examine and reflect toxicity in animal models but don’t be held to this completely.
  – Did you get unanticipated feedback that led to changes in the plans? Yes. At all times.

• Funding of the project: projections vs. realities. Early success critical

• Lessons learned
  – What advice would you give to projects headed down a similar development path? Trust your biologic intuition and experimental evidence; early data dictates subsequent studies..
  – Mistakes or missteps that you will avoid in future projects? Define biologic endpoints that are credible and push to efficacy or unacceptable toxicity.
DAMP Lab 2007
Why Women Live Longer Than Men