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IMMUNOTHERAPY FOR CANCER

A scientific activity sponsored by Montefiore Medical Center, the University Hospital for the Albert Einstein College of Medicine.

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Faculty Chairperson:
Nora Disis, MD
Associate Professor, University of Washington, Associate Member, Fred Hutchinson Cancer Research Center Seattle, WA

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Assistant Professor of Medicine, Duke University Medical Center Durham, NC

Michael Bishop, MD, FACP
Senior Investigator, Experimental Transplantation and Immunology Branch, National Cancer Institute Bethesda, MD

Jeffrey Weber, MD, PhD
Associate Professor of Medicine, University of Southern California, Norris Cancer Center Los Angeles, CA

Supported by an unrestricted Educational Grant from EMD Pharmaceuticals.
Welcome

Howard Kaufman, MD, FACS
Assistant Professor of Surgery, Medical Oncology, & Microbiology & Immunology, Albert Einstein College of Medicine, & Chief, Division of Surgical Oncology, Albert Einstein College of Medicine & Montefiore Medical Center Bronx, NY
History Of Cancer Therapy

- 1600 BC  Surgical excision
- 1809  Surgical oncology
- 1896  Radiation therapy
- 1942  Chemotherapy
- 1976  Immunotherapy
9 Year-old Girl with Ocular Melanoma

Before IL-2  
11/22/99

After 3 courses of high-dose IL-2  
1/31/01
Overview of Tumor Immunology

Nora Disis, MD

Associate Professor, University of Washington, Associate Member, Fred Hutchinson Cancer Research Center
Seattle, WA
Overview of Tumor Immunology

I. Immunity & Antigen Recognition

II. Human Tumors Are Immunogenic

III. Design of Immunotherapeutic Strategies
Immunity & Antigen Recognition
Immunity & Cancer

- Immunosuppression increases cancer risk
  - Lymphoma (x 90), skin (x 29), cervix (x 14)
- Spontaneous regressions occur
  - Melanoma (69), GI (34), lung (25), breast (22)
- Tumors can have infiltrating lymphocytes
- Human tumors are immunogenic
  - Tumor antigens have been defined

Antibodies

- Soluble proteins
- Produced by B cells
- Bind intact proteins
- Have Fc receptors
- Can kill cells by binding
  - Complement
  - Cytotoxic-APC
- Can block cell growth
  - Breast cancer
Cytotoxic T Cells (CD8+)

- Peptide antigen
- Class I MHC
- Directly kills the cell
  - Injects enzymes
  - Death signal
- Clonal expansion
- Helped by T helper cells
T Helper Cells (CD4+)

- Peptide antigen
- Class II MHC
- Secrete cytokines
  - Depending on many factors
- T-helper 1: IL-2, IFN- CTL
- T-helper 2: IL-10, 5, 4-Ab
- Immunologic memory
Antigen Recognition

- **MHC II**
  - IL-10
  - IL-5
  - IL-4

- **MHC I**
  - IL-2
  - IFN

- **Antibodies**
- **Cell**
- **Death**

- **Helper T Cells**
  - CD4+
  - **IL-2**
  - **IFN**

- **Cytotoxic T Cells**
  - CD8+
  - **IL-2**

- **Others**
  - **CD8+**
  - **CD4+**
Immunotherapy for Cancer

**Advantages**
- Tumors are immunogenic
- Single cell kill
- Migrate to tissue
- Memory
- Life-long protection

**Types of Immunotherapy**
- Cytokines
- Antibody infusion
- Vaccines
- Adoptive immunotherapy
- Immunomodulators
Human Tumors Are Immunogenic
Human Cancer is Immunogenic

Solid Tumor Antigens
Mage, 1,2,3
MART
gp100
CEA
HER2
Mucin
PSA
PAP
(shared between multiple tumor types)

Tumor Antigens

- Viral proteins: HBV, EBV, HPV
- Oncogenic proteins: p53, ras, HER2
- Glycosylated proteins and carbohydrates: mucin, Tn
- Antigens defined by immunity: MAGE
Immunity & Tumor Growth

- Proteins expressed by cancers elicit immunity
- Tumor-specific T cells and antibodies are found in cancer patients
- Cancer grows despite existent tumor-specific immunity
Inefficient Immune Response

• Cytokine environment
• Ineffective antigen-presenting cells
• Immunosuppressive factors
  • Direct modulation, e.g. virus
  • Secreted factors
• Antigen weakly immunogenic
  • Tolerance
Design of Immunotherapeutic Strategies
Immunotherapy for Cancer

Passive Immunity
- Supply the response
- Short lived
- Example: MoAb

Active Immunity
- Endogenous response
- Depends on the host
- Example: Vaccine
Cancer Patients Can Be Vaccinated

**Recall Antigen**

- Breast Cancer Patients (Stage III/IV)
- DTH pre tt
- DTH post tt

**Neo-Antigen**

- KLH Ab
- KLH T cell

Schiffman et al, 2001
Make “Self” More Immunogenic

**ANTIBODIES**

**CD4+**

IL-10
IL-5
IL-4

**IL-2**

**IFN**

**ANTIGEN-PRESENTING CELLS**

**KILLER T CELLS**

**PEPTIDES**

**CD8+**

**DEATH**

**HELPER T CELLS**

**IMMUNE SYSTEM ACTIVATORS**
Disease Burden & Immunotherapy

Disease Burden

Clinically Undetectable
1:10,000 T cells

Localized Disease
1:50 T cells

Advanced Disease
1:2 T cells?
MONOCLONAL ANTIBODIES

Michael R. Bishop, MD, FACP
Senior Investigator, Experimental Transplantation & Immunology Branch, National Cancer Institute
Bethesda, MD
Clinical Applications of Monoclonal Antibodies

- Diagnosis
- Monitoring of disease progression
- Therapy
Considerations for the Use of Monoclonal Antibodies for Immunotherapy

- Choice of target antigen
- Immunogenicity of MoAb
- MoAb half-life
- Manufacturing
- Unconjugated vs. Conjugated
  - Radioisotopes
  - Toxins
  - Chemotherapy
Therapeutic Effects of Monoclonal Antibodies

● Direct Effects
  - Induction of apoptosis
  - Block growth factor receptors
  - Anti-idiotype Ab formation

● Indirect Effects
  - Ab-dependent cell-mediated cytotoxicity (ADCC)
  - Complement-mediated cellular cytotoxicity

ADCC: Recruitment of natural killer (NK) cells, macrophages and monocytes by MoAB through its binding to their Fcγ receptors

Complement Dependent Cytotoxicity (CDC)

CDC: Induced by MoAB binding to C1q, resulting in activation of the complement cascade and generation of the membrane attack complex

Antibody-Based Cancer Therapy

1. ADCC
   - Complement pathway
   - Antidiotype
   - IL-1
   - TNF
   - Tumor cell
   - Mac

2. 
   - I-131
   - I-131
   - I-131

3. 
   - A

4. 
   - D

Obstacles to the Effectiveness of Monoclonal Antibodies

- Heterogeneity of antigen distribution on malignant cells
- Non-homogenous blood flow to tumors
- High interstitial pressure in tumors
- Unbound antigen-binding Ab
- Human anti-mouse Ab (HAMA)
- Cross reactivity with normal tissue antigen

## Monoclonal Antibodies for Treatment of B cell Malignancies

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Type</th>
<th>Investigational Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>Rituximab (Rituxan)</td>
<td>Chimeric</td>
<td>FDA approved</td>
</tr>
<tr>
<td></td>
<td>Tositumomab (Bexxar)</td>
<td>$^{131}$I-Murine</td>
<td>Submitted</td>
</tr>
<tr>
<td></td>
<td>Ibritumomab (Zevalin)</td>
<td>$^{90}$Y-Murine</td>
<td>Submitted</td>
</tr>
<tr>
<td>CD52</td>
<td>Alemtuzumab (Campath)</td>
<td>Humanized</td>
<td>FDA approved</td>
</tr>
<tr>
<td>CD22</td>
<td>Epratuzumab (Lymphocide)</td>
<td>Humanized</td>
<td>Phase II/III</td>
</tr>
</tbody>
</table>
CD20 Structure

- 297 amino acids
- 4 transmembrane domains
- Intracellular phosphorylation consensus sequences for serine/threonine kinases
  - Protein kinase C (orange)
  - Calmodulin/calcium (green)
  - Casein kinase II (yellow)

CD20: A Putative Calcium Channel

- Structural homology with calcium channels\(^1\)
- Increased intracellular calcium in stably transfected cell lines\(^1,2\)
- Calcium flux detected upon stimulation of CD20 with moAbs crosslinked with antimouse secondary antibodies\(^3\)
- Calcium chelators block apoptosis induced by CD20 stimulation\(^3\)

Effects of CD20 Crosslinking

- Increase in intracellular calcium
- Activation of
  - Src family of tyrosine kinases
  - Serine/threonine kinases
- Phosphorylation of
  - CD20
  - Phospholipase Cγ
- Upregulation of
  - c-myc and b-myb mRNA
  - Adhesion molecule expression
  - MHC II protein expression

CD20 Expression in B cell Malignancies

- Hairy Cell
- Burkitt's Lymphoma
- Large Cell
- Mantle Cell
- Marginal Zone
- Follicular Small Cell
- Small-Cleaved
- CLL/PLL
- CLL

Mean Channel Fluorescence
t(14;18) Translocation

14

J_H E_H C_\mu C_8

18

bcl-2

14;18

bcl-2 J_H E_H C_\mu C_8

Coding Region
Untranslated (noncoding)

Courtesy of John C. Reed, MD, PhD.
Bcl-2 Protein

- Overproduced in most follicular lymphomas and many diffuse lymphomas
- Localized mitochondria, endoplasmic reticulum, nuclear envelope
- Physiologic function: confers longevity on memory B and T cells
- Prolongs cell survival by blocking apoptosis
- Implicated in chemoresistance

Apoptotic Cell Death Pathway

Chemotherapy → p53 → Bcl-2 Expression → Bax Expression

γ-irradiation → Bcl-2 in excess of Bax (cells viable)

Bax in excess of Bcl-2 (cell death)

Bax

Bcl-2

Courtesy of John C. Reed, MD, PhD.
Rituximab: An Anti-CD20 Monoclonal Antibody

- Genetically engineered chimeric murine/human monoclonal antibody
  - Variable light- and heavy-chain regions from murine anti-CD20 antibody IDEC-2B8
  - Human IgGk constant regions
Rituximab: Mechanisms of Action

- **Direct**
  - Stimulation of apoptosis by inducing Ca\(^{2+}\) flux
    - Caspase activation
    - DNA fragmentation
    - Cell death

- **Indirect**
  - Antibody-dependent cell-mediated cytotoxicity (ADCC)
  - Complement-mediated cytotoxicity
Rituximab for Initial Treatment of LGNHL: Treatment Schema

- **Rituximab 375 mg/m², slow I.V. infusion**

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR</td>
<td>PR</td>
<td>PR</td>
<td>SD</td>
<td>PD</td>
<td>OFF</td>
</tr>
</tbody>
</table>

**Reevaluate**

**Rituximab Maintenance**
- Rituximab 375 mg/m² weekly x 4 every 6 months
- x 2 years

**Off Study**

Rituximab for Initial Treatment of LGNHL: Response Data

Rituximab for Initial Treatment of LG NHL: Duration of Response

Phase II Trial Rituximab Plus CHOP: Overall Survival

GELA Phase III Trial of Rituximab/CHOP

- Stage II-IV diffuse large B cell lymphoma (DLCL)
- Previously untreated
- Elderly (60 to 80 years)
- ECOG PS ≤ 2
- No history of indolent NHL or CNS lymphoma
- No contraindication for doxorubicin or vincristine

GELA Phase III Trial of Rituximab/CHOP

Untreated DLCL Elderly Patients

CHOP
Cyclophosphamide 750 mg/m², day 1
Doxorubicin 50 mg/m², day 1
Vincristine 1.4 mg/m², day 1
Prednisone 40 mg/m², days 1-5
G-CSF days 5-12
Every 3 weeks for 8 cycles

CHOP with Rituximab
Same CHOP schedule
plus
Rituximab 375 mg/m², day 1
Every 3 weeks for 8 cycles

GELA Phase III Trial of Rituximab/CHOP: Survival

Ongoing Phase III Trials: Intergroup Treatment Schema

Elderly; Diffuse large-cell NHL

Stratify: Number of risk factors: 0, 1 vs. 2, 3, 4

Randomization

CHOP
Every 21 days (6-8 cycles)

CHOP + Rituximab
Every 21 days (6-8 cycles)

Restaging
Stratify: CR vs. PR

Randomization

Rituximab Maintenance
Rituximab 375 mg/m² weekly x 4 every 6 months x 2 years

Observation

Habermann, Rituximab Investigator’s Meeting, September 2000
Rituximab Investigator Newsletter 2000; 2:6-9
### Monoclonal Antibodies Used for Immunotherapy in Solid Tumors

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity (antigen)</th>
<th>Target cell / disease</th>
<th>Type (chimerized, etc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endrecolomab (Panorex®)</td>
<td>17 – 1A antigen</td>
<td>Colon / rectal cancer</td>
<td>Murine IgG2a</td>
</tr>
<tr>
<td>Trastuzumab (Herceptin®)</td>
<td>HER2 oncoprotein</td>
<td>Mammary cancer</td>
<td>Humanized murine IgG1</td>
</tr>
</tbody>
</table>

Herceptin® (Trastuzumab): Humanized Anti-HER2 Antibody

Targets HER2
- High affinity \( (K_d=5 \text{ nM}) \) and specificity
- Humanized
  - 95% human
  - 5% murine
Herceptin®: Potential Mechanism of Action

- Down regulates HER2 receptor expression
- Inhibits proliferation of human tumor cells that overexpress HER2 protein
- Enhances immune recruitment and antibody-dependent cellular cytotoxicity (ADCC) against HER2 protein overexpressing cancer cells
- Down regulates angiogenesis factors
Herceptin® Monotherapy in Relapsed MB: Schema

**Design:** single-arm, open-label

Herceptin

4 mg/kg loading

Week 1

Herceptin

2 mg/kg qw maintenance

Week 2

Assessments

- **Primary end points**
  - Tumor assessments at weeks 8, 12, 24, then q12w thereafter
  - Objective tumor response (REC)*

- **Secondary end points**
  - Duration of response, TTP, time to treatment failure assessments, survival

* Independent Response Evaluation Committee

### Herceptin® Monotherapy in Relapsed MBC: Response Rates by REC*

<table>
<thead>
<tr>
<th>% of Patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>3.6</td>
</tr>
<tr>
<td>PR</td>
<td>12</td>
</tr>
<tr>
<td>ORR</td>
<td>15</td>
</tr>
<tr>
<td>SD (6 mos)</td>
<td>4</td>
</tr>
<tr>
<td>Clinical benefit†</td>
<td>19</td>
</tr>
</tbody>
</table>

* Response Evaluation Committee.
† CR + PR + SD ≥ 6 mo.

Herceptin® Monotherapy in Relapsed MBC: Median Duration of Response in Responders by REC*

Median time to disease progression = 9.1 mo

* Response Evaluation Committee.
Herceptin® First-Line Monotherapy in MBC: Schema

- Eligible patients (n = 114)
- MBC
- HER2 overexpression (2+/3+)
- No prior CT for MBC
- Measurable disease
- KPS > 60%

Primary end point: ORR
Secondary end points: RD, TTP and survival

# Herceptin® First-Line Monotherapy in MBC: Evaluable Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2 mg/kg n (%)</th>
<th>4 mg/kg n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>58 (100)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>CR</td>
<td>3 (6)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>PR</td>
<td>11 (19)</td>
<td>11 (21)</td>
</tr>
<tr>
<td>ORR</td>
<td>14 (24)</td>
<td>15 (28)</td>
</tr>
<tr>
<td>95% CI</td>
<td>13-35</td>
<td>16-40</td>
</tr>
<tr>
<td>SD ≥ 6 mo</td>
<td>6 (12)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Clinical Benefit*</td>
<td>20 (34)</td>
<td>22 (42)</td>
</tr>
</tbody>
</table>

*CR + PR + SD ≥ 6 mo.

## Herceptin® Monotherapy in Relapsed MBC: FISH Clinical Outcome Analysis

<table>
<thead>
<tr>
<th>IHC 2+/3+ combined</th>
<th>FISH+ n (%)</th>
<th>FISH- n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients evaluable</td>
<td>173 (100)</td>
<td>36 (100)</td>
</tr>
<tr>
<td>CR</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>ORR (%)</td>
<td>33 (19)</td>
<td>0</td>
</tr>
<tr>
<td>95% CI</td>
<td>14–20</td>
<td>0–10</td>
</tr>
<tr>
<td>Clinical Benefit*</td>
<td>41 (24)</td>
<td>0</td>
</tr>
</tbody>
</table>

* CR + PR + SD > 6 mo.

Herceptin® First-Line Monotherapy in MBC: Time to Disease Progression

- Nonresponders (n = 71) - TTP (1.8 mo)
- Stable disease < 6 mo (n = 13) - TTP (15.3 mo)
- Responders (n = 30) - TTP (18.8 mo)

# Herceptin® Monotherapy in MBC

**Studies: Summary**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>First-Line</th>
<th>Relapsed</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (n)</td>
<td>114</td>
<td>222</td>
</tr>
<tr>
<td>ORR (%)</td>
<td>26</td>
<td>15*</td>
</tr>
<tr>
<td>SD &gt; 6 mo (%)</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Clinical benefit† (%)</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>Median time to disease progression (mo)</td>
<td>3.5–3.8‡</td>
<td>3.1</td>
</tr>
<tr>
<td>Median DR (mo)</td>
<td>&gt; 12</td>
<td>9.1</td>
</tr>
<tr>
<td>Median survival (mo)</td>
<td>24.4</td>
<td>13</td>
</tr>
</tbody>
</table>

* Intent-to-treat population.
† CR + PR + MR + SD > 6 mo.
‡ 2 mg/kg and 4 mg/kg dose.


## Summary of Monotherapy Trials: Response

<table>
<thead>
<tr>
<th>Prior Therapy</th>
<th>N</th>
<th>RR (%)</th>
<th>Median DR (mo)</th>
<th>Median TTP (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2</td>
<td>46</td>
<td>11</td>
<td>6.6</td>
<td>NA</td>
</tr>
<tr>
<td>1–2</td>
<td>222</td>
<td>15</td>
<td>9.1</td>
<td>3.1</td>
</tr>
<tr>
<td>None</td>
<td>114</td>
<td>26</td>
<td>~18.0</td>
<td>3.5–3.8</td>
</tr>
</tbody>
</table>

**Herceptin® Combination Pivotal Trial in First-Line MBC (H0648g): Schema**

- Eligible patients (n = 469)
- MBC
- HER2-positive
- No prior CT for MBC
- Measurable disease
- KPS 60%

**Stratification**

- **No prior adjuvant AC**
  - Randomization
  - Herceptin + AC (n = 143)
  - AC (n = 138)

- **Prior adjuvant AC**
  - Randomization
  - Herceptin + Taxol® (n = 92)
  - Taxol (n = 96)

AC = doxorubicin (60 mg/m²) or epirubicin (75 mg/m²) + cyclophosphamide (600 mg/m²) q3w for 6 cycles; Taxol® (175 mg/m² x 3 h) q3w for 6 cycles; Herceptin (4 mg/kg IV) loading dose, 2 mg/kg qw until progression.

Herceptin® Combination Pivotal Trial: End Points

Primary
- Time to disease progression (TTP)

Secondary
- Objective response rate (ORR)
- Duration of response (DR)
- Time to treatment failure (TTF)
- 1-year survival
- Quality of life (QOL)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Herceptin + CT (n = 235)</th>
<th>CT (n = 234)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PR</td>
<td>43</td>
<td>28</td>
</tr>
<tr>
<td>ORR (%)</td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td>95% CI</td>
<td>44–57</td>
<td>26–38</td>
</tr>
</tbody>
</table>

$p$ value = $< 0.001$

Herceptin® Combination Pivotal Trial: Time to Progression

- Herceptin + CT (n = 235)
- CT alone (n = 234)

$\textit{Median follow-up: 30 mo}$

$p < 0.001$

Herceptin® Combination Pivotal Trial: Overall Survival*

**RR = 0.80**

\( p = 0.046 \)

% of patients crossing over to Herceptin at progression*:

<table>
<thead>
<tr>
<th>Month</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>15</td>
<td>62</td>
</tr>
<tr>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td>40</td>
<td>72</td>
</tr>
</tbody>
</table>

Median follow-up: 35 mo (range: 30–51)

† These patients are still reported in the CT arm.

Herceptin® Combination Pivotal Trial: Overall Survival

FISH+
- Herceptin + CT (n = 176)
- CT (n = 169)
  RR = 0.71
  \( p = 0.007 \)

FISH-
- Herceptin + CT (n = 50)
- CT (n = 56)
  RR = 1.11
  \( p = \text{NS} \)

Probability of survival

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity (Antigen)</th>
<th>Target Cell / Disease</th>
<th>Type (Chimerized, etc.)</th>
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<td>17 - 1A antigen</td>
<td>Colon / rectal cancer</td>
<td>Murine IgG2a</td>
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<tr>
<td>Trastuzumab (Herceptin®)</td>
<td>HER2 oncoprotein</td>
<td>Mammary cancer</td>
<td>Humanized murine IgG1</td>
</tr>
<tr>
<td>Anti-idiotypic antibodies</td>
<td>Individual patients’ B-cell tumor antigens</td>
<td>B-cell lymphoma</td>
<td>Customized human Mab</td>
</tr>
<tr>
<td>CAMPATH-1</td>
<td>CD52 antigen</td>
<td>CLL</td>
<td>Humanized IgG1</td>
</tr>
<tr>
<td>Rituximab (Rituxan ®)</td>
<td>CD20 antigen</td>
<td>NHL</td>
<td>Chimeric human / murine IgG1</td>
</tr>
<tr>
<td>Anit-B1 (Tositumomoab)a</td>
<td>B1 antigen</td>
<td>NHL</td>
<td>Mouse</td>
</tr>
<tr>
<td>LYM-1a</td>
<td>HLA-DR antigen</td>
<td>NHL</td>
<td>Murine IgG2a</td>
</tr>
<tr>
<td>LL2 (Epratuzumab) a, b</td>
<td>CD22 antigen</td>
<td>NHL</td>
<td>Murine IgG2a</td>
</tr>
<tr>
<td>Anti-CD33 (Hu-M195) a</td>
<td>CD-33 antigen</td>
<td>Acute / chronic myelogenous leukemia</td>
<td>Humanized Murine Mab</td>
</tr>
<tr>
<td>Ibritumomab Tiuxetanb,c(TM IDEC-Y2B8)</td>
<td>CD20 antigen</td>
<td>NHL</td>
<td>Chimeric human / murine IgG1</td>
</tr>
</tbody>
</table>

*a131I-conjugated Mab: monoclonal antibody; **90Y-conjugated B-cell; **11In-conjugated In-conjugated Mab: monoclonal antibody; CLL; chronic lymphocytic leukemia; NHL; non-Hodgkin's lymphoma

White, Weaver, Grillo-Lopez, Ann Rev Med 52:125
Cytokines and Cancer Therapy

Jeffrey Weber, MD, PhD

Associate Professor of Medicine, University of Southern California, Norris Cancer Center
Los Angeles, CA
What are Cytokines?

- Cytokines are substances (generally proteins and glycoproteins) which are secreted by immune cells and have autocrine and paracrine functions; they may function locally, or they may function at a distance, either to suppress or augment immunity.

- Cytokines function to regulate the innate (inborn NK/macrophage/neutrophil) immune response and adaptive (flexible, T and B cell) immune response
What Cytokines Don’t Work as Anticancer Agents?

- IL-1 beta had minimal anticancer activity and in fact may account for the severe side effects of fever, hypotension and anorexia with IL-2
- TNF caused severe hypotension used systemically
- IL-4 had little anticancer activity and was toxic
- IL-6 had minimal anticancer activity and is a growth factor for myeloma cells
- GM-CSF and IL-3 have no anticancer activity
What Cytokines are Important for Cancer Therapy?

- IL-2 and IFN-alfa 2b are FDA approved
- IL-2 has known activity in renal cell, melanoma, lymphoma, leukemia; IFN also has activity in the above diseases plus CML, KS and Hairy Cell Leukemia
- IL-12 and GM-CSF are likely effective adjuvants
- TNF and IL-4 may play a “niche” role in therapy in vitro
- IL-1 and IL-6 play a role in cancer-related toxicity
- IL-2 substitutes like BAY-50-4798 are designed to reduce its “capillary leak” toxicity
What is Alfa-Interferon?

- A superfamily of closely related genes on chromosome 9 that encode variably glycosylated proteins in the 150-160 amino acid range; they bind type I IFN receptors.
- All interferons have profound and pleiotropic effects on gene expression.
- Gamma interferon binds a type II IFN receptor and mediates different effects than type I IFNs.
- Gamma interferon has no anticancer effects, but is FDA-approved for Chronic Granulomatous Dis.
What Does Alfa-Interferon Do?

- Gene upregulation
  - MHC class I
  - Tumor antigens
  - Adhesion molecules
- Anti-angiogenesis
- Immune
  - B cell activity
  - T cell activity
  - Macrophages
  - Dendritic cells
  - Fc receptors
- Antiviral
Alfa-Interferon: Renal Cell Cancer

- Small but consistent response rates in the 5-10% range in a number of studies
- Randomized controlled trial of IFN-alfa vs. Megace® showed a survival of 8.5 for IFN vs. 6 months in 335 patients with PFS <5% at 2 years
- Not clear what the optimal dose, route and schedule for IFN are
Alfa-Interferon: Renal Cell Cancer

- In a compilation of 551 patients on phase II IFN trials given IM or SC, overall response rates were 8-29% with a median of 11%

- Interferon is the second choice treatment for RCC, and is intended for palliation only; eligible patients should be offered FDA approved IL-2 if they agree and are eligible
Does Interferon-alfa 2b Have Anti-Melanoma Activity?

- Kirkwood: 10-100 MU IV daily X 28 days with 23 pts; 22% RR
- Dorval: 10 MU/M² T.I.W. SC with 22 pts; 27% RR
- Robinson: 30 MU/M² T.I.W. SC X 12 weeks with 40 pts; 25% RR
- Sertoli: 10 MU/M² T.I.W. IM with 21 pts; 14% RR
- Overall, 106 eligible pts; 22.6% RR

Does Interferon-alfa 2b Have Anti-Melanoma Activity with DTIC?

- Five trials of IFN-alfa 2b with cimetidine; 19/111 or 17% RR; no advantage seen

- Eight trials of IFN-alfa with DTIC; 613 total pts; 144/613 = 23% RR

- Median survival of groups receiving IFN+TAM, DTIC+IFN, DTIC+TAM or DTIC in a 2X2 matrix trial was 9.1 months with median time to progression of 3.7 months; no advantage for IFN plus DTIC

Adjuvant Interferon for Melanoma

- The ECOG 1684 trial of 280 randomized patients who were observed or received one year of interferon-alpha 2b suggested a disease-free and overall survival advantage for high-dose IFN leading to FDA approval.

- IFN yielded a 3.8 year versus a 2.78 year OS and 0.8 vs. 1.8 year RFS; the advantage was 27% survival prolongation at p<0.04

Adjuvant Interferon for Melanoma

- The SWOG 9111/ECOG 1690 trial of 642 patients who received one year of HDI, two years of LDI or OBS showed no overall survival differences, with HR=1.0 for HDI vs. OBS but DFS was improved in the HDI arm.

- Median OS in the observed arm was 6.0 years, better than the treatment arm of the prior study.

- The HR for RFS in HDI vs. OBS arm was 1.28.

- Was high-dose IFN still the standard of care?
Adjuvant Interferon for Melanoma

- The post relapse survival in the 1690 trial was 4.3 years vs. 1.8 years in the 1684 trial. Why?
- Post relapse survival in the two HDI arms was similar: 2.6 yrs for 1690 vs. 2.1 yrs for 1684.
- More patients in the OBS arm received biochemotherapy than HDI pts (17% vs. 6 %) after relapse. Intensity of treatment was same.
- Does this suggest that IFN works, but can be given early or late after relapse?

Adjuvant Interferon for Melanoma

- Was adjuvant IFN ineffective and too toxic?
- The recent SWOG/ECOG 1694 IFN vs. GM-K vaccine trial showed a clear DFS and OS advantage for the IFN arm early after accrual was finished and was prematurely halted.
- 774 eligible patients accrued
- RFS was prolonged with HR=1.6; OS prolonged with HR=1.28; stage IIB patients also benefited; OS p value was = 0.04
E1694/S9512/C509801
Relapse-Free Survival, Eligible Patients

Probability vs. Months

GMK vs. IFN

$p = 0.0027$
Overall Survival, Eligible Patients

E1694/S9512/C509801

Probability

Months

GMK
IFN

$p = 0.015$
Relapse-Free Survival for IFN Trials
E1684, E1690, E1694 High-Dose IFN Arms

What Randomized Controlled Trials Have Been Done with Postoperative Adjuvant Interferon-Alfa?

<table>
<thead>
<tr>
<th>Trial</th>
<th>Interferon Type</th>
<th>Participants</th>
<th>Outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG 1684</td>
<td>alfa-2b</td>
<td>287</td>
<td>DFS and OS</td>
</tr>
<tr>
<td>NCCTG 83-7052</td>
<td>alfa-2a</td>
<td>273</td>
<td>DFS stage III</td>
</tr>
<tr>
<td>EORTC 18871</td>
<td>alfa-2a</td>
<td>900</td>
<td>negative low dose</td>
</tr>
<tr>
<td>WHO 16</td>
<td>alfa-2a</td>
<td>426</td>
<td>negative low dose</td>
</tr>
<tr>
<td>EORTC 18952</td>
<td>alfa-2b</td>
<td>1000</td>
<td>closed ????</td>
</tr>
<tr>
<td>ECOG 1690</td>
<td>alfa-2b</td>
<td>660</td>
<td>DSF not OS</td>
</tr>
<tr>
<td>ECOG 1694</td>
<td>alfa-2b</td>
<td>771</td>
<td>DSF and OS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(stopped early)</td>
</tr>
</tbody>
</table>
Does Interferon-Alfa 2b Have an Effect on Concurrent Vaccinations?

- ECOG 3694 trial: 107 patients randomized to GMK vaccine, IFN + GMK concurrent, or GMK started 5 weeks after hi-dose IFN

- Primary end point was anti-GM2 IgG titre

- There were no differences in probability of or the peak value of anti-GM2 IgG or IgM between arms

- No correlation between RFS and antibody titre

- At 23 mo. median follow-up, RFS was prolonged in the IFN groups vs no IFN!
Adjuvant Interferon for Melanoma

Once again, IFN-alpha is the standard adjuvant treatment for stage III melanoma, and should be the standard against which future adjuvant therapies are measured.

Prophylactic Paxil may decrease IFN toxicity, as can IV hydration during hi-dose therapy.

The current SWOG trial randomizes stage IIB/III patients to receive HDI versus three cycles of a modified Legha chemobiotherapy.

### Alfa Interferon for Hairy Cell Leukemia

<table>
<thead>
<tr>
<th>Study</th>
<th>Pt #</th>
<th>CR</th>
<th>PR</th>
<th>MR</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quesada, et al.</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Foon, et al.</td>
<td>14</td>
<td>7</td>
<td>86</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Rai, et al.</td>
<td>25</td>
<td>28</td>
<td>24</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>Golomb, et al.</td>
<td>195</td>
<td>4</td>
<td>78</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>264</strong></td>
<td><strong>9</strong></td>
<td><strong>71</strong></td>
<td><strong>10</strong></td>
<td><strong>10</strong></td>
</tr>
</tbody>
</table>

Concentration: IFN is an active, although second line, agent for Hairy Cell Leukemia.
Alfa Interferon for CML

- At MD Anderson, there is a large experience with IFN for CML:
  - Patients treated: 274
  - Complete hematologic response: 219 (80%)
  - Cytogenetic responses: 159 (58%)
    - Complete: 72 (26%)
    - Partial <35%: 32 (12%)
    - Minor: 55 (20%)

Median survival is greater than 89 months with a significant number of projected 10 year survivors.

Alfa Interferon for CML

Four randomized trials of alfa-interferon versus chemotherapy have been performed indicating superior survival for alfa-IFN:

<table>
<thead>
<tr>
<th>Study</th>
<th># pts</th>
<th>IFN</th>
<th>Chemo</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian Cooperative</td>
<td>322</td>
<td>72</td>
<td>52</td>
<td>0.002</td>
</tr>
<tr>
<td>Hehlmann, et al.</td>
<td>513</td>
<td>66</td>
<td>45</td>
<td>0.008</td>
</tr>
<tr>
<td>Allan, et al.</td>
<td>587</td>
<td>61</td>
<td>41</td>
<td>0.001</td>
</tr>
<tr>
<td>Ohnishi, et al.</td>
<td>159</td>
<td>54</td>
<td>32</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Alfa-IFN is an established nontransplant therapy for CML
Interleukin-2: T Cell Growth Factor

- A glycoprotein T-cell growth factor that binds to a receptor on T-cells
- First tested at the NCI in the early 1980s, it was found that surprisingly RCC responded to IL-2
- Higher doses of IL-2 appeared to induce significant clinical responses, with severe toxicity
- A trial of IL-2 at high doses intravenously was expanded and resulted in a 24% response rate
Side Effects: Interleukin-2

- Toxicities of IL-2 stem from a capillary leak
- IL-2 therapy in high dose is like a controlled state of septic shock with low BP, low SVR, high CO
  - Grade III/IV hypotension present in 74%
  - Grade III/IV cardiac toxicity present in 11%
  - Grade III/IV hematologic present in 39%
  - Grade III/IV hepatic in 39%
  - Grade III/IV renal in 80%
  - Grade III/IV pulmonary in 19%
Interleukin-2: Renal Cell Cancer

- High-dose bolus IL-2 in renal cell CA:
  - Cycle 1 rIL-2 720,000 IU/kg I.V. over 15 minutes every 8 hours for a maximum of 14 doses
  - Then a 5-9 day rest period
  - Cycle 2 rIL-2 720,000 IU/kg I.V. over 15 minutes every 8 hours for a maximum of 14 doses
**Interleukin-2: Renal Cell Cancer**

High dose rIL-2 for renal cell CA: 255 patients

<table>
<thead>
<tr>
<th>ECOG Performance Status</th>
<th># of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>166</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Prior Nephrectomy</td>
<td>218</td>
</tr>
<tr>
<td>Less than 1 year</td>
<td>143</td>
</tr>
<tr>
<td>More than 1 year</td>
<td>112</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time from Diagnosis</th>
<th># of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 year</td>
<td>143</td>
</tr>
<tr>
<td>More than 1 year</td>
<td>112</td>
</tr>
</tbody>
</table>
Interleukin-2: Renal Cell Cancer

- High dose bolus IL-2 for RCC
- Complete responses 7%, 80+ month duration
- Range of complete response durations: 7-131+
- Partial responses 8%, 20 month duration
- Range of partial response durations: 3-126+
- Overall 37/255 or 15% response rate
IL-2 And LAK Cells: Renal Cell Cancer

- 1985 trial showed that LAK cells plus high dose IL-2 gave a 35% response rate in RCC
- LAK cells were grown from pheresis specimens of PBMC incubated for 72 hours in IL-2 *ex vivo*
- Adoptive transfer of LAK cells caused profound pulmonary toxicity and hypotension
- No compelling evidence that IL-2 + LAK better than IL-2 alone
Renal Cell Cancer: Interleukin-2

- Management of IL-2 toxicities:
  - Dopamine used for low urine output; dosing continues until creatinine is 3.5-4.0
  - Neosynephrine® for hypotension resistant to fluid
  - Indocin® and Tylenol® around the clock for fevers and chills induced by IL-2
  - Cimetidine used for increased acidity and prophylactic Oxacillin for neutrophil dysfunction that led to a 27% rate of sepsis in one series before antibiotics were used

Low Dose Interleukin-2: Renal Cell Cancer

- Lissoni, et al.: 91 patients; 23% RR, 2% CR
- Buter, et al.: 47 patients; 19% RR, 3% CR
- Tourani, et al.: 39 patients; 18% RR, 3% CR
- Yang & Rosenberg: 53 patients; 11% RR, 6% CR
- Crecy study: Negrier, et al.: 425 patients; got SC IL-2 vs. SC IFN vs. SC IL-2+IFN; there was a 6.5% vs. 8% vs. 18.6% RR. No difference in overall survival was observed.
Interleukin-2 for Renal Cell Cancer: Conclusions

- Response rate N=255 is 15%, 7% CR, 8% PR
- Median overall duration of response is 54 months
- Responders can have a substantial tumor load
- Sites of regression include lungs, lymph nodes, bone, bowel disease
- Side effects manageable and virtually always reversible upon stopping therapy
- Low-dose IL-2 has fewer CRs and lower OS
High-Dose IL-2 for Melanoma

- Retrospective data from 266 patients in 7 trials from 17 institutions
- 17% overall response rate; 16 (6%) CR and 30 (11%) PR
- At a median 5-year follow-up, 69% of CRs are alive, 47% of all responders
- Median duration of response: 6.5 mos.
- 10/16 CRs, and 6/30 PRs remain progression-free 5-15 years later
Interleukin-2 in Combination Treatment for Melanoma

- 7 trials of IL-2 with anti-GD3 antibodies:
  - 145 total patients with only 5 responses, 1 CR and 4 PR or 3% RR
  - 16 trials of bolus, continuous or low-dose SC IL-2 with alfa-interferon: 217 got bolus, 224 got CIV, 41 got SC
  - 48/217=22% RR for bolus, 28/224=12% RR for CIV, and 3/41=8% RR for SC IL-2

- Conclusion: IFN does not add to IL-2
What Correlates with Survival After IL-2 Treatment?

- Performance status
- Development of vitiligo and/or autoimmune thyroiditis
- Amount of IL-2 given during first course
- Height of the rebound lymphocytosis
- Vitiligo seen in 11/74 melanoma pts with response, in no non-responders, and in no RCC patients receiving high dose IL-2
# High-Dose IL-2 for Lymphoma

IL-2 has activity in stage IV non-Hodgkin’s lymphoma.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Pts Evaluable</th>
<th>CR</th>
<th>PR</th>
<th>CR+PR</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>82</td>
<td>5</td>
<td>9</td>
<td>14/82</td>
<td>(17%)</td>
</tr>
<tr>
<td>IL-2 + LAK</td>
<td>39</td>
<td>2</td>
<td>5</td>
<td>7/39</td>
<td>(17%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>121</td>
<td>7</td>
<td>14</td>
<td>21/121</td>
<td>(17%)</td>
</tr>
</tbody>
</table>
Interleukin-2 for Lymphoma & Leukemia Post Stem Cell Transplant

- 25 patients post-ASCT for NHL at Seattle:
- 80% completed induction IL-2; all tolerated maintenance well.
- Disease-free survival is 60% at 30 months- compares favorably if not better than historical controls at Seattle
- 46 patients with AML in CR1 received IL-2 median of 36 days post transplant; at 2 years, DSF is 82%, which is quite favorable compared to historical controls
- Currently there are large SWOG trials underway of ASCT +/- IL-2 for NHL and AML
Interleukin 2 as an Adjunct in Acute Leukemia

Seattle regimen:

- Induction: $9 \times 10^6 \text{ IU/M}^2/\text{day} \times 4\text{d CIV}$
- Rest: 4 days
- Maintenance: $1.6 \times 10^6 \text{ IU/M}^2/\text{day} \times 10\text{d}$

In a phase II trial of AML patients in relapse, 13/24 CR were observed in relapses 2-4.

- Several trials of post CR1 IL-2 treatment have been done: In one 18 patient trial, 6/18 pts (33%) were in CR at 4 years vs. 5/36 pts (14%) without IL-2 retrospectively

- Median CR duration for 10/23 patients in CR1 who stayed on IL-2 post-remission was 22 months at Dana Farber.
IL-2 Plus Histamine

- In animal models, histamine compounds decrease the inhibition of the action of IL-2 by macrophages.

- A randomized open label trial was conducted of 305 stage IV melanoma patients who received low-dose IL-2 vs. low-dose IL-2 + histamine.

- No differences in response rate were seen; overall survival was increased in the subgroup of patients with liver metastases with p<0.004; overall survival for all pts. increased from 5.0 to 9.1 months but was not significantly different (unadjusted p=0.052).

- The FDA’s ODAC declined to approve Maxamine.
BAY 50-4798: IL-2 Without Toxicity?

- A genetically engineered IL-2 molecule with a two amino-acid change to eliminate the “toxic” region.
- In animal models induces less capillary leak which suggests it will have fewer side effects.
- Now being evaluated in a phase I trial; the dose escalation continues and we are now at roughly a dose equivalent to IL-2 without dose limiting toxicity, and will less need for pressors.
Is GM-CSF Effective as Adjuvant Therapy for Stages III/IV Melanoma?

- 48 patients with resected stages III/IV melanoma received GM-CSF at 125 µg/M² days 1-14 every 28d X 12 cycles
- Median survival was 37.5 mos. vs. 12.2 mos. in the matched UAB database from 1960-1988
- 14 Stage III pts had 35.8 mos. median survival, worse than that seen with IFN (46 mos.) but 4 or more LNs or > 3 cm LN was required in this trial.
- Rationale? What are the pitfalls in interpreting this trial?

IL-12: Clinically Active Cytokine?

- A heterodimeric protein of 70,000 MW that promotes both NK and cytolytic T cell activity
- It has antitumor activity systemically in mouse models and when delivered as a gene therapy
- In phase I, of 40 patients 1 CR, 1 PR, 4 SD with IV IL-12
- In another phase I, of 50 pts 1 CR, 34 SD with SC IL-12
- IL-12 is being tested with IL-2 and with IFN in melanoma, and in T cell leukemia-lymphoma; it also has preclinical activity as a adjuvant in murine models
gp100/tyrosinase Peptides/IFA Vaccine +IL-12 and +/- GM-CSF: Schema

- There was a rationale for testing both cytokines as adjuvants so two trials were done with a peptide vaccine
- All patients were HLA-A201 positive with completely resected stages II or III/IV melanoma
- Intervals were q 2 weekly X 4, then q 4 weekly X 3 then q 8 weekly X 1; 8 injections over 26 weeks
- Pheresis and skin testing were performed prior to and after 6 months of vaccine therapy
- IL-12 was given intradermally once and GM-CSF subcutaneously for 5 days both at the vaccine site
T-Cell Immune Response to gp100-2M at Day 10 by Cytokine Release

pg/ml of Gamma Interferon per 10^5 Effectors

Treatment

- pre no IL-12
- post no IL-12
- pre with IL-12
- post with IL-12
Each line represents an individual patient. Solid lines indicate treatment with IL-12; dashed lines indicate no IL-12.
Conclusions: gp100/tyrosinase Peptides/IFA Vaccine +/- IL-12:

- Well tolerated: only grade III local toxicity, no grade IV, 3 patients of 48 with ulceration
- The IL-12 group so far has had greater DTH reactivity to gp100; none seen to tyrosinase
- The IL-12 group seems to have greater across the board cytokine release reactivity to both gp100-WT and -2M but not tyrosinase.
- 85% of patients had an immune response
T cell Immune Response to gp100-2M at Day 10 by Cytokine Release

pg/ml of Gamma Interferon per 10^5 Effectors

Treatment

pre no GM-CSF  post no GM-CSF  pre with GM-CSF  post with GM-CSF

0 500 1000 1500 2000 2500
Conclusions: gp100/tyrosinase Peptides/IFA Vaccine +/- GM-CSF:

- Well tolerated: only grade II local toxicity, no grade III/IV, no patients of 43 with ulceration
- The GM group so far has had similar DTH reactivity to gp100; none seen to tyrosinase
- The GM group seems to have increased across the board cytokine release reactivity to gp100-WT and -2M but not tyrosinase.
- 85% of patients had an immune response
Conclusions: Cytokine Therapy for Cancer

- IL-2 has modest antitumor activity in metastatic renal cell and melanoma, is FDA approved for both, primarily because of the 5-10% long term survival “tail on the curve,” but is quite toxic
- IL-2 at low doses may have promising activity posttransplant as an immune “restorative”
- Alfa-interferon had a modest effect on survival when used as adjuvant therapy for resected melanoma, but is toxic
- IL-12 and GM-CSF are promising adjuvants for vaccines
Dendritic Cell Vaccine Approaches Against Cancer
Michael Morse, MD
Assistant Professor of Medicine
Duke University Medical Center
Durham, NC
Acknowledgments

I would like to thank the following for their contributions to this research:

- H. Kim Lyerly
- Paul Mosca
- Tim Clay
- Amy Hobeika
- Ian Cumming
- Michelle St. Peter
- Shubi Khan
- Tracey Kerby
- Terry Kruger
- Sharon Peplinski
- NCI
  - Jeff Schlom
  - Larry Kwak
- Immunex, Inc.
  - Dania Caron
  - Elaine Thomas
- Schering Plough, Inc.
  - Mary Ellen Ryback
- Coulter, Inc.
  - Pat Roth
- Becton Dickinson
  - Skip Maino
- Duke
  - Eli Gilboa
  - Smita Nair
  - Dave Boczkowski

Funding from the National Cancer Institute.
Processing and presentation of class I antigens

Protein synthesized

Protein fragmented

Class I MHC and beta 2 microglobulin synthesized

Class I MHC bind peptides

MHC-peptide complex transported

MHC-peptide complex recognized by antigen specific T cells

Dendritic cell

Accessory molecules produced
Peptide & Protein Vaccines

Dendritic cell

Protein/peptide

T cell

Adjuvant (QS21, GM-CSF)
## Peptide & Protein Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immune Response</th>
<th>Clinical Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp100+ tetanus tox + Montanide ISA-51 or QS-21</td>
<td>14% with gp100+ CTL</td>
<td>75% survival at 4.5yrs; (resected melanoma)</td>
<td><em>Clin Cancer Res.</em> 2001;7:3012-3024.</td>
</tr>
<tr>
<td>gp100, IL-2, IL-12</td>
<td>7/7 alone</td>
<td>Greater tumor regression with IL-2</td>
<td><em>J Immunol.</em> 1999;163:6292-6300.</td>
</tr>
<tr>
<td>HER2/neu helper peptides</td>
<td>14/18 with prolif to one Ag</td>
<td>NR</td>
<td><em>J Clin Invest.</em> 2001;107:477-484.</td>
</tr>
</tbody>
</table>
Tumor Cell Vaccines

Genetically Modified or Virally-infected Tumor cell

Viral proteins

HLA molecule
Costimulatory molecule

IL-2
GM-CSF

T cell
Monocyte
DC
# Tumor Cell Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immune Response</th>
<th>Clinical Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CancerVax (melanoma)</td>
<td>82% + complmnt dependent cytotox (CDC)</td>
<td>Median survival 54 mo, if deltaCDC &gt; 10%</td>
<td><em>Ann Surg Oncol.</em> 1998;5:595-602.</td>
</tr>
<tr>
<td>Autologous colon CA + BCG</td>
<td>Increased DTH in all patients</td>
<td>No overall survival benefit</td>
<td><em>Vaccine</em> 2001;19:2576-2582.</td>
</tr>
<tr>
<td>Autologous GBM+ Newcastle Virus</td>
<td>DTH increased from 1.67 to 4.05 cm2</td>
<td>Median survival was 46 weeks</td>
<td><em>J Neurooncol.</em> 2001;53:39-46.</td>
</tr>
</tbody>
</table>
Viral Vector & Plasmid Vaccines

Muscle cell → Expressed antigen → DC → Expressed antigen
Viral Vector & Plasmid Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immune Response</th>
<th>Clinical Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALVAC-CEA B7.1</td>
<td>5/9 CEA+ T cell precursor frequency</td>
<td>0 PR; 4/23 with decreased CEA</td>
<td><em>Clin Cancer Res.</em> 2001;7:1181-1191.</td>
</tr>
</tbody>
</table>
Dendritic Cells

- Considered professional antigen-presenting cells
- Derived from bone marrow precursors, but multiple lineages can generate antigen-presenting cells
- Comprise approximately 0.3%-0.5% of the total circulating peripheral blood mononuclear cells
- Clinical utility limited by low numbers of dendritic cells
Variables Associated with DC Vaccination

- **Source- lineage**
  - DC1 (myeloid, CD11c+, CD123 dim)
  - DC2 (lymphoid, CD11c-, CD123 bright)
  - CD8a in murine system

- **Type of antigen loading**

- **Maturation and/or activation**

- **Route of administration**

- **Clinical evaluation**
  - Clinical endpoints
  - Immunologic endpoints
Clinical Sources of Dendritic Cells

- Peripheral blood- direct isolation
- CD34+ progenitors- BM or PBST
- Monocyte progenitors following *in vitro* expansion (GM-CSF and IL-4, etc.)
- Mobilization of progenitors (G-CSF, GM-CSF, G-CSF + GM-CSF, GM-CSF+ IL-4, Flt3L, or Flt3L-G-CSF)
- Converting non-DC to DC-like cells
DC Precursors

Immature DC
- ↑ intracellular MHC
- ↑ endocytosis
- ↑ CCR1, CCR5, CCR6
- ↓ CCR7
- ↓ CD54, CD58, CD80, CD86
- ↓ CD40
- ↓ CD83
- Tolerize?

Hours
Pathogens
Cytokines
T Cells

Mature DC
- ↑ surface MHC
- ↓ endocytosis
- ↓ CCR1, CCR5, CCR6
- ↑ CCR7
- ↑ CD54, CD58, CD80, CD86
- ↑ CD40, DC-LAMP
- ↑ CD83
- Activate

Cytokines- such as GM-CSF+IL-4, GM-CSF+IL-13, GM-CSF+TNFα, GM-CSF+IFNα, etc.

Days to weeks
Antigen Loading of Dendritic Cells

- **Peptides that can bind to MHC directly:**
  - often defined, often class I, commonly HLA A*0201 (can use unfractionated peptides)

- **Antigens that require intracellular processing:**
  - Protein (endocytosed and processed)
  - DNA/RNA - (protein expressed and processed)
  - Viral vectors- (protein expressed and processed)
  - Cell lysate- (endocytosed and processed)
  - DC - tumor cell fusions
  - Apoptotic bodies, exosomes
Maturation of DC: Effects on Phenotype and Antigen Loading & Processing


Pre- TNF-a treatment

Post-TNF-a treatment

Stimulators
- DC+CEA ep+TNF-a
- DC+CEA RNA+TNF-a
- DC+TNF-a+CEA pep
- DC+TNF-a+CEA RNA
Only a Subset of CD83+ DC can be Matured to Produce IL-12

Intravenous Administration of In 111-labeled DC

Subcutaneous & Intradermal Administration of In 111-labeled DC

Tumor Antigens for Clinical Trials

- Lymphoma — targeting Id protein
- Melanoma — targeting melanoma antigens- Mage-1, Mart, gp 100, Mage-3
- GI — targeting CEA, ras, p53
- Breast — targeting CEA, Her-2/neu, p53
- Prostate — targeting PSA, PSA-M
- Lung — targeting CEA, Mage-3
- Telomerase
Recognition of Malignant Myeloma Cells by Id Specific T cells

# Phase I Trial of DC in Patients with Metastatic Disease

**Direct comparison of pre- and post-immunization blood samples**

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**Legend:**
- Screen
- Entry
- Leukapheresis

**Events:**
- DC Injection
- Direct comparison of pre- and post-immunization blood samples
Clinical Responses

- Tumor response/shrinkage
- Tumor marker response - ?PSA, etc.
- Other clinical markers - ? vitiligo
- Delay in time to progression
- Delay in time to recurrence
- Increase in overall survival
- Adverse effects — ? autoimmunity
Immunologic Responses

**In Vivo**
- DTH
- Vitiligo
- Tumor infiltration

**In Vitro**
- T cell proliferation
- T cell cytokine release
- ELISPOT (cytokine release)
- Intracellular cytokine
- Peptide-MHC tetramer
- Cytotoxic T-lymphocyte function
- CTLp frequency
Magnitude and Duration of Antigen-Specific Immune Response May Affect Clinical Response

"Resting Immune Response CTLp 1 in 5,000 by LDA"

"Clinically effective" Cell response

Did not achieve sufficient frequency of antigen-specific T cells

"Clinically ineffective" Cell response

Did not achieve sufficient duration of Antigen-specific T cells

Immunotherapy

Cellular Immune Function

Time (Months)
Results of Phase I Study of DC Loaded with CEA Peptide (CAP-1)

- Safe and feasible to administer up to $1 \times 10^8$ DC IV
- 1 minor response
- T cell infiltration of DC injection sites but minimal DTH response
- Minimal detectable CEA specific T cell activity using CTL activity as read-out
Direct Assessment of Peripheral Blood Immune Function Minimizes *In Vitro* Artefact

*In Vitro* Expansion of Antigen-specific T cells

(EBV specific CD8+ T cells)
ELISPOT Response After Vaccination with DC Based Vaccines
Normal Donor PBMC (EBV+)
ELISPOT Analysis

IaCu (Tetramer #7) ELISPOT
(MSPs values)

IFNγ secreting spots/200K Responders

PWM, EBV, CMV, Mart-1, Media w/ cells, Media w/o cells

Antigen

Well 1, Well 2, Well 3, Well 4, Well 5, Well 6, Mean
Peptide-MHC Tetramer Detection of EBV specific CD8+ T cells in PBMC EBV+
Intra-cellular Cytokine Staining of Antigen-specific T cells in PBMC EBV+ (CD8+ T cells)

- Untreated
- CMV 1 µg/ml
- Mart-1 1 µg/ml
- PWM 5 µg/ml
- EBV 1 µg/ml

CD69

IFNγ
Intracellular IFN-gamma Analysis of Vaccine Response

Pre-Vaccination

Post-Vaccination

CD69

Tet Tox

IFN-γ
Intra-cellular IFN-γ Analysis of CD4+ and CD8+ T cells in PBMC

![Graph showing the percentage of T Cells CD69+/INF-γ+ for CD4 and CD8 over weeks.](image)
### Phase I Trials of Her2/neu ICD Protein-pulsed DC in Patients with Metastatic Disease

**Direct comparison of pre- and post immunization blood samples**

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**In vitro generation of DC**

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**Administration of DC**

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**Time of analysis**

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- Screen
- Entry
- Leukapheresis
- Fresh DC Injection
- DC Injection
- Direct comparison of pre- and post immunization blood samples
Generation of Her2/neu ICD specific T cells *In Vitro* with Her2/neu ICD Protein-pulsed DC

**Experiment #1**

- Targets: 1 = DC+CMV lysate
- n = DC+HER2/neu ICD

**Experiment #2**

- Targets: 1 = DC+HER2/neu ICD protein
- n = DC+varicella lysate

**Experiment #3**

- Stimulators: 1 = DC+varicella lysate
- n = DC+HER2/neu ICD
ELISPOT Response after Vaccination with Her2/neu ICD DC Vaccines

Pre-immunization Her2/neu ICD= 7 in 100,000 PBMC
ELISPOT Response after Vaccination with Her2/neu ICD DC Vaccines

Post immunization

Her2/neu ICD= 22 in 100,000 PBMC
Flt3L-mobilized DC-ELISPOT Results

Tet Tox  Cntrl

Flt3L

Wks  -2  0  1  3  5  9

Spots per 67,000 Cells

Wks  -2  0  1  3  5  9

Spots per 67,000 Cells
Intra-cellular IFN-gamma Analysis of PBMC after Flt3L DC Immunization
## Dendritic Cell Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immune Response</th>
<th>Clinical Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34 + DC +gp100 MART-1, MAGE-3, tyrosinase, gp100</td>
<td>16/18 with Ag+ T cell activity</td>
<td>Regression of &gt;1 melanoma met in 7/18</td>
<td>Cancer Res. 2001;61:6451.</td>
</tr>
<tr>
<td>Flt3L mobilized DC + CAP1-6D (CEA+)</td>
<td>5/12 tetramer+ T cells</td>
<td>2/12 PR, 2/12 MR</td>
<td>PNAS. 2001;98:8809.</td>
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<tr>
<td>DC + tumor RNA, KLH (colon CA)</td>
<td>11/13 KLH+ responses</td>
<td>7/13 decreased CEA; 0 PRs</td>
<td>Hepatogastro. 2001;48:347.</td>
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<tr>
<td>DC + mouse PAP (prostate Ca)</td>
<td>100% IFN-g+ T cells</td>
<td>NR</td>
<td>J Immunol. 2001;166:4254.</td>
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## Dendritic Cell Vaccines (continued)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immune Response</th>
<th>Clinical Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC+ glioma peptides</td>
<td>4/7 with Ag-specific CTL</td>
<td>T cell infiltration into tumor of 2/4</td>
<td>Cancer Res. 2001;61:842-847.</td>
</tr>
<tr>
<td>DC+ MART-1 and GP-100 iv</td>
<td>1/5 with Ag specific CTL</td>
<td>1/7 PR</td>
<td>J Immunother. 2000;23:487-498.</td>
</tr>
<tr>
<td>DC + Id (myeloma)</td>
<td>24/26 KLH+ 4/26 Id+ T cells</td>
<td>17/26 alive at 30 mo post ABMT</td>
<td>Biol Blood Marrow Transpl. 2000;6:621-627.</td>
</tr>
</tbody>
</table>
Induction and Expansion of Antigen-specific Immune Response

Cellular Immune Function

“Resting Immune Response”

Immunotherapy

“Responding Immune Response”

Expansion

“Clinically Detectable”

“Clinically Effective”

“Clinically Ineffective”

Time (Months)