Antibody Therapy:
Biology, Immunocytokines, and Hematologic Malignancy

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University of Wisconsin
Madison
DISCLOSURE STATEMENT

P. Sondel has disclosed the information listed below. Any real or apparent conflict of interest related to the content of the presentation has been resolved.

**Organization**  
EMD-Pharmaceuticals  
Quintesence  
Medimmune

**Affiliation**  
Scientific Advisor  
Scientific Advisor  
Scientific Advisor
1. T-cell Recognition

2. Innate Immunity

3. Passive Immunity

4. Tumor Induced Immune Suppression

5. Cellular Therapy
Making Monoclonal Antibody (mAb)

Abbas and Lichtman: 2003
Underlying principle of mAb therapy

SELECTIVE recognition of tumor cells, but not most normal cells by therapeutic mAb
<table>
<thead>
<tr>
<th></th>
<th>LEUKEMIA</th>
<th>SOLID TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-20</td>
<td>B</td>
<td>GD-2</td>
</tr>
<tr>
<td>CD-19</td>
<td>B</td>
<td>Her2</td>
</tr>
<tr>
<td>CD-5</td>
<td>T</td>
<td>EpCAM</td>
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</tbody>
</table>
From Genes to Antibodies

Antibody Genes

Gene components scattered through one chromosome

Rearranged gene components encoding a heavy chain

Assembled antibody molecule

Heavy chain
Light chain
Antigen-binding region

Constant region
Antibody Engineering

• **First step - development of monoclonal antibodies**
  – Fusion of antibody-producing B cell with myeloma
  – Results in immortalized monospecific Ab-producing cell line

• **Second step - ability to clone and re-express Abs**
  – Initially done with cloned, rearranged genes from hybridomas
  – Parallel work with isolated Fab fragments in bacteria

• **Third step - re-engineering for desired properties**
  – Reducing immunogenicity of mouse antibodies
  – Tailoring size and half-life for specific need
  – Adding or removing functions

• **Engineered diversity – phage display approach**

From S. Gillies
Chimeric Mouse-human antibodies

e.g. Erbitux

From S. Gillies
V Region “humanization”

This approach has worked but often leads to mis-folding of CDRs requiring additional FR back-mutations

From S. Gillies
Mechanisms of mAb mediated anti-tumor effects

Delivery of Toxic Agent

Toxin, Drug, Radionuclide, etc

Death
$^{131}$I-3F8 binding to melanoma

Cheung et al.
Biol Ther. Of Cancer, 1995

(Anti-EGFR mAb)

\[ p = 0.03 \]
CDC

C'1-9 Cascade

Tumor

Osmotic Lysis

ADCC

NK

FcR

Tumor

Necrosis & Apoptosis
Mechanisms of anti-CD20 therapy: CDC

From Dr. John Leonard - Cornell

CD20

Complement fixation
membrane attack complex

lipid raft

Rituximab

B cell
CD59 and S protein Inhibit MAC

Abbas and Lichtman: 2003
Complement mediated destruction is inhibited in vitro by CD59 (blocks MAC)

<table>
<thead>
<tr>
<th>Tumor cell type</th>
<th>CD59</th>
<th>CD20</th>
<th>Viability</th>
<th>Viability</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rituxan</td>
<td>Rituxan + C’</td>
</tr>
<tr>
<td>NHL</td>
<td>++++</td>
<td>++++</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>NHL</td>
<td>-</td>
<td>++++</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

BUT, there is no correlation in vivo with CD59 and Rituxan response!

Mechanisms of anti-CD20 therapy: ADCC

From Dr. John Leonard - Cornell
Human Fcγ Receptor Family

FcγRI  FcγRIIA  FcγRIIB  FcγRIIIA  FcγRIIIB

CD32  CD16

Dr. R. Clynes – Columbia U
2 Major Types of Activating FcR for IgG

- **FcγRIIA (CD32)**
  - **Expressed on:**
    - Macrophages
    - PMNs
  - **Functions:**
    - Phagocytosis
    - ADCC

- **FcγRIIIA (CD16)**
  - **Expressed on:**
    - NK Cells
  - **Functions:**
    - ADCC
Efficacy of FcR influences in vivo Rituxan Effects

AA #158 of FcRIII

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>In Vitro ADCC</th>
<th>In Vivo to Rituxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/V</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>V/F</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>F/F</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

V → Higher Affinity for huIgG
F → Lower Affinity

Reponse Rate


\[ p < .05 \]
Importance of FcγRIIIA on NK cells in Rituxan Therapy

Fig. 2

Kaplan-Meier estimates of progression-free survival by immunoglobulin G fragment C receptor IIIα (Fc RIIIα) 158 valine (V)/phenylalanine (F) polymorphism.


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Importance of FcgRIIA on Mφs and PMNs cells in Rituxan Therapy

Fig. 3

Kaplan-Meier estimates of progression-free survival (PFS) by immunoglobulin G fragment C receptor IIa (Fc RIIa) 131 histidine (H)/arginine (R) polymorphism.

Activate cells with FcγRIIA (PMNs and Mφs) with GM-CSF

- Treat with murine anti-GD2 mAb (3F8 or ch14.18) AND GM-CSF
- 3F8 is a murine IgG3 mAb:
  - Murine IgG3 mAb binds better to the FcγRIIA-R131 than to the FcγRIIA-H131 allele
- Does the FcγRIIA allele status impact on outcome?

Cheung et al, JCO, 24:2885, 2006
The FcγRIIA allele status impacts outcome ONLY if GM-CSF is given (based on historical controls receiving 3F8 without GM-CSF.
Activate NK cells to mediate ADCC

In Vivo IL-2/Rituximab Trial
CCG-0935/0935A-
Pilot Phase-I study of ch14.18 + IL2 + GM-CSF following ABMT for NBL

- Day 0: ABMT
- Day 35: Ch14.18 + GM-CSF
- Day 56: Ch14.18 + IL2
- Day 77: Ch14.18 + GM-CSF
- Day 98: Ch14.18 + IL2
- Day 119: Ch 14.18 + GM-CSF

- Ozkaynak et al, JCO 18:4077, 2000
- Gilman et al, Submitted 2007
High Risk NBL

Induction

Ablation + Stem cell Rescue

Randomize

Observe

ch14.18 + GM-CSF + IL2

Cis-retinoic acid
Tumor Cell

Anti-GD2+IL2 Immunocytokine (IC)

GD2 Antigen

hu14.18-IL2

IL-2 Receptor

IL-2

S. Gillies and R. Reisfeld
Clinical material made by NCI-BRB

T Cell
or
NK Cell
Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases


![Graph showing the number of liver mets for PBS control, ch14.18 + IL-2, and ch14.18-IL-2 treatments. The numbers of liver mets are 123 ± 69 for PBS control, 34 ± 21 for ch14.18 + IL-2, and 0 ± 0 for ch14.18-IL-2.](image)
Assays with Patient Effectors (C1D8) And Serum Pre and Post hu14.18-IL2 Infusion (1144 Blood samples from 33pts)


ADCC of NBL targets
Soluble IL-2 receptor $\alpha$ level determined on days 1, 2, 3, 4, 8 and 15 of each course.

Conclusions from Phase I Trials  
**hu14.18-IL2**

- Dose, Schedule and MTD with acceptable toxicity found in pts. with NBL and MEL.

- MTD in children with NBL (heavily pretreated with chemo) is higher than MTD in adults, as expected. Similar PK.

- Hu14.18-IL2 induces immune activation in vivo (PBL, sIL2R, ADCC).
Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases

Hu14.18-IL2 (10ug/d) for 5 days starting on day 5, 7, 9, or 11 following $5 \times 10^5$ NXS2 cells injected on day 0, and harvested on day 28.

ANBL0322: A Phase II Trial of the HU14.18-IL2 Fusion Protein in Children with Refractory Neuroblastoma

Paul Sondel Chair
Suzy Shusterman Vice Chair
**ANBL0322 Patient Accrual Goals**

- **Stratum 1 (n=20):** residual/refractory NBL measurable by standard radiographic criteria

- **Stratum 2 (n=20):** residual/refractory NBL not measurable by standard radiographic criteria, but evaluable by MIBG scanning or by bone marrow histology

- **Stratum 3 (n=20):** residual/refractory neuroblastoma in clinical remission but with disease identified by BM ICC (>5 NBL cells per 1,000,000 cells)

*MRD Strata*
<table>
<thead>
<tr>
<th>Generic</th>
<th>Brand</th>
<th>Target</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>Rituxan</td>
<td>CD20</td>
<td>B cell NHL</td>
</tr>
<tr>
<td>Trastutumab</td>
<td>Herceptin</td>
<td>HER-2</td>
<td>HER-2 Breast CA</td>
</tr>
<tr>
<td>Gemtuzumab</td>
<td>Mylotarg</td>
<td>CD33</td>
<td>AML (mAb-toxin)</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>Campath</td>
<td>CD52</td>
<td>B-CLL, CTCL</td>
</tr>
<tr>
<td>Ibritumomab/Tosifumomab</td>
<td>Zevalin/Bexxar</td>
<td>CD2</td>
<td>Refractory B NHL (Radiolabeled mAb)</td>
</tr>
<tr>
<td>Basiliximab/Daclizumab</td>
<td>Anti-TAC</td>
<td>CD25</td>
<td>Anti-Graft Rejection/GVH</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Avastin</td>
<td>VEGF</td>
<td>GI Malignancies</td>
</tr>
<tr>
<td>Edrecolomab</td>
<td>17-1A</td>
<td>EpCam</td>
<td>GI Malignancies</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Erbitux</td>
<td>EGFR</td>
<td>GI Malignancies</td>
</tr>
</tbody>
</table>
Potential role for mAbs in standard therapy – clinical goal

- Include a mAb-containing regimen (possibly combined with other therapy) in the standard care for patients with high-risk cancers (i.e. likely to relapse)

- **Goal – to prevent recurrence**
Antitumor applications of mAb

- Some are already working! (i.e., FDA approved components of standard therapy)
- Continued development and efficacy appear likely.
Important issues not covered

1. The IMMUNOGENICITY of mAbs in patients

• Does the patient’s immune response (HAMA, HACA, anti-id) HELP or HINDER the anti-tumor effect?
Patient Anti-id Antibody Inhibits Binding of hu14.18 to Tumor cells (Flow Cytometry Assay)
Mimicry of a TAA determinant by anti-id antibodies

(Adapted from S. Ferrone)
Important issues not covered

2. The ability of tumors to ESCAPE from immunotherapy (ie: antigen loss or MHC modulation)

• Equivalent to selecting cancer cells with multi-drug resistance
Suboptimal hu14.18-IL2 treatment causes transient antitumor effects in mice with palpable neuroblastomas
Neal et al. NXS2 NBL express increased MHC-I upon recurrence from NK dependent immunotherapy. Cancer Imm. and Imm. 53:41-52, 2004

Results: a > 9-fold increase in H2Kk expression and a > 10-fold increase in H2Dd expression
NXS2 Tumors modulate class I, down OR up, to escape T or NK mediated Immunotherapy

Neal et al. Canc. Imm. And Imm. 53:41, 2004
Collaborators in UWCCC
Immunocytokine Research-2007

- UWCCC
  - J Hank
  - M Albertini
  - J Gan
  - A Rakhmilevich
  - I Buhtoivarov
  - H Lum
  - H Schalch
  - D Mahvi
  - KM Kim
  - J Eickhoff
  - A Sternberg
  - S Dean
  - R Cassaday

- C.O.G and N.A.N.T.
  - Many Pediatric Oncologists

- Lexigen-EMD
  - S Gillies and colleagues

- EMD-Merck
  - B Clements
  - Karl Joseph Kallen
  - Many others

- Scripps
  - R Reisfeld

- NCI-BRB
  - Toby Hecht and colleagues
PROOF THAT CANCER RESEARCH MAKES A DIFFERENCE!
Important issues not covered

3. The potential for combining mAb therapy with other immunotherapies to prevent ESCAPE from immunotherapy

• Combine mAbs, or use mAbs together with cytokines or vaccines
Important issues not covered

4. The potential for combining mAb therapy with other treatments (ChemoRx, RadioRx)

- Timing may be key. Should mAb be given during chemo cycle or after immune recovery?
Anti-tumor applications of mAb

Adapted from Cheung and Sondel 2005