Antibody-Based Immunotherapy of Lymphoma

Immunology Department Student Lecture

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Myron S. Czuczman, MD
Chief, Lymphoma/Myeloma Service
Head, Lymphoma Translational Research Lab
Roswell Park Cancer Institute
**NHL: Incidence and Mortality**

- **United States:**
  - 54,370 new cases
  - 20,730 deaths
  - Sixth most common type of cancer
  - Increasing since early 1970s

![Incidence and Mortality Chart](chart_image)

**Rate per 100,000**

- Incidence
- Mortality

<table>
<thead>
<tr>
<th>Year</th>
<th>Incidence</th>
<th>Mortality</th>
</tr>
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<tbody>
<tr>
<td>1973</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>1978</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>1983</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>19.3</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>19.4</td>
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NHL: Risk Factors

- Cause of NHL unknown
- Inherited Familial: accounts for a small percentage of cancers
- Environmental
  - Certain chemical suspected (e.g., certain pesticides/herbicides)
  - High-dose radiation exposure suspected
- Immunosuppression
  - Immune deficiency (AIDS, post–organ transplant)
- Viral and Bacterial
  - Infections (HTLV-1 virus, EBV, *H pylori* bacteria)
Lymphocytes

- **T cells**
  - Release cytokines
- **B cells**
  - Produce antibodies
- **Natural killer (NK) cells**
  - Kill infected cells
  - Attack cancer cells
- **Non-Hodgkin lymphoma**
  - 85% B cells
  - 15% T cells
Treating Non-Hodgkin Lymphoma
Features of an Ideal Anticancer Target

- Crucial to the malignant phenotype
- Not significantly expressed in vital organs / tissues
- A biologically relevant molecular feature
- Reproducibly measurable in readily obtainable clinical samples
- Correlated with clinical outcome
- Clinical response in significant % of target-positive patients when target is interrupted, interfered with, or inhibited
- Minimal effects in target –negative patients
Monoclonal Antibody Therapy

- Biotherapy targeted treatment
- Effective, low toxicity
- Targets tumor cells
- Two types
  - Unconjugated
  - Conjugated
Limitations of early mAbs

• Poor target selection
• Limited biological activity of unlabeled mAbs
• Poor tumor cell penetration of mAbs
• Immunogenicity (i.e. high HAMA titers)
• Infusional toxicity (i.e. purity)
• “Biotechnology to the Rescue” – 1980’s / early 1990’s
B-Cells: Express Many Surface Antigens That May Serve as Targets for mAbs

- Antigen expression variable\textsuperscript{1,2}
- Most involved in B-cell growth, differentiation, proliferation, and activation; other functions include\textsuperscript{1,2}:
  - Immune regulation
  - Complement inhibition
- Many are targets of therapeutic mAbs for current or potential use in B-cell malignancies\textsuperscript{1,2}

\textsuperscript{1}Bello C, Sotomayor EM. Hematology Am Soc Hematol Educ Program. 2007;2007:233-242
\textsuperscript{2}Hotta T. Acta Histochem Cytochem. 2002;35(4):275-279
Rationale for mAb / RIT of NHL

- B-cell lymphomas
  - Express tumor-associated antigens
  - Accessible to the vascular system
  - Rx of minimal residual disease may alter natural history

- mAbs
  - Greater tumor specificity and less non-specific toxicities
  - Unique MOA
  - Demonstrated activity alone and in combination therapy

- Radioimmunoconjugates (RIC)
  - B-cell NHL is radiosensitive
  - “Cross-fire” effect
  - Not dependent on host-immune function
Anti-CD20 MAbs: Mechanism-of-Action

Complement-mediated lysis

Clq binding

MAC

Cell lysis

Ofatumumab binding site

Rituximab, tositumomab, obinutuzumab binding site

CD20

Cell membrane

ADCC

FcγRIIIa

Effector cell

CD20 antigen

Direct effects

Antibody binding induces antiproliferative signaling, apoptosis, and cell-growth inhibition

Antibody structure

Murine variable sequence

Chimeric antibody (rituximab)

Human sequence

Human antibody (ofatumumab)
Rituximab: First mAb approved by FDA for Cancer Therapy

- Fab binds CD20 antigen present in B-cells
- Crosslinking of the Fc portion mediates rituximab antitumor activity
- Human κ constant regions
- CD20 protein in cell membrane
- Follicular lymphoma

Diagram showing cellular interaction and mechanisms:
- Active Immune Response
- Complement
- B Cell Lymphoma
- Apoptosis
- ADCC
- CMC
- PBMC
- FC Receptor
Anti-CD20 Monoclonal Antibodies Induce ADCC

- Fc region of CD20-bound MAb binds to Fc receptor (FcR) on effector cell (e.g. macrophage, NK cell, neutrophil, etc)
- Effector cell releases mediators that damage and destroy CD20-positive cell
- CD20-positive cell is phagocytosed
CD20-Bound mAbs Activate the Complement Cascade

- CD20-bound mAbs bind to the first complement component, activating the complement cascade.
Complement Activation Causes MAC Formation and B-Cell Lysis

- Activation of complement components on the B-cell surface leads to their incorporation into the membrane attack complex (MAC)

- MAC forms a pore through target cell membrane, causing osmotic cell lysis
Anti-CD20 MAbs May Directly Induce B-Cell Apoptosis

- mAb binding to CD20 may induce transmission of intracellular signals that trigger cell cycle arrest and programmed cell death
FDA-approved indications for rituximab

- Relapsed/refractory, low-grade or follicular, CD20+ B-cell NHL as a single agent
- Previously untreated FL in combination with ... or following... CVP
- As maintenance Rx for FL pts who achieve a response to R + chemo
- Previously untreated DLBCL (CD20+) in combination with CHOP or other anthracycline chemo regimens
- CLL (R + FC): either Rx-naïve or previously treated
# Next Generation anti-CD20 mAbs (+ more)

<table>
<thead>
<tr>
<th>Name</th>
<th>Comparison to Rituximab</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofatumumab(^1,2)</td>
<td>• Human mAb</td>
<td>• FDA-approved in r/r CLL</td>
</tr>
<tr>
<td></td>
<td>• Novel membrane proximal CD20 epitope</td>
<td>• S/P Ph III in rituximab-refractory FL</td>
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<tr>
<td></td>
<td>• Stronger CDC</td>
<td>• Ph III: in CLL, FL, DLBCL</td>
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<tr>
<td></td>
<td>• Slower dissociation rate</td>
<td>• Several Ph II trials (also RA and MS)</td>
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<tr>
<td></td>
<td>• Stronger binding to B-cells</td>
<td></td>
</tr>
<tr>
<td>GA101(^1) = Obinutuzumab</td>
<td>• Type II anti-CD20 (glycol-engineered Fc Region)</td>
<td>• S/P Ph I trials</td>
</tr>
<tr>
<td></td>
<td>• Increased ADCC/Apoptosis</td>
<td>• Ph III Benda vs. Benda + GA101 in rituximab-refractory indolent NHL</td>
</tr>
<tr>
<td></td>
<td>• Stronger binding to effectors</td>
<td>• Several Ph II trials</td>
</tr>
<tr>
<td></td>
<td>• Limited CDC</td>
<td></td>
</tr>
<tr>
<td>Veltuzumab(^1)</td>
<td>• Humanized IgG1 mAb</td>
<td>• S/P Ph I/II studies (IV)</td>
</tr>
<tr>
<td></td>
<td>• Single a.a. change in CDR3-(V_H) (Asn to Asp)</td>
<td>• Phase I/II sub q in NHL/CLL</td>
</tr>
<tr>
<td></td>
<td>• Epratuzumab framework</td>
<td>• Phase I subq in ITP</td>
</tr>
<tr>
<td></td>
<td>• Slower dissociation rate</td>
<td>• Phase I combo with Milatuzumab (anti-CD74): Christian et al; ASH 2011, Abstr # 3707</td>
</tr>
<tr>
<td></td>
<td>• Stronger CDC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Enhances epratuzumab activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Low-dose subq formulation</td>
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<table>
<thead>
<tr>
<th>CD20: Type I and Type II mAbs</th>
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<tr>
<td><strong>Type I mAbs</strong></td>
</tr>
<tr>
<td>Localize CD20 to lipid rafts</td>
</tr>
<tr>
<td>High CDC</td>
</tr>
<tr>
<td>ADCC activity</td>
</tr>
<tr>
<td>Full number of binding sites / B-cell</td>
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<tr>
<td>Weak homotypic aggregation</td>
</tr>
<tr>
<td>Limited direct apoptosis</td>
</tr>
<tr>
<td>Rituximab</td>
</tr>
<tr>
<td>Ofatumumab</td>
</tr>
<tr>
<td>Veltuzumab</td>
</tr>
<tr>
<td>Ocrelizumab</td>
</tr>
<tr>
<td>AME-133</td>
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<td>PRO131921</td>
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GA101: Type II Glycoengineered anti-CD20 mAb (Obinutuzumab)

Increased Direct Cell Death
Type II vs. Type I antibody

Lower CDC
Type II vs. Type I antibody

Enhanced ADCC
Glycoengineering for increased affinity to FcγRIIIa

ADCC, antibody-dependent cell-mediated cytotoxicity
CDC, complement-dependent cytotoxicity

Mössner et al. Blood 2010
Radioimmunotherapy

- Targets tumor cell
- Monoclonal antibody and radioisotope conjugate
Efficacy of Radioimmunotherapy Enhanced Through the Crossfire Effect

Unlabeled “cold” Antibody

Radiolabeled Antibody

Courtesy of Andrew Zelenetz, M.D.
Conjugated

- **Yttrium-90 ibritumomab tiuxetan (Zevalin®)**
  - β only
  - shorter t1/2 (64 hrs)
  - dosing based on wt + PLTs

- **Iodine-131 tositumomab (Bexxar®)**
  - β and γ
  - longer t1/2 (192 hrs)
  - γ-emission allows dosing
  - need SSKI to protect thyroid
BiTE® Technology: Blinatumomab

An investigational bispecific single-chain antibody construct with dual specificity for the CD19 and CD3 antigens on B cells


Apoptosis of tumor cells
Membrane blebbing
Activation of caspases
Cleavage of PARP
Fragmentation of DNA
Morphological changes
BiTE® Technology: Blinatumomab

- Pharmacodynamic analysis in NHL patients showed complete depletion of B lymphocytes from the circulation at blinatumomab doses ≥5 μg/m²/d, the depletion being faster at higher doses.

- Encouraging single-agent activity in both adult and pediatric patients with ALL, as well as adult patients with NHL

- Currently under investigation in 5 trials:
  - Phase 1 trial for adult patients with relapsed/refractory NHL
  - Two Phase 2 trials for adult patients with relapsed/refractory ALL
  - Phase 1/2 trial for pediatric patients with relapsed/refractory ALL
  - Phase 2 trial for adult ALL patients with minimal residual disease (MRD)
Blinatumomab: Safety

- **CNS-related adverse events** resulting in discontinuation

- **Most common clinical adverse events** are **flu-like** and are of grade 1 or 2 (pyrexia, headache, chills, fatigue)
  - Transient: Seen only during first days following start of infusion
  - Caused by onset of **T cell activation** (first dose reaction)

- **Most common laboratory abnormalities** are lymphopenia and leukopenia
  - Related to mode of action: Initial T cell redistribution and sustained B target cell depletion
Antibody-Drug Conjugates (ADCs)

- Arose as an effort to combine cytotoxic chemotherapy and antibody specificity in order to obtain the benefit of their complementarity

- The antibody can be used to direct the cytotoxic agent to the tumor cell and thereby accomplish 2 objectives:
  - Diminish the side effect profile of the cytotoxic agent
  - Enable delivery of a more potent therapeutic because of the ability to control the target and the side effects
Components of an ADC

- The cancer, or target, antigen
- The antibody to that target
- The linker that connects the drug to the antibody
- The drug itself
The Target Antigen

• Should have high expression on a tumor
• Should have little or no expression in normal tissue
• Should be present on the cell surface
• Should be an internalizing antigen
Optimal Target Antigen for ADC

Target antigen

- Tumor cell: Expressed abundantly on tumor cells
- Normal healthy cell: Limited or no expression on normal or vital tissues
The Linker: Cleavable vs Noncleavable

• The linker of an ADC should be stable in the circulation so that the cytotoxic agent is not released systemically where it can be internalized into normal, nontarget cells
  • The linker should also maintain attachment of the cytotoxic agent (the conjugate to the antibody) until the ADC reaches the tumor and is internalized\(^1\)

• The early, cleavable linkers were too labile\(^2\) which led to release of free drug in the circulation and consequent off-target toxicity

• Approximately 10 years ago, a non-cleavable linker was developed
  • This type of linker is extremely stable in the circulation, and it prevents premature release of the cytotoxic agent into the circulation\(^2\)

Early cleavable linkers used in ADC

Release of drug into circulation with consequent off-target toxicity
Development of a non-cleavable linker for ADC

“Stable” in circulation and prevents premature release of cytotoxic agent into circulation
Cytotoxic Agents

- The most common cytotoxic agents currently used in ADCs—maytansinoids and monomethyl auristatin E—have IC50s that are 100-1,000-fold more potent than those of conventional chemotherapeutic agents from the same or a similar class.

- Most current ADCs use a ratio of cytotoxic drug to antibody in the range of 2:1 to 4:1.
Brentuximab Vedotin (SGN35)

- Antibody-drug conjugate (ADC) directed to CD30
- Expressed on virtually all Reed Sternberg and ALCL cells
- Present in several T-cell lymphoproliferative diseases
- In healthy tissue: limited to activated B and T lymphs and NK cells
- Granted accelerated FDA approval in August 2011 for 2 indications:
  - Hodgkin lymphoma patients who relapse after autologous transplant or fail at least two prior multi-agent chemotherapy regimens if transplant ineligible
  - Systemic anaplastic large cell lymphoma (ALCL) patients who fail at least one prior multi-chemo regimen
Mechanism of action of brentuximab vedotin

Diffusible MMAE responsible for bystander cell killing

Cytoplasmic membrane

MMAE is cleaved from SGN35

MMAE potently disrupts microtubule polymerization

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CCR Drug Updates

Significant Adverse Events

- Grade 3-4 (from Phase II studies)
  - Peripheral neuropathy 8-10%
  - Neutropenia 20%
  - Febrile neutropenia 0%
  - Thrombocytopenia 8-14%

- Progressive Multifocal Leukoencephalopathy

- Pulmonary Toxicity when given in combination with Bleomycin
### Pulmonary Toxicity

<table>
<thead>
<tr>
<th>Preferred term</th>
<th>ABVD with brentuximab vedotin N=25</th>
<th>AVD with brentuximab vedotin N=26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event</td>
<td>11 (44)</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary toxicity</td>
<td>9 (36)</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial lung disease</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>1 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

- **Events generally occurred during Cycles 3–4**
- **Two patient deaths** were associated with pulmonary toxicity
- **Events resolved in 9 of 11 patients (82%)**
- **8 of 11 patients with events discontinued bleomycin and were able to complete treatment with AVD combined with brentuximab vedotin**

Other ADCs in Clinical Trials for Lymphoid Malignancies

- Inotuzumab ozogamicin (CMC-544), a humanized anti-CD22 antibody conjugated to calicheamicin, a potent DNA-binding antibiotic

- SAR3419, a humanized IgG1 anti-CD19 monoclonal antibody conjugated to the maytansinoid derivative DM4

- Anti-CD22 or -CD79b conjugated to MMAE
Structure of CMC-544, a CD22-targeted immunoconjugate of CalichDMH

Anti-CD22 and -CD79b: MOA

**Step 1**
ADC specifically binds to corresponding BCR

**Step 2**
Once bound, ADC internalized into target cell

**Step 3**
Cytotoxic gent released inside target cell, leading to microtubule disruption and cell death

Anti-CD79b (DCDS4501A) and -CD22 (DCDT2980S) in NHL: Phase 1 Safety

Common AEs (all grades)
- Diarrhea
- Fatigue
- Nausea
- Neutropenia (59% for anti-CD79b and 26% for CD22)

Decreased appetite
- Vomiting
- Peripheral edema

≥3 AEs in ≥ 10% of patients
- Anti-CD22: Neutropenia (24%)
- Anti-CD79b: Neutropenia (39%) and leukopenia (12%)
Conclusion/Future

• **Exciting era of biotechnology:** Continue to advance our understanding of mAb structure vs function and lead to the production of even “more effective” mAbs and innovative immunoconjugates in the future.

• **Future:** “**Individualized Rx**”: Choose which specific mAb(s) to use based on: target (e.g. epitope) and the predominant MOA (unlabeled biologically-augmented vs drug vs toxin vs radiolabel) we wish to achieve.

• **Major challenge:** Determining these novel agents true value and how to optimize their use in today’s clinical arena.