Antibody-Based Immunotherapy of Lymphoma

Immunology Department Student Lecture

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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
NHL: Risk Factors

• Cause of NHL unknown
• Inherited Familial: accounts for a small percentage of cancers
• Environmental
  – Certain chemical suspected (eg, 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\(^1,\,^2\)
- Most involved in B-cell growth, differentiation, proliferation, and activation; other functions include\(^1,\,^2\):
  - Immune regulation
  - Complement inhibition
- Many are targets of therapeutic mAbs for current or potential use in B-cell malignancies\(^1,\,^2\)

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

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

ADCC
- Effector cell
- FcγRIIIa

Ofatumumab binding site
- Rituximab, tositumomab, obinutuzumab binding site

CD20 antigen

Murine variable sequence
- Chimeric antibody (rituximab)
- 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

- Follicular lymphoma

- Cell membrane

- Complement

- CMC

- APOPTOSIS

- ADCC
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
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>
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</table>
| Ofatumumab\(^1,2\)   | • Human mAb  
• Novel membrane proximal CD20 epitope  
• Stronger CDC  
• Slower dissociation rate  
• Stronger binding to B-cells                                                                                                                             | • FDA-approved in r/r CLL  
• S/P Ph III in rituximab-refractory FL  
• Ph III: in CLL, FL, DLBCL  
• Several Ph II trials (also RA and MS)                                                                                                                  |
| GA101\(^1\) = Obinutuzumab | • Type II anti-CD20 (glycol-engineered Fc Region)  
• Increased ADCC/Apoptosis  
• Stronger binding to effectors  
• Limited CDC                                                                                                             | • S/P Ph I trials  
• Ph III Benda vs. Benda + GA101 in rituximab-refractory indolent NHL  
• Several Ph II trials                                                                                                                                          |
| Veltuzumab\(^1\)     | • Humanized IgG1 mAb  
• Single a.a. change in CDR3-\(V_H\) (Asn to Asp)  
• Epratuzumab framework  
• Slower dissociation rate  
• Stronger CDC  
• Enhances epratuzumab activity  
• Low-dose subq formulation                                                                                                                                   | • S/P Ph I/II studies (IV)  
• Phase I/II sub q in NHL/CLL  
• Phase I subq in ITP  
• Phase I combo with Milatuzumab (anti-CD74): Christian et al; ASH 2011, Abstr # 3707                                                                |

## CD20: Type I and Type II mAbs

<table>
<thead>
<tr>
<th></th>
<th>Type I mAbs</th>
<th>Type II mAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localize CD20 to lipid rafts</td>
<td>Do not localize CD20 to lipid rafts</td>
<td></td>
</tr>
<tr>
<td>High CDC</td>
<td>Minimal CDC</td>
<td></td>
</tr>
<tr>
<td>ADCC activity</td>
<td>ADCC activity</td>
<td></td>
</tr>
<tr>
<td>Full number of binding sites / B-cell</td>
<td>Half number of binding sites / B-cell</td>
<td></td>
</tr>
<tr>
<td>Weak homotypic aggregation</td>
<td>Strong homotypic aggregation</td>
<td></td>
</tr>
<tr>
<td>Limited direct apoptosis</td>
<td>Strong direct apoptosis</td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>Tositumumab (B1)</td>
<td></td>
</tr>
<tr>
<td>Ofatumumab</td>
<td>GA101=Obinutuzumab</td>
<td></td>
</tr>
<tr>
<td>Veltuzumab</td>
<td></td>
<td></td>
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<tr>
<td>Ocrelizumab</td>
<td></td>
<td></td>
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<tr>
<td>AME-133</td>
<td></td>
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<tr>
<td>PRO131921</td>
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</table>
GA101: Type II Glycoengineered anti-CD20 mAb (Obinutuzumab)

Increased Direct Cell Death
Type II vs. Type I antibody

Enhanced ADCC
Glycoengineering for increased affinity to FcγRIIIa

Lower CDC
Type II vs. Type I antibody

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

Mössner et al. Blood 2010
Ofatumumab versus Rituximab Salvage Chemoimmunotherapy in r/r DLBCL: The Orcharrd Study*

- Response to salvage Rx is critical for a durable PFS post-autologous SCT
  - 3 yr EFS in pts receiving R-based induction, followed by R-containing salvage Rx was only 21% (CORAL Study)
  - Ofatumumab: efficacy in RRCL + activity in pts with r/r DLBCL
  - Orcharrd Study: Compare efficacy of O vs R in combo with DHAP in r/r DLBCL

- Methods:
  - Randomize r/r DLBCL pts 1:1 to 3 cycles of R-DHAP vs D-DHAP
  - 1° end-point: PFS

*van Imhoff et al. ASH 2014. Abstract 630
Ofatumumab versus Rituximab Salvage Chemoimmunotherapy in r/r DLBCL: The Orchard Study*

• Results:
  – n=447 pts randomized
  – Pt characteristics were evenly distributed

<table>
<thead>
<tr>
<th>Rx-Arm</th>
<th>%ORR (CR)</th>
<th>2 yr-PFS</th>
<th>2 yr-EFS</th>
<th>2 yr-OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Arm</td>
<td>38% (15%)</td>
<td>21%</td>
<td>14%</td>
<td>41%</td>
</tr>
<tr>
<td>R-Arm</td>
<td>42% (22%)</td>
<td>26%</td>
<td>17%</td>
<td>36%</td>
</tr>
</tbody>
</table>

• Conclusions:
  – No significant diff between O-DHAP vs R-DHAP
  – “Better salvage Rx options needed for upfront R-CHOP failures”

*van Imhoff et al. ASH 2014. Abstract 630
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.
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.

- The early, cleavable linkers were too labile, 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.

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

Brentuximab Vedotin

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
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

SGN-CD19A in R/R NHL: Study Schema

- Ongoing phase I open-label, dose-escalation study of SGN-CD19A (anti-CD19 antibody drug conjugate) in R/R B-cell NHL
- Primary endpoints: safety, MTD
- Secondary endpoints: efficacy, PK, antitumor activity

R/R B-cell NHL (n = 44)
- ≥12 years of age
- Confirmed DLBCL or FL grade 3
- ECOG 0-1
- ≥1 prior systemic Rx
- Prior intensive salvage therapy ± ASCT (DLBCL or FL3 only)

SGN-CD19A 0.5-6 mg/kg IV, day 1 of 21-day cycles

MTD Response by IWG 2007

Moskowitz et al. ASH 2014. Abstract 1741
SGN-CD19A in R/R NHL: Safety

<table>
<thead>
<tr>
<th>TEAEs (≥ 10%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blurred vision</td>
<td>59%</td>
</tr>
<tr>
<td>Dry eye</td>
<td>39%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>39%</td>
</tr>
<tr>
<td>Constipation</td>
<td>32%</td>
</tr>
<tr>
<td>Keratopathy</td>
<td>23%</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>20%</td>
</tr>
</tbody>
</table>

- Median duration of treatment was 9.2 weeks (range, 3-36)
- Low incidence of grade 3/4 AEs: 10% thrombocytopenia, 8% anemia, and 2% neutropenia
- MTD not yet; escalating to 6 mg/kg
- 1 DLT of corneal epithelia keratopathy (3 mg/kg)
  - “Ocular AEs were most common in 35 (67%) patients”
  - Time to onset of grade 3+ ocular events was ~2 cycles; improved in most patients through dose modification

Moskowitz et al. ASH 2014. Abstract 1741
SGN-CD19A in R/R NHL: Summary

- SGN-CD19A has shown encouraging clinical activity, with a 35% ORR and 20% CR
  - 55% ORR (32% CR) in relapsed patients (n = 22)
  - 21% ORR (10% CR) in refractory patients (n = 29)
- SGN-CD19A is well tolerated; MTD not exceeded at 6 mg/kg
- Observed ocular AEs are manageable with steroid eye drops and dose modifications
- Activity in DLBCL with minimal myelosuppresion provides rationale for randomized phase II study of RICE +/- SGN-CD19A in pre-ASCT patients

Moskowitz et al. ASH 2014. Abstract 1741
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

Potential Strategies to Improve on Rituximab Monotherapy Results

Novel anti-CD20 constructs
Enhancing Effector cell #’s and function
Targeting different surface Ags
Immunotoxins/Immunoconjugates/RIT
“Restoring” Pro-Apoptotic Potential (ABT-199)
Restoring/Augmenting T-cell function (anti-PD1)
Immunomodulatory drugs (IMiDs)

Replace “R” Maintenance with short course of novel consolidation!!!
Effects of Lenalidomide on Tumor Cells and their Microenvironment

Lenalidomide:
- ↓ ICAM
- ↓ VEGF
- ↓ TNF-α

Tumor Cell:
- TNF-α
- PDGF
- IL-10
- TGF-β

Stromal Cell:
- VEGF
- Lenalidomide:
  - ↓ Proliferation
  - ↑ Apoptosis
  - ↓ pAkt
  - ↓ pErk

T - Cell:
- TGF-β

NK Cell:
- Lenalidomide:
  - Activates NK cells
  - NK cell proliferation

FL-001: Phase 3 Study Design

![Diagram]

- **R²** = Rituximab + Lenalidomide
- **R-Chemo** (investigator choice of R-CHOP, R-CVP, R-B)
- Lenalidomide 20 mg x 6 cycles, if CR then 10 mg
- Co-primary endpoints
  - surrogate endpoint (for initial approval): a) CR/CRu rate at 1.5 years; b) PFS

1st line FL n = 1000

Primary end-point: PFS

- **R²** = Rituximab + Lenalidomide
- **R-Chemo** (investigator choice of R-CHOP, R-CVP, R-B)
- Lenalidomide 20 mg x 6 cycles, if CR then 10 mg
- Co-primary endpoints
  - surrogate endpoint (for initial approval): a) CR/CRu rate at 1.5 years; b) PFS
Preliminary Results of a Phase I Study of Nivolumab (BMS-936558) in Patients With Relapsed or Refractory Lymphoid Malignancies

Lesokhin et al. Abstract 291
Nivolumab (BMS) in R/R NHL: Study Schema

- Phase I open-label study of nivolumab (anti–PD-1 receptor antibody) in R/R lymphoid malignancies (B-NHL, T-NHL, and MM)
- Primary endpoint: safety and tolerability
- Secondary endpoints: best ORR (investigator-assessed), objective response, DOR, PFS, biomarkers

R/R patients HM (n = 105)
- No autoimmune disease
- No prior organ or stem cell allografting
- No prior checkpoint blockade

Dose Escalation
Nivolumab
1 mg/kg to 3 mg/kg
wks 1, 4 then q2w
n = 13
B cell NHL (n = 8)
CML (n = 1)
MM (n = 4)

Dose Expansion
(3 mg/kg)
Hodgkin (n = 23)
B cell lymphoma (n = 23)
T cell lymphoma (n = 23)
MM (n = 23)

Lesokhin et al. ASH 2014. Abstract 291
### Nivolumab in R/R NHL: Efficacy

<table>
<thead>
<tr>
<th>Types</th>
<th>n</th>
<th>ORR, n (%)</th>
<th>CR, n (%)</th>
<th>PR, n (%)</th>
<th>SD, n (%)</th>
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</thead>
<tbody>
<tr>
<td>B cell lymphoma</td>
<td>29</td>
<td>8 (28)</td>
<td>2 (7)</td>
<td>6 (21)</td>
<td>14 (48)</td>
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<tr>
<td>DLBCL</td>
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<td>4 (36)</td>
<td>1 (9)</td>
<td>3 (27)</td>
<td>3 (27)</td>
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<tr>
<td>FL</td>
<td>10</td>
<td>4 (40)</td>
<td>1 (10)</td>
<td>3 (30)</td>
<td>6 (60)</td>
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<tr>
<td>T cell lymphoma</td>
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<td>Mycosis fungoides</td>
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<td>9 (69)</td>
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<tr>
<td>PTCL</td>
<td>5</td>
<td>2 (40)</td>
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<td>2 (40)</td>
<td>0</td>
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<tr>
<td>Multiple myeloma</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18 (67)</td>
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<tr>
<td>Primary mediastinal B-cell lymphoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (100)</td>
</tr>
</tbody>
</table>

Lesokhin et al. ASH 2014. Abstract 291
Nivolumab in R/R NHL: Summary

- Nivolumab has a safety profile similar to that reported in other nivolumab trials, in patients with r/r heme neoplasm.
- Activity was demonstrated across multiple malignancies with ORRs of 40% and 36% in FL and DLBCL, respectively.
- In MM, stable disease was seen in the absence of an ORR.
- Genetic alterations of 9p24.1 were uncommon in this small NHL series.
- Multicenter, phase 2 studies are ongoing in DLBCL and FL.

Abstract 289: Nivolumab: 87% ORR in r/r cHL (n=23); PFS=86% @ 6m; median OS=NR
Abstract 290: Pembrolizumab (Merck): 53% ORR in r/r cHL (n=15)
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

Nagorsen and Baueuerle. Exp Cell Res 2011
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
Targeting Leukemia with Chimeric Antigen Receptor Modified T cells

- CARs combine an Ag recognition domain of antibody with intracellular signaling domain into single chimeric protein
- Gene transfer (lentivirus vector) to stably express CAR on T cells confers novel Ag specificity

Courtesy: John Gribben
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.