AME-133: A Next-Generation Anti-CD20 Engineered for Enhanced Killer Function

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Antibody Therapy of Lymphoma

• Rituximab as first-generation biotherapeutic
  – Efficacious in chemoresistant NHL
  – Less toxic than most chemotherapies
  – Multiple potential antitumor activities
    • CDC- Complement dependent cytotoxicity
    • ADCC- Antibody dependent cytotoxicity
    • Direct apoptotic effect (with cross linking)

• Opportunities for improvement
  – Chimeric mouse/human structure
  – Modest affinity for CD20
  – Role of host immune system in efficacy
    • FcR influences response
Influence of FCγRIIIa Polymorphism on Rituximab Efficacy

• Clinical and molecular response to rituximab in chemo-naive follicular NHL
  – Cartron, Blood. 2002; 99:754-758
• Clinical response to rituximab in relapsed follicular NHL
  – Weng, JCO 2003; 21:3940-47
• Clinical response to rituximab in Waldenstrom’s macroglobulinemia
  – Treon, ASH 2002, Poster #2002
• B cell depletion in SLE
  – Anolik, Arth Rheum. 2003; 48:455-459
### FCγRIIIa 158 Genotype

<table>
<thead>
<tr>
<th>Prevalence n (%)</th>
<th>VV</th>
<th>VF or FF</th>
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<tbody>
<tr>
<td>PR+CR M1-3 %</td>
<td>92*</td>
<td>59</td>
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<tr>
<td>PR+CR M6 %</td>
<td>85*</td>
<td>45</td>
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<td>PR+CR M9 %</td>
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<td>PR+CR M12 %</td>
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*p < 0.05 VV vs. VF+FF

Progression Free Survival After Rituximab Correlates with FcγRIIIa Genotype

AME & Antibody Engineering Opportunities

- Potency
  - Association rate
  - Dissociation rate
  - Complement dependent cytotoxicity
  - Cell mediated cytotoxicity (ADCC)
  - Half-life
- Immunogenicity
  - Framework chimeric residues
  - Somatic mutations (framework, V/C)
- Specificity (epitope, cross-reactions)
- Pharmaceutical properties
  - Oxidation sites
  - Deamidation sites
  - Glycosylation sites
  - Protease sites
  - Solubility
  - Production
  - Cost of facilities and goods

Addressed in anti-CD20 engineering
AME Antibody Optimization Technology

- Generate DNA library with directed variability
- Express protein library
- Screen for desired activity
- Iterative cycles to optimize

Increase potency by codon substitution in the antigen-binding region (CDRs)

Murine → Increase potency by codon substitution in the antigen-binding region (CDRs) → Human
Anti-CD20 Antibodies with Fully Human Frameworks and Increased Affinity

- Multiple high affinity variants identified
  - Variants provide tool for characterizing impact of affinity on ADCC, CDC, and apoptosis
- Fully human, common germline frameworks
Fc Optimization - Cell Based Primary Screening with Human PBMC

- >2,400 variants screened
- Multiple novel variants identified

<table>
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<tr>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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AME-133 is More Potent than Rituxan in ex vivo ADCC

Human PBMCs and Wil-2S Target cells; mean +/- 1 SD
AME-133 Retains CDC Activity

![Graph showing the comparison between Rituximab and AME-133 with RFU (Relative Fluorescence Units) on the y-axis and Ab Conc. (µg/ml) on the x-axis. The graph indicates that AME-133 retains CDC Activity.]
Apoptosis

• Rituxan induces apoptosis of Ramos cells (weakly) in the absence of antibody cross-linking
• Induction of apoptosis is enhanced significantly by secondary cross-linking reagents
• AME anti-CD20 variants induce apoptosis in the presence of secondary cross-linking reagents
Summary

• Multiple characteristics of proteins can be improved significantly through optimization
  – Improved efficacy, safety, and potency
  – Enhanced convenience
  – Decreased manufacturing costs
  – Broadened intellectual property
  – Increased understanding of biology

• AME-133, an optimized anti-CD20, will be tested for clinical activity in CD20⁺ oncology indications
Humanization of Antibodies

- Reduces immunogenicity associated with murine Iggs
  - CDR grafting typically diminishes affinity
  - Structural modeling is used to predict framework residues key for maintaining affinity
AME- Applied Molecular Evolution

- San Diego directed evolution company founded in 1989
  - All classes of protein therapeutics engineered
- Partnerships with MedImmune, Centocor, Eli Lilly, Bristol-Myers Squibb, Chiron, Seattle Genetics, CancerVax, Biosynexus
- Application of the protein engineering to in-house projects
  - A development function was added to go from gene to clinic
  - AME-527 (TNF, inflammation) & AME-133 (CD-20, oncology)
- AME and Eli Lilly and Company performed multiple collaborative projects
  - Successes led to discussion on broadened collaboration
  - AME was acquired by Lilly in 2004