Case Study of Prior Knowledge: Development and Implementation of a Binding Assay to Assess the Potency of Synagis® (palivizumab)
Outline

• What is prior knowledge?
• Mechanism and epidemiology of RSV disease
• What is Synagis® and how does it prevent RSV disease?
• Potency assay assessment
• Use of the F protein binding ELISA
• How can prior knowledge be used for "next generation" products?
What is Prior Knowledge?

- Published literature
- Non-clinical information
- Clinical information

Overall Knowledge
Influence of Prior Knowledge

- Sponsor’s clinical experience
- Published clinical experience
- Sponsor’s pre-clinical experience
- Published pre-clinical experience
- Published literature*

*for example, manufacturing/QC publication from another company
Credibility Index

Most credible

Peer-reviewed published papers

QA-reviewed company reports

Witnessed lab notebooks

Trade journal articles*

*not peer-reviewed
Respiratory Syncytial Virus (RSV)

- Isolated in 1956
- Orthopneumovirus family (ssRNA viruses)
- Most important viral agent of serious respiratory tract disease in the pediatric population
- No fully effective anti-viral therapy or approved vaccine
Orthopneumovirus Structure

- HN (Hemagglutinin-Neuraminidase)
- F (Fusion protein)
- Lipid bilayer
- M (Matrix protein)
- SH (Small Hydrophobic protein)
- Viral RNA
- L (Large-Polymerase)
- N (Nucleocapsid)
- P (Phosphoprotein)
- RNAP
- V (Cysteine rich)
RSV Life Cycle
Mab Binding to F Protein Antigen

WHO meeting on RSV in High Risk Infants (2016)
RSV Prophylaxis

• Anti-viral therapies and vaccines are not currently an effective option for infants at risk of infection

• Passive immunotherapy using intravenous immune globulins has proven to be safe and effective (RespiGam®)

• Passive immunotherapy using monoclonal antibody (Synagis®--palivizumab) at a reduced dose has proven to be safe and effective
Synagis® (palivizumab)

- Humanized monoclonal antibody (MAb)
- Launched September 1998
- Prevention of serious lower respiratory tract disease caused by RSV in high risk infants
- 300,000+ infants annually in U.S.
Why is Synagis® Important?

• RSV is the most common cause of lower respiratory infections in infants and children worldwide (estimated 3.4 million episodes of severe RSV infection resulting in hospital admission)
• Each year in the United States, more than 90,000 infants are hospitalized with RSV disease
• There are approximately 325,000 infants at high risk of acquiring severe RSV disease in the U.S.
Palivizumab clinical studies for approval

IMPact-RSV Trial 1996-1997
Synagis Approved US 1998, EU 1999
Synagis CHD Study 1998-2002
Synagis Label US CHD 2003

Phase 1: adults
Phase 1/2: high risk infants

55% Reduction $p < 0.001$
45% Reduction $p = 0.003$

Assays for Potency

• Microneutralization
• F protein binding ELISA
• Fusion inhibition
• Cotton rat prophylaxis
Binding Assay Used for Potency Test?

- Mechanism of action is well-established* (preclinical)
- Development of animal models, cell culture based assays, and binding assays (preclinical)
- Accelerated stability studies show parallel or comparable results (preclinical)
- Clinical data shows no significant adverse events (clinical)
- Parallel real-time stability data shows no adverse trends (clinical)
- Continue monitoring animal models/bioassays as a characterization test (marketed product)

Mechanism of Action

- Binding to the F protein (specific neutralizing epitope) on the surface of the virion
- Glycosylation of the antibody plays no significant role
- Complement plays no significant role
- Molar equivalent of Fab has comparable binding
- Fc region plays no significant role in potency of antibody
# Effects of Deglycosylation

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>F protein ELISA (% of Reference Standard)</th>
<th>Microneutralization (ED50 of sample/ED50 of Ref. Std.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNGase treated Ref. Std.*</td>
<td>93%</td>
<td>0.76</td>
</tr>
<tr>
<td>Reference Standard Control</td>
<td>95%</td>
<td>1.13</td>
</tr>
</tbody>
</table>

*Reference Standard lot was digested with PNGase (8 Units of enzyme per mg of Synagis™) for 22 hours at 37°C. The control digestion was performed under identical conditions without enzyme. The completeness of digestion was monitored by observing a shift in molecular weight of the heavy chain on SDS-PAGE and CGE.
Effect of Complement

- Complement has no effect on neutralization
Accelerated Stability

• F protein ELISA and microneutralization showed parallel trends (no significant decline)
• HPSEC showed significant decrease
• Potency assays may not be as sensitive an indicator of stability as biochemical tests
• Rate of decline for assays is product- and assay-specific
Accelerated Stability of Synagis®

- Neither assay showed significant change during accelerated stability

Red bars indicate 95% confidence intervals.
Real-time vs. Accelerated Stability

- HPSEC showed significant change during accelerated stability but ELISA did not.
Similar Clinical Results Between Lots

• Multiple lots (> 12) from different scales made at different locations showed comparable animal and human PK results
• Real-time stability data on multiple lots showed no significant differences
• If clinical lots showed differences, these would need to be discussed with the regulatory authorities because binding alone might not be detecting the changes
Cotton Rat Prophylaxis

- No difference in potency in animals using different batches
• No difference in human PK, regardless of dose or method of administration
# Validation Comparisons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ELISA</th>
<th>Microneut.</th>
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</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td>&lt; 10% CV</td>
<td>&lt; 20% CV</td>
</tr>
<tr>
<td>Linearity</td>
<td>&gt; 0.990</td>
<td>&gt; 0.970</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Specific for Synagis</td>
<td>Specific for Synagis</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>&lt; 20% CV</td>
<td>&lt; 30% CV</td>
</tr>
<tr>
<td>Robustness</td>
<td>&lt; 20% CV</td>
<td>&lt; 30% CV</td>
</tr>
</tbody>
</table>
Conclusions

• Synagis® potency assay developed based on understanding of the mechanism, effectiveness of passive immunotherapy, and parallel stability data

• All results suggested that Fc function played no significant role in product potency

• Microneutralization and F protein binding ELISA behaved the same during accelerated and real-time stability testing over multiple lots

• F protein binding ELISA could be used for potency testing
Application of Prior Knowledge to New Products

• Understanding of mechanism of action (MoA) can enable faster development of next generation products
  – Must demonstrate same MoA in new product

• Simplified control strategy may be achieved sooner, if product is in the same class (e.g., monoclonal antibody)

• For antibodies that have more complex mechanisms of action, using binding assays as the sole measure of potency may not be suitable
Acknowledgements (MedImmune)

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- Gail Folena-Wasserman (Development)
- Julie Lanahan (QC)
- Dave Pfarr (Research)
- Julia Goldstein (Regulatory)
- Franco Piazza (Clinical)
- Filip Dubovsky (Clinical)
Johns Hopkins University (JHU) proposal to study correlation between quality data and immunogenicity

- Mark Schenerman, Ph.D.
  President, CMC Biotech-MAS Consulting

Confidential information
30 June 2017
Hypothesis

Monoclonal dimer levels have no significant correlation with patient immunogenicity
Principle Investigator

Caleb Alexander,
Associate Professor
John Hopkins University
Bloomberg School of Public Health

Departmental Affiliations
- Epidemiology (Primary)
  - Division: Cardiovascular Disease and Clinical Epidemiology
- School of Medicine (Joint)

Center & Institute Affiliations
- Center for Drug Safety and Effectiveness
- Center for Health Services and Outcomes Research
- Center for Mental Health and Addiction Policy Research
- Center of Excellence in Regulatory Science and Innovation (CERSI)
How will the study be done?

• Assemble product quality (dimer levels) and clinical safety (immunogenicity) data on monoclonal antibodies to either prove or disprove the hypothesis (see mock database slide)
  – Data includes different monoclonal antibodies that are IgG1, IgG2, or IgG4 isotypes and have been evaluated in the clinic (Phase 2-3)
• Database is owned by JHU (or CERSI)
• Solicit other monoclonal manufacturers to contribute data
• Sponsors would anonymize the data (lot numbers) so it could be publically studied for trends
Data compilation by JHU

• Proprietary information will be protected by JHU
• Only JHU will know the identities of the companies that participate; sponsors must give permission to be named in public presentations
• Database will eventually have a public-facing view so that other global epidemiologists can analyze the data
Data analysis by JHU Epidemiologist (Caleb Alexander)

• JHU epidemiologists at the Bloomberg School of Public Health will analyze the data for trends in safety (immunogenicity)
• Through the collaborative interaction with industry sponsors, various sub-set analyses may be performed based on patient population, indication, and Ig subtype.
• Findings will be published in a high impact journal (e.g., Nature)
Benefits of the Study

• Builds industry collaboration with a world renowned epidemiology group at JHU
• JHU group lends credibility to the study; makes subsequent publication more impactful
• JHU group is part of joint FDA/JHU foundation (CERSI) that is dedicated to product safety monitoring
• Delivers on the challenge presented by regulators to analyze and correlate quality and safety data
Risks

- New safety signal identified through the study; would need to be reported to regulators
Possible outcomes 1
Hypothesis is proven

• Opens the opportunity for discussion with regulators whether dimer could be considered less risk (non-CQA)
• May enable greater manufacturing flexibility, while maintaining appropriate control of all aggregates
• Science-driven approach to challenging the Health Authority stereotype that “all aggregates are a high risk of immunogenicity”
Possible outcomes 2
Hypothesis is disproven

• Maintains current state
• All aggregates considered to be the highest immunogenicity risk
• No change to current approach to process development or product control
Possible outcomes 3

No clear conclusion

• Maintains current state
• All aggregates considered to be the highest immunogenicity risk
• No change to current approach to process development or product control
What is the Center for Regulatory Science & Innovation (CERSI)?

- FDA's Centers of Excellence in Regulatory Science and Innovation (CERSIs) are collaborations between FDA and academic institutions to advance regulatory science through innovative research, education, and scientific exchanges. Evolving areas of science are promising new approaches to improving our health while demanding new ways to evaluate the safety and effectiveness of the products FDA regulates. FDA's Strategic Plan for Advancing Regulatory Science describes how FDA is harnessing these new technologies in collaboration with academia, industry, and other governmental agencies to develop the tools, standards, and approaches required to assess the safety, efficacy, quality, and performance of innovative products.
- CERSI centers:
  - University of Maryland
  - Georgetown University
  - University of California at San Francisco (UCSF) in a joint effort with Stanford University (UCSF-Stanford)
  - Johns Hopkins University
  - Yale University in joint effort with Mayo Clinic
- [https://www.fda.gov/ScienceResearch/SpecialTopics/RegulatoryScience/ucm301667.htm](https://www.fda.gov/ScienceResearch/SpecialTopics/RegulatoryScience/ucm301667.htm)