Recombinant, Insect Cell-Derived RSV Nanoparticle Vaccine

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Agenda for RSV Discussion

- Overview of Insect Cell Technology
- Respiratory Syncytial Virus Burden of Disease and Pathophysiology
- The RSV Nanoparticle Vaccine
- Preclinical Evaluation
- Clinical Evaluation
- Overview of Future Development
Insect Cell Vaccine Technology for Manufacture of Recombinant Nanoparticle Vaccines

- Select genetic sequences encoding vaccine antigens
- Genes cloned into baculovirus
- Baculovirus infects insect (Sf9) cells
- Antigens expressed and purified as multimeric nanoparticles
- Immunogenic particle with antigens in native configuration

Genes coding for antigens cloned into baculovirus

Baculovirus-infected insect (Sf9) cells

Proteins form nanoparticles
Clinical Applications for and RSV Vaccine

• Major new target for vaccine developers

• Infects all children by age 2 years

• Is the leading cause of bronchiolitis and pneumonia in infants <1 year of age
  – 75,000 to 125,000 hospitalizations annually (in US)

• A leading cause of pneumonia in older adults
  – 14,000 deaths and 175,000 hospitalizations annually

• No vaccine currently available
Respiratory Syncytial Virus (RSV)

- **Fusion (F) protein**
  - Virus entry and syncytia formation
  - Type I trimeric glycoprotein
  - F protein conserved across RSV-A/B strains
  - Target of palivizumab (Synagis®)
  - Ideal vaccine target

- **Attachment (G) protein**
  - Cell attachment
  - Type II tetrameric glycoprotein
  - Ga and Gb subgroups, accounts for year to year strain variation
  - More challenging vaccine target
Recombinant Nanoparticle Vaccines: RSV F Nanoparticle Antigen

1. **F Protein Gene**
2. Insert into Baculovirus
3. Infect Sf9 Insect Cells
4. F Protein expressed BV/SF9 System
5. Trimers Form RSV F Nanoparticles
6. EM of Insect cell-derived RSV F nanoparticles

**Legend:**
- DNA symbol: F Protein Gene
- Arrow: Insert into Baculovirus
- Arrow: Infect Sf9 Insect Cells
- Arrow: F Protein expressed BV/SF9 System
- Arrow: Trimers Form RSV F Nanoparticles
- EM image: EM of Insect cell-derived RSV F nanoparticles
Recombinant RSV F Nanoparticle (F2+F1): Modifications in Fusion Peptide and Cleavage Site

RSV F nanoparticles present antigen in its native configuration, which is important for induction of functional antibodies.
Electron Microscopy: Purified RSV F Forms Trimer-Based Nanoparticles
Preclinical Summary
- RSV F Nanoparticles were administered with and without Adjuphos at day 0 and 21.
- Immune evaluations were performed after the second dose at day 49.
- RSV F with AdjuPhos resulted in higher titers compared to no alum groups (p <0.0001)
- RSV-Infection groups had similar neutralizing titers to vaccine groups
Cotton Rat Immunogenicity – Anti-RSV F IgG

• Robust RSV F specific IgG response in vaccine groups

• FI-RSV group did not induce antibodies against RSV F nanoparticle

• AdjuPhos Adjuvant groups had higher responses compared to unadjuvanted groups (p >0.0001)

• RSV Infection group log$_2$ titer is significantly lower compared to the vaccine groups (p>0.0001)

**Keys**
PBS = phosphate buffered saline,
PBS+Chal = PBS
RSV+Chal = prior infection with RSV
FI-RSV = Formalin-inactivated RSV vaccine
RSV F = RSV F nanoparticle vaccine
Cotton Rat Immunogenicity – Palivizumab Competitive ELISA

- Palivizumab competitive ELISA using pooled sera
- FI-RSV and RSV IN challenge did not induce palivizumab competitive antibodies
- RSV F vaccine induced high levels of palivizumab competing antibodies
Cotton Rat– Lung Viral Titers/Histopathology

Challenge Outcomes
- RSV/A virus was detected only in the PBS control and FI-RSV groups after (IN) challenge with RSV/A Long virus at $10^6$ pfu/0.1mL

Histopathology:
- Adjuphos groups and placebo had lowest histopathology scores
- No evidence of disease exacerbation
- With upper doses of RSV F/Adjuphos, the vaccine antigen shows protection similar to prior RSV infection

* No virus detected at the lowest dilution tested.
Clinical Data from Phase 1 Trial
Study Design

• Randomized, observer-blind, placebo-controlled design

• 150 healthy adults
  – Male and female
  – 18 to 49 years of age inclusive

• Two-dose series at Days 1 and 30, IM administration

• Dose escalation under SMC supervision, with placebo recipients embedded in each cohort
  – 5, 15, 30, and 60 µg of F-protein with adjuphos (n= 20 per group)
  – 30 and 60 µg of F-protein without adjuvant (n= 20 per group)
  – Placebo (cumulative n= 30)

• 7-day reactogenicity diary + pre/post clinical safety labs + standard AE follow-up for 6 months

• Serologic sampling at Days 1, 30, and 60
Safety Summary

• Overall vaccine was well tolerated
• Majority of AEs were local pain and tenderness and the majority were mild
  – Local AEs were higher in vaccine group compared to placebo
  – No dose effect or AE trend based on dose
• No systemic signal in the vaccine groups
• No vaccine-related serious adverse advents (SAEs)
ELISA plates were coated with 2μg/ml RSV F nanoparticle antigen. Palivizumab at 10 μg/ml concentration serially diluted four fold and reacted to RSV F on the plate. Anti–human HRP reaction was used to determine the Palivizumab binding to RSV F nanoparticles. An unweighted four parameter logistic regression curve is presented for both assays.
F Protein ELISA: Anti-F Specific IgG Geometric Mean Titers
F Protein ELISA: Fold-Rise Dose Response for Adjuphos Groups Only

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>5 µg+</th>
<th>15 µg+</th>
<th>30 µg+</th>
<th>60 µg+</th>
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</thead>
<tbody>
<tr>
<td><strong>Day 30</strong></td>
<td>1.2</td>
<td>4.4</td>
<td>4.7</td>
<td>8</td>
<td>14</td>
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<tr>
<td><strong>Day 60</strong></td>
<td>1.2</td>
<td>7.1</td>
<td>7.6</td>
<td>11.1</td>
<td>19.1</td>
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</table>
Relevance of Palivizumab

- Palivizumab is a monoclonal antibody that binds to a key antigenic site on the F protein.
- Palivizumab has been shown to prevent RSV disease in infants in multiple studies.
- Palivizumab has provided an important window into the functionality/efficacy of the immune responses to the RSV F nanoparticle vaccine.

Antigenic site II: Palivizumab epitope
Amino acids 254-278
NSELLSLINDMPITNDQKKLMSNNV

Peptide ELISA: Assay Methodology

Binding Palivizumab to Palivizumab peptide

Binding of palivizumab mAb to palivizumab epitope peptide. ELISA plates were coated with streptavidin at 5ug/ml. Palivizumab peptide at 1ug/ml was bound on to Streptavidin. Palivizumab at 10ug/ml was serially diluted four fold and incubated to the peptide on the plate. Palivizumab binding was detected using anti-human HRP reaction.
Peptide ELISA:
Anti-Palivizumab Peptide IgG

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day 1</th>
<th>Day 30</th>
<th>Day 60</th>
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</thead>
<tbody>
<tr>
<td>1 Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 5ug + Alum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 15ug + Alum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 30ug + Alum</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5 30ug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 60ug + Alum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 60ug</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ELISA Unit
Palivizumab-Competitive ELISA: Assay Methodology

Biotin Synagis + Human Sera

Competitive Binding

Avidin HRP

Colored Substrate
Competitive ELISA with anti-RSV F Vaccinated Mouse Serum and Palivizumab. ELISA plates were coated with RSV F nanoparticle at 2 ug/ml. Pre immune and a pool of day 28 serum from mice immunized on day 0 with 30 ug RSV F nanoparticle with aluminum phosphate were mixed with 50 ng/ml biotin-Palivizumab then serially diluted and incubated with purified RSV F coated ELISA plate. Streptavidin was used to determine Palivizumab bound to the plate. An unweighted four parameter logistic regression curve is presented.
Palivizumab-Competitive ELISA: Fold-Rise of Palivizumab-like Antibodies Induced by Vaccine

Vaccine-induced antibodies competing with palivizumab for palivizumab epitope on F Protein:
Fold rise of the 50% inhibitory titer
### Palivizumab-Competitive ELISA: Translating Palivizumab-like Antibody Concentration (µg/mL)

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Placebo (N=26)</th>
<th>5 µg + AlP0₄ (N=17)</th>
<th>15 µg + AlP0₄ (N=16)</th>
<th>30 µg + AlP0₄ (N=18)</th>
<th>30 µg (N=17)</th>
<th>60 µg + AlP0₄ (N=13)</th>
<th>60 µg (N=18)</th>
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<tr>
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<td>N 26</td>
<td>17</td>
<td>16</td>
<td>18</td>
<td>17</td>
<td>13</td>
<td>18</td>
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<tr>
<td>GMT</td>
<td>11</td>
<td>12</td>
<td>14</td>
<td>10</td>
<td>14</td>
<td>14</td>
<td>20</td>
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<tr>
<td>95% CI</td>
<td>10, 12</td>
<td>10, 16</td>
<td>11, 20</td>
<td>NA, NA</td>
<td>21, 20</td>
<td>10, 20</td>
<td>13, 31</td>
</tr>
<tr>
<td>Study Day 30</td>
<td>N 26</td>
<td>17</td>
<td>16</td>
<td>18</td>
<td>17</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>GMT</td>
<td>13</td>
<td>70</td>
<td>81</td>
<td>105</td>
<td>112</td>
<td>227</td>
<td>174</td>
</tr>
<tr>
<td>95% CI</td>
<td>11, 17</td>
<td>41, 120</td>
<td>61, 107</td>
<td>80, 137</td>
<td>69, 180</td>
<td>167, 308</td>
<td>117, 260</td>
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<tr>
<td>Study Day 60</td>
<td>N 26</td>
<td>17</td>
<td>16</td>
<td>18</td>
<td>17</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>GMT</td>
<td>14</td>
<td>111</td>
<td>128</td>
<td>142</td>
<td>100</td>
<td>337</td>
<td>157</td>
</tr>
<tr>
<td>95% CI</td>
<td>11, 18</td>
<td>75, 162</td>
<td>103, 161</td>
<td>114, 175</td>
<td>69, 145</td>
<td>254, 448</td>
<td>108, 230</td>
</tr>
</tbody>
</table>

- Palivizumab-like activity (µg/ml) determined using conversion factor of 2.1 µg/ml per 50% inhibition unit
- In cotton rats, levels of ~30 µg/ml provided a 100-fold reduction in viral load
- MedImmune used a trough level of 40 µg/ml to guide efficacy studies for palivizumab
Concordance between Palivizumab-Competitive and Anti-F IgG ELISA’s

As anti-F antibodies rise in response to immunization, functional Palivizumab-like antibodies also rise in concordant manner.
Neutralizing Antibody Assay: Methodology Performed by Baylor College of Medicine

Neutralizing Titers

- RSV Virus
- Virus + Serum (1:10)
- Virus + Hep-2
- Serum + Virus + Hep-2

Plaque Positive
- Plaque
- Virus Neutralized
Neutralization Antibody Assay: RSV-A Microneutralization Titers

- Minimum protective titer in MN assay is log$_2$ 6 (Piedra et al, Vaccine)
- Pre-existing neutralizing antibodies above the protective titer, as expected
- Post-immunization neutralizing antibodies increased by ~2 fold
Neutralizing Antibody Assay: Reverse Cumulative Distribution of RSV-A Microneutralization Titers
Neutralizing Antibody Assay: Reverse Cumulative Distribution of RSV-A Microneutralization Titers

Eliminating low titer subjects
MN Fold Rise by Starting Titer

Baseline MN Titer Level (log2)

Geo. Mean Fold Rise (95%CI)

- <=6: n=11
- <=7: n=33
- <=8: n=61
- <=10: n=94
- <=12: n=99
Study Conclusions

• The vaccine was well-tolerated, with no dose related toxicity
• The vaccine was immunogenic as measured by:
  – Anti-F IgG
  – Palivizumab epitope binding antibodies
  – Rise in neutralization titers detected against both RSV-A and RSV-B strains
• Concordance seen with anti-F IgG and palivizumab epitope binding antibodies
• Palivizumab-like antibodies are functional and may be predictive for vaccine efficacy
  – Quantitation indicate antibodies above protective levels (>40ug/ml)
• Neutralization titers above protective levels
  – Minimal protective titer log₂ 6
  – No low titer subjects after immunization
Conclusion

• Novavax RSV F recombinant nanoparticle vaccine is a conserved, immunogenic antigen, in its native confirmation
  – Induces functional, high affinity immunity, critical for an RSV vaccine
  – Baculovirus/insect cell system appears ideal to produce natively configured antigen
    – Recombinant RSV F nanoparticle made via viral infection in eukaryotic cell

• Clinical immunogenicity data may be predictive of protection and de-risks development program
  – MN compare well with naturally induced, protective levels
    • Protective in both elderly and pediatric sero-epidemiology studies
  – Palivizumab-like antibodies compare well with mAb protective levels
    – Effective levels established RSV infant prophylaxis

Clinical data indicates RSV F nanoparticle vaccine warrants further development