RSV F Nanoparticle Vaccine: Improving on Nature to Generate an Effective Vaccine

Virus-like Particles as Vaccines, Vectors and Adjuvants

Foundation Merieux, 1 April, 2014

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Novavax, Gaithersburg, Maryland, USA
Topics For Discussion

• Clinical significance of RSV
• The puzzling ineffectiveness of naturally induced immunity
• The Fusion protein in viral pathogenesis, a “universal” vaccine target
• The RSV F nanoparticle displays the palivizumab “Site II” binding site
• The vaccine induces palivizumab-like antibodies (PCA), natural infection does not!
• The efficacy of palivizumab and motavizumab: unprecedented de-risking for a novel vaccine
• Clinical plans forward
Respiratory Syncytial Virus (RSV): Important Facts

- Most common cause of infant LRTI Globally
  - Mortality exceeded only by Pneumo, HiB, and highest in LDCs¹

- Most frequent cause of infant hospitalization
  - 132,000–172,000 US hospitalizations annually²

- Severe disease often leads to ongoing wheezing
  - Associated with recurrent wheezing for years³

- Complex pathophysiology=challenging vaccine puzzle

2. MMWR, 2013, 263:141
RSV: A Predictable Epidemic

Monthly distribution of RSV A and RSV B infections in Israel.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0090515
Maternal Antibody Transfer

Active transport of mother’s antibodies into baby’s blood
- Mother’s antibodies from decades of infections and vaccines
- At full term baby has >100% of mother’s antibody levels.

Antibodies from natural RSV infection of mothers over decades are actively transported to infants blood

Physiologic mechanism for protecting the infants, assuming the antibodies are effective

Peak at term, relative concentration compared to mothers
Antibody Transfer

The Very Young Receive the Mother’s RSV Antibodies: Decades of RSV Exposure, Yet and Immune Response That is Not Protective

Suara et al., CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 1996, p. 477–479
RSV Disease - The Great Puzzle

- RSV infections induce a robust immune response
- Infants who receive maternal antibodies are most vulnerable to serious disease
- Recurrent infections are the norm
- Two F-protein based mabs directed to Site II have been demonstrated to be protective in 5 randomized clinical trials
  - Palivizumab (Synagis), licensed for prophylaxis in premature infants
  - Motavizumab, higher affinity than palivizumab
RSV: Genetic Stability of Surface Glycoproteins is a Clue to The Importance of Their Structure in Infection

- Strain changes due to the G protein variability
- The Fusion (F) protein changes less
- Site II on the Fusion protein does not change in nature, is the target of Palivizumab

Frequency of Amino Acid Changes In Glycoproteins G and F
In a natural infection, F is a “moving target”

In the course of a natural infection, what form of F do we make antibodies against?
Points in Pathogenesis Where Palivizumab/Motavizumab Disrupt Viral Proliferation

Huang et al., JOURNAL OF VIROLOGY, Aug. 2010, p. 8132–8140
Proposed Pre and Post Fusion Forms of RSV F

AA 254-278 (Antigenic site II) is the Target of motavizumab, palivizumab

Antigenic Site II present in both crystal structures

In the course of a natural infection, what form of F do we make antibodies against?

We can take advantage of the clinical efficacy of palivizumab and motavizumab.
Antigenic Site Ø on the F Protein

- Antigenic site Ø, a metastable site located at the membrane-distal apex of the prefusion RSV F trimer\(^1\).
- Antigen retained the C-terminal trimerization domain and combined it with other means of stabilization, including the introduction of cysteine pairs or cavity-filling hydrophobic substitutions\(^1\).
- Antigenic site II on the F gene demonstrated high conservation between genotypes. However, the newly defined antigenic site Ø in the pre-fusion F appeared variable especially among contemporary HRSV-A strains compared to prototype viruses\(^2\).

1. Structure-Based Design of a Fusion Glycoprotein Vaccine for Respiratory Syncytial Virus, Mclellan, Science 1 November 2013

RSV: Genetic Stability of Surface Glycoproteins is a Clue to The Importance of Their Structure in Infection

- RSV Surface Glycoproteins
  - Attachment Protein (G)
  - Fusion Protein (F)

Frequency of Amino Acid Changes In Glycoproteins G and F

Strain changes due to the G protein variability

The Fusion (F) protein changes less

Site II on the Fusion protein does not change in nature, is the target of Palivizumab
Virus-Like Particles (VLP) Seasonal & Pandemic Influenza

- HA, NA Protein
- Empty - No genetic material
- M1 Matrix Protein
- Configuration and size of the virus without RNA genome

Recombinant Protein Nanoparticles RSV, Rabies & Others

- Hydrophilic head of protein particle
- Hydrophobic tail of protein particle
- Protein particles form micelles for efficient antigen presentation:
  - Single antigen
  - Repeating unit

Novavax RSV F Nanoparticles
RSV F Nanoparticle Vaccine: A Near Full Length, Stabilized F Protein

- Purified RSV F forms nanoparticles comprising multiple F protein trimers in hydrophobic interactions
- Deletions stabilize the F protein; manifested by lack of toxicity to the expression system (insect cells)
- Preserves antigenic Site II

Smith, et al. 2012. PLOS. 7(11), e50852
F Protein Nanoparticle Vaccine Displays Site II: Palivizumab Binding

- Palivizumab binds avidly to the F protein nanoparticle vaccine

Antigenic site II: Amino acids 254-278
NSELLSLINDMPITNDQKKLMSNNV

RSV F Constructs

Unstable Wild Type

Wild type, membrane bound

Vaccine Candidate

NVAX vaccine F683, membrane bound, micelle, insect Sf9

Soluble F

NVAX soluble/secerted, insect cell Sf9
Palivizumab mAb Affinity To Different Forms of RSV F: Avid Binding to the RSV F nanoparticle vaccine

A. RSV F Nanoparticle
   KD = 0.542 nM

B. RSV F 710 (soluble form)
   KD = 12.47 nM

C. RSV F 683 (262M) Escape Mutant
   No binding

D. RSV F 683 tetra Escape Mutant
   No binding

Palivizumab was immobilized on the chip surface.
The Cotton Rat Model: Safety and Efficacy Evaluations

Immunization
- Day 0: I.M.
- Day 28: I.M.

Challenge
- Day 49: I.N.

Terminal Bleed
- Day 54

Serum
Serum
Serum
Serum, Nasal, BALT

Predictive Model for Respigam, Palivizumab and Motavizumab
Serum RSV F IgG ELISA Titers

Day 49 Pre-challenge

<table>
<thead>
<tr>
<th></th>
<th>FI-RSV 1/25 Lot 100</th>
<th>RSV-Infection 10E5 pfu</th>
<th>RSV F-Alum 30 µg</th>
<th>RSV F 30 µg</th>
<th>Placebo</th>
<th>Pali-Passive 15mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean serum F IgG</td>
<td>26,647</td>
<td>6,881</td>
<td>1,469,000</td>
<td>147,962</td>
<td>100</td>
<td>31,926</td>
</tr>
</tbody>
</table>
## RSV A Neutralizing and Fusion Inhibiting Antibody

### RSV A Long Neutralization

![Bar chart showing RSV A Long Neutralization](chart.png)

### Table: Day 49 Pre-challenge Neutralizing (Neut-GMT) and Fusion Inhibiting (FI-GMT) GMTs

<table>
<thead>
<tr>
<th></th>
<th>FI-RSV</th>
<th>RSV-Infection</th>
<th>RSV F-Alum</th>
<th>RSV F</th>
<th>Placebo</th>
<th>Pali-Passive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neut-GMT</td>
<td>&lt;10</td>
<td>73</td>
<td>452</td>
<td>40</td>
<td>&lt;10</td>
<td>160</td>
</tr>
<tr>
<td>FI-GMT</td>
<td>&lt;10</td>
<td>95</td>
<td>697</td>
<td>51</td>
<td>&lt;10</td>
<td>320</td>
</tr>
</tbody>
</table>
Palivizumab-Competitive ELISA (PCA)

Vaccine-induced antibodies compete with palivizumab for binding to Site II on the F Protein

Protective levels elucidated by the cotton rat studies: 30μg/ml
Palivizumab Competing Antibody (PCA)

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<thead>
<tr>
<th></th>
<th>FI-RSV 1/25 Lot 100</th>
<th>RSV-Infection 10E5 pfu</th>
<th>RSV F-Alum 30 µg</th>
<th>RSV F 30 µg</th>
<th>Placebo</th>
<th>Pali-Passive 15mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean</td>
<td>&lt;10</td>
<td>24</td>
<td>884</td>
<td>100</td>
<td>&lt;10</td>
<td>84</td>
</tr>
</tbody>
</table>

Day 49 Pre-challenge

Competitive Inhibition
50% GMT (log2)

884 µg/ml

84 µg/ml

LLOQ
Lung and Nasal Titers to RSV A

**Day 54 – 4 days post-challenge**

<table>
<thead>
<tr>
<th></th>
<th>Lung Viral Titer log₁₀ pfu/g</th>
<th>Nasal Viral Titer log₁₀ pfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fl-RSV</td>
<td>2.5</td>
<td>4.5</td>
</tr>
<tr>
<td>RSV Infection</td>
<td>2.5</td>
<td>4.5</td>
</tr>
<tr>
<td>RSV F-AlPO4</td>
<td>2.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Palivizumab</td>
<td>6.5</td>
<td>6.5</td>
</tr>
</tbody>
</table>

**GMT**

<table>
<thead>
<tr>
<th></th>
<th>Lung</th>
<th>Nasal Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMT-Lung</td>
<td>2,357</td>
<td>&lt;100</td>
</tr>
<tr>
<td>GMT-Nasal</td>
<td>71,105</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

**Titers**

- Lung: Fl-RSV 1/25 Lot 100, RSV Infection 10E5 pfu, RSV F Alum 30 µg, RSV F 30 µg, Placebo Palivizumab
- Nasal Wash: Fl-RSV, RSV Infection, RSV F-AlPO4, RSV F, Placebo Palivizumab
Passive Immunization with Immune Sera vs Palivizumab: Validation of the PCA Assay

Figure 6. RSV A challenge of passively immunized cotton rats:
Cotton rats (n=5) were passively immunized on day -1 with 5.6; 1.6; 0.6 mg/kg PCA antibodies, or 5; 1.25; 0.625 mg/kg palivizumab antibody. Control rats received 0.15 mL of pooled pre-immune serum (NCS). Panel A. Sera were obtained from all the groups on day 0 (24 hrs post transfer) to determine palivizumab competitive titers by ELISA as described in the method section. The geometric mean titers (GMT) for each group are represented with the bar graph shown on figure 6A. *p<0.01 when compared with 5.0 mg/kg group and +p<0.01 when compared to 1.3mg/Kg group by two-tailed student T test. Panel B. All groups were challenged on day 0 (24 hrs post transfer) with RSV/A/Long. Lung tissue was collected on day 4 post challenge and lung vial titer was determined by plaque assay. The mean log_{10} pfu/g titers for each group are represented with the bar graph shown on figure6B. *p<0.01 when compared with negative control (NCS) group by two-tailed student T test. NS: not significant difference with negative control group.
Cotton Rat as a Model of Protection Against RSV: Respigam

Preclinical Basis for Respigam

Comparison of Antibody Concentrations and Protective Activity of Respiratory Syncytial Virus Immune Globulin and Conventional Immune Globulin

George R. Sibor, Donna Leonbruno, Jeanne Leszcynski, James McIver, Dinah Bodkin, René Gonin, Claudette M. Thompson, Edward E. Walsh, Pedro A. Piedra, Val C. Hemming, and Gregory A. Prince

RSV challenge/passive immunization with RSVIG
- 99% lung reduction at PRNT 390
- 99% nasal titer reduction at PRNT 3500

Figure 2. Relationship between serum RSV titer by complement-enhanced plaque-reduction neutralization at time of challenge and RSV concentrations recovered from lung homogenates (A) or nose tissue homogenates (B) 4 days after challenge.
Cotton Rat as a Model of Protection Against RSV: palivizumab

Preclinical Basis for clinical evaluation of palivizumab

Protective level 25-30 μg/ml

Johnson, s. et al. JID, 176:1215-1223
Efficacy in Term Infants: Effects of Site II Binding by Monoclonal Antibodies (Motavizumab)

6.4.2.1 Incidence of RSV Hospitalization

The incidence of RSV hospitalization in the MI-CP117 ITT population is presented in Table 6.4.2.1-1. The efficacy of motavizumab was confirmed, with motavizumab demonstrating a statistically significant 83% relative reduction in the incidence of RSV hospitalization (RR: 0.168, 95% CI: [0.086, 0.308]; p < 0.001) as compared to placebo.

Table 6.4.2.1-1

<table>
<thead>
<tr>
<th>Population</th>
<th>Placebo</th>
<th>Motavizumab</th>
<th>Fisher’s Exact Test p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relative Risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT population</td>
<td>39/472 (8.3%)</td>
<td>13/938 (1.4%)</td>
<td>&lt; 0.001</td>
<td>0.168</td>
<td>(0.086, 0.308)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Prespecified interim analysis alpha spending level was 0.032

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Target Populations for an RSV Vaccine

Young Infants via Maternal Immunization

- Provide protection for infants younger than six months and most at risk of serious RSV disease, prevent hospitalization, medical care, wheezing

Pediatric

- Decrease respiratory disease burden in children, prevent medical care, wheezing

Elderly

- Mitigate RSV disease burden that results from waning immunity and immunosenescence, prevent hospitalization and death
Summary of Clinical Studies to Date

• Study 101: Safety and immunogenicity in healthy adults
  – Stimulated robust immune responses
  – Induced production of palivizumab-neutralizing antibodies (n=120)

• Study M201: Safety and immunogenicity in women of childbearing age
  – Confirmation of safety and immunogenicity in target population (n=330)

• Study E101: Safety and Immunogenicity in elderly adults
  – Confirmation of safety and immunogenicity in target population (n=220)

• Study M202: Safety dose finding in women of childbearing age (Data Q2 2014)
  – Selection of dose and schedule for pregnant women (n=720)
  – Safety
Study M201: Anti-F IgG in Women of Childbearing Age

- 2 dose, alum adjuvanted vaccines induced the highest titer
- ~15 fold higher than naturally induced F antibodies
Palivizumab-Competitive ELISA (PCA)

Vaccine-induced antibodies compete with palivizumab for binding to Site II on the F Protein

Protective levels elucidated by the cotton rat studies: 30μg/ml
PCA in Women of Childbearing Age

- Titers up to >300ug/ml
- Palivizumab ‘protective’ at 30 ug/ml
Vaccine Induced Anti-F Antibodies Differ From Post-Infection Antibodies

Anti-F IgG responses closely tied to palivizumab-like antibodies

Almost no PCA at day 0!

Concordance slope w/o placebo = 1.08 (0.95–1.22)
Alum Adjuvant Enhances Anti F, PCA Antibodies
Modeling the Effect of PCA via Maternal Immunization: Potential for Protection of infants up to 5-6 months
RSV/A Neutralizing Responses

- Day 0 titers $\sim \log_2 8$
- Day 28 titers $\sim \log_2 10$
Study M201: Safety and immunogenicity in women of childbearing age
Microneutralization Data

- 1- and 2-dose groups are pooled (identical at Day 28)
- 60 and 90µg groups are pooled to show overall impact of alum adjuvant at Day 28
- Peak GMT log₂ 10.0-10.5
Women with Lowest Baseline MN Titers Show the Greatest Increase in MN Titers

RSV/A Microneutralization Titer Response After One Dose: Effect of Baseline Titer and Presence of Adjuvant

- **Unadjuvanted**
- **Adjuvanted**

Numbers = N for the group analyzed

Fold Increase in RSV/A Microneutralization Titer at Day 28

<table>
<thead>
<tr>
<th>RSV/A Microneutralization Titer at Baseline (Day 0, expressed as Log2)</th>
<th>&lt;=7</th>
<th>&lt;=8</th>
<th>&lt;=9</th>
<th>&lt;=10</th>
<th>&lt;=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjuvanted</td>
<td>10</td>
<td>31</td>
<td>67</td>
<td>93</td>
<td>106</td>
</tr>
<tr>
<td>Adjuvanted</td>
<td>16</td>
<td>46</td>
<td>95</td>
<td>126</td>
<td>135</td>
</tr>
</tbody>
</table>
On (Ka) and Off (Kd) Measurements: Representative RSV F Binding Curves

Palivizumab
KD = 0.95E-10

Vaccinee day 30; ID 1112
KD = 2.29E-12
### Anti-F IgG Avidity Measurements: RSV Nanoparticle Vaccinees (60µg + AdjuPhos)

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>KD</th>
<th>Anti F EU*</th>
<th>Palivizumab-like IgG</th>
<th>KD</th>
<th>Anti F EU*</th>
<th>Palivizumab-like IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Titer (µg/ml) **</td>
<td></td>
<td></td>
<td>Titer (µg/ml) **</td>
</tr>
<tr>
<td>1104</td>
<td>1.76 E-12</td>
<td>4,249</td>
<td>57</td>
<td>8.26 E-13</td>
<td>6,141</td>
<td>66</td>
</tr>
<tr>
<td>1105</td>
<td>3.92 E-13</td>
<td>4,492</td>
<td>85</td>
<td>8.23 E-14</td>
<td>6,300</td>
<td>106</td>
</tr>
<tr>
<td>1107</td>
<td>3.91 E-13</td>
<td>4,348</td>
<td>208</td>
<td>2.24 E-14</td>
<td>9,633</td>
<td>312</td>
</tr>
<tr>
<td>1108</td>
<td>6.80 E-13</td>
<td>9,400</td>
<td>169</td>
<td>1.94 E-15</td>
<td>7,360</td>
<td>116</td>
</tr>
<tr>
<td>1109</td>
<td>9.92 E-10</td>
<td>21,402</td>
<td>177</td>
<td>NA</td>
<td>17,930</td>
<td>253</td>
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<tr>
<td>1110</td>
<td>1.35 E-10</td>
<td>7,969</td>
<td>106</td>
<td>1.62 E-10</td>
<td>8,555</td>
<td>134</td>
</tr>
<tr>
<td>1112</td>
<td>2.29 E-12</td>
<td>8,032</td>
<td>190</td>
<td>3.64 E-13</td>
<td>7,528</td>
<td>203</td>
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<tr>
<td>1113</td>
<td>2.72 E-13</td>
<td>11,037</td>
<td>337</td>
<td>9.02 E-14</td>
<td>9,058</td>
<td>315</td>
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<tr>
<td>1115</td>
<td>4.21 E-13</td>
<td>2,587</td>
<td>109</td>
<td>6.75 E-10</td>
<td>3,207</td>
<td>131</td>
</tr>
<tr>
<td>1117</td>
<td>4.93 E-13</td>
<td>9,505</td>
<td>76</td>
<td>4.18 E-13</td>
<td>16,923</td>
<td>261</td>
</tr>
<tr>
<td>1119</td>
<td>1.11 E-13</td>
<td>6,463</td>
<td>96</td>
<td>1.55 E-13</td>
<td>7,435</td>
<td>140</td>
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<tr>
<td>1120</td>
<td>1.23 E-13</td>
<td>5,047</td>
<td>77</td>
<td>1.70 E-13</td>
<td>8,205</td>
<td>122</td>
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<td>11024</td>
<td>6.18 E-13</td>
<td>6,041</td>
<td>94</td>
<td>4.79 E-13</td>
<td>10,798</td>
<td>124</td>
</tr>
<tr>
<td>Palivizumab</td>
<td>0.95 E-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vaccine induced antibodies behave like Motavizumab Preimmune sera are negative
Conclusions

• An RSV Vaccine has to do something that nature does not
• Site II on the F protein is highly conserved and relatively cryptic in natural infection
• There is little to no site II specific PCA…but people are primed
  – Ideal prime boost regimen
• The RSV F nanoparticle induces robust PCA
• Palivizumab and motavizumab clinical efficacy inform a complex vaccine development program, derisk the program
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  - Ziping Wei
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  - Monique Malou-Williams
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  - Tim Hahn
  - Malek Masoud
  - Mervyn Hamer
  - Cast of thousands
- Quality Systems
  - Jody Hatch
  - Mike Sowers
  - James Wong
  - Konnie Taylor
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  - Nigel Thomas
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