Recombinant Nanoparticle Vaccines: Respiratory and Emerging Viruses

Lou Fries, MD
Novavax Inc.
25 May 2016
The Novavax nanoparticle platform and Matrix-M™ adjuvant

Recombinant Nanoparticles:
- Sequences cloned into baculovirus
- Expressed in Sf9 cells
  - glycosylated
  - properly folded
- Detergent extraction
- Chromatographically purified

Protein particles form micelles for efficient antigen presentation:
- Single antigen
- Repeating unit

Matrix-M Adjuvant Saponin Based Nanoparticle

Nanoparticle size (40 nm) particles composed of Quillaja saponins, cholesterol and phospholipid

Activates innate immunity and results in:
- Increased antibody responses
- Increase CD4+ and CD8+ responses
- Dose sparing

Novavax RSV F Nanoparticles
Respiratory Syncytial Virus (RSV)
The RSV problem

• RSV is a major cause of ALRI in children worldwide\(^1,2\) :
  • 33.8 x 10\(^6\) (19.3-46.2 x 10\(^6\)) cases of ALRI annually in children under 5
  • ~22% of all ALRI, 28.8% of pneumonias, and 22.6% of severe pneumonia
  • 3.4 x 10\(^6\) (2.8-4.3 x 10\(^6\)) hospitalizations
  • 66-199,000 deaths; 99% in developing countries

• RSV is also major cause of community and hospitalized lower respiratory tract disease in older adults, often equaling influenza\(^3,4\).

• Naturally-occurring neutralizing antibodies are associated with a decreased risk of severe disease and pneumonia\(^5,6\), but...

• Recurrent infection occurs throughout life, despite substantial neutralizing titers.

**RSV F protein is highly conserved – an ideal vaccine target**

### Structure of RSV

- **Attachment protein (G)**
- **Small hydrophobic Protein (SH)**
- **Nucleoprotein (N)**
- **Lipid bilayer**
- **Matrix protein (M)**
- **Phosphoprotein (P)**
- **RNA polymerase (L)**
- **Fusion protein (F)**

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**RSV is an evolving RNA virus**

- Primary surface glycoproteins evaluated for vaccines are the attachment protein (G) and the fusion protein (F)
- Evolutionary changes occur predominantly in the G protein
- The F protein, in contrast, is highly conserved, so no vaccine strain change is necessary
- Site II on the F protein is the target of palivizumab, and is highly conserved from year to year and across isolates since at least the 1980’s

### Frequency of Amino Acid Changes

Novavax’ RSV F vaccine is a full-length F protein stabilized with two changes:

- (1) a mutation in furin cleavage site II,
- (2) a 10 amino acid deletion (Phe137 - Val146) in fusion domain.

Modification of the furin cleavage site blocks full transition to post-fusion form of RSV F, and exposes cryptic, neutralizing epitopes not consistently recognized on pre-fusion F.

The hydrophobic C-terminal transmembrane region is intact, so the RSV F oligomers forms protein-protein nanoparticles.

Source: Smith, et al. 2012. PLOS. 7(11), e50852
RSV F nanoparticle induces antibodies that compete with palivizumab for binding to RSV F

• Mice (unsurprisingly) and men (surprisingly) have little or no serum antibody competing with palivizumab (PCA) pre-immunization.
• Immunization with RSV F nanoparticles evokes PCA
  • Men are primed (one dose works), mice are not
• PCA can be quantified using a palivizumab standard curve
Immunized cotton rats are protected from RSV challenge

- RSV F vaccine groups (± aluminum) were compared to live challenge formalin-activated vaccine (FI-RSV) and passive palivizumab.

- RSV F Vaccine induced anti-F IgG, PCA and neutralizing antibodies.

- The RSV F Vaccine eliminated virus replication in the lungs without disease enhancement (unlike FI-RSV immunized animals).
Immunity elicited by the RSV F nanoparticle vaccine targets multiple broadly neutralizing epitopes

- Immune responses to conserved bnMAb epitopes are absent/low after years of infection, suggesting these epitopes are important to virus fusion function and are cryptic in natural infection

- Novavax RSV F vaccine induces antibodies to Site I, II and IV.

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RSV F nanoparticle vaccine induces statistically-significant protection in Phase II trial in adults ≥60 y.o.

**RT-PCR confirmed RSV Events Product-Limit Survival Estimate**

Log-Rank test of equality over strata; p=0.039

Proportion of Group With No Symptomatic RSV Infection

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<th>Time to RSV Onset (days)</th>
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<th>100</th>
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<td>Last case:</td>
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4.9% of placebo with symptomatic RSV infections

<table>
<thead>
<tr>
<th>Number of Symptoms and Signs Reported*</th>
<th>Vaccine Efficacy</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Any acute respiratory symptoms + RSV</td>
<td>41%</td>
<td>0.041</td>
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<tr>
<td>≥3 lower respiratory tract signs or symptoms + RSV</td>
<td>64%</td>
<td>0.047</td>
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<tr>
<td>≥least 4 lower respiratory tract signs or symptoms + RSV</td>
<td>75%</td>
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Ebola candidate – a construct displaying neutralizing protective epitopes defined by MAb binding
### 2014 ZEBOV/Makona GP – Candidate antigen probed with protective MAbs

![Image of virus with antibodies and epitopes](image.png)

<table>
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<tr>
<th>mAb</th>
<th>EBOV GP Epitope</th>
<th>SPR Analysis</th>
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<tr>
<td>KZ52</td>
<td>aa 42-43, 513, 550-553, 556</td>
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<td>13F6</td>
<td>aa 401-417 ATQVEQHHRRTDNDSTA ATQVGQHHRRAADNDSTA(^1)</td>
<td>Linear Neutralizing</td>
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\(^1\)Two amino acid substitutions occurred in 2014 Guinea GP amino acids compared to 1976 Mayinga GP 401-417 epitope.
Sf9/baculovirus nanoparticle technology provides rapid response to an episodic “emerging” disease

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<th>November</th>
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<td>Publication of Gire <em>et al.</em> in <em>Science</em></td>
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<td>EBOV GP gene plasmid construction</td>
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<td>Production – drug substance, downstream</td>
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<td>Production – drug product</td>
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<td>Development – purification process</td>
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<td>Development – EBOV GP ELISA</td>
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<td>QC – drug substance release testing</td>
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<td>QC – drug product release testing</td>
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<td>QA – batch and QC record review</td>
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<td>QA – GMP batch release</td>
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*Primary Critical Path  Secondary Critical Path*
**Human Antibody Responses to ZEBOV GP Nanoparticle Vaccine**

- **Placebo doses, no MXM**
- **1 dose w/ MXM**
- **2 dose w/ MXM**
- **2 doses w/ MXM**
- **6.5 µg, 2 doses w/ MXM**

**Whole Virus ELISA**
**Mayinga nab titer**
**ZEBOV PsVNA80**

**Pooled Survival Data 3 EBOV Challenge Studies in Cynomolgus Macaques**

- **Vaccine**
- **Placebo**

- **Non-EBOV death**

- **Median for protected macaques**

- 6.5 to 50µg of GP +/- Matrix-M adjuvant
- One or two doses (21 day interval)
- Day 35 antibody titers

- 5µg EBOV GP + 50µg Matrix-M adjuvant on days 0 and 21 or 42
- Challenge 3-6 weeks post dose 2 with 100 pfu EBOV Kikwit
Influenza
Why not influenza; the ultimate re-emerging virus?

• Seasonal influenza vaccine formulations change on a yearly basis in search of the best match with predicted circulating strains.

• This is not a sure process; vaccine effectiveness may be compromised by:
  • Unanticipated antigenic drift in the selected viruses; e.g, A(H3N2) in recent seasons,
  • Sequence changes in hemagglutinin genes induced by egg adaptation during manufacture,
  • Co-circulation of two antigenically distinct B virus lineages in variable proportions,
  • Waning of immunity late in the season.

• Influenza HA, the prime protective antigen, is known to contain broadly neutralizing epitopes, but these don’t dominate the immune response.

• Can nanoparticle HA-based influenza vaccines (+/- Matrix-M adjuvant) provide broadly neutralizing antibodies (or other key responses)?
Broadly neutralizing monoclonal antibodies generated with nanoparticle influenza HA

- Pathway mirrors the approach to RSV vaccine
- Serial immunization of mice with several different strains of influenza and Matrix-M adjuvant, screen for broadly neutralizing monoclonal antibodies (bnMAbs)
- Clone and produce bnMAbs for Group 1, 2 and B strains (including Victoria and Yamagata lineages)
- Measure neutralization and calculate potency of bnMAb
- Screen candidate vaccine antigens for binding of bnMAb to the nanoparticles
- Immunize animals, with and without Matrix-M adjuvant; look for functional immunity and protection
- Evaluate induction of antibodies that bind to the same site(s) as bnMAb(s) via a competition assay
## Broad A(H3N2) neutralizing activity from nanoparticle-induced MAbs

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</table>
bnMAbs recognize nanoparticle HA antigens and neutralize virus at very low concentration

A/Brisbane/10/07 HA  A/Switz/9715293/13 HA

A2.91.3

K\textsubscript{D} = 9.8 nM

A2.36.1

K\textsubscript{D} = 3.8 nM

K\textsubscript{D} = 1.9 nM

K\textsubscript{D} = 2.8 nM

\begin{table}
\centering
\begin{tabular}{|c|cc|}
\hline
\textbf{Virus} & \textbf{A2.91.3} & \textbf{A2.36.1} \\
\hline
A/HK/4801/14 & 3.1 & 21.8 \\
A/S.Aust/55/14 & 2.1 & 14.8 \\
A/Switz/9715293/13 & 7.1 & 13.3 \\
A/Tx/50/12 & 14.1 & 2.2 \\
A/Vic/36/11 & 0.5 & 4.5 \\
A/Perth/09 & 1.8 & 2.9 \\
A/Brisbane/10/07 & 6.3 & 430 \\
\hline
\end{tabular}
\end{table}
# Broad A(H1N1) neutralizing activity from bnMAbs

## A/H1N1 Microneutralization (100 TCID<sub>50</sub>)

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A(H1N1) neutralization and HAI by selected neutralizing bnMAbs demonstrates high avidity binding.
Nanoparticle vaccine with Matrix-M adjuvant provides enhanced neutralization and antibodies to conserved neutralizing epitopes.

### Ferret Immunogenicity of A/California/07/09 Nanoparticles

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<thead>
<tr>
<th></th>
<th>A/Cal/07/09 HAI</th>
<th>mAb A1.27.1 IC50</th>
<th>A/Cal/07/09 MN</th>
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<tr>
<td>A/Cal NP</td>
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<td>A/Cal NP + MXM</td>
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<tr>
<td>A/Cal NP + MXM</td>
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GMT (95% CI)
Superior clearance of A/California/07/09 challenge by ferrets immunized with vaccine and Matrix-M adjuvant
High-avidity, cross-lineage influenza B virus neutralization by nanoparticle-induced bnM Ab
Combination respiratory vaccine: Influenza+ RSV

• Single Vaccine to cover two key respiratory pathogens commonly responsible for lower respiratory tract disease

• Co-formulate nanoparticle Influenza and RSV vaccines

• Induce broadly neutralizing influenza antibodies, to address:
  • Unanticipated antigenic drift in the selected viruses, e.g. A(H3N2) in recent seasons
  • Sequence changes in hemagglutinin genes induced by egg adaptation
  • Potential for improved efficacy

• Build on the RSV efficacy data

• Explore leveraging of Matrix-M adjuvant:
  • Enhance both the magnitude and affinity of antibodies
  • Enhance the induction of broadly neutralizing antibodies
  • Dose spare
Co-formulation of RSV and influenza nanoparticles

**Balb/C mice**

- 1.5µg RSV-F +/- 1.5µg HA per strain +/- 5µg MXM, 0 and 21 days

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**Influenza HAI and RSV Antibody Responses to a Combination Respiratory Vaccine**

<table>
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<th>Vaccine</th>
<th>bnMAb</th>
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<td>Quad NP</td>
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<td>Combo</td>
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<td>RSV F + Matrix-M</td>
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<td>Quad NP + Matrix-M</td>
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<td>Combo + Matrix-M</td>
<td>34.6</td>
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* H3 bnMAb
Nanoparticle vaccines and Matrix-M adjuvant

- Nimble manufacturing platform
- Can generate fully glycosylated and properly-folded membrane glycoproteins
- Antigens display neutralizing epitopes that are cryptic in natural infection
  - Demonstrated efficacy against RSV illness in older adults
  - Demonstrated ZEBOV neutralizing responses in human and protection in NHPs
  - Murine and ferret neutralizing responses to influenza HA, and induction of antibodies to broadly neutralizing epitopes, which may translate into improved protection across strains
- Matrix-M adjuvant provides:
  - Dose-sparing
  - Enhanced neutralizing antibody responses
  - Enhanced CD4+ and CD8+ T cell responses

Next steps in 2016-17

- RSV F nanoparticle vaccine is in Phase 3 efficacy trials:
  - Infant protection via maternal immunization
  - Older adults ≥ 60 years
- Influenza nanoparticle vaccine / respiratory combination vaccine:
  - Optimizing process for GMP manufacture
  - Targeting Q1 2017 Phase 1 clinical trial to assess:
    - Induction of broadly neutralizing HA antibodies in heavily primed humans
    - Optimal formulation for immunogenicity of all components in a multivalent influenza vaccine with RSV F
    - Contribution of, and optimal dose for, Matrix-M adjuvant.
- Other projects:
  - MERS
    - Antigen validated by high neutralizing titers in animal sera
    - Targeting vaccine Phase 1 in 2017
  - Zika – pre-clinical

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