Respiratory Syncytial Virus (RSV) Pre-fusogenic F Nanoparticle Vaccine Compared to Pre-fusogenic F and Post-fusion F Proteins

G Smith¹, N Patel¹, H Zhou¹, M Massare¹, JH Tian¹, D Scott¹, M Guebre-Xabier¹, H Lu¹, A Portnoff², Q Wang², Q Xie², G Glenn¹
¹Novavax, Inc., Gaithersburg, MD; ²Baylor College of Medicine, Houston, TX

BACKGROUND

RSV is the leading viral cause of severe lower respiratory tract disease in infants and young children worldwide. RSV fusion (F) envelope glycoprotein is a major target for vaccine development. RSV F is subject to significant rearrangements; precursor (P0) is cleaved by furin forming a fusion inactive F2-p27F1 intermediate (Pre-fusogenic F). A second furin cleavage releases a p27 peptide to form metastable pre-fusion F (Pre-F), which upon rearrangement from a 3-helix to 6-helix bundle configuration (Post-F) drives fusion of viral and cellular membranes. Novavax has developed a near-full-length RSV Pre-fusogenic F vaccine composed of purified F2-p27F1 trimers formulated with polysorbate 80 (PS80) as stable 40nm protein – detergent nanoparticles. ResVax (Pre-fusogenic F nanoparticle vaccine) is currently being assessed in the Prepar™ trial for the protection of infants via maternal immunization in healthy third trimester pregnant women.

OBJECTIVES

- Characterization of RSV Pre-fusogenic F nanoparticle vaccine, RSV Pre-F, and Post-F produced in Sf9 cells in Sf9 cells:
  - Hydrodynamic properties AUC and DLS.
  - Single particle CryoEM at 5 Å resolution.
  - Monoclonal antibody binding to antigenic sites Ø and VIII (pre-fusion specific) and sites II, IV, II/IV, and p27.
  - FACS analyses of recombinant, membrane associated RSV F:
    - Insect Sf9 cells: RSV Pre-fusogenic F and Pre-F.
    - Human 293T: RSV Pre-fusogenic F and wild type F.
    - RSV Pre-fusogenic, Pre-F, and Post-F immunogenicity in mice.

METHODS

- RSV Pre-fusogenic F (GMP drug substance), Pre-F (single-chain triple mutant; SCTM), and Post-F were produced in Sf9 cells.
- Hydrodynamic properties purified RSV F antigens were measured using analytical ultracentrifugation (AUC) and dynamic light scattering (DLS).
- Single particle CryoEM of a 72-aa G-terminal truncated Pre-fusogenic F was compared to Pre-F and Post-F at 5 Å resolution.
- Antigen binding of mAbs specific for pre-fusion sites Ø and VIII and sites II, IV, II/IV and p27 using Octet 384 Bio-Layer Interferometry and dot blot.
- FACS cell surface expressed RSV Pre-fusogenic F and Pre-F on Sf9 cells and Pre-fusogenic F and wild type RSV/A2 F on human 293T cells.
- Immunogenicity 1µg and 10µg antigens with/wo AlPO4 adjuvant in mice.

RESULTS

Fig 1. RSV Pre-fusogenic, Pre-F, Post-F Proteins

[Diagram showing different proteins and their properties]

Fig 2. Hydrodynamic parameters RSV F constructs: AUC and DLS

[Graph showing AUC and DLS values for different constructs]

Fig 3. Single Particle CryoEM RSV Pre-fusogenic F 5 Å Resolution

[Image showing cryoEM structure at 5 Å resolution]

Fig 4. Bio-layer interferometry and dot blot of RSV F mAb binding

[Graph showing binding characteristics of different antibodies]

Fig 5. A. FACS Pre-fusogenic F and Pre-F Sf9 cells

[Graph showing FACS analysis for different constructs]

Fig 6. RSV ELISA and Microneutralization responses in mice

[Graph showing ELISA and microneutralization responses for different constructs]

CONCLUSIONS

- Pre-fusogenic F forms a distinct protein – PS80 detergent nanoparticle.
- Pre-fusogenic F with a 72aa truncation of C-terminal forms trimers with a 3helix-bundle stem and a length intermediate between Pre-fusion and Post-fusion.
- Pre-fusogenic F expressed on Sf9 and human 293T cells and when purified bind pre-fusion-specific, pre/post-fusion, and p27 monoclonal antibodies.
- Pre-fusogenic F nanoparticle vaccine without and with AlPO4 adjuvant was significantly more immunogenic in mice than Pre-F and Post-F.

REFERENCES

4. Swanson KA, et al. Structural basis for immunization with postfusion respiratory syncytial virus fusion F glycoprotein (RSV F) to elicit high neutralizing antibody titers. 2011. JPAS. DOI:10.1073/pnas.1106536108.