Poster #69

In-depth Analytical Characterization and Structural Modeling of Novavax RSV F Nanoparticle Vaccine

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BACKGROUND

ResVax (Pre-fusogenic RSV F nanoparticle vaccine) is currently being assessed in the Prepare™ (Phase 3) trial for the protection of infants via maternal immunization in healthy third trimester pregnant women. The vaccine is based on recombinant near full-length RSV F protein, produced using a baculovirus/Sf9 cell culture platform. RSV F drug substance (DS) contains highly-purified F protein trimers that are formulated with polysorbate 80 (PS80) and assemble into nanoparticles. Structurally, RSV F DS has been characterized by a plethora of biophysical particle characterization methods, including dynamic light scattering (DLS), Analytical Ultracentrifugation (AUC), and Small Angle Scattering (SANS and SAXS) methods, to further our understanding of the nanoparticle assembly of the RSV F-PS80 complex.

OBJECTIVES

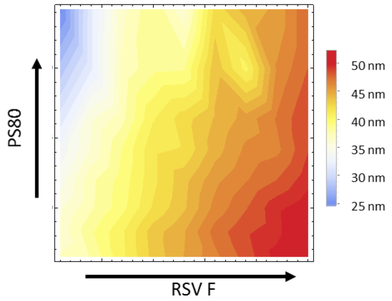
To utilize biophysical methods to characterize RSV F nanoparticle in order to understand how RSV F trimers and PS80 interact and influence nanoparticle structure.

METHODS

- Dynamic light scattering (DLS) was used to investigate the relationship between PS80-to-RSV F protein ratio and hydrodynamic diameter of the nanoparticle. RSV F was formulated with varying amounts of PS80 in the Drug Substance (DS) formulation buffer. DLS measurements were performed in a multi-well plate format using a Wyatt DynaPro PlateReader II DLS instrument. (Fig. 1)
- Analytical ultracentrifugation (AUC) is orthogonal to DLS and was used to gain further insight into the RSV F-PS80 nanoparticle. Sedimentation of RSV F at varying PS80 concentrations and in the DS formulation buffer was studied using absorbance and interference detection with a Beckman XL-I instrument (Fig. 2)
- Small angle neutron scattering (SANS) with contrast variation and small angle X-ray scattering (SAXS) were used to understand the spatial relationship between RSV F protein trimers and PS80 in the nanoparticle. SANS/SAXS was performed as a collaboration with National Institute of Standards and Technology (NIST). RSV F samples were buffer exchanged to different target D_2O concentrations to achieve contrast matching conditions for protein and detergent. (Fig. 3 and Table 1)

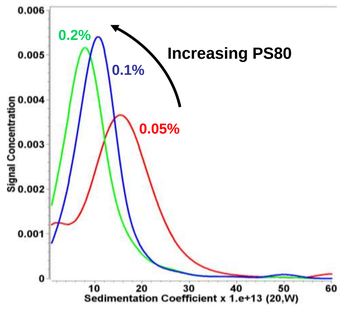
RESULTS

Fig. 1. Contour plot of DLS data (D_h) as a function of PS80 and RSV F concentration highlights dynamic vaccine nanoparticle.



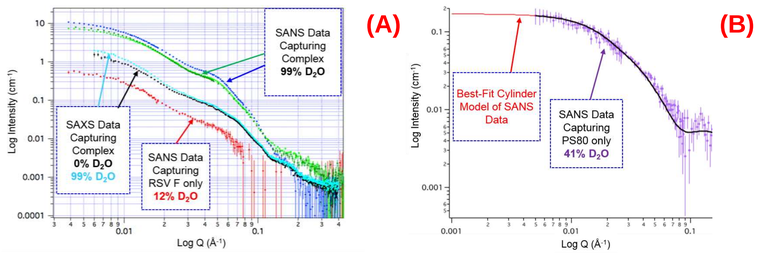
- Changing the molar ratio of PS80 to RSV F protein modulates hydrodynamic diameter (D_h) of the vaccine nanoparticle.
- PS80 competes with RSV F trimer-trimer interactions and reduces nanoparticle size.
- Nanoparticle size in the Drug Substance is controlled by the RSV F manufacturing process.

Fig. 2. AUC analysis of RSV F. **(A)** Sedimentation velocity AUC data of the RSV F nanoparticle vaccine at increasing concentrations of PS80. **(B)** Absorbance and interference detection of Drug Substance supports a complex between RSV F and PS80.



- AUC is a native separation technique that is based on first principles of sedimentation and diffusion.
- RSV F in DS exists as a discrete population of sedimenting species.
- Increasing PS80 concentration shifts the nanoparticle population to smaller size, consistent with DLS.
- Interference and absorbance sedimentation data overlap.
- No free PS80 micelles (s = 2 S).
- RSV F and PS80 co-sediment in the nanoparticle complex.
- The complex contains on average 4 to 9 RSV F trimers, with average mass of 1.2 MDa.

Fig. 3. SANS and SAXS data for RSV F DS in formulation buffer containing different concentrations of D_2O. **(A)** 0%, 12%, 99% D_2O. **(B)** 41% D_2O (fit to an ellipsoid model)



RESULTS

Continued, for Fig. 3.

- SAXS scattering profiles for RSV F sample at 0% and 99% D_2O (**Fig. 3A**) suggest no impact on structure.
- D_2O match points chosen based on scattering-length density calculations for protein (41% D_2O) and detergent (12% D_2O).
- Modeling of the 41% D_2O data (**Fig. 3B**) is consistent with an ellipsoidal-shaped detergent core having radius of 40 Å, length of 280 Å, and a radius of gyration of about 9 nm.
- Ten different SANS/SAXS data sets were analyzed by Guinier fitting to obtain mass (from I(0)) and radial size of RSV F and PS80 components as well as the RSV F nanoparticle complex. Results are shown in **Table 1**.

Table 1: Component analysis of the RSV F-PS80 nanoparticle vaccine by contrast matching with SANS.

Component	Mass	$R_{gyration}$
RSV F in complex	1 MDa	15 nm
PS80 in complex	0.5 MDa	9 nm
RSV F-PS80 complex	1.5 MDa	15 nm

CONCLUSIONS

- DLS, AUC, and SANS/SAXS data indicate that the RSV F nanoparticle vaccine is a stable colloidal system consisting of highly-purified RSV F protein trimers in complex with PS80 detergent.
- Hydrodynamic size of the RSV F nanoparticle, measured by DLS, was shown to be dependent on the molar ratio of PS80 to RSV F and is controlled through the vaccine manufacturing process.
- AUC and SAXS/SANS measurements of the RSV F nanoparticle mass produced similar results in the range of 1.2 – 1.5 MDa. This mass corresponds to an average of 5 RSV F trimers and 300 PS80 molecules per nanoparticle.
- Modeling of the SANS data indicates PS80 is organized in an ellipsoidal shell at the core of the RSV F nanoparticle. Detailed modeling of the arrangement of RSV F trimers in the nanoparticle is in progress.

REFERENCES

1. August, A, Glenn, GM, et al. A Phase 2 randomized, observer-blind, placebo-controlled, dose-ranging trial of aluminum-adjuvanted respiratory syncytial virus F particle vaccine formulations in healthy women of childbearing age. Vaccine 2017; 35(30), 3691-3796.

Conflict of Interest Disclosure: ELM, DS, OB, and GS are employees of Novavax, Inc. JEC, AG and SK are employees of NIST. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter of materials discussed in the poster apart from those disclosed.