Poster # 70

Antigenic Characterization of RSV Pre-fusogenic F Nanoparticle Vaccine, Pre-F, and Post-F Proteins against a Broad Range of Neutralizing Monoclonal Antibodies and Epitope Responses in Cotton Rats

N Patel, JH Tian, H Lu, K Jacobson, M Guebre-Xabier, G Glenn, G Smith
Novavax, Inc., Gaithersburg, MD

Contact:
Nita Patel, MS, CRP
npatel@novavax.com
Phone: 240.268.2000

NOVAVAX
Creating Tomorrow's Vaccines Today

BACKGROUND

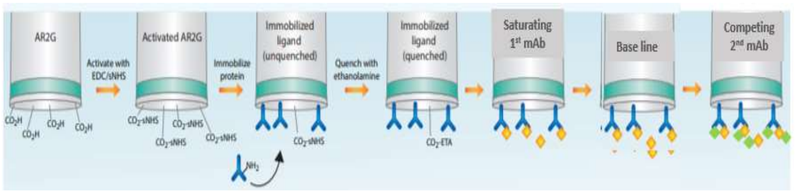
Respiratory Syncytial Virus (RSV) is one of the most common causes of lower respiratory tract infections (LRTI) among infants and young children worldwide. Severe cases of LRTI can result in inflammation of the small airways of the lungs (bronchiolitis) or pneumonia. ResVax (Pre-fusogenic RSV F nanoparticle 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. In animal and clinical studies, pre-fusogenic F nanoparticle vaccine has been shown to induce broadly neutralizing antibodies. Here, we characterize the binding efficiency and competitive binning of a panel of well characterized neutralizing monoclonal antibodies.

OBJECTIVES

To study and compare antigenic characterization of different forms of the RSV F protein against a broad range of neutralizing monoclonal antibodies and epitope responses in cotton rats.

METHODS

- Cotton rats were immunized intramuscularly (IM) with Pre-fusogenic F nanoparticle vaccine, Post-F, novel stable (NVAX) Pre-F or single-chain triple mutant (SCTM) Pre-F with or without $AlPO_4$ on Days 0 and 21. Intranasal RSV challenge was performed on Day 42 with 10^5pfu of RSV A Long strain. Lung tissue was collected on Day 46.
- RSV-F specific serum IgG titers were measured by ELISA to RSV F protein on day 42.
- Lung viral titers were measured by plaque assay on HEp2 cells.
- Serum RSV Neutralization titers were measured on RSV/A Long strain. RSV replication was determined by using anti-RSV NP mouse antibody. The 50% neutralization titer was calculated by 4-parameter curve fitting.
- Immune human serum adsorption study was performed using biotinylated RSV F linked to streptavidin magnetic beads. Anti-RSV F titers to Pre-F were determined by ELISA.
- Antibody epitope binning and Real-time competition binding studies were conducted using Octet QK 384 Bio-Layer Interferometry. RSV F proteins bound to biosensor tips were reacted with first antibody or cotton rat sera followed by Pre and Post-fusion F specific RSV mAbs. Binding or competition was analyzed using Octet HT 10 software. Competing antibody equivalent (CAE, µg/ml) was calculated based on percentage competition and concentration of competing antibody.



RESULTS

Fig 1. Antibody Epitope Binning on RSV F Proteins

A. Pre-fusogenic F Nanoparticle Vaccine

1st	2nd mAb	Pre-fusion F: Ø D25	Ø hRSV106	Ø hRSV90	Pre-fusion and Post-fusion F: IIa Pali	IIb Mota	IIb RSV3J20	IV R1.42	IV RSHZ19	II/IV 4C6	Pre-fusogenic F P27
Ø	D25	0	57	50	42	50	41	23	32	23	25
	hRSV106	15	0	51	14	18	15	12	9	14	18
VIII	hRSV90	28	109	0	47	63	62	27	53	0	32
IIa	Pali	0	0	23	0	12	3	69	16	23	71
IIb	Mota	0	0	19	0	0	0	70	7	22	73
	RSV3J20	5	33	23	32	40	0	64	14	17	81
IV	R1.42	0	0	0	86	107	96	0	25	40	29
	RSHZ19	15	76	36	79	98	65	24	0	21	76
II/IV	R4.C6	0	0	0	26	32	31	33	22	0	85
P27	RSV.7.10	0	0	0	52	62	60	33	34	81	0

- mAbs to Site Ø and IV strongly compete for binding.
- Antigenic site Ø and IV are very close or overlapping.
- Site II and IV are distinct .
- Presence of unique P27 antigenic site.

B. NVAX Pre-fusion F

1st	2nd mAb	Pre-fusion F: Ø D25	Ø hRSV106	Ø hRSV90	Pre-fusion F and Post-fusion F: IIa Pali	IIb Mota	IIb RSV3J20	IV R1.42	IV RSHZ19	II/IV R4.C6	Pre-fusogenic F P27
Ø	D25	5	9	22	95	91	87	97	73	78	-
	hRSV106	10	8	81	75	87	81	76	71	69	-
VIII	hRSV90	0	107	4	2	20	67	78	75	5	-
IIa	Pali	95	75	38	4	13	10	86	72	5	-
IIb	Mota	97	77	54	0	2	2	83	75	0	-
	RSV3J20	96	71	84	1	6	4	87	80	2	-
IV	R1.42	84	80	84	83	89	86	0	0	75	-
	RSHZ19	88	79	89	87	108	90	8	1	80	-
II/IV	R4.C6	90	70	26	10	22	15	84	73	7	-
P27	RSV.7.10	-	-	-	-	-	-	-	-	-	-

- mAbs to Site Ø and VIII strongly compete for binding.
- Antigenic site Ø and VIII are close or overlapping.
- Site Ø, II and IV are distinct from each other.
- Antigenic site P27 is absent.

C. Post-fusion F

1st	2nd mAb	Pre-fusion F: Ø D25	Ø hRSV106	Ø hRSV90	Pre-fusion F and Post-fusion F: IIa Pali	IIb Mota	IIb RSV3J20	IV R1.42	IV RSHZ19	II/IV R4.C6	Pre-fusogenic F RSV7.10
Ø	D25	-	-	-	-	-	-	-	-	-	-
	hRSV106	-	-	-	-	-	-	-	-	-	-
VIII	hRSV90	-	-	-	-	-	-	-	-	-	-
IIa	Pali	-	-	-	0	9	11	74	57	6	-
IIb	Mota	-	-	-	0	0	4	73	59	2	-
	RSV3J20	-	-	-	3	7	0	67	42	6	-
IV	R1.42	-	-	-	21	26	16	0	0	2	-
	RSHZ19	-	-	-	32	44	42	26	0	15	-
II/IV	R4.C6	-	-	-	0	7	7	24	6	0	-
P27	RSV7.10	-	-	-	-	-	-	-	-	-	-

- mAbs to Site II and IV do not compete for binding.
- Site II and IV are distinct from each other.
- Antigenic Site Ø, VIII and P27 are absent.

No Competition | Moderate Competition | Strong Competition | Self binding

Fig 2. Immune Responses to RSV F Proteins on Day 42

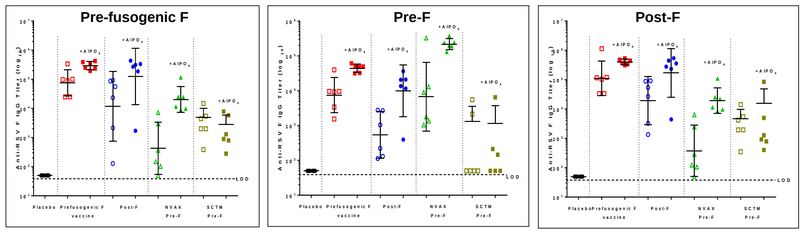
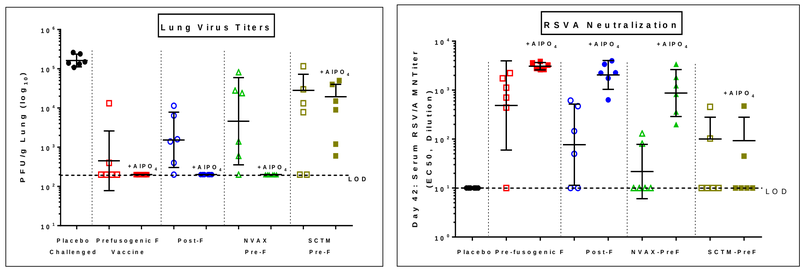


Fig 3. RSV Lung Virus and RSVA Neutralization Titers



RESULTS

Fig 4. CAE Responses and Anti-F IgG vs RSV/A Neutralization Titer

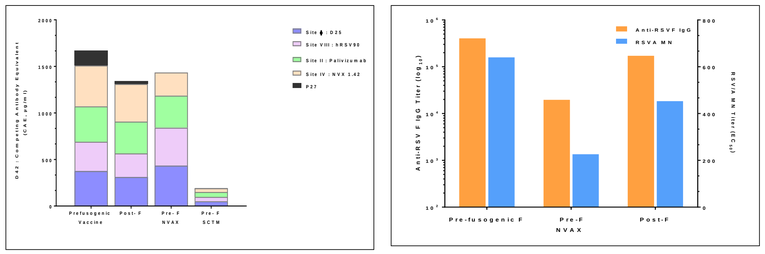
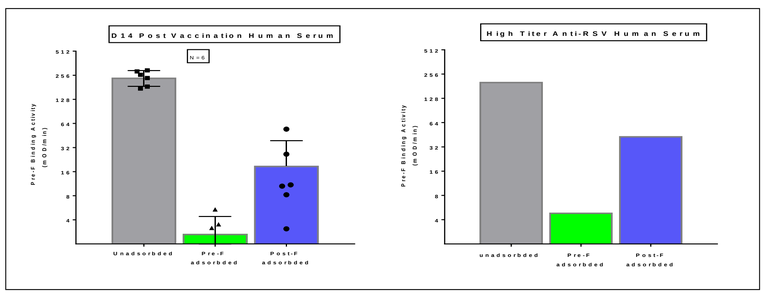


Fig 5. Human Serum Adsorption with Pre-F and Post-F Proteins



CONCLUSIONS

- Antibody epitope binning reveals the differences of antigenic sites present on Pre-fusogenic F, Pre-F and Post-F proteins.
- Epitope binning shows presence of both Pre-F and Post-F specific antigenic sites on Pre-fusogenic F nanoparticle vaccine.
- Pre-fusogenic F nanoparticle vaccine with adjuvant elicited robust anti-RSV F IgG responses, Pre-F and Post-F antigenic site responses, and potent RSV neutralization in cotton rats.
- Increased CAE to all RSV F antigenic sites specific to Pre-F, Post-F and p27 was observed with Pre-fusogenic F nanoparticle vaccine compared to Pre-F and Post-F protein.
- Adsorption studies with post vaccinated human serum on RSV Pre-F and Post-F proteins confirms that Pre-fusogenic F nanoparticle vaccine induce antibodies against Pre-F epitopes.

REFERENCES

1. Smith G, et al. Respiratory syncytial virus fusion glycoprotein expressed in insect cells form protein nanoparticles that induce protective immunity in cotton rats. PLOS ONE2012;7:e50852.
2. Raghunandan R, et al. An insect cell derived respiratory syncytial virus (RSV) F nanoparticle vaccine induces antigenic site II antibodies and protects against RSV challenge in cotton rats by active and passive immunization. Vaccine 32; 2014 6485–6492.
3. Mousa J,etal. A novel pre-fusion conformation-specific neutralizing epitope on the respiratory syncytial virus fusion protein. Nature microbiology 2017; 2:16271.
4. Ngwuta JO,etal. Prefusion F–specific antibodies determine the magnitude of RSV neutralizing activity in human sera. Sci.Transl Med. 2015; 7(309).

Conflict of Interest Disclosure: NP, JHT, HL, KJ, MGX, GG and GS are employees of Novavax, Inc. 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.