Novavax
(240)-268-2000
IR@novavax.com

Development and Characterization of Recombinant RSV F Nanoparticle Vaccine Induced Monoclonal Antibody

Hanxin Lu, Ramadevi Raghunandan, David Flyer, Yingyun Wu, Mike Massare, Gale Smith, and Gregory Glenn. Novavax, Inc. , Gaithersburg, MD, 20878

Background: Respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory infection in infants and young children. The recombinant RSV F protein nanoparticle vaccine (RSV F nanoparticle vaccine) induces high levels of anti-RSV F IgG competitive for binding palivizumab (Synagis®). A site II binding, vaccine-derived mAb would confirm that the RSV F vaccine generates antibody specificities of similar binding affinities as palivizumab.

Methods: Using murine immunization with RSV F vaccine, a mAb, NVX4C6, was generated that binds to antigenic site II on the extracellular domain of RSV F protein, the target of palivizumab. Binding affinities were compared using Surface Plasmon Resonance (SPR).

Results: NVX4C6 was found to have neutralizing activity for RSV A, RSV B and a palivizumab escape mutant. Association and dissociation kinetic analyses using SPR indicate NVX4C6 binds with higher affinity to RSV F vaccine than palivizumab (Kd 0.13nM versus Kd 0.47nM).

INTRODUCTION RSV, a single-stranded, RNA virus of the paramyxovirus family, is the leading cause of severe lower respiratory tract disease in infants and young children worldwide and recognized as an important illness in elderly and high risk adults. Despite decades of research there is no vaccine or specific therapeutic agent for RSV and human RSV infection does not always induce fully protective immunity. Currently the monoclonal antibody palivizumab (Synagis®) against RSV Fusion protein antigenic site II prevent severe disease by passive immunoprophylaxis in high risk newborns. An RSV vaccine is in development by Novavax based on a recombinant, near-full length Fusion glycoprotein produced *Spodoptera frugiperda* (Sf9) insect cells, resulting 40 nanometer protein-protein micelles (nanoparticles). In both animal studies and clinical trials, Novavax' RSV F nanoparticle vaccine has been shown to induce palivizumab competing antibody. The current study aim is to evaluate mAbs derived from RSV F vaccine and compare it with palivizumab.

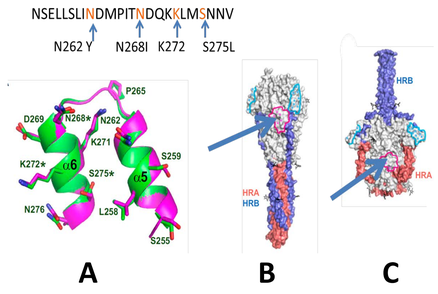

Fig. 1. Model of RSV F protein either in pre-fusion (B) or post-fusion (C) conformation with antigenic site II (A). The palivizumab interacting amino acid residues within antigenic site II peptide are indicated by arrows (Adapted from Swanson *et al*. PNAS 2011).

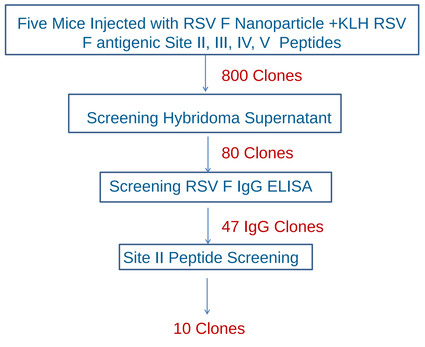

Fig. 2. Development of anti-RSV F (Fusion) protein mAb.

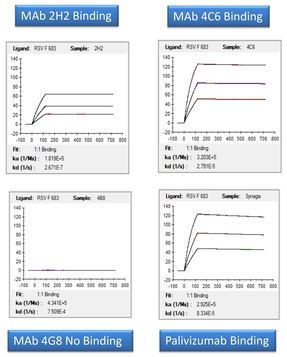

Fig. 3. Kinetics summary of RSV F protein binding to various mAbs. CM5 chips were amine coupled with RSV F vaccine Serial dilution of mAbs at 40nM, 20nM, 10nM, 5nM, and buffer control were used to bind RSVF on the chip. After 180 minutes of binding, dissociation was initiated with buffer. Association and dissociation rates were calculated based on 1:1 fitting model.

RSV F MAb	PCA	MN RSV- B	MN RSV- A	RSV F Protein KD(M)	PFP KD(M)	RSV F (K272M) KD(M)
NVX4C6	+	+	+	8.7E-11	8.40E-11	2.97E-09
Palivizumab	+	+	+	2.8E-10	8.98E-10	-
2GB	-	+	+	NA*	-	+
2H2	+	+	+	1.47E-12	-	+
4B10	-	+	+	NA*	-	-
4G8	-	+	+	-	-	-
5B7	-	-	+	NA*	-	-
5C4	-	-	+	NA*	-	+

Table 1. Summary of RSV F mAb binding kinetics and neutralizing activity. PCA: palivizumab competition antibody. MN: microneutralization assay. PFP: purified fusion protein from RSV A virus. RSV F(K272M) includes the point mutation at amino acid residue 272 as compared to RSV F protein.

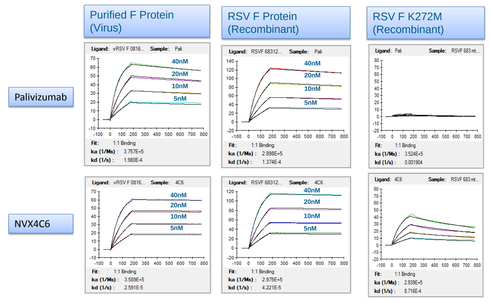

Fig. 4. Both RSV F protein and PFP (Purified F protein) from RSV virus bind to either palivizumab and NVX4C6 with high affinity. In contrast to palivizumab, NVX4C6 binds to a mutant form of RSV F (K272M). CM5 chips were coupled with PFP, RSV F vaccine or RSV F mutant protein. Serial dilution of mAbs at 40nM, 20nM, 10nM, 5nM, and buffer control were used to bind RSVF on the chip. After 180 minutes of binding, dissociation was initiated with buffer. Association and dissociation rates were calculated based on 1:1 fitting model.

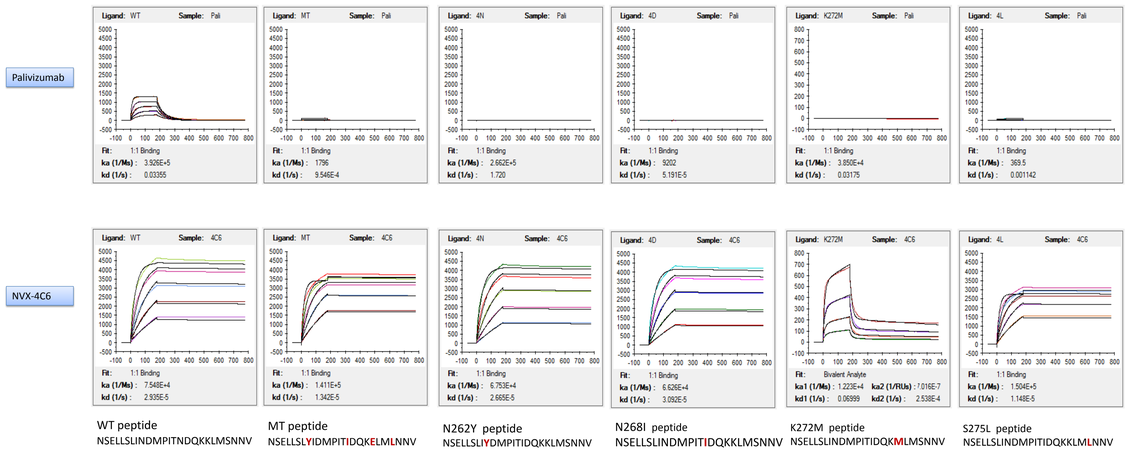

Fig. 5. Palivizumab and mAb NVX4C6 binding antigenic peptide kinetics. In contrast to palivizumab, NVX4C6 binds to all mutant peptide derived palivizumab escape mutant RSV virus with high affinity. SA chip was applied to capture biotin-RSVF site II antigenic wild type or mutant peptide as indicated above. Serial dilution of palivizumab or NVX4C6 at 400 nM, 200 nM, 100 nM, 50 nM, 25 nM and buffer control were used to bind RSVF on the chip. After 180 minutes of binding, dissociation was initiated with buffer. Association and dissociation rates were calculated based on 1:1 fitting model.

Palivizumab antigenic peptide fragments		NVX4C6 binding	Palivizumab binding
WT	NSELLSLINDMPITNDQKKLMSNNV	+++	++
2-1	NSELLSLINDMPITNDQKKL	+/-	-
2-2	LINDMPITNDQKKLMSNNV	++	-
3-1	NSELLSLINDMPITN	-	-
3-2	SLINDMPITNDQKKL	+/-	-
3-3	MPITNDQKKLMSNNV	++	-

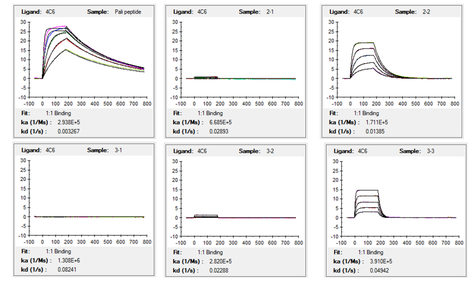

Fig. 6. Amino acid residue MSNNV within palivizumab antigenic peptide is critical for binding NVX4C6. A series of peptides were synthesized to evaluate peptide binding kinetics to NVX4C6. Either NVX4C6 or palivizumab was directly coupled to a CM5 chip. Serial dilution of peptide indicated at 400 nM, 200 nM, 100 nM, 50 nM, 25 nM and buffer control were used to bind monoclonal antibody on the chip. After 180 minutes of binding, dissociation was initiated with buffer. Association and dissociation rates were calculated based on 1:1 fitting model.

CONCLUSIONS

- Novavax's RSV F nanoparticle vaccine induces a high frequency of B cells with site II specificity.
- Some monoclonal antibodies generated from RSV F vaccine immunized mice demonstrate higher binding affinity to either RSV F protein antigen or antigenic peptide compared to palivizumab.
- The site II epitope specificity of monoclonal antibody NVX4C6 is different than palivizumab.
- NVX4C6 recognizes mutated site II peptide not recognized by palivizumab suggesting NVX4C6 may neutralize palivizumab escape mutant virus.