Immunogenicity of a Recombinant RSV F Protein Nanoparticle Vaccine Manufactured in Insect Cells: Induction of Palivizumab-like Activity

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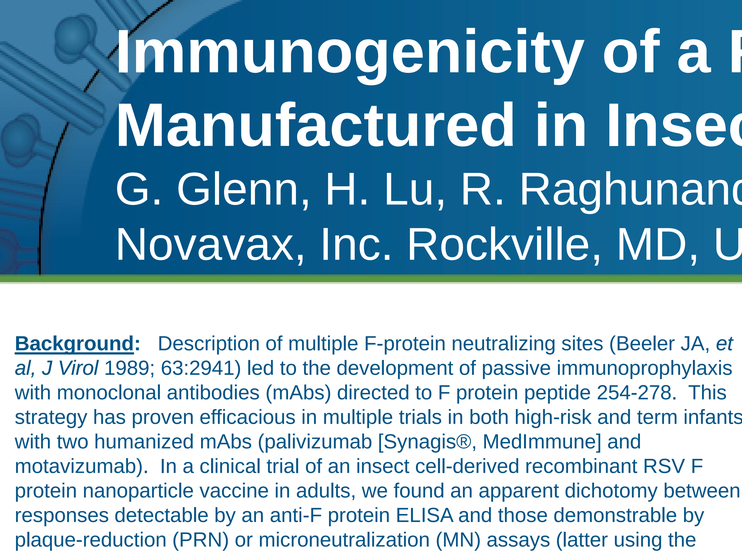
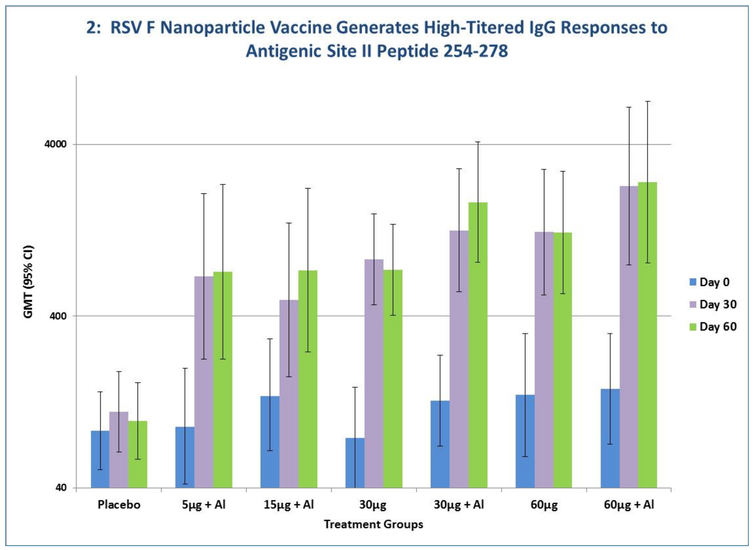
Background: Description of multiple F-protein neutralizing sites (Beeler JA, *et al, J Virol* 1989; 63:2941) led to the development of passive immunoprophylaxis with monoclonal antibodies (mAbs) directed to F protein peptide 254-278. This strategy has proven efficacious in multiple trials in both high-risk and term infants with two humanized mAbs (palivizumab [Synagis®, MedImmune] and motavizumab). In a clinical trial of an insect cell-derived recombinant RSV F protein nanoparticle vaccine in adults, we found an apparent dichotomy between responses detectable by an anti-F protein ELISA and those demonstrable by plaque-reduction (PRN) or microneutralization (MN) assays (latter using the method of Piedra PA, *et al*. *Pediatr Infect Dis J* 1996; 15:23; **see Panel 1**). In order to better understand this result, we examined the background levels of antibodies to the 254-278 peptide in these adults, induction of responses to this peptide by the recombinant F protein vaccine, and the induction of antibodies competitive with palivizumab for binding to F protein trimers *in vitro.*

The Vaccine: The vaccine comprises nanoparticles composed of near-full length F protein, assembled into trimers. F protein is produced in *Sf*9 insect cells infected with a recombinant baculovirus. The cells are lysed and solubilized with detergent, and the F protein is purified chromatographically. The antigen is delivered by intramuscular injection, with or without adsorption to $AlPO_4$.

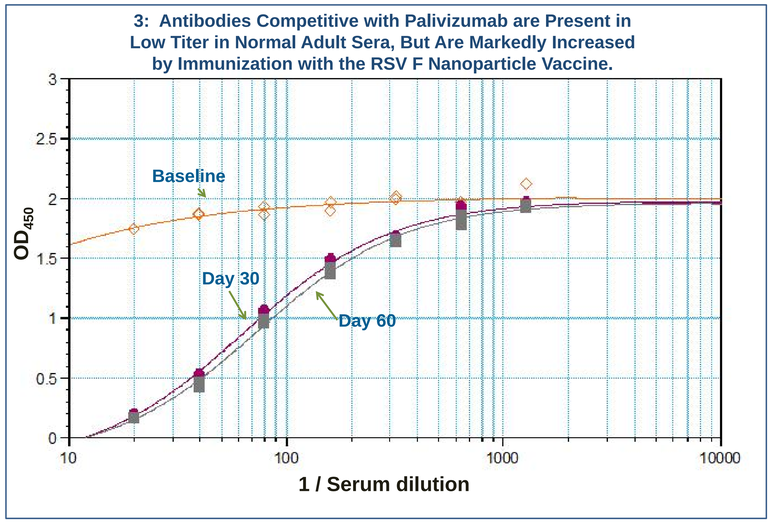
The Clinical Trial: Healthy adults (N = 150; mean age 31.3 years; 59% female) were enrolled in 6 cohorts, each including 20 active vaccine and 5 placebo recipients. Test articles were given as 2-dose series at a 30 day interval; RSV F antigen was tested at doses of 5, 15, 30 and 60µg adsorbed to $AlPO_4$, and 30 and 60µg without adjuvant. Safety was monitored by soliciting local and systemic symptoms for 7 days after each dose, and ascertainment of unsolicited adverse events for 6 months. Functional antibodies were assessed using PRN and MN assays (which gave similar results) and ELISA assays for multiple antigens as below. The trial is described elsewhere at this symposium (session VII).



Panel 1. RSV Neutralizing Responses Appear to Lag anti-F Responses: MN antibody responses occur in the active groups, but anti-F responses are much more dynamic Whereas baseline anti-F IgG titers spanned a more limited range, baseline microneutralization titers varied over a >32-fold range. The vaccine induced substantial MN responses in subjects with low MN baselines (geometric mean 3.9-fold rises in those in the lowest 1/3 of the population), but overall fold- rises were limited by the 1/3 of subjects with high baseline values (where <2-fold responses were noted).



Panel 2. RSV F Nanoparticle Vaccine Elicits Antibody Responses to Antigenic site II Peptide 254-278: Synthetic RSV-F peptide 254-278, which harbors the palivizumab and motavizumab epitopes, was biotinylated and immobilized to streptavidin-coated plates. Serial dilutions of sera were incubated with the peptide coated plates. Bound IgG was detected after washing with enzyme-conjugated goat anti-human IgG. Pre-immunization titers were uniformly low, but increased 5- to 15-fold with receipt of RSV-F nanoparticle vaccine.



Panel 3. Palivizumab Competition ELISA with Human Serum Before and After RSV-F Nanoparticle Immunization: ELISA plates were coated with RSV F protein antigen at 2µg/mL. Pre- and post-immunization sera were serially diluted, spiked with 50ng/mL biotinylated palivizumab, and then incubated in the coated plates. Enzyme-conjugated streptavidin was used to detect palivizumab bound to the plate. A four-parameter fit was generated and the serum dilution yielding 50% palivizumab binding inhibition was interpolated.

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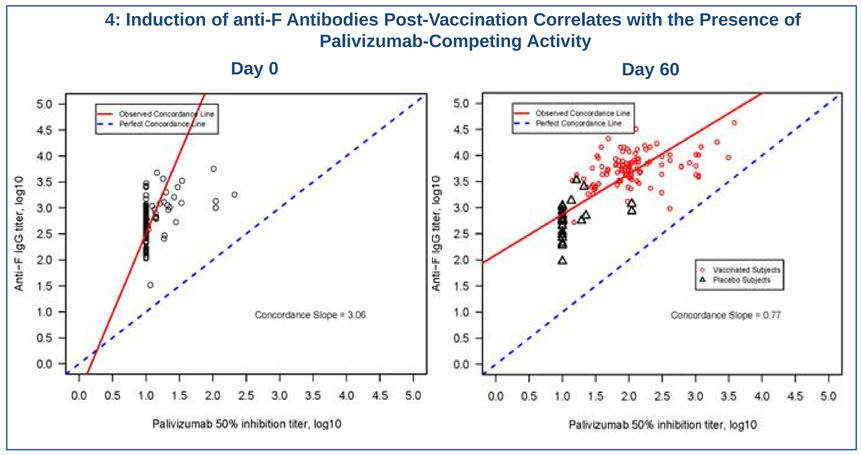

Panel 4. Increases in RSV F Nanoparticle Vaccine-induced Anti-F Antibodies Correlate with Enrichment of Antibodies that Compete for the Palivizumab Binding Site: Despite anti-F titers present at Day 0, most healthy young adult sera yielded palivizumab-like antibody levels near the competition assay LLOQ. On Day 60, placebo recipients had an unchanged distribution, but active vaccinees (in red) showed an increase in anti-F antibodies with enrichment of palivizumab-like specificities

Unlabeled palivizumab, added to normal serum, achieved 50% inhibition in the competitive ELISA at 2.1µg/mL. This was used in **Table 1** to calculate an approximate equivalent of "palivizumab-like" activity in vaccinee serum. In parallel, palivizumab was also spiked into 5 normal adult sera at three different levels, and its impact on MN titer was determined; increases in GMTs based on 2 replicates on each of 2 different days are shown in **Table 2**.

Table 1: 50% Inhibitory GMTs and Calculated Palivizumab-like Activity in Subject Sera

	Placebo	5µg + Al	15µg + Al	30µg only	30µg + Al	60µg only	60µg + Al
Day 0 GMT (95% CI)	6 (5-6)	6 (5-8)	7 (5-10)	7 (5-10)	5 (-)	10 (7-15)	7 (5-10)
Day 0 "palivizumab equivalent"(µg/mL)	11	13	15	15	10	21	15
Day 60 GMT (95% CI)	7 (6-9)	55 (38-81)	64 (51-80)	50 (34-73)	71 (57-88)	79 (54-115)	169 (127-224)
Day 60 "palivizumab equivalent"(µg/mL)	15	116	135	106	149	165	337

Table 2: Impact of Defined Palivizumab Inputs on MN Activity in Human Adult Sera

	Baseline MN GMT	Fold-increase of MN GMT with Added Palivizumab		
Palivizumab	0	40µg/mL	80µg/mL	120µg/mL
Serum 1	64	2.2	3.8	4.8
Serum 2	136	1.3	2.1	2.7
Serum 3	192	1.5	2.0	2.1
Serum 4	342	1.1	1.3	1.4
Serum 5	456	0.9	1.0	1.3

Conclusions:

- **Robust anti-F protein immune responses to the insect cell-derived RSV F protein nanoparticle vaccine are enriched in antibodies to the highly-conserved and clinically important F protein antigenic site II, which contains the palivizumab and motavizumab binding sites.**
- **Levels of palivizumab-like activity achieved by the vaccine are commensurate with those that have been efficacious when passively administered to infants, and vaccine-induced increases in serum MN activity are closely similar to those produced by spiking sera with comparable concentrations of palivizumab.**
- **Further clinical development of this RSV F protein vaccine candidate is warranted.**

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