Development of Protein Capsular Matrix Vaccine (PCMV) Technology

Thanawastien, A., Griffin, T., and K. P. Killeen

atrivax

650 Albany Street
Boston, MA USA

1. Introduction

Background:

Matrivax R&D Corp. is a start-up biotechnology company with R&D operations located in Boston, USA and full access to a vaccine pilot manufacturing facility in Haikou, China. We are developing a proprietary vaccine process that entraps polysaccharides in a cross-linked protein 'carrier' or matrix, termed Protein Capsular Matrix Vaccine (PCMV), as an alternative to conjugate vaccine technology. Despite highly efficacious pneumococcal conjugate vaccines , eg., Prevnar®, *S. pneumoniae* causes > 1 million deaths worldwide annually. Likewise, typhoid fever afflicts ~16 million people, resulting in 600,000 deaths despite effective vaccines such as Typhim Vi® and Vivotif ® (Ty21a). Inexpensive, efficacious polysaccharide vaccines that elicit T_H-cell 'memory' will actively displace their unconjugated and conjugated vaccine counterparts. Towards this end, Matrivax is actively research and developing pneumococcal, enteric fever, and meningococcal PCMV candidates.

Summary of Results:

1) 'Optimized' PCMV chemical reaction conditions improve polysaccharide incorporation into protein matrices,

2) Fractionation of PCMV particles by size-exclusion chromatography significantly increases immunopotency **(Section 3),**

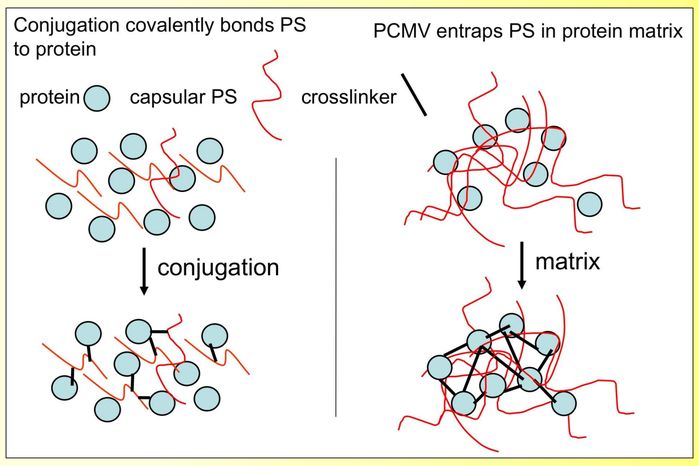
3) 'Optimized' PPS14 PCMVs containing 1.5% or 6% the amount of PPS14 antigen contained in Prevnar® elicits a comparable anti-PPS14 antibody response in a murine immunogenicity model **(Section 4),**

4) PCMV immunization elicits an immune response suggestive of memory **(Section 5),**

5) S. Typhi Vi antigen is captured in a DNI PCMV,

6) Immunization with Vi PCMV elicited ~8 fold higher specific response compared to immunization with Typhim Vi® **(Section 6).**

2. PCMV Concept



Issues associated with conjugate vaccines:

1) relatively high cost of goods (COGs),

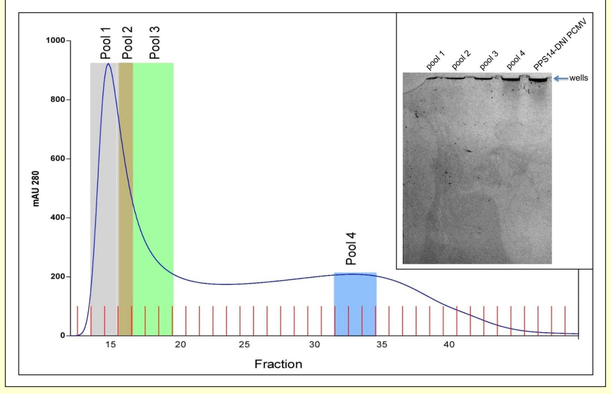
2) limitation in the number of PS serotypes that can be incorporated (reduced breadth of protection); and

3) each PS require different conjugation chemistry with carrier protein

Alternative approach: Protein Capsular Matrix Vaccine (PCMV) technology may allow multiple PS incorporation 'cocktail' which will streamline manufacture, enabling addition of increased serotypes, and reduce COGs.

3. Optimization of PCMV process

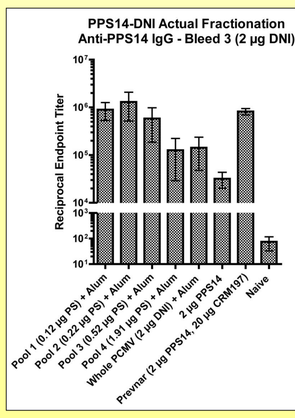
Chromatogram and SDS PAGE of PPS14:DNI PCMV reaction for immunopotency experiment



Reaction parameters & particle sizing:
1) alter reactant proportions and reaction time
2) new cross-linking agents
3) alternative homologous and heterologous protein matrices
4) size fractionate particles

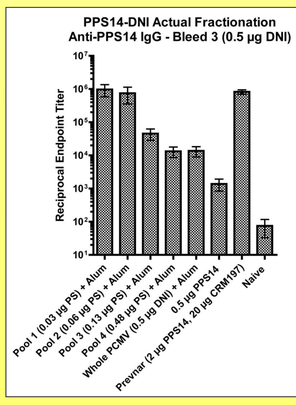
4. Preclinical comparison of PCMV to Prevnar®

Day 39 anti-PPS14 IgG titers showing mean/standard error of the mean (left panel) and GMT (right panel) from mice immunized with PCMVs and Prevnar.



PPS14 dose (2µg DNI)	Day 38 GMT
Pool 1 (0.12 µg PS)	619077
Pool 2 (0.22 µg PS)	586094
Pool 3 (0.52 µg PS)	256531
Pool 4 (1.91 µg PS)	36933
Whole PCMV	13846
PPS14 only (2 µg)	12335
Prevnar (2 µg PS, 20 µg CRM197)	776047
Naive	50

Day 39 anti-PPS14 IgG titers showing mean/standard error of the mean (left panel) and GMT (right panel) from mice immunized with PCMVs and Prevnar.



PPS14 dose (0.5 µg DNI)	Day 38 GMT
Pool 1 (0.03 µg PS)	501103
Pool 2 (0.06 µg PS)	228543
Pool 3 (0.13 µg PS)	27691
Pool 4 (0.48 µg PS)	4615
Whole PCMV (0.5 µg DNI)	9413
PPS14 only (0.5 µg)	800
Prevnar (2 µg PS, 20 µg CRM197)	776047
Naive	50

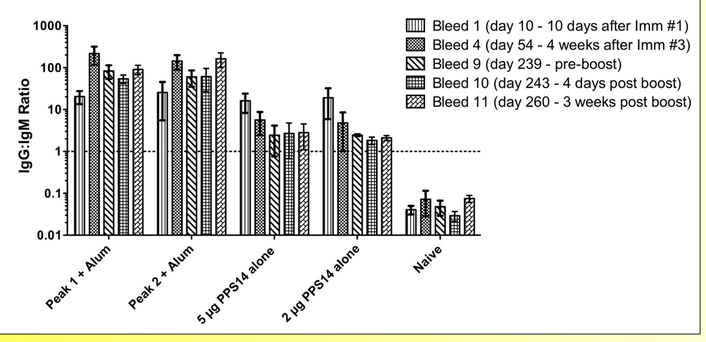
Result: Anti-PPS14 IgG GMT from PPS14-DNI PCMV-immunized animals is comparable to that of Prevnar-immunized animals at either 6% the dose (top panel) or 1.5% the dose (bottom panel) of PPS14 contained in Prevnar.

5. PPS14 PCMV Elicits Memory Immune Response

Mice were immunized on Day 0, 14, and 28 and then boosted on Day 239. Anti-PPS14 IgG and IgM responses were assayed 4 days and 21 days post-boost.

Immunization Group	Boosted with:	Pre-Boost GMT (day 239)	4 days post-boost GMT (day 243)	3 weeks post-boost GMT (day 260)
Peak 1 + Alum (5 µg DNI and 2.4 µg PPS14)	**(5 µg DNI and 0.6 µg PPS14) + Alum**	**334531**	**408445**	**816890**
Peak 2 + Alum (5 µg DNI and 5 µg PPS14	**(5 µg DNI and 0.6 µg PPS14) + Alum**	**152691**	**266251**	**863756**
5 µg PPS14	**5 µg PPS14**	**2652**	**2652**	**3046**
2 µg PPS14	**2 µg PPS14**	**11314**	**11314**	**11314**
Naive		**119**	**100**	**71**

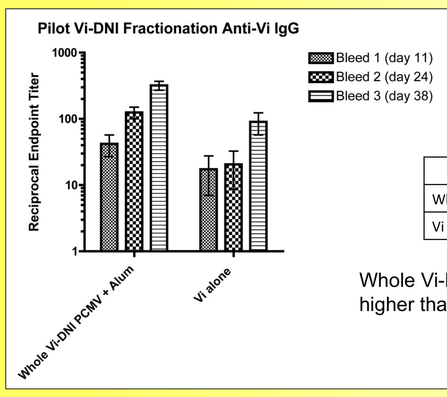
PPS14-DNI PCMV-Induced Anti-PPS14 IgG-to-IgM Ratio



Following a boost ~ 7 months after PCMV primes there is an increase in GMT at 4 days which continues to rise at 21 days (top panel, table) compared to PS immunized animals. More specific IgG is induced in PCMV-immunized animals compared to PS immunized animals (bottom panel, graph).

6. Unoptimized Vi-DNI PCMV Immunogenicity

Mice were immunized on Day 0, 14, and 28. Sera was collected 10-12 days after each immunization and assayed for anti-Vi responses by ELISA.



10 µg DNI dose	Day 38 GMT
Whole Vi-DNI PCMV	303
Vi (10 µg)	40

Whole Vi-DNI PCMV GMT is ~8-fold higher than Vi alone

Immunization with an unoptimized Vi-DNI PCMV elicits ~8 fold higher anti-VI IgG GMT than immunization with Vi PS alone.

7. Vi-PCMV Typhoid Fever Vaccine Next Steps

- Apply optimized reaction conditions to Vi-PCMV
- Evaluate addional matrix proteins, e.g. CRM197
- Size-fractionate Vi-CRM197 PCMV particles and evaluate immunogenicity
- Finalize 10L scale expression conditions and purify *S.* Typhi-derived Vi and *C. diphtheriae* CRM197 and technology transfer to Haikou

8. Vaccine Technologies Institute (VTI)

Haikou China pilot vaccine facility

1) 3,000 square meters facility
2) Three parallel production suites (each certified for BL2 containment), 50 and 150 L fermentation capacity, polysaccharide purification, formulation, and filling
3) Projected completion date February 2010/ Validation underway

9. Conclusions and Future Direction

- High-titer antibody response elicited against particle sized PPS14- and unoptimized Vi-PCMVs; anamnestic/memory immune response induced
- In vitro and in vivo 'proof-of concept' demonstrated with anthrax and tularemia PCMV (functional antibody elicited with pneumococcal and meningococcal antigen PCMV; data not shown)
- Developing Vi, pneumococcal, and CRM197 expression and purification processes for technology transfer to Haikou
- GMP manufacture scheduled 4Q10 in Haikou
- Enteric fever Vi antigen based PCMV Phase 1 trial 2011