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Issues and Challenges in the Development of Pneumococcal Protein Vaccines: A Two Day International Symposium

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Abstract

In view of the increasing licensure and use of pneumococcal conjugate vaccines (PCVs), their relatively high cost, and growing issues with serotype emergence, there is a need to re-evaluate the role of pneumococcal protein vaccines (PPVs) and pathways to their licensure. This paper summarizes the discussion and viewpoints from an expert meeting regarding the development of PPVs. A wide spectrum of pneumococcal vaccine researchers, developers, and regulators met to review the state of PPVs, identify research and development needs, and provide consensus opinions to support the introduction of new PPVs where possible. They also discussed clinical and regulatory aspects pertinent to these vaccines and generated a series of recommendations for moving the field forward.

Keywords

Streptococcus pneumoniae; pneumococcal vaccine; protein; animal models

1. Introduction

The US National Institutes of Health (NIH) and PATH, an international nonprofit organization engaged in vaccine development, organized a symposium on the development of pneumococcal protein vaccines (PPVs), which took place in June 2010 in Dulles, Virginia. Researchers and vaccine developers in this field were invited to review the rationale and design of PPVs, as well as the current status of and challenges for their development and licensure. In addition to vaccine resources, participants identified research and development needs, and discussed regulatory requirements and pathways to vaccine licensure. Of note, a deliberate attempt was made to avoid discussing any particular PPV candidate in detail, but to discuss the requirements and challenges in moving a generic PPV through development, clinical evaluation and licensure. Issues relevant to developing country manufacturers were emphasized. In an effort to keep the discussion focused, other important issues such the role of mucosal immunity, vector-based approaches, alternate

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Expert Commentary

(the author's expert view on the current status of the field under discussion)

Five-year view

(a speculative viewpoint on how the field will evolve in 5 years time)

Key points:

(8–10 bullet points summarizing the review)

routes of administration, needle-free delivery and vaccine stabilization were beyond the scope of this symposium and were either not discussed or discussed superficially. What follows is a summary of the symposium, points of consensus, and recommendations for moving forward.

2. Rationale for development of a PPV

With more than 90 different and regionally variable serotypes, *Streptococcus pneumoniae* (pneumococcus) is a leading cause of pneumonia, meningitis, sepsis, and acute otitis media (OM) worldwide.^{1,2} To protect against the bacterium, currently available pneumococcal conjugate vaccines (PCVs) elicit antibodies to the capsule type of the most prevalent pneumococcal serotypes.³ Since the successful introduction of a 7-valent conjugate vaccine (PCV-7) in the United States in 2000, the prevalence of invasive pneumococcal disease (IPD) has greatly decreased in the country.⁴ Ten and 13-valent PCVs have achieved licensure with the latter expected to be widely used for routine childhood vaccinations in the United States.^{5,6}

While PCVs are highly effective at preventing IPD caused by serotypes included in the vaccines, they are extremely complex and relatively costly vaccines to manufacture, and do not offer protection against all disease-causing pneumococcal serotypes. In contrast, with a more efficient and less expensive manufacturing process, PPVs have the potential to provide broad coverage across all serotypes, and thereby prevent serotype emergence or replacement. In preclinical models, PPVs have demonstrated diverse immunological mechanisms of protection and have the potential to protect against all stages of pneumococcal disease, including nasopharyngeal carriage, OM, pneumonia and invasive disease.

Although PCVs are very effective among the general population in the United States and certain other industrialized countries, there is a concern that among susceptible populations, particularly in developing countries, PCVs may have less overall impact. For example, following the introduction of PCV-7, there has been a steady increase in pneumococcal disease caused by non-vaccine serotypes among Alaskan natives.⁷ Thus, the overall impact on pneumococcal disease in this population has been minor. As a result, the need for vaccines with broad, serotype-independent coverage may be greater in certain parts of the world. Due to differing serotype epidemiologies and/or issues of cost and availability, the demand for and use of a PPV will depend on the region.

3. PPVs

To be optimally effective, a PPV must induce protective functional antibodies and/or CD4⁺ T cell and IL-17A dependent immune responses. Anti-protein antibodies are important for eliciting protection by blocking critical steps in pneumococcal pathogenesis and/or promoting opsonophagocytosis. Recent evidence suggests that in addition to antibodies, T helper 17 (Th17) cells play an important role in protective immunity against *S. pneumoniae*.⁸ In particular, evidence from preclinical models suggests that Th17 cells specific for protein antigens are important in protection against nasopharyngeal carriage, whereas antibodies to proteins are critical for protection against pneumonia and IPD. In addition, target antigens for eliciting protective immune responses against invasive disease may differ from those protective against pneumonia, though there is likely to be some overlap. Importantly, T cell-dependent vaccines are highly immunogenic in infants, though the spectrum of T helper cell phenotypes and cytokines produced may be different from those in adults.

Pneumococcal proteins could potentially play a role either alone or together with capsular polysaccharide as a means to broaden the protection offered by PCVs. Three such strategies were discussed by symposium participants:

a. Proteins as stand-alone vaccines

While less complex and potentially cheaper to manufacture than PCVs, a stand-alone multivalent protein vaccine approach has a yet-to-be defined regulatory pathway and will require human efficacy data for licensure. Furthermore, a stand-alone PPV may also have to demonstrate a reduction in nasopharyngeal carriage and impact on herd protection, without which it may be viewed as an inferior vaccine to PCVs. One concern of a stand-alone PPV, based upon experience with preclinical animal models, is that it may not be as efficacious as a PCV, though its potential for broader protection against all serotypes may compensate. Another challenge is that correlates of protection are often unknown and a potency assay for protein vaccines may be difficult to develop.

b. Pneumococcal protein(s) as a carrier for a PCV

Employing conserved pneumococcal protein(s) as a carrier for a PCV is an alternative approach to broadening coverage that has generated some interest. In the case of a limited valency PCV, a high yield conjugation technology that produces a PCV with a sufficient number of serotypes (a minimum of four to five) to enable at least 50 percent coverage could be utilized and combined with common pneumococcal proteins for a combination PCV-PPV vaccine. An effective carrier protein could provide the T cell epitopes required of the conjugate carrier effect, and induce serotype-independent protective immunity distinct from the serotype-specific antibody response directed towards the included capsular polysaccharides. An example comes from SynflorixTM, where utilization of protein D from nontypable *Haemophilus influenzae* (NTHi) as a carrier protein offered protection against OM caused by NTHi.⁹ A small number of serogroups (1, 5, 6, 14, 19) are responsible for most IPD occurring globally, which supports a rationale for targeting these serogroups through a limited valency PCV-PPV. Understanding that conjugate vaccine manufacture is complex and costly, the target populations for a limited valency PCV-PPV combination vaccine would likely be in the developing world.

Since the protein(s) is viewed as a carrier protein(s), this approach may be able to use the same regulatory pathway as conventional PCVs, provided the carrier function promotes immune responses to the polysaccharides that meet the standard of non-inferiority to licensed PCVs. The ability of the protein carrier to offer additional protection against serotypes not included in the PCV would then need to be demonstrated in post-licensure clinical trials.

c. Proteins combined with a PCV

Another approach is to extend protection by simply adding one or more pneumococcal proteins to a PCV. While capable of potentially broadening coverage and minimizing serotype emergence, this approach would require human efficacy data to show that the protein(s) contributes to protection before the vaccine could be licensed.

4. Protein antigen discovery

Studies during the 1980s on pneumococcal pathogenesis identified initial protein antigen targets; however, access to the *S. pneumoniae* genome sequence in the late 1990s permitted a more systematic approach to protein antigen discovery. As a result, researchers have since identified over 20 pneumococcal proteins as protective antigens using animal models of pneumococcal disease. These candidate vaccine antigens are involved in a variety of cellular

functions, but, in some cases, have no known functions. While the symposium was not designed to discuss specific PPVs in detail, participants did highlight some protein candidates, some of which are briefly discussed below:

a. Pneumococcal surface protein A (PspA)

PspA is a choline-binding protein that inhibits complement-mediated clearance of pneumococci from the blood, and binds and protects against bactericidal *apo*-lactoferrin. Despite significant sequence heterogeneity among pneumococcal isolates, some regions of PspA are conserved and PspA is cross-protective against multiple serotypes. Interestingly, researchers conducted a Phase 1 clinical trial with a PspA vaccine a number of years ago and demonstrated that it was safe and immunogenic; however, low sequence homology with human cardiac myosin has led to theoretical concerns about PspA eliciting autoantibodies that might promote cardiac inflammation or autoimmune disease. Current strategies with PspA focus on regions of the molecule that do not contain the homology with myosin.

b. Pneumococcal histidine triad protein D (PhtD)

PhtD is a surface-exposed protein suggested to be involved in the invasion process of the pneumococcus and the inhibition of complement deposition. It is highly conserved among various pneumococcal strains and has been shown to elicit protective immune responses in a number of preclinical animal models, including invasive disease and pneumonia.

c. Pneumolysin

Pneumolysin is a multifunctional hemolytic cytolysin that activates the classical complement pathway and is highly conserved among pneumococcal isolates. Pneumolysin contributes to the early pathogenesis of invasive pneumococcal pneumonia by facilitating intrapulmonary bacterial growth and invasion into the blood. Various genetic mutants of pneumolysin (pneumolysoids) that have reduced or no remaining toxin activity have been developed as vaccine candidates and have been assessed in preclinical models. Pneumolysoids have also been developed as potential carrier proteins for PCVs.

d. IC47 antigens

Intercell AG has developed a PPV candidate consisting of the following three highly conserved protein antigens: serine/threonine kinase protein (StkP), a surface protein needed for cell wall separation (PcsB); pneumococcal surface adhesin protein A (PsaA) with roles in cell division and signaling, cell wall metabolism and division; and adhesion (Mn^{2+} -binding lipoprotein component of ABC transporter). The IC47 vaccine has demonstrated protection against sepsis, pneumonia, and nasopharyngeal carriage in animal models and is currently in early stage clinical evaluation.

e. RrgB

Existing in three variants, RrgB constitutes the backbone of the pneumococcal pilus and is protective in three different mouse models of pneumococcal infection. Novartis Vaccines and Diagnostics has developed a RrgB chimera of the three variants, which is protective against all piliated pneumococcal strains implicated in adhesion to epithelial cells, lung infection, and virulence.

f. Whole cell vaccine

Made from killed, non-encapsulated autolysin-deficient pneumococcal cells, a whole cell vaccine (WCV) candidate with the pneumolysin gene knocked out and replaced with pneumolysoid is currently in early stage clinical development. The pneumolysoid has three point mutations known to abolish both cytolytic activity and complement activation. The

WCV is inactivated using beta-propiolactone, formulated in aluminum hydroxide adjuvant, and administered by either the subcutaneous or intramuscular route. WCV protection against nasopharyngeal carriage is dependent upon Th17 cells and IL-17A, whereas protection against pneumococcal sepsis is dependent upon antibodies.

g. Selection of proteins

It remains unclear which proteins and in what combination will offer the best protection in a vaccine against pneumococcal disease in humans. A single antigen is vulnerable to antigenic and genomic variations, and no single protein antigen is able to completely protect against lethal challenge by all strains in preclinical animal models. The consensus at the symposium was that a combination of proteins has the highest chance of success in the clinic. Participants agreed that important questions remain about whether the proteins chosen are variable or present in all strains, or if they will work against carriage and different forms of pneumococcal disease.

The symposium participants also acknowledged that a globally representative collection of strains will be required to assess the diversity of antigens of interest as a means of determining the presence of protein antigens across strains with a high degree of sequence conservation. They agreed that seeking evidence of protective immunity against the protein antigen and knowledge about the relative roles of humoral and cellular immunity for the anti-protein response is necessary. They also emphasized the need for enhanced knowledge of possible human (mammalian) protein homology and the probable biological function of the protein. A genome-derived PPV candidate could have unanticipated toxicities and it may be important to confirm correct protein folding and conformation. Furthermore, horizontal gene exchange may influence a PPV's ability to protect. Also critical is the selection of an appropriate adjuvant, particularly if the vaccine is designed to elicit both B and T cell immunity. In order to elicit Th17 responses in immunologically naïve individuals, for example, an adjuvant more potent than aluminum salts may be necessary. The discussion of particular adjuvants was beyond the scope of the symposium though it was noted that novel adjuvants pose a particular regulatory concern for use in infants, one of the prime target populations for a PPV.

5. Preclinical activities

a. Animal models

Animal models are important to provide evidence regarding vaccine potential and to help elucidate the mechanisms of protection elicited by individual protein vaccine candidates. Several animal models of pneumococcal carriage, OM, sepsis (aspiration, intraperitoneal, and intravenous), meningitis, and pneumonia exist, yet the most predictive model of protective potential in humans is still not clear. Comparison of different animal models can provide insight into general protective mechanisms, and evaluation of protein candidates individually and in combinations is also useful. Preferred animal species and strains, pneumococcal challenge strains, disease models and routes of challenge, positive protective controls, endpoints for effectiveness measures, and other factors are all important to consider. Different proteins work best in different models supporting the notion that combinations of antigens may provide the most comprehensive protective immunity. Protective mechanisms for antibody-mediated immunity may include toxin neutralization, inhibition of adhesion, and opsonization. In addition, recent evidence suggests that Th17 cells play a role in protection against nasopharyngeal carriage in murine models. Overall, researchers proposed that PPVs with the greatest likelihood of success need to demonstrate significant protection in several animal models of pneumococcal disease utilizing a variety of challenge strains and serotypes. In this regard, models using standardized challenge

strains that can be reproduced in multiple laboratories are important to develop and disseminate.

b. *In vitro* functional assays and correlates of protection

To turn candidate pneumococcal proteins into useful vaccines, their immunogenicity needs to be monitored. The most common way of doing this is to measure the levels of antibody elicited. Even so, antibody levels can sometimes be misleading. For example, in assessing the protective capacity of PCV-7 against serotype 19A, enzyme-linked immunosorbent assay (ELISA) measurements of antibody levels did not correlate well with protection, but opsonic capacity did.¹⁰ Thus, it is important to have an assay measuring functional capacity of antibodies to these candidate antigens. Efficient assays measuring antibody function or opsonophagocytic capacity of anti-capsule antibodies induced with PCVs have been developed, although traditionally they had been considered to be technically difficult and impractical to standardize across laboratories.

In developing functional assays for the proteins impacting complement fixation (e.g., PspA), adaptation of opsonophagocytosis assays made for anti-capsule antibodies may be possible. Vaccines based on toxins such as pneumolysin should be assessed with toxin neutralization assays. However, many candidate proteins mediate different or unknown functions. In cases of unknown functions, one may adapt an epitope inhibition assay using monoclonal antibodies known to be protective. Merck used a similar approach to evaluate a human papillomavirus vaccine.¹¹ This analytical approach is adaptable to different antigen types, yielding an efficient multiplexed assay. In addition to these considerations, any assay useful for vaccine evaluations should use standardized protocols and reagents. Also, once clinical efficacy has been established, a correlate of protection needs to be defined and utilized for further clinical evaluation.

c. Human challenge studies

The further development of human carriage models was mentioned by some participants as a possible means to assess pneumococcal carriage as an endpoint for assessment of PPVs. Several PPV candidates have been shown to protect in preclinical models of nasopharyngeal carriage, although no data is currently available in humans. It was noted by symposium participants, however, that the requirements to protect healthy adults against experimental carriage may differ from those needed to protect infants and children from natural exposure to pneumococcus.

6. Clinical trials

Intercell AG, Sanofi Pasteur, and GlaxoSmithKline Biologicals are all developing vaccine candidates containing pneumococcal proteins that have strong preclinical data demonstrating protective immunity and that have been found to be safe and immunogenic in early phase clinical trials. It is now important to determine the criteria for proceeding to larger clinical trials, and then to design these subsequent trials appropriately.

In pediatric clinical trials looking at the development of OM, tympanocentesis may be required to assess a microbiologic outcome. In recognition of the practical and ethical difficulties associated with conducting tympanocenteses in a large number of children, it may be preferable to demonstrate a clinical endpoint not requiring tympanocentesis wherein extensive quantitative carriage data shows an impact on non-PCV serotypes. It will be important to determine the acceptability of using statistically powered composite endpoints to reduce the necessarily large sample sizes in clinical studies. A major concern in pediatric clinical trials investigating the development of pneumonia and IPD is whether a stand-alone PPV arm is ethically justifiable. Discussants mentioned that head-to-head trials of PPVs

with licensed PCVs are not ethical unless a strong case has been built for protection by the PPV. In the end, licensure of PPVs will likely require large-scale efficacy trials which may include composite endpoints such as IPD and pneumonia.

7. Nasopharyngeal carriage

With the introduction of PCVs, studies have found nasopharyngeal carriage of PCV serotypes to decrease, and non-PCV serotypes to emerge as major causes of OM and respiratory illness.^{12–14} Such observations make the assessment of PPVs for their impact on nasopharyngeal carriage and herd protection all the more important. PPVs may carry the risk of negatively disturbing the nasopharyngeal ecology by targeting highly conserved streptococcal antigens, resulting in reduction of carriage of *S. pneumoniae* as well as its close relatives *Streptococcus mitis* and *Streptococcus oralis*. The specificity as well as the quantification of carriage reduction will need to be considered in vaccine clinical trial designs. While care must be taken to weigh the benefit of herd protection against the risk of disturbing the nasopharyngeal ecology, it is also important to consider that efficacy against OM may be less likely if there is no decrease in carriage. A reduction in carriage would provide proof-of-concept and generate confidence for pursuing large and expensive clinical trials for IPD and/or pneumonia. It is likely that when PPVs are eventually assessed in infants, they will be initially administered concomitantly with a licensed PCV, for ethical reasons. As a result the impact of the proteins on non-PCV serotypes will be measured. If a PPV demonstrates a significant reduction in carriage of non-PCV serotypes, the vaccine candidate could undergo testing for impact on OM. It remains important to consider, however, that methodologies for evaluating carriage are not yet standardized. Since PPVs may reduce rather than eliminate carriage, carriage may need to be measured quantitatively.

8. Regulatory aspects

Symposium participants discussed a possible clinical and regulatory strategy for licensure of a PPV for use in infants:

Correlates of protection capable of predicting clinical benefits will presumably be necessary for licensure of PPVs, but are not well defined to date. Furthermore, whether a vaccine could be licensed on the basis of its impact on nasopharyngeal carriage rather than on disease is unclear. The question remains if reduction in nasopharyngeal carriage and/or transmission would be sufficient for licensure, along with pneumonia efficacy. Would proof of efficacy against OM be adequate for licensure or would a full IPD efficacy trial be needed? Market approval for the indication of OM may make it possible to then perform post-marketing studies for pneumonia and/or IPD.

Urgency to address unmet medical needs could provide an opportunity for PPVs to gain conditional approval from regulatory authorities, similar to the way influenza vaccine licensure occurred with demonstration of efficacy post-approval. For instance, early registration in an at-risk group(s) or the threat of accelerated serotype emergence post-PCV introduction could fuel an accelerated pathway to licensure for PPVs.

9. Target product profile

The World Health Organization (WHO) facilitated the development of a target product profile (TPP) for PCVs that would make them eligible for WHO pre-qualification and for procurement through the Advance Market Commitment (AMC) vaccine financing mechanism supported by the GAVI Alliance and other groups.¹⁵ The TPP helps guide the development of products tailored to address unmet health needs. Symposium participants

discussed the critical need for a TPP for PPVs and the potential role that the WHO could play in this process.

10. Resources

a. WHO

Through its global mandate, the WHO works to develop international consensus for vaccine regulation and introduction. By means of the WHO's activities and resources available through its reference laboratories and collaborating centers, the organization helps to standardize clinical trial outcomes including assay development, the establishment of reference standards and reagents, and the radiological diagnosis of pneumonia and nasopharyngeal carriage. It also works to establish guidelines for production, quality control, and pre-clinical and clinical evaluation of vaccines.¹⁶ Further, the WHO facilitates the availability of a panel of seed strains, reagents, and standards for universal access and helps with worldwide distribution of reference materials. Building on experience gained from other vaccine development programs (e.g., PCVs and meningococcal A and C vaccines), the WHO guides manufacturers and regulators worldwide, and enables United Nations agencies to procure and distribute vaccines for use in developing countries.

b. NIH

The NIH extramural program fosters vaccine development programs through a number of mechanisms. Foremost, the NIH provides a variety of grants that support the spectrum of activities ranging from basic research to clinical trials. Most of these grants are available to the research community worldwide, while a few are targeted toward US-based trainees and small businesses.¹⁷ The NIH also provides in-kind material support, such as custom reagents, type strains, and genomic services to qualified requestors at no cost.¹⁸ Currently, the NIH supports a reference laboratory that provides method development, method validation and related pre-clinical and clinical support services to researchers engaged in pneumococcal vaccine studies and trials.¹⁹ Plans are underway to provide small animal model services for vaccine evaluation, vaccine serological support services to external clinical studies and trials, and early stage clinical trial services through the NIH's contractual mechanisms, at no cost to the requester.²⁰

c. PATH

PATH is an international nonprofit organization based in Seattle, Washington, that creates sustainable, culturally relevant solutions, enabling communities worldwide to break longstanding cycles of poor health. The organization manages a pneumococcal vaccine project that aims to accelerate the development of high-quality, low-cost, and accessible vaccines against *S. pneumoniae* that are optimal for children in low-income countries.²¹ To this end, PATH works in collaboration with public- and private-sector partners to advance research activities in support of pneumococcal vaccine development and to help the pneumococcal research community advance the field of pneumococcal vaccine development. One such partnership has resulted in the organization of a multi-site animal model consortium that assesses vaccine candidates in different models relevant to clinical disease. Another project includes establishing a global pneumococcal strain library to house both multi-locus sequence typing (MLST) and geographically diverse isolates, publicly available genome sequencing to potentially identify new vaccine targets and sequence information on existing vaccine candidate, defining the natural human immune response to pneumococcal protein antigens and developing diagnostic assays for pneumococcal disease are underway. The intention behind each of these activities is to provide resources for the greater pneumococcal research community.

11. Recommendations

Symposium participants acknowledged the many challenges that lie ahead for the development and licensure of PPVs and provided the following recommendations for moving forward:

- a. Since all antigens cannot be tested in humans, preclinical studies must be the basis for prioritizing vaccine candidates. Protection must be:
 1. Broad, preferably against all serotypes and multiple MLST types;
 2. Robust and non-inferior to PCVs for included serotypes; and
 3. Effective against both invasive and non-invasive disease, including carriage.
- b. Under the WHO's leadership, it will be essential to develop a TPP and to define preferred performance and presentation standards for PPVs. To ensure the inclusion of PPVs in the WHO's priorities and work plans, vaccine developers should engage early with the WHO pre-qualification group.
- c. Appropriate development and standardization of assays is critical to establishing defined immunological correlates of protection and to supporting timely regulatory decisions. Work on identifying potential assays, standardizing assays, conducting multi-laboratory studies, creating of reference reagents and sera, and establishing reference laboratories should start while vaccines are in early phase clinical trials. Development of production and quality control guidelines is also important.
- d. Uncertainty regarding the regulatory pathway remains. All relevant stakeholders need to be engaged to come to a consensus on the path forward.

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Appendix A. Participant list

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Possible strategy to licensure of a PPV

1. Demonstrate mechanism of protection *in vitro* and/or *in vivo*;
2. Develop assay(s) for immune response that measures or correlates with functional activity;
3. Demonstrate protection in one or more animal models;
4. Demonstrate biological effect in early phase clinical trials with reduction in carriage and impact on OM; and
5. Conduct a Phase 3 clinical trial investigating the outcomes of IPD and/or pneumonia.