Review: Current and new generation pneumococcal vaccines

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Summary

Pneumococcal polysaccharide vaccines (PPVs) and conjugate vaccines (PCVs), of which PPV23 and PCV13 are the current front runners, have had a significant, beneficial impact on public health. With regard to PPV23, there has been some debate, however, about its protective efficacy against all-cause pneumonia, as opposed to invasive pneumococcal disease, in high-risk

cases. PCVs, on the other hand, have been included in many national immunisation programmes for prevention of severe pneumococcal disease in infants and young children, as well as for adults in various high-risk categories. Although innovative and effective, the protective efficacy of PCVs, the composition of which is based on the geographic prevalence and virulence of pneumococcal serotypes, is limited due to colonisation of the nasopharynx with non-vaccine serotypes. This phenomenon of serotype replacement has provided the impetus for development of new generation recombinant protein and whole cell pneumococcal vaccines with the potential to provide serotype-independent protection. In addition to an overview of the successes and limitations of PPVs and PCVs, this review is focused on emerging and pipeline protein-based and whole cell vaccines, preceded by a consideration of conserved pneumococcal virulence factors which are potential vaccine candidates.

Keywords: Pneumococcal conjugate vaccines; pneumococcal polysaccharide vaccines; pneumococcal choline binding protein A; pneumococcal surface protein A; pneumococcal surface protein C; pneumolysin; polyhistidine triad proteins; pneumolysin; recombinant protein vaccines; *Streptococcus pneumoniae*; whole cell vaccines.

Streptococcus pneumoniae **(pneumococcus) – the organism**

The pneumococcus is a Gram-positive diplococcus, of which there are more than 90 serotypes known, not all of which are pathogenic in man.[1,2] The natural reservoir for the microorganism is the human nasopharynx, and nasopharyngeal carriage is not only an essential precursor of active infection, but also a source of transmission of the pneumococcus.[1-3] The organism has a myriad of virulence factors that allow it on one hand to successfully colonise the nasopharynx of the human host, evading the host's immune response, and on the other hand to cause active infection, including invasive disease.[1,2]

One of the most important virulence factors of the pneumococcus is its polysaccharide capsule.[1,2] Each of the serotypes has a chemically distinct polysaccharide capsule and the overall contribution of the capsule to virulence of the pneumococcus appears to vary with its composition.[1,3] The capsule is a primary virulence factor, enabling the organism to evade phagocytosis .[2,3] The serotype of the organism affects various aspects of pneumococcal disease pathogenesis, as well as likely susceptibility or resistance of the isolate to antimicrobial agents, and while serotypes differ in prevalence and in their tendency to cause either mucosal colonisation or invasive disease the most common serotypes associated with carriage or invasive disease are geographically fairly consistent.[3] Importantly, the capsular polysaccharides are immunogenic, inducing antibodies which are protective against pneumococcal infection, this being the basis of current vaccines.[4]

The burden of pneumococcal disease

It is clearly evident from a number of recent reviews that infections with *Streptococcus pneumoniae* (pneumococcus) continue to be associated with considerable morbidity and mortality worldwide, and that despite significant advances in medicine, a number of challenges remain with regard to diagnosis, treatment and prevention.[5-9]

The pneumococcus can cause non-invasive or invasive disease (organism isolated from a normally sterile body site)[5,8] and is a common cause of community-acquired pneumonia (CAP), meningitis and bacteraemia in both children and in adults.[10] With regard to all-cause CAP, it is clear that the pneumococcus is the most commonly isolated pathogen, irrespective of whether the infection is either mild enough to be treated at home or requires hospitalisation or even intensive care unit admission, also irrespective of the severity of infection such as assessed by the Pneumonia Severity Index (PSI).[8,11] However, in a meta-analysis of studies from Europe it is clear that there are regional differences in the prevalence of the pneumococcus as a cause of CAP, depending on both the treatment setting and diagnostic approach.[12] However, since the pneumococcus is the most important

pathogen in most situations, the data on the epidemiology of CAP to a large extent mirrors that of pneumococcal pneumonia.[8]

A number of additional reviews confirm the enormous clinical and economic burden caused by CAP in North America, Europe, the Asia Pacific region, Latin America, and elsewhere,[2, 13-15] with pneumococcal disease *per se* having been documented to carry high economic costs in the United States and in Europe.[8,16] There have certainly been changes in the epidemiology of invasive pneumococcal disease (IPD) in adults in the era of paediatric pneumococcal conjugate vaccines, with their use in children certainly having benefitted older adults (decreases in the incidence of IPD in adults > 50 years of age), indirectly through herd protection, more so in healthier persons than in adults with certain comorbid conditions.[17]

Yet the true burden of pneumococcal pneumonia is likely to be considerably underestimated since many studies reporting on the incidence of pneumococcal disease report on invasive disease, given the difficulties in diagnosing non-invasive pneumococcal infections.[7] Said and colleagues conducted a systematic literature review of studies providing information on the performance of various laboratory tests used for detecting pneumococcal pneumonia (urine, sputum, blood culture).[7] The authors concluded that for every case of bacteraemic pneumococcal pneumonia there were likely to be at least 3 additional cases of non-invasive pneumococcal pneumonia. One additional factor that needs to be considered with regard to the burden of pneumococcal infections is the increasing occurrence of antimicrobial resistance, although there is some debate as to the true impact of the outcome of infections with antibiotic-resistant serotypes, at least in the case of the beta-lactam agents commonly used.[2,6,18,19]

Risk Factors for Pneumococcal Infection

The risk factors for pneumococcal infection, including CAP and IPD, have been well characterised and are extensively reviewed elsewhere.[2,8,10,11,20] Major risk factors include:

- demographic factors (age, gender)
- ethnic and socioeconomic factors
- living circumstances
- substance use (alcohol and smoking)
- comorbid medical conditions
- viral respiratory infections, especially influenza
- immunosuppression (including human immunodeficiency virus (HIV) infection and organ transplant recipients)
- various malignancies
- asplenia or splenic dysfunction (including sickle cell disease)
- certain medications

With regard to age as a risk factor, both the young and the elderly are at increased risk of pneumococcal infections and with the aging of the population in countries such as the United States, decision analytical models suggest that there is likely to be significant increases in pneumococcal pneumonia hospitalisations and costs into the future.[21] Clearly, many of the risk factors for infection are also risk factors for mortality, identifying those patients for whom pneumococcal vaccination is clearly beneficial.[22]

Mortality from pneumococcal infection

CAP and pneumococcal infections are associated with mortality, both short-term and long-term, which varies somewhat in different countries.[8] For CAP, the short-term mortality was reported as being between <1% and 48% in Europe, the significant variability being explained by a number of factors related to the different studies.[8] Case fatality rates for IPD have been reported as being between 11 and 30% in the western world, with reports from Asia describing corresponding rates of between 26-30%.[8] Interestingly, some authors have indicated that the case fatality rates for patients hospitalised with IPD have remained largely unchanged since the mid-1950s at around 12%.[8] With regard to bacteraemic pneumococcal pneumonia, one study which

investigated host, bacterial and treatment factors impacting on outcome suggested that a number of host factors were more important than specific pneumococcal serotypes in determining mortality, which has implications not only for prognostication, but also for targeting certain individuals for preventive strategies.[23] In addition, it has been documented that early administration of appropriate antibiotic therapy is a critical determinant of survival in patients with bacteraemic pneumococcal CAP.[24]

Prevention of pneumococcal infections – Vaccination

Currently there are two types of vaccine available for the prevention of pneumococcal infections, namely the polyvalent pneumococcal polysaccharide vaccine (PPV) and the pneumococcal conjugate vaccines (PCV).[25-27] The most commonly used vaccines are the PPV containing polysaccharide from 23 serotypes (PPV23) and the PCV containing polysaccharide from 13 serotypes (PCV13), conjugated to a carrier protein that is non-toxic and nearly identical to diphtheria toxin (CRM197).[25]

Pneumococcal polysaccharide vaccine (PPV23) in adults

PPV23 has been recommended since the mid-1980s for use in adults > 65 years and in younger adults (19-64 years) who have any of the risk conditions for IPD or pneumococcal pneumonia described above.[25] In 2010, the United States Advisory Committee on Immunization Practices (ACIP) added asthma as one of the chronic pulmonary disease risk factors and also indicated that adults between the ages 19-64 years who smoked should be given a single dose of PPV23 in addition to smoking cessation guidance.[28]

In 2012, the ACIP began recommending both PPV23 and PCV13 for individuals with immunocompromising conditions, functional or anatomic asplenia, cerebrospinal fluid (CSF) leaks or cochlear implants, with PCV13 being given first and PPV23 being given at least 8 weeks later[25,29] (see registration of PCV13 for adults and dosing scheduling in relationship to PPV23 vaccination below). It was recommended that subsequent dosing of

PPV23 should follow current recommendations, namely, that a second dose is recommended after 5 years in persons 19-64 years with functional or anatomic asplenia, but not in those with cochlear implants or CSF leaks.[28] The ACIP also indicated that all persons should be vaccinated at age 65 years and those who had received PPV23 before the age of 65 years for any indication should receive a second dose at 65 years of age or older, provided at least 5 years had lapsed between the two doses.[28]

There is considerable debate regarding the efficacy of PPV23 in preventing non-invasive pneumococcal pneumonia, although the general belief is that there is evidence of protection against IPD, at least in healthy young adults and in the healthy older population.[28] However, efficacy against IPD has not been demonstrated amongst immunocompromised or very old persons.[28] There is also evidence that in immunocompetent older adults that vaccine effectiveness may decrease with age and with time since vaccination.[30] While there are some studies documenting benefit against non-bacteraemic infections, others have failed to demonstrate efficacy in preventing non-bacteraemic infections or in reducing mortality.[25] There is inadequate evidence to support a protective effect of PPV23 against pneumococcal infections in high-risk patients with HIV infection.[31] Evidence for and against benefit of PPV23 in these settings has been extensively discussed elsewhere.[6,25,32-34]

There is also some controversy with regard to efficacy in IPD. Generally meta-analyses of PPV23 efficacy studies that include both randomised controlled studies (RCT) and non-randomised observational studies suggest there is some efficacy against IPD (74% in one meta-analysis); furthermore efficacy in analysis of observational studies alone appears good (overall effectiveness estimate of 52% in another meta-analysis), whereas those from analysis of RCT studies alone show relatively poor, if any, benefit (efficacy of 10% with wide confidence intervals (-77%-54% in a third analysis).[28] Clearly, there are a number of potential reasons for these conflicting study outcomes, including differences in the quality of the studies and in study designs, differences in patient populations recruited to the studies, difficulties in accurately diagnosing pneumococcal pneumonia

without bacteraemia, differences in endpoints measured, the inclusion of different studies in the various analyses, and possibly other reasons.[25,28,33]

Several more recent studies bear mention. Huss and colleagues performed a meta-analysis of efficacy of pneumococcal vaccination in adults, including studies that compared PPV23 with control, taking into account the quality of the individual investigations.[35] The end-points measured were presumptive pneumococcal pneumonia, pneumonia from all-causes, and death from all causes. There was considerable heterogeneity among the studies. Trial quality, particularly double-blinding, explained a considerable proportion of the heterogeneity.[35] Benefit of the vaccine against the former two end-points was found when considering all the studies together. However, when only studies of higher methodological quality were analysed there appeared to be little benefit of the PPV23 against the measured end-points.[35] There was also little evidence of protection in the elderly and in adults with chronic illnesses.

Moberly and colleagues concluded that their most recent met-analysis supported the recommendation for PPV23 in protecting adults against IPD, with the evidence from RCTs in adults with chronic illnesses being less clear, and no evidence to support routine PPV23 use to prevent all-cause pneumonia or mortality.[36] The latest study of PPV23, a population-based cohort study, was undertaken in Spain among adults > 60 years of age.[37] End-points were pneumococcal (invasive and non-invasive) and all cause CAP. In the primary analysis of the complete cohort of patients, PPV23 did not appear to be effective against any of the outcomes. Further detailed analysis with multivariate adjustments suggested that recent vaccination (<5 years ago) was associated with a reduced risk of pneumococcal and all-cause CAP.[37] Interestingly, a nested case-control study suggested that PPV23 protected elderly patients from hospitalisation due to pneumococcal CAP, especially female patients, and future studies should consider the impact of gender.[38]

There are additional considerations with regard to PPV23. In the first instance, this vaccine cannot be used in children under two years of age due to poor immunogenicity of polysaccharides in this age group.[25] Furthermore, there is some concern that repeated doses of PPV23 may induce hyporesponsiveness rather than having a booster effect as reported in one study undertaken in 61 elderly patients (median age 75 years), who had a history of hospitalisation for pneumonia, and were revaccinated on average 5.3 years after initial vaccination with PPV23.[39] In these cases a significant increase occurred in the geometric mean antibody concentration following vaccination and in the geometric mean antibody fold increase, but to lower levels than after the primary vaccination, with age alone seeming to play a very minor role.[39] Another study reported that the hyporessponsiveness noted following booster immunisations with polysaccharides is caused by apoptosis of memory B-cells.[40] Overall, point effectiveness of PPV23 has been shown to decline with aging and to be further associated with decreasing efficacy over 5-7 years following vaccination, which is more pronounced in the elderly.[30] Lastly, in older adults vaccination with PPV23 may induce antibodies with low opsonic capacity and reduced antibody potency.[41]

Pneumococcal conjugate vaccines (PCVs) in adults

Lack of efficacy PPV23 in neonates and infants < 2 years of age led to the development of PCVs. While PPV23 elicits a T-cell independent, humoral immune response, covalent conjugation of capsular polysaccharides to a carrier protein activates a T-cell dependent antibody response in the setting of mucosal immunity and immunological memory.[25,27] Mucosal immunity mediates decreased nasopharyngeal carriage, thus being associated with, among other factors, herd protection. This has been confirmed in many studies in which the use of childhood PCV was associated not only with a sustained decrease in IPD and declines in US hospitalisations for pneumonia in children, but also significant decreases in IPD in older adults.[25,42,43] As indicated, the T-cell dependent response also elicits immunological memory, which primes the immune system for future natural exposure or for a subsequent booster effect with re-vaccination (see below).[44]

Currently, the commonest vaccine used in children is PCV13,, introduced into the US in February 2010 and replacing PCV7.[29] Thereafter, the FDA licenced PCV13 for use as a single dose alone in adults > 50 years in December 2011 for the prevention of pneumococcal pneumonia and IPD. This recommendation was based on comparative immunogenicity studies against PPV23, but has not yet been included in the guidelines of the ACIP.[25,29] It has also been registered for use in the European Union and various other countries. In June 2012, the ACIP recommended routine use of PCV13 for adults > 19 years with immunocompromising conditions, functional or anatomic asplenia, CSF leaks, cochlear implants and indicated that this should be given followed by PPV23, as indicated above.[29] With regard to dosing schedules, PCV13 can be given immediately to vaccine-naïve adults followed by PPV23 a minimum of 8 weeks later. In individuals previously immunised with PPV23, PCV13 should be given > 1 years following PPV23 vaccination. Revaccination schedules thereafter for PPV23, follow the usual guideline recommendations.[29]

The initial recommendations for PCV13 in adults were based on immunogenicity studies (serotype-specific opsonophagocytic activity (OPA)), much of which has been reviewed elsewhere.[27,44] PCV13 has also been found to have similar safety and tolerability compared to PPV23.[27] Immunogenicity studies have documented the following:

- PCV13 elicits a greater functional immune response than PPV23 for most serotypes covered by PCV13, in vaccine naïve adults aged 60-64 years.[45]
- In adults aged 50-64 years, initial vaccination with PCV13 appeared to result in an immune state that results in recall antibody responses upon subsequent vaccination with either conjugate or polysaccharide vaccines, whereas initial vaccination with PPV23 results in an immune state whereby subsequent vaccination with PPV23 leads to lower responses.[46]

- In adults aged 60-64 years an initial PCV13 vaccination augments the immune response to subsequent administration of PPV23 for many serotypes common to both vaccines. In contrast, prior vaccination with PPV23 was associated with a diminished response to PCV13 with regard to all serotypes.[47]

- In adults 70 years of age or older previously vaccinated with PPV23, subsequent vaccination with PCV13 was more immunogenic than with PPV23 for most serotypes, suggesting that a prior dose of PPV23, but not PCV13, diminishes the subsequent response to PCV13.[48]

In terms of clinical efficacy, data from two studies are available. Best available evidence is from a randomised, placebo-controlled study of the benefit of PCV7 in adults in Malawi, many of whom were HIV-infected, who received two doses of the vaccine.[49] The study documented a lower number of episodes of IPD caused by vaccine serotypes, with vaccine efficacy being 85% in the first year. There was no difference in all-cause mortality or mortality between the study and control groups. More recently, the CAPiTA study has been completed (Community-Acquired Pneumonia Immunization Trial in Adults). The study design has been fully described elsewhere.[50] Topline results document that compared to the placebo group adults 65 years and older who received PCV13 had 45.56% fewer first episodes of vaccine-type CAP (primary outcome parameter), 45% fewer episodes of non-bacteraemic vaccine-type CAP (secondary objective), and 75% fewer episodes of vaccine-type IPD.[51]

Furthermore, studies utilising existing data for analysis suggested that PCV13 may be more cost-effective than PPV23 with respect to use in adults.[25,52] Modelling studies of PCV13 in older adults, including immunocompromised adults, have suggested that a single dose of PCV13 may be more practical economically than previous vaccine recommendations.[25,53,54] In addition, a microsimulation model in which it was assumed that PCV13 efficacy in adults would be comparable to PCV7 in

children, together with reasonable assumptions regarding risks and costs of IPD and non-bacteraemic pneumonia, projected that routine PCV13 in place of PPV23 would result in a greater reduction in burden of pneumococcal disease in US adults.[55] The question remains however, as to whether the success of the PCVs can be convincingly extrapolated to adults.[56] Caution has also been recommended with regard to modelling studies which predict cost-effectiveness since there is limited data on vaccine efficacy in the setting of reliable epidemiological and economic information, so that extensive assumptions need to be made.[57]

As described below, one aspect of vaccination with PCV13 that needs highlighting is the possibility of serotype-replacement disease in which serotypes not present in the current vaccines emerge to cause pneumococcal infection fairly soon after introduction of the conjugate vaccines for use in children.[58-60] Similar studies and comprehensive ongoing surveillance need to be undertaken in adults.

New generation vaccines

The remaining sections of this review are focused on "new generation" pneumococcal vaccines, some of which are in a fairly advanced stage of development. The following topics are covered:

- the rationale for new vaccine development

- pneumococcal protein virulence factors as priority vaccine candidates

- types of protein vaccine (recombinant fusion/hybrid protein and DNA vaccines, native and attenuated virulence factors, whole cell vaccines).

Rationale for new pneumococcal vaccine development

Despite its apparent efficacy in reducing the incidence of IPD in the healthy elderly, which has been challenged,[61,62] the prototype 23-valent PPV has a number of perceived limitations, most importantly lack of immunogenicity in young children as mentioned above.[34,63-65] Additional limitations include: i) transient efficacy in the healthy elderly; ii) lack of efficacy in the frail elderly; iii) trivial impact on nasopharyngeal carriage of vaccine serotypes; iv) no effect on herd protection; and v) failure to induce mucosal immunity.[34,63-65]

These limitations of the 23-valent PPV prompted the development of PCVs which has been characterised by a progressive increase in the number of vaccine serotypes (PCV-7, -10, -13, and -15). PCVs have overcome many of the limitations of the 23-valent PPV. Most importantly, these innovative vaccines are immunogenic and impressively protective against IPD in young children, in the setting of significant reductions in nasopharyngeal carriage of vaccine serotypes, induction of herd protection, and improved efficacy in the immunocompromised, including the frail elderly and the HIV-infected.[66]

Despite significantly improved efficacy in preventing IPD and pneumonia, the inclusion of PCVs into public immunisation programmes has highlighted a number of challenges confronting the sustained use of these vaccines.[34,62-65] These include:

- protective immunity is limited to the vaccine serotypes contained in a given PCV

- the increase in nasopharyngeal colonisation with non-vaccine serotypes and the accompanying risk of development of IPD with these serotypes. This process of "serotype replacement" is the major limitation of PCVs, countering the secondary benefit of herd protection

- increased frequency of nasopharyngeal colonisation with other respiratory pathogens such as *Staphylococcus aureus* and *Haemophilus influenzae*, as well as non-pneumococcal streptococci and anaerobic bacteria[65,67]

- variable induction of mucosal immunity and protection against development of acute otitis media, especially with earlier vaccines. This may be due to the absence of pneumococcal protein epitopes which activate cell-mediated (Th1/Th17-dependent) immune responses,[65,68-71] as well as the low number of serotypes in PCV7[63]

- limited efficacy against serotypes 1 and 5 in the second generation expanded coverage PCVs (PCV10 and PCV13) possibly due to the intransigence of these serotypes and/or shortcomings of the conjugation procedures used in vaccine manufacture[72]

- pre-immunisation nasopharyngeal colonisation of infants with vaccine serotypes results in serotype-specific immune hypo-responsiveness[73-75]

- although concerns about vaccine-induced selective pressure resulting in an increased frequency of vaccine serotype escape mutants due to capsular switching appear to be unfounded,[76,77] as stated recently, "it is highly likely that the proliferation of recently-generated NVT (non-vaccine type) capsular switch variants will continue to be favored by PHiD-CV and PCV13 vaccination programs, as was the case after PCV7 implementation." [77] While the potential impact of these events is uncertain, ongoing vigilance is essential

- vaccine-induced selective pressure may also favour nasopharyngeal colonisation by non-vaccine type, penicillin-nonsusceptible transformants which have undergone capsular

switching together with simultaneous transfer of closely associated penicillin-binding protein genes (*pbp2x* and *pbp1a*)[77]

- high costs of manufacture of PCVs with the pipeline vaccine, PCV15,[72,78] possibly representing the pinnacle of expanded coverage vaccines in terms of cost-effectiveness[34,65,72]

These limitations of PCVs, as well as those of PPV23, both real and perceived, have provided the impetus for development of novel vaccines based on broadly representative, serotype-independent, highly-conserved pneumococcal protein antigens. Several such vaccine development strategies have reached varying stages of advancement, including vaccines based on the following: i) recombinant protein antigens individually or in combination; ii) recombinant protein antigens individually or in combination as carriers of pneumococcal capsular polysaccharides; iii) recombinant pneumococcal protein carriers combined with traditional carriers; iv) traditional conjugate vaccines with added pneumococcal proteins; v) recombinant pneumococcal fusion proteins; vi) DNA vaccines; and vii) attenuated whole cell vaccines with low-level expression of capsular polysaccharides.

Prior to a more detailed consideration of these emerging pneumococcal vaccines, candidate protein antigens, predominantly virulence factors, are updated in the following section, albeit briefly as many of these have been covered extensively in several recent reviews.[2,79,80]

Pneumococcal protein virulence factors

For the purposes of this review the major protein virulence factors of the pneumococcus, all of which are immunogenic, have been grouped in either of two categories (in some cases both): i) those, mainly surface adhesins and enzymes which promote attachment to, and invasion of respiratory epithelial cells and other types of host cell; and ii) those which suppress host defences.

Pneumococcal pro-adhesive and pro-invasive protein virulence factors

Pneumococcal adhesins mediate attachment of the pneumococcus to both host cells and components of the extracellular matrix, as well as to microbial DNA, processes which necessitate a reduction in capsular size.[81] The major protein adhesins which promote attachment of the pneumococcus to respiratory epithelial cells are described briefly here.

Pneumococcal surface adhesin A (PsaA)

Notwithstanding its metal-binding and –transporting activities, PsaA is a common lipoprotein antigen of the pneumococcus which promotes the attachment of the pathogen to nasopharyngeal epithelial cells via interaction with E-cadherin, the Ca^{2+}-dependent, transmembrane cell adhesion molecule.[82] This surface protein is considered to be a promising vaccine candidate antigen.

Pneumococcal surface protein C (PspC)

This choline-binding protein of the pneumococcus, also known as CbpA and SpsA, is a major adhesin, mediating binding of the pathogen to the ectodomain of the polymeric Ig receptor expressed on human respiratory epithelial cells, resulting in colonisation and epithelial activation, as well as invasion via transcytosis across the epithelial layer.[83] In addition, PspC has also been reported to bind to, and activate, epithelial and endothelial cells,[84-86] probably via its interaction with the heparin-binding FH20 domain of complement regulator factor H (FH),[87] which is present not only in the fluid phase, but also on endothelial and epithelial cell surfaces.[84,88] More recently, PspC has been reported to interact with the C-terminal heparin-binding domain of the extracellular matrix glycoprotein, vitronectin,[89] which is also expressed on airway epithelium,[90] favouring colonisation of the pneumococcus.

Choline-binding protein E (CbpE)

CbpE, via its interaction with plasminogen/plasmin, has been reported to promote the migration of the pneumococcus across the extracellular matrix, again favouring dissemination of the pathogen.[91] Plasminogen is also located on the surface of various types of host cell, including epithelial and endothelial cells.[92,93]

Enolase, plasmin- and fibronectin-binding protein (PfbA) and pneumococcal adherence and virulence factors A and B (Pav A/B)

Like CbpE, pneumococcal α-enolase and the cell surface proteins, PfbA and PavB, have all been reported to mediate the attachment of the pathogen to plasminogen, favouring colonisation and dissemination.[91-97] In addition, PavB and PfbA, as well as PavA, promote the attachment of the pneumococcus to fibronectin, a component of the extracellular matrix.[91-97]

Polyhistidine triad (Pht) proteins

This is the collective term for a family of conserved pneumococcal surface-expressed proteins characterised by a histidine triad motif *viz.* PhtA, PhtB, PhtD, and PhtE. Two of these, PhtD and PhtE, are adhesins which promote attachment of the pneumococcus to airway epithelial cells via interactions that remain to be established.[98,99] Because of their broadly serotype-independent expression, immunogenicity and involvement in pneumococcal colonisation, these proteins are considered to be priority vaccine candidates.[98,99]

Pneumococcal serine-rich repeat protein (Psrp)

Psrp is a lung-specific pneumococcal adhesin which promotes binding to the intermediary filament protein, KR10, expressed on the surface of the lung, but not on nasopharyngeal cells.[100,101] Expression of the *psrP* gene was detected in 49.3% of strains isolated from children with pneumococcal pneumonia <2 years of age and in 65.4% of older children, indicating the potential of Psrp as

a component of a multivalent, as opposed to a monovalent, protein-based vaccine.[102]

Pneumococcal choline-binding protein A (PcpA)

This choline-binding protein, which is structurally similar to PspC, is expressed on the surface of almost all virulent pneumococcal strains and is considered to be a promising candidate vaccine antigen.[103] PcpA has been reported to mediate the adhesion of the pneumococcus to nasopharyngeal and lung epithelial cells.[103]

Pneumococcal pili

Two types of pneumococcal pili known as pilus islet 1 (PI-1, and *rlrA* islet pilus) and pilus islet 2 (PI-2) have been described.[104-106] Like Psrp, both belong to the family of peptidoglycan-binding, LPXTG-motif expressing proteins. PI-1, via its RrgA subunit, mediates adhesion of the pneumococcus to respiratory epithelial cells, as well as to components of the extracellular matrix, specifically collagen, fibronectin and laminin[104,105] and has been shown to enhance virulence in a murine model of experimental infection.[107-109] PI-2 has also been reported to promote adhesion of the pneumococcus to various types of respiratory epithelial cells.[106] However, given their relatively low level distribution on pneumococcal clinical isolates (approximately 30% and 16% for PI-1 and PI-2 respectively) recombinant PI-1 and PI-2 should only be considered as candidate antigens for inclusion in a multivalent protein-based vaccine at best.[104-106,110]

Recently, the pneumococcus has been reported to possess a novel pilus similar in structure to the Type IV pili of Gram-negative bacteria.[111] This pilus binds directly to DNA and has been proposed to be the "primary DNA receptor on the bacterial cell during transformation in *S. pneumoniae*," promoting acquisition of antibiotic resistance and vaccine escape.[111]

Pneumococcal surface enzymes which promote adhesion and invasion

Notwithstanding enolase mentioned above, several other pneumococcal surface enzymes, all of which are potential candidates for inclusion in protein-based vaccines, have been reported to contribute to pneumococcal virulence via their pro-adhesive and/or pro-invasive properties. These are: i) neuraminidases A and B (NanA and NanB);[112] ii) a pneumococcus-specific glycosyl hydrolase 25 (GHIP);[113] iii) hyaluronate lyase (SpnHL);[114] iv) NADH oxidase;[115] v) a novel pneumococcal protein endopeptidase O (PepO);[116] vi) the large zinc metalloproteinase, ZmpB, one of 4 such enzymes produced by the pneumococcus;[117] and vii) the cell wall-associated serine protease, Prta.[117]

NanA, NanB and GHIP, via their enzymatic activities, appear to promote pneumococcal adhesion to, and invasion of, respiratory epithelial cells by unmasking cryptic adhesion receptors,[112,113] while hyaluronate lyase promotes dissolution of the extracellular matrix.[114] NAD oxidase, PepO, ZmpB, and Prta on the other hand appear to function as adhesins. NAD oxidase promotes attachment of the pneumococcus to epithelial cells via interaction with contactin 4, chondroitin 4 sulphotransferase and laminin,[115] while PepO interacts with plasminogen and fibronectin.[116] Both ZmpB and Prta have been reported to interact with collagen IV,[112] with the former, which is expressed by all isolated pneumococcal strains, considered to be a promising candidate vaccine antigen,[118,119] and Prta less so.[120]

Pro-invasive activity of pneumolysin (Ply)

While apparently lacking pro-adhesive properties, the pneumococcal cholesterol-binding, pore-forming toxin, Ply, is a potent pro-invasive virulence factor of the pneumococcus Ply.[121] The toxin, which is released predominantly extracellularly following hydrolysis of the cell-wall of the pneumococcus mediated by the action of autolysin, LytA, a member of the family of choline-binding proteins.[121,122] A Ply export mechanism has also been described, which may account for the presence of the toxin on the cell wall of the

Table 1: Pneumococcal protein adhesins and invasins with vaccine potential

Adhesin	Ligand
Pneumococcal surface adhesin A (PspA)	E-cadherin [82]
Pneumococcal surface protein C (PspC)	Polymeric Ig receptor [83]
	FH domain of complement factor H [84-86]
	Vitronectin [89]
Choline-binding protein E (CbpE)	Plasminogen [91]
α-Enolase	Plasminogen [93]
Fibronectin-binding protein A (PfbA)	Fibronectin and plasminogen [94]
Pneumococcal adherence and virulence factors A and B (Pav A/B)	Fibronectin and plasminogen [95-97]
Polyhistidine triad (Pht) proteins	Unknown [98,99]
Pneumococcal serine-rich repeat protein (Psrp)	The intermediary filament protein, KR10 [100,101]
PcpA	Uncertain [103]
Pneumococcal pili, PI-1 and PI-2	Collagen, fibronectin and laminin [104,105]
Type IV pilus	DNA [111]
Neuraminidases A and B (Nan A/B)	Expose cryptic adhesion sites on target cells [112]
Pneumococcus-specific glycosyl hydrolase 25 (GHIP)	Exposes cryptic adhesion sites on target cells [113]
Hyaluronate lyase (SpnHL)	Hydrolyses extracellular matrix [114]
NADH oxidase	Contactin 4, chondroitin 4, sulphotransferase, laminin [115]
Pneumococcal protein endopeptidase O (PepO)	Plasminogen and fibronectin [116]
Zinc metalloproteinase B (ZmpB)	Collagen IV [117]
Cell wall-associated serine protease (PtrA)	Collagen IV 117
Autolysin A (LytA)	Promotes autolysis and release of Ply [121,122]
Pneumolysin (Ply)	Facilitates invasion via pore-forming activity [121,125]

pneumococcus.[123,124] The pore-forming, cytolytic actions of Ply favour the invasion and disruption of epithelial and endothelial barriers, promoting dissemination of the pneumococcus.[121,125]

Ply, which is produced by all clinical isolates of the pneumococcus, is also considered to be a priority candidate antigen for inclusion in pneumococcal vaccines.[80]

These various pneumococcal protein adhesin and invasin vaccine candidates are summarised in Table 1.

Pneumococcal protein virulence factors which subvert host defences

These virulence factors of the pneumococcus target both innate and adaptive host defence mechanisms, especially complement activation, phagocytosis, oxygen-dependent and lactoferrin-mediated antimicrobial systems, neutrophil migration and extracellular trap (NET) formation, and IgA1-mediated immunity.

Subversion of complement activation by pneumococcal surface proteins and pneumolysin.

Several pneumococcal proteins, specifically PspC, pneumococcal surface protein A (PspA), and Ply enable the pathogens to evade the protective activities of both the alternative and classical pathways of complement activation. As mentioned above, the pneumococcal adhesin, PspC, mediates the binding of the alternative pathway regulatory factor, FH, to the pneumococcal surface, thereby preventing deposition of the complement-derived opsonins C3b and C3bi, resulting in impaired phagocytosis.[126] Several mechanisms enable the pneumococcus to subvert the activity of the classical pathway of complement activation. Firstly, PspA has been reported to interfere with the binding of C-reactive protein (CRP), an activator of the classical pathway,[127] to its ligand, phosphocholine, on the surface of the pneumococcus,[128] which may underpin the inhibitory effects of PspA on

phagocytosis of the pneumococcus.[129] Secondly, the adhesins, PspC and enolase, have also been reported to bind the inhibitor of the classical pathway, C4b-binding protein.[130,131] Thirdly, Ply by mechanisms distinct from pore-forming activity and which have been described in detail elsewhere, mediates activation of the classical pathway.[121] Extracellular release of the toxin is therefore likely to represent a diversionary strategy by which the pneumococcus evades innate and adaptive host defences.

Virulence factors which confer protection against antimicrobial reactive oxygen species (ROS) and lactoferrin

The pneumococcus utilises several strategies to confer protection against not only phagocyte-derived ROS, but also hydrogen peroxide (H_2O_2) generated endogenously by the pneumococcal enzyme, pyruvate oxidase.[132] In the latter scenario, H_2O_2 production by the pneumococcus not only inhibits the growth of competing commensals and pathogens, but also interferes with the protective activities of ciliated respiratory epithelium.[132,133] Anti-oxidative protein virulence factors which enable the catalase-negative pneumococcus to withstand endogenously-generated and phagocyte-derived ROS include: i) the thioredoxin-family lipoproteins, Etrx1 and Etrx2;[134] and ii) the heat shock protein, high-temperature requirement A (HtrA).[120,135] These, as well as other types of pneumococcal hsp, such as the caseinolytic protease (Clp, hsp100), GroEL (hsp60), and DnaJ (hsp40), have also been identified as potential candidate antigens for inclusion in a protein-based vaccine.[136]

The pneumococcus is also adept at hijacking the anti-oxidant defences of the host. It does so by utilising the aforementioned adhesins and invasins to invade erythrocytes, thereby evading host defences both by concealment and by accessing erythrocyte catalase which protects against membrane-permeable, phagocyte-derived H_2O_2.[137]

In addition to its pro-adhesive properties and inhibitory effects on complement activation mentioned above, PspA, a priority vaccine candidate, has also been reported to interact with, and antagonise the antimicrobial

activity of the iron-binding, neutrophil secondary granule protein, apolactoferrin.[138]

Virulence factors which subvert neutrophil migration and extracellular traps (NETs)

Notwithstanding indirect inhibitory effects of PspA, PspC, Ply and enolase on neutrophil migration via interference with complement activation, as well as cytolysis of these cells at high concentrations of Ply, the zinc metalloproteinase, ZmpC, has been reported to interfere with neutrophil adhesion to vascular endothelium both *in vitro* and in an experimental murine model of pneumococcal pneumonia.[139] In the latter setting, ZmpC was found to cleave the N-terminal domain of the glycoprotein endothelial adhesion molecule, P-selectin (PSGL-1), interfering with initial events in neutrophil transendothelial migration.[139]

NET formation is a strategy used not only by activated neutrophils, but also eosinophils and monocytes/macrophages, to immobilise and kill microbial pathogens by ensnaring and exposing them extracellularly to an array of antimicrobial granule proteins immersed in a web of citrullinated histones and DNA.[140] Enolase, as well as IgA and IgG antibodies of undetermined specificity, have been reported to induce NET formation.[141,142] The pneumococcus, however, appears to exploit NET formation as a strategy to potentiate invasion and dissemination, particularly in the setting of co-infection with influenza virus.[142,143] Evasion and escape from NETs is mediated by the virulence factor endonuclease A (EndA), which degrades NETs,[144] while excessive NET formation poses the potential risk of histone-mediated epithelial and endothelial cytotoxicity, favouring dissemination.[145]

Subversion of IgA1-mediated mucosal immunity by the pneumococcal IgA1 protease.

The IgA1 protease, a member of the pneumococcal family of zinc metalloproteinases,[117] cleaves the Fcα1 heavy chain in the hinge region of

Table 2: Pneumococcal protein virulence factors which subvert host defences

Virulence factor	Mechanism of subversion
PspC	Interference with complement activation via the alternative and classical pathways via binding of factor H and C4b-binding protein to the pneumococcus respectively [126,130]
PspA	Interference with the classical pathway of complement activation via interference of binding of CRP to the pneumococcus [128,129]
	Neutralises the antimicrobial activity of apolactoferrin [138]
Enolase	Interferes with complement activation via interaction with C4b-binding protein [131]
Ply	Activates classical pathway of complement activation causing inappropriate consumption of complement proteins [121]
Thioredoxin family lipoproteins (Etrx1/2)	Protect against phagocyte-derived antimicrobial ROS [134]
High-temperature requirement A (HtrA) and other heat shock proteins	Protects against antimicrobial ROS [120,135]
ZmpC	Antagonises binding of neutrophils to PSGL-1 on endothelial cells [139]
Endonuclease A (EndA)	Disrupts neutrophil extracellular traps [144]
IgA1 protease	Neutralises the effector functions of Ig1 [146]

IgA1 anti-capsular antibodies, attenuating protective effector functions.[146] Because of its involvement in successful colonisation and infection in a murine model, the IgA1 protease may have vaccine potential.[146]

These various pneumococcal virulence factors which promote evasion of host defences and which in many cases have proven vaccine potential

based on immunogenicity in animal models [reviewed in 65] are summarised in Table 2.

Protein-based pneumococcal vaccines

Notwithstanding, affordability, safety, regulatory aspects and stringent design of vaccine trials,[72,147,148] priority pneumococcal protein vaccine candidates must satisfy the following criteria: i) they must be highly conserved and "universally" representative of all clinical isolates; ii) there must be compelling evidence of immunogenicity and protective efficacy in animal models of experimental infection; iii) immunogenicity should encompass both antibody (IgG)- and cell-mediated (Th1 and Th17) immune responses; and iv) immunity should be of long duration.

Given these criteria, 5 pneumococcal virulence factors, PspA, PspC, PcpA, Ply, and PhtD, individually and in combination or as fusion proteins, are currently at the forefront of protein-based vaccine development and are the focus of this section of the review. All of these activate potentially protective antibody- and cell-mediated immune responses.[149-165] It is also noteworthy that PspA and PspC have been reported to promote pneumococcal invasiveness in children.[166]

PspA-based vaccines

This pneumococcal virulence factor, which is expressed on all clinical isolates of the pneumococcus, exists as 3 structurally distinct families consisting of 2, 3 and 1 clades in families 1, 2 and 3 respectively, with family 3 being very rarely expressed.[167] Because they induce protective antibodies reactive with clades 1-5 in mice, Psp4 and PspA5 appear to be the optimum immunogenic variants for inclusion in a protein-based vaccine.[168] Additionally, a combination of one or both of these with a clade 1 PspA protein has also been proposed, either as the individual recombinant proteins or as a hybrid protein consisting of the immunogenic N-terminal regions of each.[167,168]

The PspA-based vaccines which have reached the most advanced stage of development are DNA vaccines utilising 3 different recombinant, attenuated recombinant *Salmonella typhi* vectors producing family 1, clade 2 PspA, which have recently undergone phase I dose-escalation trials in adults.[153] Although safety and tolerability issues were satisfactory, immunogenicity based on production of PspA antibodies of the IgA and IgG classes was, however, disappointing, necessitating modifications of vaccine design.[153]

Several other types of PspA vaccine based on inclusion of the recombinant protein are in the pre-clinical stages of development, all of which have demonstrated immunogenicity, and, in most cases, efficacy in murine models of experimental infection. These include: i) a nanogel-based, intranasal family 1, clade 2 vaccine which induces high levels of anti-PspA serum IgG and nasal and bronchial IgA antibodies, as well as circulating and mucosal Th17 responses in the setting of reduced pneumococcal colonisation and invasion;[154] ii) a vaccine based on combining PspA5 with diphtheria and tetanus toxoids together with low levels of *Bordetella pertussis* lipopolysaccharide, an adjuvant for PspA, resulting in high levels of systemic IgG and protection against lethal challenge, supporting development of a combined PspA-DTP vaccine;[155] iii) a conjugate vaccine utilising PspA1 as a carrier for pneumococcal capsular polysaccharides, which has been demonstrated in a recent study to evoke production of antibodies to both antigens;[156] and iv) a series of hybrid proteins based on fusion of PspA1 or PspA2 with either of two variants of attenuated Ply (pneumolysoids PID1 and PID2) generated by site-directed mutagenesis, which have demonstrated increased immunogenicity and protection relative to co-administration of the corresponding native proteins.[157]

PcpA-based vaccines

Protein-based vaccines consisting of recombinant PcpA lacking the choline-binding domain individually or in combination with the full-length histidine triad protein, PhtD without the signalling sequence, have been developed in

collaboration with Sanofi-Pasteur.[158] In a recently reported phase I stepwise, dose-escalation study in adults, both the monovalent and bivalent vaccines demonstrated good safety and immunogenicity profiles, the latter based on significant increases in circulating concentrations of PcpA and PhtD antibodies of the IgG class.[158] In addition, the safety and immunogenicity of a Sanofi-Pasteur investigational protein vaccine containing recombinant PhtD, PcpA and the pneumolysoid, PlyD1 (PID1), has been demonstrated in a recently completed phase I clinical trial.[159]

PspC-based vaccines

A pneumolysoid (L460D)/PspC peptide recombinant fusion protein, is in the pre-clinical stages of evaluation, having demonstrated immunogenicity and protection in murine models of nasopharyngeal carriage, otitis media, pneumonia and sepsis, with the hybrid protein being superior to the pneumolysoid alone.[160]

Pneumolysin (Ply)-based vaccines

In addition to the aforementioned recombinant pneumolysoid-containing fusion and combination whole protein vaccines mentioned above, all of which are in the pre-clinical stages of assessment,[157,159,160] 2 other pneumolysoid-based vaccines have recently completed phase I evaluation. The first of these is the single protein vaccine consisting of the pneumolysoid PID1, developed in association with Sanofi-Pasteur, which was evaluated in a dose-escalation study in adults.[161] The vaccine was found to be both safe and immunogenic with respect to production of PlyD1 IgG antibodies with toxin-neutralising activity.[161]

The second vaccine, developed in association with GlaxoSmithKline (GSK) Biologicals is a trivalent vaccine consisting of recombinant, formaldehyde-detoxified Ply in combination with full-length PhtD lacking the signal peptide, and recombinant, non-typeable *Haemophilus influenzae*

(NTH1) protein D (PD) expressed by both encapsulated and non-encapsulated variants.[162] In a recently reported phase I study, the vaccine was administered to healthy adults as 2 doses 60 days apart and found to be safe. Following immunisation, serum levels of antibodies against vaccine antigens increased markedly and remained elevated at day 420, while cell-mediated immune responses (Th1 and Th17) were transiently activated.[162] Immunogenicity was enhanced by inclusion of the adjuvant, AS03 (vitamin E + squalene in an oil-in-water emulsion).

In addition to these, a recombinant fusion protein constructed from the heat shock protein, DNA J, and the pneumolysoid, ΔA146Ply is in the pre-clinical stages of evaluation.[169]

PhtD

Although both histidine triad proteins, PhtD and PhtE, evoke protective antibody- and cell-mediated immune responses in mice,[152,163] the former is currently the preferred vaccine antigen candidate, not only because of immunogenicity, but also due to its high level of conservation across pneumococcal serotypes.[163]

In addition to the 2 PhtD-containing vaccines mentioned above, those being the Sanofi-Pasteur PcpA/PhtD bivalent vaccine and the GSK Biologicals trivalent PlyD1/PhtD/PD vaccine, a third vaccine has recently completed phase I/II evaluation. This is the PhtD monovalent vaccine developed in association with GSK Biologicals which has been evaluated for safety and immunogenicity in young (18-45 years) and older (≥65 years) healthy adults following administration of 2 doses 2 months apart with and without the adjuvant ASO2v (an oil-in-water emulsion combined with the immunostimulants 3-O-desacyl-4'-monophosphoryl lipid A and saponin QS21).[164] In the absence of adjuvant, the production of antibodies to PhtD was found to be higher in the younger group in the setting of acceptable safety and tolerability, while inclusion of adjuvant was associated with comparable antibody responses in both groups. Inclusion of ASO2v was also

associated with increased efficiency of PhtD-specific cell-mediated immune responses in both groups measured according to the frequency of circulating anti-specific CD4$^+$ T cells.[164]

On a cautionary note, it has recently been reported that truncated derivatives of PhtD are more effective vaccine antigens than the full-length protein when tested in mice.[165] However, the relevance of these findings to vaccine efficacy in humans has not been established.

Others

Other vaccines in the pre-clinical/early clinical stages of assessment include: i) the Novartis Vaccines/Intercell/Path Partners vaccine containing PsaA, PcsB (essential surface hydrolase) and StkB (serine/threonine kinase protein);[170] ii) the Novartis Vaccines RrgB pneumococcal pilus vaccine protective against pilus expressing strains, whch has proved disappointing in pre-clinical studies of experimental otitis media;[170,171] iii) the NasVax Ltd./Protea Vaccine Technologies 8-component protein vaccine;[170,172] iv) the multi-component pneumococcal heat shock protein vaccine alluded to earlier;[136] and v) those in much earlier stages of development.

Novel approaches to identification of vaccine protein antigen candidates

Although the above-mentioned protein-based vaccines are those which are in the most advanced stages of development, the acquisition of novel technologies including reverse vaccinology,[173] pan-surfomic analysis[174] and Genocea Biosciences T cell vaccine technology[175] will undoubtedly result in the identification of novel vaccine contenders.

Combined PCV/protein vaccines

GSK Biologicals has developed a combined PCV/pneumococcal recombinant protein vaccine, containing PVC10 (Synflorix™ with serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F conjugated to PD) with added dPly and PhtD.[72,176] This

vaccine has recently successfully completed phase II evaluation in young children aged 2-4 years and 23-23 months in the Gambia and Czech Republic respectively,[177,178] as well as in healthy adults aged 18-40 years.[179] A variant thereof has also successfully completed safety and immunogenicity studies in elderly adults aged 65-85 years.[180] The investigational vaccine evaluated is a PCV containing 8 serotypes differentially conjugated to PD (serotypes 4, 5, 7F, 14), dPly and PhtD (serotypes 19A and 22F), tetanus toxoid (serotype 13C) and diphtheria toxoid (serotype19F).

Live attenuated whole cell-based vaccines

Several whole cell pneumococcal vaccines are currently in the pipeline, the most advanced being the PATH Vaccine Development/Harvard Medical Shool/Butanton Institute vaccine.[181,182] This is an unencapsulated Rx1 strain of the pneumococcus (a rough derivative of the serotype 2 strain, D39), which is inactivated by treatment with beta-propiolactone, defective in autolysin production, expresses attenuated Ply, and is antibiotic-susceptible.[181,182] According to the originators of this capsule-negative vaccine it would be expected "to present many surface proteins in native configuration unoccluded by capsule" and "would elicit immune responses to pneumococci of varying capsular type, isolation site, and genetic background." [181] In murine models of experimental infection, the vaccine has been found to induce both IgG- and Th17-mediated immune responses to common protein antigens and to be broadly protective against 23 clinical isolates of the pneumococcus (15 causing invasive disease) encompassing 13 serotypes.[181,182] Pilot studies have demonstrated the feasibility of bulk production of the vaccine in a developing country (Brazil) in compliance with current Good Manufacturing Practices (cGMP),[182] paving the way for phase I evaluation.

Other whole cell pneumococcal vaccines based on deletion of the signal recognition pathway component, *ftsy*, or the calcium/magnesium transporter, *caxP*, which are necessary for colonisation and/or invasive disease, have recently been described.[183] In preliminary studies using models of experimental infection, these vaccines were found to induce serotype-

independent IgG responses and to protect against development of acute otitis media, sinusitis, pneumonia, and invasive pneumococcal disease.[183]

Recent studies using a model of experimental pneumococcal carriage in healthy adult human volunteers based on intranasal infection with live, encapsulated serotype 6B of the pneumococcus have demonstrated the potential for mucosal immunisation with whole cell vaccines.[184] Experimental colonisation with the pneumococcus was associated with production of protective mucosal and systemic IgA and IgG antibodies reactive with both capsular and protein antigens which promoted both eradication and prevention of re-colonisation on subsequent re-challenge.[184] Interestingly, passive transfer of post-colonisation serum to mice was associated with serotype-independent protection against experimentally-induced invasive disease, consistent with the involvement of antibodies reactive with pneumococcal proteins.[184] While highlighting the potential of viable, whole cell mucosal vaccines, the safety of these in high-risk, immunosuppressed groups is of potential concern.[185]

The various types of protein-based pneumococcal vaccine which have reached phase I/II development, as well as currently licensed and pipeline PCV vaccines are shown in Table 3.

In conclusion, although a number of promising protein-based and whole cell pneumococcal vaccines are currently undergoing phase I/II evaluation, the protective efficacy of these in the public health setting, particularly in comparison with conventional PCVs, remains to be established.

Table 3: Pneumococcal conjugate and new generation vaccines which are either licensed or have progressed to phase I/II evaluation.

Vaccine	Type	Serotypes covered	Current status
PCV7 (Prevenar®, Pfizer)	Conjugate with CRM197 as carrier	4, 6B, 9V, 14, 18C, 19F, 32F	Licensed
PCV10 (Synflorix™, GSK Biologicals)	Conjugate with protein D, diphtheria and tetanus toxoids as carriers	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	Licensed
PCV13 (Prevenar™, Pfizer)	Conjugate with CRM197 as carrier	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	Licensed
PCV15 (Merck)	Conjugate with CRM197 as carrier	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F	Phase I/II
PCV8 (Path Partners)	Conjugate with CRM and PlyD1 as carriers	Broad coverage	Phase I/II
PCV10 + proteins (GSK Biologicals)	Conjugate with protein D, diphtheria and tetanus toxoids as carriers with added PlyD1 and PhtD	Broad coverage	Phase I/II
Protein-based (Sanofi-Pasteur)	PcpA	Broad coverage	
Protein-based (Sanofi-Pasteur)	PcpA + PhtD	Broad coverage	Phase I
Protein-based (Sanofi-Pasteur)	PcpA + PhtD + dPly	Broad coverage	Phase I
Protein-based (Sanofi-Pasteur)	PlyD1 monovalent	Broad coverage	Phase I
Protein-based (GSK Biologicals)	Protein D + dPly + PhtD trivalent	Broad coverage	Phase I
Protein-based (GSK Vaccines)	PhtD monovalent	Broad coverage	Phase I/II
Live, attenuated whole cell (PATH Vaccines/ Harvard Medical School/ Butantan Institute)	Whole cell	Broad coverage	Phase I

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