Future immunisation strategies to prevent *Streptococcus pneumoniae* infections in children and adults

Bernice Ramos, Nirma Khatri Vadlamudi, Crystal Han, Manish Sadarangani

Streptococcus pneumoniae **is a major respiratory pathogen, causing 1·2 million deaths and 197 million pneumonia episodes globally in 2016. The spread of *S pneumoniae* to sterile sites, such as the blood and brain, leads to invasive pneumococcal disease. The best approach available for prevention of invasive pneumococcal disease in children and, more recently, adults is the use of pneumococcal conjugate vaccines (PCVs). PCVs are also highly effective at preventing colonisation and, thus, transmission, offering indirect protection to non-target immunisation groups such as adults—a characteristic that has been crucial in their success. However, PCVs only include and protect up to 20 of the 100 serotypes that can cause disease. The rise in adult cases of invasive pneumococcal disease from serotypes included in PCVs suggests indirect protection might be limited. Additionally, non-vaccine serotypes and some vaccine types that persist, some linked to antibiotic resistance, continue to cause disease. Future vaccine strategies include increasing the number of serotypes covered in PCVs for use in children and adults, broader vaccine use in adults, the development of adult-specific conjugate vaccines containing serotypes different from those covered in PCVs used in children, and protein vaccines, all of which will be explored in this Review. These strategies are expected to help mitigate the global burden of invasive pneumococcal disease in future years.**

Lancet Infect Dis 2025; 25: 330–44

Published Online March 17, 2025 https://doi.org/10.1016/S1473-3099(24)00740-0

Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada (B Ramos BSc); **Vaccine Evaluation Center, BC Children's Hospital Research Institute, Vancouver, BC, Canada** (B Ramos, N K Vadlamudi PhD, C Han BSc, M Sadarangani PhD); **Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada** (M Sadarangani)

Correspondence to: Dr Manish Sadarangani, Vaccine Evaluation Center, BC Children's Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada **msadarangani@bcchr.ubc.ca**

Streptococcus pneumoniae

Microbiology

Streptococcus pneumoniae is a Gram-positive bacterium characterised by the presence of lancet-shaped diplococci.[1] *S pneumoniae* is encapsulated by a capsule polysaccharide that is attached to the outer surface of the bacterium's cell wall peptidoglycan.[2] The capsule polysaccharide is structurally and serologically distinct, and has been used to define *S pneumoniae* serotypes—historically with the Quellung reaction test and more recently in the past decade with genome sequencing.[3,4] There are approximately 100 identified serotypes, all of which can cause disease. The capsule polysaccharide is also the most dominant virulence factor and plays a crucial role in pathogenesis and immune evasion.[2,5]

Surface-exposed pneumococcal proteins, such as LPxTG cell wall anchors, choline-binding proteins, and lipoproteins, aid *S pneumoniae* in colonising the nasopharynx and causing disease.[6] Choline-binding proteins increase binding to nasopharyngeal epithelial cells. They have specific motifs that bind to receptors on the nasopharyngeal mucosa and the blood–brain barrier, enabling bacterial translocation into these sites.[7,8] Surface proteins mediate bacterial binding to the nasopharyngeal mucosa and can also inhibit complement deposition and activation, interfering with phagocytosis.[9,10] Furthermore, the composition of choline-binding proteins can change, a process known as phase variation. Bacteria with so-called transparent phenotypes commonly occupy the nasopharynx and are less virulent, expressing fewer capsule polysaccharide proteins and more choline-binding proteins, contributing to their adhesion to the nasopharynx.[11] Bacteria with opaque phenotypes are dominant in cases of invasive pneumococcal disease and have higher numbers of capsule polysaccharide and surface proteins and fewer choline-binding proteins, increasing their survival in the blood by helping them resist phagocytosis.[11]

Pathogenesis

Colonisation of the nasopharynx by *S pneumoniae* can be asymptomatic, often referred to as nasopharyngeal carriage.[8] Upon infection, the bacteria can invade the upper respiratory tract, resulting in mild-to-moderate disease, such as middle ear infections (otitis media) and sinusitis.[7,8] When bacteria enter the lower respiratory tract, they can migrate and adhere to the alveolar epithelium where they can secrete pore-forming cytotoxins, such as pneumolysin, leading to pneumonia, one of the major disease manifestations.[12,13] Invasion of the respiratory epithelium, such as the alveoli, allows access to the bloodstream. Once in the bloodstream (bacteraemia), the bacteria can travel to other sterile sites of the body such as the brain, which can lead to meningitis, an infection of the lining around the brain.[6] Invasion from the blood into the cerebrospinal fluid is believed to occur through the choroid plexus or via the blood–brain barrier.[14] Bacteria can also bind to receptors on the brain microvascular epithelium, facilitating penetration of the blood–brain barrier.[15]

Epidemiology

Invasive pneumococcal disease is commonly reported in children younger than 2 years and adults aged 65 years or older.[16] In 2000, 600 000 deaths were estimated to have occurred in children younger than 5 years globally.[17] Following the introduction of pneumococcal vaccines, this number has decreased substantially. The 2016 Global Burden of Disease Study (GBD) estimated that *S pneumoniae* was responsible for 300 000 deaths in children younger than 5 years. Although the global incidences of infections and invasive pneumococcal disease in older adults in the early 1990s and 2000s vary

widely, invasive pneumococcal disease has been estimated to have been responsible for 50 or more cases per 100 000 adults aged 65 years or older in high-income countries (HICs).[18] The 2016 GBD study reported that *S pneumoniae* was responsible for 29·43 million pneumonia episodes and 494 340 deaths among adults older than 70 years, indicating that this infection remains a notable burden within this population. Despite vaccine efforts, overall *S pneumoniae* was the leading cause of lower respiratory tract infections, causing 1·2 million deaths and 197 million pneumonia episodes in 2016.[19] Individuals with specific underlying health conditions such as immunosuppression (ie, transplant recipients or individuals with HIV, malignancies, or sickle cell disease) and chronic medical conditions (ie, chronic lung and heart disease) are also at higher risk for developing invasive pneumococcal disease.[20,21]

Current pneumococcal vaccines

Current pneumococcal vaccines include the purified polysaccharide vaccine (PPV) and the pneumococcal conjugate vaccine (PCV), both of which target the capsule polysaccharide. Generally, bacterial polysaccharides such as the capsule polysaccharide are poor immunogens due to their inability to be processed and expressed adequately by antigen-presenting cells as they do not fit into the major histocompatibility complex.[22] Thus, PPVs only engage B cells in a T-cell independent manner. In some instances, zwitterionic polysaccharides, a unique class of polysaccharides, can engage T cells as they can be processed by antigen-presenting cells, as has been found in the case of *S pneumoniae* serotype 1.[23,24] PPVs such as PPV23 were developed in the 1970s and are moderately immunogenic in older children and adults and ineffective in young children.[22] Antibody responses in immunocompetent adults who receive PPVs are generally satisfactory, but protection can vary, as has been shown in cases of *S pneumoniae*-associated pneumonia. Distinguishing between primary *S pneumoniae* pneumonia, which is caused directly by the bacterium, and secondary pneumonia, which can arise from co-infections, such as with influenza, is important.[25,26] A study of *S pneumoniae* infection in individuals with influenza found that *S pneumoniae* was the most common co-infection species at 35% of co-infections.[26] A review of 25 randomised clinical trials showed strong evidence of the efficacy of PPVs against invasive pneumococcal disease and against all-cause pneumonia in low-income countries, but not in HICs.[27] Although the results showed a protective effect against pneumococcal pneumonia, this conclusion was based on only two trials and seven events, resulting in wide confidence intervals and limiting the certainty of these findings.[27] A meta-analysis of 22 adult clinical trials reported statistically significant heterogeneity in protection against all-cause pneumonia from PPVs, a reduced risk of pneumococcal pneumonia, and some evidence of protection in adults aged 60 years or older or adults with chronic illness.[28] PPV23 has limited effectiveness and does not elicit a strong immune response in infants and immunocompromised individuals due to their underdeveloped or compromised immune systems.[22] A study looking at immune responses to a 14-valent PPV in children younger than 5 years showed that responses did increase with age, but were poor in children aged up to 4·5 years.[29] No notable anamnestic responses were observed in a subset of children aged 2 years or younger who received booster doses after 6 months.[29]

PCVs produce a more robust immune response than PPVs in all populations. PCVs covalently conjugate the capsule polysaccharide to a carrier protein, such as the non-toxic diphtheria toxin CRM_{197}.[30] When processed, the peptides from the carrier protein can be loaded onto the major histocompatibility complex and presented alongside the capsule polysaccharide to T cells.[22] This T-cell activation, which provides the help necessary to start B-cell differentiation, affinity maturation, memory B-cell and T-cell responses, and production of plasma cells that produce capsule polysaccharide-specific antibodies, elicits an improved antibody response that is long lived.[22] PCVs also prevent nasopharyngeal colonisation, lowering the carriage rate of bacteria and reducing transmission, providing indirect protection in unvaccinated individuals.[22] The mechanisms behind colonisation prevention are not yet fully understood, but high levels of IgG in the nasopharynx have been postulated to reduce colonisation via bacterial agglutination.[31,32] The primary considerations for new PCVs commonly involve assessing local and systemic reactions, immunogenicity, and correlates of protection to show non-inferiority or bioequivalence to existing vaccines. This assessment can include IgG geometric mean concentrations measured by ELISA and opsonophagocytic activity geometric mean titres.[33] An IgG antibody concentration threshold of at least 0·35 µg/mL against capsule polysaccharide has been used globally as an accepted correlate of protection to support the licensure of PCVs.[34]

Impact of pneumococcal conjugate vaccines

The 7-valent vaccine (PCV7) includes capsule polysaccharides from seven *S pneumoniae* serotypes (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F; figure 1A), and was the first PCV licensed (in 2000) for children younger than 5 years in the USA.[35] After almost 10 years (by December 2009), PCV7 was licensed in 100 of 193 WHO member countries, the majority of which were HICs.[36] By 2012, a total of 33 low-income and middle-income countries (LMICs) had implemented PCV7 as part of their routine childhood immunisation schedules.[37] PCV7 led to a substantial decrease in invasive pneumococcal disease incidence rates due to PCV7 vaccine types in children younger than 5 years, including in the USA (a decrease of 100%), the UK, (a decrease of 95%), and

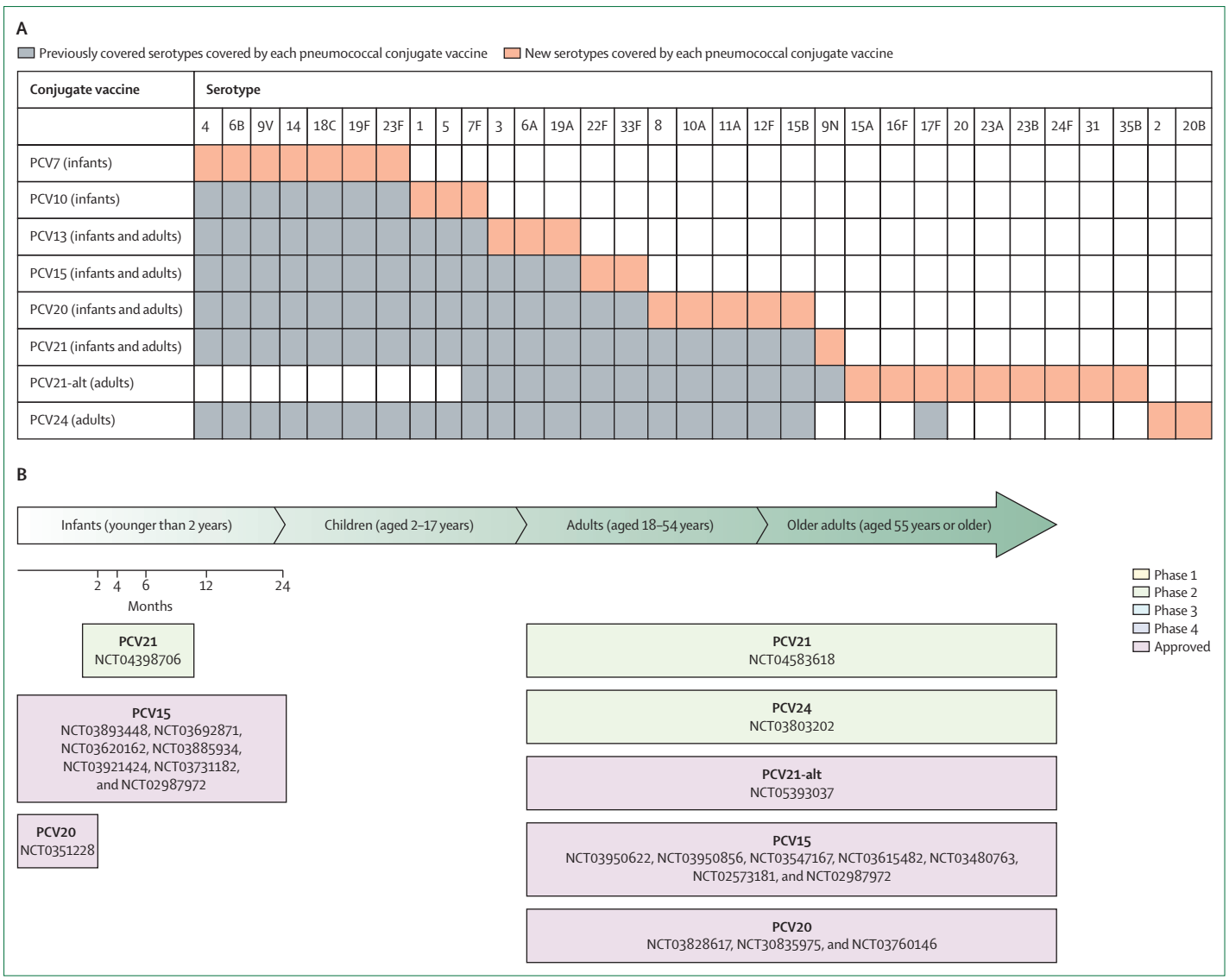

Figure 1: **Serotype coverage (A) and licensure or clinical trial stage (B) of pneumococcal conjugate vaccines in infants, children, and adults**
PCV7=7-valent pneumococcal conjugate vaccine. PCV10=10-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine. PCV15=15-valent pneumococcal conjugate vaccine. PCV20=20-valent pneumococcal conjugate vaccine. PCV21=21-valent pneumococcal conjugate vaccine. PCV21-alt=21-valent pneumococcal conjugate vaccine with alternative serotypes. PCV24=24-valent conjugate vaccine.

Canada (a decrease of 63%); overall invasive pneumococcal disease reductions were 76%, 52%, and 47%, respectively.[38–40] In adults aged 18 years or older, invasive pneumococcal disease incidence rates due to PCV7 vaccine types decreased between 87% and 92% in the USA, 74% and 83% in the UK, and 23% and 78% in Canada.[38–40] Overall invasive pneumococcal disease rates declined by 18–40% in the USA and 19–42% in the UK, but in Canada, this decrease ranged from a 34% decrease in adults aged 65–84 years to a 73% increase in individuals aged 16–64 years. This finding was attributed to an outbreak of invasive pneumococcal disease due to serotype 5; when this serotype is excluded, the increase for all serotypes is 15%.[38–40] Other countries have reported varying levels in the reduction of overall invasive pneumococcal disease following PCV7 introduction.[41]

The 10-valent (PCV10) and 13-valent (PCV13) vaccines were the next PCVs to be licensed. PCV10 includes the PCV7 serotypes plus serotypes 1, 5, and 7F, whereas PCV13 contains the PCV10 serotypes and adds serotypes 3, 6A, and 19A (figure 1A). Most countries opted to replace PCV7 with PCV13 (including the USA, the UK, and Canada) whereas others used PCV10 (eg, Brazil, the Netherlands, and Kenya).[42–47] Countries differed in their vaccine schedules for PCV10 and PCV13, which can be used in different regimens (ie, 3+1, 3+0, 2+1, and 2+0 dose schedules) and catch-up programmes, making direct comparisons between countries difficult.[48]

A study of direct vaccine effectiveness against invasive pneumococcal disease from various countries (including the USA, Germany, and Brazil) in children younger than 5 years with a 3+1 dose schedule found vaccine effectiveness ranged between 86% and 96% for PCV13 and between 73% and 100% for PCV10, but both were ineffective against serotype 3.[49]

Direct vaccination of children with PCV13 had variable indirect effects on invasive pneumococcal disease rates among adults older than 60 years globally. Initial studies indicated a substantial decline in cases of invasive pneumococcal disease among adults.[50,51] In France, PCV13 vaccination in children showed a substantial decrease in invasive pneumococcal disease in adults from 67·7% to 25·2% from 2009 to 2015.[50] In the UK, the incidence rate of invasive pneumococcal disease in adults aged 65 years or older decreased from 10·3 cases per 100 000 population during 2008–10 to 3·7 cases per 100 000 during 2013–14, but reductions were generally smaller when looking at each additional PCV13 serotype.[51] However, the indirect protection afforded by PCV13 in unvaccinated groups was poor in some countries. After PCV13 introduction in Germany in 2009, there was an initial decrease to 2·4 cases per 100 000 adults older than 60 years; this rate steadily increased to 3·2 cases per 100 000 in 2017–18 for PCV13 vaccine types.[52] In Uruguay, the incidence of invasive pneumococcal disease due to PCV13 vaccine types in adults aged 60 years or older increased from 1·6 cases per 100 000 in 2003–07 to 5·4 cases per 100 000 in 2011–12.[53]

Limitations of current pneumococcal conjugate vaccines

Emergence of non-vaccine serotypes

The emergence of non-vaccine serotypes has limited the overall impact of PCV programmes. After the introduction of PCV7 in the USA, invasive pneumococcal disease incidence rates due to non-vaccine serotypes increased from 16·3 cases per 100 000 population in 1998–99 to 19·9 cases per 100 000 in 2004 in children younger than 5 years, and from 27·0 cases per 100 000 in 1998–99 to 29·8 cases per 100 000 in 2004 in adults aged 65 years or older, with substantial increases in serotypes 3, 15, 19A, 22F, and 23F.[54] Between 2000 and 2010 in the UK, there was a 68% increase in invasive pneumococcal disease due to non-vaccine serotypes in children younger than 2 years and a 48% increase in adults aged 65 years or older, with serotypes 7F, 19A, and 22F as the key contributors.[55] The number of invasive pneumococcal disease cases increased due to serotypes 1, 7F, and 19A in Portugal from 2006 to 2008.[56] In Canada, the incidence of invasive pneumococcal disease due to serotype 5 increased from 2005 to 2008.[57] PCV7 was initially believed to provide cross-protection against serologically similar serotypes in the vaccine, such as 19F and 19A and 6A and 6B. However, the presence of serotype 19A increased considerably in the USA following the introduction of PCV7.[54,58] Although serotype 6A decreased in the USA,[59] the prevalence of 6A increased in Europe following this introduction.[60]

After introduction of PCV13, serotypes 22F and 33F accounted for 8% of invasive pneumococcal disease cases from 2010 to 2012 in Canada and 20% of cases from 2010 to 2014 in the USA in children younger than 5 years.[61,62] From 2012 to 2015, Spain reported an increase in a multidrug-resistant serotype 8, accounting for 16% of invasive pneumococcal disease cases.[63,64] An increase in serotype 8 was also reported in Denmark from 2011 to 2019, particularly in adults older than 85 years.[65] A study of the serotype distribution of invasive pneumococcal disease in the UK, France, Germany, Belgium, Sweden, and Israel reported increases in serotypes 15B/C, 10A, and 12F in children younger than 15 years.[66] In Asian countries that implemented PCV13 (ie, South Korea, China, Malaysia, the Philippines, Singapore, and Thailand), there was an increase in multidrug-resistant non-vaccine serotypes, including serotypes 10, 11A, 15A, 23A, and 35B.[67] Newer PCVs have tried to address this issue by including some of these emerging serotypes: the 15-valent vaccine (PCV15) includes serotypes 22F and 33F, and the 20-valent vaccine (PCV20) includes serotypes 8, 10A, 11A, 12F, and 15B/C (figure 1A).

Persistence of vaccine types

There is increasing concern around vaccine types still capable of causing severe disease despite being included in PCVs, particularly serotypes 3, 19A (covered by PCV13), and 19F (covered by PCV7). In Canada, the proportion of cases of invasive pneumococcal disease due to serotype 3 has remained unchanged in children younger than 5 years (from 6·6% to 8·6% between 2016 and 2019).[68] Consequently, this trend has resulted in little indirect protection against serotype 3 in adults older than 65 years, among whom the proportion of cases increased from 8·6% to 12·6% between 2016 and 2019.[68] In the UK, serotype 3 incidence rates increased from 1·6 per 100 000 population in 2010–11 to 3·6 per 100 000 in 2014–15.[69] Serotype 3 persistence could be due to the bacterium's thick capsule surrounded by a mucoid layer or due to capsule polysaccharide ejection from the synthase, both of which can interfere with antibody-mediated killing and opsonophagocytic activity, potentially reducing the protective effects of anti-capsule polysaccharide antibodies.[70,71]

Serotypes such as 19F and 19A have persisted despite their inclusion in PCVs.[72] Between 2010 and 2015 in the UK, the incidence rate of invasive pneumococcal disease due to serotype 19F increased from 0·3 cases per 100 000 population to 1·6 cases per 100 000 in children younger than 5 years and remained unchanged in adults aged 18–64 years.[69] Serotype 19F was initially thought to provide cross-protection against serotype 19A. Although studies show some direct protection, this cross-protection was insufficient in preventing 19A colonisation and a net

decrease in invasive pneumococcal disease due to 19A in children.[73,74] In Ireland from 2007–08 to 2017–18, serotype 19A was responsible for 10% of total invasive pneumococcal disease cases, with the highest number of cases observed in adults aged 65 years or older.[75] The USA reported initial substantial declines in invasive pneumococcal disease due to serotype 19A,[76] but a multicentre study of hospitalisation due to invasive pneumococcal disease between 2014 and 2017 showed that serotype 19A accounted for 33 (29%) of 115 PCV13 serotypes.[77] Serotypes 19A and 19F are challenging because they are highly resistant to antibiotics, which might contribute to their persistence.[78]

Poor geographic coverage and vaccine implementation

The largest impact of PCVs was observed in HICs in North America and Europe. The selection of serotypes was largely based on circulating serotypes in these regions, and as such, PCV coverage in other parts of the world differ.[79] A systematic review on serotype distribution and vaccine coverage in the South Asian Association for Regional Cooperation countries reported that PCV13 serotypes accounted for only 55% of invasive pneumococcal disease cases in children.[80] In nations supported by the Global Alliance for Vaccines and Immunizations (GAVI; including the countries with the lowest incomes globally), serotypes 2, 35B, and 15B were the most prevalent.[81] Surveillance of invasive pneumococcal disease isolates from a paediatric population in Argentina from 2006 to 2019 reported an increased proportion of serotype 24 post PCV13.[82]

There is interest in developing region-specific PCVs, but this approach might be hindered by the high cost of PCVs.[83] This issue is particularly crucial in LMICs, where the disease burden is highest and serotype distribution differs. Although the countries with the lowest incomes receive financial support from GAVI, middle-income countries that are self-procuring or that have recently transitioned from low-income country status struggle to keep up with PCV costs.[84] WHO's Market Information Access for Vaccines reported that the high price of PCVs is a substantial barrier to the implementation of immunisation programmes in many middle-income countries.[85] A study found that PCVs disproportionately benefit HICs, generating greater net economic benefits compared with LMICs, which face low health-care budgets and competing health-care needs.[84] Tailoring PCVs to region-specific serotypes and establishing and promoting local vaccine manufacturing in LMICs could encourage their inclusion in childhood immunisation programmes to mitigate other related health-care costs.

Future conjugate vaccine strategies

Higher-valent conjugate vaccines in infants and children

Higher valent conjugate vaccines, including 15-valent, 20-valent, and 21-valent PCVs for infants, were approved for use between 2021 and 2024 (figure 1B). The 24-valent PCV is still in clinical trials (figure 1B). Clinical trial studies for PCVs frequently report geometric mean titre ratios, the proportion of participants showing a 4-fold or greater increase from baseline for each serotype (table 1), and safety and tolerability (appendix p 1).

See Online for appendix

A PCV15 vaccine for infants was approved by the US Food and Drug Administration (FDA) for children aged 6 weeks to 17 years on the basis of seven clinical trials.[95] In one of the trials, adjuvanted and non-adjuvanted PCV15 was evaluated as a four-dose series in infants and a single dose in children aged 2–17 years.[96] Both formulations were non-inferior to PCV13 for ten of 13 shared serotypes but did not meet non-inferiority for serotypes 6A, 6B, and 19A on the basis of the proportion of infants who had IgG concentrations of 0·35 µg/mL or higher (table 1).[96] PCV15 also had a significantly increased antibody response to serotypes 3, 22F, and 33F compared with PCV13.[96] As a catch-up series in children aged 7 months to 17 years, 83–100% of participants met the 0·35 µg/mL IgG concentration threshold, but the study was not powered to assess non-inferiority or superiority in immune responses.[97] Most local and systemic reactions reported were injection site pain, erythema, and irritability.[96,97]

A 20-valent vaccine (PCV20) was assessed in a phase 2 clinical trial in infants in the USA as a four-dose series.[98] PCV20 IgG geometric mean concentrations and opsonophagocytic activity geometric mean titres were generally similar to those with PCV13 for the 13 shared serotypes, although modestly numerically lower in the PCV20 group, but displayed a strong boosting response after dose four.[98] Robust immune responses were observed after dose three for the additional eight serotypes. Local and systemic reactions were similar between PCV20 and PCV13.[98]

A 21-valent vaccine (PCV21) is being developed (NCT04398706 and NCT04583618) for infants aged 42–49 days, children aged 12–15 months, and adults aged 50–64 years in the USA and Canada, and will include the PCV20 serotypes and serotype 9N. Three formulations will be compared with PCV13 to assess the safety profile and immunogenicity of a three-dose versus four-dose schedule in infants and a one-dose schedule in toddlers.

Increasing serotype valency in new PCVs might reduce immunogenicity. Differences in immunogenicity have been observed for shared serotypes across various PCVs, which might stem from changes in the capsule polysaccharide–protein ratio.[99] Increasing the number of serotypes in PCVs could lead to immune interference, in which capsule polysaccharide-specific B cells compete for T-cell help from the carrier protein, resulting in diminished immune responses.[99] Additionally, carrier-induced suppression could occur if capsule polysaccharide-specific B-cell and antibody responses are hindered by anticarrier antibodies that compete for T-cell help.[99] This risk might be heightened with an increase in carrier protein dose as the PCV valency is increased, and

	Location	Vaccines	Cohort	Participants (n)	Dose	Schedule	Timepoints measured	Final dose IgG OPA GMTR for STs (investigational vaccine *vs* control)	Proportion of participants with ≥4-fold IgG concentration increase
Platt et al (2022)[86]	USA, Japan, Spain, Canada, and Taiwan	PCV15 (PCV13 control)	Adults ≥50 years	1202	1+0	Dose 1: day 0	Day 1 and 30 days post-D1	PCV15 >1·00: 3, 6B, 18C, 23F, 22F, and 33F; PCV15 ≤1·00: 1, 4, 5, 14, 19A, and 19F	PCV15: 52% (ST14) to 85% (ST4); control: 6% (ST33F) to 81% (ST6B)
Hammitt et al (2021)[87]	USA	PCV15 + PPV23 (PCV13 + PPV23 control)	Adults ≥18 years	1515	1+1	Dose 1: day 0 (PCV15); dose 2: day 180 (PPV23)	Pre-dose 1; 30 days post dose 1; pre-dose 2; and 30 days post dose 2	PCV15 + PPV23 >1·00: 1, 5, 6A, 6B, 14, 18C, 19A, 19F, and 23F; PVC15 + PPCV23 ≤1·00: 3, 4, 7F, 9V, 22F, and 33F	PCV15 + PPV23: 8% (ST6A) to 88% (ST1); control: 60% (ST7F) to 87% (ST5)
Stacey et al (2019)[88]	USA	PCV15A and PCV15B (PCV13 control)	Adults ≥50 years	690	1+0	Dose 1: day 0	Pre-dose 1 and 30 days post dose 2	PCV15A >1·00: 1, 3, 4, 5, 6B, 9V, 14, 18C, 19F, and 22F; PCV15A ≤1·00: 6A, 7F, 19A, 23F, and 33F; PCV15B >1·00: 1, 3, 5, 6A, 6B, 18C, 9A, 19F, 23F, and 22F; PCV15B ≤1·00: 4, 7F, 9V, 14, 33F	PCV15A: 54% (ST33F) to 87% (ST6B); PCV15B: 50% (ST14) to 87% (6B); control: 9% (ST33F) to 87% (6B)
Mohapi et al (2022)[89]	France, Peru, South Africa, Thailand, and USA	PCV15 + PPV23 (PCV13 + PPV23 control)	Adults ≥18 years	302	1+1	Dose 1: day 0 (PCV15); dose 2: day 60 (PPV23)	Pre-dose 1; 30 days post dose 1; and 30 days post dose 2	PCV15 >1·00: 1, 3, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19, 23F, 22, and 33F; PCV15 ≤1·00: 4	PCV15 + PPV23: 42% (ST9V) to 85% (ST4); control: 45% (ST9V) to 81% (ST4)
Song et al (2021)[90]	USA, South Korea, Spain, and Taiwan	PCV15 + PPV23 (PCV13 + PPV23 control)	Adults ≥50 years	652	1+1	Dose 1: day 0 (PCV15 or PCV13) ; dose 2: month 12 (PPV23)	Pre-dose 1; 30 days post dose 1; pre-dose 2; and 30 days post dose 2	PCV15 >1·00: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 18C, 19A, 19F, 23F, and 22F; PCV15 ≤1·00: 33F	PCV15 + PPV23: 56% (ST44F) to 88% (ST1); PCV13 + PPV23: 51% (ST9V) to 84% (ST4)
Klein et al (2021)[91]	USA	PCV20 (PCV13 control)	Adults aged 18–49 years	1710	1+0	Dose 1: day 0	Pre-dose 1 and 30 days post dose 1	PCV20 >1·00: NR; PCV20 ≤1·00: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F	PCV20: 42% (ST11A) to 94% (12F); PCV13: 4% (ST12F) to 95% (ST6A)
Cannon et al (2021)[92]	USA and Sweden	Previous PPV23 only + PCV20; previous PCV13 only + PCV20; and previous PCV13 + PPV23 + PCV20	Adults ≥65 years	875	1+0	Dose 1: day 0	Pre-dose 1 and 30 days post dose 1	NR	Previous PPV23 only + PCV20: 15% (ST33F) to 61% (ST6A); previous PCV13 only + PCV20: 24% (ST14) to 82% (ST22F); previous PCV13 + PPV23 + PCV20: 14% (ST7F) to 54% (22F)
Chichili et al (2022)[93]	USA	PCV24*; PCV13; and PCV13 + PPSV23	Adults ≥18 years	271	1+0	Dose 1: day 0	Pre-dose 1; 20 days post dose 1; and 180 days post dose 1	PCV24 >1·00: 3, 5, 7F, 9V, 15, 18C, 19A, 19F, 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F, and 33F; PCV24 ≤1·00: 6A, 6B, and 23F	NR
Platt et al (2023)[94]	USA	PCV21 and PPV23	Adults ≥18 years	506	1+0	..	Pre-dose 1 and 30 days post dose 1	PCV21 >1·00: 3, 6A, 7F, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19A, 20, 22F, 23A, 23B, 24F, 31, and 35B; PCV21 ≤1·00: 33F	NR

NR=not reportable. OPA GMTR=opsonophagocytic activity geometric mean titre ratio. PCV=pneumococcal conjugate vaccine. PPV=purified polysaccharide vaccine. PPSV=pneumococcal polysaccharide vaccine. ST=serotype. *PCV24 was analysed in a dose escalation study (1 µg, 2 µg, and 5 µg) and only the highest dose was reported for this table.

Table 1: **Clinical trial characteristics and immunogenicity results for pneumococcal conjugate vaccines**

if other vaccines share the same carrier protein as PCVs (ie, diphtheria toxin in diphtheria, tetanus, and pertussis vaccines) that are co-administered in childhood immunisation programmes.[99,100] A possible solution is to adjust the capsule polysaccharide-to-carrier protein ratio to reduce immune interference or to use different carrier proteins, as in the case of PCV10 (protein D), or more conserved *S pneumoniae* proteins.[99]

Adult vaccination

The indirect protection of adults against invasive pneumococcal disease through vaccination of infants and children has plateaued both due to the moderate level of protection against vaccine types and also due to an increase in invasive pneumococcal disease and community-acquired pneumonia deaths due to non-vaccine serotypes.[101] A meta-analysis[102] consisting of observational studies from countries across Europe, the Americas, Africa, and Australia assessed the impact of PCV13 in adults. The incidence rate ratio of overall invasive pneumococcal disease in adults older than 18 years was 0·82 (95% CI 0·78–0·86; p<0·0001) following the introduction of PCV13.[102] Thus, the direct vaccination of adults with PCVs is strongly recommended

in situations in which protection is needed (eg, in populations at high risk for invasive pneumococcal disease) and higher-valent PCVs have been approved or are in clinical trials. However, the high cost of PCVs might preclude universal use in publicly funded programmes for all adults.

In July, 2021, PCV15 was approved for use in adults aged 18 years or older in the USA on the basis of data from seven randomised, double-blind clinical trials in healthy adults aged 18 years or older.[86–89,90,103,104] In one trial, one dose of PCV15 was assessed in adults older than 50 years. The non-inferiority criteria for all 13 shared serotypes were met according to opsonophagocytic activity geometric mean titre ratios, ranging from 0·68 to 1·60, and superiority was shown for serotypes 22F and 33F.[86] A study comparing PCV15 plus PPV23 versus PCV13 plus PPV23 in PCV-naive adults older than 50 years[87] reported that PCV15 plus PPV23 resulted in similar immune responses to PCV13 plus PPV23 at 30 days post PPV23 vaccination. Opsonophagocytic activity geometric mean titre ratios ranged from 1·03 to 1·38, and opsonophagocytic activity geometric mean titre ratios were 1·63 for serotype 22F and 0·95 for serotype 33F.[87] From these studies, most reported local and systemic reactions for PCV15 included injection site pain and fatigue.[86,87]

A PCV20 vaccine was approved by the FDA in 2021 and Health Canada in 2022 for use in adults aged 18 years or older on the basis of three clinical trials.[105,106] A phase 3 study in vaccine-naive adults aged 18–49 years assessed PCV20 versus PCV13.[91] Opsonophagocytic activity geometric mean titres for all 20 vaccine serotypes showed robust immune responses; 42·2–94·5% of participants had a 4-fold rise from baseline across three PCV20 lots.[91] For shared PCV13 serotypes, opsonophagocytic activity geometric mean titre ratios were similar, ranging from 0·71 to 1·00.[91] Opsonophagocytic activity geometric mean titres were significantly higher for the additional seven PCV20 serotypes. Most local and systemic reactions to PCV20 were mild and similar to those of PCV13.[91] In adults older than 65 years receiving PCV20 who had previously received PCV13, PPV23, or PPV23 plus PCV13, opsonophagocytic activity geometric mean titres were numerically higher in an initial PCV13 vaccination cohort following PCV20 vaccination for the shared 13 serotypes, and participants who had PCV13 plus PPV23 vaccination had higher opsonophagocytic activity geometric mean titres for the additional seven serotypes, followed by those who had an initial PCV13 vaccination.[92]

A phase 1/2 study in vaccine-naive adults aged 18–64 years and 65–85 years assessed a 24-valent vaccine (PCV24) containing serotypes shared between PCV13 and PPV23 plus serotype 20B.[93] This formulation also contains a unique carrier fusion protein consisting of rhizavadin fused to two protein segments from genetically conserved surface protein genes *sp1500* and *sp0785*.[93] Adults aged 18–64 years had significantly greater opsonophagocytic activity geometric mean titres for the shared PCV13 serotypes and all unique PCV24 serotypes; those who received 2 µg and 5 µg of PCV24 had statistically significantly greater titres.[93] For adults aged 65–85 years, all three PCV24 doses were significantly greater for the PCV13 shared serotypes and unique PCV24 serotypes, with significant increases reported for serotypes 5 and 19F in the 5-µg-dose group.[93]

A complementary approach could involve pairing infant PCVs with adult-specific PCVs that contain serotypes specific to the adult population. A 21-valent pneumococcal vaccine with alternative serotypes (PCV21-alt) containing serotypes specific to the adult population was recently approved in the USA.[107] In a phase 2 study in the USA, which compared PCV21-alt with PPV23,[94] PCV21-alt met the non-inferiority opsonophagocytic activity geometric mean titre ratios for the 11 (of 12) serotypes shared with PPV23, ranging from 1·00 to 2·57. Opsonophagocytic activity geometric mean titre ratios were also superior for the nine unique serotypes, ranging from 3·00 to 34·90.[94] Injection site pain (118 [46%] of 254 participants) and fatigue (49 [19%] of 254 participants) were the most common local and systemic reactions, respectively.[94]

Future purified protein vaccine strategies

Pneumococcal protein vaccines use highly conserved protein sequences across all serotypes, offering a serotype-independent strategy, in contrast to capsule polysaccharide-based vaccines. A study of 25 *S pneumoniae* strains from different phylogenetic lineages and serotypes found up to 26 (40%) of the 65 identified highly conserved genes encoding for virulence factors were being constitutively expressed without substantial sequence variation in all strains.[108] The overwhelming success of PCVs is due to their prevention of bacterial colonisation, leading to lower nasopharyngeal carriage. The success or failure of protein vaccines might therefore depend on their ability to prevent colonisation and invasive pneumococcal disease. Surface-based proteins and cytolysins are current vaccine candidates that have been heavily studied in preclinical studies.[109–111] Few have progressed to clinical trials (figure 2) and been studied and little knowledge exists on whether protein vaccines prevent nasopharyngeal carriage.

Monovalent protein vaccines

Monovalent protein vaccines use a single protein and served as the basis for early clinical trials (table 2; appendix p 4). Pneumococcal surface protein A (PspA) is a highly variable surface protein. Despite this highly variable nature, antibody responses in mice have been shown to offer cross-protection irrespective of capsule type.[120] In a phase 1 study of recombinant attenuated *Salmonella enterica* serovar Typhimurium vectors expressing heterologous *S pneumoniae* PspA, 0% showed

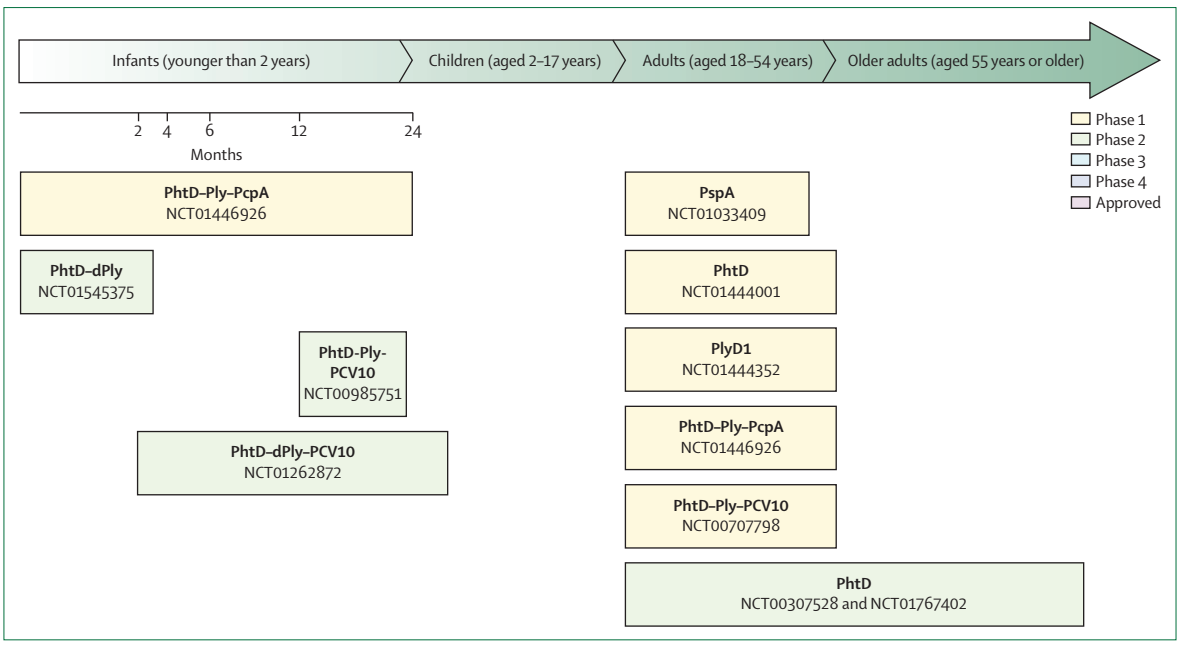

Figure 2: **Protein vaccines in clinical trials in infants and children**
dPly=detoxifed Ply variant. PcpA=pneumococcal choline binding protein A. PCV10=10-valent pneumococcal conjugate vaccine. PhtD=pneumococcal histidine triad protein D. Ply=pneumolysin. PlyD1=detoxifed Ply variant. PspA=pneumococcal surface protein A.

a 4-fold or greater increase for IgG and 3% showed this increase for IgA.[121]

Pneumococcal histidine triad protein D (PhtD) is expressed on the surface of *S pneumoniae* and is involved in the inhibition of the complement system and adherence of bacteria to host epithelial cells.[122] In a phase 1 study of a two-dose PhtD vaccine in healthy adults in Switzerland, 14 (82%) of 17 participants in the 25-µg and 14 (82%) of 17 participants in the 100-µg group had a 4-fold rise post dose 2 from baseline, whereas only seven (35%) of 20 participants showed this rise in the 6-µg group.[112] Two phase 1/2 studies in healthy young (ie, aged 18–45 years) and older (ie, older than 65 years) adults evaluated a PhtD vaccine with or without an adjuvant.[123] $AS02_V$ or aluminum phosphate (alum) was given with either two doses of 10 µg or 30 µg of PhtD compared with PPV23. Solicited local and general adverse events were more commonly observed in young participants and in those who received adjuvanted vaccines. PhtD-specific antibodies' geometric mean concentrations were consistently lower in older adults than younger adults. In younger adults, geometric mean concentrations were higher in participants who received PhtD with the $AS02_V$ adjuvant compared with those who received PhtD alone or 30 µg PhtD with alum.[123]

Pneumolysin (Ply) is an exotoxin integral in the early pathogenesis of invasive pneumococcal disease. It has also been well studied in clinical trials.[124] In a phase 1 study, a genetically mutated Ply protein candidate (PlyD1) was evaluated in 100 adults in a two-dose series.[113] The vaccine was safe and well tolerated. Antibodies were functional, as confirmed in an in-vitro assay by neutralising wild-type Ply activity. Furthermore, geometric mean concentrations were substantially higher in participants who received the 25 µg dose than those who received the 50 µg dose after injections one and two.

Multivalent protein vaccines

Although monovalent protein vaccines served as the foundation to establish safety and reactogenicity, variability at the amino acid sequence level expressed among different serotypes is a concern.[125] Some regions within a protein have sequence variability and heterogeneity, making them less than ideal for use in routine vaccination as they might not be able to provide higher serotype coverage.[125] Multivalent vaccines use different proteins simultaneously to address the issue of heterogeneity and variability, thus broadening their coverage against different serotypes.

A recombinant trivalent candidate vaccine (PPrV) that combined PhtD, Ply, and pneumococcal choline-binding protein A (PcpA) has been evaluated.[121] PcpA mediates the adherence of *S pneumoniae* to nasopharyngeal and lung epithelial cells and has also been found to elicit functional antibody responses in humans.[122] This was a phase 1 study that included infants, toddlers, and adults receiving different doses of the vaccine with or without an adjuvant.[114] At least 75% of participants in the PPrV plus adjuvant groups experienced a 2-fold or greater increase in antibody concentrations against PhtD, Ply, and PcpA. The safety profile of each group was acceptable,

	Trial phase	Valency	Formulation	Location	Cohort	Participants (n)	Dosing groups	Dose	Schedule	Timepoints measured	GMC (95% CI) prevaccination	GMC (95% CI) post final dose
Seiberling et al (2012)[112]	Phase 1	Monovalent	PhtD	Switzerland	Adults aged 18–40 years	63	6 µg; 25 µg; and 100 µg	2+0	Dose 1: day 0; dose 2: day 30	Baseline and days 0, 30, and 60	Anti-PhtD: 129 EU/mL (95–175) for 6 µg; 96 EU/mL (55–166) for 25 µg; and 194 EU/mL (122–305) for 50 µg	Anti-PhtD: 378 EU/mL (276–519) for 6 µg; 837 EU/mL (539–1300) for 25 µg; and 1569 EU/mL (1083–2272) for 50 µg
Kamtchoua et al (2013)[113]	Phase 1	Monovalent	PlyD1	Switzerland	Adults aged 18–50 years	100	10 µg; 25 µg; and 50 µg	2+0	Dose 1: day 0; dose 2: day 30	Days 0, 30, and 60	Anti-PlyD1: 1558 EU/mL (925–2622) for 10 µg; 2313 EU/mL (1674–3198) for 25 µg; and 2499 EU/mL (1574–3966) for 50 µg	Anti-PlyD1: 6989 EU/mL (4188–11 664) for 10 µg; 17 542 EU/mL (11 047–27 856) for 25 µg; and 2901 EU/mL (2122–3965) for 50 µg
Brooks et al (2015)[114]	Phase 1	Multivalent	PhtD–dPly–PcpA	Bangladesh	Adults aged 18–50 years	30	50 µg + adj	1+0	Dose 1: day 0	Days 0 and 30	Anti-PhtD: 108 EU/mL (76–154); anti-PcpA: 13 173 EU/mL (10 203–17 008); and anti-PlyD1: 2223 EU/mL (1590–3138)	Anti-PhtD: 376 EU/mL (261–542); anti-PcpA: 47 247 EU/mL (37 639–59 309); and anti-PlyD1: 14 963 EU/mL (11 289–19 834)
Brooks et al (2015)[114]	Phase 1	Multivalent	PhtD–PcpA–dPly	Bangladesh	Toddlers aged 12–23 months	30	50 µg + adj	1+0	Dose 1: day 0	Days 0–30	Anti-PhtD: 105 EU/mL (68–62); anti-PcpA: 12 999 EU/mL (7524–22 457); and anti-PlyD1: 1039 EU/mL (714–1513)	Anti-PhtD: 597 EU/mL (420–848); anti-PcpA: 62244 EU/mL (42 308–81 573); and anti-PlyD1: 5552 EU/mL (3603–8557)
Brooks et al (2015)[114]	Phase 1	Multivalent	PhtD–dPly–PcpA	Bangladesh	Infants aged 42–49 days	220	10 µg + adj; 25 µg + adj; and 50 µg + adj	3+0	Dose 1: week 6; dose 2: week 10; dose 3: week 14	Days 0, 30, and 90	Anti-PhtD: 35 EU/mL (29–42) for 10 µg + adj; 33 EU/mL (27–40) for 25 µg + adj; 33 EU/mL (27–40) for 25 µg; and 40 EU/mL (32–50) for 50 µg + adj; anti-PcpA: 3938 EU/mL (2989–5189) for 10 µg + adj; 4284 EU/mL (3611–5083) for 25 µg + adj; 3780 EU/mL (2887–4950) for 25 µg; and 4190 EU/mL (3309–5306) for 50 µg + adj; and anti-PlyD1: 945 EU/mL (736–1213) for 10 µg + adj; 842 EU/mL (632–1122) for 25 µg + adj; 789 EU/mL (527–1182) for 25 µg; and 789 EU/mL (527–1182) for 50 µg + adj	Anti-PhtD: 134 EU/mL (105–170) for 10 µg + adj; 182 EU/mL (153– 216) for 25 µg + adj; 47 EU/mL (33–67) for 25 µg; and 165 EU/mL (121–223) for 50 µg + adj; anti-PcpA: 14 347 EU/mL (10 386–19 818) for 10 µg + adj; 17 928 EU/mL (4520–22 135) for 25 µg + adj; 3450 EU/mL (2057–5785) for 25 µg; and 17 633 EU/mL (13 221–23 518) for 50 µg + adj; and anti-PlyD1: 5322 EU/mL (3510–8071) for 10 µg + adj; 6667 EU/mL (5036–8826) for 25 µg + adj; 5286 EU/mL (3609–7743) for 25 µg; and 5286 EU/mL (3609–7743) for 50 µg + adj
Berglund et al (2014)[115]	Phase 1	Multivalent	dPly–PhtD–PD (HiP)	Sweden	Adults aged 18–49 years	40	60 µg (HiP + adj) and 60 µg (HiP)	2+0	Dose 1: day 0; dose 2: day 60	Days 0, 14, 30, 60, 74, and 90	Anti-PhtD: 21 128 LU/mL (14 753–30 259) for HiP + adj and 23 633 LU/mL (15 895–35 139) for HiP; anti-dPly: 14 298 LU/mL (10 113–20 216) for HiP + adj and 14 060 LU/mL (9489–20 833) for HiP; and anti-PD: 128 LU/mL (84–196) for HiP + adj and 35 (14–62) for HiP	Anti-PhtD: 87 194 LU/mL (52 102–94 706) for HiP + adj and 58 420 LU/mL (36 278–94 706) for HiP; anti-dPly: 274 366 LU/mL (206 602–364 358) for HiP + adj and 163 653 LU/mL (92 215–290 435) for HiP; and anti-PD: 2142 LU/mL (1225–3748) for HiP + adj and 100 LU/mL (81–100) for HiP

(Table 2 continues on next page)

	Trial phase	Valency	Formulation	Location	Cohort	Participants (n)	Dosing groups	Dose	Schedule	Timepoints measured	GMC (95% CI) prevaccination	GMC (95% CI) post final dose
(Continued from previous page)												
Prymula et al (2014)[116]	Phase 2	Combination	PhtD–dPly–PCV10	Czech Republic	Toddlers aged 12–23 months	257	PhtD–dPly: 10 µg and 30 µg; and PhtD–dPly–PCV10: 10 µg and 30 µg	3+1	Dose 1: 6–14 weeks; dose 2: 3 months; dose 3: 4 months; and booster: 12–15 months	Pre-dose 1; 30 days post dose 2; pre-booster; and 30 days post booster	Anti-PhtD for PhtD–dPly: 1923 LU/mL (1131–3270) for 10 µg and 2695 LU/mL (1563–4646) for 30 µg; anti-PhtD for PhtD–dPly–PCV10: 2743 (1536–4897) for 10 µg and 1362 (765–2426) for 30 µg; anti-dPly for PhtD + dPly: 3104 LU/mL (1938–4971) for 10 µg and 2741 LU/mL (1972–4493) for 30 µg; and anti-dPly for PhtD–dPly–PCV10: 3104 LU/mL (1938–4971) for 10 µg and 2200 LU/mL (1369–3536) for 30 µg	Anti-PhtD for PhtD + dPly: 30 403 LU/mL (20 724–44 603) for 10 µg and 35 044 LU/mL (24 164–50 822) for 30 µg; anti-PhtD for PhtD–dPly–PCV10: 23 438 LU/mL (16 618–33 057) for 10 µg and 36 098 LU/mL (26 621–48 949) for 30 µg; anti-dPly for PhtD + dPly: 135 704 LU/mL (95 377–193 081) for 10 µg and 148 448 LU/mL (101 600–216 897) for 30 µg; and anti-dPly for PhtD–dPly–PCV10: 135 704 LU/mL (95 377–193 081) for 10 µg and 571 750 LU/mL (44 909–72 728) for 30 µg
Leroux-Roels et al (2014)[117]	Phase 1	Combination	PhtD–dPly–PCV10	Belgium	Adults aged 18–49 years	157	dPly: 10 µg and 30 µg; dPly–PhtD: 10 µg and 30 µg; and PhtD–dPly–PCV10: 10 µg and 30 µg	dPly–PhtD: 2+0; and dPly–PhtD–PCV10: 2+1	Dose 1: day 0 and dose 2: day 60	Pre-dose 1 and days 0, 30, 60, and 90	Anti-PhtD for dPly: 9997 LU/mL (6609–15 121) for 10 µg and 17 716 LU/mL (13 161–23 849) for 30 µg; anti-PhtD for dPly–PhtD: 16 454 LU/mL (11 491–23 561) for 10 µg and 16 801 LU/mL (13 271–21 271) for 30 µg; anti-PhtD for PhtD–dPly–PCV10: 13 959 LU/mL (10 650–18 297) for 10 µg and 16 771 LU/mL (11 893–23 650) for 30 µg; anti-dPly for dPly: 9198 LU/mL (5993–14 115) for 10 µg and 15 333 LU/mL (10 751–21 867) for 30 µg; anti-dPly for dPly–PhtD: 13 060 LU/mL (9621–17 729) for 10 µg and 14 905 LU/mL (11 128–19 963) for 30 µg; and anti-dPly for PhtD–dPly–PCV10: 12 239 LU/mL (8648– 17 320) for 10 µg and 19 988 LU/mL (15 149–26 371) for 30 µg	Anti-PhtD for dPly: 8054 LU/mL (500–12 961) for 10 µg and 14 378 LU/mL (10 120–20 429) for 30 µg; anti-PhtD for dPly–PhtD: 37 671 LU/mL (29 311–48 414) for 10 µg and 58 531 LU/mL (49 364–69 401) for 30 µg; anti-PhtD for PhtD–dPly–PCV10: 24 312 LU/mL (18 801–31 439) for 10 µg and 40 142 LU/mL (29 095–55 382) for 30 µg; anti-dPly for dPly: 47 010 LU/mL (30 960–71 379) for 10 µg and 87 300 LU/mL (58 202–130 947) for 30 µg; anti-dPly for dPly–PhtD: 63 999 LU/mL (48 406–84 614) for 10 µg and 143 923 LU/mL (106 150–195 138) for 30 µg; and anti-dPly for PhtD–dPly–PCV10 28 560 LU/mL (21 331–38 239) for 10 µg and 73 597 LU/mL (52 250–103 665) for 30 µg
Odutola et al (2016)[118]	Phase 2	Combination	PhtD–dPly–PCV10	The Gambia	Toddlers aged 2–4 years	120	30 µg	1+0	Dose 1: day 0	Pre-dose 1 and days 0 and 30	Anti-PhtD: 19 758 LU/mL (16 525– 236 324); and anti-dPly: 10 833 LU/mL (8583–13 673)	Anti-PhtD: 31 326 LU/mL (26 294–37 322); and anti-dPly: 22 795 LU/mL (17 570–29 573)
Odutola et al (2017)[119]	Phase 2	Combination	PCV10–dPly–PhtD	The Gambia	Infants aged 8–10 weeks	1200	10 µg and 30 µg	3+0	Dose 1: day 0; dose 2: day 30; and dose 3: day 90	Ages 2 months, 3 months, and 4 months	NR	Anti-PhtD for PCV10–dPly–PhtD: 2578 EU/mL for 10 µg and 2193 EU/mL for 30 µg; and anti-dPly for PCV10-dPly-PhtD: 13 951 EU/mL for 10 µg and 16 793 EU/mL for 30 µg
Odutola et al (2017)[119]	Phase 2	Combination	PCV10–dPly–PhtD	The Gambia	Infants aged 8–10 weeks	1200	30 µg	2+1	Dose 1: day 0 and dose 2: day 30	Ages 2 months, 4 months, and 9 months	NR	NR

Adj=adjuvant. dPly=detoxifed Ply variant. EU=ELISA units. GMC=geometric mean concentration. HiP=*Haemophilus influenzae* and pneumococcal. LU=Luminex units. NR=not reportable. PcpA=pneumococcal choline binding protein A. PCV=pneumococcal conjugant vaccine. PD=protein D. PhtD=pneumococcal histidine triad protein D. Ply=pneumolysin. PlyD1=detoxifed Ply variant.

Table 2: **Study characteristics and immunogenicity results for protein vaccines**

with most reactions being mild or moderate, resolving within 3 days. These were adjuvant and dose independent. Immunogenicity in infants was improved when adjuvanted without changing the safety profile, and antibody concentrations were higher at three vaccinations. No statistically significant differences in nasopharyngeal carriage were observed between vaccinated and unvaccinated groups.[114]

Another trivalent vaccine (HiP) with Ply, PhtD, and a non-typeable *Haemophilus influenzae* protein D was assessed in a phase 1 study in adults with or without an adjuvant in a two-dose series.[115] Statistically significant increases in anti-protein D and anti-Ply geometric mean concentrations were observed after dose one and dose two, respectively. Furthermore, the two doses of HiP with an adjuvant induced humoral immunity and $CD4^+$ T-cell responses for each antigen versus the non-adjuvanted vaccine.[115] However, this formulation appeared to be reactogenic—the frequency and intensity of local and systemic reactions increased after the second dose of HiP with an adjuvant.[115]

Conjugate and protein vaccine combinations

The combination of PCVs and protein vaccines is a compelling avenue to explore as such formulations have the potential to target nasopharyngeal carriage through PCVs while offering broader protection through protein vaccines. To date, four clinical trials have assessed the combination of PCVs and protein vaccines.

A phase 2 clinical trial in toddlers aged 12–23 months in the Czech Republic assessed a Ply–PhtD vaccine given alone or in combination with PCV10 at either 10 µg or 30 µg doses.[116] Rates of local and systemic reactions were similar between the investigational group and PCV10. Despite toddlers displaying seropositivity for PhtD and Ply prevaccination, those who received the investigational vaccine had an 8-fold to 16-fold anti-PhtD and 8-fold to 34-fold anti-dPly geometric mean concentration increase from baseline.[116] This vaccine candidate was also assessed in adults aged 18–40 years, showing immunogenicity in both formulations.[117] PhtD and Ply geometric mean concentrations increased after each vaccination, with notably higher responses observed in the 30 µg cohort.[117] Irrespective of vaccine formulation, 9% or fewer doses administered during primary and booster vaccination were followed by local pain, redness, swelling, or systemic reactions, such as headache and fatigue.[117]

Studies in The Gambia are the only trials to date that have included nasopharyngeal carriage as a primary outcome.[118,119] A phase 2 pilot assessment was conducted in toddlers aged 2–4 years. A single dose of 30 µg PhtD–dPly–PCV10 was administered and compared with PCV13, but was not powered to detect differences in immune responses between the study groups.[118] Due to high *S pneumoniae* carriage rates in The Gambia, measurable dPly and PhtD antibody titres were detected prevaccination.[118] There was a substantial increase in anti-dPly and anti-PhtD geometric mean concentrations after PhtD–dPly–PCV10 administration, whereas no increase was observed with PCV13.[118] Overall, vaccine tolerability was similar for PhtD–dPly–PCV10 and PCV13, and no safety concerns were raised.[118] PhtD–dPly–PCV10 has also been given as a three-dose series together with the WHO Expanded Program on Immunization in healthy infants aged 8–10 weeks in The Gambia.[119] When looking at non-PCV10 vaccine type nasopharyngeal carriage, there was no difference between groups receiving PhtD–dPly–PCV10 and the control group beyond the protection provided by PCV10, regardless of dose or schedule.[119] Acquisition of non-PCV10 vaccine types was higher than clearance after primary vaccination, suggesting an increase in non-PCV10 vaccine type prevalence.[119]

Conclusion

Current PCVs have been successful in reducing the burden of invasive pneumococcal disease caused by vaccine types, but the emergence of non-vaccine serotypes and persisting vaccine types has limited their effectiveness in some populations and the ability to provide comprehensive indirect protection. Increasing the valency of PCVs for routine use in children could help reduce the invasive pneumococcal disease burden caused by circulating non-vaccine serotypes. Direct vaccination in adults can help reduce invasive pneumococcal disease burden in this population. Protein vaccines are composed of highly conserved antigens have the potential to overcome some of the limitations of PCVs. However, future studies should aim to include evaluation of amino acid heterogeneity and variation to establish the potential effectiveness of protein vaccines against different serotypes. If protein vaccines were to replace PCVs, assessing how they and their dosing regimens fit into existing immunisation strategies to minimise health-care visits and potential immune interference with other co-administered vaccines should be considered.

In general, future clinical studies should aim to include further evaluation and standardised methods for correlates of protection. These methods could include opsonophagocytic killing assays or serum bactericidal assays, which could measure the killing of multiple *S pneumoniae* serotypes by immune cells.[126,127] Opsonophagocytic killing assays and serum bactericidal assays can be used to test against multiple *S pneumoniae* serotypes. Another key consideration, especially in the case of protein vaccines, is to evaluate the ability of future vaccines to prevent nasopharyngeal colonisation, which can be done through swab collections and measuring bacterial load pre-immunisation and post-immunisation or through mucosal assays.[119,128] The expansion of correlates of protection measures does require rigorous discussion by developers, regulators, and policy makers to identify and prioritise the most important roles these

Search strategy and selection criteria

A literature search of PubMed, Google Scholar, ClinicalTrials.gov, and the University of British Columbia Online Library was conducted between Jan 1, 2000, and Oct 4, 2024, for articles with the terms "*Streptococcus pneumoniae*", "invasive pneumococcal disease", "pneumococcal vaccines", "pneumococcal polysaccharide vaccines", "pneumococcal conjugate vaccines", "PPSV23", "PCV7", "PCV13", "PCV15", "PCV20", and "protein vaccines". Only articles published in English were included.

methods could add.[128] Developing a serotype-independent vaccine while also reducing nasopharyngeal carriage is a challenging but important goal that could lead to substantial global invasive pneumococcal disease reductions and associated health-care costs.

Contributors

BR, NKV, and MS conceptualised and designed this Review. BR conducted the literature search, data extraction, data analysis, and data interpretation, and prepared the draft manuscript. CH conducted data extraction for conjugate vaccines and protein vaccines clinical trials. NKV and MS provided input on data interpretation, critical reviews, and feedback to improve the overall manuscript. All authors approved submitting the manuscript for publication.

Declaration of interests

MS has been an investigator on projects funded by GSK, Merck, Moderna, Pfizer, and Sanofi Pasteur. All funds were paid to his institute, and he has not received any personal payments. MS is Chair and Deputy Chair of two data safety monitoring boards for COVID-19 vaccine trials, involving different vaccines. NKV has worked on unrelated projects funded by Pfizer, Merck, and Sanofi Pasteur. All funds were paid to her institute and she received a stipend. NKV has consulted on unrelated projects for Merck, Broadstreet, WHO, and Sanofi Pasteur. All payments were issued to NKV directly. NKV has received honoraria for presentations from Sanofi Pasteur; payments were issued to NKV directly. All other authors declare no competing interests.

Acknowledgments

MS is supported by salary awards from the BC Children's Hospital Foundation and Michael Smith Health Research BC. NKV is supported by a Canadian Immunization Research Network postdoctoral fellowship. BR is supported by the Canadian Immunization Research Network doctoral Trainee Scholarship programme.

References

1 Henriques-Normark B, Tuomanen EI. The pneumococcus: epidemiology, microbiology, and pathogenesis. *Cold Spring Harb Perspect Med* 2013; **3:** a010215.
2 Paton JC, Trappetti C. *Streptococcus pneumoniae* capsular polysaccharide. *Microbiol Spectr* 2019; published online April 12. https://doi.org/10.1128/microbiolspec.gpp3-0019-2018.
3 Habib M, Porter BD, Satzke C. Capsular serotyping of *Streptococcus pneumoniae* using the Quellung reaction. *J Vis Exp* 2014; **84:** e51208.
4 Epping L, Van Tonder AJ, Gladstone RA, et al. SeroBA: rapid high-throughput serotyping of *Streptococcus pneumoniae* from whole genome sequence data. *Microb Genom* 2018; **4:** e000186.
5 Hyams C, Camberlein E, Cohen JM, Bax K, Brown JS. The *Streptococcus pneumoniae* capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. *Infect Immun* 2010; **78:** 704–15.
6 Loughran AJ, Orihuela CJ, Tuomanen EI. *Streptococcus pneumoniae*: invasion and inflammation. *Microbiol Spectr* 2019; published online March 15. https://doi.org/10.1128/microbiolspec.gpp3-0004-2018.
7 Zhang J-R, Mostov KE, Lamm ME, et al. The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. *Cell* 2000; **102:** 827–37.
8 Orihuela CJ, Gao G, Francis KP, Yu J, Tuomanen EI. Tissue-specific contributions of pneumococcal virulence factors to pathogenesis. *J Infect Dis* 2004; **190:** 1661–69.
9 Khan MN, Sharma SK, Filkins LM, Pichichero ME. PcpA of *Streptococcus pneumoniae* mediates adherence to nasopharyngeal and lung epithelial cells and elicits functional antibodies in humans. *Microbes Infect* 2012; **14:** 1102–10.
10 Mukerji R, Mirza S, Roche AM, et al. Pneumococcal surface protein A inhibits complement deposition on the pneumococcal surface by competing with the binding of C-reactive protein to cell-surface phosphocholine. *J Immunol* 2012; **189:** 5327–35.
11 Li J, Zhang J-R. Phase variation of *Streptococcus pneumoniae*. *Microbiol Spectr* 2019; published online Feb 8. https://doi.org/10.1128/microbiolspec.gpp3-0005-2018.
12 Gilbert RJC, Jiménez JL, Chen S, et al. Two structural transitions in membrane pore formation by pneumolysin, the pore-forming toxin of *Streptococcus pneumoniae*. *Cell* 1999; **97:** 647–55.
13 Rubins JB, Duane PG, Clawson D, Charboneau D, Young J, Niewoehner DE. Toxicity of pneumolysin to pulmonary alveolar epithelial cells. *Infect Immun* 1993; **61:** 1352–58.
14 Ring A, Weiser JN, Tuomanen EI. Pneumococcal trafficking across the blood-brain barrier. Molecular analysis of a novel bidirectional pathway. *J Clin Invest* 1998; **102:** 347–60.
15 van Sorge NM, Doran KS. Defense at the border: the blood–brain barrier versus bacterial foreigners. *Future Microbiol* 2012; **7:** 383–94.
16 Lynch JP 3rd, Zhanel GG. *Streptococcus pneumoniae*: epidemiology, risk factors, and strategies for prevention. *Semin Respir Crit Care Med* 2009; **30:** 189–209.
17 Wahl B, O'Brien KL, Greenbaum A, et al. Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. *Lancet Glob Health* 2018; **6:** e744–57.
18 Fedson DS, Scott JA, Scott G. The burden of pneumococcal disease among adults in developed and developing countries: what is and is not known. *Vaccine* 1999; **17** (suppl 1): S11–18.
19 Troeger C, Blacker B, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* 2018; **18:** 1191–210.
20 Feldman C, Anderson R. Review: current and new generation pneumococcal vaccines. *J Infect* 2014; **69:** 309–25.
21 Lynch JP 3rd, Zhanel GG. *Streptococcus pneumoniae*: epidemiology, risk factors, and strategies for prevention. *Semin Respir Crit Care Med* 2009; **30:** 189–209.
22 Rappuoli R, De Gregorio E, Costantino P. On the mechanisms of conjugate vaccines. *Proc Natl Acad Sci USA* 2019; **116:** 14–16.
23 Zhang Q, Overkleeft HS, van der Marel GA, Codée JD. Synthetic zwitterionic polysaccharides. *Curr Opin Chem Biol* 2017; **40:** 95–101.
24 Kaushal N, Kumari S, Jhelum H, Sehgal D. In vitro and in vivo characterization of the interaction, proinflammatory, immunomodulatory and antigenic properties of capsular polysaccharide from *Streptococcus pneumoniae* serotype 1. *Int J Biol Macromol* 2020; **143:** 521–32.
25 Grant LR, Meche A, McGrath L, et al. Risk of pneumococcal disease in US adults by age and risk profile. *Open Forum Infect Dis* 2023; **10:** ofad192.
26 Klein EY, Monteforte B, Gupta A, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. *Influenza Other Respir Viruses* 2016; **10:** 394–403.
27 Moberley S, Holden J, Tatham DP, Andrews RM. Vaccines for preventing pneumococcal infection in adults. *Cochrane Database Syst Rev* 2013; published online Jan 31. https://doi.org/10.1002/14651858.CD000422.pub3.
28 Huss A, Scott P, Stuck AE, Trotter C, Egger M. Efficacy of pneumococcal vaccination in adults: a meta-analysis. *CMAJ* 2009; **180:** 48–58.
29 Douglas RM, Paton JC, Duncan SJ, Hansman DJ. Antibody response to pneumococcal vaccination in children younger than five years of age. *J Infect Dis* 1983; **148:** 131–37.

30 Bröker M, Costantino P, DeTora L, McIntosh ED, Rappuoli R. Biochemical and biological characteristics of cross-reacting material 197 CRM197, a non-toxic mutant of diphtheria toxin: use as a conjugation protein in vaccines and other potential clinical applications. *Biologicals* 2011; **39:** 195–204.

31 Simell B, Nurkka A, Ekström N, Givon-Lavi N, Käyhty H, Dagan R. Serum IgM antibodies contribute to high levels of opsonophagocytic activities in toddlers immunized with a single dose of the 9-valent pneumococcal conjugate vaccine. *Clin Vaccine Immunol* 2012; **19:** 1618–23.

32 Zangari T, Wang Y, Weiser JN. *Streptococcus pneumoniae* transmission is blocked by type-specific immunity in an infant mouse model. *MBio* 2017; **8:** e00188–17.

33 Abghari PF, Poowuttikul P, Secord E. Pneumococcal antibody titers: a comparison of patients receiving intravenous immunoglobulin versus subcutaneous immunoglobulin. *Global Pediatric Health* 2017; **4:** 2333794X1668963.

34 WHO. Recommendations for the production and control of pneumococcal conjugate vaccines. Geneva: World Health Organization, 2005.

35 Centers for Disease Control and Prevention. Progress in introduction of pneumococcal conjugate vaccine—worldwide, 2000. *MMWR Morb Mortal Wkly Rep* 2008; **57:** 1148–51.

36 Reinert RR, Paradiso P, Fritzell B. Advances in pneumococcal vaccines: the 13-valent pneumococcal conjugate vaccine received market authorization in Europe. *Expert Rev Vaccines* 2010; **9:** 229–36.

37 Fitzwater SP, Chandran A, Santosham M, Johnson HL. The worldwide impact of the seven-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2012; **31:** 501–08.

38 Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010; **201:** 32–41.

39 Ladhani SN, Collins S, Djennad A, et al. Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000–17: a prospective national observational cohort study. *Lancet Infect Dis* 2018; **18:** 441–51.

40 Kellner JD, Vanderkooi OG, MacDonald J, Church DL, Tyrrell GJ, Scheifele DW. Changing epidemiology of invasive pneumococcal disease in Canada, 1998–2007: update from the Calgary-area *Streptococcus pneumoniae* Research (CASPER) study. *Clin Infect Dis* 2009; **49:** 205–12.

41 McIntosh EDG, Reinert RR. Global prevailing and emerging pediatric pneumococcal serotypes. *Expert Rev Vaccines* 2011; **10:** 109–29.

42 Metcalf BJ, Gertz RE Jr, Gladstone RA, et al. Strain features and distributions in pneumococci from children with invasive disease before and after 13-valent conjugate vaccine implementation in the USA. *Clin Microbiol Infect* 2016; **22:** 60.e9–29.

43 Ricketson LJ, Bettinger JA, Sadarangani M, Halperin SA, Kellner JD, and the Canadian Immunization Monitoring Program, Active (IMPACT) investigators. Vaccine effectiveness of the 7-valent and 13-valent pneumococcal conjugate vaccines in Canada: an IMPACT study. *Vaccine* 2022; **40:** 2733–40.

44 Andrews N, Kent A, Amin-Chowdhury Z, et al. Effectiveness of the seven-valent and thirteen-valent pneumococcal conjugate vaccines in England: the indirect cohort design, 2006–2018. *Vaccine* 2019; **37:** 4491–98.

45 de Oliveira LH, Camacho LAB, Coutinho ESF, et al. Impact and effectiveness of 10 and 13-valent pneumococcal conjugate vaccines on hospitalization and mortality in children aged less than 5 years in Latin American countries: a systematic review. *PLoS One* 2016; **11:** e0166736.

46 Peckeu L, van der Ende A, de Melker HE, Sanders EAM, Knol MJ. Impact and effectiveness of the 10-valent pneumococcal conjugate vaccine on invasive pneumococcal disease among children under 5 years of age in the Netherlands. *Vaccine* 2021; **39:** 431–37.

47 Silaba M, Ooko M, Bottomley C, et al. Effect of 10-valent pneumococcal conjugate vaccine on the incidence of radiologically-confirmed pneumonia and clinically-defined pneumonia in Kenyan children: an interrupted time-series analysis. *Lancet Glob Health* 2019; **7:** e337–46.

48 Grant LR, Slack MPE, Theilacker C, et al. Distribution of serotypes causing invasive pneumococcal disease in children from high-income countries and the impact of pediatric pneumococcal vaccination. *Clin Infect Dis* 2023; **76:** e1062–70.

49 Berman-Rosa M, O'Donnell S, Barker M, Quach C. Efficacy and effectiveness of the PCV-10 and PCV-13 vaccines against invasive pneumococcal disease. *Pediatrics* 2020; **145:** e20190377.

50 Hays C, Vermee Q, Agathine A, et al. Demonstration of the herd effect in adults after the implementation of pneumococcal vaccination with PCV13 in children. *Eur J Clin Microbiol Infect Dis* 2017; **36:** 831–38.

51 Waight PA, Andrews NJ, Ladhani SN, Sheppard CL, Slack MPE, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. *Lancet Infect Dis* 2015; **15:** 535–43.

52 van der Linden M, Imöhl M, Perniciaro S. Limited indirect effects of an infant pneumococcal vaccination program in an aging population. *PLoS One* 2019; **14:** e0220453.

53 García Gabarrot G, López Vega M, Pérez Giffoni G, et al, and the Uruguayan SIREVA II Group. Effect of pneumococcal conjugate vaccination in Uruguay, a middle-income country. *PLoS One* 2014; **9:** e112337.

54 Hicks LA, Harrison LH, Flannery B, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *J Infect Dis* 2007; **196:** 1346–54.

55 Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. *Lancet Infect Dis* 2011; **11:** 760–68.

56 Aguiar SI, Brito MJ, Gonçalo-Marques J, Melo-Cristino J, Ramirez M. Serotypes 1, 7F and 19A became the leading causes of pediatric invasive pneumococcal infections in Portugal after 7 years of heptavalent conjugate vaccine use. *Vaccine* 2010; **28:** 5167–73.

57 Leal J, Vanderkooi OG, Church DL, Macdonald J, Tyrrell GJ, Kellner JD. Eradication of invasive pneumococcal disease due to the seven-valent pneumococcal conjugate vaccine serotypes in Calgary, Alberta. *Pediatr Infect Dis J* 2012; **31:** e169–75.

58 Whitney CG, Pilishvili T, Farley MM, et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. *Lancet* 2006; **368:** 1495–502.

59 Løchen A, Croucher NJ, Anderson RM. Divergent serotype replacement trends and increasing diversity in pneumococcal disease in high income settings reduce the benefit of expanding vaccine valency. *Sci Rep* 2020; **10:** 18977.

60 McIntosh EDG, Reinert RR. Global prevailing and emerging pediatric pneumococcal serotypes. *Expert Rev Vaccines* 2011; **10:** 109–29.

61 Demczuk WHB, Martin I, Griffith A, et al. Serotype distribution of invasive *Streptococcus pneumoniae* in Canada after the introduction of the 13-valent pneumococcal conjugate vaccine, 2010-2012. *Can J Microbiol* 2013; **59:** 778–88.

62 Moore MR, Link-Gelles R, Schaffner W, et al. Effectiveness of 13-valent pneumococcal conjugate vaccine for prevention of invasive pneumococcal disease in children in the USA: a matched case-control study. *Lancet Respir Med* 2016; **4:** 399–406.

63 Sanz JC, Rodríguez-Avial I, Ríos E, García-Comas L, Ordobás M, Cercenado E. Increase of serotype 8, ST53 clone, as the prevalent strain of *Streptococcus pneumoniae* causing invasive disease in Madrid, Spain (2012–2015). *Enferm Infecc Microbiol Clin* 2020; **38:** 105–10.

64 Ardanuy C, de la Campa AG, García E, et al. Spread of *Streptococcus pneumoniae* serotype 8-ST63 multidrug-resistant recombinant clone, Spain. *Emerg Infect Dis* 2014; **20:** 1848–56.

65 Hansen CB, Fuursted K, Valentiner-Branth P, Dalby T, Jørgensen CS, Slotved H-C. Molecular characterization and epidemiology of *Streptococcus pneumoniae* serotype 8 in Denmark. *BMC Infect Dis* 2021; **21:** 421.

66 Levy C, Ouldali N, Caeymaex L, Angoulvant F, Varon E, Cohen R. Diversity of serotype replacement after pneumococcal conjugate vaccine implementation in Europe. *J Pediatr* 2019; **213:** 252–53.

67 Kim SH, Chung DR, Song J-H, et al. Changes in serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates from adult patients in Asia: emergence of drug-resistant non-vaccine serotypes. *Vaccine* 2020; **38:** 6065–73.

68 Golden A, Griffith A, Demczuk W, et al. Invasive pneumococcal disease surveillance in Canada, 2020. *Can Commun Dis Rep* 2022; **48:** 396–406.

69 Kandasamy R, Voysey M, Collins S, et al. Persistent circulation of vaccine serotypes and serotype replacement after 5 years of infant immunization with 13-valent pneumococcal conjugate vaccine in the United Kingdom. *J Infect Dis* 2019; **221:** 1361–70.

70 Lapidot R, Shea KM, Yildirim I, Cabral HJ, Pelton SI, The Massachusetts Department Of Public Health. Characteristics of serotype 3 invasive pneumococcal disease before and after universal childhood immunization with PCV13 in Massachusetts. *Pathogens* 2020; **9:** 396.

71 Choi EH, Zhang F, Lu Y-J, Malley R. Capsular polysaccharide (CPS) release by serotype 3 pneumococcal strains reduces the protective effect of anti-type 3 CPS antibodies. *Clin Vaccine Immunol* 2015; **23:** 162–67.

72 Ruiz García Y, Nieto Guevara J, Izurieta P, Vojtek I, Ortega-Barría E, Guzman-Holst A. Circulating clonal complexes and sequence types of *Streptococcus pneumoniae* serotype 19A worldwide: the importance of multidrug resistance: a systematic literature review. *Expert Rev Vaccines* 2021; **20:** 45–57.

73 Hausdorff WP, Hoet B, Schuerman L. Do pneumococcal conjugate vaccines provide any cross-protection against serotype 19A? *BMC Pediatr* 2010; **10:** 4.

74 Moore MR, Gertz RE Jr, Woodbury RL, et al. Population snapshot of emergent *Streptococcus pneumoniae* serotype 19A in the United States, 2005. *J Infect Dis* 2008; **197:** 1016–27.

75 Corcoran M, Mereckiene J, Cotter S, et al. Using genomics to examine the persistence of *Streptococcus pneumoniae* serotype 19A in Ireland and the emergence of a sub-clade associated with vaccine failures. *Vaccine* 2021; **39:** 5064–73.

76 Isturiz R, Sings HL, Hilton B, Arguedas A, Reinert R-R, Jodar L. *Streptococcus pneumoniae* serotype 19A: worldwide epidemiology. *Expert Rev Vaccines* 2017; **16:** 1007–27.

77 Kaplan SL, Barson WJ, Lin PL, et al. Invasive pneumococcal disease in children's hospitals: 2014–2017. *Pediatrics* 2019; **144:** e20190567.

78 Hackel M, Lascols C, Bouchillon S, Hilton B, Morgenstern D, Purdy J. Serotype prevalence and antibiotic resistance in *Streptococcus pneumoniae* clinical isolates among global populations. *Vaccine* 2013; **31:** 4881–87.

79 Tvedskov ESF, Hovmand N, Benfield T, Tinggaard M. Pneumococcal carriage among children in low and lower-middle-income countries: a systematic review. *Int J Infect Dis* 2022; **115:** 1–7.

80 Jaiswal N, Singh M, Das RR, et al. Distribution of serotypes, vaccine coverage, and antimicrobial susceptibility pattern of *Streptococcus pneumoniae* in children living in SAARC countries: a systematic review. *PLoS One* 2014; **9:** e108617.

81 Johnson HL, Deloria-Knoll M, Levine OS, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS Med* 2010; **7:** e1000348.

82 Zintgraff J, Gagetti P, Napoli D, et al. Invasive *Streptococcus pneumoniae* isolates from pediatric population in Argentina for the period 2006–2019. Temporal progression of serotypes distribution and antibiotic resistance. *Vaccine* 2022; **40:** 459–70.

83 Kumar R, Arora N, Santosham M. South Asia symposium on pneumococcal disease and the promise of vaccines—meeting report. *Vaccine* 2016; **34:** 2622–26.

84 Niyibitegeka F, Russell FM, Jit M, Carvalho N. Inequitable distribution of global economic benefits from pneumococcal conjugate vaccination. *Vaccines* 2024; **12:** 767.

85 WHO. WHO pneumococcal vaccines global market study June 2020. World Health Organization, 2020. https://www.who.int/publications/m/item/who-pneumococcal-vaccines-global-market-study-june-2020 (accessed Sept 23, 2022).

86 Platt HL, Cardona JF, Haranaka M, et al. A phase 3 trial of safety, tolerability, and immunogenicity of V114, 15-valent pneumococcal conjugate vaccine, compared with 13-valent pneumococcal conjugate vaccine in adults 50 years of age and older (PNEU-AGE). *Vaccine* 2022; **40:** 162–72.

87 Hammitt LL, Quinn D, Janczewska E, et al. Immunogenicity, safety, and tolerability of V114, a 15-valent pneumococcal conjugate vaccine, in immunocompetent adults aged 18-49 years with or without risk factors for pneumococcal disease: a randomized phase 3 trial (PNEU-DAY). *Open Forum Infect Dis* 2021; **9:** ofab605.

88 Stacey HL, Rosen J, Peterson JT, et al. Safety and immunogenicity of 15-valent pneumococcal conjugate vaccine (PCV-15) compared to PCV-13 in healthy older adults. *Hum Vaccin Immunother* 2019; **15:** 530–39.

89 Mohapi L, Pinedo Y, Osiyemi O, et al. Safety and immunogenicity of V114, a 15-valent pneumococcal conjugate vaccine, in adults living with HIV. *AIDS* 2022; **36:** 373–82.

90 Song J-Y, Chang C-J, Andrews C, et al, and the V114-016 (PNEU-PATH) study group. Safety, tolerability, and immunogenicity of V114, a 15-valent pneumococcal conjugate vaccine, followed by sequential PPSV23 vaccination in healthy adults aged ≥50 years: a randomized phase III trial (PNEU-PATH). *Vaccine* 2021; **39:** 6422–36.

91 Klein NP, Peyrani P, Yacisin K, et al. A phase 3, randomized, double-blind study to evaluate the immunogenicity and safety of 3 lots of 20-valent pneumococcal conjugate vaccine in pneumococcal vaccine-naive adults 18 through 49 years of age. *Vaccine* 2021; **39:** 5428–35.

92 Cannon K, Elder C, Young M, et al. A trial to evaluate the safety and immunogenicity of a 20-valent pneumococcal conjugate vaccine in populations of adults ≥65 years of age with different prior pneumococcal vaccination. *Vaccine* 2021; **39:** 7494–502.

93 Chichili GR, Smulders R, Santos V, et al. Phase 1/2 study of a novel 24-valent pneumococcal vaccine in healthy adults aged 18 to 64 years and in older adults aged 65 to 85 years. *Vaccine* 2022; **40:** 4190–98.

94 Platt H, Omole T, Cardona J, et al. Safety, tolerability, and immunogenicity of a 21-valent pneumococcal conjugate vaccine, V116, in healthy adults: phase 1/2, randomised, double-blind, active comparator-controlled, multicentre, US-based trial. *Lancet Infect Dis* 2023; **23:** 233–46.

95 Merck. US FDA approves Merck's VAXNEUVANCE™ (pneumococcal 15-valent conjugate vaccine) for the prevention of invasive pneumococcal disease in infants and children. Merck, June 22, 2022. https://www.merck.com/news/u-s-fda-approves-mercks-vaxneuvance-pneumococcal-15-valent-conjugate-vaccine-for-the-prevention-of-invasive-pneumococcal-disease-in-infants-and-children/ (accessed July 29, 2022).

96 Greenberg D, Hoover PA, Vesikari T, et al. Safety and immunogenicity of 15-valent pneumococcal conjugate vaccine (PCV15) in healthy infants. *Vaccine* 2018; **36:** 6883–91.

97 Banniettis N, Wysocki J, Szenborn L, et al. A phase III, multicenter, randomized, double-blind, active comparator-controlled study to evaluate the safety, tolerability, and immunogenicity of catch-up vaccination regimens of V114, a 15-valent pneumococcal conjugate vaccine, in healthy infants, children, and adolescents (PNEU-PLAN). *Vaccine* 2022; **40:** 6315–25.

98 Senders S, Klein NP, Lamberth E, et al. Safety and immunogenicity of a 20-valent pneumococcal conjugate vaccine in healthy infants in the United States. *Pediatr Infect Dis J* 2021; **40:** 944–51.

99 Feemster K, Buchwald UK, Banniettis N, et al. Immunogenicity of current and next-generation pneumococcal conjugate vaccines in children: current challenges and upcoming opportunities. *Open Forum Infect Dis* 2024; **11:** ofae220.

100 Pobre K, Tashani M, Ridda I, Rashid H, Wong M, Booy R. Carrier priming or suppression: understanding carrier priming enhancement of anti-polysaccharide antibody response to conjugate vaccines. *Vaccine* 2014; **32:** 1423–30.

101 Tomczyk S, Bennett NM, Stoecker C, et al. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among adults aged ≥65 years: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2014; **63:** 822–25.

102 Vadlamudi NK, Chen A, Marra F. Impact of the 13-valent pneumococcal conjugate vaccine among adults: a systematic review and meta-analysis. *Clin Infect Dis* 2019; **69:** 34–49.

103 McFetridge R, Meulen AS, Folkerth SD, et al. Safety, tolerability, and immunogenicity of 15-valent pneumococcal conjugate vaccine in healthy adults. *Vaccine* 2015; **33:** 2793–99.

104 Severance R, Schwartz H, Dagan R, et al. Safety, tolerability, and immunogenicity of V114, a 15-valent pneumococcal conjugate vaccine, administered concomitantly with influenza vaccine in healthy adults aged ≥50 years: a randomized phase 3 trial (PNEU-FLU). *Hum Vaccin Immunother* 2022; **18:** 1–14.

105 Pfizer. Advisory Committee on Immunization Practices votes to recommend routine use of Pfizer's PREVNAR 20™ (pneumococcal 20-valent conjugate vaccine) in adults. Pfizer, Oct 20, 2021. https://www.pfizer.com/news/press-release/press-release-detail/advisory-committee-immunization-practices-votes-recommend# (accessed Aug 17, 2022).

106 Pfizer. Pfizer announces PREVNAR 20™ (pneumococcal 20-valent conjugate vaccine) is now available in Canada for the prevention of pneumonia and invasive pneumococcal disease in adults 18 years of age and older. Pfizer, July 21, 2022. https://www.pfizer.ca/pfizer-announces-prevnar-20%E2%84%A2-pneumococcal-20-valent-conjugate-vaccine-now-available-canada# (accessed Aug 17, 2022).

107 Kobayashi M, Leidner AJ, Gierke R, et al. Use of 21-valent pneumococcal conjugate vaccine among US adults: recommendations of the Advisory Committee on Immunization Practices—United States, 2024. *MMWR Morb Mortal Wkly Rep* 2024; **73:** 793–98.

108 Gámez G, Castro A, Gómez-Mejia A, et al. The variome of pneumococcal virulence factors and regulators. *BMC Genomics* 2018; **19:** 10.

109 Lagousi T, Basdeki P, Routsias J, Spoulou V. Novel protein-based pneumococcal vaccines: assessing the use of distinct protein fragments instead of full-length proteins as vaccine antigens. *Vaccines* 2019; **7:** 2–18.

110 Pichichero ME, Khan MN, Xu Q. Next generation protein-based *Streptococcus pneumoniae* vaccines. *Hum Vaccin Immunother* 2016; **12:** 194–205.

111 Duke JA, Avci FY. Emerging vaccine strategies against the incessant pneumococcal disease. *NPJ Vaccines* 2023; **8:** 122.

112 Seiberling M, Bologa M, Brookes R, et al. Safety and immunogenicity of a pneumococcal histidine triad protein D vaccine candidate in adults. *Vaccine* 2012; **30:** 7455–60.

113 Kamtchoua T, Bologa M, Hopfer R, et al. Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults. *Vaccine* 2013; **31:** 327–33.

114 Brooks WA, Chang L-J, Sheng X, Hopfer R, and the PPR02 Study Team. Safety and immunogenicity of a trivalent recombinant PcpA, PhtD, and PlyD1 pneumococcal protein vaccine in adults, toddlers, and infants: a phase I randomized controlled study. *Vaccine* 2015; **33:** 4610–17.

115 Berglund J, Vink P, Tavares Da Silva F, Lestrate P, Boutriau D. Safety, immunogenicity, and antibody persistence following an investigational *Streptococcus pneumoniae* and *Haemophilus influenzae* triple-protein vaccine in a phase 1 randomized controlled study in healthy adults. *Clin Vaccine Immunol* 2014; **21:** 56–65.

116 Prymula R, Pazdiora P, Traskine M, Rüggeberg JU, Borys D. Safety and immunogenicity of an investigational vaccine containing two common pneumococcal proteins in toddlers: a phase II randomized clinical trial. *Vaccine* 2014; **32:** 3025–34.

117 Leroux-Roels G, Maes C, De Boever F, Traskine M, Rüggeberg JU, Borys D. Safety, reactogenicity and immunogenicity of a novel pneumococcal protein-based vaccine in adults: a phase I/II randomized clinical study. *Vaccine* 2014; **32:** 6838–46.

118 Odutola A, Ota MO, Ogundare EO, et al. Reactogenicity, safety and immunogenicity of a protein-based pneumococcal vaccine in Gambian children aged 2-4 years: a phase II randomized study. *Hum Vaccin Immunother* 2016; **12:** 393–402.

119 Odutola A, Ota MOC, Antonio M, et al. Efficacy of a novel, protein-based pneumococcal vaccine against nasopharyngeal carriage of *Streptococcus pneumoniae* in infants: a phase 2, randomized, controlled, observer-blind study. *Vaccine* 2017; **35:** 2531–42.

120 Briles DE, Ades E, Paton JC, et al. Intranasal immunization of mice with a mixture of the pneumococcal proteins PsaA and PspA is highly protective against nasopharyngeal carriage of *Streptococcus pneumoniae*. *Infect Immun* 2000; **68:** 796–800.

121 Frey SE, Lottenbach KR, Hill H, et al. A Phase I, dose-escalation trial in adults of three recombinant attenuated *Salmonella* Typhi vaccine vectors producing *Streptococcus pneumoniae* surface protein antigen PspA. *Vaccine* 2013; **31:** 4874–80.

122 Plumptre CD, Ogunniyi AD, Paton JC. Polyhistidine triad proteins of pathogenic streptococci. *Trends Microbiol* 2012; **20:** 485–93.

123 Leroux-Roels I, Devaster J-M, Leroux-Roels G, et al. Adjuvant system AS02V enhances humoral and cellular immune responses to pneumococcal protein PhtD vaccine in healthy young and older adults: randomised, controlled trials. *Vaccine* 2015; **33:** 577–84.

124 Lawrence SL, Feil SC, Morton CJ, et al. Crystal structure of *Streptococcus pneumoniae* pneumolysin provides key insights into early steps of pore formation. *Sci Rep* 2015; **5:** 14352.

125 Davies MR, McIntyre L, Mutreja A, et al. Atlas of group A streptococcal vaccine candidates compiled using large-scale comparative genomics. *Nat Genet* 2019; **51:** 1035–43.

126 Kuroda E, Koizumi Y, Piao Z, et al. Establishment of a modified opsonophagocytic killing assay for anti-pneumococcal surface protein A antibody. *J Microbiol Methods* 2023; **212:** 106804.

127 Bahadori Z, Shafaghi M, Sabzevari J, et al. Design, development, and assessment of a novel multi-peptide vaccine targeting PspC, PsaA, and PhtD proteins of *Streptococcus pneumoniae*. *Int J Biol Macromol* 2024; **258:** 128924.

128 King DF, Groves H, Weller C, et al. Realising the potential of correlates of protection for vaccine development, licensure and use: short summary. *NPJ Vaccines* 2024; **9:** 82.