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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

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ABSTRACT

Background: Pneumococcal diseases remain prevalent despite available polysaccharide and conjugate vaccines. This phase 1/2 study evaluated safety/tolerability and immunogenicity of a novel 24-valent pneumococcal vaccine (ASP3772) based on high-affinity complexing of proteins and polysaccharides.
Methods: Pneumococcal vaccine–naïve adults aged 18–85 years were randomized to receive either ASP3772 or PCV13 (13-valent conjugate vaccine). Participants received a single intramuscular injection of ASP3772 (1-, 2-, or 5-μg dose per polysaccharide) or PCV13. A separate, nonrandomized group of PCV13-vaccinated participants (65–85 years) received PPSV23 (23-valent polysaccharide vaccine). Assessments were obtained through Day 7 for reactogenicity, through Day 30 for safety and tolerability, and through Month 6 for serious adverse events. Immunogenicity was measured at Day 30 using assays for functional opsonophagocytic activity (OPA) and pneumococcal serotype-specific anticapsular polysaccharide immunoglobulin G for each serotype.
Results: In both age cohorts, the most frequently reported local reactions were self-limited tenderness and pain after ASP3772 at all dose levels or after PCV13, occurring within 2–3 days. Fatigue, headache, and myalgia were the most frequently reported systemic reactions following either vaccine. Robust OPA responses for all serotypes were observed across all ASP3772 dose groups in both age cohorts. Older adults (aged 65–85 years) who received ASP3772 had significantly higher immune responses to several PCV13 serotypes and all non-PCV13 serotypes than participants who received PCV13. OPA responses to the ASP3772 5-μg dose were significantly higher for several serotypes in naïve participants than in older adults with prior exposure to PCV13 who were administered PPSV23 in this study.
Conclusions: These results demonstrate that ASP3772 is well tolerated, highly immunogenic, and in adults may offer significantly broader protection than existing pneumococcal vaccines.
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1. Introduction

Pneumococcal vaccines have substantially decreased the burden of pneumococcal disease; however, *Streptococcus pneumoniae* remains a significant cause of morbidity and mortality in older adults and young children [1]. *S. pneumoniae* is responsible for approximately 27% of pneumonia cases worldwide and over 36,000 cases in the USA of invasive pneumococcal disease, which includes meningitis, bacteremic pneumonia, and bacteremia [1–4]. Increasingly, the burden of pneumococcal disease is caused by vaccine serotypes not included in licensed vaccines.

Conjugate and polysaccharide vaccines, such as PCV13 (13-valent pneumococcal conjugate vaccine) and PPSV23 (23-valent pneumococcal polysaccharide vaccine), are recommended for routine use in adults in many countries worldwide. Conjugate vaccines induce T-cell–dependent antibody responses to the serotypes contained in the vaccine whereas polysaccharide vaccines generate T-cell–independent antibody responses, which can be diminished in older adults [5]. Results of preclinical studies indicated that memory Th17 cells are important in protecting against pneumonia and colonization, independent of serotypes, suggesting that generating mucosal Th17 cell response may be a feasible approach to vaccine development [6,7]. Strategies to achieve broader

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protection include the addition of more capsular serotypes or pneumococcal proteins that protect by inducing antibodies or T-cell responses that reduce colonization [8]. An inactivated *S. pneumoniae* whole cell vaccine, which has been evaluated in healthy adults and toddlers in a phase 1 trial, could potentially prevent new serotypes from spreading by reducing pneumococcal colonization in the nasopharynx [9,10]. Vaccines based on protein antigens that are recognized by the immune system, including pneumococcal histidine triad protein D, pneumolysin, pneumococcal surface protein A, and pneumococcal choline-binding protein A, have been evaluated in clinical trials [10].

ASP3772, which has since been renamed AFX3772, is a novel 24-valent pneumococcal vaccine that was developed based on a Multiple Antigen Presenting System (MAPS) platform, which has been shown to induce robust B-cell and T-cell immunity in animal models [11]. The MAPS platform takes advantage of the high-affinity noncovalent binding between biotin and rhizavidin, a biotin-binding protein that has no significant predicted homology with human proteins [11]. ASP3772 contains 24 polysaccharides, including the 13 serotypes contained in PCV13 plus an additional 10 contained in PPSV23. ASP3772 serotypes are 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (these are shared with PCV13 as well as with PPSV23, with the exception of 6A) plus 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 22F, and 33F (these are shared only with PPSV23) and 20B. Of note, serotype 20B is the predominant circulating strain of serotype 20; serotype 20A rarely occurs in nature [12,13]. Each serotype is individually biotinylated and complexed with a unique fusion protein consisting of rhizavadin fused to two pneumococcal protein segments derived from genetically conserved surface protein genes (*sp*1500 and *sp*0785) [14]. Deletion of *sp*1500 and *sp*0785 resulted in significant reduction in virulence of a type 3 pneumococcus in a preclinical model [14]. In addition, fusion of the two proteins conferred protection against colonization and generated opsonic antibodies that assisted in the killing of pneumococcal strains.

Nonclinical data from challenge studies suggested that ASP3772 has the potential to be clinically effective at protecting against not only vaccine serotype pneumococcal infection but also non-vaccine serotype disease. This first-in-human phase 1/2 clinical study evaluated the safety, tolerability, and immunogenicity of ASP3772, compared with PCV13, and the immunogenicity of ASP3772, compared with PPSV23, in adults previously administered PCV13 (NCT03803202).

2. Methods

2.1. Study design and participants

This was a combined phase 1, first-in-human, dose-escalation and phase 2, dose-finding study (Fig. 1). Phase 1 enrolled healthy adults aged 18 to 64 years from January to April 2019. After completion of phase 1 of the study, phase 2 was initiated, which enrolled adults aged 65 to 85 years from June 2019 to April 2020. The sample size for phase 1 was targeted at 120 participants aged 18 to 64. In phase 2, approximately 495 participants aged 65 to 85 years were planned for enrollment. Three groups were enrolled based on age and vaccine status. All vaccines were administered as a single intramuscular injection into the deltoid muscle of the right or left arm. In pneumococcal vaccine–naïve participants aged 18 to 64 years (Group 1), safety and immunogenicity were established by dose-escalation of ASP3772 at polysaccharide dose levels of 1, 2, or 5 μg. Specifically, participants were randomly assigned 3:1 to receive either ASP3772 1 μg or PCV13 (Prevnar 13®, Wyeth Pharmaceuticals, LLC, a subsidiary of Pfizer Inc, Philadelphia, PA). After safety was confirmed for ASP3772 1 μg, participants in the next group were randomized to receive either ASP3772 2 μg or PCV13. Then, after safety was confirmed for ASP3772 2 μg, participants in the next group were randomly assigned to receive either ASP3772 5 μg or PCV13. Once safety was confirmed in all participants aged 18 to 64 years, these doses were then evaluated in pneumococcal vaccine–naïve adults aged 65 to 85 years, who were healthy or had chronic controlled stable disease (Group 2) and were randomized 3:1 to receive either ASP3772 (1-, 2-, or 5-μg doses) or PCV13. Any potential participant aged 65 to 85 years who had previously received PCV13 was allowed to enter the study to receive PPSV23. Thus, a separate group of participants aged 68 to 85 years who had received PCV13 approximately 10 to 24 months before enrollment (Group 3) were not randomized and received PPSV23 (Pneumovax®23, Merck & Co., Inc, Whitehouse Station, NJ). This active-controlled, double-blinded study was conducted at 23 locations in the United States. This study was approved by the Institutional Review Board and conducted in accordance with Good Clinical Practice, International Council for Harmonisation (ICH) guidelines, and relevant regulations and guidelines on study conduct and ethical principles with origins in the Declaration of Helsinki. All participants provided written informed consent prior to any study-related procedures.

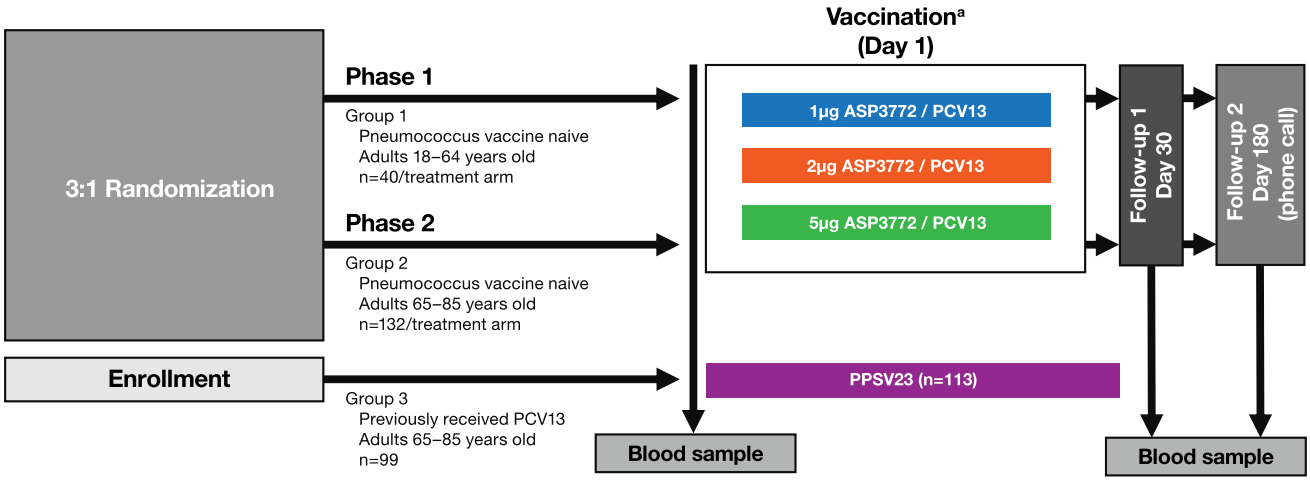

Fig. 1. Study Schema. Phase 2 Groups 2 and 3 commenced after completion of phase 1 Group 1. [a]Solicited local and systemic reactions were reported through 7 days postimmunization by the participant in an electronic diary. A dose-escalation committee reviewed safety through Day 7 from all participants within a dose cohort before initiating the next dose cohort. Abbreviations: PCV, pneumococcal conjugate vaccine; PPSV, pneumococcal polysaccharide vaccine.

2.2. Objectives

The primary objective was to evaluate the safety and tolerability of three dose levels of ASP3772 compared with PCV13 in participants aged 18 to 85 years (Group 1 and Group 2). The secondary objective was to evaluate the immunogenicity of three dose levels of ASP3772 compared with PCV13 (Group 1 and Group 2). Immune responses in ASP3772 recipients were also evaluated relative to responses with PPSV23 in recipients aged 65 to 85 years previously vaccinated with PCV13 (Group 3), with an interest in those serotypes not included in PCV13.

2.3. Assessments

Safety and tolerability were assessed by recording treatment-emergent adverse events (TEAEs). Solicited local and systemic reactions were reported through 7 days postimmunization by the participant in an electronic diary. A dose-escalation committee reviewed safety through Day 7 from all participants within a dose cohort before initiating the next dose cohort. TEAE reporting continued through Day 30 postimmunization. Safety assessments for serious adverse events (AEs) including potentially immune-mediated medical conditions, new onset chronic diseases (NOCDs), and medically attended AEs continued through Day 180 postimmunization. Anti-biotin antibodies were measured from serum samples on Day 1 prior to study immunization, at the Day 30 follow-up visit, and repeated on Day 180 if positive on Day 30. If anti-biotin antibodies were detected at either Day 1, Day 30, or Day 180, the titer was measured, and biotin levels were evaluated.

Immunological responses were assessed by serotype-specific functional opsonophagocytic activity (OPA) and pneumococcal serotype-specific anticapsular polysaccharide IgG from serum samples collected on Day 1 and Day 30. Antibody responses specific to the pneumococcal fusion protein *sp*1500 and *sp*0785 and the protein-specific T-cell responses, including measurement of elicited cytokines interleukin (IL)-17A and –22, were evaluated at Day 1 and Day 30. Additional samples were collected on Day 7 for T-cell responses in phase 2 only.

Functional OPA was measured using the multiplex OPA (MOPA) assay [15]. The MOPA strains of *S. pneumoniae* serotypes (obtained from Professor Moon Nahm's laboratory, University of Alabama, Birmingham, AL) were selected and propagated based on their unique antibiotic resistance [16,17]. Functional OPA for each serotype characterized by an OPA titer^{-1} response was expressed as the reciprocal of the serum dilution that causes a 50% reduction in the colony-forming units.

Pneumococcal serotype-specific anticapsular polysaccharide IgG concentrations were measured using Luminex-based multiplex direct immunoassay [18]. Both the MOPA assay and the Luminex-based multiplex direct immunoassay were developed and qualified according to Good Clinical Laboratory Practice guidelines.

Fusion protein-specific antibody levels were measured using MSD ELISA. Briefly, MSD GOLD streptavidin plates (Meso Scale Diagnostics, Rockville, MD) were coated with biotinylated *sp*1500 + *sp*0785 capture protein. Following the addition of serum samples, SULFO-TAG labeled anti-human antibody (Meso Scale Diagnostics) was used as secondary antibody for detection. The plates were analyzed in MSD Sector Imager 600.

For Th17 response assessment, cryopreserved peripheral blood mononuclear cells (PBMCs) for ELISpot assays were rested for 24 h at 37 °C, 5% CO_2 in 10% fetal bovine serum in RPMI culture medium. Cells were then stimulated with 5-μg/mL *sp*1500-0785 fusion protein present in ASP3772 (without rhizavidin) in ELISpot plates (MABTECH, Stockholm, Sweden) pre-coated with a specific monoclonal anti-IL-17 antibody, at 2.0×10^5 cells/well in the presence of the specific protein conjugate. Following stimulation for 48 h, cells were washed, and IL-17 spots were detected using a monoclonal IL-17 detection antibody, alkaline phosphatase, and a substrate solution. The plates were dried for 12 h at room temperature in the dark prior to spot counting using the CTL Immunospot® Analyzer. To measure cytokine responses, PBMCs were cultured in RPMI medium supplemented with 10% fetal bovine serum in the presence of 5-μg/mL *sp*1500-*sp*0785. Supernatants from cultured plates were collected and cytokine concentrations were measured using an MSD V-PLEX Plus Th17 Panel 1 Human Kit (Meso Scale Diagnostics).

2.4. Analyses

The safety population comprised all participants who received an immunization in this study with either ASP3772, PCV13, or PPSV23. All participants in the safety population who had at least one postimmunization measurement were included in the primary analysis of immunogenicity data. Recipients of PCV13 within each cohort were pooled for analysis. No pre-specified hypotheses were tested in this early phase study.

Continuous data were summarized with means (SD) or geometric means and 95% confidence intervals (CIs). Categorical data were summarized with frequency and percentage. The geometric mean titers (GMTs) (or geometric mean concentrations [GMCs]) were calculated.

In phase 2, the relative immunogenicity to PCV13 or PPSV23 was evaluated by comparing the ratio of the Day 30 OPA GMTs and the 95% CIs for each ASP3772 dose level over the active-comparator responses for each serotype. If the lower bound of the 95% CI was above 1, the immunogenicity of the ASP3772 dose was significantly higher than PCV13. If the upper bound of the 95% CI was below 1, the immunogenicity of PCV13 was significantly higher than the ASP3772 dose. The same assessment was conducted for the comparison of ASP3772 dose levels and PPSV23.

3. Results

3.1. Participants

In Group 1 (pneumococcal vaccine–naïve, aged 18 to 64 years), 126 participants were randomly assigned to and received ASP3772 or PCV13 (Fig. S1A). In Group 2 (pneumococcal vaccine–naïve, aged 65 to 85 years), 390 participants were randomly assigned to and received ASP3772 or PCV13 (Fig. S1B). In Group 3 (prior PCV13, aged 65 to 85 years), 113 participants received PPSV23 (Fig. S1C).

Baseline characteristics were generally balanced across the groups (Tables S1 and S2). Most participants were female and White. In Group 1, the median ages were 29 years (range, 18 to 64) and 28 years (range, 18 to 63) across the ASP3772 groups and in the PCV13 group, respectively. The median (range) age in Group 2 was 67 (65–84), 68 (65–85), and 66 (65–80) in the ASP3772 1-μg, 2-μg, and 5-μg dose groups and 67 (65–81) in the PCV13 group. In the overall ASP3772 group and in the PCV13 group, 12.3% and 13.5% of patients, respectively, were aged 75 to 85 years. In Group 3, the median (range) age was 66 (65–78). In addition, underlying co-morbidities were generally similar across the groups (Tables S3 and S4).

3.2. Safety

(a) Reactogenicity: Tenderness and pain occurring within the first 2–3 days were reported most often following administration of either ASP3772 or PCV13 in Groups 1 and 2 (Figs. 2A and 3A). In Group 2, there was an increase in pain and tenderness with increasing ASP3772 dose; however, all events were reported to

be mild to moderate in intensity (grade 1–2). Fatigue, headache, and myalgia were the most commonly reported systemic events after ASP3772 or PCV13 administration (Figs. 2B and 3B). In Groups 1 and 2, there was an increase in reported fatigue and myalgia with increasing ASP3772 dose; however, even at the highest dose, the incidence of events was comparable to PCV13 and were mostly grade 1–2.

(b) Other adverse events: In Groups 1 and 2 there was no apparent pattern of TEAEs associated with increasing ASP3772 dose and the incidence was generally comparable across ASP3772 and PCV13 treatment arms (Table S5). Across dose groups, four (4.3%) participants in Group 1 and 18 (6.1%) participants in Group 2 had AEs considered to be related to ASP3772 by the investigator; none were serious and all resolved without sequelae. In Group 2 PCV13 recipients, there were three (3.1%) vaccine-related AEs; two (1.8%) AEs in Group 3 were considered related to PPSV23.

In Group 2, there were four NOCDs reported with ASP3772 (n = 2 [2.0%] for 1 μg; n = 1 [0.9%] for 2 μg; n = 1 [1.2%] for 5 μg), all of which were considered unrelated to the vaccine (Table S5). One participant in the ASP3772 2-μg dose group died

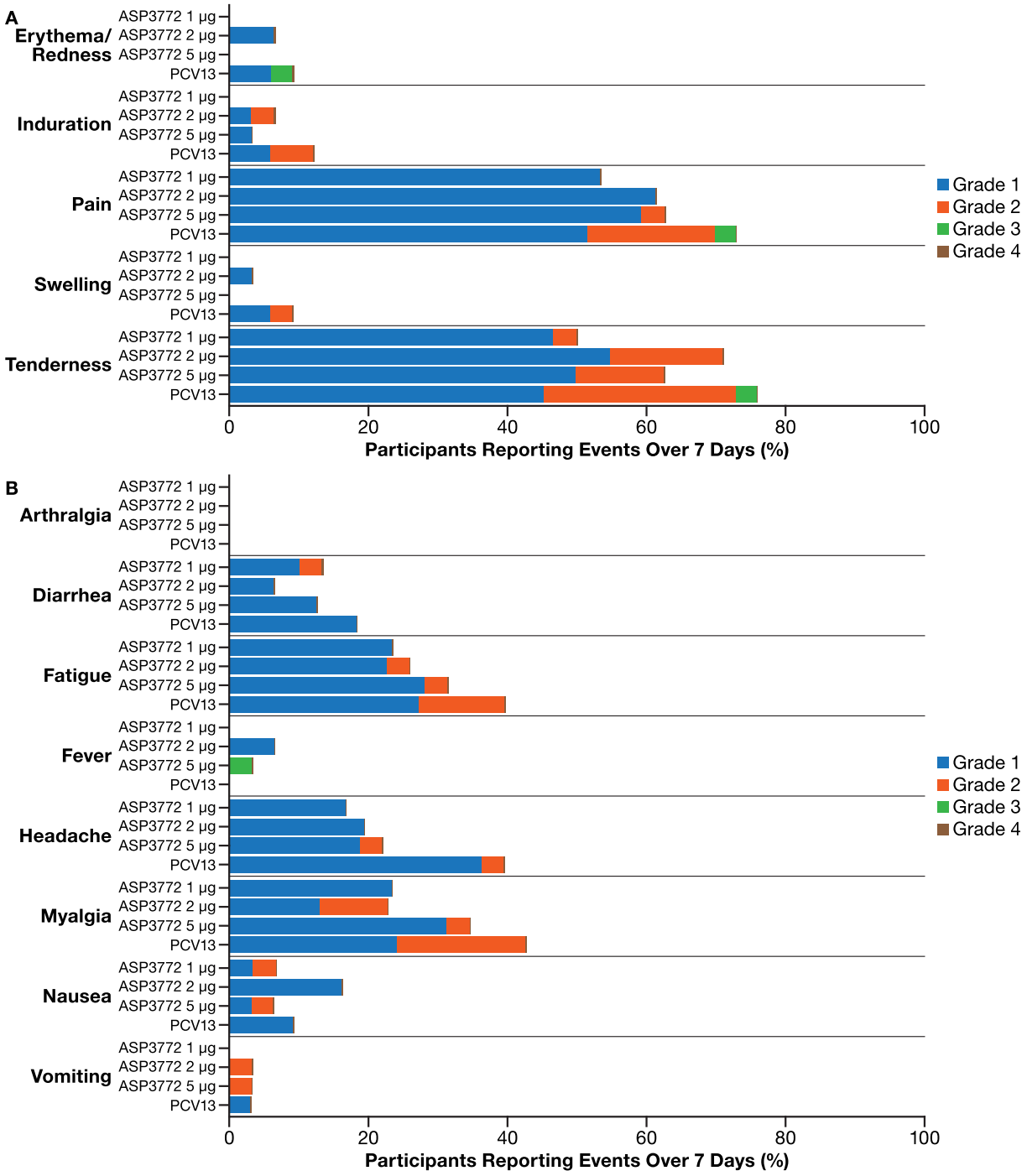

Fig. 2. (A) Local and (B) Systemic Reactions in the ASP3772 Dose Groups and in the PCV13 Group for Participants Aged 18–64 Years. Abbreviation: PCV, pneumococcal conjugate vaccine.

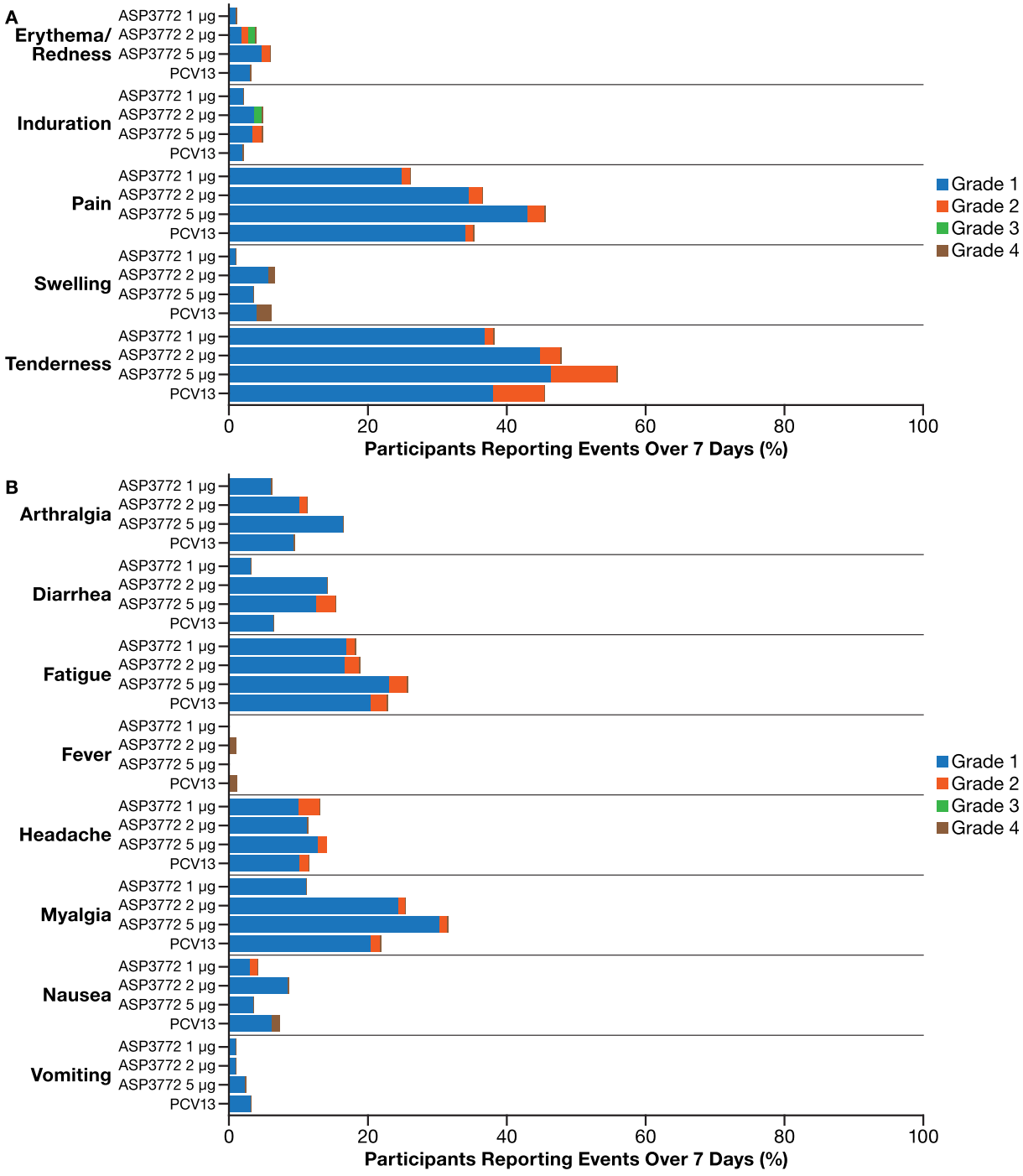

Fig. 3. (A) Local and (B) Systemic Reactions in the ASP3772 Dose Groups and in the PCV13 Group in Pneumococcal Vaccine–Naïve Participants Aged 65–85 Years. Abbreviation: PCV, pneumococcal conjugate vaccine.

during the follow-up period due to exacerbation of an underlying condition; this death was deemed by the investigator to be unrelated to the vaccine.

No clinically relevant abnormalities were observed in vital signs, electrocardiograms, and laboratory parameters during either phase of the study.

(c) Biotin levels and anti-biotin antibody measurements: Anti-biotin antibodies were infrequently detected pre- and post-vaccination in both age groups in recipients of either ASP3772 or PCV13 (Table S6). There were no significant differences in percentage of participants with anti-biotin antibody, mean levels of anti-biotin antibody, or serum biotin levels before and after immunization across vaccine groups.

3.3. Immunogenicity: Polysaccharide responses

(a) Group 1: ASP3772 was immunogenic across all three dose levels. OPA titers increased from Day 1 to Day 30 for all 24 serotypes. When comparing ASP3772 doses and PCV13, OPA GMTs at Day 30 were comparable for most common serotypes and greater for serotypes unique to ASP3772 (Fig. S2A and C). At all ASP3772 dose levels, the Day 30 OPA GMTs were significantly greater than

PCV13 for shared serotype 3 and all unique ASP3772 serotypes (Fig. S2B and D). Robust IgG responses were observed across the ASP3772 dose groups for serotypes shared with PCV13; these responses were generally similar to the PCV13 responses for most serotypes but were statistically significantly greater following ASP3772 2 μg and 5 μg for serotype 3. Day 30 IgG responses were significantly greater with all doses of ASP3772 compared with PCV13 for all unique serotypes.

(b) Group 2: OPA GMTs at Day 30 were comparable for most common serotypes and significantly greater for serotypes unique to ASP3772 (Fig. 4A and C). At all ASP3772 dose levels, the Day 30 OPA GMTs were significantly greater than PCV13 for shared serotype 3 and all unique ASP3772 serotypes (Fig. 4B and D; Table S7). Day 30 OPA titers were also significantly greater for serotypes 5 and 19F at the ASP3772 5-μg dose, compared with PCV13 (Fig. 4B). Day 30 IgG concentrations across the ASP3772 dose groups and the PCV13 group were similar for most serotypes shared with PCV13; IgG GMCs were significantly greater following ASP3772, for the 5-μg dose, than with PCV13 for serotypes 3, 7F, 9V, and 19F (Fig. 5A and B). Day 30 IgG concentrations were significantly greater with all doses of ASP3772 than with PCV13 for all unique serotypes (Fig. 5C and D).

(c) Group 3: Baseline OPA GMTs for PCV13 serotypes in PCV13-naïve participants who received any dose of ASP3772 were significantly less than baseline OPA GMTs for participants who received PPSV23 in this study since the latter had previously received PCV13 (not shown). However, at Day 30, OPA GMTs were significantly greater for most serotypes in PCV13-naïve ASP3772 recipients compared to PCV13-experienced PPSV23 recipients (Fig. 6A-D and Table S8).

Similar to OPA, baseline IgG GMCs for PCV13 serotypes in PCV13-naïve participants who received any dose of ASP3772 were significantly less than baseline IgG GMCs for participants who received PPSV23 because of their prior PCV13 exposure. Day 30 IgG GMCs were significantly greater following ASP3772 than PPSV23 for most serotypes, including 10 of the 11 serotypes not found in PCV13 (data not shown).

3.4. Fusion protein immune responses

The antibody responses to the *sp*1500 + *sp*0785 fusion protein to which the polysaccharides are complexed were evaluated. While these adult populations had preexisting antibody to the pneumococcal components of the *sp*1500 + *sp*0785 fusion protein, the GMC of the *sp*1500 + *sp*0785 fusion protein-specific IgG increased from Day 1 to Day 30 for all ASP3772 doses in participants aged 18 to 64 (Fig. S3A); the geometric mean fold rise (GMFR) of the *sp*1500 + *sp*0785 fusion protein-specific IgG at Day 30 was 5- to 6-fold across ASP3772 doses. In participants aged 65 to 85 years, the GMC of the *sp*1500 + *sp*0785 fusion protein-specific IgG increased at Day 30 (Fig. S3B) and the GMFR was 9- to 13-fold for all three ASP3772 doses. There were no increases in *sp*1500 + *sp*0785 fusion protein-specific IgG in the groups that received PCV13.

3.5. Th-17 induction

PBMCs collected on Days 7 (phase 2 only) and 30 were stimulated *ex vivo* with the *sp*1500 + *sp*0785 fusion protein to measure induction of Th17 responses after vaccination. In Group 1, partici-

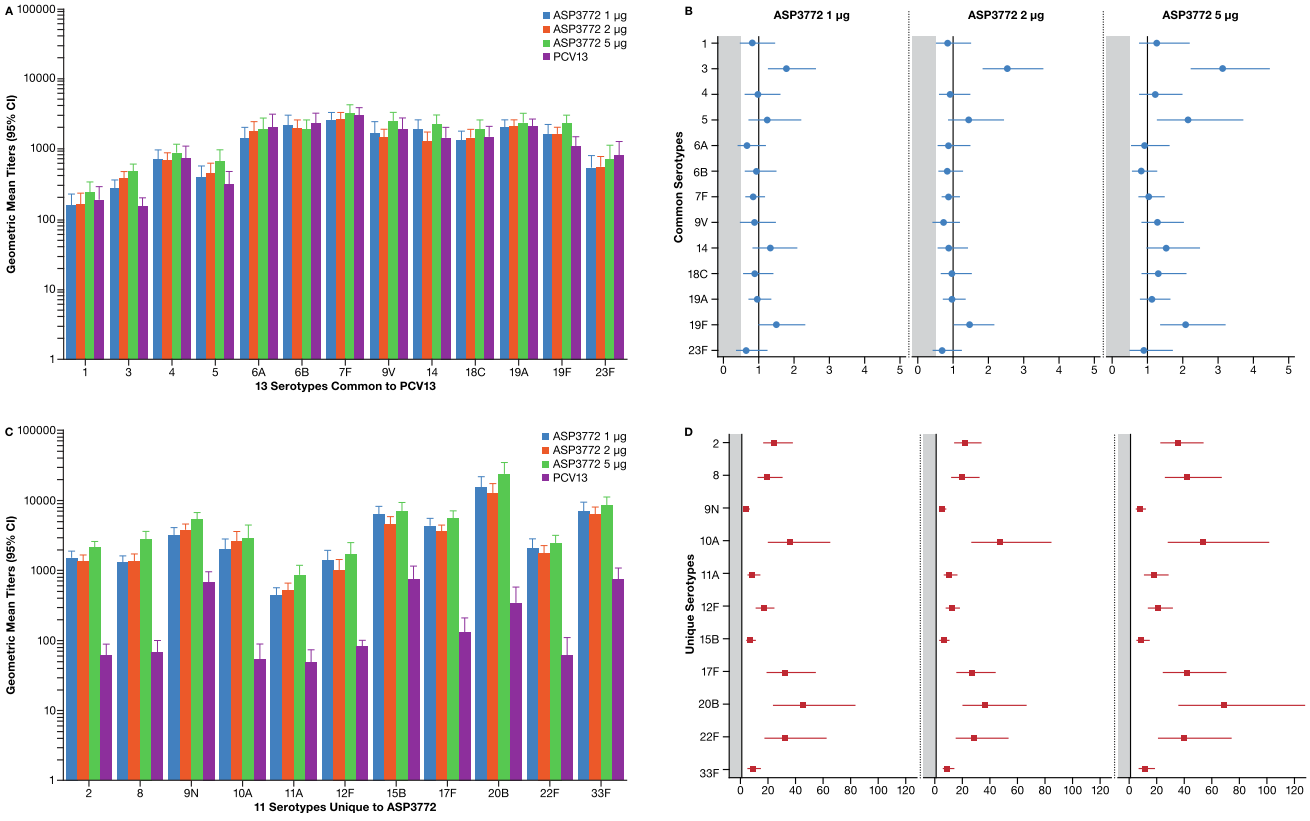

Fig. 4. Postimmunization OPA Geometric Mean Titers at Day 30 for ASP3772 and PCV13 and Postimmunization Ratios of OPA Geometric Mean Titers (ASP3772 Dose Level to PCV13) in Participants Aged 65 to 85 Years. (A) Titers and (B) Ratios for 13 Serotypes Common to PCV13. (C) Titers and (D) Ratios for 11 Serotypes Unique to ASP3772. Abbreviations: CI, confidence interval; OPA, opsonophagocytic activity; PCV, pneumococcal conjugate vaccine.

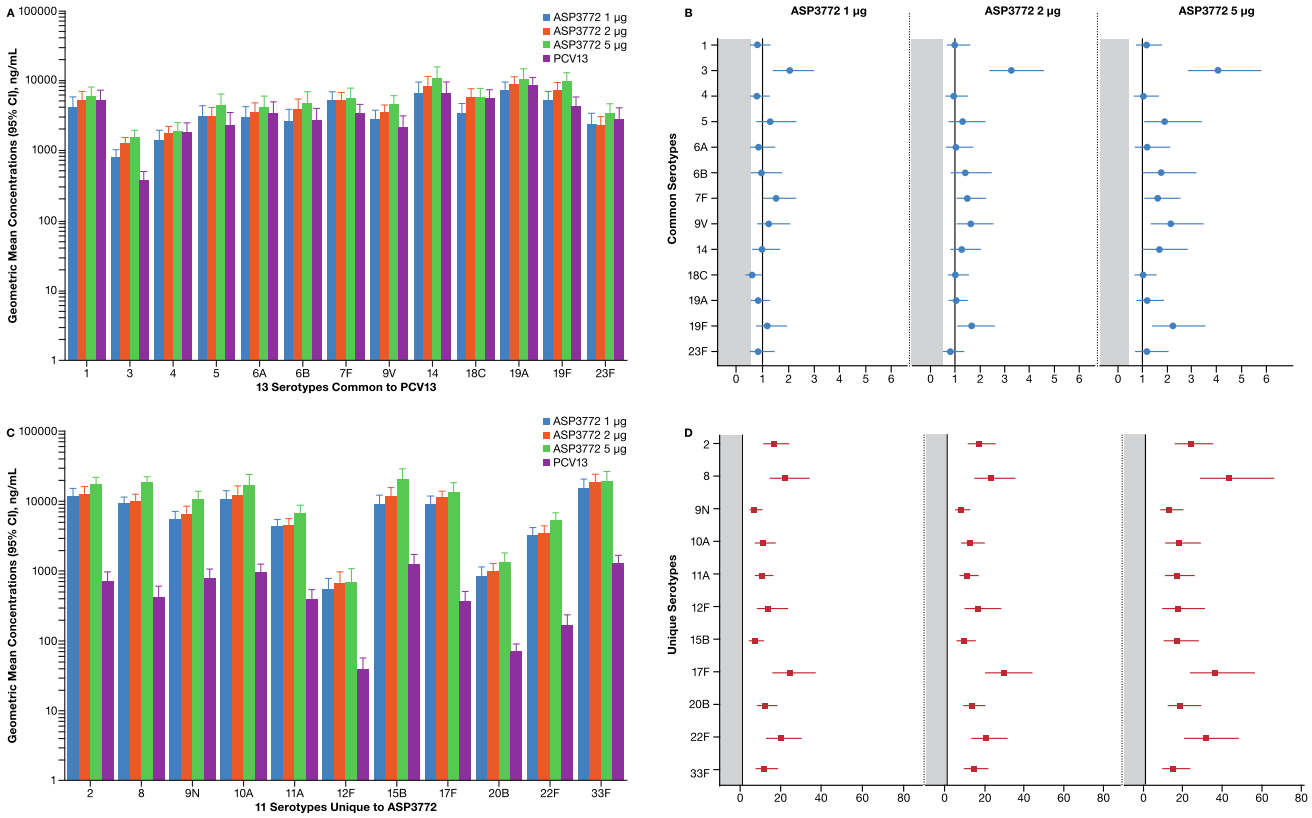

Fig. 5. Postimmunization IgG Geometric Mean Concentrations (ng/mL) at Day 30 for ASP3772 and PCV13 and Postimmunization Ratios of IgG Geometric Mean Concentrations (ASP3772 Dose Level to PCV13) in Participants Aged 65 to 85 Years. (A) Concentrations and (B) Ratios for 13 Serotypes Common to PCV13. (C) Concentrations and (D) Ratios for 11 Serotypes Unique to ASP3772. The point is the ratio of the geometric means. Whiskers extend to the upper limit of the 95% CI of the ratio. Abbreviations: CI, confidence interval; PCV, pneumococcal conjugate vaccine.

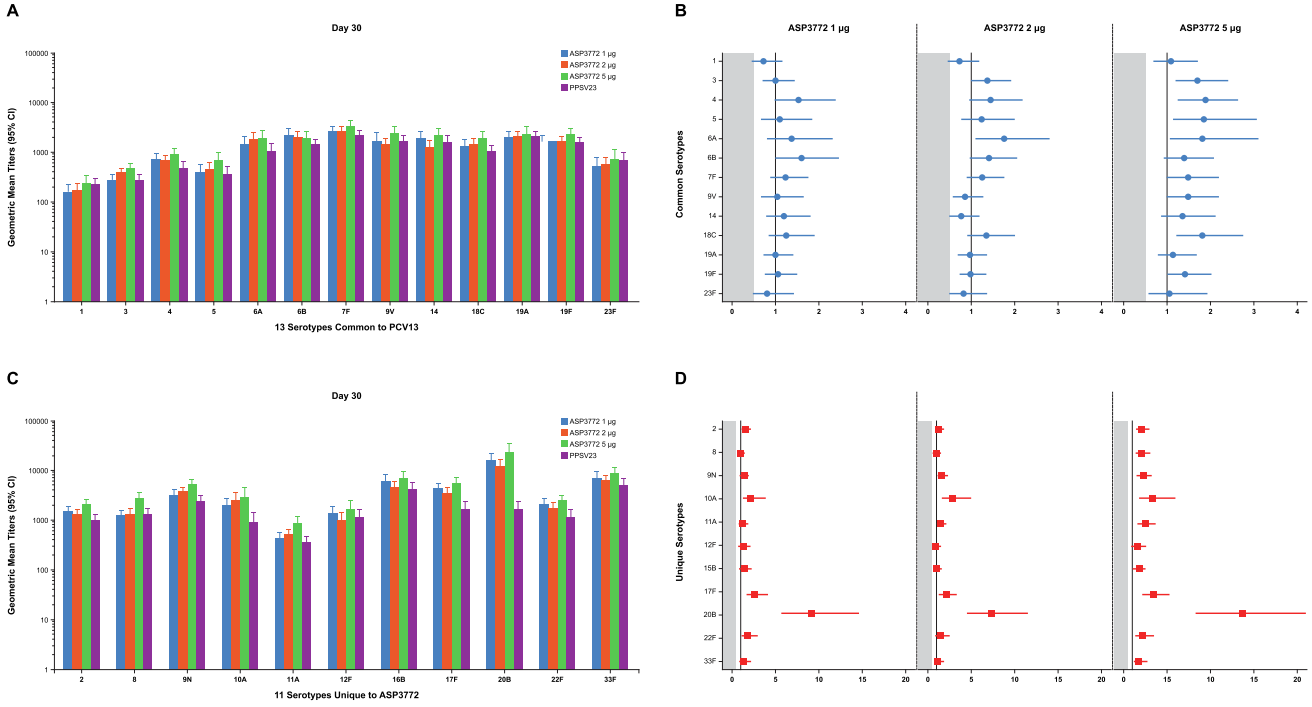

Fig. 6. Postimmunization OPA Geometric Mean Titers at Day 30 for ASP3772 and PPSV23 and Postimmunization Ratios of OPA Geometric Mean Titers (ASP3772 Dose Level to PPSV23) in Participants Aged 65 to 85 Years. (A) Titers and (B) Ratios for 13 Serotypes Common to PCV13. (C) Titers and (D) Ratios for 11 Serotypes Unique to ASP3772. 3. The point is the ratio of the geometric means. Whiskers extend to the upper limit of the 95% CI of the ratio. Abbreviations: CI, confidence interval; OPA, opsonophagocytic activity; PCV, pneumococcal conjugate vaccine; PPSV, pneumococcal polysaccharide vaccine.

pants who received ASP3772 5 μg had significantly more *sp*1500 + *sp*0785-specific Th17 cells (P = 0.021) and significantly higher IL-17 production by cultured PBMCs post stimulation (P = 0.018) at Day 30 than participants who received PCV13 (Table S9). Change from baseline at Day 30 within each treatment group was significantly greater for IL-17 (P = 0.001) and IL-22 (P = 0.001) in the ASP3772 5-μg group. It should be noted that the IL-17 and IL-22 baseline values were significantly lower in the ASP3772 5-μg group than the PCV13 group (P = 0.046 and P = 0.024, respectively). No significant increases were observed in Th17-related responses in the ASP3772 1-μg and 2-μg groups compared with PCV13.

In Group 2, mean *sp*1500 + *sp*0785-specific Th17 cell numbers at Day 7 were not significantly different for any of the ASP3772 dose groups compared with PCV13 (Table S10). At Day 30, the number of Th17 cells and IL-17 and of IL-22 cytokine production were significantly greater in the ASP3772 5-μg dose group than in the PCV13 group (P = 0.012, P = 0.031, and P = 0.005, respectively). At baseline (Day 1) and Day 7, IL-17 and IL-22 cytokine production measured in culture supernatants showed no significant difference for any of the ASP3772 dose groups compared with PCV13.

In paired analysis of change from baseline in participants with available data, Day 7 Th17 cell numbers and IL-17 cytokine production were significantly higher in the ASP3772 1-μg dose group (P < 0.001 and P = 0.001, respectively). Change from baseline to Day 30 was marginally greater for Th17 cell number and significantly greater for IL-22 cytokine production (P = 0.001) in the ASP3772 5-μg dose group. Change from baseline to Day 30 IL-17 cytokine production was significantly higher in the ASP3772 2- and 5-μg dose groups (P = 0.032 and P = 0.011, respectively).

4. Discussion

Protection against pneumococcal diseases has been enhanced by the development of pneumococcal polysaccharide and conjugate vaccines. Despite these advances, vaccine-type pneumococcal diseases persist in vulnerable populations, and replacement disease by nonvaccine serotypes has occurred in many settings [19]. The 24-valent ASP3772 vaccine reported here has the ability to provide the broadest coverage of any pneumococcal vaccine in clinical development. Beyond the broad serotype coverage, this vaccine incorporates a fusion protein consisting of two highly conserved pneumococcal proteins (*sp*1500 and *sp*0785) that have been shown in preclinical studies to be important for pneumococcal virulence *in vivo* and protective immunity *in vivo* [14,20]. Thus, this vaccine may offer a further advantage in terms of potential impact on pneumococcal carriage of both vaccine and nonvaccine serotypes. Importantly, this vaccine is formulated using an aluminum-based adjuvant and without the need for a novel adjuvant.

In these first-in-human studies, ASP3772 was well tolerated in healthy adults aged 18 to 85 years across 1-, 2-, and 5-μg dose levels per serotype polysaccharide. The safety profile described in this phase 1/2 study demonstrates similar frequencies of solicited local and systemic reactions as PCV13 and as reported in other pneumococcal vaccine clinical trials [21,22]. Acute reactogenicity through 7 days postimmunization was similar to that of PCV13 for both local and systemic reactions, in spite of the greater number of serotypes included and overall higher polysaccharide and protein content. Local reactions peaked on Days 2 to 3 and were mostly grade 1–2. There were no vaccine-related serious AEs reported in the study and no evidence of potential immune-mediated TEAEs or new onset medical diagnoses related to any ASP3772 dose. Vaccine-related TEAEs after Day 7 were self-limited, generally mild, and infrequent, occurring in less than 10% of ASP3772 recipients. Because a core component of the MAPS technology is the interaction between biotin-rhizavidin, it is noteworthy that the vaccine did not induce antibody to biotin and had no impact on serum biotin concentrations. In fact, when low levels of anti-biotin antibodies were detected in a participant, whether pre- or post-immunization with either ASP3772 or PCV13, there was no evidence of subsequent impact on serum biotin levels.

The criteria for marketing approval of pneumococcal vaccines for adults have focused primarily on levels of functional antibody measured by OPA as a surrogate of efficacy in comparison with PCV13 and PPSV23. In this study we compared participants' immunologic responses to three ASP3772 dose levels with PCV13 alone and with the two-dose series of PCV13 followed by PPSV23 10 to 24 months later. All ASP3772 dose levels generated functional antibody to all 24 serotypes at levels similar to or greater than the responses to PCV13 and PPVS23, with the highest responses seen with the 5-μg dose of ASP3772. It is noteworthy that significantly higher immune responses were generated by all ASP3772 dose levels for serotypes 3 and 19F, as well as serotype 5 by ASP3772 5 μg, compared with PCV13. In addition, across both age groups of the study, there was clear evidence of antibody and Th17 cell responses to the fusion protein, including 5- to 13-fold GMFR in the concentrations of antibody directed against the pneumococcal fusion protein following vaccination with each of the three doses of ASP3772. While the clinical significance of antibody and Th17 responses to the pneumococcal fusion protein has yet to be determined, it is noteworthy that pneumococcal protein-specific immune responses are not generated with either conjugate vaccines or PPSV23 [5,8].

Some limitations should be considered. This study enrolled a group of older adults who had previously been immunized with PCV13 to receive PPSV23. The same inclusion and exclusion criteria were applied to these participants to obtain a contemporaneously enrolled group with similar characteristics as those who received ASP3772. The comparison in this study of PPSV23 to ASP3772 provides preliminary evidence of the relative immunogenicity profiles; however, the participants who received PPSV23 were not a randomized group because of their previous PCV13 immunization. Therefore, the comparison performed here may need to be confirmed in phase 3 randomized, controlled studies. Of note, this was a phase 2 dose-finding study and the immunogenicity findings should be considered preliminary, given the variability of data across the groups (especially in Group 1). Th17 response to fusion protein varied considerably at baseline between treatment groups. Clinical relevance of the Th17 response has not been established. Further, the generalizability of these data to demographic groups not enrolled or enrolled in small numbers is limited.

In conclusion, results of this phase 1/2 study showed that a single dose of the MAPS-based ASP3772 vaccine was well tolerated at all dose levels, has a safety profile comparable to PCV13, and exhibits robust immunogenicity. In older adults, the functional antibody responses to ASP3772 were comparable or higher than those shared with PCV13 and PPSV23. As such, the data from this phase 1/2 study provide strong evidence for further clinical development of ASP3772 (now renamed AFX3772).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Gurunadh R. Chichili reports financial support was provided by Astellas Pharma Global Development Inc. Ronald Smulders reports financial support was provided by Astellas Pharma Global Development Inc. Vicki Santos reports financial support was provided by Astellas Pharma Global Development Inc. Beth Cywin

reports financial support was provided by Astellas Pharma Global Development Inc. Laura Kovanda reports financial support was provided by Astellas Pharma Global Development Inc. Charles Van Sant reports financial support was provided by Astellas Pharma Global Development Inc. Frank Malinoski reports a relationship with Affinivax that includes: employment. Shite Sebastian reports a relationship with Affinivax that includes: employment. George Siber reports a relationship with Affinivax that includes: consulting or advisory and employment. Richard Malley reports a relationship with Affinivax that includes: consulting or advisory and employment. Richard Malley has issued and pending patents on MAPS vaccines, Rhizavidin-fusion proteins, and Multivalent Pneumococcal Vaccine. Shite Sebastian has issued and pending patents on Multivalent Pneumococcal Vaccine.

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Role of the Funding Source

This study was funded by Astellas Pharma Global Development, Inc. The sponsor participated in the study design, collection, analysis and interpretation of data, writing of the report, and in the decision to submit the article for publication, as detailed below in Author Contributions.

Data Sharing Statement

Researchers may request access to anonymized participant level data, trial level data and protocols from Astellas sponsored clinical trials at www.clinicalstudydatarequest.com.

For the Astellas criteria on data sharing see: https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Astellas.aspx.

Author Contributions

G. Chichili, R. Smulders, V. Santos, B. Cywin, L. Kovanda, C. Van Sant, F. Malinoski, S. Sebastian, G. Siber, R. Malley contributed to the study conception and design, analyzed and interpreted the data, critically revised the manuscript, and approved the final version.

Author Disclosures

G. Chichili, R. Smulders, V. Santos, B. Cywin, L. Kovanda, C. Van Sant are employees of Astellas Pharma Global Development, Inc. F. Malinoski, S. Sebastian, R. Malley are employees of Affinivax, which received funding from Astellas Pharma, Inc. G. Siber is a consultant to Affinivax and both G. Siber and R. Malley are Affinivax board members. R. Malley has issued and pending patents on MAPS vaccines, Rhizavidin-fusion proteins, and Multivalent Pneumococcal Vaccine. S. Sebastian has issued and pending patents on Multivalent Pneumococcal Vaccine.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.05.079.

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