SUPPLEMENTARY APPENDIX

Additional Methods

Ethical Conduct of Study

This study was conducted in accordance with all legal and regulatory requirements and the ethical principles set forth in the Declaration of Helsinki, the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences), and the International Council for Harmonisation Good Clinical Practice guidelines.

Written informed consent was obtained from each participant before performance of any study-specific activity. The final protocol and informed consent were approved by the institutional review boards and/or independent ethics committees at each participating site.

Randomization and Blinding

Participants were randomly allocated to vaccine group by site personnel via an interactive response technology system. Study site personnel, including the principal investigator (PI), were blinded to the investigational product assignments during the study. The first vaccination (20-valent pneumococcal conjugate vaccine [PCV20] or 13-valent pneumococcal conjugate vaccine [PCV13]) was administered in a double-blind fashion, while the second vaccination (≥60 years) was prepared and administered by an unblinded site staff member who did not participate in any study evaluations; all participants and other study personnel, including the PI, were blinded. Sponsor personnel directly involved in evaluating participant data were blinded to vaccine assignment until the database lock for the final analysis of all safety and immunogenicity data. All investigator site staff remained blinded until study completion.

Exclusion Criteria

Exclusion criteria included previous vaccination with any licensed or investigational pneumococcal vaccine or planned receipt through study participation; history of severe adverse reaction associated with a vaccine or severe allergic reaction to any component within PCV20, PCV13, 23-valent pneumococcal polysaccharide vaccine (PPSV23), or any other diphtheria toxoid-containing vaccine; serious chronic disorder or other acute or chronic medical or psychiatric condition that would require participant exclusion based on the judgement of the investigator; history of microbiologically proven pneumococcal invasive disease; known or suspected immunodeficiency or other condition associated with immunosuppression; and receipt or planned receipt of treatment with immunosuppressive therapy (ie, cytotoxic agents or systemic corticosteroids).

Safety Endpoints and Analyses

Prompted local reactions (ie, redness, swelling, and pain at the injection site) and systemic events (ie, fatigue, headache, muscle pain, and joint pain) were recorded in an electronic diary for 10 or 7 days, respectively, after initial vaccination. Reactogenicity events were categorized as either mild, moderate, or severe based on a grading scale of increasing severity. Redness and swelling were assessed in measuring device units with severity graded by size of the reaction. Pain at the injection site and all systemic events were graded based on interference with activity, with a severe event defined as those preventing daily activity. Fever (≥38.0°C) was recorded as a systemic event, with the highest temperature recorded daily by electronic diary. The active collection period for adverse events (AEs) was from the signing of informed consent through 28–42 days after second vaccination (for participants ≥60 years) or 28–42 days after initial vaccination (for participants 18–59 years). Serious AEs (SAEs) and newly diagnosed chronic medical conditions were recorded from informed consent through 6 months after initial vaccination. Immediate events occurring within the first 30 minutes after vaccine administration were assessed and appropriately recorded as AEs or SAEs.

Evaluable Immunogenicity Populations

Evaluable immunogenicity populations were the primary analysis populations used in the analysis of immunogenicity results. Participants were grouped as randomized.

The evaluable 13-matched immunogenicity population was the primary analysis population for immunogenicity results of the 13 matched serotypes in participants 60 years and older. It included participants who received the first study vaccine (PCV13 or PCV20) as randomized, were enrolled in the appropriate age-specific cohort (≥60 years cohort) based on age at first vaccination, had at least 1 valid OPA titer from a blood sample collected from 27–49 days after initial vaccination, and had no other major protocol deviations.

The evaluable 7-additional immunogenicity population was the primary analysis population for immunogenicity results of the 7 additional serotypes in participants 60 years and older. It included participants who received PCV20 if randomized to the PCV20/saline group or PCV13 then PPSV23 if randomized to the PCV13/PPSV23 group, were enrolled in the appropriate age-specific cohort (≥60 years cohort) based on age at first vaccination, had at least 1 valid OPA titer from a blood sample collected either at 27–49 days after initial vaccination (PCV20/saline group) or 27–49 days after second vaccination (PCV13/PPSV23 group), and had no other major protocol deviations.

The evaluable-20 immunogenicity population was the primary analysis population for bridging analysis comparing the immune responses to PCV20 between participants aged 18–49 and 50–59 years to those aged 60–64 years. This population was limited to those participants receiving PCV20 as randomized, were enrolled in the appropriate age-specific cohort, had at least 1 valid OPA titer from a blood sample drawn 27–49 days after PCV20, and had no other major protocol deviations.

Hypothesis Testing

Hypothesis testing was performed to assess the noninferiority of immune response to PCV20 compared with the control vaccine PCV13 for the 13 matched serotypes and to PPSV23 for the additional 7 serotypes in participants aged ≥60 years. The null hypothesis for each serotype-specific OPA titer was as follows:

$$H_0: \ln(\mu_A) - \ln(\mu_B) \leq \ln(0.5)$$

where: ln(0.5) corresponded to a 2-fold margin for noninferiority, $\ln(\mu_A)$ was the natural log of the OPA geometric mean titer (GMT) 1 month after PCV20, and $\ln(\mu_B)$ was the natural log of the OPA GMT 1 month after PCV13 for any of the 13 matched serotypes or after PPSV23 for any of the 7 additional serotypes. Noninferiority was evaluated by a 2-sided 95% confidence interval (CI) for the ratio of the serotype-specific OPA GMTs (PCV20 over PCV13 or PPSV23). Noninferiority was declared if the lower bound of the 2-sided 95% CI for the geometric mean ratio (GMR) of GMT_A to GMT_B was >0.5 (2-fold criterion). The GMR and the 2-sided CI for each serotype were calculated based on a regression model of log-transformed OPA titers including terms of vaccine group, sex, smoking status, age at vaccination in years (continuous), and baseline log-transformed OPA titers.

Noninferiority of immune responses to PCV20 in each of the 2 younger groups of participants (50–59 years or 18–49 years) to response in participants 60–64 years in the ≥60-year cohort were evaluated in a similar way, with $\ln(\mu_C) - \ln(\mu_A) \leq \ln(0.5)$ as the null hypothesis statement for each serotype being the natural log of the OPA GMTs for 1 month after PCV20 in the younger participants (50–59 years or 18–49 years) and in participants 60–64 years in the ≥60-year cohort, respectively. Noninferiority was declared if the lower bound of the 2-sided 95% CI for the GMR of GMT_C to GMT_A was >0.5 (2-fold criterion). The GMR and the 2-sided CI for each serotype were calculated based on a regression model of log-transformed OPA titers including terms of cohort, sex, smoking status, and baseline log-transformed OPA titers.

Table S1. Participant Demographics (Safety Population)

	≥60 Years		50–59 Years[a]		18–49 Years[b]	
Demographic	PCV20/ Saline (N=1507)	PCV13/ PPSV23 (N=1490)	PCV20 (N=334)	PCV13 (N=111)	PCV20 (N=335)	PCV13 (N=112)
Sex, n (%)						
Male	610 (40.5)	611 (41.0)	139 (41.6)	42 (37.8)	121 (36.1)	35 (31.3)
Female	897 (59.5)	879 (59.0)	195 (58.4)	69 (62.2)	214 (63.9)	77 (68.8)
Race, n (%)						
White	1295 (85.9)	1237 (83.0)	278 (83.2)	90 (81.1)	274 (81.8)	101 (90.2)
Black or African American	177 (11.7)	212 (14.2)	35 (10.5)	15 (13.5)	34 (10.1)	7 (6.3)
Asian	19 (1.3)	15 (1.0)	10 (3.0)	2 (1.8)	11 (3.3)	1 (0.9)
American Indian or Alaska Native	6 (0.4)	9 (0.6)	0	3 (2.7)	1 (0.3)	1 (0.9)
Native Hawaiian or other Pacific Islander	1 (0.0)	1 (0.0)	0	0	3 (0.9)	1 (0.9)
Multiracial	7 (0.5)	9 (0.6)	6 (1.8)	1 (0.9)	8 (2.4)	1 (0.9)
Not reported	2 (0.1)	7 (0.5)	5 (1.5)	0	4 (1.2)	0
Ethnicity, n (%)						
Non-Hispanic/ non-Latino	1324 (87.9)	1308 (87.8)	319 (95.5)	101 (91.0)	300 (89.6)	102 (91.1)
Hispanic/Latino	167 (11.1)	169 (11.3)	12 (3.6)	8 (7.2)	24 (7.2)	7 (6.3)
Not reported	16 (1.1)	13 (0.9)	3 (0.9)	2 (1.8)	11 (3.3)	3 (2.7)
Age at vaccination, y						
Mean ± SD	64.6±4.8	64.6±4.8	54.9±2.8	55.0±3.1	34.0±8.8	33.9±8.0
Median (range)	63.0 (60–91)	63.0 (60–89)	55.0 (50–59)	56.0 (48–59)	34.0 (18–60)	32.0 (19–49)
Age group, y, n (%)						
60–64	993 (65.9)	992 (66.6)	–	–	–	–
65–69	319 (21.2)	305 (20.5)	–	–	–	–
70–79	160 (10.6)	159 (10.7)	–	–	–	–
≥80	35 (2.3)	34 (2.3)	–	–	–	–

Abbreviations: PCV13, 13-valent pneumococcal conjugate vaccine; PCV20, 20-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; SD, standard deviation.

[a]One participant (18–49 years) was incorrectly enrolled with participants 50–59 years and was included in that safety population; this participant was not included in any evaluable population.

[b]One participant (≥60 years) was incorrectly enrolled with participants 18–49 years and was included in that safety population; this participant was not included in any evaluable population.

Table S2. Summary of Adverse Events (Safety Population)

	≥60 Years		50–59 Years		18–49 Years	
Time Point AE Type	PCV20/Saline (N=1507[a]/1461[b])	PCV13/PPSV23 (N=1490[a]/1445[b])	PCV20 (N=334[a])	PCV13 (N=111[a])	PCV20 (N=335[a])	PCV13 (N=112[a])
Through 1 mo after PCV20 or PCV13						
Any AE	148 (9.8)	166 (11.1)	34 (10.2)	9 (8.1)	51 (15.2)	13 (11.6)
Immediate	3 (0.2)	3 (0.2)	0	1 (0.9)	0	1 (0.9)
Related	14 (0.9)	23 (1.5)	3 (0.9)	1 (0.9)	4 (1.2)	1 (0.9)
Severe	12 (0.8)	12 (0.8)	2 (0.6)	1 (0.9)	5 (1.5)	2 (1.8)
SAE	8 (0.5)	8 (0.5)	1 (0.3)	1 (0.9)	1 (0.3)	0
NDCMC	6 (0.4)	14 (0.9)	1 (0.3)	1 (0.9)	2 (0.6)	0
Through 1 mo after saline or PPSV23						
Any AE	108 (7.4)	176 (12.2)	–	–	–	–
Immediate	2 (0.1)	8 (0.6)	–	–	–	–
Related	6 (0.4)	93 (6.4)	–	–	–	–
Severe	7 (0.5)	18 (1.2)	–	–	–	–
SAE	10 (0.7)	8 (0.6)	–	–	–	–
NDCMC	7 (0.5)	6 (0.4)	–	–	–	–
Throughout study[c]						
SAE[d]	36 (2.4)	29 (1.9)	1 (0.3)	1 (0.9)	2 (0.6)	1 (0.9)
NDCMC	34 (2.3)	35 (2.3)	5 (1.5)	1 (0.9)	5 (1.5)	2 (1.8)

Abbreviations: AE, adverse event; NDCMC, newly diagnosed chronic medical condition; PCV13, 13-valent pneumococcal conjugate vaccine; PCV20, 20-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; SAE, serious adverse event.

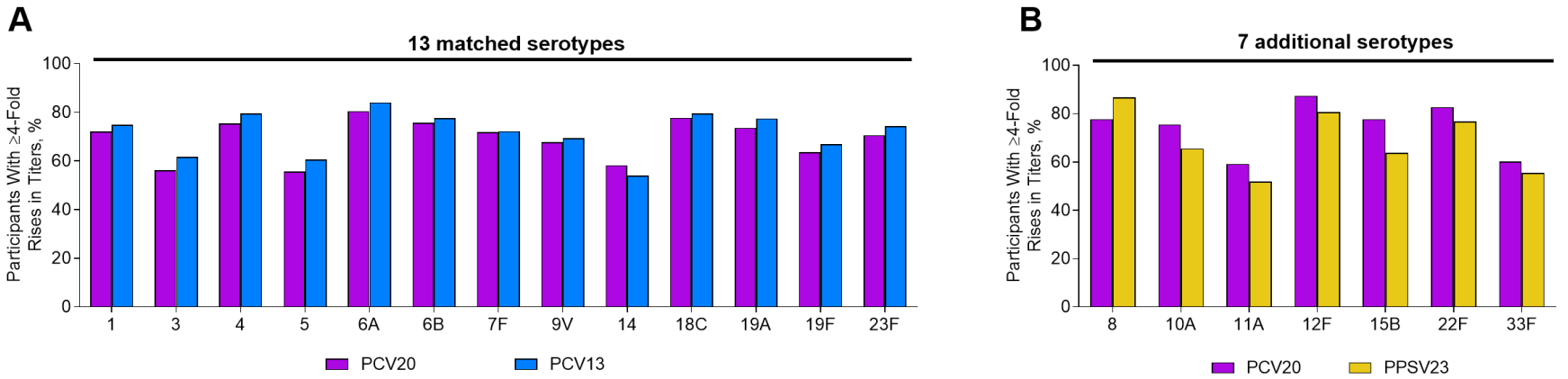
[a]Number of participants included in the safety population; denominator used in the percentage calculations for AEs reported through 1 month after PCV20 or PCV13 or throughout study.

[b]Number of participants included in the safety population; denominator used in the percentage calculations for AEs reported through 1 month after saline or PPSV23.

[c]Through 6 months after initial vaccination.

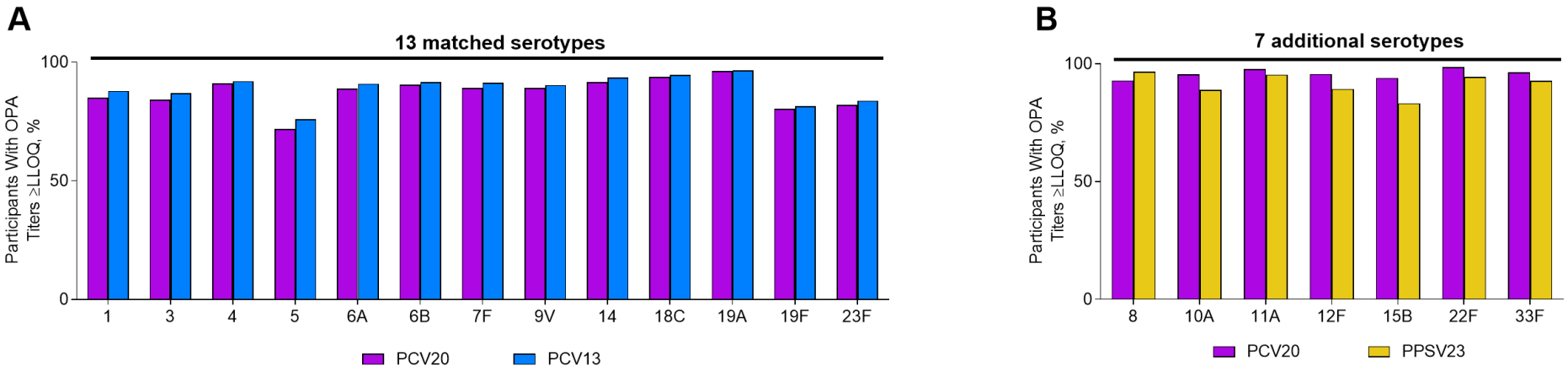
[d]No significant imbalances between reported events in the PCV20 and control groups. The only SAE reported by >2 subjects in the PCV20 group within any of the age groups was coronary artery disease, reported by 3 participants in the PCV20 group of adults ≥60 years old and by 1 participant in the PCV13/PPSV23 group in the same age group. Data on AEs and SAEs reported in this trial are available at: https://clinicaltrials.gov/ct2/show/results/NCT03760146.

Supplementary Figure 1. Percentages of participants ≥60 years with a ≥4-fold rise in OPA titers from before vaccination to 1 month after vaccination. Assay results below the LLOQ were set to 0.5 x LLOQ in the analysis. LLOQ, lower limit of quantitation; OPA, opsonophagocytic activity; PCV13, 13-valent pneumococcal conjugate vaccine; PCV20, 20-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.



Supplementary Figure 2. Percentages of participants ≥60 years with OPA titers ≥LLOQ at 1 month after vaccination.

LLOQs for individual serotypes were as follows: serotype 1, 18; serotype 3, 12; serotype 4, 21; serotype 5, 29; serotype 6A, 37; serotype 6B, 43; serotype 7F, 113; serotype 9V, 141; serotype 14, 35; serotype 18C, 31; serotype 19A, 18; serotype 19F, 48; serotype 23F, 13; serotype 8, 30; serotype 10A, 66; serotype 11A, 96; serotype 12F, 48; serotype 15B, 30; serotype 22F, 18; serotype 33F, 325. LLOQ, lower limit of quantitation; OPA, opsonophagocytic activity; PCV13, 13-valent pneumococcal conjugate vaccine; PCV20, 20-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.



Supplementary Figure 3. Model-based OPA GMRs of (A) participants 50–59 years to 60–64 years and (B) participants 18–49 years to 60–64 years for the 20 vaccine serotypes at 1 month after vaccination. Noninferiority for each serotype was declared if the lower 2-sided 95% CI for the serotype-specific OPA GMR exceeded 0.5. Assay results below the LLOQ were set to 0.5 × LLOQ in the analysis. GMRs (ratio of younger cohort to 60–64 years) and 2-sided CIs were calculated by exponentiating the difference in least-squares means and the corresponding CIs based on analysis of log-transformed OPA titers using a regression model including terms of cohort, sex, smoking status, and baseline log-transformed OPA titers. GMR, geometric mean ratio; LLOQ, lower limit of quantitation; OPA, opsonophagocytic activity.