Statistical Analysis Plan

Study ID: 219659

Official Title of Study: A Phase 1/2, Randomized, Single Ascending Dose Study in Adults (Stage 1) and Randomized, Single Ascending Dose-Finding Study (Stage 2) in Elderly Subjects with ASP3772, a Pneumococcal Vaccine

NCT number: NCT03803202

Other Identifier: 3772-CL-1001

Date of Document: 30-SEP-2020

STATISTICAL ANALYSIS PLAN

Final Version 3.0, dated 23-SEP-2020

A Phase 1/2, Randomized, Single Ascending Dose Study in Adults (Stage 1) and Randomized, Single Ascending Dose-Finding Study (Stage 2) in Elderly Subjects with ASP3772, a Pneumococcal Vaccine

ISN/Protocol 3772-CL-1001
IND number: 18657

Sponsor name
Astellas Pharma Global Development, Inc.
(APGD) 1 Astellas Way
Northbrook, IL 60062

Table of Contents

I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
AE	adverse event
ALT	alanine aminotransferase
anti-HBc	hepatitis B core antibody
anti-HCV	hepatitis C virus antibodies
APGD	Astellas Pharma Global Development Inc
AST	aspartate aminotransferase
CRF	case report form
CRO	contract research organization
DEC	dose escalation committee
DILI	drug-induced liver injury
DLIA	direct Luminex immunoassays
ECG	electrocardiogram
ESV	end of study visit
eCRF	electronic case report form
ePRO	electronic patient reported outcome
FAS	full analysis set
FIH	first-in-human
GCP	good clinical practice
GMC	geometric mean concentration
GMFR	geometric mean fold rise
GMT	geometric mean titer
GSD	geometric standard deviation
HBsAg	hepatitis B surface antigen
HIV	human immunodeficiency virus
IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonization of technical requirements for registration of pharmaceuticals for human use
IgG	immunoglobulin G
IND	investigational new drug
INR	international normalized ratio
ISN	international study number
MAAE	medically attended adverse event
MedDRA	medical dictionary for regulatory activities
NOCD	new onset chronic disease
OPA	opsonophagocytic activity
PCV13	Prevnar 13
PE	Physical exam
CCI [redacted]	

Abbreviations	Description of abbreviations
PIMMC	potential immune-mediated medical condition
PKAS	pharmacokinetic analysis set
PPS	per protocol set
PPSV23	Pneumovax 23
RBC	red blood cell
SAE	serious adverse event
TEAE	treatment emergent adverse event
TBL	total bilirubin
TLFs	table, listing, figures
ULN	upper limit of normal
WBC	white blood cell

Definition of Key Study Terms

Terms	Definition of terms
Baseline	Assessments of subjects as they enter a study before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study.
Enroll	To register or enter a subject into a clinical study. NOTE: Once a subject has received the study drug or placebo, the clinical study protocol applies to the subject.
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test drug or comparative drug (sometimes without randomization) is usually given to a subject and continues until the last assessment after completing administration of the test drug or comparative drug.
Post investigational period	Period of time after the last assessment of the protocol. Follow-up observations for sustained adverse events and/or survival are done in this period.
Randomization	The process of assigning study subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential subjects for enrollment in a study.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a study.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

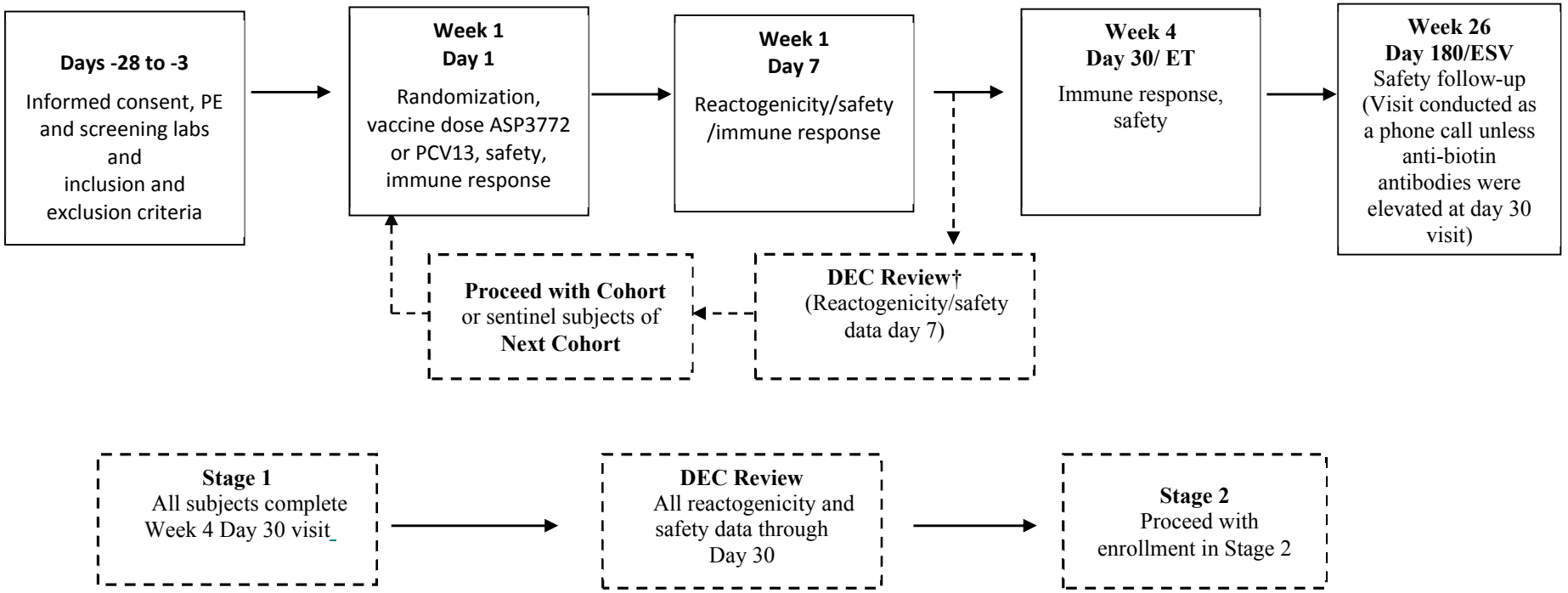
This Statistical Analysis Plan (SAP) contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes detailed procedures for executing the statistical analysis of the primary and secondary endpoints and other data.

Prior to database hard lock, a final review of data and TLFs meeting will be held to allow a review of the clinical trial data and to verify the data that will be used for analysis set classification. If required, consequences for the statistical analysis will be discussed and documented. A meeting to determine analysis set classifications may also be held prior to database hard lock.

2 FLOW CHART AND VISIT SCHEDULE

Flow Chart A

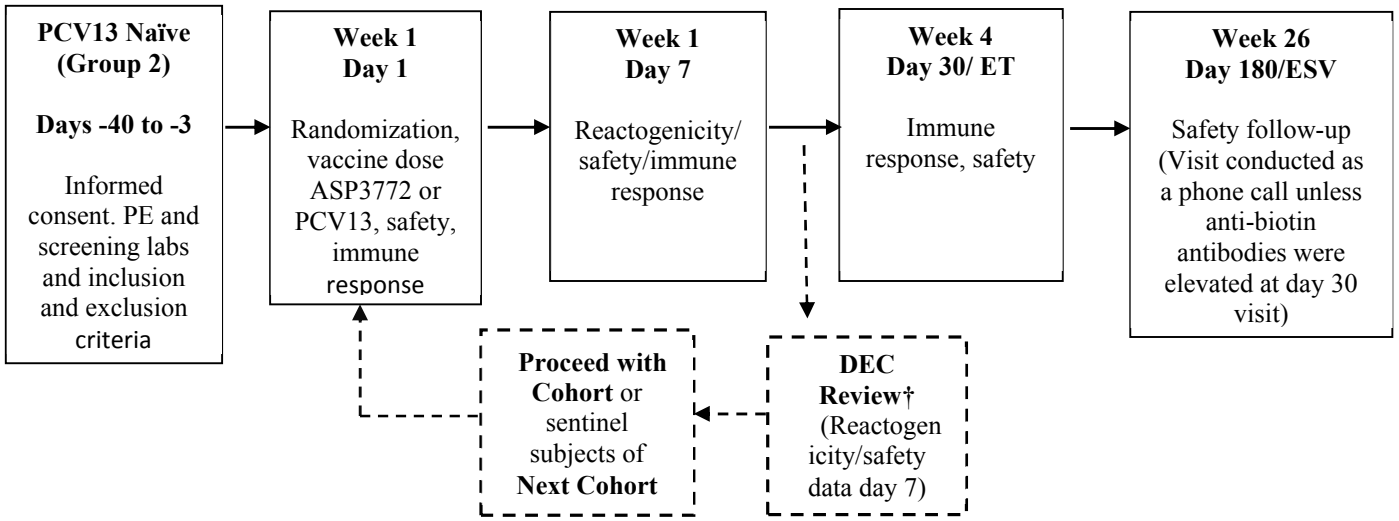
Stage 1



DEC: Dose Escalation Committee; ESV: end-of-study visit; ET: early termination; PCV13: Prevnar 13; PE: physical examination.
† DEC will review day 7 data for sentinel subjects before opening full cohort.

Flow Chart B

Stage 2



DEC: Dose Escalation Committee; ESV: end-of-study visit; ET: early termination; PCV13: Prevnar 13; PE: physical examination; PPSV23: Pneumovax 23

† DEC will review day 7 data for sentinel subjects before opening full cohort

Table 1 Schedule of Assessments - Stage 1: Subjects 18 to 64 Years of Age (Group 1)

Assessments	**Screening[a]**	**Dosing**	**Follow-up**		
Visit Number	**1**	**2**	**3**	**4**	**5**
Visit Days (Window)	**-28 to -3**	**1**	**7 (+1)**	**30 (±2)***	**180 (±14)§**
Informed Consent[b]	X				
Inclusion/Exclusion Criteria	X	X†			
Randomization[c]		X†			
Demographics	X				
Medical History[d]	X	X†			
Physical Examination[e]	X	X†		X	
Routine 12-lead ECG	X	X†	X	X	
Virology[f]	X				
Urine Drug Screen[g]	X	X†			
Pregnancy Test[h]	X	X†		X	
Height, Body Weight	X				
Study Immunization		X			
Vital Signs (blood pressure, pulse rate, body temperature)[i]	X	X†	X	X	
Clinical Laboratory Tests (hematology biochemistry, coagulation profile, hepatic profile, urinalysis)[j]	X	X†‡	X	X	
CCI					
Serum sample for Immunogenicity		X†		X	
Serum sample for Anti-biotin Antibodies		X†		X	X[l]
Whole-blood sample for T-cell Immune Response Measurement		X†	X	X	
CCI					
Prior/Concomitant Medication[n]	←			→	
Reactogenicity[o]		←	→		
AEs[p]	←			→	
SAEs[p]	←				→
MAAEs including PIMMCs and NOCDs[p]		←			→

Footnotes appear on next page

AE: adverse event; ECG: electrocardiogram; MAAE: medically attended adverse event; NOCD: new-onset chronic disease; CCI ; PIMMC: potential immune-mediated medical conditions; SAE: serious adverse event; WBC: white blood cell.

† To be performed prior to administration of study vaccine. **Note:** Vital signs will also be performed after the administration of the study vaccine.

‡ Not required if collected at a screening visit, which is ≤ 7days from dosing visit.

§ Visit conducted as a phone call unless anti-biotin antibodies were elevated at day 30 visit.

* If a subject has received any systemically absorbed antibiotics between day 1 and day 30, the visit should take place at least 7 days after the last dose of the antibiotics.

a. Screening will be completed up to 28 days prior to study immunization. Subjects will be assigned a subject number for use throughout the study at the screening visit via the Interactive Response Technology (IRT) system

b. Informed consent must be obtained prior to the performance of any study-related procedure and before Randomization.

c. Subjects will be randomized via the IRT at the dosing visit.

d. Medical history will be collected at screening in order to assess Inclusion/Exclusion criteria and will be reconfirmed prior to study immunization.

e. A physical examination will be performed at screening visit (days -28 thru -3), prior to study immunization (day 1) and day 30 visits.

f. Perform tests for Hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), hepatitis A virus antibodies (immunoglobulin M), HCV antibody (anti-HCV) confirmed by reflex testing (HCV-RNA) and HIV antigen/antibody (type 1 and/or type 2) at screening.

g. Perform screening test for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates at screening visit. Repeat urine drug screening before study immunization.

h. Serum pregnancy test will be done at the screening visit. Urine pregnancy test will be done at study immunization (day 1) and day 30 visits. Not necessary for female subjects who are not of childbearing potential (e.g., post-menopausal [defined as at least 1 year without any menses]) or documented surgically sterile) at screening and dosing visit.

i. Body temperature, blood pressure and pulse rate will be assessed in a sitting position. At the dosing visit, body temperature, blood pressure, pulse rate and respiratory rate will be assessed predose and approximately 30 min and 1 hour postdose. Predose vital signs should be collected close to the vaccination and should not be more than 30 minutes prior to dose administration. Body temperature will be collected by the subject daily through day 7 following the study immunization.

j. Perform test for hematology (hemoglobin, hematocrit, erythrocytes, leukocytes, differential WBC, platelets), biochemistry (sodium, potassium, calcium, chloride, glucose, total cholesterol, triglycerides, blood urea nitrogen, creatinine, total protein, albumin, c-reactive protein), coagulation profile (prothrombin time, activated partial thromboplastin time, fibrinogen), hepatic profile (alkaline phosphatase, aspartate transaminase, alanine transaminase, γ-glutamyltransferase, total bilirubin, lactate dehydrogenase), urinalysis (using dipstick - protein, glucose, pH, blood, leukocytes, urobilinogen, bilirubin, ketones, nitrite, urine microscopy [optional]).

k. CCI

l. Sample collection to test for serum anti-biotin antibodies should be repeated at the day 180 visit if tested positive on day 30. Biotin levels will be measured in the same sample if the sample is positive for anti-biotin.

m. CCI . Please see Laboratory Manual for more details.

n. All concomitant medications or therapies administered from the time the informed consent form (ICF) is signed through day 30 follow up visit will be collected.

Footnotes continued on next page

o. Subject must remain at site for the first 60 minutes following the injection. Local reactogenicity will be evaluated at 1 hour by site staff and entered into an electronic diary by the subject while on site. The subject will evaluate local and systemic reactogenicity symptoms except for joint pain/arthralgia as per Appendix B daily and record the results in the electronic diary daily through day 7 following study immunization. On day 7, subject will be questioned if he/she experienced any joint pain/arthralgia or other illness which is not part of reactogenicity assessment in the electronic diary.

p. All observed or spontaneously reported AEs, inclusive of MAAEs, PIMMCs and NOCDs will be documented. AEs will be assessed at the study immunization visit, before and approximately 60 minutes after immunization. MAAEs will be reported through day 180. The investigator will avoid leading questions to influence reporting of AEs.

Table 2 Schedule of Assessments - Stage 2: Subjects 65 to 85 Years of Age, PCV13 Naïve (Group 2)

Assessments	**Screening[a]**	**Dosing**	**Follow-up**		
Visit Number	**1**	**2**	**3**	**4**	**5**
Visit Day (Window)	**-40 to -3**	**1**	**7 (+1)**	**30 (±2)***	**180 (±14)§**
Informed Consent[b]	X				
Inclusion/Exclusion Criteria	X	X†			
Randomization[c]		X†			
Demographics	X				
Medical History[d]	X	X†			
Physical Examination[e]	X	X†		X	
Routine 12-lead ECG	X	X†	X	X	
Virology[f]	X				
Height, Body Weight	X				
Study Immunization		X			
Vital Signs (blood pressure, pulse rate, body temperature)[g]	X	X†	X	X	
Clinical Laboratory Tests (hematology, biochemistry, coagulation profile, hepatic profile, urinalysis)[h]	X	X†‡	X	X	
CCI					
Serum sample for Immunogenicity[j]		X†		X	
Serum sample for Anti-biotin Antibodies		X†		X	X[k]
Whole-blood sample for T-cell Immune Response Measurement		X†	X	X	
Prior/Concomitant Medication[l]	←	—	—	→	
Reactogenicity[m]		←	—	→	
AEs[n]	←	—	—	→	
SAEs[n]	←	—	—	—	→
MAAEs including PIMMCs and NOCDs[n]		←	—	—	→

Footnotes appear on next page

AE: adverse event; ECG: electrocardiogram; MAAE: medically attended adverse event; NOCD: new-onset chronic disease; PCV13: Prevnar 13; CCI [redacted]; PIMMC: potential immune-mediated medical conditions; SAE: serious adverse event; WBC: white blood cell.

† To be performed prior to administration of study vaccine. **Note:** Vital signs will also be performed after the administration of the study vaccine.

‡ Not required if collected at a screening visit, which is ≤ 7days from dosing visit.

§ Visit conducted as a phone call unless anti-biotin antibodies were elevated at day 30 visit.

* If a subject has received any systemically absorbed antibiotics between day 1 and day 30, the visit should take place at least 7 days after the last dose of the antibiotics.

a. Screening will be completed up to 40 days prior to study immunization. Subjects will be assigned a subject number for use throughout the study at the screening visit via the Interactive Response Technology (IRT) system.

b. Informed consent must be obtained prior to the performance of any study-related procedure and before randomization.

c. Subjects will be randomized via the IRT system at the dosing visit.

d. Medical history will be collected at screening in order to assess Inclusion/Exclusion criteria and will be reconfirmed prior to study immunization.

e. A physical examination will be performed at the screening visit (days -40 thru -3), prior to study immunization (day 1) and day 30 visits.

f. Perform tests for Hepatitis B surface antigen (HBsAg), hepatitis A virus antibodies (immunoglobulin M), HCV antibody (anti-HCV) confirmed by reflex testing (HCV-RNA) and HIV antigen/antibody (type 1 and/or type 2) at screening.

g. Body temperature, blood pressure and pulse rate will be assessed in a sitting position. At the dosing visit, body temperature, blood pressure, pulse rate and respiratory rate will assessed predose at approximately 30 min and 1 hour postdose. Predose vitals should be collected close to the immunization and should not be more than 30 minutes prior to the dose administration. Body temperature will be collected by the subject daily through day 7 following the study immunization.

h. Perform test for hematology (hemoglobin, hematocrit, erythrocytes, leukocytes, differential WBC, platelets), biochemistry (sodium, potassium, calcium, chloride, glucose, total cholesterol, triglycerides, blood urea nitrogen, creatinine, total protein, albumin, c-reactive protein), coagulation profile (prothrombin time, activated partial thromboplastin time, fibrinogen), hepatic profile (alkaline phosphatase, aspartate transaminase, alanine transaminase, γ-glutamyltransferase, total bilirubin, lactate dehydrogenase), urinalysis (using dipstick - protein, glucose, pH, blood, leukocytes, urobilinogen, bilirubin, ketones, nitrite, urine microscopy [optional]).

i. CCI [redacted]

j. Immunogenicity data will be provided to the investigative site when available to support decision-making for the standard of care.

k. Sample collection to test for serum anti-biotin antibodies should be repeated at the day 180 visit if tested positive on day 30. Biotin levels will be measured in the same sample if the sample is positive for anti-biotin.

l. All concomitant medications or therapies administered from the time the informed consent form (ICF) is signed through day 30 follow-up visit will be collected.

m. Subject must be under direct supervision for the first 60 minutes following the injection. Local reactogenicity will be evaluated at 1 hour by site staff and entered into the electronic diary by the subject while on site. The subject will evaluate local and systemic reactogenicity symptoms except for joint pain/arthralgia as per Appendix B daily and record the results on an electronic diary daily through day 7 following the study immunization. On day 7, subject will be questioned if he/she experienced any other illness, which is not incorporated into the reactogenicity assessment, in the electronic diary.

n. All observed or spontaneously reported AEs, inclusive of MAAEs, PIMMCs and NOCDs will be documented. AEs will be assessed at the study immunization visit, before and approximately 60 minutes after immunization. MAAEs will be reported through day 180. The investigator will avoid leading questions to influence reporting of AEs.

Table 3 Schedule of Assessments - Stage 2: Subjects 65 to 85 Years of Age and Received PCV13 Within a Year of Randomization (Group 3)

Assessments	**Screening[a]**	**Dosing**	**Follow-up**
Visit Number	**1**	**2**	**3**
Visit Day (Window)	**-40 to -3**	**1**	**30 (±2)**
Informed Consent[b]	X		
Inclusion/Exclusion Criteria	X	X†	
Vaccine assignment[c]		X†	
Demographics	X		
Medical History[d]	X	X†	
Physical Examination[e]	X	X†	X
Routine 12-lead ECG	X	X†	X
Virology[f]	X		
Height, Body Weight	X		
Study Immunization		X	
Vital Signs (blood pressure, pulse rate, body temperature)[g]	X	X†	X
Clinical Laboratory Tests (hematology, biochemistry, coagulation profile, hepatic profile, urinalysis)[h]	X	X†‡	X
CCI [redacted]			
Serum sample for Immunogenicity		X†	X
Prior/Concomitant Medication[j]	←	—	→
AEs[k]	←	—	→
SAEs[k]	←	—	→
MAAEs including PIMMCs and NOCDs[k]	←	—	→

AE: adverse event; ECG: electrocardiogram; MAAE: medically attended adverse event; NOCD: new-onset chronic disease; PCV13: Prevnar 13; CCI [redacted]; PIMMC: potential immune-mediated medical conditions; SAE: serious adverse event; WBC: white blood cell.

† To be performed prior to administration of study vaccine. **Note:** Vital signs will also be performed after the administration of the study vaccine.

‡ Not required if collected at a screening visit, which is ≤ 7days from dosing visit.

Footnotes continued on next page

a. Screening will be completed up to 40 days prior to study immunization. Subjects will be assigned a subject number for use throughout the study at the screening visit via the Interactive Response Technology (IRT) system.
b. Informed consent must be obtained prior to the performance of any study-related procedure and before assigning vaccine via the IRT system.
c. Subjects will be assigned a vaccine via the IRT system at the dosing visit.
d. Medical history will be collected at screening in order to assess Inclusion/Exclusion criteria and will be reconfirmed prior to study immunization.
e. A physical examination will be performed at screening visit, prior to study immunization (day 1) and day 30 visits.
f. Perform tests for Hepatitis B surface antigen (HBsAg), hepatitis A virus antibodies (immunoglobulin M), HCV antibody (anti-HCV) and HIV antigen/antibody (type 1 and/or type 2) at screening.
g. Body temperature, blood pressure and pulse rate will be assessed in a sitting position.
h. Perform test for hematology (hemoglobin, hematocrit, erythrocytes, leukocytes, differential WBC, platelets), biochemistry (sodium, potassium, calcium, chloride, glucose, total cholesterol, triglycerides, blood urea nitrogen, creatinine, total protein, albumin, c-reactive protein), coagulation profile (prothrombin time, activated partial thromboplastin time, fibrinogen), hepatic profile (alkaline phosphatase, aspartate transaminase, alanine transaminase, γ-glutamyltransferase, total bilirubin, lactate dehydrogenase), urinalysis (using dipstick - protein, glucose, pH, blood, leukocytes, urobilinogen, bilirubin, ketones, nitrite, urine microscopy [optional]).
i. CCI [redacted]
j. All concomitant medications or therapies administered from the time the informed consent form (ICF) is signed through day 30 follow-up visit will be collected.
k. All observed or spontaneously reported AEs, inclusive of MAAEs, PIMMCs and NOCDs will be documented. AEs will be assessed at the study immunization visit, before immunization and through day 30. The investigator will avoid leading questions to influence reporting of AEs.

3 STUDY OBJECTIVE(S) AND DESIGN

3.1 Study Objective(s)

Stage 1:

Primary Objective: To evaluate the safety and tolerability of 3 different dose levels of ASP3772 in comparison to the active comparator Prevnar 13® (PCV13) in adults 18 to 64 years of age.

Secondary Objective: To evaluate the immunogenicity of 3 different dose levels of ASP3772 in comparison to the active comparator PCV13 in adults 18 to 64 years of age.

Stage 2:

Primary Objective: To evaluate the safety and tolerability of 3 different dose levels of ASP3772 in comparison to the active comparator PCV13 in elderly 65 to 85 years of age.

Secondary Objectives:

To evaluate the immunogenicity of 3 different dose levels of ASP3772 in comparison to the active comparator PCV13 in elderly 65 to 85 years of age.

To evaluate the immunogenicity of 3 different dose levels of ASP3772 relative to the response seen following administration of PPSV23 for the serotypes not included in PCV13.

3.2 Study Design

This is a combined phase 1, first-in-human (FIH), dose-escalation (Stage 1) and phase 2, dose-finding (Stage 2) study to evaluate the safety, tolerability and immunogenicity of ASP3772 in adult subjects and in elderly subjects.

The objectives of this study are to evaluate safety, tolerability including reactogenicity and immunogenicity in adult subjects 18 to 64 years of age in Stage 1 and in elderly subjects

65 to 85 years of age in Stage 2, who are naïve to licensed or investigational pneumococcal vaccine.

The study population will consist of 3 different groups: **Group 1 -** Stage 1 subjects randomized within 3 sequential cohorts to ASP3772 or PCV13; **Group 2 -** Stage 2 PCV13 naïve subjects randomized within 3 sequential cohorts to ASP3772 or PCV13; and **Group 3 -** Stage 2 subjects previously vaccinated with PCV13 will receive PPSV23.

To minimize the risk to subjects, a sentinel group of subjects (n = 2; 1 ASP3772:1 PCV13) will be vaccinated in each cohort prior to initiating enrollment of the full cohort. Safety data through day 7 from sentinel subjects will be evaluated by the Dose Escalation Committee (DEC) prior to enrolling the remainder of the cohort. Safety data through day 7 from all subjects within a dose cohort will be evaluated by the DEC prior to escalating to the next dose cohort within the adult and within elderly subjects. Based on the safety data through 30 days after immunization from the first 3 cohorts, the DEC will determine if Stage 2 can

commence, and if sentinel dosing is required for Stage 2. A DEC charter will detail the roles, responsibilities and procedures for decision-making.

Stage 1, Group 1: Stage 1 of the phase 1/2 design is a randomized, active-controlled, observer-blinded, dose- escalation study of the safety, tolerability and immunogenicity of the ASP3772 pneumococcal vaccine in adult subjects (18 to 64 years of age) compared with PCV13 with a 3:1 randomization (ASP3772:PCV13). The syringe used to administer PCV13 is distinct. The vaccine administrator will differ from the person evaluating reactogenicity to remove reporting bias for any reactogenicity events. Additionally, treatment assignment will be unknown to the full study team.

Subjects will be enrolled sequentially into 1 of 3 cohorts designated by escalating ASP3772 dose levels. A single dose of ASP3772 will be administered as a 0.5 mL intramuscular (IM) injection on day 1 at 1 of 3 dose levels: 1, 2 or 5 μg of ASP3772. The subjects randomized to PCV13 will receive a single injection of the standard dose of PCV13 on day 1.

Approximately 120 subjects will be enrolled in Stage 1 of the study, 40 subjects per cohort (ASP3772 n = 30 and PCV13 n = 10).

After all subjects complete dosing in Cohort 1 (low dose, 1 μg of ASP3772 or PCV13), safety (including reactogenicity) and tolerability through 7 days postimmunization will be assessed by the DEC in a blinded manner. Assessments of safety and tolerability by the DEC will occur prior to initiating the next dose group, Cohort 2 (2 μg of ASP3772 or PCV13) and then Cohort 3 (5 μg of ASP3772 or PCV13), based on safety and tolerability data through 7 days postimmunization. The DEC Charter will provide details for revealing the treatment assignment for individual subjects to DEC members, if necessary.

Safety and tolerability assessments will continue through 30 days postimmunization. Safety assessments for serious adverse events (SAEs) and medically attended adverse events (MAAEs) including potential immune-mediated medical conditions (PIMMCs) and new-onset chronic disease (NOCD) will continue through day 180 postimmunization. Data will be collected in an observer-blinded manner (vaccine recipients and those responsible for evaluation of any study endpoint will be unaware of which vaccine the subject received).

Serum samples to measure pneumococcal serotype-specific anticapsular polysaccharide (PS) immunoglobulin G (IgG) and functional opsonophagocytic activity (OPA) will be collected prior to the first study vaccination on day 1 and on day 30 postimmunization.

The design schematic for Stage 1 is shown in the table below. There will be a total of approximately 120 evaluable adults 18 to 64 years of age.

Number of Subjects per Cohort by Immunization

Cohort	Stage 1 (Group 1)			
	ASP3772	Number of Subjects	Comparator	Number of Subjects
1	1 μg	30	PCV13	10
2	2 μg	30		10
3	5 μg	30		10

PCV13: Prevnar 13

Stage 2, Group 2:

Stage 2 of the study is a dose-finding (sequentially higher doses), active-controlled, observer-blinded study with a 3:1 randomization (ASP3772:PCV13) in elderly subjects 65 to 85 years of age (Group 2). For Stage 2, Group 2 subjects the vaccine administrator will differ from the person evaluating reactogenicity to remove reporting bias for these events. Treatment assignment will also be unknown to the blinded study team.

After screening, PCV13 naïve subjects will be randomized within each cohort to receive ASP3772 or PCV13. Subjects randomized to ASP3772 will receive a single dose of ASP3772 administered as a 0.5 mL intramuscular (IM) injection on day 1, at 1 of 3 dose levels: 1, 2 or 5 µg of ASP3772 for Cohorts 4, 5 and 6, respectively. The subjects randomized to PCV13 will receive a single injection of the standard dose of PCV13.

Stage 2 will commence after the DEC has evaluated safety (including reactogenicity) data through 30 days from all cohorts in Stage 1. Dose escalation to Cohort 5 (intermediate dose, 2 µg) in Stage 2 will commence once the DEC has evaluated Stage 2 Cohort 4 (1 µg) safety (including reactogenicity) and tolerability through 7 days postimmunization. Dose escalation into Cohort 6 (high dose, 5 µg) in Stage 2 will commence once the DEC has evaluated Stage 2 Cohort 5. The DEC will review the data for each cohort in Stage 2, Group 2 in a blinded manner. The DEC Charter will provide details for revealing the treatment assignment for individual subjects to DEC members, if necessary. Randomization for Stage 2 subjects will be stratified by age (65 to 74 years of age and 75 to 85 years of age) to ensure there is a balanced number of subjects within each age cohort immunized with either ASP3772 or PCV13.

Safety and tolerability assessments including adverse events (AEs) will continue through 30 days postimmunization. Safety assessments for SAEs and MAAEs including PIMMCs and NOCDs will continue through day 180 postimmunization. Data will be collected in an observer-blinded manner (vaccine recipients and the site staff who are responsible for evaluation of any study endpoint will be unaware of which vaccine the subject received).

Serum samples to measure IgG and OPA will be collected prior to study vaccination on day 1 and postimmunization on day 30. The primary immunogenicity evaluation for dose finding is 30 days following the initial immunization.

The design schematic for Stage 2, Group 2 is shown in the table below. There will be a total of approximately 396 evaluable adult subjects from 65 to 85 years of age.

Number of Subjects per Cohort by Treatment

Cohort	Stage 2 (Group 2)			
	ASP3772	**Number of Subjects**	**Comparator**	**Number of Subjects**
4	1 µg	99	PCV13	33
5	2 µg	99		33
6	5 µg	99		33

PCV13: Prevnar 13

Stage 2, Group 3:

Elderly subjects previously immunized with PCV13 will be enrolled and receive the recommended booster immunization with PPSV23 only (n = 99). Prior immunization

(PCV13) should have taken place no less than 10 months and no more than 2 years prior to study drug vaccination. Immunogenicity to the 11 non-PCV13 serotypes contained in PPSV23 will be evaluated. Subjects who are given PPSV23 without previous exposure to PCV13 will not be included in the immunogenicity summaries.

Safety assessments will continue through 30 days postimmunization.

Serum samples to measure IgG and OPA levels will be collected prior to study immunization on day 1 and postimmunization on day 30.

The design schematic for Stage 2, Group 3 is shown in the table below. There will be a total of approximately 99 evaluable adult subjects from 65 to 85 years of age.

Study Treatment Group	Stage 2 (Group 3) Number of Subjects
PPSV23	99

PPSV23: Pneumovax 23

3.3 Randomization

A central randomization will be used for this study. Stage 1 subjects will be randomized to receive either ASP3772 (1, 2 or 5 μg) or PCV13 with a 3:1 ratio.

Stage 2, Group 2 subjects will be randomized to receive either ASP3772 (1, 2 or 5 μg) or PCV13 with a 3:1 ratio. The randomization for Group 2 will be stratified by age (65 to 74 years of age and 75 to 85 years of age).

The randomization assignment for Stage 1 and Stage 2, Group 2 will be unavailable to anyone involved with the study with the exception of the pharmacist and vaccine administrator until all Day 30 visits have been completed. After all subjects in Stage 1 have completed Day 30, there will be a data cutoff and an interim analysis. Similarly, once all Day 30 visits for subjects in Stage 2, Group 2 have completed, there will be a data cutoff and interim analysis. Additional safety information will be collected at the Day 180 visit.

Stage 2, Group 3 subjects will have been previously given PCV13 before entering the study and will be enrolled to receive a booster vaccination with PPSV23 only on Day 1.

4 SAMPLE SIZE

In Stage 1, approximately 120 evaluable adults 18 to 64 years of age are planned to be enrolled. This sample size is considered sufficient to obtain preliminary estimates in a phase 1 study of safety and reactogenicity in the adult population, ages 18 to 64. In Stage 2, approximately 396 evaluable adult subjects from 65 to 85 years of age are planned to be enrolled to receive either ASP3772 or PCV13. For each PCV13 serotype the OPA titer^{-1} responses, Geometric Mean Titer (GMT) and Geometric Standard Deviation (GSD) in subjects 60 to 64 years of age administered PCV13 (Source Prevnar13® label) were assumed

to be representative of the response following immunization with ASP3772. The width of 95% confidence intervals for each serotype with 30, 100 and 150 subjects were calculated. A sample size of approximately 100 subjects at each dose level treated with ASP3772 under these assumptions was considered sufficient to provide good estimates of OPA titer^{-1} responses. The sample size and dose level for the phase 3 study will be determined based on the responses in stage 2 elderly subjects. In Stage 2, Group 3 approximately 99 subjects are planned to be enrolled to receive PPSV23 to provide estimates of the response to non-PCV13 serotypes in an elderly population.

5 ANALYSIS SETS

In accordance with International Conference on Harmonization (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

5.1 Full Analysis Set (FAS)

The Full Analysis Set (FAS) will consist of all subjects who receive an immunization in this study with either ASP3772, PCV13 or PPSV23 and have at least 1 post immunization measurement.

The FAS will be used for summaries and primary analyses of immunogenicity data.

5.2 Per Protocol Analysis Set (PPS)

The PPS will be used to assess immunologic response and will serve as a supportive analysis. The PPS will exclude the following subjects:

- Subjects who are given antibiotics within 7 days prior to the 30-day blood draw to assess immunogenicity in the ASP3772 immunization group since these may interfere with the immunogenicity assay
- Subjects who have their 30-day blood draw 14 or more days after the 30 day post immunization scheduled blood collection
- Subjects who receive immunosuppressants as this may interfere with their ability to mount a response to the vaccine
- Subjects who have newly diagnosed immunologic abnormalities within the first two weeks after receiving the immunization.

5.3 Safety Analysis Set (SAF)

The Safety Analysis Set (SAF) consists of all randomized subjects who received a vaccination in this study with either ASP3772, PCV13, or PPSV23.

The SAF will be used for most summaries of demographic and baseline characteristics and all safety and tolerability related variables.

5.4 Pharmacokinetic Analysis Sets (PKAS)

Not applicable for this protocol.

5.5 Pharmacodynamic Analysis Set (PDAS)

Not applicable for this protocol.

6 ANALYSIS VARIABLES

In this Phase 1/2 study, the primary endpoints are safety-related. All immunogenicity endpoints are secondary endpoints.

6.1 Efficacy Endpoints

6.1.1 Serotypes

The 13 serotypes contained in PCV13 are: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F. The 23 serotypes contained in PPSV23 are: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20A, 22F, 23F, and 33F. ASP3772 is a 24-valent MAPS vaccine and contains the following serotypes: 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F. The tables below illustrate the commonalities.

	Serotypes Common to PCV13												
PCV13	1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F
PPSV23	1	3	4	5		6B	7F	9V	14	18C	19A	19F	23F
ASP3772	1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F

	Serotypes Not Contained in PCV13										
PCV13											
PPSV23	2	8	9N	10A	11A	12F	15B	17F	20A	22F	33F
ASP3772	2	8	9N	10A	11A	12F	15B	17F	20B	22F	33F

Serotype 20A is unique to PPSV23 and serotype 20B is unique to ASP3772. In this study there is only an assay for 20B. Serotype 20A has shown some cross reactivity with 20B.

6.1.2 Secondary Endpoint(s)

Stage 1, Group 1:

Surrogate markers used to assess the immunological response on day 30 for the 13 serotypes common to ASP3772 and PCV13 are:

- Functional OPA activity for each serotype characterized by an OPA titer^{-1} response expressed as the reciprocal of the serum dilution that causes a 50% reduction in the colony-forming units
- Pneumococcal serotype-specific anticapsular polysaccharide PS IgG for each serotype.

Summaries of immune response 30 days after immunization in adults 18 to 64 years of age will be provided for each serotype by treatment group (ASP3772 (1 µg, 2 µg, 5 µg) or PCV13) and stage. All adult subjects treated with PCV13 will be combined into a single group. To assess similarity between the 3 cohorts of PCV13 subjects, summaries will be provided by cohort for the PS IgG levels and the OPA titers.

Endpoints used to assess the immunological response on day 30 for the 11 serotypes unique to ASP3772 (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F, 33F) are:

- Geometric mean fold rise (GMFR) in anticapsular PS IgG at 30 days relative to pre-immunization
- Proportion of subjects with a ≥ 4-fold increase in anticapsular PS IgG
- Functional OPA activity for each serotype characterized by an OPA titer^{-1} response

Stage 2; Group 2:

Endpoints used to assess the immunological response on day 30 are:

- Functional OPA activity for each serotype characterized by an OPA titer^{-1} response
- Pneumococcal serotype-specific anticapsular PS IgG for each serotype for the 13 serotypes contained in PCV13
- For each of the 11 serotypes unique to ASP3772, the GMFR in anticapsular PS IgG at 30 days relative to pre-immunization
- For each of the 11 serotypes unique to ASP3772, the proportion of subjects with a ≥4-fold increase relative to baseline in anticapsular PS IgG for ASP3772 and PCV13-treated subjects

For Stage 2, Group 2 summaries will be provided by treatment group (ASP3772 (1 µg, 2 µg, 5 µg) or PCV13) for all subjects age 65-85, for subjects 65-74 and for those 75-85. Subjects treated with PCV13 in cohorts 4, 5 and 6 will be combined into a single dose group and summarized by age (65-85, 65-74 and 75-85). To assess similarity between the 3 cohorts of PCV13 subjects, summaries will be provided by cohort for the PS IgG levels and the OPA titers.

In addition, for ASP3772, the following endpoints will be assessed.

- Dose response in the OPA titer^{-1} for ASP3772
- Dose response in the PS IgG antibodies for ASP3772

Stage 2; Group 3:

For the 11 serotypes contained in PPSV23 and not in PCV13, immunological response will be evaluated by the following endpoints at 30 days after administration of PPSV23:

- Functional OPA activity characterized by an OPA titer^{-1} response
- Pneumococcal serotype-specific anticapsular PS IgG

6.1.3 Exploratory Efficacy Endpoints

CCI

6.1.4 Other Efficacy Variables: Non-Vaccine-Type (NVT) Pneumococcal Immunogenicity

In both Stage 1 Group 1 and Stage 2 Group 2, blood draws to determine SP1500+0785 levels (ng/mL) will be collected for subjects given either ASP3772 or PCV13. Levels of TH17 will be determined using an ELISpot assay and IL17 will be measured using a standard MSD ELISA. Levels of the following cytokines will also be assayed: IL-17, IL-22, CCI

6.2 Safety Endpoints

6.2.1 Adverse Events, Laboratory Values, Vital Signs, ECGs and Reactogenicity

For this study the primary endpoints address safety and tolerability of ASP3772. Safety and tolerability of ASP3772 will be assessed by:

- Treatment-emergent adverse events (TEAEs) including MAAEs, PIMMCs and NOCDs
- Laboratory assessments

- Vital signs (body temperature, blood pressure and pulse rate)
- Physical examination
- ECG
- Reactogenicity

6.2.1.1 Adverse Events

AE collection begins after the ICF has been signed and continues until screen failure, or for those enrolled, 30 days after study vaccine administration. A TEAE is defined as an AE observed after study immunization and up to 30 days post immunization. Medically attended adverse events (MAAEs) are AEs for which the subject has received medical attention by medical personnel, or in an emergency room, or which led to hospitalization. A new-onset chronic disease (NOCD), is defined as a MAAE which a) was absent at baseline; b) has not resolved at the follow-up telephone call; c) requires continuous medical care or attention.

PIMMCs are defined as any AEs of autoimmune or auto inflammatory nature. SAEs, MAAEs, which include PIMMCs and NOCDs, will be collected throughout the study, from vaccine administration on Day 1 up to the last follow-up visit (day 30 for Group 3; day 180 for Groups 1 and 2).

6.2.1.2 Laboratory Values

Laboratory safety assessments will be collected at the screening visit and at study immunization if screening was performed > 7 days from study immunization [day 1]. Assessments will be repeated at follow-up visits on day 7 and day 30. These include the following:

- Hematology (hemoglobin, hematocrit, erythrocytes, leukocytes, differential WBC, platelets),
- Biochemistry (sodium, potassium, calcium, chloride, glucose, total cholesterol, triglycerides, blood urea nitrogen, creatinine, creatine kinase, uric acid, total protein, albumin, c-reactive protein),
- Coagulation profile (prothrombin time, activated partial thromboplastin time, fibrinogen)
- Hepatic profile (alkaline phosphatase, aspartate transaminase, alanine transaminase, γ-glutamyltransferase, total bilirubin, lactate dehydrogenase),
- Urinalysis (using dipstick - protein, glucose, pH, blood, leukocytes, urobilinogen, bilirubin, ketones, nitrite)
- Urine microscopy [optional] (casts, crystals, epithelial cells, leucocytes, erythrocytes, bacteria).
- Pregnancy Testing (Human Chorionic Gonadotropin (hCG))

Laboratory assessments assayed at baseline to determine eligibility are:

- Drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids and cocaine)
- Virology (Anti-HAV (IgM), HBsAG, Anti-HBc, Anti-HCV, Anti-HIV-1 and 2

Laboratory values collected on Days 7 and 30 will be categorized programmatically using the definitions in Appendix D: Grading Laboratory Abnormalities for Healthy Adult Subjects.

These post vaccination laboratory values are categorized into 4 grads of reactogenicity.

6.2.1.3 Vital Signs

Vital sign measurements will be taken for one hour post immunization to assess systemic reactions to the immunization. Each of these will be graded and reported according to the definitions contained in the table below.

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Fever (°C)‡ (°F)‡	38.0-38.4 100.4-101.1	38.5-38.9 101.2-102.0	39.0-40 102.1-104	>40 >104
Tachycardia (bpm)	101-115	116-130	>130	ER Visit or hospitalization for arrhythmia
Bradycardia (bpm)	50-54	45-49	<45	ER Visit or hospitalization for arrhythmia
Hypertension (systolic) (mm Hg)	141-150	151-155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) (mm Hg)	91-95	96-100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) (mm Hg)	85-89	80-84	<10	ER visit or hospitalization for hypotensive shock
Respiratory Rate (breaths per min)	17-20	21-25	>25	Intubation

bpm: beats per minute; ER: emergency room

† Subject should be at rest for all vital sign measurements.

‡ Oral temperature; no recent hot or cold beverages or smoking.

§ When resting heart rate is between 60 and 100 bpm. Use clinical judgment when characterizing bradycardia among some healthy subject populations, e.g., conditioned athletes.

6.2.1.4 Physical Examinations

Physical examinations will be performed prior to dosing and at Day 30 post immunization.

6.2.1.5 ECGs

ECGs will be collected at screening for all groups at Screening for all groups and at Day 7 for Groups 1 and 2, those randomized to PCV13 or ASP3772. ECGs will be graded as ‘normal’, ‘abnormal-not clinically significant’, and ‘abnormal-clinically significant’. Any abnormal ECGs that have worsened since screening/baseline will be recorded as an adverse event.

6.2.1.6 Reactogenicity

Reactogenicity will be assessed using daily solicited adverse reactions and the daily maximum value will be recorded in an electronic diary for up to 7 days post immunization. These include local and systemic reactions with a predefined grading scale (see Section 10.2,

Appendix B: **Local Reactogenicity Grading** and Section 10.3, Appendix C: **Grading Signs of Systemic Reactogenicity**; and Section 10.4, Appendix D: **Grading of Laboratory Abnormalities**). Local reactions are pain, tenderness, redness/erythema, swelling and induration. Systemic reactions are nausea/vomiting, diarrhea, headache, fever, fatigue and muscle discomfort or pain/myalgia. Subjects in stage 1 will be asked to report joint pain/arthralgia through day 7 postimmunization during the day 7 visit. This addition was requested by the FDA after the electronic diary was being used at the sites. The ePRO device will be revised for Stage 2 so that subjects will be asked to report symptoms of arthralgia daily for 7 days post immunization.

Reactogenicity reactions will be reported as Clinical Events. If the reactogenicity reaction extends beyond 7 days or if serious, it will also be recorded as an AE.

6.3 Pharmacokinetic Variables

Not applicable.

6.4 Pharmacodynamic Variables

Not applicable.

6.5 Other Variables

Samples for anti-biotin antibodies will be collected before immunization, at Day 30 and if positive at Day 30, at Day 180 for subjects in Stage 1, Group 1 and Stage 2, Group 2. Biotin levels will be measured in the same sample if the sample is positive for anti-biotin.

7 STATISTICAL METHODOLOGY

7.1 General Considerations

For continuous variables, descriptive statistics will include the number of subjects (n), mean, standard deviation, median, minimum, maximum, when applicable, or the geometric mean (GM) and geometric standard deviation (GSD). The geometric mean ratio (GMR) and 95% confidence interval (CI) will be used to compare the response following each dose of ASP3772 (1, 2, or 5 μg) to PCV13 within each stage. Calculations for the GM, the GMR and the geometric mean fold rise (GMFR) with the associated GSD and 95% CI can be found in Section 10.1, Appendix A: **Instructions to calculate the Summary statistics for the secondary endpoints**. Frequencies and percentages will be displayed for categorical data by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage. Percentages by category will be based on the number of subjects without missing data. Subjects with missing data will be treated as a separate category and not included in the denominator, unless specific

methods for imputation are stated.

Summaries based on FAS and PPS (if needed) (e.g. disposition, baseline and efficacy data) will be presented by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage. All subjects treated with PCV13 will be combined according to their age group (Stage 1: Adults,

Stage 2: Elderly 65-85, 65-74 and 75-85). Data for all PPSV23 enrolled subjects will be summarized separately.

For serotypes common to PCV13 and ASP3772, the following treatment group comparisons for immunogenicity are of interest to determine the magnitude of the response following ASP3772 relative to PCV13 for each serotype within adults 18-64, within elderly 65-85 and within elderly subgroups 65-74 and 75-85.

- ASP3772 1 µg vs PCV13
- ASP3772 2 µg vs PCV13
- ASP3772 5 µg vs PCV13

For all serotypes unique to ASP3772, the comparison will be post-dose relative to pre-dose.

Safety analyses and other summaries based on the SAF will be presented by treatment group (ASP3772 (1 µg, 2 µg, 5 µg) or PCV13) and stage (adults 18-64 years, elderly 65-85 years). Within the elderly cohort summaries will also be provided where indicated for those 65-74 and those 75-85. All adult PCV13 subjects will be combined into a single group and all elderly PCV13 subjects will be combined into a single group consistent with the age group being analyzed. Summaries for all PPSV23 enrolled subjects will be provided separately.

All statistical comparisons will be made using two sided tests at the α=0.05 significance level unless specifically stated otherwise and 95% confidence intervals will be provided. All null hypotheses will be of no treatment difference; all alternative hypotheses will be two-sided, unless specifically stated otherwise.

All data processing, summarization, and analyses will be performed using SAS®Version 9.1.3 or higher on Unix. Specifications for table, figures, and data listing formats can be found in the TLF specifications for this study.

7.2 Study Population

7.2.1 Disposition of Subjects

The following subject data will be presented:

- Disposition: number of subjects with informed consent, discontinued before randomization, randomized and immunized;
- Analysis sets: number and percentage of subjects in the safety analysis set (randomized and received an immunization, full analysis set (randomized, immunized, and at least
- 1 post baseline endpoint evaluation) and per protocol analysis set (if needed) (subjects who did not receive antibiotics within 7 days of their Day 30 blood draw; subjects who have their Day 30 blood draw between Day 30 and Day 44; subjects who have not received immunosuppressants; subjects who do not have newly diagnosed immunologic abnormalities within the first 2 weeks after receiving the immunization);
- Treatment discontinuation: number and percentage of randomized subjects who completed and who discontinued treatment, by primary reason for treatment discontinuation (adverse event, death, lost to follow-up, protocol deviation, withdrawal by subject, other);

- Discontinuation up to 30 days: number and percentage of randomized subjects who completed and who discontinued the 30-day investigational period, by primary reason for discontinuation (adverse event, death, lost to follow-up, protocol deviation, withdrawal by subject, other);
- Discontinuation after Day 30 up to Day 180: Number and percentage of randomized subjects who completed and who discontinued during the follow-up period (post Day 30 through Day 180), by primary reason for discontinuing during the follow-up period (adverse event, death, lost to follow-up, protocol deviation, withdrawal by subject, other); subjects in Group 3 will not be followed beyond Day 30.
- Per protocol exclusions: Number and percentage of subjects excluded from PPS by reason for exclusion defined in Section 5.2, by treatment group.

7.2.2 Protocol Deviations

The number and percentage of subjects who met the following protocol deviation criteria will be summarized for each criterion and by study site. Subjects deviating from a criterion more than once will be counted once for the corresponding criterion. Any subjects who have more than one protocol deviation will be counted once in the overall summary. A data listing will be provided by site and subject.

The protocol deviation criteria will be uniquely identified in the summary table and listing. The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

7.2.3 Demographic and Other Baseline Characteristics

Demographic and other baseline characteristics will be summarized using descriptive statistics.

Descriptive statistics for age, height, weight, and body mass index (BMI) at study entry will be presented. Frequency tabulations for sex, ethnicity and race will be shown. These summaries will be displayed for the SAF.

Medical history is coded in MedDRA and will be summarized by System Organ Class (SOC) and Preferred Term (PT) as well as by PT alone, for the SAF.

7.2.4 Concomitant Medications

Concomitant medications will be summarized by therapeutic subgroup (ATC 2nd level), chemical subgroup (ATC 4th level) and preferred WHO name. Subjects taking the same medication multiple times will be counted once per medication. A medication is classified into a single ATC based on the indication. Previous and concomitant medications will be listed.

7.3 Study Drug Exposure and Compliance

7.3.1 Exposure

The number of subjects immunized and whether or not the full dose was administered will be summarized by treatment group (ASP3772 (1 µg, 2 µg, 5 µg) or PCV13) and stage. Since this is a single dose vaccine study, duration of exposure does not apply.

7.3.2 Treatment Compliance

Since this is a single dose vaccine trial, compliance does not apply.

7.4 Analysis of Immunogenicity

7.4.1 Analysis of Immunogenicity Endpoints

Assessment of immunological response following ASP3772 administration relative to PCV13 is a secondary objective for this study.

Stage 1, Group 1 and Stage 2, Group 2:

The objective in Stage 1, Group 1 and Stage 2, Group 2 is to determine if ASP3772 stimulates an immunogenic response to the 13 pneumococcal serotypes that are also contained in PCV13 and to determine if ASP3772 stimulates an immunogenic response to an additional 11 serotypes contained in ASP3772. The following summaries will be given by treatment group (ASP3772: 1, 2, 5 µg vs. PCV13) and stage for all serotypes.

OPA Titer

- GMT, GSD and 95% CI
- The ratio of the OPA GMT (each ASP3772 dose level/PCV13), the GSD and 95% CI for the ratio
- The GMFR relative to baseline, GSD and 95% CI
- The proportion of subjects with a > 4-fold increase in OPA titer^{-1}

IgG Concentration

- The GMC, GSD and 95% CI
- The ratio of the IgG GMC (each ASP3772 dose level/PCV13), the GSD and 95% CI
- The GMFR relative to baseline, GSD and 95% CI
- The proportion of subjects with a >= 4-fold increase in anticapsular PS IgG

Values below the LLOQ will be set to ½ LLOQ for calculation of geometric means and set to LLOQ for calculation of fold changes.

Summaries for Stage 2 subjects will be given for 65-85, 65-74 and 75-85-year olds.

An analysis of covariance (ANCOVA) model with age (65 to 74 years, 75 to 85 years) as the covariate and treatment (ASP3772 1, 2, 5 µg) as the factor of interest will be conducted on the natural logarithm (log) of pneumococcal OPA titers and on the natural log of the IgG concentrations for each serotype to examine dose response. Provided the treatment effect is

significant, paired comparisons between the 5 µg vs 1 µg, followed by 5 µg vs 2 µg, followed by 2 µg vs 1 µg will be conducted. There are no adjustments to control for the Family-Wise Error Rate (FWER) for 3 comparisons for each of the 24 serotypes. A Cochran-Armitage trend test will be used to test for trend.

CCI [redacted]

Stage 2; Group 3:

The objective for Stage 2, Group 3 is to obtain estimates of the immune response following PPSV23 for the 11 serotypes contained in PPSV23 and not in PCV13. Immunological response 30 days after administration of PPSV23 will be summarized as follows:

- The GMT, GSD and 95% CI for the OPA titers
- The GMC, GSD and 95% CI for serotype-specific IgG levels.
- The GMFR, GSD and 95% CI in anticapsular polysaccharide IgG and OPA titer
- The number and percentage of subjects with a ≥ 4-fold increase in anticapsular PS IgG and OPA relative to baseline.

7.4.2 Analysis of Non-Vaccine-Type (NVT) Pneumococcal Immunogenicity

In both Stage 1 Group 1 and Stage 2 Group 2, blood draws to determine SP1500+0785 levels (ng/mL) will be collected for subjects given either ASP3772 or PCV13. Levels of TH17 will be determined using an ELISpot assay and IL17 will be measured using a standard MSD ELISA. Levels of the following cytokines will also be assayed: IL-17, IL-22, CCI [redacted]. Summaries of the levels at baseline, Day 7 and Day 30 will be provided. The fold increase relative to baseline at Days 7 and 30 will also be summarized. Paired comparisons between each of the ASP3772 dose levels and PCV13 will be completed using a Wilcoxon Test. These comparisons will be made for subjects in Stage 1, Group 1 and separately for subjects in Stage 2, Group 2.

7.4.3 Dose Response

CCI [redacted]

CCI

[redacted]

7.4.4 Analysis of Biotin and Anti-Biotin

Samples for anti-biotin antibodies will be collected before immunization, at Day 30 and if positive at Day 30, at Day 180 for subjects in Stage 1, Group 1 and Stage 2, Group 2. Biotin levels will be measured in the same sample if the sample is positive for anti-biotin.

Paired comparisons between each of the ASP3772 dose levels and PCV13 will be completed using a Wilcoxon Test. These comparisons will be made for subjects in Stage 1, Group 1 followed by subjects in Stage 2, Group 2.

7.5 Analysis of Safety

All analysis of safety will be presented by treatment group (ASP3772 (1 µg, 2 µg, 5 µg) or PCV13) and stage for SAF. All adult subjects who receive PCV13 will be combined into a single group and all elderly subjects who receive PCV13 will be combined into a single group. Safety data for subjects given PPSV23 will not be compared with any other treatment group since these subjects were not a randomized treatment group.

7.5.1 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). An overview table will include the following:

- Number and percentage of subjects with TEAEs
- Number and percentage of subjects with drug related TEAEs
- Number and percentage of subjects with serious TEAEs
- Number and percentage of subjects with serious drug related TEAEs
- Number of subjects with medically attended TEAEs (MAAEs)
- Number of subjects with potential immune mediated TEAEs (PIMMCs)
- Number of subjects with new onset chronic disease TEAEs (NOCDs)
- Number of deaths.

The number and percentage of subjects with TEAEs, classified by SOC, and PT will be summarized. Summaries (number and percentage of subjects) with each specific type of TEAE classified by SOC and preferred term will be provided for the following:

- TEAEs
- Drug-Related TEAEs,
- Serious TEAEs,
- Drug-Related serious TEAEs,
- TEAEs by Severity
- TEAEs leading to permanent discontinuation of study drug,
- Medically attended TEAEs (MAAEs)
- Potential immune mediated TEAEs (PIMMCs)
- New onset chronic disease TEAEs (NOCDs)
- Deaths

- Common (>=5% in any treatment group) Treatment-Emergent Adverse Events excluding Serious Adverse Events
- If a subject has multiple TEAEs with the same SOC or PT, the subject will be counted only once for the SOC and for an individual preferred term. For summaries by relationship, but with differing relationship (Yes, No), the subject will be counted only once for events with relationship equal to 'Yes'. If any of the relationship values are missing then the subject will be counted as having a related AE. A subject will be counted once under maximum severity. If severity is missing for all episodes of the event, the subject will be counted under the Missing category. Serious Adverse Events should not only include SAEs identified by the investigator on the eCRF, but also AEs upgraded by the Sponsor based on the Always Serious List (SAE flag on eCRF may not always be updated, but there will always be an SAE number in the eCRF database).

The following adverse events of special interest are defined:

- Reactogenicity

Each local and systemic solicited reaction that continues beyond the first 7 days after the immunization or if it is serious will be recorded as an adverse event. The percentage of subjects who report pain, tenderness, redness/erythema, swelling or hardness/induration at the vaccination site, nausea, vomiting, diarrhea, headache, tiredness (fatigue), myalgia/muscle pain and arthralgia or other AE (not specified) will be summarized by grade (n, %) and overall regardless of grade (n,%).

Reactogenicity for each dose of ASP3772 will be compared to PCV13. The percentage of subjects who had 0, 1, 2, 3, 4 or 5 local reactions using a nonparametric CMH rank statistic. The systemic reactions will be compared in a similar way. The severity for each type of reaction will be compared for each dose of ASP3772 to PCV13 after assigning an increasing score to each severity and comparing the severity using a CMH statistic with scores=modridit.

7.5.2 Clinical Events

Clinical events capturing reactogenicity will be collected by the subject in the electronic daily diary. Specific local reactions requiring responses are pain, tenderness, redness/erythema, swelling, hardness, and induration at the immunization site. Systemic reactions are nausea, vomiting, diarrhea, headache, fever, tiredness (fatigue), myalgia/muscle pain, and arthralgia with grades 1-4 defined for each. Subjects in stage 1 will be asked to report joint pain/arthralgia through day 7 postimmunization during the day 7 visit. This addition was requested by the FDA after the electronic diary was being used at the sites. The ePRO device will be revised for Stage 2 so that subjects will be asked to report symptoms of arthralgia daily for 7 days post immunization. Additionally, elevated daily body temperature will be summarized by grade: grade 1 mild (100.4-101.1), grade 2 moderate (101.2 – 102.0), grade 3 severe (102.1 – 104) grade 4 potentially life threatening (>104).

Data will be summarized for each study day collected in the diary (day 1 thru day 7) by grade and overall. The number of subjects and percentage who experience each type of reaction will

be provided. The maximum grade for each type of reaction (number and percentage of subjects) will be provided.

At the Day 7 visit the investigator will assess each type of reactogenicity and based on the discussion with the subject will assess these as related or not. Those that are assessed as not related by the investigator will be excluded from a second table summarizing related reactogenicity events. The maximum grade for each type of related reaction (number and percentage of subjects) will be provided. In the event there are none judged as not related, this summary will not be produced.

7.5.3 Clinical Laboratory Evaluation

The baseline value is the last measurement taken prior to vaccine administration.

Quantitative clinical laboratory variables, i.e. hematology and biochemistry will be summarized using mean, standard deviation, minimum, maximum and median for each treatment group at each visit. Additionally, a within-subject change will be calculated as the post-baseline measurement minus the baseline measurement and summarized in the same way. Each laboratory result will be classified as low (L), normal (N), or high (H) at each visit according to the laboratory supplied reference ranges and laboratory data will be listed with flags for low or high.

Frequency tabulations of qualitative clinical laboratory variables (urinalysis) will be

presented for treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage at each visit.

For hematology and biochemistry a summary of shifts (shift from normal or high to low, shift from normal or low to high) from baseline to worst finding during the treatment period will be presented for treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage.

The following data will be presented graphically by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage:

- Box plot of Quantitative Laboratory Test Results will be displayed by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage with PCV13 as a single group. The lower box will represent the 25th percentile, the middle line as the median, and the upper box the 75th percentile. A line showing the upper limit normal (ULN) and lower limit of normal (LLN) will be shown along with the minimum, maximum and outliers.
- Box plot of the maximum change from baseline for laboratory values will be displayed by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage with PCV13 as a single group. A line showing the ULN and LLN will be shown. The lower box will be the 25th percentile, the upper box the 75th percentile, and the middle line the median. Minimum, maximum and outliers will be shown.

7.5.3.1 Potentially Clinically Significant Laboratory Values

The frequency of Grades 1 through 4 hematology, biochemistry and urinalysis values and Grades 1 through 4 change from baseline as defined in the Guidance for Industry titled

"**Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials**" (Appendix D) will be provided by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage. Subjects treated with PCV13 will be combined into one group by stage.

7.5.3.2 Liver Enzymes and Total Bilirubin

The percentage of subjects with the following potentially clinically significant elevations in liver tests will be summarized for Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Total Bilirubin, Aspartate Transaminase (AST) and their combination. These parameters will be measured from a central laboratory.

The subject's highest value during the investigational period (day of vaccination and through Day 30) will be used.

- ALT > 3xULN, > 5xULN, > 10xULN, >20xULN
- AST > 3xULN, > 5xULN, > 10xULN, >20xULN
- ALT or AST > 3xULN, > 5xULN, > 10xULN, >20xULN
- ALP > 1.5xULN
- Total Bilirubin >2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN
- (ALT and/or AST > 3xULN) and ALP< 2xULN and Total Bilirubin > 2xULN

The last 2 criteria where 2 or more parameters are evaluated will be with the measurements on the same day or up to a maximum of 1 day apart.

The denominator for each criterion will be the number of subjects who have at least one value during the investigational period. The number and percentage of subjects meeting the criteria during the investigational period will be summarized by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage.

7.5.4 Vital Signs

The baseline visit is the last measurement taken prior to vaccine administration.

Vital signs (body temperature, systolic blood pressure, diastolic blood pressure, respiratory rate and pulse rate) will be summarized using the mean, standard deviation, minimum, maximum and median. Additionally, a within-subject change will be calculated per visit and or timepoint as the post-baseline measurement minus the baseline measurement.

Tables for potentially clinically significant vital signs will be generated using the definitions shown in Appendix C: **Grading Signs of Systemic Reactogenicity**. Subjects who have a change in grade from baseline to post baseline during the first hour after immunization (Table C1) and have a numeric change that is consistent with Table C2 will be considered to have a clinically meaningful change vital sign.

Summaries will be provided by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage using the maximum value obtained post immunization. Subjects treated with PCV13 will be combined into one group by stage.

Reactogenicity events defined in Section 6.2.1.3 will be summarized by treatment group (ASP3772 (1 μg, 2 μg, 5 μg) or PCV13) and stage. All subjects within a stage given PCV13 will be combined.

7.5.5 Electrocardiograms (ECGs)

Subjects with normal, not clinically significant abnormal, and clinically significant abnormal results as assessed by investigator for the 12 lead ECG will be listed.

7.5.6 Physical Exam

Any physical examination findings will be listed.

7.6 Interim Analysis

Safety data will be provided for DEC review after the sentinel subjects and after each dose cohort for the adult and elderly populations after the Day 7 visit for dose escalation decisions. Prior to initiating enrollment of the elderly subjects, the DEC will review the available data through 30 days post immunization in adult subjects. Details are provided in the DEC Charter.

The immunogenicity endpoint will occur 30 days after immunization. Once all subjects in Stage 1 have completed 30 days, there will be a database cut-off to conduct a formal analysis of the immunogenicity data. All data will be cleaned and the treatment assignment will be unblinded to Astellas. Treatment assignment will continue to be unavailable to the sites until after all subjects have completed the 180-day follow-up, all data has been cleaned and the database has been locked.

Similarly for subjects in Stage 2 once Stage 2 subjects have completed 30 days, there will be a database cut-off to conduct an interim analysis of the immunogenicity data. All data will be cleaned and the treatment assignment will be unblinded to Astellas. Treatment assignment will remain unavailable to the sites until after all subjects have completed the 180-day follow-up and the database has been locked.

In addition, there will be an expedited assessment of whether there is an immune response to ASP3772 in the Stage 1, adult subjects. The vendor responsible for analyzing the immunogenicity data will provide a file of the immunogenicity results with the patient numbers to an unblinded member of Bioanalytics team who will then add the treatment assignment and remove the subject numbers. The data will be sorted based on treatment assignment, which will further obscure the possibility of identifying the individual treatment assignment for an individual subject. Summaries of the IgG and OPA titers will be provided by ASP3772 dose and for combined Prevnar 13 subjects. The purpose of this exercise is to determine if any doses should be eliminated from the planned toddler study due to insufficient immunogenicity in the healthy adult population.

7.7 Handling of Missing Data, Outliers, Visit Windows, and Other Information

7.7.1 Clinical Laboratory (Hematology, Biochemistry, and Urinalysis) Analysis Windows

Analysis Visits	Scheduled Day in Protocol	Analysis Windows (day)
Baseline	Day 1	Last non-missing value between -28 and 1 (inclusive)
Visit 3	Day 7	Closest non-missing post immunization value to study day 7
Visit 4	Day 30	Closest non-missing post immunization value to study day 30

7.7.2 Blood Sampling for Immunogenicity, Anti-biotin and T-cell Immune Response Analysis Windows

Analysis Visits	Scheduled Day in Protocol	Analysis Windows (day)
Baseline	Day 1	Last non-missing value on day 1 prior to immunization dosing
Visit 3	Day 7	Closest non-missing post immunization value to study day 7
Visit 4	Day 30	Closest non-missing post immunization value to study day 30
Visit 5	Day 180	Closest non-missing post immunization value to study day 180

7.7.3 Visit Windows

The study protocol gives the overall study schedule and the permissible intervals for these visits expressed as the number of days relative to Visit 2.

Analyses will not exclude subject data due to the subject's failure to comply with the visit schedule.

All the assessments will be allocated to CRF visit based on the table below:

CRF visit	Target day	Planned assessment day	Analyzed visit
Visit 1	D-21	D-28 to D-3	Screening
Visit 2	D1	D1	Baseline
Visit 3	D7	D4 to D11	Day 7
Visit 4	D30	D16 to D46	Day 30
Visit 5	D180	D150 to D210	Day 180

The value with the assessment day that is the closest to the defined target day within the windows is used. If two values are equally close, one before and one after the target date, the value with the later date is used in the analysis.

7.7.4 Imputation Rules for Incomplete Dates

In case of missing partial start and stop dates for concomitant medications, the following rules will be used:

If the start date is missing or partial:

- if the month is missing, use January
- if the day is missing, use the first day of the month under consideration
- if the year is missing, use year of the informed consent date
- if the entire date is missing, use informed consent date

If the stop date is missing or partial:

- if the month is missing, use December
- if the day is missing, use the last day of the month under consideration
- if the year or the entire date is missing, set the stop date to December 31st, of the current year.

If the imputed start date is after the stop date, then the imputed start date will be 1 day prior to the stop date.

For AEs, a missing or incomplete onset date will be imputed according to the following conventions.

If an onset date is missing or only the year is known, the imputed onset date will be the date of first dose of study drug.

If only the year is known for the AE onset date, the imputed onset date will be the latest of the following non-missing dates:

- Date of first dose of study drug
- January 1 of the year of AE onset date

If only the month and year is known for the onset date, set the imputed onset date to the first day of that month and then apply the following rules.

- If the month and year of the onset date is prior to the month and year of the first dose of study drug, then the imputed onset date will be the imputed onset date.
- If the month and year of the onset date is on or after the month and year of the first dose of study drug, then the imputed onset date will be the **latest** of the following non-missing dates:
    - Date of first dose of study drug
    - Surrogate onset date

If the imputed onset date is after the adverse event end date, the imputed onset date will be the same as the adverse event end date.

8 REVISION AND RATIONALE

Version 1 of the SAP has been updated to include planned interim analyses, provide greater details of treatment assignment blinding for study personnel, update the analysis of exploratory endpoints and to correct assorted typographical errors.

Version 2 of the SAP has been updated to

1. Increased screening for Stage 2 to be consistent with the protocol from -28 days to -40 days.
2. Added: "Subjects who are given PPSV23 without previous exposure to PCV13 will not be included in the immunogenicity summaries." These are subjects in Stage 2, Group 3 who should have received PCV13 before receiving PPSV23.
3. CCI [redacted]
4. Changed the algorithm for setting the value for LLOQ for the calculation of fold change in response to feedback from the FDA. Values below the LLOQ will be set to ½ LLOQ for calculation of geometric means and set to LLOQ for calculation of fold changes.
5. CCI [redacted]
6. CCI [redacted]
7. Added a CMH rank test to examine the difference between ASP3772 and PCV13 in the number of reactogenicity reactions. Added a CMH statistics with scores=modridit to compare the severity for each type of reaction between each dose of ASP3772 and PCV13 after assigning an increasing score to each severity.
8. Added details for determining systemic reactogenicity events during the 1st hour post vaccination.

9 REFERENCES

(1) Guidance for Industry titled "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" issued by US Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research on September 2007.

(2) Jozef Nauta. Statistics in Clinical Vaccine Trials, Heidelberg, Germany: Springer-Verlag 2010.

10 APPENDICES

10.1 Appendix A: Instructions to calculate the Summary statistics for the secondary endpoints

Geometric Mean (GM) and Geometric Standard Deviation (GSD)

To obtain the GM and GSD, first calculate the natural log of each titer or IgG concentration using the LOG function in SAS. Using PROC FREQ on the log transformed values obtain the mean and standard deviation of the log transformed values. Exponentiate mean and standard deviation to obtain the geometric mean and geometric standard deviation.

To obtain the lower confidence limit of the 95% confidence interval of the GM obtain the critical value of the student's distribution using TINV(0.975, n-1) where n=the number of non-zero titers or concentrations. For example, TINV(0.975, 19) = 2.093. The lower 95% confidence interval is then:

$GM / GSD^{(TINV\ (0.975,\ n-1)\ /SQRT(n))}$

and the upper 95% confidence is calculated using: $GM \times GSD^{(TINV\ (0.975,\ n-1)\ /SQRT(n))}$

where SQRT is the square root.

Geometric Mean Ratio (GMR)

To obtain the geometric ratio, calculate the GM for each treatment group. The geometric mean ratio can then be calculated as;

$GM_{ASP3772} / GM_{PCV13}$

Alternately, calculate the natural log of each titer or IgG concentration using the LOG function in SAS to transform the titers or IgG concentrations. Obtain the mean of the log transformed values for each treatment group, μ_1 and μ_0. To calculate the geometric mean ratio:

$GMR = e^{(\mu_{1.} - \mu_{0.})}$

where $\mu_{1.}$ is the arithmetic mean of the log transformed ASP3772 titers (or concentrations) and $\mu_{0.}$ is the arithmetic mean of the log transformed PCV13 titers (or concentrations).

To test that the null hypothesis that the GMR = 1, use the two sample t-test for the treatment difference of the log transformed titers (or concentrations). This is equivalent to testing if the GMR is equal to 1.

To obtain the 95% CI for the geometric mean ratio:

(1) Obtain the standard error of the difference for the log transformed values SE_{DIFF}.
(2) Calculate the lower CI:

$$e^{GMR - (TINV(0.975, n(1)+n(2)-2)) * SE_{DIFF}}$$

(3) Calculate the upper CI:

$$e^{GMR + (TINV(0.975, n(1)+n(2)-2)) * SE_{DIFF}}$$

Geometric Mean Fold Increase (GMFR)

To obtain the GMFR, calculate the fold rise for each subject. Take the natural log using LOG function in SAS. Using PROC FREQ on the log transformed values obtain the mean and standard deviation of the log transformed values. Exponentiate mean and standard deviation to obtain the geometric mean and geometric standard deviation.

To obtain the lower confidence limit of the 95% confidence interval of the GMFR obtain the critical value of the student's distribution using TINV (0.975, n-1) where n=the number of subjects. For example, with 20 subjects TINV (0.975, 19) = 2.093. The lower 95% confidence interval is then:

$$GM / GSD^{(TINV(0.975, n-1) /SQRT(n))}$$

and the upper 95% confidence is calculated using: $GM \times GSD^{(TINV (0.975, n-1) /SQRT(n))}$

where SQRT is the square root.

Comparing the Geometric Mean Fold Rise (GMFR) between two groups

To compare the GMFR between the two treatment groups (each dose of ASP3772 vs. PCV13) use an analysis of covariance with the log-transformed post immunization value as the dependent variable and the log-transformed pre vaccination value as the independent value. With log-transformed antibody values (titer or IgG levels) the variability usually decreases with increasing antibody values (heteroscedasticity).

Assume heteroscedasticity exists and use the following model to analyze the data.

```
proc mixed;
   model y=group x group*x/ solution; repeated / local=exp ( x x2);
run;
```

Where: y= log-transformed post immunization antibody titer (or IgG concentration)

x= log-transformed pre immunization antibody titer (or IgG concentration)

x2=(log-transformed pre immunization antibody titer (or IgG concentration))^2 group= treatment group

group*x= treatment group by pre immunization log-transformed antibody titer (or IgG concentration). If group*x is significant then there is heteroscedasticity.

Use the ESTIMATE and CL options in proc mixed to obtain the estimate of the baseline corrected mean fold ratio and the 95% confidence interval for the baseline corrected mean fold ratio. The ratio is the GMFI for ASP3772 / PCV13. The baseline-corrected geometric mean ratio of the two treatment groups is:

2^{β_1} where β_1 is the estimate of the treatment group effect obtained from the ANCOVA. The 95% lower confidence limit is then $2^{\beta(LCL)}$ and the 95% upper confidence interval is then $2^{\beta(UCL)}$ LCL and UCL are the 95% confidence limits obtained from the CL option in Proc Mixed.

Proportion of subjects with a > 4-fold increase in OPA titer

SAS PROC FREQ can be used to obtain the proportions and 95 % CI for each serotype.

```
PROC FREQ data=xxxx order=freq;
    tables fourfold / binomial (wilson) alpha=0.05;
    weight count;
```

10.2 Appendix B: Local Reactogenicity Grading

Stage 1 Group 1 and Stage 2 Group 2 subjects will record the local reactogenicity in an electronic diary along with body temperature through day 7 following study immunization.

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life-threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness†	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling‡	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

ER: emergency room

† In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable. A measurement tool will be provided to the subjects to measure the size of the erythema/redness.

‡ Induration/Swelling should be evaluated and graded using the functional scale, as well as the actual measurement. A measurement tool will be provided to the subjects to measure the induration/swelling.

10.3 Appendix C: Grading Signs of Systemic Reactogenicity

Vital signs measurements will be monitored for reactogenicity through 1 hour postdose in all Stage 1 Group 1 and Stage 2 Group 2 subjects. Oral temperature will be recorded by the subjects daily through day 7 post immunization.

Table C1.

Vital Signs†	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Fever (°C)‡ **(°F)‡**	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia (bpm)	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia (bpm)§	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) (mm Hg)	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) (mm Hg)	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) (mm Hg)	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate (breaths per min)	17 – 20	21 – 25	> 25	Intubation

bpm: beats per minute; ER: emergency room

† Subject should be at rest for all vital sign measurements.

‡ Oral temperature; no recent hot or cold beverages or smoking. Subjects will be provided with an oral thermometer to record temperature daily though day 7 postimmunization.

§ When resting heart rate is between 60 and 100 bpm. Use clinical judgment when characterizing bradycardia among some healthy subject populations, e.g., conditioned athletes.

Table C2.

	Absolute change to include in calculations
Fever (°C)‡	As in Table above (change in Grade)
Tachycardia (bpm)	As in Table above (change in Grade)
Bradycardia (bpm)§	>10 bpm
Hypertension (systolic) (mm Hg)	>20 mmHg
Hypertension (diastolic) (mm Hg)	>15 mmHg
Hypotension (systolic) (mm Hg)	As in Table above (change in Grade)
Respiratory Rate (breaths per min)	>4 breaths/min

Stage 1 Group 1 and Stage 2 Group 2 subjects will be asked to record the below systemic reactogenicity symptoms through day 7 following study immunization.

Table C3.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Nausea/ Vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 g/24 hours	4 – 5 stools or 400 – 800 g/24 hours	6 or more watery stools or > 800 g/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia*	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Illness or clinical AE (as defined according to applicable regulations) †	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

AE: adverse event; ER: emergency room; IV: intravenous.

*: Stage 1, subjects will be questioned on day 7 visit if they experienced any join pain/arthralgia through day 7 and if yes, the site personnel will report it as a clinical event using this grading scale. Stage 2 subjects will report Arthralgia daily on electronic diary through day 7.

† : Subjects will be questioned on day 7 if they experienced any other illness through day 7 post immunization and if any site personal will report it as a clinical event using this grading scale.

10.4 Appendix D: Grading Laboratory Abnormalities for Healthy Adult Subjects

Clinical laboratory test results will be graded and displayed using these grades. Grading values are based on ICON Central Laboratories, and will be adjusted if the reference ranges change.

Chemistry	ICON Reference range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)†
Sodium – Hyponatremia (mEq/L)	**136-145***	**133 – 135**	**131 – 132**	**126 – 130**	**< 126**
Sodium – Hypernatremia (mEq/L)		**146 – 147**	**148 – 149**	**150 – 151**	**> 151**
Potassium – Hypokalemia (mEq/L)	**3.5-5.1***	**3.3 – 3.4**	**3.1 – 3.2**	**2.9 – 3.0**	**< 2.9**
Potassium – Hyperkalemia (mEq/L)		**5.2 – 5.3**	**5.4 – 5.5**	**5.6 – 5.7**	**> 5.7**
Glucose – Hypoglycemia (mg/dL)		**60 – 64**	**50 – 59**	**40 – 49**	**< 40**
Glucose – Hyperglycemia Fasting (mg/dL)	**Fasting 74-99**	**100 – 110**	**111 – 125**	**> 125**	**Insulin requirements or hyperosmolar coma**
Random (mg/dL)	**Random 74-139**	**140 – 155**	**156 – 230**	**> 230**	
Blood Urea Nitrogen (mg/dL)	**7-19 (F) [18-50] 10-20 (F) [>50] 9-21 (M) [18-50] 8-26 (M) [>50]**	**22 – 25**	**26 – 30**	**> 30**	**Requires dialysis**
Creatinine (mg/dL)	**0.55-1.02 (F) 0.72-1.18 (M)**	**1.5 – 1.7**	**1.8 – 2.1**	**2.2 – 2.5**	**> 2.5 or requires dialysis**
Calcium – Hypocalcemia (mg/dL)	**8.4-10.2**	**7.9 – 8.3**	**7.4 – 7.8**	**6.9 – 7.3**	**< 6.9**
Calcium – Hypercalcemia (mg/dL)		**10.3 – 11.0**	**11.1 – 11.5**	**11.6 – 12.0**	**> 12.0**
Magnesium – Hypomagnesemia (mg/dL)	**1.3-2.1***	**1.1-1.2**	**0.9 – 1.0**	**0.7 – 0.8**	**< 0.7**
Phosphorous – Hypophosphatemia (mg/dL)	**2.3-4.7**	**2.0 – 2.2**	**1.7 – 1.9**	**1.3 – 1.6**	**< 1.3**
Creatine Kinase (mg/dL)	**29-168 (F)**	**1.25 – 1.5 ×**	**1.6 – 3.0**	**3.1 –10**	**> 10**
	30-200 (M)	**ULN*****	**× ULN**	**× ULN**	**× ULN**
Albumin – Hypoalbuminemia (g/dL)	**3.5-5.0 [18-61] 3.4-4.8 [>61]**	**2.8 – 3.1**	**2.5 – 2.7**	**< 2.5**	**NA**
Total Protein – Hypoproteinemia (g/dL)	**6.4-8.3**	**5.5 – 6.0**	**5.0 – 5.4**	**< 5.0**	**NA**
ALP (Increase by Factor)	**40-150**	**1.1 – 2.0 × ULN**	**2.1 – 3.0 × ULN**	**3.1 – 10 × ULN**	**> 10 × ULN**
Liver Function Tests – ALT (Increase by Factor)	**≤55**	**1.1 – 2.5 × ULN**	**2.6 – 5.0 × ULN**	**5.1 – 10 × ULN**	**> 10 × ULN**
Liver Function Tests – AST (Increase by Factor)	**5-34**	**1.1 – 2.5 × ULN**	**2.6 – 5.0 × ULN**	**5.1 – 10 × ULN**	**> 10 × ULN**
Bilirubin – When Accompanied by Any Increase in Liver Function Test (Increase by Factor)	**≤1.20**	**1.1 – 1.25 × ULN**	**1.26 – 1.5 × ULN**	**1.51 – 1.75 × ULN**	**> 1.75 × ULN**
Bilirubin – When Liver Function Test is Normal (Increase by Factor)	**≤1.20**	**1.1 – 1.5 × ULN**	**1.6 – 2.0 × ULN**	**2.0 – 3.0 × ULN**	**> 3.0 × ULN**

Table continued on next page

Chemistry	ICON Reference range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)†
Total Cholesterol (mg/dL)	**Desirable:** **< 200** mg/dL **Borderline:** **200 – 239** mg/dL **High:** **≥ 240** mg/dL	**201 – 210**	**211 – 225**	**> 226**	**NA**

*** The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.**

**** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.**

*****ULN" is the upper limit of the normal range.**

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; NA: not applicable; ULN: upper limit of normal

Hematology	ICON Reference range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Hemoglobin (Female Subjects) (g/dL)	**11.5-15.5**	**10.0 – 10.9**	**8.5 – 9.9**	**7.0 – 8.4**	**< 7.0**
Hemoglobin (Female Subjects) Change From Baseline Value (g/dL)		**Any decrease – 1.5**	**1.6 – 2.0**	**2.1 – 5.0**	**> 5.0**
Hemoglobin (Male Subjects) (g/dL)	**13.2-17.0**	**11.4 – 12.4**	**10.0 – 11.3**	**8.0 – 9.9**	**< 8.0**
Hemoglobin (Male Subjects) Change From Baseline Value (g/dL)		**Any decrease – 1.5**	**1.6 – 2.0**	**2.1 – 5.0**	**> 5.0**
White Blood Cell Increase (k/uL)	**3.50-11.10***	**11 – 15**	**15.1 – 20**	**20.1 – 25**	**> 25**
White Blood Cell Decrease (k/uL)	**3.50-11.10***	**2.5 – 3.5**	**1.5 – 2.4**	**1 – 1.4**	**< 1**
Lymphocytes Decrease (k/uL)	**1.2-4.0**	**0.5 - 0.6**	**0.3 - 0.4**	**0.1 - 0.2**	**< 0.1**
Neutrophils Decrease (k/uL)	**1.8-7.0**	**1.3 - 1.6**	**0.8 - 1.2**	**0.5 - 0.7**	**< 0.5**
Eosinophils (k/uL)	**0.0-0.5**	**0.8 – 1.2**	**1.3 - 5**	**> 5**	**Hypereosinophilic**
Platelets Decreased (k/uL)	**150-400**	**125 – 140**	**100 – 124**	**25 – 99**	**< 25**
Prothrombin Time (Increase by Factor)	**11.4-14.9 sec**	**1.01 – 1.10 × ULN†**	**1.11 – 1.20 × ULN**	**1.21 – 1.25 × ULN**	**> 1.25 ULN**
Partial Thromboplastin Time (Increase by Factor)	**24.7-34.4 sec**	**1.01 – 1.2 × ULN**	**1.21 – 1.4 × ULN**	**1.41 – 1.5 × ULN**	**> 1.5 × ULN**
Fibrinogen Increase (mg/dL)	**228-439**	**476 – 600**	**601 – 700**	**> 700**	**NA**
Fibrinogen Decrease (mg/dL)		**150 – 199**	**125 – 149**	**100 – 124**	**< 100 or associated with gross bleeding or DIC**

*** The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.**

DIC: disseminated intravascular coagulation; NA: not applicable; ULN: upper limit of normal

† Upper limit of the normal range.

Urine	ICON Reference range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Protein	**Negative**	**Trace**	**1+**	**2+**	**Hospitalization or dialysis**
Glucose	**Negative**	**Trace**	**1+**	**2+**	**Hospitalization for hyperglycemia**
Blood (Microscopic) – RBC per HPF (rbc/hpf)	**None**	**1 - 10**	**11 – 50**	**> 50 and/or gross blood**	**Hospitalization or packed RBC transfusion**

RBC: red blood cell; HPF: high power field

11 APPENDICES

11.1 Appendix 1: Author and Approver Signatures

Prepared by: _____ Date: _____

PPD
PPD

Date (DD Mmm YYYY)

Approved by: _____ Date: _____

PPD
PPD , Biostatistics

Date (DD Mmm YYYY)

Approved by: _____ Date: _____

PPD
PPD

Date (DD Mmm YYYY)

11.2 Appendix 2: Adverse Events of Special Interest (Preferred Terms; MedDRA 18.0)

11.2.1 Adverse Events of Interest

None have been defined at this time.

AUTHOR AND APPROVER SIGNATORIES

(E-signatures are attached at end of document)

<Name/Degree, Title, CRO Name/Global Data Science> was the study statistician for this study.

<Name/Degree, Title, CRO Name/ Global Data Science> was the biostatistics peer reviewer of this Statistical Analysis Plan

This Statistical Analysis Plan was approved by: Name, Degree Title, Department <Global Medical Lead or equivalent or designee>

ELECTRONIC SIGNATURE PAGE

Document Type : Statistical Analysis Plan

Document Control Number : MGC1900389

Amendment Number : N/A

International Study Number : 3772-CL-1001

Departmental Study Number : N/A

Actual Version Number : Version 3.0

Document Version : 4.0

Nonclinical Initial SD Approved Date (UTC) : N/A

Date (UTC)	Signed by	Sign Off Meaning
09/29/2020 22:37:09	PPD	Responsible Officer
Full Name / Legal Name	PPD	
09/30/2020 04:10:03	PPD	Principal Reviewer Approval
Full Name / Legal Name	PPD	
09/30/2020 15:53:35	PPD	Signature as Document Originator
Full Name / Legal Name	PPD	
Full Name / Legal Name		
Full Name / Legal Name		
Full Name / Legal Name		
Full Name / Legal Name		
Full Name / Legal Name		
Full Name / Legal Name		
Full Name / Legal Name		

*UTC: Coordinated Universal Time