Protocol Amendment 2

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: 04-DEC-2019

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

Version 2.1

Incorporating Nonsubstantial Amendment 2 [see Section 13]

02 Dec 2019

IND 18657

Sponsor:

Astellas Pharma Global Development, Inc. (APGD)

1 Astellas Way
Northbrook, IL 60062

Protocol History:
Version 1.0 [Original, 07Aug2018]
Version 1.1 [Nonsubstantial amendment 1, 16Nov2018]
Version 2.0 [Substantial amendment 1, 28Feb2019]

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I. SIGNATURES

1. SPONSOR'S SIGNATURES

Required signatures (e.g., Protocol authors and contributors, etc.) are located in [Section 15 Sponsor Signature].

2. COORDINATING INVESTIGATOR'S SIGNATURE

The Coordinating Investigator's signature can be found in Section 14 Coordinating Investigator's Signature, located at the end of this document.

3. INVESTIGATOR'S SIGNATURE

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

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Incorporating Nonsubstantial Amendment 2

02 Dec 2019

I have read all pages of this clinical study protocol for which Astellas is the sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations. I will also ensure that subinvestigator(s) and other relevant members of my staff have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: ----------

Date (DD Mmm YYYY) ----------

Printed Name: ----------

Address: ----------

II. CONTACT DETAILS OF KEY SPONSOR'S PERSONNEL

24h-Contact for Serious Adverse Events (SAEs) See [Section 5.5.5 Reporting of Serious Adverse Events] for SAE Fax Number and Email	**Please fax or email the SAE Worksheet to:** **Astellas Pharma Global Development, Inc.** **Pharmacovigilance** **North America Fax number: 888-396-3750** North America Alternate Fax number: 847-317-1241 **International Fax Number : +44-800-471-5263** **Email: safety-us@astellas.com**
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III. LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

Abbreviations	Description of abbreviations
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
anti-HCV	hepatitis C virus antibodies
APGD	Astellas Pharma Global Development Inc.
AST	aspartate aminotransferase
AT	aminotransferase
CA	Competent Authorities
cEC	concerned Ethics Committee
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CP1	carrier protein
CRF	case report form
CRO	contract research organization
DEC	dose escalation committee
ECG	electrocardiogram
eCRF	electronic case report form
EEA	European Economic Area
ESV	end-of-study visit
FAS	full analysis set
FIH	first-in-human
FSH	follicle stimulating hormone
GCP	good clinical practice
GMC	geometric mean concentration
GMFR	geometric mean fold rise
GMP	good manufacturing practices
GMT	geometric mean titer
GSD	geometric standard deviation
HBsAg	hepatitis B surface antigen
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation of technical requirements for registration of pharmaceuticals for human use
IEC	independent ethics committee
IgG	immunoglobulin G
IL	interleukin
IND	investigational new drug

Abbreviations	Description of abbreviations
INR	international normalized ratio
IPD	invasive pneumococcal disease
IRB	institutional review board
IRT	interactive response technology
LA-CRF	liver abnormality case report form
MAAE	medically attended adverse event
MAPS	multiple antigen-presenting system
MedDRA	Medical Dictionary for Regulatory Activities
CCI [redacted]	
NOCD	new-onset chronic disease
NSAID	nonsteroidal anti-inflammatory drug
OPA	opsonophagocytic activity
PBMC	peripheral blood mononuclear cells
PCV13	Prevnar 13®
CCI [redacted]	
PIMMC	potentially immune mediated medical condition
PPS	per protocol set
PPSV23	Pneumovax®23
PS	polysaccharide
RBC	red blood cell
(S)AE	serious adverse event or adverse event
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reactions
TBD	to be decided
TEAE	treatment-emergent adverse event
TBL	total bilirubin
Th17	T helper 17 cells
ULN	upper limit of normal
USM	urgent safety measure
WBC	white blood cell
WOCBP	woman of childbearing potential

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.
Intervention	The drug, device, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety and pharmacoeconomics).
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.

IV. SYNOPSIS

Date and Version No of Protocol Synopsis:	02 Dec 2019, Version 2.1
Sponsor: Astellas Pharma Global Development Inc. (APGD)	**Protocol Number:** 3772-CL-1001
Name of Study Drug: ASP3772	**Phase of Development:** Phase 1/2

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

Planned Study Period:
Stage 1: 1Q2019 – 2Q2019
Stage 2: 3Q2019 – 3Q2020

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 Pneumovax®23 (PPSV23) for the serotypes not included in PCV13.

Planned Total Number of Study Centers and Location(s):
Stage 1: Approximately 10 sites in United States
Stage 2: Approximately 25 sites (includes sites from Stage 1) in United States

Study Population:
Stage 1: Adults:18 to 64 years of age
Stage 2: Elderly: 65 to 85 years of age

Number of Subjects to be Enrolled/Randomized:
Stage 1: 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: A total of approximately 495 evaluable adult subjects from 65 to 85 years of age.

Number of Subjects per Cohort by Immunization

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

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

PPSV23: Pneumovax 23

Study Design Overview:

This is a combined phase 1, first-in-human (FIH), dose-escalation (Stage 1) and phase 2, dose-finding (Stage 2), observer-blinded study to evaluate the safety, tolerability and immunogenicity of ASP3772 in adult and 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 PCV13 naïve 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 that 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 Dose Escalation Committee (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. All subjects within each cohort will remain under observation for approximately 1 hour postimmunization, provided no reactions have occurred that require additional observation in the opinion of the investigator. The DEC membership may include representatives from Astellas Medical Science, Pharmacovigilance and Statistics personnel, as well as external independent safety physician(s). The external safety physician will be selected based on expertise in vaccine clinical studies and other considerations such as the disease area. A DEC charter will detail the roles, responsibilities and procedures for decision-making.

See the Schedule of Assessments for details on procedures performed at each study visit for each group.

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). After screening, subjects will be enrolled sequentially into 3 cohorts designated by escalating ASP3772 dose levels. A single dose of ASP3772 will be administered as a 0.5 mL intramuscular

Study Design Overview** (continued)**:

injection into the deltoid muscle of the right or left arm on day 1 at 1 of 3 dose levels: 1, 2 or 5 μg of ASP3772 for cohorts 1, 2 and 3 respectively. The subjects randomized to PCV13 will receive a single injection of the standard dose of PCV13 on day 1.

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. 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.

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 (NOCDs) 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 immunoglobulin G (IgG) and opsonophagocytic activity (OPA) will be collected prior to the first study immunization on day 1 and on day 30 postimmunization.

Stage 2 Group 2:

Stage 2 of the study is a dose-finding (sequentially higher doses), active-controlled, observer-blinded study with 3:1 randomization (ASP3772:PCV13) in elderly subjects 65 to 85 years of age.

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 injection into the deltoid muscle of the right or left arm 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.

Approximately 396 subjects will be enrolled in Stage 2 of the study; 132 subjects per cohort (ASP3772 n = 99 and PCV13 n = 33).

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. 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 immunized with PCV13 and ASP3772 with immunogenicity data across the elderly population.

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 those 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 immunization on day 1 and postimmunization on day 30.

Primary immunogenicity evaluation for dose finding is 30 days following the initial immunization.

Stage 2 Group 3:

A separate group of elderly subjects previously immunized with PCV13 will be enrolled and receive the recommended booster immunization with PPSV23 only (n = 99) **(Group 3)**.

Study Design Overview** (continued)**:

Prior immunization (PCV13) should have taken place no less than 10 months and no more than 2 years prior to study vaccine administration. Immunogenicity to the 11 non-PCV13 serotypes in PPSV23 will be evaluated. 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.

Inclusion/Exclusion Criteria:

Inclusion:

A subject is eligible for the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] Authorization for US sites) must be obtained from the subject prior to any study-related procedures (including withdrawal of prohibited medications, if applicable).
2. Stage 1: Subject is healthy male or female between 18 and 64 year of age inclusive, at screening.
3. Stage 2: Subject is a male or female between 65 and 85 years of age, inclusive, at screening who is healthy or has chronic controlled, stable disease with no change in disease severity, medical therapy and no hospitalization records in last 12 weeks as determined by medical history, physical examination and laboratory data and the clinical judgment of the investigator.
4. A female subject is eligible to participate if she is not pregnant (see [Appendix 12.3 Contraception Requirements]) and at least 1of the following conditions applies:
    a. Not a woman of childbearing potential (WOCBP) as defined in [Appendix 12.3 Contraception Requirements]
    OR
    b. WOCBP who agrees to follow the contraceptive guidance as defined in [Appendix 12.3 Contraception Requirements] at screening and for at least 28 days after the study vaccine administration.
5. Female subject must agree not to breastfeed starting at screening and for 28 days after the study vaccine administration.
6. Female subject must not donate ova starting at screening and for 28 days after the study vaccine administration.
7. A male subject with female partner(s) of childbearing potential must agree to use contraception as detailed in [Appendix 12.3 Contraception Requirements] at screening and for at least 28 days after the study vaccine administration.
8. Male subject must not donate sperm starting at screening and for 90 days after the study vaccine administration.
9. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding at screening and for 28 days after the study vaccine administration.
10. Subject agrees not to participate in another interventional study while participating in the present study.

Waivers to the inclusion criteria will **NOT** be allowed.

Exclusion:

A subject will be excluded from participation if any of the following apply:

1. Subject has a known or suspected hypersensitivity to ASP3772, its comparators or any components of the formulations used.
2. Subject has had previous exposure with ASP3772.
3. Subject has had known previous exposure with PPSV23.
4. Subject has received PCV13 or any other licensed or investigational pneumococcal vaccine at any time. (**Note**: This exclusion criterion is not applicable to Group 3; those subjects 65 to 85 years of age who previously received immunization with PCV13. Prior PCV13 immunization should have taken place no less than 10 months and no more than 2 years prior to study immunization. These subjects are eligible to be enrolled in the nonrandomized arm of Stage 2, Group 3).
5. Subject has a history of microbiologically-proven invasive disease caused by *Streptococcus pneumoniae*.
6. Subject has an immune disorder(s) (including autoimmune disease) and/or clinical conditions requiring immunosuppressive drugs.
7. Subject has any evidence of any unstable or active clinically significant cardiovascular, gastrointestinal, endocrine, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, renal and/or other major disease or malignancy, as judged by the investigator, e.g., uncontrolled hypertension, uncontrolled diabetes, heart failure, uncontrolled chronic obstructive pulmonary disease, end-stage renal disease.
8. Subject has history of illicit drug(s) or alcohol abuse that the investigator believes will interfere with the protocol requirements and/or a positive urine drug test (for Stage 1 subjects only) at screening.
9. Subject has any clinically significant history of allergic conditions including drug allergies, asthma or anaphylactic reactions, but excluding untreated asymptomatic seasonal allergies prior to vaccine administration.
10. Subject has a coagulation disorder contraindicating intramuscular immunization.
11. Subject has a positive serology test for hepatitis B surface antigen (HBsAg), hepatitis A virus antibodies (immunoglobulin M), hepatitis C virus antibodies (anti-HCV) confirmed by reflex testing (HCV-RNA) or antibodies to human immunodeficiency virus (HIV) type 1 and/or type 2 at screening.
12. Subject has/had febrile illness (> 100.4°F oral equivalent) or symptomatic, viral, bacterial (including upper respiratory infection) or fungal (noncutaneous) infection within 1 week prior to day 1.
13. Subject has any clinically significant abnormality following the investigator's review of the physical examination, electrocardiogram (ECG) and protocol defined clinical laboratory tests during screening.
14. Subject is unlikely to adhere to study procedures, keep appointments, is planning to relocate during the study or cannot be adequately followed for safety according to the protocol.
15. Subject has any other condition, which, in the opinion of the investigator, precludes the subject's participation in the study.

16. Subject has received any vaccines within 30 days prior of receipt of the study vaccine (exception: Inactivated influenza virus vaccine given according to recommended guidelines must have been given at least 7 days prior to receiving study vaccine).
17. Subject has had significant blood loss, donated 1 unit (450 mL or more) or received transfusion of any blood or blood products within 60 days or donated plasma within 7 days prior to day 1.
18. Subject has received any systemically absorbed antibiotics during the 7-day period prior to day 1.

Waivers to the exclusion criteria will **NOT** be allowed.

Investigational Product(s):

Study Vaccine:
ASP3772 is a suspension containing multiple pneumococcal capsular polysaccharide (PS) serotypes

Dose(s):
A single injection of 0.5 mL ASP3772 at either 1, 2 or 5 μg of each capsular PS serotype

Mode of Administration:
Intramuscular injection into the deltoid muscle of the right or left arm.

Comparative Vaccine(s):

Prevnar 13 (PCV13):
PCV13 pneumococcal capsular PS serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F in each 0.5 mL dose.

Dose(s):
A single injection of 0.5 mL suspension of PCV13 supplied in a single-dose prefilled syringe.

Mode of Administration:
Intramuscular injection into the deltoid muscle of the right or left arm.

Pneumovax 23 (PPSV23):
Pneumovax 23 (pneumococcal vaccine polyvalent) capsular PS from *S. pneumoniae* types 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, 33F in each 0.5 mL dose.

Dose(s):

A single injection of 0.5 mL solution of PPSV23 supplied in a single-dose prefilled syringe.

Mode of Administration:

Intramuscular injection into the deltoid muscle of the right or left arm.

Concomitant Medication Restrictions or Requirements:
Subjects will not be allowed to take immunizations, blood product, immunosuppressants including systemic steroids (topical steroids will be allowed) from first study vaccine administration until 30 days postimmunization. Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are allowed during the study, but not within 24 hours prior to immunization and collection of serum samples for immunogenicity. Subjects who are taking concomitant medications (3 months prior to study enrollment) to treat pre-existing conditions (e.g., hypertension) must remain on stable doses of these medications throughout the study. The doses of all concomitant medications should remain stable throughout the study, but may be changed for medical reasons as determined by the clinical investigator.

If during the study, a subject's health condition necessitates the use of any medication from those listed above, the investigator and medical monitor or designee(s), will discuss the case and determine if the subject should be withdrawn from the study. The excluded concomitant medication will be recorded as a protocol deviation. All concomitant medication including prescription and nonprescription, vitamins and natural and herbal remedies (e.g., St. John's Wort) will be documented.

Use of systemically absorbed antibiotics during the 30-day period prior to collection of the immunogenicity samples should be restricted unless clinically warranted (e.g., avoid prophylaxis) to avoid interference with the immunoassay. If a subject has been treated with any systemically absorbed antibiotics between day 1 and day 30, the day 30 follow-up visit should take place at least 7 days after the last dose of the antibiotics.

Subjects may receive other routine immunizations at the 30-day follow-up visit after the completion of all study procedures at each site's discretion; however, routine vaccines should not be given in less than 30 days poststudy immunization.

Duration of Study:

Stage 1:

Adults 18 to 64 years of age - PCV13 Naïve Subjects (Group 1):

Subjects will receive 1 immunization of ASP3772 or PCV13 administered on day 1. Follow-up study visits will be conducted at 7, 30 and 180 days postimmunization. The final study visit at day 180 may be conducted via telephone call.

Stage 2:

Elderly 65 to 85 years of age - PCV13 Naïve Subjects (Group 2):

Subjects will receive 1 immunization of ASP3772 or PCV13 administered on day 1.

Follow-up study visits will be conducted at 7, 30 and 180 days postimmunization. The final study visit at day 180 may be conducted via telephone call.

Elderly 65 to 85 years of age - Previous PCV13 Exposed Subjects (Group 3):

A third group of nonrandomized subjects previously vaccinated with PCV13 will be enrolled and receive a booster immunization with PPSV23 only on day 1. A follow-up visit will be conducted at 30 days postimmunization and this visit will also serve as the final study visit.

Formal Stopping Rules:

Early Termination of Subjects:

Individual subjects will be discontinued from the study if:

- Subject is lost-to-follow-up despite reasonable efforts by the investigator to locate the subject.
- Subject voluntarily withdraws from the study at any time for any reason.

Formal Stopping Rules for Cohorts:

No additional subjects will be randomized or dosed in the study until the events below occurring in a subject who received ASP3772 are reviewed by the DEC and the DEC recommends that it is acceptable to resume cohort immunization.

- Unexplained death of a subject.
- Acute hypersensitivity reaction in a subject.
- One of the sentinel subjects experiences an AE of severe intensity related to ASP3772, when sentinel subjects are required.
- An SAE of the same character occurs in at least 2 subjects or 1 subject experiences a vaccine-related SAE.

- Grade 3 or higher lab abnormality considered related to the immunization occurs in 2 or more subjects [FDA Guidance for Industry, 2007].
- The same type of reactogenicity event assessed as grade 3 or higher occurs in 2 or more subjects, or 1 of the sentinel subjects experiences a reactogenicity event assessed as grade 3 or higher, using the FDA toxicity grading scale for preventative vaccines [FDA Guidance for Industry, 2007].
- An imbalance of unsolicited AEs between ASP3772 and PCV13 is observed.

Decision Process for Dose Escalation:

After both sentinel subjects in a cohort have completed study procedures on day 7, the decision to open enrollment for the full cohort will be determined by DEC based on review of the Day 7 reactogenicity and safety data of those 2 sentinel subjects.

After all vaccinated subjects in a cohort have completed study procedures on day 7, the decision to dose escalate to next cohort within both stages will be determined by DEC based on the review of the following:

- Day 7 reactogenicity and safety data (AEs, ECG, clinical laboratory tests, vital signs) from the current cohort.
- Day 7 reactogenicity and safety data from all subjects of the preceding cohort(s).

To evaluate safety by DEC, day 7 data is needed from a minimum of 38 subjects in each cohort for Stage 1.

Stage 2 will commence based on DEC review of the following:

- Day 7 reactogenicity and safety data (AEs, ECG, clinical laboratory tests, vital signs) from all subjects in Stage 1.
- Day 30 safety data (AEs, ECG, clinical laboratory tests, vital signs) from all subjects in Stage 1.

To evaluate safety by DEC, day 7 data is needed from a minimum of 129 subjects in each cohort for Stage 2. Further details around the dose escalation process are included in the DEC charter.

Dose Escalation Criteria:

Depending on the nature, frequency and severity of the safety profile observed in the study, and the study vaccine received, the DEC will decide whether to:

- Proceed with dose escalation.
- Proceed from Stage 1 to Stage 2.
- Determine need of sentinel dosing in Stage 2 cohorts.
- Stop dose escalation.

Dose escalation may proceed unless 1 (or more) of the following apply in subject(s) receiving ASP3772:

- Two or more subjects experience an AE of severe intensity related to ASP3772 or 2 or more subject(s) experience a reactogenicity event of grade 3 or higher intensity related to ASP3772.
- One or more subject(s) experience a SAE related to ASP3772.

Endpoints for Evaluation:

Stage 1:

Primary Endpoints

Safety and tolerability of ASP3772 will be assessed by:

- Treatment-emergent adverse events (TEAEs) including MAAEs, PIMMCs, NOCDs
- Vital signs (body temperature, blood pressure and pulse rate)
- Reactogenicity assessed using solicited adverse reactions recorded daily by the subject through day 7 postimmunization. These include local and systemic reactions with a predefined grading scale. Local reactions are pain, tenderness, redness/erythema and swelling/induration. Systemic reactions are nausea/vomiting, diarrhea, headache, fever, fatigue, and muscle discomfort or pain/myalgia. Joint pain/arthralgia through day 7 postimmunization will be assessed once by subject recall at the day 7 visit.

Additional safety assessments include laboratory values, ECGs and physical examinations.

Secondary Endpoints

Endpoints used to assess the immunological response on day 30:

- 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 PS IgG for each serotype

Endpoints used to assess the immunological response on day 30 for the 11 serotypes unique to ASP3772:

- 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 expressed as an OPA titer^{-1}.

Stage 2 Group 2:

Primary Endpoints

Safety and tolerability of ASP3772 will be assessed by:

- TEAEs including MAAEs, PIMMCs, NOCDs
- Vital signs (body temperature, blood pressure and pulse rate)
- Reactogenicity assessed using solicited adverse reactions recorded daily by the subject through day 7 postimmunization. These include local and systemic reactions with a predefined grading scale. Local reactions are pain, tenderness, redness/erythema and swelling/induration. Systemic reactions are nausea/vomiting, diarrhea, headache, fever, fatigue, joint pain/arthralgia, and muscle discomfort or pain/myalgia.

Additional safety assessments include laboratory values, ECGs and physical examinations.

Secondary Endpoints

Endpoints used to assess the immunological response on day 30:

- Functional OPA activity for each serotype characterized by an OPA titer^{-1} response
- Pneumococcal serotype-specific anticapsular PS IgG for each serotype
- For each of the serotypes unique to ASP3772, the GMFR in anticapsular PS IgG at 30 days relative to pre-immunization
- For each of the 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
- Dose response in the OPA titer^{-1} for ASP3772
- Dose response in the 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 30 days after administration of PPSV23:

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

Statistical Methods:

Sample Size Justification

The sample size in Stage 1 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 495 evaluable adult subjects from 65 to 85 years of age are planned to be enrolled. 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 100 subjects treated with ASP3772 under these assumptions was considered sufficient to provide good estimates of OPA titer^{-1} responses.

Analysis of Safety and Tolerability

Stage 1 and Stage 2 (Group 2):

Safety analyses will be performed on the safety population; all subjects who receive study immunization. Data from PCV13 subjects in each cohort will be pooled for comparison with the ASP3772 groups. For each immunization arm, the frequencies and percentages will be displayed for the following TEAEs (coded using the Medical Dictionary for Regulatory Activities [MedDRA]) by system organ class and preferred term:

- Overall
- Serious
- Related (yes or no) to study vaccine

Descriptive statistics for each laboratory test (e.g., hematology, biochemistry and urinalysis) and vital signs will be tabulated by immunization group, dose level for ASP3772 and scheduled time point. MAAEs, if any, will be summarized.

For each local and systemic solicited reaction the percentage of subjects who reported each symptom by grade (None, Grade 1, 2, 3 or 4) will be summarized (n, %) by immunization group (PCV13 vs ASP3772) and dose level (ASP3772: 1, 2, 5 µg) and the percentage of subjects who reported any symptom.

Analysis of Immunogenicity

Stage 1:

The following measures in adults 18 to 64 years of age will be used to characterize the immunological response 30 days following administration of either ASP3772 or PCV13. The following measures will be provided for each immunization group (PCV13 vs ASP3772) and dose level (ASP3772: 1, 2, 5 µg).

- The GMT, GSD and 95% confidence interval (CI) for functional OPA for each serotype will be calculated.
- The ratio of the OPA GMT (each ASP3772 dose level/PCV13) and 95% CI will be provided.
- The geometric mean concentration (GMC), GSD and 95% CI for serotype-specific IgG for each of the serotypes will be calculated.
- The ratio of the IgG GMC (each ASP3772 dose level/PCV13) and 95% CI will be provided.
- The GMFR, GSD and 95% CI will be calculated for each of the serotypes unique to ASP3772.
- The proportion of subjects with a ≥ 4-fold increase in anticapsular PS IgG and OPA relative to baseline will be calculated.

Stage 2; Group 2:

- The GMT, GSD and 95% CI for functional OPA activity for each serotype will be calculated.
- The ratio of the GMT for functional OPA (each ASP3772 dose level/PCV13) for each serotype and the 95% CI will be provided.
- The GMC, GSD and 95% CI for serotype-specific IgG for each of the serotypes will be calculated.
- The ratio of the GMC (each ASP3772 dose level/PCV13) and 95% CI will be provided for each serotype.
- The GMFR, GSD and 95% CI will be calculated for each of the serotypes unique to ASP3772.
- The proportion of subjects with a ≥ 4-fold increase in anticapsular PS IgG and OPA relative to baseline will be calculated.
- To assess the impact of ASP3772 dose, an analysis of covariance (ANCOVA) model with age (65 - 74, 75 - 85) as the covariate and immunization (ASP3772 1, 2, 5 µg or PCV13) as the factor of interest will be conducted on the natural logarithm (log) of pneumococcal OPA titers.
- To assess the impact of ASP3772 dose, an ANCOVA model with age (65 - 74, 75 - 85) as the covariate and immunization (ASP3772 1, 2, 5 µg or PCV13) as the factor of interest will be conducted on the natural logarithm (log) of the IgG concentrations.

In addition, for both Stage 1 Group 1 and Stage 2 Group 2, SP1500+0785 levels (ng/mL) will be collected for those subjects given either ASP3772 or PCV13. Levels of the following cytokines will be collected for subjects given ASP3772 or PCV13: Th17, IL-17, CCI IL-22, CCI [redacted] The units for all are pg/mL. Non-parametric methods will be used for these analyses. A Kruskal-Wallis test will be used to test for overall differences between the 3 dose levels and PCV13. All treatment groups in Stage 1 will be compared, followed by paired comparisons between each of the ASP3772 dose levels and PCV13 using a Wilcoxon Test. The same set of comparisons will be made for subjects in Stage 2, Group 2.

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:

- The GMFR, GSD and 95% CI
- The proportion of subjects with a ≥ 4-fold increase in anticapsular PS IgG and OPA relative to baseline
- The GMT, GSD and 95% CI for the OPA titers

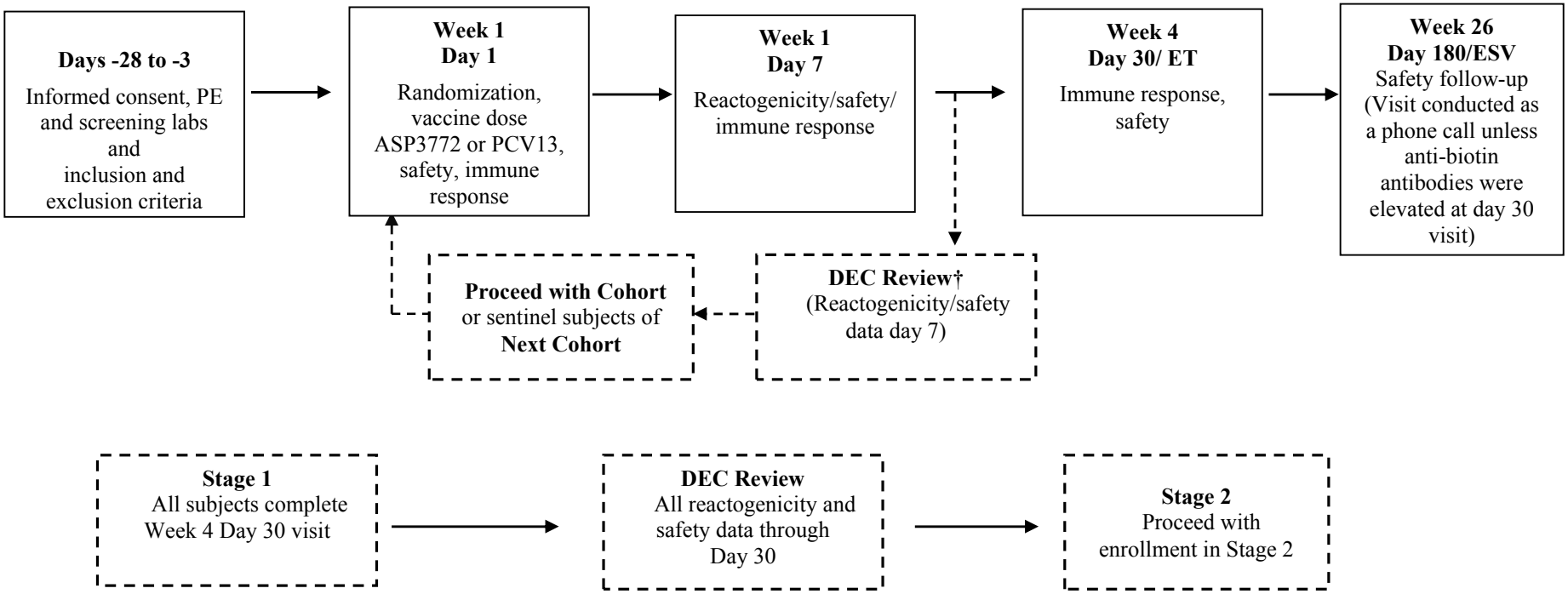
The GMC, GSD and 95% CI for serotype-specific IgG levels.

Pharmacokinetics: Not applicable.
Pharmacodynamics: Not applicable.
Interim analyses: No formal interim analysis for efficacy is planned. Safety data will be provided for DEC review after each dose cohort for the adult and elderly populations for dose escalation decisions. Prior to initiating the enrollment of the elderly subjects, the DEC will review the available data through 30 days post immunization in adult subjects. Details will be provided in the DEC Charter.

V. FLOW CHART AND SCHEDULE OF ASSESSMENTS

Flow Chart A

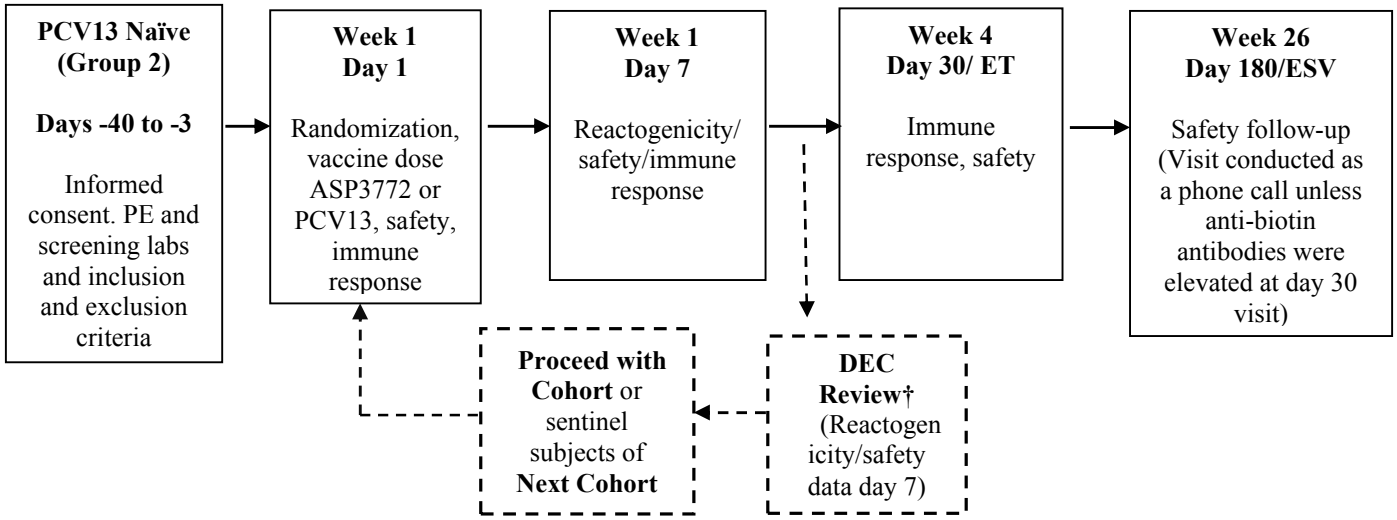
Stage 1

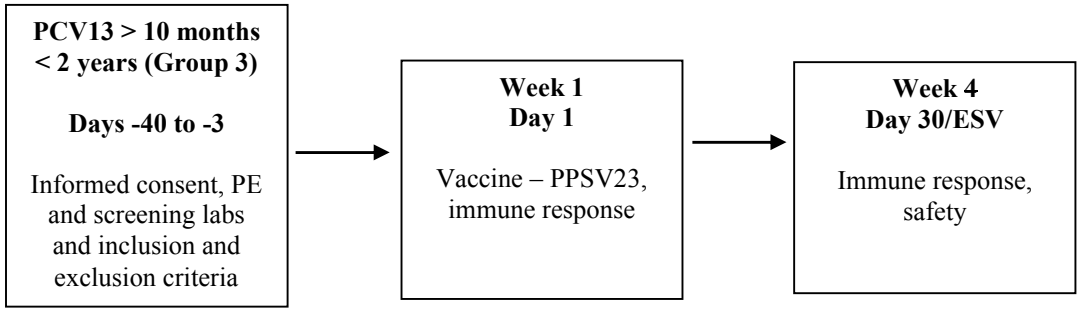


DEC: Dose Escalation Committee; ESV: end-of-study visit; ET: early termination; PCV13: Prevnar 13; PE: physical examination.
† DEC will review day 7 data for sentinel subjects before opening full cohort.

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]		←	—	—	→

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.

Footnotes continued on next page

† 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 the 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, 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 the 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 [redacted]

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 [redacted] 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 12.7 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 incorporated into the 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 ; 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

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 12.7 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			
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 ; 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.

1 INTRODUCTION

Streptococcus (S.) pneumoniae is a significant cause of morbidity and mortality among infants, young children, the elderly and individuals who have certain underlying medical conditions. *S. pneumoniae* remains a leading cause of serious illness, including bacteremia, meningitis and pneumonia among children and adults worldwide. It is also the most frequent cause of sinusitis and acute otitis media [CDC, 2010]. Pneumococcal disease can cause both invasive and noninvasive disease. The most common form of noninvasive disease, nonbacteremic pneumococcal pneumonia, remains one of the most frequent disease manifestation accounting for pneumonia hospitalizations. Invasive pneumococcal disease (IPD) is defined as *S. pneumoniae* isolated from a normally sterile site (e.g., cerebrospinal fluid, blood, joint fluid, pleural fluid or peritoneal fluid). The highest incidence of IPD is found at the extremes of age – in elderly adults and in young children < 2 years of age. *S. pneumoniae* causes approximately 17000 cases of invasive disease each year among children younger than 5 years of age, including 700 cases of meningitis and 200 deaths [CDC, 2000]. The highest morbidity and mortality rates have been reported in developing countries, but the disease burden is also considerable in industrialized countries.

Two different vaccines for *S. pneumoniae* are currently available in the US. PCV13, a 13-valent conjugate vaccine, has been approved for the prevention of IPD caused by the 13 serotypes contained in the vaccine in children and for the prevention of pneumonia and IPD in adults. In this vaccine, conjugation of pneumococcal saccharides to the CRM197 protein creates a saccharide-protein complex, which is capable of inducing a T-dependent immune response; thus, T helper cells are stimulated, leading to a substantial primary response and a strong booster response at re-exposure [PREVNAR 13 prescribing information, 2017]. However, there are > 90 serotypes of *S. pneumoniae*, and PCV13 provides protection only against those 13 serotypes contained in the vaccine. While infections with *S. pneumoniae* of multidrug-resistant serotypes, which are contained in PCV13 decreased after availability of this vaccine, an increase of infections with multidrug-resistant serotypes 35B, 23A, 23B and 15B, which are not included in PCV13, was noted. Also, PCV13 appeared to have marginal activity against serotype 3, as its prevalence persists in the population [Richter et al, 2014].

The second vaccine, Pneumovax®23 (PPSV23), is a 23-valent polysaccharide (PS) vaccine and is indicated for the prevention of pneumococcal disease in adults ≥ 50 years of age or in persons ≥ 2 years of age at increased risk of pneumococcal disease. It is composed of purified capsular PS from 23 pneumococcal serotypes. While this vaccine protects against more serotypes when compared to PCV13, it does not provide protection against the emerging serotypes 35B, 23A and 23B. In addition, PPSV23 elicits a T-independent PS immune response that stimulates mature B-lymphocytes, but not T-lymphocytes, and induces an immune response that is neither long-lasting nor anamnestic upon subsequent challenge. In addition, PS-type vaccines are not used in infants and children < 2 years of age, because these children respond poorly to T-independent antigens [Pneumovax 23 prescribing information, 2017; CDC, 2010]. Data suggest that PPSV23 protects adults and the elderly against IPD; however, no consistent effect has been observed in the prevention of pneumonia [Gruber et al, 2008].

Thus, there is a medical need for a vaccine that provides T-cell dependent immunity against a broad range of serotypes of *S. pneumoniae*.

1.1 Background

ASP3772 is an investigational vaccine that contains 24 serotypes of *S. pneumoniae* and a unique carrier protein (CP1) created by the fusion of 2 pneumococcal proteins (SP1500 and SP0785), which has the potential to induce a T- and B-cell response providing extended protection (refer to details in the [ASP3772 Investigator's Brochure]). This novel pneumococcal vaccine is based on multivalent multiple antigen-presenting system (MAPS) platform containing each of the 13 pneumococcal capsular PS contained in PCV13 (serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F and 23F) and 11 additional pneumococcal capsular PS (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F), individually biotinylated and complexed with a CP1 carrier protein. In nonclinical studies, MAPS vaccines have also been shown to induce antigen-specific Th1/Th17 responses, which efficiently protect against pneumococcal disease [Zhang et al, 2013].

The MAPS platform takes advantage of the high affinity (dissociation constant [KD] $\approx 10^{-15}$M) noncovalent binding between biotin and rhizavidin, a biotin-binding protein, which has no significant predicted homology with human proteins. Rhizavidin, a naturally occurring dimeric protein in the avidin protein family, was first discovered in *Rhizobium etli*, a symbiotic bacterium of the common bean. Rhizavidin has only a 22% amino acid identity with chicken avidin, a protein commonly found in eggs, but with high conservation of amino acid residues involved in biotin binding. No cross-reactivity to rhizavidin was observed in human serum samples obtained from subjects exposed to avidin [Helppolainen et al, 2007], suggesting that rhizavidin antibodies may not cross-react with chicken avidin. Biotin conjugates have been used in several clinical applications without any reported adverse events (AEs) [Buller et al, 2014; Paty et al, 2010; Lazzeri et al, 2004]. The biotinylation of the PS and MAPS complexing process in ASP3772 has been optimized to consistently show no free biotin, thus reducing the potential for generating anti-biotin antibodies.

The total amount of aluminum per dose in ASP3772 vaccine formulation is expected to be 0.625 mg, which is below the FDA/WHO maximum recommended dose of 0.85 mg to 1.25 mg.

CCI

1.2 Nonclinical and Clinical Data

The nonclinical pharmacology data demonstrated that ASP3772 is highly immunogenic in New Zealand White rabbits. The literature reported for conjugated pneumococcal vaccines suggests that an immunogenic response elicited in nonclinical programs is translatable to an immunogenic response in humans. The successful translation between the rabbit immunogenicity studies and human efficacy observed for conjugated pneumococcal vaccines supports the position that ASP3772 has the potential to be clinically effective. There were no treatment-related effects on survival, clinical signs, skin reaction, body weight/food consumption, ophthalmology, hematology parameters, serum biotin, anti-biotin antibodies or organ weights. With the exception of the injection sites, there were no treatment-related macroscopic post-mortem or histopathological findings in the tissues/organs examined. Analysis of post-dose sera confirmed that animals treated with ASP3772 had robust serum immunoglobulin G (IgG) antibody responses to all pneumococcal polysaccharides included in the ASP3772.

In the pharmacology studies, intramuscular administration of ASP3772 in rabbits resulted in a higher immune response (antibody production) in the ASP3772 group in comparison to the PCV13 group not only for a majority of the PCV13 serotypes, but also for non-PCV13 serotypes contained in ASP3772. Antibody responses to the pneumococcal proteins were also robust in the ASP3772 group compared to the levels seen in the PCV13 group in rabbits.

Intranasal administration with either purified pneumococcal proteins (components of carrier protein) in mice induced a strong antigen-specific Th17 response and a significant reduction in pneumococci colony-forming units recovered from the nasopharyngeal wash following challenge with type 6B pneumococci (603 strain). Similar responses were observed in subcutaneous immunization of 12-valent and 6-valent MAPS with CP1 carrier protein in mice. These data suggest that ASP3772 has the potential to be clinically effective at protecting against pneumococcal infection following immunization.

Please refer to the current Investigator's Brochure (IB) for the most recent data.

1.3 Summary of Key Safety Information for Study Drugs

No clinical studies of ASP3772 have been conducted to date.

In the 5-cycle intramuscular dose toxicity study in rabbits (3772-TX-0001), a total of 5 intramuscular injections of ASP3772 at dose level of 5 µg of each PS per animal given once every 2 weeks to male and female New Zealand White rabbits over a period of 57 days was well-tolerated. There were no treatment-related effects on survival, clinical signs, skin reaction, body weight/food consumption, ophthalmology, hematology parameters, serum biotin, anti-biotin antibodies or organ weights. With the exception of the injection sites, there were no treatment-related macroscopic post-mortem or histopathological findings in the tissues/organs examined. Analysis of postdose sera confirmed that animals treated with ASP3772 had robust serum IgG antibody responses to all pneumococcal PS included in the ASP3772.

In animals treated with ASP3772, slightly elevated body temperature, changes in clinical pathology and histopathological findings at the injection sites were observed. The elevated body temperature and changes in clinical pathology such as reductions in prothrombin time and/or activated partial thromboplastin time, as well as increased fibrinogen concentrations, and decreased albumin and albumin/globulin ratio, and increased globulin, creatine kinase and C-reactive protein levels were considered to be consistent with the immune and/or inflammatory reactions induced by ASP3772 administration, which are expected responses in intramuscular immunization.

Microscopic findings at the intramuscular injection sites included ASP3772-related findings consisting of minimal to moderate myofiber degeneration/necrosis, minimal to mild histiocytic inflammation (muscle) and minimal histiocytic inflammation (subcutis), as well as ASP3772-related exacerbation of local injection site findings that included minimal to moderate mixed cell inflammation (subcutis), minimal to marked mixed cell inflammation (muscle), minimal to mild mixed cell inflammation (dermis), minimal to moderate hemorrhage (subcutis), minimal to mild hemorrhage (muscle) and minimal serocellular crusts (epidermis). These changes recovered or tended to recover after a 4-week recovery period suggesting that the ASP3772-related findings were reversible.

In clinical studies, measurement of body temperature, monitoring of subjective symptoms such as pain and soreness, observation of injection sites, evaluation of clinical pathology parameters including coagulation, clinical chemistry and C-reactive protein will be monitored [Table 4].

Table 4 Summary of Potential Safety Concerns from Nonclinical Studies

Potential Safety Concern	Relevance to Human Usage
Elevation of body temperature	Monitoring of body temperature
Changes in clinical pathology (coagulation parameters, creatine kinase and C-reactive protein)	Monitoring of clinical laboratory measures
Changes at the injection sites	Observation of local injection reactions

Please refer to the current IB for key safety information.

1.4 Risk Benefit Assessment

Subjects participating in this clinical study may not benefit from administration of ASP3772. In contrast, subjects might experience AEs related to study vaccines (e.g., allergic reactions) or procedural complications (e.g., blood draws or slight skin irritation from the adhesive on the electrocardiogram [ECG] electrodes).

A summary of the nonclinical safety findings and risk minimization and characterization actions are given in Table 4. Overall, the toxicological target organs and AEs that were identified in the nonclinical toxicology studies will be carefully monitored so that subjects will not be exposed to undue risks. However, since this is the first study of this vaccine in humans, the risk profile in humans is unknown.

2 STUDY OBJECTIVE(S), DESIGN AND ENDPOINTS

2.1 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.

2.2 Study Design and Dose Rationale

2.2.1 Study Design

Study Design Overview:

This is a combined phase 1, first-in-human (FIH), dose-escalation (Stage 1) and phase 2, dose-finding (Stage 2), observer-blinded 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 PCV13 naïve 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 that will receive PPSV23.

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: A total of approximately 495 adult subjects from 65 to 85 years of age.

Number of Subjects per Cohort by Immunization

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

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

PPSV23: Pneumovax 23

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. All subjects within each cohort will remain under observation for approximately 1 hour post immunization, provided no reactions have occurred that require additional observation in the opinion of the investigator. The DEC membership will include Astellas Medical Science, Pharmacovigilance and Statistics personnel, as well as external independent safety physician(s). The external safety physician will be selected based on expertise in vaccine clinical studies and other considerations such as the disease area. A DEC charter will detail the roles, responsibilities and procedures for decision-making.

See the Schedule of Assessments [Table 1, Table 2 and Table 3] for details on procedures performed at each study visit for Group 1, Group 2 and Group 3, respectively.

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). After screening, subjects will be enrolled sequentially into 3 cohorts designated by escalating ASP3772 dose levels. A single dose of ASP3772 will be administered as a 0.5 mL intramuscular injection into the deltoid muscle of the right or left arm on day 1 at 1 of 3 dose levels: 1, 2 or 5 μg of ASP3772 for cohorts 1, 2 and 3 respectively. 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. 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.

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 NOCDs will continue through day 180 postimmunization. Data will be collected in an observer-blinded manner (vaccine recipients and any site staff who is responsible for evaluation of any study endpoint will be unaware of which vaccine the subject received).

Serum samples to measure IgG and opsonophagocytic activity (OPA) will be collected prior to the first study immunization on day 1 and on day 30 postimmunization.

Stage 2 Group 2:

Stage 2 of the study is a dose-finding (sequentially higher doses), active-controlled, observer-blinded study with 3:1 randomization (ASP3772:PCV13) in elderly subjects 65 to 85 years of age.

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 injection into the deltoid muscle of the right or left arm 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.

Approximately 396 subjects will be enrolled in Stage 2 of the study; 132 subjects per cohort (ASP3772 n = 99 and PCV13 n = 33).

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. 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 immunized with ASP3772 or PCV13 with immunogenicity data across the elderly population.

Safety and tolerability assessments including 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 is 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 immunization on day 1 and postimmunization on day 30.

Primary immunogenicity evaluation for dose finding is 30 days following the initial immunization.

Stage 2 Group 3:

A separate group of 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 vaccine administration. Immunogenicity to the 11 non-PCV13 serotypes contained in PPSV23 will be evaluated.

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.

2.2.2 Dose Rationale

The proposed initial doses of 1, 2 and 5 μg for this FIH clinical study were chosen based on the PS levels contained in marketed PS conjugate vaccines and the ASP3772 nonclinical pharmacology and toxicology studies.

The 3 currently licensed pneumococcal conjugated vaccines vary in the number of serotypes they contain, the carrier proteins used and the methods of conjugation employed. The 7-valent formulation (Pfizer's Prevnar 7/Prevnar 7) with all serotypes conjugated to diphtheria toxoid mutant (CRM197), using a reductive animation conjugation method, was first licensed in the year 2000. The 10-valent formulation (GlaxoSmithKline Biologicals' Synflorix), with 8 serotypes conjugated to Protein D, a recombinant non-lipidated form of a highly conserved 40 kDa cell-surface lipoprotein of non-typeable *Haemophilus influenzae,* serotype 18C conjugated to tetanus toxoid and serotype 19F conjugated to diphtheria toxoid via bifunctional spacer, was licensed in Europe in 2009. The 13-valent formulation (Pfizer's PCV13/PCV13), with all serotypes conjugated to CRM197 using reductive animation, was licensed in 2010. In addition, Merck initiated the development of a 15-valent formulation (V114), using the PS dose ranges used previously by the commercially available pneumococcal conjugated vaccines. In all of these cases, the PS doses ranged from 1 μg to 4.4 μg for each of the serotypes. A nonclinical immunogenicity study with ASP3772 and PCV13 at one-tenth of the human PCV13 dose for each PS showed that ASP3772

serotype-specific responses to ASP3772 were comparable to those elicited by PCV13 for the 13 serotypes in common between the vaccines. For the 11 noncommon serotypes, there was a robust response elicited in rabbit for ASP3772 and no response for PCV13. In the toxicology study in New Zealand White rabbits, ASP3772 was administered at 5 μg of each of the 24 PS every 2 weeks, for a total of 5 intramuscular injections (5 cycles). The findings were mild in nature, and monitorable and reversible.

The above information, coupled with the literature showing successful translation between the rabbit immunogenicity studies and human efficacy for pneumococcal vaccines, support the position that ASP3772 has the potential to be clinically effective at the proposed doses (1 μg, 2 μg and 5 μg).

2.3 Endpoints

Stage 1:

Primary Endpoints

Safety and tolerability of ASP3772 will be assessed by:

- Treatment-emergent adverse events (TEAEs) including MAAEs, PIMMCs and NOCDs
- Vital signs (body temperature, blood pressure and pulse rate)
- Reactogenicity assessed using solicited adverse reactions recorded daily by the subject through day 7 postimmunization. These include local and systemic reactions with a predefined grading scale. Local reactions are pain, tenderness, redness/erythema and swelling/induration. Systemic reactions are nausea/vomiting, diarrhea, headache, fever, fatigue, and muscle discomfort or pain/myalgia. Joint pain/arthralgia through day 7 postimmunization will be assessed once by subject recall at the day 7 visit.

Additional safety assessments include laboratory values, ECGs and physical examinations.

Secondary Endpoints

Endpoints used to assess the immunological response on day 30 for the 13 serotypes contained in PCV13:

- 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 PS IgG for each serotype

Endpoints used to assess the immunological response on day 30 for the 11 serotypes unique to ASP3772:

- 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 expressed as an OPA titer^{-1}

Stage 2 Group 2:

Primary Endpoints

Safety and tolerability of ASP3772 will be assessed by:

- TEAEs including MAAEs, PIMMCs, and NOCDs
- Vital signs (body temperature, blood pressure and pulse rate)
- Reactogenicity assessed using solicited adverse reactions recorded daily by the subject through day 7 postimmunization. These include local and systemic reactions with a predefined grading scale. Local reactions are pain, tenderness, redness/erythema and swelling/induration. Systemic reactions are nausea/vomiting, diarrhea, headache, fever, fatigue, joint pain/arthralgia and muscle discomfort or pain/myalgia.

Additional safety assessments include laboratory values, ECGs and physical examinations.

Secondary Endpoints

Endpoints used to assess the immunological response on day 30:

- Functional OPA activity for each serotype characterized by an OPA titer^{-1} response
- Pneumococcal serotype-specific anticapsular PS IgG for each serotype
- For each of the serotypes unique to ASP3772, the GMFR in anticapsular PS IgG at 30 days relative to pre-immunization
- For each of the 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
- Dose response in the OPA titer^{-1} for ASP3772
- Dose response in the 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}
- Pneumococcal serotype-specific anticapsular PS IgG

3 STUDY POPULATION

3.1 Selection of Study Population

The study population will consist of adult subjects 18 to 64 years of age in Stage 1 and elderly subjects 65 to 85 years in Stage 2.

The study population will consist of 3 different groups:

- **Group 1** (approximately 120 evaluable subjects) **-** Stage 1 PCV13 naïve subjects randomized within 3 sequential cohorts to ASP3772 or PCV13;

- **Group 2** (approximately 396 evaluable subjects) - Stage 2 PCV13 naïve subjects randomized within 3 sequential cohorts to ASP3772 or PCV13; and
- **Group 3** (approximately 99 evaluable subjects) - Stage 2 subjects previously vaccinated with PCV13 that will receive PPSV23.

The verification of inclusion and exclusion criteria for a subject's participation in this study is to be determined by the investigator or a subinvestigator.

3.2 Inclusion Criteria

A subject is eligible for the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] Authorization for US sites) must be obtained from the subject prior to any study-related procedures (including withdrawal of prohibited medications, if applicable).
2. Stage 1: Subject is healthy male or female between 18 and 64 year of age inclusive, at screening.
3. Stage 2: Subject is a male or female between 65 and 85 years of age, inclusive, at screening who is healthy or has chronic controlled, stable disease with no change in disease severity, medical therapy and no hospitalization records in last 12 weeks as determined by medical history, physical examination and laboratory data and the clinical judgment of the Investigator.
4. A female subject is eligible to participate if she is not pregnant (see [Appendix 12.3 Contraception Requirements]) and at least 1 of the following conditions applies:
    a. Not a woman of childbearing potential (WOCBP) as defined in [Appendix 12.3 Contraception Requirements]

       OR

    b. WOCBP who agrees to follow the contraceptive guidance as defined in [Appendix 12.3 Contraception Requirements] at screening and for at least 28 days after the study vaccine administration.
5. Female subject must agree not to breastfeed starting at screening and for 28 days after the study vaccine administration.
6. Female subject must not donate ova starting at screening and for 28 days after the study vaccine administration.
7. A male subject with female partner(s) of childbearing potential must agree to use contraception as detailed in [Appendix 12.3 Contraception Requirements] at screening and for at least 28 days after the study vaccine administration.
8. Male subject must not donate sperm starting at screening and for 90 days after the study vaccine administration.

9. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding at screening and for 28 after the study vaccine administration.
10. Subject agrees not to participate in another interventional study while participating in the present study.

Waivers to the inclusion criteria will **NOT** be allowed.

3.3 Exclusion Criteria

A subject will be excluded from participation if any of the following apply:

1. Subject has a known or suspected hypersensitivity to ASP3772 its comparators or any components of the formulations used.
2. Subject has had previous exposure with ASP3772.
3. Subject has had known previous exposure with PPSV23.
4. Subject has received PCV13 or any other licensed or investigational pneumococcal vaccine at any time. (**Note**: This exclusion criterion is not applicable to Group 3; those subjects 65 to 85 years of age who previously received immunization with PCV13. Prior PCV13 immunization should have taken place no less than 10 months and no more than 2 years prior to study vaccine administration. These subjects are eligible to be enrolled in the nonrandomized arm of Stage 2, Group 3.
5. Subject has a history of microbiologically-proven invasive disease caused by *S. pneumoniae*.
6. Subject has an immune disorder(s) (including autoimmune disease) and/or clinical conditions requiring immunosuppressive drugs.
7. Subject has any evidence of any unstable or active clinically significant cardiovascular, gastrointestinal, endocrine, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, renal and/or other major disease or malignancy, as judged by the investigator, e.g., uncontrolled hypertension, uncontrolled diabetes, heart failure, uncontrolled chronic obstructive pulmonary disease, end-stage renal disease.
8. Subject has history of illicit drug(s) or alcohol abuse that the investigator believes will interfere with the protocol requirements and/or a positive urine drug test (for Stage 1 subjects only) at screening.
9. Subject has any clinically significant history of allergic conditions including drug allergies, asthma or anaphylactic reactions, but excluding untreated asymptomatic seasonal allergies prior to study vaccine administration.
10. Subject has a coagulation disorder contraindicating intramuscular immunization.
11. Subject has a positive serology test for hepatitis B surface antigen (HBsAg), hepatitis A virus antibodies (immunoglobulin M), hepatitis C virus antibodies (anti-HCV) confirmed

by reflex testing (HCV-RNA) or antibodies to human immunodeficiency virus (HIV) type 1 and/or type 2 at screening.

12. Subject has/had febrile illness (> 100.4°F oral equivalent) or symptomatic, viral, bacterial (including upper respiratory infection) or fungal (noncutaneous) infection within 1 week prior to day 1.
13. Subject has any clinically significant abnormality following the investigator's review of the physical examination, ECG and protocol defined clinical laboratory tests during screening.
14. Subject is unlikely to adhere to study procedures, keep appointments, is planning to relocate during the study or cannot be adequately followed for safety according to the protocol.
15. Subject has any other condition, which, in the opinion of the investigator, precludes the subject's participation in the study.
16. Subject has received any vaccines within 30 days prior of receipt of the study vaccine (exception: Influenza virus vaccine given according to recommended guidelines must be given at least 7 days prior to receiving study vaccine).
17. Subject has had significant blood loss, donated 1 unit (450 mL or more) or received transfusion of any blood or blood products within 60 days or donated plasma within 7 days prior to day 1.
18. Subject has received any systemically absorbed antibiotics during the 7-day period prior to day 1.

Waivers to the exclusion criteria will **NOT** be allowed.

4 IMMUNIZATION(S)

4.1 Identification of Investigational Products

4.1.1 Study Vaccine (ASP3772)

ASP3772 is a suspension containing 24 pneumococcal capsular PS serotypes. It consists of biotinylated PS complexed with a carrier protein. ASP3772 is supplied as a sterile suspension in single-dose vials and appears as grey/white suspension. It is administered as a single intramuscular injection via needle and syringe into the deltoid muscle of the right or left arm.

Each 0.5 mL dose of ASP3772 drug product is formulated to contain 1, 2 or 5 µg of each *S. pneumoniae* serotype 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 PS as MAPS complexes. ASP3772 will be supplied by Astellas as single-use vials with respective dose. ASP3772 should be stored in refrigerator at 2°C to 8°C protected from exposure to light. Aseptic technique must be used for the preparation of ASP3772.

Detailed instructions for the storage, handling, preparation and administration of the ASP3772 injectable solution along with instructions for storage, handling and use of syringe and needle are provided in the pharmacy manual.

4.1.2 Comparative Vaccine(s)

4.1.2.1 Prevnar 13

Prevnar 13 (PCV13) is a suspension of pneumococcal capsular antigens of *S. pneumoniae* PS serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F individually linked to a non-toxic diphtheria CRM197 protein. It is administered as a single intramuscular injection of 0.5 mL into the deltoid muscle of the right or left arm. PCV13 is supplied in a single-dose prefilled syringe by Astellas.

Detailed instructions for the storage, handling, preparation and administration of the Prevnar 13 prefilled syringe along with instructions for storage, handling and use of preferred syringe and needle are provided in the pharmacy manual or in the package insert.

4.1.2.2 Pneumovax 23

PPSV23 (pneumococcal vaccine polyvalent) is a capsular PS from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, 33F in each 0.5 mL dose. It is administered as a single intramuscular injection of 0.5 mL solution into the deltoid muscle of the right or left arm. PPSV23 is supplied in a single-dose prefilled syringe by Astellas.

Detailed instructions for the storage, handling, preparation and administration of the PPSV23 prefilled syringe along with instructions for storage, handling and use of preferred syringe and needle are provided in the pharmacy manual or in the package insert.

4.2 Packaging and Labeling

All study vaccine(s) (ASP3772, PCV13 and PPSV23) used in this study will be prepared, packaged and labeled under the responsibility of qualified staff at Astellas Pharma Global Development Inc. (APGD) or sponsor's designee in accordance with APGD or sponsor's designee Standard Operating Procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, ICH Good Clinical Practice (GCP) guidelines and applicable local laws/regulations.

Each vial will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as investigational vaccine. The study centers should store ASP3772, PCV13 and PPSV23 in original provided carton or packing, protected from sunlight and at a temperature indicated in pharmacy manual.

4.3 Study Vaccine Handling

Current ICH GCP Guidelines require the investigator to ensure that study vaccine deliveries from the sponsor are received by the investigator/or designee and that:

- Such deliveries are recorded,
- Study vaccine is handled and stored according to labeled storage conditions,
- Study vaccine with appropriate expiry/retest and is only dispensed to study subjects in accordance with the protocol, and
- Any unused study vaccine is returned to the sponsor.

Study vaccine inventory and accountability records will be kept by the study pharmacist. Study vaccine accountability throughout the study must be documented and reconciled. The following guidelines are therefore pertinent:

- The investigator, study pharmacist agrees not to supply study vaccines to any persons except the eligible subjects in this study in accordance with the protocol.
- The study pharmacist or designee will keep the study vaccines in a pharmacy or other locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense these study vaccines.
- A study vaccine inventory will be maintained by the study pharmacist or designee. The inventory will include details of material received and a clear record of when they were dispensed and to which subject.
- At the conclusion or termination of this study, study pharmacist or designee agrees to conduct a final vaccine supply inventory and to record the results of this inventory on the Vaccine Accountability Record or equivalent form in Interactive Response Technology (IRT) system. It must be possible to reconcile delivery records with those of used and/or returned study vaccine. Any discrepancies must be accounted for and documented. Appropriate forms of deliveries and returns must be signed by the site staff delegated this responsibility.
- The site staff must return study vaccine to the sponsor or designee at the end of the study or upon expiration unless otherwise approved by the sponsor.

4.4 Blinding

4.4.1 Blinding Method

This is an observer-blind study since the comparator has a unique syringe that the sponsor cannot duplicate. Subjects will be randomized at a 3:1 ratio (ASP3772:comparator vaccine PCV13) in a blinded fashion such that the investigator, clinical staff, nor the subject will know which agent is being administered. Only the pharmacist and designated staff, including the person(s) injecting the vaccine will be unblinded to study vaccine. The syringes will be masked prior to dosing to maintain the blind for subjects and all other personnel. The details regarding study blinding will be described in the pharmacy manual. The randomization number will be assigned based on information obtained from the IRT. The study will be unblinded to the investigator after the database lock.

The safety and tolerability data for dose escalation by the DEC will be reviewed under blinded conditions. If there are AEs that meet the criteria that could prohibit dose escalation, (i.e., a SAE, an AE of severe intensity, or 2 or more subjects experience a reactogenicity event of grade 3 or higher intensity), the study vaccine assignment for these subjects will be revealed to determine if these events are related to ASP3772. If 10% or more of the subjects within a cohort experience the same or related AE, the study vaccine assignment for those subjects will be revealed to determine if there is an imbalance in unsolicited AEs between ASP3772 and PCV13. The DEC can request the study vaccine assignment for 1 or more subjects.

4.4.2 Confirmation of the Indistinguishability of the Study Vaccines

Not applicable, as the comparator vaccines (PCV13 and PPSV23) are available in the form of pre-filled syringes and it is not possible to make the study vaccines indistinguishable. The investigational product and comparator will be sent to the site in an unblinded fashion for the unblinded staff at the site to handle.

4.4.3 Retention of the Assignment Schedule and Procedures for Study Vaccine Assignment Code Breaking

The randomization list and study medication blind will be maintained by the IRT system. The study pharmacist (or investigator's designee) will be unblinded to prepare vaccine for administration according to randomization code.

4.4.4 Breaking the Study Vaccine Assignment Code for Emergency

The study vaccine assignment code for each randomized subject will be provided by the IRT in the event of a medical emergency requiring knowledge of the study vaccine assigned to the subject. The IRT will be programmed with blind-breaking instructions that may only be requested by the investigator or subinvestigators designated to have access to perform blind-break. In case of a medical emergency, the investigator has the sole responsibility for determining if unblinding of subject's vaccine assignment is warranted. Subject safety must always be the first consideration in making such determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's vaccine assignment unless this could delay emergency treatment for the subject.

The investigator must have confirmed functionality to access code-break through the IRT system and must have a designated back up (e.g., redundant processes) to support emergency unblinding requirements.

Prior to randomization, subjects should be provided with information that includes the site emergency contact number and back-up contact number in case of a medical emergency. Any unblinding by the investigational staff must be reported immediately to the sponsor and include an explanation of why the study vaccine was unblinded. If unblinding is associated with a SAE the investigator is to follow the instructions in [Section 5.5.5 Reporting of SAE].

Care should be taken to limit knowledge of the randomization arm, in case this could affect the blinding of other subjects or future trial assessment for the subject.

4.4.5 Breaking the Study Vaccine Assignment Code by the Sponsor

The sponsor and DEC may break the study vaccine assignment code for subjects who experience a Suspected Unexpected Serious Adverse Reaction (SUSAR), in order to determine if the individual case or a group of cases requires expedited regulatory reporting. Individual Emergency Codes will be provided to the limited staff who are responsible to break the codes for all SUSAR cases for reporting purposes.

4.5 Assignment and Allocation

At the Screening Visit, after the informed consent form (ICF) has been signed, site staff will access the IRT system, enter the subject's required information, and the subject will receive a subject number assignment through the IRT for use throughout the study.

Each cohort will be initiated with randomization of 1 sentinel subject each assigned to either ASP3772 or PCV13. Sentinel subjects need to be replaced if discontinued or unable to follow up for at least 7 days. The safety data from sentinel subjects will be reviewed by the DEC to make a decision to proceed with enrollment of the rest of the cohort. Subjects in the full cohort who meet the inclusion/exclusion criteria will be randomized in a 3:1 ratio (ASP3772:PCV13) according to the randomization schedules through IRT. In Stage 2, the randomization to study vaccine assignment will be stratified by age. Prior to study immunization, the site staff will access the IRT system in order to determine the randomly assigned study vaccine. The site personnel will dispense the study vaccine according to the IRT system's assignment. Specific procedures for randomization through the IRT are contained in the study procedures manual.

Subjects who subsequently meet the inclusion/exclusion criteria will be randomly assigned to receive either ASP3772 or PCV13. The randomization to study vaccine will be stratified by age.

The IRT vendor will generate the randomization schedule. To obtain the randomized study vaccine assignment for a subject, the pharmacist or designee will utilize an IRT system, which is available 7 days a week, 24 hours a day. After submitting required information about the eligible subject, the vaccine kit number assignment will be provided.

Study vaccine assignment will remain blinded to all site staff except the pharmacist, designated staff and the unblinded administrator.

If a subject is randomized but does not receive study vaccine, the kit number will not be used again. In this instance, the study vaccine assignment should only be known by the pharmacist and designated staff (i.e., the blind must be maintained as it is with all other subjects).

5 IMMUNIZATIONS AND EVALUATION

5.1 Dosing and Administration of Study Vaccine and Other Medications

5.1.1 Dose/Dose Regimen and Administration Period

Stage 1:

Adults 18 to 64 years of age - PCV13 Naïve Subjects (Group 1):

Subjects randomized to ASP3772 will receive a single immunization of ASP3772 as a 0.5 mL intramuscular injection on day 1 at 1 of 3 dose levels: 1, 2 or 5 µg of ASP3772 for Cohorts 1, 2 and 3, respectively. The subjects randomized to PCV13 will receive a single injection of the standard dose of PCV13 on day 1. Follow-up study visits will be conducted at 7 and 30 days postimmunization. The final study visit at day 180 may be conducted via telephone call.

Stage 2:

Elderly 65 to 85 years of age - PCV13 Naïve Subjects (Group 2):

Subjects randomized to ASP3772 will receive a single immunization of ASP3772 administered as a 0.5 mL intramuscular 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 0.5 mL intramuscular injection of the standard dose of PCV13. Follow-up study visits will be conducted at 7 and 30 days postimmunization. The final study visit at day 180 may be conducted via telephone call.

Elderly 65 to 85 years of age - Previous PCV13 Exposed Subjects (Group 3):

A third group of nonrandomized subjects previously vaccinated with PCV13 will be enrolled and receive a booster immunization (single 0.5 mL intramuscular injection) with PPSV23 on day 1. A follow-up visit will be conducted at 30 days postimmunization and this visit will also serve as the final study visit.

5.1.2 Increase or Reduction in Dose of the Study Vaccine(s)

Decision Process for Dose Escalation:

After both sentinel subjects in a cohort have completed study procedures on day 7, the decision to open enrollment for the full cohort will be determined by DEC based on review of the Day 7 reactogenicity and safety data of those 2 sentinel subjects.

After all vaccinated subjects in a cohort have completed study procedures on day 7, the decision to dose escalate to next cohort within both stages will be determined by DEC based on the review of the following:

- Day 7 reactogenicity and safety data (AEs, ECG, clinical laboratory tests and vital signs) from the current cohort.
- Day 7 reactogenicity and safety data from all subjects of the preceding cohort(s).

To evaluate safety by DEC, day 7 data is needed from a minimum of 38 subjects in each cohort for Stage 1.

Stage 2 will commence based on DEC review of the following:

- Day 7 reactogenicity and safety data (AEs, ECG, clinical laboratory tests and vital signs) from all subjects in Stage 1.
- Day 30 safety data (AEs, ECG, clinical laboratory tests and vital signs) from all subjects in Stage 1.

To evaluate safety by DEC, day 7 data is needed from a minimum of 129 subjects in each cohort for Stage 2. Further details around the dose escalation process are located in the DEC charter.

Dose Escalation Criteria:

Depending on the nature, frequency and severity of the safety profile observed in the study, and the study vaccine received, the DEC will decide whether to:

- Proceed with dose escalation.
- Proceed from Stage 1 to Stage 2.
- Determine need of sentinel dosing in Stage 2 cohorts.
- Stop dose escalation.

Dose escalation may proceed unless 1 (or more) of the following apply in subject(s) receiving ASP3772:

- Two or more subjects experience an AE of severe intensity related to ASP3772 or 2 or more subject(s) experience a reactogenicity event of grade 3 or higher intensity.
- One or more subject(s) experience a SAE related to ASP3772.

Stopping criteria for study immunization are described in detail in [Section 5.1.5 Criteria for Continuation of Immunization].

5.1.3 Previous and Concomitant Treatment (Medication and NonMedication Therapy)

Subjects will not be allowed to take immunizations, blood product, immunosuppressants including systemic steroids (topical steroids will be allowed) from first study vaccine administration until 30 days postimmunization. Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are allowed during the study, but not within 24 hours prior to immunization and collection of serum samples for immunogenicity. Subjects who are taking concomitant medications (3 months prior to study enrollment) to treat pre-existing conditions (e.g., hypertension) must remain on stable doses of these medications throughout the study. The doses of all concomitant medications should remain stable throughout the study, but may be changed for medical reasons as determined by the clinical investigator.

If during the study, a subject's health condition necessitates the use of any medication from those listed above, the investigator and medical monitor or designee(s), will discuss the case and determine if the subject should be withdrawn from the study. The excluded concomitant medication will be recorded as a protocol deviation. All concomitant medication including prescription and nonprescription, vitamins and natural and herbal remedies (e.g., St. John's Wort) will be documented.

Use of systemically absorbed antibiotics during the 30-day period prior to collection of the immunogenicity samples should be restricted unless clinically warranted (e.g., avoid prophylaxis) to avoid interference with the immunoassay. If a subject has been treated with any systemically absorbed antibiotics between day 1 and day 30, the day 30 follow-up visit should take place at least 7 days after the last dose of the antibiotics.

Subjects may receive other routine immunization at the 30-day follow-up visit after the completion of all study procedures at each site's discretion; however, routine vaccines should not be given in less than 30 days after study immunization.

A list of excluded concomitant medications is provided in [Appendix 12.4 List of Excluded Concomitant Medications].

5.1.4 Immunization Compliance

Immunization compliance will be recorded at the time of study immunization on day 1 at visit 2. Study immunization will take place in the clinical unit. The exact day and time of study vaccine administration will be documented.

Observation Period: Subjects will remain under observation for 1 hour postimmunization, (provided no reactions have occurred). If a subject experiences significant clinical symptoms, in the opinion of the investigator, then additional direct supervision may be required as needed for management of the symptoms.

5.1.5 Criteria for Continuation of Immunization

Formal Stopping Rules for Individual Subjects:

Individual subjects will be discontinued from the study if:

- Subject is lost-to-follow-up despite reasonable efforts by the investigator to locate the subject.
- Subject voluntarily withdraws from the study at any time for any reason.

Formal Stopping Rules for Cohorts:

No additional subjects will be randomized or dosed in the study until the events below occurring in a subject who received ASP3772 are reviewed by the DEC and it is acceptable to resume cohort immunization.

- Unexplained death of a subject.
- Serious acute hypersensitivity reaction in a subject.
- One of the sentinel subjects experiences an AE of severe intensity related to ASP3772, when sentinel subjects are required.
- An SAE of the same character occurs in at least 2 subjects or 1 subject experiences a vaccine-related SAE.
- Grade 3 or higher lab abnormality considered related to the immunization occurs in 2 or more subjects [FDA Guidance for Industry, 2007].
- The same type of reactogenicity event assessed as Grade 3 or higher occurs in 2 or more subjects, or 1 of the sentinel subjects experiences a reactogenicity event assessed as Grade 3 or higher, using the FDA toxicity grading scale for preventative vaccines [FDA Guidance for Industry, 2007].
- An imbalance of unsolicited AEs between ASP3772 and PCV13 is observed.

5.1.6 Restrictions During the Study

See [Section 5.1.3 Previous and Concomitant Treatment] for prohibited medications.

5.2 Demographics and Baseline Characteristics

5.2.1 Demographics

Demographic information will be collected for all subjects (where permitted) at the screening visit and will include age, date of birth, sex, race and ethnicity.

5.2.2 Medical History

Medical history will be collected at the screening visit in order to assess Inclusion/Exclusion criteria and will be reconfirmed prior to study immunization (if screening was performed > 7 days from study immunization [day 1]).

5.3 Immunogenicity Assessments

The immunogenicity assessments described below are accepted surrogates for efficacy in pneumococcal vaccine studies.

5.3.1 Immunological Response

Considering its mechanism of action, ASP3772 is expected to induce the immune response to pneumococcal PS and carrier proteins, the antigens present in ASP3772. Blood samples to assess immunogenicity will be collected as indicated in the Schedule of Assessments [Table 1, Table 2 and Table 3]. Immunogenicity assessments will be performed from blood samples collected at the dosing visit (day 1) prior to study immunization and at the 7-day and 30-day follow-up visits. Immunogenicity data will be provided to the investigative site when available.

Immunogenicity of ASP3772 will be evaluated by measuring serotype-specific anticapsular IgG antibody concentrations by multiplex direct Luminex immunoassays and OPA titers against the 24 vaccine serotypes in serum samples. OPA measures the functional killing activity of antibodies against the pneumococcal serotypes. In addition, pneumococcal carrier protein (SP1500+0785)-specific immune response will be assessed by measuring specific IgG in serum and activity against non-vaccine serotypes. T helper 17 cells (Th17) response to SP1500+0785 will be evaluated by measuring interleukin (IL)-17, CCI IL-22, CCI [redacted] induction in peripheral blood mononuclear cells (PBMCs) as exploratory assessments.

Details on sampling, processing and storage will be described in the laboratory manual. Samples will be shipped on dry ice to a designated contract research organization (CRO) for the analyses.

5.4 Safety Assessment

5.4.1 Adverse Events

All observed or spontaneously reported AEs will be documented. TEAEs and MAAEs (including PIMMCs and NOCDs) will be assessed throughout the study, from the time of dosing (day 1) up to the last follow-up visit (day 30 for Group 3; day 180 for Groups 1 and

2). Details of definitions and reporting of AEs (including TEAEs, MAAEs, PIMMCs and NOCDs) and SAEs are provided in [Section 5.5 Adverse Events and Other Safety Aspects].

5.4.2 Vital Signs

Vital signs (body temperature, blood pressure and pulse rate) will be measured at screening, the dosing visit (day 1) and at each subsequent visit (day 7 and day 30 [all groups]). On day 1, vital signs (body temperature, blood pressure, pulse rate and respiration rate) will be collected pre-dose and 30 min and 1 hour post-dose. Vital signs will be collected after the subject has been in seated position for at least 5 minutes. When vital signs are scheduled for the same time as blood sample or study vaccine administration, then vital signs will be taken first. Blood pressure should be obtained using the same cuff size and the same arm. Abnormal findings will be documented as clinical events through day 7 and AEs post day 7.

Subjects will be instructed to measure and record in the electronic diary their body temperature daily through day 7. Subjects will also be instructed to measure and record their body temperature in case of illness and contact the site if elevated.

5.4.3 Reactogenicity

Subjects will remain at the study site 60 minutes after the injection for safety evaluation. Subjects may be discharged after 60 minutes at the discretion of the investigator. Local and systemic reactogenicity will also be reported for each of 7 consecutive days following the study injection.

Reactogenicity will be assessed using solicited adverse reactions from the time of immunization (day 1) up to 7 days postimmunization. These include local and systemic reactions with a protocol-specified predefined grading scale (see [Appendix 12.6 Grading of Local Reactogenicity; Appendix 12.7 Grading of Signs of Systemic Reactogenicity; and Appendix 12.8 Grading of Laboratory Abnormalities]). Local reactions are pain, tenderness, redness/erythema and swelling/induration. Systemic reactions are nausea/vomiting, diarrhea, headache, fever, fatigue, joint pain or arthralgia and muscle discomfort or pain/myalgia. The daily maximum measurement of local and systemic reactogenicity signs will be recorded by the subject during the 7-day period using an electronic subject electronic diary. For Stage 1, subjects will be questioned at the day 7 visit if they experienced any joint pain/arthralgia through their day 7 visit postimmunization. If their response is yes, they will be asked to provide the degree of severity using a 4-category grading scale from 1 (no interference with activity) to 4 (ER visit or hospitalization) as per Appendix 12.7. For Stage 2, assessments of joint pain/arthralgia will be collected daily through day 7 using the electronic diary. All subjects will be questioned if they experienced any other illness through day 7 postimmunization on their day 7 visit. Reactogenicity reactions will be reported as Clinical Events. If the reactogenicity reaction extends beyond 7-day period or if serious, it will be recorded as an AE, and followed until resolution or deemed medically stable. Subjects will be instructed to contact the site during the 7 consecutive days following an injection to report any reaction ≥ grade 2. All local and systemic reactions ≥ grade 3 require confirmation by a health care professional.

5.4.4 Laboratory Assessments

Laboratory assessments will be performed 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. See [Appendix 12.5 Laboratory Assessments] for details of laboratory tests.

Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator/subinvestigator who is a qualified physician.

5.4.5 Anti-biotin Antibodies

Anti-biotin antibodies will be measured from serum samples on day 1 prior to study immunization and at day 30 follow-up visit for Group 1 and Group 2 subjects. Serum anti-biotin antibodies will be repeated on day 180 if tested positive on day 30. In subjects with positive serum anti-biotin antibodies, serum biotin levels will be measured in the same sample.

5.4.6 Physical Examination

A physical examination consisting of an examination of general appearance, eyes, nose throat, neck (including thyroid), lymph nodes, chest, lungs, cardiovascular, abdomen, skin, extremities, musculoskeletal and neurological system including mental status will be conducted at the screening visit, prior to study immunization (day 1) and at the day 30 follow-up visit. A symptom-directed physical exam may be performed at any other time, if necessary. The screening physical examination also includes evaluation of significant, ongoing medical conditions. Any changes between screening and randomization will be captured in the medical/surgical history.

Any abnormal finding(s) at screening must not be exclusionary per the eligibility criteria and must be assessed and documented as not clinically significant if a subject is to be enrolled in the study. After receiving the study vaccine, new abnormal findings or a worsening of an ongoing abnormal condition will be recorded as an SAE or AE ([S]AE).

5.4.7 Electrocardiogram

Standard 12-lead ECG recordings will be used for the purposes of safety assessment and subject management by the Investigator.

A 12-lead, resting ECG is to be recorded at each timepoint as indicated in Table 1, Table 2 and Table 3 (Schedule of Assessments). Subjects should remain supine for at least 5 minutes prior to all ECGs. Dates and times may be generated by the machine's internal clock and are considered source data. The results are to be interpreted by qualified personnel in real time for the management of the subjects' clinical condition. The principal investigator/designee will initial and date, and provide his/her clinical interpretation on each report. The results (normal, abnormal not clinically significant, abnormal clinically significant) are to be recorded in the electronic case report forms (eCRFs).

The ECG recorded during screening will be used to determine eligibility for study participation. Subjects who have a clinically significant abnormal ECG will not be eligible

for the study. The interpretation of the ECG from screening will be the baseline to which post-study vaccine dosing ECGs will be compared.

Unscheduled ECGs will be performed if clinically indicated.

If a machine-read corrected QT interval exceeds 500 msec after repeat measurement, this should be reported as an AE.

The original print-out and an electronic copy of all scheduled and unscheduled ECG tracings should be maintained on site as source data.

5.5 Adverse Events and Other Safety Aspects

5.5.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a subject administered a study vaccine, and which does not necessarily have to have a causal relationship with this immunization. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product whether or not considered related to the medicinal product.

In order to identify any events that may be associated with study procedures and could lead to a change in the conduct of the study, Astellas collects AEs even if the subject has not received study product. AE collection begins after the signing of the ICF and will be collected until 30 days after study vaccine administration or the subject is determined to be a screen failure.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be identified as an AE only if the abnormality meets 1 of the following criteria:

- Induces clinical sign or symptoms
- Requires active intervention
- Requires interruption or discontinuation of study medication
- The abnormality or investigational value is clinically significant in the opinion of the investigator.

MAAEs are AEs for which the subject has received medical attention by medical personnel, or in an emergency room, or which led to hospitalization. For each reported AE, the investigator will ask the subject if such medical attention has been received. In addition, the investigator will assess each MAAE if it constitutes a new-onset chronic disease (NOCD), defined as a MAAE which a) was absent at baseline, b) is not resolved at the follow-up telephone call, c) requires continuous medical care or attention. The investigator will also assess if any MAAE constitutes a PIMMC. PIMMCs are defined as any AEs of autoimmune

or auto inflammatory nature. The investigator will record MAAEs, NOCDs and PIMMCs in the AE eCRF.

5.5.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, clinical chemistry, or urinalysis) or other safety assessment (e.g., ECGs, radiographic scans, vital signs measurements, physical examination), including those that worsen from baseline, that is considered to be clinically significant in the medical and scientific judgment of the investigator and not related to underlying disease, is to be reported as an unsolicited (S)AE.

Any clinically significant abnormal laboratory finding or other abnormal safety assessment, which is associated with the underlying disease does not require reporting as an (S)AE, unless judged by the investigator to be more severe than expected for the subject's condition.

Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

5.5.1.2 Potential Cases of Drug-Induced Liver Injury

Refer to [Appendix 12.5 Liver Safety Monitoring and Assessment] for detailed instructions on drug-induced liver injury. Abnormal values in aspartate transaminase (AST) and/or alanine transaminase (ALT) concurrent or with abnormal elevations in total bilirubin that meet the criteria outlined in [Appendix 12.5 Liver Safety Monitoring and Assessment], in the absence of other causes of liver injury, are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and are always to be considered important medical events and reported per [Section 5.5.5 Reporting of Serious Adverse Events].

5.5.2 Definition of Serious Adverse Events (SAEs)

An AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in death
- Is life-threatening (an AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death)
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly, or birth defect
- Requires inpatient hospitalization (except for planned procedures as allowed per study) or leads to prolongation of hospitalization (except if prolongation of planned hospitalization is not caused by an AE). Hospitalization for treatment/observation/examination caused by AE is to be considered as serious.)
- Other medically important events (defined in paragraph below)

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent 1 of the other outcomes listed in the definition above. These events, including those that may result in disability/incapacity, usually are considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

5.5.2.1 Always Serious Adverse Events

The sponsor has a list of events that they classify as "always serious" events. If an AE is reported that is considered by the sponsor to be an SAE per this classification as "always serious", additional information on the event (e.g., investigator confirmation of seriousness, causality) will be requested.

5.5.3 Criteria for Causal Relationship to Study Vaccine

A medically qualified investigator is obligated to assess the relationship between the study vaccine and each occurrence of each (S)AE. This medically qualified investigator will use medical judgment as well as the Reference Safety Information to determine the relationship. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

The medically qualified investigator is requested to provide an explanation for the causality assessment for each (S)AE and must document in the medical notes that he/she has reviewed the (S)AE and has provided an assessment of causality.

Following a review of the relevant data, the causal relationship between the study vaccine and each (S)AE will be assessed by answering '**yes**' or '**no**' to the question "**Do you consider that there is a reasonable possibility that the event may have been caused by the study vaccine**".

When making an assessment of causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a 'reasonable possibility' that an (S)AE may have been caused by the study vaccine (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Plausible temporal relationship between exposure to the study vaccine and (S)AE onset and/or resolution. Has the subject actually received the study vaccine? Did the (S)AE occur in a reasonable temporal relationship to the administration of the study vaccine?
- Plausibility; i.e., could the event been caused by the study vaccine? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug/vaccine class, preclinical and clinical study data, etc.
- Laboratory or other test results; a specific lab investigation supports the assessment of the relationship between the (S)AE and the study vaccine (e.g., based on values pre-, during and post-immunization).

- Available alternative explanations independent of study vaccine exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation.

There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the medically qualified investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the sponsor. With limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of 'no' is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise his/her assessment of causality in light of new information regarding the SAE and shall send an SAE follow-up report and update the eCRF with the new information and updated causality assessment.

5.5.4 Criteria for Defining the Severity of an Adverse Event

The investigator will use the following definitions to rate the severity of each AE:

- Mild: No disruption of normal daily activities
- Moderate: Affect normal daily activities
- Severe: Inability to perform daily activities
- Potentially life threatening: Emergency room visit or hospitalization

5.5.5 Reporting of Serious Adverse Events (SAEs)

The collection of AEs will continue until 30 days after study vaccine administration in all groups. The expedited reporting of SAEs will start following receipt of the ICF and will continue until 180 days after study vaccine administration in Groups 1 and 2 and until 30 days after study vaccine administration in Group 3, or until the subject is determined to be a screen failure or withdraws from the study.

In the case of an SAE, the investigator must contact the sponsor by fax or email immediately (within 24 hours of awareness).

The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the sponsor by email or fax immediately (within 24 hours of awareness).

The SAE worksheet must be signed by a medically qualified investigator (as identified on Delegation of Authority Log). Signature confirms accuracy and completeness of the SAE data as well as the investigator causality assessment including the explanation for the causality assessment.

If the SAE is associated with emergency unblinding as outlined in [Section 4.4.4 Breaking the Study Vaccine Assignment Code for Emergency], this is to be recorded on the SAE

worksheet. Within the SAE worksheet, the investigator is to include when unblinding took place in association with the SAE.

For contact details, see [Section II Contact Details of Key Sponsor's Personnel]. Fax or email the SAE/Special Situations Worksheet to:

Astellas Pharma Global Development – United States
Pharmacovigilance
Fax number 847-317-1241
Email: safety-us@astellas.com

The following minimum information is required:

- International Study Number/Study number,
- Subject number, sex and age,
- The date of report,
- A description of the SAE (event, seriousness criteria),
- Causal relationship to the study vaccine (including reason), and
- The drug provided (if any) (blinded regimen is also an option)

The sponsor or sponsor's designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality and expectedness of the events (e.g., Suspected Unexpected Serious Adverse Reaction [SUSAR] reporting) according to current local/regional regulatory requirements in participating countries. The sponsor or sponsor's designee will submit expedited safety reports (e.g., Investigational New Drug [IND] Safety Reports, SUSAR, Council for International Organizations of Medical Sciences [CIOMS-I]) to Competent Authorities (CA) and concerned Ethics Committee (cEC) per current local regulations, and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports as required by their IRB within timelines set by regional regulations (e.g., EMA, FDA) where required. Documentation of the submission to and receipt by the IRB of expedited safety reports should be retained by the site.

The sponsor will notify all investigators responsible for ongoing clinical studies with the study vaccine of all SUSARs, which may require submission per local requirements to the site IRB.

The investigators or designee should provide written documentation of IRB notification for each report to the sponsor.

The investigator may contact the sponsor's medical monitor/study physician for any other problem related to the safety, welfare, or rights of the subject.

5.5.6 Follow-up of Adverse Events

All AEs occurring during or after the subject has discontinued the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized by the investigator.

If after the protocol defined AE collection period [Section 5.5.1 Definition of Adverse Event], an AE progresses to an SAE, or the investigator learns of any (S)AE including death,

where he/she considers there is reasonable possibility it is related to the study vaccine treatment or study participation, the investigator must promptly notify the sponsor.

5.5.7 Monitoring of Common Serious Adverse Events

For this phase 1/2 clinical study, there is no list of common SAEs for which a single occurrence will be excluded from IND safety reporting.

5.5.8 Special Situations

Certain Special Situations observed in association with the study vaccine(s), such as incorrect administration (e.g., wrong dose of study vaccine, comparator, or background therapy) are collected in the eCRF, as Protocol Deviation per [Section 8.3 Major Protocol Deviations] or may require special reporting, as described below. These Special Situations are not considered AEs, but do require to be communicated to Astellas as per the timelines defined below.

If a Special Situation is associated with, or results in, an AE, the AE is to be assessed separately from the Special Situation and captured as an AE in the eCRF. If the AE meets the definition of a SAE, the SAE is to be reported as described in [Section 5.5.5 Reporting of Serious Adverse Events] and the details of the associated Special Situation are to be included in the clinical description on the SAE worksheet.

The Special Situations are:

- Pregnancy
- Medication error, overdose and “off-label use”
- Misuse/abuse
- Occupational exposure
- (Suspicion of) Transmission of infectious agent
- Suspected drug-drug interaction

5.5.8.1 Pregnancy

If a female subject becomes pregnant within 28 days of immunization, the investigator is to report the information to Astellas according to the timelines in **[**Section 5.5.5 Reporting of Serious Adverse Events] using the Pregnancy Reporting Form and in the eCRF.

The investigator will attempt to collect pregnancy information on any female partner of a male subject who becomes pregnant within 28 days of immunization and report the information to Astellas according to the timelines in [Section 5.5.5 Reporting of Serious Adverse Event**s**] using the Pregnancy Reporting Form.

The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data, etc., should be included in this information.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female study subject as an AE in the eCRF or SAE per [Section 5.5.5 Reporting of Serious Adverse

Events]. For (S)AEs experienced by a female partner of a male subject, (S)AEs are to be reported via the Pregnancy Reporting Form.

Additional information regarding the outcome of a pregnancy when also categorized as an SAE is mentioned below:

- "Spontaneous abortion" includes miscarriage, abortion and missed abortion.
- Death of a newborn or infant within 1 month after birth is to be reported as an SAE regardless of its relationship with the study vaccine.
- If an infant dies more than 1 month after the birth, is to be reported if a relationship between the death and intrauterine exposure to the study vaccine is judged as "possible" by the investigator.
- Congenital anomaly (including anomaly in miscarried fetus)

Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination. (S)AEs experienced by the newborn/infant should be reported via the Pregnancy Reporting Form. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date.

5.5.8.2 Medication Error, Overdose and "Off-Label Use"

If a medication error, overdose or "off-label use" (i.e., use outside of what is stated in the protocol) is suspected, refer to [Section 8.3 Major Protocol Deviations]. Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in [Section 5.5.5 Reporting of Serious Adverse Events] together with the details of the medication error, overdose and/or "off-label use".

In the event of suspected ASP3772 overdose, the subject should receive supportive care and monitoring. The medical monitor/expert should be contacted as applicable.

5.5.8.3 Misuse

If misuse of the study vaccine(s) is suspected, the investigator must forward the Special Situation worksheet Astellas Pharma Inc. by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in [Section 5.5.5 Reporting of Serious Adverse Events] together with details of the misuse or abuse of the study vaccine(s).

5.5.8.4 Occupational Exposure

If occupational exposure (e.g., inadvertent exposure to the study vaccine(s) of site staff whilst preparing it for administration to the patient) to the study vaccine(s) occurs, the investigator must forward the Special Situation worksheet to Astellas Pharma Inc. by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs occurring to the individual associated with or resulting from the Special Situation are to be reported on the Special Situations worksheet.

5.5.8.5 (Suspicion of) Transmission of infectious agent

If transmission of an infectious agent associated with the study vaccine(s) is suspected, the investigator must forward the Special Situation worksheet to Astellas Pharma Inc. by fax or email immediately (within 24 hours of awareness) and any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in [Section 5.5.5 Reporting of Serious Adverse Events] together with the details of the suspected transmission of infectious agent.

5.5.8.6 Suspected Drug-Drug Interaction

If a suspected drug-drug interaction associated with the study vaccine(s) is suspected, the investigator must forward the Special Situation worksheet Astellas Pharma Inc. by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in [Section 5.5.5 Reporting of Serious Adverse Events] together with details of the suspected drug-drug interaction.

5.5.9 Supply of New Information Affecting the Conduct of the Study

When new information becomes available necessary for conducting the clinical study properly, the sponsor will inform all investigators involved in the clinical study as well as the regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

The investigator will also inform the subjects, who will be required to sign an updated ICF in order to continue in the clinical study.

5.5.10 Urgent Safety Measures

An Urgent Safety Measure (USM) is an intervention, which is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the sponsor, relevant CA, IRB/IEC, where applicable, in order to protect study participants from any immediate hazard to their health and/or safety. Either the investigator or the sponsor can initiate an USM. The cause of an USM can be safety, product or procedure related.

5.5.11 Reporting Urgent Safety Measures

In the event of a potential USM, the investigator must contact the Astellas study physician (within 24 hours of awareness). Full details of the potential USM are to be recorded in the subject's medical records. The sponsor may request additional information related to the event to support their evaluation.

If the event is confirmed to be an USM the sponsor will take appropriate action to ensure the safety and welfare of the patients. These actions may include, but are not limited to, a change in study procedures or study treatment, halting further enrollment in the trial, or stopping the study in its entirety. The sponsor or sponsor's designee will notify CA and cEC within the timelines required per current local regulations, and will inform the investigators as required.

When required, investigators must notify their IRB/IEC within timelines set by regional regulations.

5.6 Test Drug Concentration

Measurement of study vaccine concentrations is not applicable to this study.

5.7 Other Measurements, Assessments or Methods

Not applicable.

CCI [redacted]

5.8 Total Amount of Blood

The approximate total amount of blood to be collected for central laboratory assessment per subject is presented in below tables. Repeat and additional blood samples may be taken if required, however the maximum blood volume drawn from the subject will not exceed 550 mL over the course of the study. Refer to laboratory manual for more details regarding blood collection.

Stage 1

Sample Type	Number of Samples	Sample Volume (mL)	Total Volume (mL)
Clinical Laboratory Tests	4	10.0	40.0
CCI [redacted]			
T-cell response (PBMCs)	3	20.0	60.0
Anti-biotin	2	10.0	20.0
Anti-1500+0785	2	10.0	20.0
IgG and OPA	2	10.0	20.0
CCI [redacted]			
Total			CCI [redacted]

IgG: immunoglobulin G; OPA: opsonophagocytic activity; PBMC: peripheral blood mononuclear cells;
CCI [redacted]

Stage 2
Group 2

Sample Type	Number of Samples	Sample Volume (mL)	Total Volume (mL)
Clinical Laboratory Tests	4	10.0	40.0
CCI			
T-cell response (PBMCs)	3	20.0	60.0
Anti-biotin	2	10.0	20.0
Anti-1500+0785	2	10.0	20.0
IgG and OPA	2	10.0	20.0
Total			CCI

IgG: immunoglobulin G; OPA: opsonophagocytic activity; PBMC: peripheral blood mononuclear cells;
CCI

Group 3

Sample Type	Number of Samples	Sample Volume (mL)	Total Volume (mL)
Clinical Laboratory Tests	3	10.0	30.0
CCI			
IgG and OPA	2	10.0	20.0
Total			CCI

IgG: immunoglobulin G; OPA: opsonophagocytic activity; CCI

6 DISCONTINUATION

6.1 Discontinuation of Individual Subject(s) From Study

Individual subjects will be discontinued from the study if:

- Subject is lost-to-follow-up despite reasonable efforts by the investigator to locate the subject.
- Subject can voluntarily withdraw from the study at any time for any reason.

The reason for discontinuation from study must be documented in the subject's medical records.

Any subject who has received immunization, but withdraws consent, will be asked to complete the day 30 study assessments at the time of their withdrawal if possible.

6.1.1 Lost to Follow Up

Every reasonable effort is to be made to contact any subject lost to follow-up during the course of the study to complete study-related assessments and record outstanding data.

6.2 Discontinuation of the Site

If an investigator intends to discontinue participation in the study, the investigator must immediately inform the sponsor.

6.3 Discontinuation of the Study

The sponsor may terminate this study prematurely, either in its entirety or at any study site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns. If the sponsor terminates the study for safety reasons, the sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

7 STATISTICAL METHODOLOGY

A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database soft lock at the latest. Any changes from the analyses planned in SAP will be justified in the clinical study report.

In general, continuous data will be summarized with descriptive statistics (number of subjects, mean, standard deviation, geometric mean and geometric standard deviation (GSD) where applicable, minimum, median and maximum), and frequency and percentage for categorical data. Percentages by categories will be based on the number of subjects with no missing data, i.e., the percentages will sum to 100%.

Baseline will be defined as the last observation prior to first dose, unless otherwise specified.

7.1 Sample Size

In Stage 1, approximately 120 evaluable adults 18 to 64 years of age are planned to be enrolled. This sample size in 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 495 evaluable adult subjects from 65 to 85 years of age are planned to be enrolled. For each PCV13 serotype the OPA titer^{-1} responses (geometric mean titer [GMT] and 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 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.

7.2 Analysis Sets

The Safety Analysis Set (SAF) will consist of all subjects who receive an immunization in this study with either ASP3772, PCV13, or PPSV23. 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 postimmunization measurement.

For each ASP3772, PCV13 or PPSV23 immunization group, the number and percentage of subjects in each analysis set will be given for all randomized (ASP3772 or PCV13) or enrolled (PPSV23) subjects.

7.2.1 Full Analysis Set (FAS)

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

7.2.2 Per Protocol 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 2 weeks after receiving the immunization.

7.2.3 Safety Analysis Set (SAF)

The SAF will be used for most summaries of demographic and baseline characteristics and all safety and tolerability related variables. There are a limited number of displays that will use all subjects who gave informed consent or all subjects who were randomized.

7.3 Demographics and Baseline Characteristics

Demographics and baseline characteristics (age, sex, race, ethnicity, weight, height and body mass index) will be summarized by dose cohort and immunization group (ASP3772 or PCV13) and for all PCV13 subjects combined. Summaries will be given separately for the adults (18 to 64 years) and elderly (65 to 84 years). A summary will be provided for the PPSV23 enrolled subjects.

7.3.1 Subject Disposition

Disposition (consented, discontinued before randomization, randomized, vaccinated) will be provided by stage (adults, elderly), dose cohort (1 µg, 2 µg, 5 µg) and immunization group (ASP3772 or PCV13) and for all PCV13 subjects combined. A summary will be provided for the PPSV23 enrolled subjects.

The number and percentage of subjects who completed the study, those who discontinued the study and the reasons for study discontinuation (AE, death, lost to follow-up, protocol deviation, withdrawal by subject, non-compliance with study, other) will be presented for SAF subjects for each stage (adults, elderly). All disposition details and dates of first and last evaluations for each subject will be listed.

7.3.2 Previous and Concomitant Medications

All previous and concomitant medications for each subject will be listed.

7.3.3 Medical History

Medical history for each subject will be listed.

7.4 Analysis of Immunogenicity

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

Stage 1:

The following measures in adults 18 to 64 years of age will be used to characterize the immunological response 30 days following administration of either ASP3772 or PCV13. These measures will be provided for each immunization group (PCV13 vs ASP3772) and dose level (ASP3772: 1, 2, 5 μg).

- The GMT, GSD and 95% confidence interval (CI) for functional OPA for each serotype will be calculated.
- The ratio of the OPA GMT (each ASP3772 dose level/PCV13) and 95% CI will be provided.
- The geometric mean concentration (GMC), GSD and 95% CI for serotype-specific IgG for each of the serotypes will be calculated.
- The ratio of the IgG GMC (each ASP3772 dose level/PCV13) and 95% CI will be provided.
- The GMFR, GSD and 95% CI in anticapsular polysaccharide IgG and OPA titer will be calculated for each of the serotypes unique to ASP3772.
- The proportion of subjects with a ≥ 4-fold increase in anticapsular polysaccharide IgG and OPA relative to baseline will be calculated.

Stage 2 Group 2:

The following measures in adults 65 to 84 years of age will be used to characterize the immunological response 30 days following vaccine administration. These measures will be provided by dose cohort for ASP3772 and for PCV13 as a single group for each serotype.

- The GMT, GSD and 95% CI for functional OPA activity for each serotype will be calculated.
- The ratio of the GMT for functional OPA (each ASP3772 dose level/PCV13) for each serotype and the 95% CI will be provided.
- The GMC, GSD and 95% CI for serotype-specific IgG for each of the serotypes will be calculated.
- The ratio of the IgG GMC (each ASP3772 dose level/PCV13) and 95% CI will be provided for each serotype.
- The GMFR, GSD and 95% CI in anticapsular polysaccharide IgG and OPA titer will be calculated for each of the serotypes unique to ASP3772.
- The proportion of subjects with a ≥ 4-fold increase in anticapsular polysaccharide IgG and OPA relative to baseline will be calculated
- To assess the impact of ASP3772 dose, an analysis of covariance (ANCOVA) model with age (65 to 74 years, 75 to 85 years) as the covariate and immunization (ASP3772 1,

2, 5 µg or PCV13) as the factor of interest will be conducted on the natural logarithm (log) of pneumococcal OPA titers and the natural log of the IgG concentrations.

In addition, for both Stage 1 Group 1 and Stage 2 Group 2, SP1500+0785 levels (ng/mL) will be collected for those subjects given either ASP3772 or PCV13. Levels of the following cytokines will be collected for subjects given ASP3772 or PCV13: Th17, IL-17, CCI , IL-22, CCI The units for all are pg/mL. Non-parametric methods will be used for these analyses. A Kruskal-Wallis test will be used to test for overall differences between the 3 dose levels and PCV13. All treatment groups in Stage 1 will be compared, followed by paired comparisons between each of the ASP3772 dose levels and PCV13 using a Wilcoxon Test. The same set of comparisons will be made for subjects in Stage 2, Group 2.

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:

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

7.5 Analysis of Safety

Safety and tolerability of ASP3772 are the primary objectives in Stage 1 and Stage 2 of this study and will be assessed by:

- TEAEs (including SAEs, MAAEs, PIMCCs and NOCDs)
- Laboratory assessments
- Vital signs (body temperature, blood pressure and pulse rate)
- Physical examination
- ECG
- Reactogenicity

Safety analyses will be performed on the SAF; all subjects who receive study immunization. Data from PCV13 subjects in each cohort will be pooled.

For each local and systemic solicited reaction the percentage of subjects who reported each symptom by grade (None, Grade 1, 2, 3 or 4) will be summarized (n, %) by immunization group (PCV13 vs ASP3772) and dose level (ASP3772: 1, 2, 5 µg) and the percentage of subjects who reported any symptom.

7.5.1 Adverse Events

A TEAE is defined as an AE observed after study immunization and up to 30 days postimmunization. SAEs will be monitored for up to 180 days postimmunization (Group 1 and Group 2).

For each immunization arm, the frequencies and percentages will be displayed for the following TEAEs (coded using the Medical Dictionary for Regulatory Activities [MedDRA]) by system organ class and preferred term:

- Overall
- Serious
- Related (yes or no) to study vaccine

The number and percentage of subjects with TEAEs, SAEs, AEs and AEs related to study vaccine will be summarized by system organ class, preferred term and immunization group. The number and percentage of AEs by severity will also be summarized. MAAEs including PIMMCs and NOCDs, if any, will be summarized. All AEs will be listed.

7.5.2 Laboratory Assessments

Descriptive statistics for each laboratory test (e.g., hematology, biochemistry and urinalysis) and vital signs will be tabulated by immunization group, dose level for ASP3772 and scheduled time point.

Shifts relative to normal ranges from baseline to each time point during the immunization period in laboratory tests will also be tabulated. Laboratory data will be listed.

7.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline for subjects in the SAF by immunization group and time point.

Vital signs data will be displayed in listings.

7.5.4 Physical Examination

Physical examination findings will be listed by immunization group.

7.5.5 Routine 12-lead Electrocardiograms

The 12-lead ECG results will be listed by immunization group.

7.6 Analysis of Pharmacokinetics

Pharmacokinetic analysis is not applicable in this study.

7.7 Analysis of Pharmacodynamics

Pharmacodynamic analysis is not applicable in this study.

7.8 Major Protocol Deviations and Other Analyses

Major protocol deviations as defined in [Section 8.3 Major Protocol Deviations] will be summarized for all randomized subjects by immunization group and total as well as by site. A data listing will be provided by site and subject.

The major 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 immunization or incorrect dose
PD4 – Received excluded concomitant treatment

7.9 Interim Analysis (and Early Discontinuation of the Clinical Study)

No formal interim analysis for efficacy is planned. Safety data will be provided for DEC review after each dose cohort for the adult and elderly populations for dose escalation decisions. Prior to initiating the enrollment of the elderly subjects, the DEC will review the available data through 30 days post immunization in adult subjects. Details will be provided in the DEC Charter.

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

As a general principle, no imputation of missing data will be done. Exceptions are the start and stop dates of AEs and concomitant medications if they are missing on day of first dose. The imputed dates will be used to assess if the AEs or concomitant medications are treatment-emergent or concomitant, respectively. Listings of the AEs and concomitant medications will present the actual partial dates; imputed dates will not be shown.

8 OPERATIONAL CONSIDERATIONS

8.1 Data Collection

The investigator or site designee will enter data collected using an electronic data capture system. In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including laboratory values, if applicable) in the eCRF within 5 days after the subject's visit. The investigator or site designee will be required to expedite recording of data in the eCRF preferably within 24 hours for data required for dose escalation decisions. The investigator or site designee will also be required to expedite recording of data in the eCRF when last subject of Stage 1 Cohort 3 completes day 30 (visit 4) in order to make the Stage 1 up-to-day-30 visit data for all subjects available for DEC review.

The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with source documents. These documents should be appropriately maintained by the site.

The monitor should verify the data in the eCRFs with source documents and confirm that there are no inconsistencies between them.

Laboratory tests are performed at a central laboratory. Central Laboratory data will be transferred electronically to the sponsor or designee at predefined intervals during the study. The Central laboratory will provide the sponsor or designee with a complete and clean copy of the data.

ECGs are performed at a site ECG reader device. ECG read data will be reviewed by the investigator for the entry in eCRF.

The investigator or designee must record all protocol-required data in the provided eCRF. In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including laboratory values, if applicable) onto the eCRF as soon as possible after the subject visit. eCRFs and any supporting documents should be available for retrieval by the sponsor/delegated CRO at any given time. The monitor should verify the data in the eCRFs with source documents to confirm that there are no inconsistencies between them. If the monitor finds no inconsistencies, the appropriate eCRFs are collected, ensuring a copy remains at site. The originals should be submitted to the sponsor or designee.

If any inconsistency is detected on the collected eCRFs, the monitor or data manager should query the investigator/subinvestigator. The investigator/subinvestigator should provide an answer to the query and provide the resolved query to the sponsor.

The monitor should verify the revised data of the eCRFs with source documents and confirm that there are no inconsistencies between them, and also check that appropriate records on the correction/addition of data are maintained.

All procedures conducted under the protocol must be documented. For screen failures, the minimum demographic data (sex, birth date, race and informed consent date), outcome of eligibility assessment (inclusion and exclusion criteria), reason for screen failure and AEs details must be documented.

The investigator or designee will be responsible for eCRF completion and that all data and queries are accurate, complete and are verifiable with the source. The source should be appropriately maintained by the clinical unit.

Electronic data sources and any supporting documents should be available for review/retrieval by the sponsor/designee at any given time.

Electronic Patient Reported Outcome:

Subject diaries and questionnaires as described in [Section 5.4.3 Reactogenicity] will be completed by the subject from day 1 through day 7 on an electronic device and the collected electronic source data will be hosted at the vendor. The investigator or site designee will

review the diaries and questionnaire data throughout the study to ensure completion and protocol compliance. The diary and questionnaire data will be transferred electronically to sponsor or designee at predefined intervals during the study. The vendor will provide the investigator with a complete and clean copy of the site's data and will provide the sponsor or designee with a complete and clean copy of the study data. The ownership of this data is with the investigator and subsequently any changes requested to the non-subject reported data will be made using a Data Clarification Form (DCF) to the vendor. The requested change must be supported by documented evidence at site. For this study, it has been decided that there are justifiable scientific reasons (e.g., recall bias) for not allowing changes after the original daily data has been entered or not allowing additional data entered after the daily 24-hour window has expired as these changes could potentially impact the data integrity of this study. Data not collected by the subject in his/her ePRO diary will be noted as missing in the dataset.

8.2 Screen Failures

For screen failures the demographic data, reason for failing, informed consent, inclusion and exclusion criteria and AEs will be collected in the eCRF.

8.3 Major Protocol Deviations

A major protocol deviation is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change. The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and must protect the rights, safety and welfare of subjects. The investigator should not implement any deviation from, or changes of, the protocol, unless it is necessary to eliminate an immediate hazard to study subjects.

A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited. The major protocol deviation criteria are described in [Section 7.8 Major Protocol Deviations and Other Analyses].

When a major deviation from the protocol is identified for an individual subject, the investigator or designee must ensure the sponsor is notified. The sponsor will follow-up with the investigator, as applicable, to assess the deviation and the possible impact to the safety and/or efficacy of the subject to determine subject continuation in the study.

If a major deviation impacts the safety of a subject, the investigator must contact the sponsor immediately. The investigator will also assure that deviations meeting IRB/IEC and applicable regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and applicable regulatory authorities will be provided to the sponsor and maintained within the trial master file.

9 END OF TRIAL

Study completion occurs with the last visit of the last subject in the study. The End of Study is defined as the date on which the independent DEC completes their assessment of the study data.

10 STUDY ORGANIZATION

10.1 Dose Escalation Committee (DEC)

For this clinical study, the Astellas DEC and the investigator/designee are responsible for dose escalation decisions. Progression to higher dose level cohorts will be determined by the DEC, which may consist of representatives from Astellas Medical Science, Pharmacovigilance and Statistics personnel, as well as external independent safety physician(s). For detailed information on the dose-escalation decision process, refer to DEC Charter.

11 REFERENCES

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12 APPENDICES

12.1 Ethical, Regulatory and Study Oversight Considerations

12.1.1 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

12.1.2 Institutional Review Board (IRB)

GCP requires that the clinical protocol, any protocol amendments, the IB, the ICF and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an IRB. The IRB will review the ethical, scientific and medical appropriateness of the study before it is conducted. IRB approval of the protocol, ICF and subject information and/or advertising, as relevant, will be obtained prior to the authorization of drug shipment to a study site.

Any substantial amendments to the protocol will require IRB approval before implementation, except for changes necessary to eliminate an immediate hazard to subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies and procedures established by the IRB
- Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

12.1.3 Protocol Amendment and/or Revision

Amendments to this protocol must be signed by the sponsor and the investigator. Written verification of IRB approval will be obtained before any amendment is implemented. Modifications to the protocol that are administrative in nature do not require IRB approval, but will be submitted to the IRB for their information, if required by local regulations.

If there are changes to the ICF, written verification of IRB approval must be forwarded to the sponsor. An approved copy of the new ICF must also be forwarded to the sponsor.

12.1.4 Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.1.5 Informed Consent of Subjects

12.1.5.1 Subject Information and Consent

The investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subject, the person who administered the informed consent and any other signatories according to local requirements. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that informed consent was obtained prior to any study-related procedures and that the subject received a signed copy.

The signed consent forms will be retained by the investigator and made available (for review only) to the study monitor and auditor regulatory authorities and other applicable individuals upon request.

12.1.5.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information

1. The investigator or his/her representative will immediately inform the subject orally whenever new information becomes available that may be relevant to the subject's consent or may influence the subject's willingness to continue to participate in the study (e.g., report of serious drug adverse drug reaction). The communication must be documented in the subject's medical records and whether the subject is willing to remain in the study or not must be confirmed and documented.
2. The investigator must update their ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the subject on all updated ICFs throughout their participation in the study. The investigator or his/her designee must re-consent subjects with the updated ICF even if relevant information was provided orally. The investigator or his/her representative who obtained the written informed consent and the subject should sign and date the ICF. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must be made in the subject's records documenting the re-consent process.

12.1.6 Source Documents

Source data must be available at the site to document the existence of the study subjects and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are hand-written on paper or entered electronically. If source data are created (first entered), modified, maintained, achieved, retrieved or transmitted electronically via computerized systems (and/or other kind of electric devices) as part of regulated clinical trial activities, such systems must be

compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol related assessments, AE tracking and/or drug accountability.

Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments and audit trail information, if applicable). All printed records must be kept in the subject file and available for archive.

12.1.7 Record Retention

The investigator will archive all study data (e.g., subject identification code list, source data, eCRFs and investigator's file) and relevant correspondence. These documents are to be kept on file for the appropriate term determined by local regulation (for US sites, 2 years after approval of the NDA or discontinuation of the IND). The sponsor will notify the site/investigator if the NDA/MAA/J-NDA is approved or if the IND/IMPD/CHIKEN TODOKE is discontinued. The investigator agrees to obtain the sponsor's agreement prior to disposal, moving, or transferring of any study-related records. The sponsor will archive and retain all documents pertaining to the study according to local regulations.

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes.

The documents of the Efficacy and Safety Evaluation Committee (minutes and SOPs and others) and the judgment committee outside the study sites (minutes and SOPs and others) shall be retained by the sponsor.

12.1.8 Subject Confidentiality and Privacy

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited unless otherwise the subject provides written consent or approval. Additional medical information may be given only after approval of the subject to the investigator or to other appropriate medical personnel responsible for the subject's well-being.

The sponsor shall not disclose any confidential information on subjects obtained during the performance of their duties in the clinical study without justifiable reasons.

Even though any individuals involved in the study, including the study monitors and auditors, may get to know matters related to subject's privacy due to direct access to source documents, or from other sources, they may not leak the content to third parties.

The sponsor affirms the subject's right to protection against invasion of privacy. Only a subject identification number will identify subject data retrieved by the sponsor. However, the sponsor requires the investigator to permit the sponsor, sponsor's representative(s), the IRB/IEC and when necessary, representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

The sponsor agrees to comply and process personal data in accordance with all applicable privacy laws and regulations, including, without limitation, the Personal Information Protection Law in Japan and Privacy laws in the US. If the services will involve the collection or processing of personal data (as defined by applicable data protection legislation) within the European Economic Area (EEA), then sponsor shall serve as the controller of such data, as defined by the EU Data Protection Directive, and investigator and/or third party shall act only under the instructions of the sponsor in regard to personal data. If sponsor is not based in the EEA, sponsor must appoint a third party to act as its local data protection representative or arrange for a co-controller established in the EU for data protection purposes in order to comply with the Directive.

12.1.9 Arrangement for Use of Information and Publication of the Clinical Study

Information concerning the study vaccine, patent applications, processes, unpublished scientific data, the IB and other pertinent information is confidential and remains the property of the sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the sponsor will use the information obtained during the clinical study in connection with the development of the drug and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide the sponsor with all data obtained during the study.

Publication of the study results is discussed in the clinical study agreement.

12.1.10 Signatory Investigator for Clinical Study Report

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final study report which forms part of a marketing authorization application be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator (s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. The representative for coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the sponsor prior to database lock.

12.2 Procedure for Clinical Study Quality Control

12.2.1 Clinical Study Monitoring

The sponsor or delegated CRO is responsible for monitoring the clinical study to ensure that subject's human rights, safety and well-being are protected, that the study is properly conducted in adherence to the current protocol and GCP, and study data reported by the investigator/subinvestigator are accurate and complete and that they are verifiable with study-related records such as source documents. The sponsor is responsible for assigning study monitor(s) to this study for proper monitoring. They will monitor the study in accordance with planned monitoring procedures.

12.2.2 Direct Access to Source Data/Documents

The investigator and the study site must accept monitoring and auditing by the sponsor or delegated CRO, as well as inspections from the IRB/IEC and relevant regulatory authorities. In these instances, they must provide all study-related records including source documents when they are requested by the sponsor monitors and auditors, the IRB/IEC, or regulatory authorities. The confidentiality of the subject's identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

12.2.3 Data Management

Data Management will be coordinated by the Astellas Global Data Manager in accordance with the SOPs for data management. All study-specific processes and definitions will be documented by Data Management. eCRF completion will be described in the eCRF instructions. Coding of medical terms and medications will be performed using MedDRA and WHO Drug Dictionary, respectively.

12.2.4 Quality Assurance

The sponsor is implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP and applicable regulatory requirement(s). Where applicable, the quality assurance and quality control systems and written SOPs of the CRO will be applied.

The sponsor or sponsor's designee may arrange to audit the clinical study at any or all investigational sites and facilities. The audit may include on-site review of regulatory documents, eCRFs and source documents. Direct access to these documents will be required by the auditors.

12.3 Contraception Requirements

WOCBP participants who choose complete abstinence must continue to have pregnancy tests, as specified in Schedule of Assessments.

WOMEN OF CHILDBEARING POTENTIAL DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile.

Women in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
- Post-menopausal

Documentation of any of these categories can come from the site personnel's review of the female subject's medical records, medical examination, or medical history interview.

A postmenopausal state is defined as at least 12 months after last regular menstrual bleeding without an alternative medical cause.

- In case the last regular menstrual bleeding cannot be clearly determined, confirmation with repeated follicle stimulating hormone (FSH) measurements of at least > 40 IU/L (or higher per local institutional guidelines), is required.

Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use 1 of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status by repeated FSH measurements before study enrollment.

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILD BEARING POTENTIAL

One of the highly effective methods of contraception listed below is required at the time of informed consent and until the end of relevant systemic exposure, defined as 28 days after the study vaccine administration.*****

Highly Effective Contraceptive Methods (Failure rate of < 1% per year when used consistently and correctly)[a]

Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation

- oral
- intravaginal

- transdermal

Progestogen-only hormonal contraception associated with inhibition of ovulation

- oral
- injectable
- implantable

Hormonal methods of contraception containing a combination of estrogen and progesterone:

- vaginal ring
- injectables
- implants
- intrauterine hormone-releasing system
- intrauterine device

Bilateral tubal occlusion/ligation is considered as a highly effective contraception method.

Vasectomized partner: *A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*

Sexual abstinence: *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study vaccine. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant. It is not necessary to use any other method of contraception when complete abstinence is elected.*

***** Local laws and regulations may require use of alternative and/or additional contraception methods.

[a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILD BEARING POTENTIAL.

Male participants with female partners of childbearing potential are eligible to participate if they agree to the following during the study and until the end of relevant systemic exposure defined as 28 days after study vaccine administration.*****

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator.
- Male participants are required to use a condom during the study and until end of relevant systemic exposure defined 28 days after study immunization.
- Female partners of male participants who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception until the end of relevant systemic exposure, defined as 28 days after study immunization.

12.4 List of Excluded Concomitant Medications

Subjects must not use any of the following:

- Vaccines, blood product, immunosuppressants including systemic steroids (topical steroids will be allowed) from the day of study administration until 30 days postimmunization.
- Subjects may receive other routine immunizations at the 30-day follow-up visit after the completion of all study procedures at each site's discretion; however routine vaccines should not be given in less than 30 days post-study immunization.
- Acetaminophen and NSAIDs are allowed during the study, but not within 24 hours prior to immunization and collection of serum samples for immunogenicity.
- Use of any systemically absorbed antibiotics during the 30-day period prior to collection of the immunogenicity samples should be restricted unless clinically warranted (e.g., avoid prophylaxis) to avoid interference with the immunoassay. If a subject has been treated with any systemically absorbed antibiotics between day 1 and day 30, the 30-day follow-up visit should take place at least 7 days after the last dose of the antibiotics.

12.5 Laboratory Assessment

Laboratory Test	Visit	Parameters to be Analyzed
Hematology	Screening (Visit 1) or Dosing (Visit 2), day 7 (Visit 3) and day 30 (Visit 4)	• Hemoglobin (HGB) • Hematocrit (HCT) • Erythrocytes (RBC) • Leukocytes (WBC) • Differential WBC • Platelets (PLT)
Biochemistry	Screening (Visit 1) or Dosing (Visit 2), day 7 (Visit 3) and day 30 (Visit 4)	• Sodium (Na) • Potassium (K) • Calcium (Ca) • Chloride (Cl) • Glucose (Gluc) • Total cholesterol (TC) • Triglycerides (TG) • Blood Urea Nitrogen (BUN) • Creatinine (Cr) • Creatine kinase (CK) • Total protein (TP) • Uric acid (UA) • Albumin (Alb) • C-reactive protein
Coagulation Profile	Screening (Visit 1) or Dosing (Visit 2), day 7 (Visit 3) and day 30 (Visit 4)	• Prothrombin Time (PT) • Activated Partial Thromboplastin Time (aPTT) • Fibrinogen
Hepatic Profile	Screening (Visit 1) or Dosing (Visit 2), day 7 (Visit 3) and day 30 (Visit 4)	• Alkaline phosphatase (ALP) • Aspartate transaminase (AST) • Alanine transaminase (ALT) • Gamma Glutamyl Transferase (GGT) • Total bilirubin (TBL) • Lactate dehydrogenase (LDH)
Urinalysis	Screening (Visit 1) or Dosing (Visit 2), day 7 (Visit 3) and day 30 (Visit 4)	Using dipstick • Protein • Glucose • pH • Blood • Leukocytes • Urobilinogen • Bilirubin • Ketones • Nitrite Microscopy (Optional) • Casts • Crystals • Epithelial cells • Leucocytes • Erythrocytes • Bacteria

Table continued on next page

Laboratory Test	Visit	Parameters to be Analyzed
Pregnancy Test (females of childbearing potential only)	Screening (Visit 1) – serum, Dosing (Visit 2) – urine, Day 30 (Visit 4) – urine	• Human Chorionic Gonadotropin (hCG)
Urine Drug Screening	Screening (Visit 1) or Dosing (Visit 2)	• Amphetamines • Barbiturates • Benzodiazepines • Cannabinoids • Cocaine
Virology	Screening (Visit 1) or Dosing (Visit 2)	• Anti-HAV (IgM) • HBsAg • Anti-HCV (reflex testing/HCV-RNA) • Anti-HIV-1 and 2
Immunogenicity	Dosing (Visit 2) and day 30 (Visit 4)	• Anti-biotin • Anti-1500+0785 • IgG and OPA
Immunogenicity	Dosing (Visit 2), day 7 (Visit 3) and day 30 (Visit 4)	• T-cell response (PBMCs)
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12.6 Grading of Local Reactogenicity

Stage 1 Group 1 and Stage 2 Group 2 subjects will be asked to record the below local reactogenicity 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.

‡ 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 diameter of the erythema/redness and induration/swelling.

12.7 Grading of 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 postimmunization.

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 a digital thermometer to record oral 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.

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

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 joint 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 postimmunization.

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

Reference

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.

12.8 Grading of Laboratory Abnormalities

Clinical laboratory test results will be graded and used for dose escalation decisions and for analysis of safety at the end of the study. The laboratory values within the grading scale provided in the FDA guidance have been modified based on ICON Central Laboratories reference ranges. These values will be provided to the DEC.

Reference

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.

12.9 Liver Safety Monitoring and Assessment

Any subject enrolled in a clinical study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × upper limit of normal (ULN) or bilirubin > 2 × ULN should undergo detailed testing for liver enzymes (including at least ALT, AST, alkaline phosphatase and total bilirubin [TBL]). Testing should be repeated within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator and study team. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN:

	ALT or AST		**Total Bilirubin**
Moderate	> 3 × ULN	Or	> 2 × ULN
Severe	> 3 × ULN	And	> 2 × ULN

In addition, the subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN.
- ALT or AST > 5 × ULN for more than 2 weeks.
- ALT or AST > 3 × ULN and International Normalized Ratio (INR) > 1.5 (If INR testing is applicable/evaluated).
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

The investigator may determine that abnormal liver function results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and laboratory tests. The site staff is to complete the liver abnormality case report form (LA-CRF). Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated 2 to 3 times weekly then weekly or less if abnormalities stabilize or the study vaccine has been discontinued and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a SAE. The

sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to study vaccine are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases is to be recorded as "AEs" within the eCRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic patients, and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications, is to be entered in the eCRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
  - Acute viral hepatitis (A, B, C, D, E or other infectious agents),
  - Ultrasound or other imaging to assess biliary tract disease,
  - Other laboratory tests including INR, direct bilirubin.
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

*Hy's Law Definition: Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10 to 50% mortality (or transplant).

The 2 "requirements" for Hy's Law are:

1) Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher 3 × ULN ("2 × ULN elevations are too common in treated and untreated patients to be discriminating").

2) Cases of increased bilirubin (at least 2 × ULN) with concurrent transaminase elevations at least 3 × ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated alkaline phosphatase [ALP]) or Gilbert's syndrome [Temple, 2006].

FDA Guidance for Industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," 2009:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (nonhepatotoxic) control drug or placebo.

2. Among trial subjects showing such AT elevations, often with ATs much greater than 3 × ULN, 1 or more also show elevation of serum TBL to > 2 × ULN, without initial findings of cholestasis (elevated serum ALP).

3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury. [Guidance for Industry titled "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" issued by FDA on July 2009]

References

Temple R. Hy's law: Predicting Serious Hepatotoxicity. Pharmacoepidemiol Drug Saf. 2006 April;15(Suppl 4):241-3.

Guidance for Industry titled "Drug-Induced Liver Injury: Premarketing Clinical Evaluation" issued by FDA on July 2009.

12.10 Common Serious Adverse Events

For this phase 1/2 clinical study, there is no list of common SAEs for which a single occurrence will be excluded from IND safety reporting.

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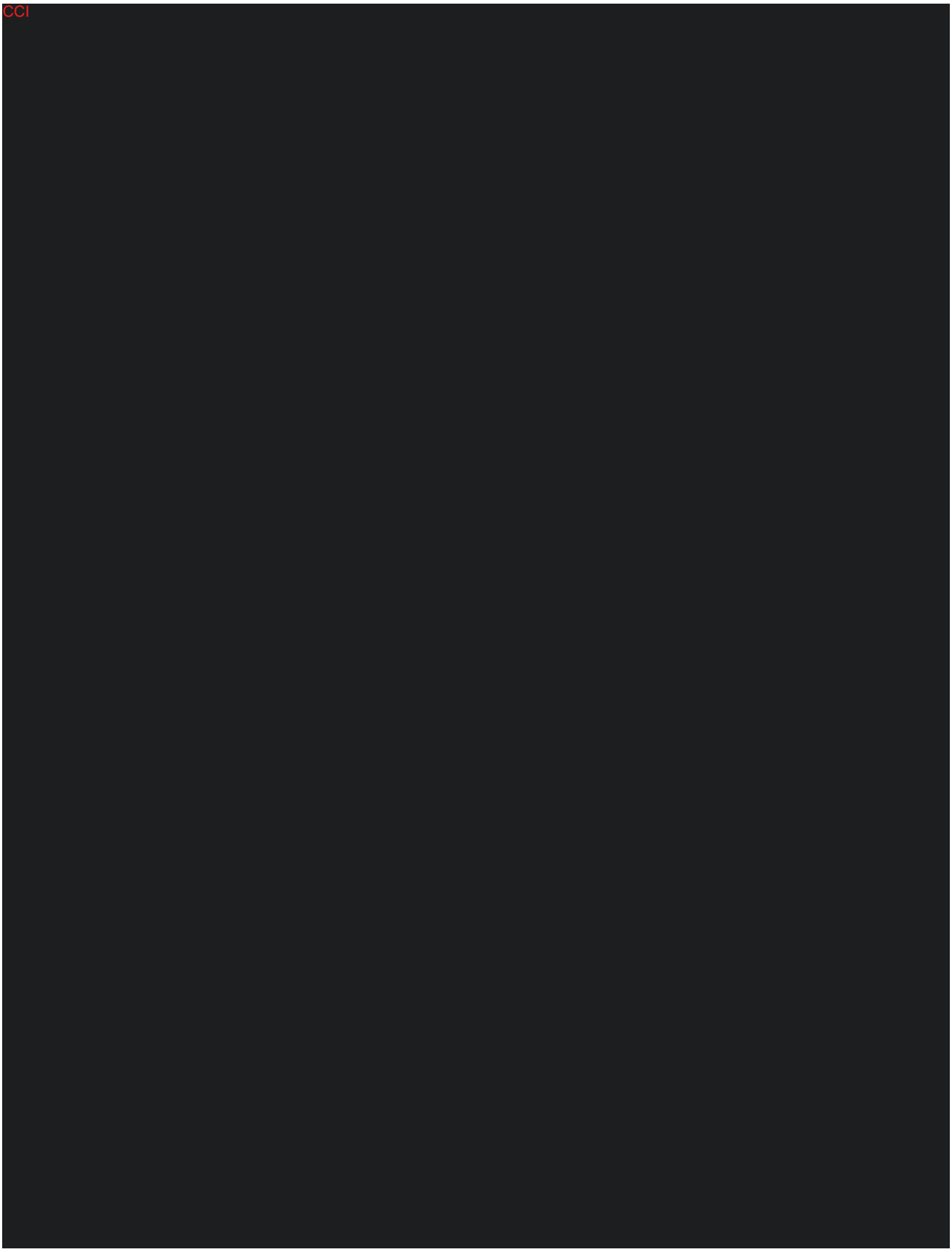

INFORMATION DISCLOSURE TO THE SUBJECTS

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13 ATTACHMENT 1: NONSUBSTANTIAL AMENDMENT 2

I. The purpose of this amendment is:

Non-Substantial Changes
1. Minor Administrative-type Changes
DESCRIPTION OF CHANGE:
Include minor administrative-type changes, e.g., typos, format, numbering, consistency throughout the protocol.
RATIONALE:
To provide clarifications to the protocol and to ensure complete understanding of study procedures.
2. Update Planned Study Period
DESCRIPTION OF CHANGE:
The planned study period for this Stage 2 of this trial is moved out a quarter.
RATIONALE:
This revision is made due to reflect the current study timelines.
3. Increase in Number of Study Sites
DESCRIPTION OF CHANGE:
The number of study sites for both Stage 1 and Stage 2 is increased.
RATIONALE:
The site numbers are increased to support subject recruitment in Stage 2 Group 2.
4. Modification to Exclusion Criterion #11
DESCRIPTION OF CHANGE:
Exclusion criterion #11 is revised to remove the requirement to exclude subjects who are positive for hepatitis B core antibody (HBc) during serology testing.
RATIONALE:
It was determined that hepatitis B surface antigen (HBsAg) is sufficient to confirm evidence of chronic hepatitis B virus infection.
5. Extension of Screening Period
DESCRIPTION OF CHANGE:
The screening period for Stage 2, Groups 2 and 3, is extended to 40 days before dosing.

RATIONALE:
The screening period is extended for Groups 2 and 3 to minimize the number of subjects who may require rescreening procedures due to the timing of cohort data reviews.
6. **Modification to Stopping Rules**
DESCRIPTION OF CHANGE:
The formal stopping rules for cohorts is updated such that enrollment/dosing will be stopped when 1 of the sentinel subjects experiences an ASP3772-related adverse event (AE) of severe intensity only when sentinel subjects are required.
RATIONALE:
Based on the dose escalation committee review of safety data in previous cohorts, it was decided that sentinel subjects will no longer be required for Cohort 5 onward.

II. Amendment Summary of Changes:

IIa. Non-substantial Changes

II. Contact Details of Key Sponsor's Personnel
WAS:
Clinical Research Contacts: PPD , MBBS, MS Clinical Study Manager Astellas Pharma Global Development, Inc. 1 Astellas Way, Northbrook, IL 60062 Tel: PPD Cell/Mobile: PPD Email: PPD PPD Project Manager ICON Clinical Research 2100 Pennbrook Parkway, North Wales, PA 19454 Tel: PPD Cell/Mobile: PPD Email: PPD
IS AMENDED TO:
Clinical Research Contacts: PPD ~~, MBBS, MS~~ PPD **Senior** Clinical Study Manager Astellas Pharma Global Development, Inc. 1 Astellas Way, Northbrook, IL 60062 Tel: PPD

Cell/Mobile: PPD
Email: PPD
PPD PPD
Project Manager
ICON Clinical Research
2100 Pennbrook Parkway, North Wales, PA 19454
Tel: PPD
Cell/Mobile: PPD
Email: PPD

IV Synopsis *Planned Study Period*
WAS:
Stage 1: 1Q2019 – 2Q2019 Stage 2: 2Q2019 – 2Q2020
IS AMENDED TO:
Stage 1: 1Q2019 – 2Q2019 Stage 2: ~~2Q2019 – 2Q2020~~ **3Q2019 – 3Q2020**

IV Synopsis *Planned Total Number of Study Centers and Location(s)*
WAS:
Stage 1: Approximately 5 sites in United States Stage 2: Approximately 10 sites (includes sites from Stage 1) in United States
IS AMENDED TO:
Stage 1: Approximately ~~5~~ **10** sites in United States Stage 2: Approximately ~~10~~ **25** sites (includes sites from Stage 1) in United States

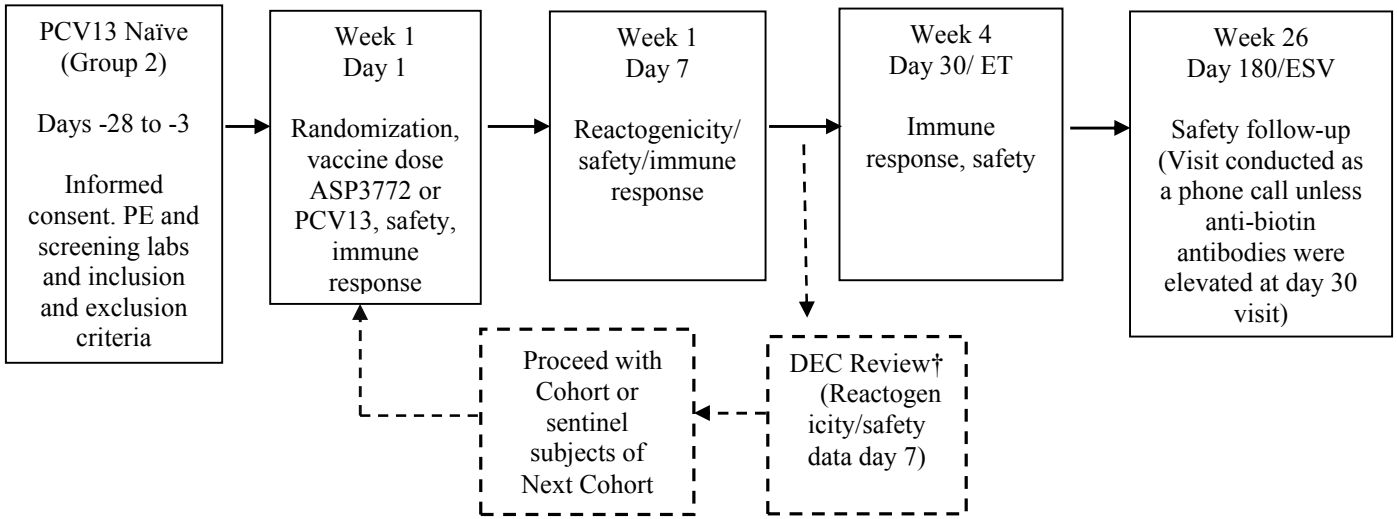
IV Synopsis, Exclusion Criteria and 3 Study Population *3.3 Exclusion Criterion #11*
WAS:
11. Subject has a positive serology test for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), hepatitis A virus antibodies (immunoglobulin M), hepatitis C virus antibodies (anti-HCV) confirmed by reflex testing (HCV-RNA) or antibodies to human immunodeficiency virus (HIV) type 1 and/or type 2 at screening.
IS AMENDED TO:
11. Subject has a positive serology test for hepatitis B surface antigen (HBsAg), ~~hepatitis B core antibody (anti-HBc),~~ hepatitis A virus antibodies (immunoglobulin M), hepatitis C virus antibodies (anti-HCV) confirmed by reflex testing (HCV-RNA) or antibodies to human immunodeficiency virus (HIV) type 1 and/or type 2 at screening.

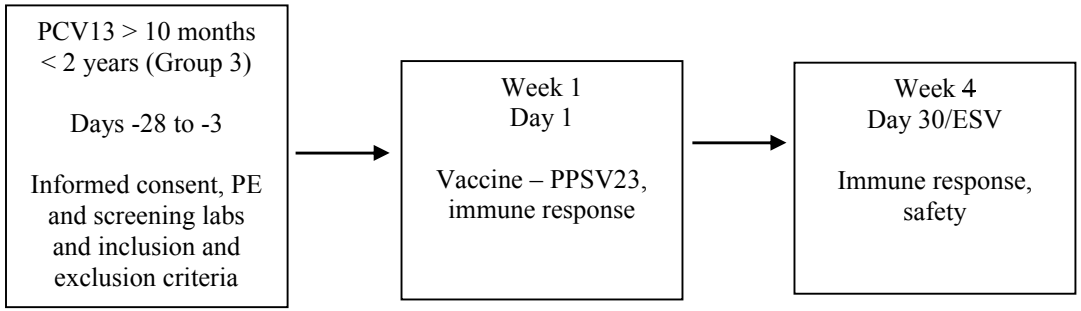
V Flow Chart and Schedule of Assessments

Flow Chart B

WAS:

Stage 2

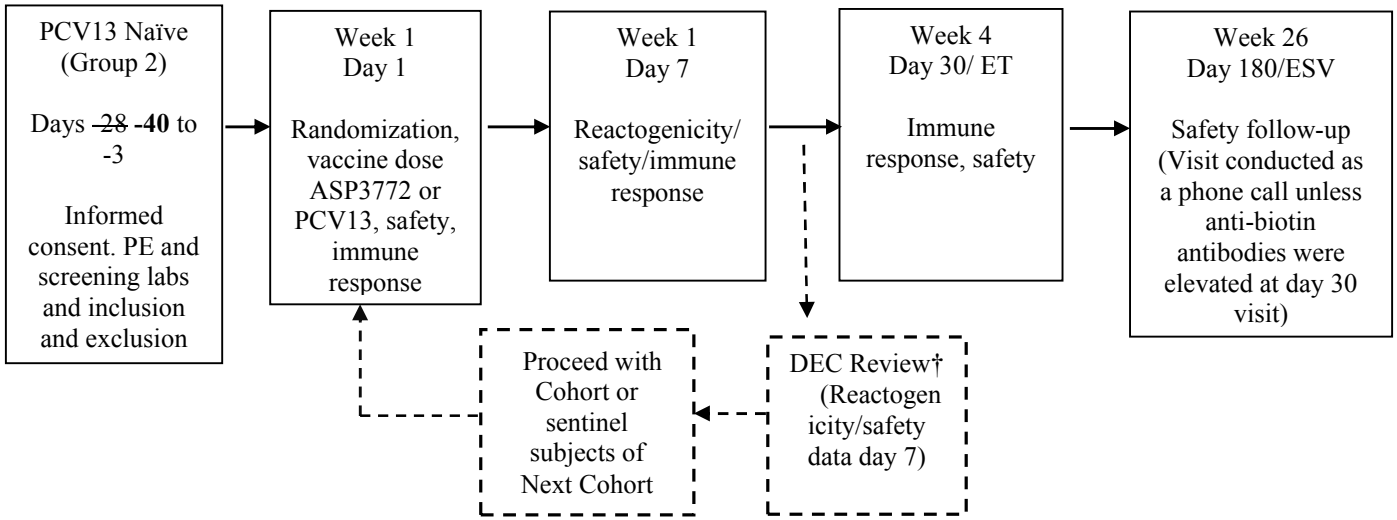


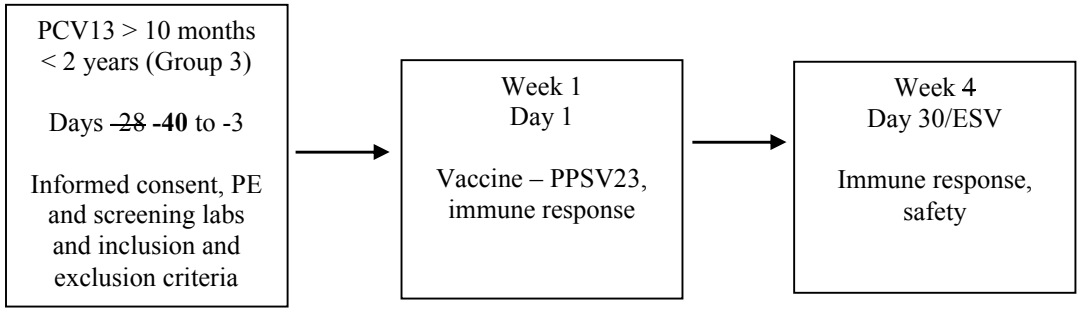


DEC: Dose Escalation Committee; ESV: end-of-study visit; ET: early termination; PCV13: Prevnar 13; PE: physical examination; PPSV23: Pneumovax 23
† DEC will review day 7 data for sentinel subjects before opening full cohort

IS AMENDED TO:

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

V Flow Chart and Schedule of Assessments

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

WAS:

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.

IS AMENDED TO:

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.

V Flow Chart and Schedule of Assessments

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

WAS:

Assessments	Screening[a]	Dosing	Follow-up		
Visit Number	1	2	3	4	5
Visit Day (Window)	-28 to -3	1	7 (+1)	30 (±2)*	180 (±14)§

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.
e. A physical examination will be performed at the 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. 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 and 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.

IS AMENDED TO:

Assessments	Screening[a]	Dosing	Follow-up		
Visit Number	1	2	3	4	5
Visit Day (Window)	~~-28~~ **-40** to -3	1	7 (+1)	30 (±2)*	180 (±14)§

a. Screening will be completed up to ~~28~~ **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.
e. A physical examination will be performed at the screening visit (days ~~-28~~ **-40** 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. 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 ~~and~~ **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.

V Flow Chart and Schedule of Assessments

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

WAS:

Assessments	Screening[a]	Dosing	Follow-up
Visit Number	1	2	3
Visit Day (Window)	-28 to -3	1	30 (±2)

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.
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) and HIV antigen/antibody (type 1 and/or type 2) at screening.

IS AMENDED TO:

Assessments	Screening[a]	Dosing	Follow-up
Visit Number	1	2	3
Visit Day (Window)	~~28~~ **-40** to -3	1	30 (±2)

a. Screening will be completed up to ~~28~~ **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.

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) and HIV antigen/antibody (type 1 and/or type 2) at screening.

5 Immunizations and Evaluation
5.1.5 Criteria for Continuation of Immunization

WAS:

Formal Stopping Rules for Cohorts:

No additional subjects will be randomized or dosed in the study until the events below occurring in a subject who received ASP3772 are reviewed by the DEC and it is acceptable to resume cohort immunization.

- Unexplained death of a subject.
- Acute hypersensitivity reaction in a subject.
- One of the sentinel subjects experiences an AE of severe intensity related to ASP3772.
- An SAE of the same character occurs in at least 2 subjects or 1 subject experiences a vaccine-related SAE.
- Grade 3 or higher lab abnormality considered related to the immunization occurs in 2 or more subjects [FDA Guidance for Industry, 2007].
- The same type of reactogenicity event assessed as Grade 3 or higher occurs in 2 or more subjects, or 1 of the sentinel subjects experiences a reactogenicity event assessed as Grade 3 or higher, using the FDA toxicity grading scale for preventative vaccines [FDA Guidance for Industry, 2007].
- An imbalance of unsolicited AEs between ASP3772 and PCV13 is observed.

IS AMENDED TO:

Formal Stopping Rules for Cohorts:

No additional subjects will be randomized or dosed in the study until the events below occurring in a subject who received ASP3772 are reviewed by the DEC and it is acceptable to resume cohort immunization.

- Unexplained death of a subject.
- ~~Acute~~ **Serious acute** hypersensitivity reaction in a subject.
- One of the sentinel subjects experiences an AE of severe intensity related to ASP3772**, when sentinel subjects are required**.
- An SAE of the same character occurs in at least 2 subjects or 1 subject experiences a vaccine-related SAE.
- Grade 3 or higher lab abnormality considered related to the immunization occurs in 2 or more subjects [FDA Guidance for Industry, 2007].
- The same type of reactogenicity event assessed as Grade 3 or higher occurs in 2 or more subjects, or 1 of the sentinel subjects experiences a reactogenicity event assessed as Grade 3 or higher, using the FDA toxicity grading scale for preventative vaccines [FDA Guidance for Industry, 2007].
- An imbalance of unsolicited AEs between ASP3772 and PCV13 is observed.

5 Immunizations and Evaluation
5.2.1 Demographics

WAS:

Demographic information will be collected for all subjects (where permitted) at the screening visit (day -28 to day-3) and will include age, date of birth, sex, race and ethnicity.
IS AMENDED TO:
Demographic information will be collected for all subjects (where permitted) at the screening visit ~~(day -28 to day-3)~~ and will include age, date of birth, sex, race and ethnicity.

5 Immunizations and Evaluation *5.2.2 Medical History*
WAS:
Medical history will be collected at the screening visit (day -28 to day-3) in order to assess Inclusion/Exclusion criteria and will be reconfirmed prior to study immunization (if screening was performed > 7 days from study immunization [day 1]).
IS AMENDED TO:
Medical history will be collected at the screening visit ~~(day -28 to day-3)~~ in order to assess Inclusion/Exclusion criteria and will be reconfirmed prior to study immunization (if screening was performed > 7 days from study immunization [day 1]).

5 Immunizations and Evaluation *5.4.6 Physical Examination*
WAS:
A physical examination consisting of an examination of general appearance, eyes, nose throat, neck (including thyroid), lymph nodes, chest, lungs, cardiovascular, abdomen, skin, extremities, musculoskeletal and neurological system including mental status will be conducted at the screening visit (days -28 to -3), prior to study immunization (day 1) and at the day 30 follow-up visit. A symptom-directed physical exam may be performed at any other time, if necessary. The screening physical examination also includes evaluation of significant, ongoing medical conditions. Any changes between screening and randomization will be captured in the medical/surgical history.
IS AMENDED TO:
A physical examination consisting of an examination of general appearance, eyes, nose throat, neck (including thyroid), lymph nodes, chest, lungs, cardiovascular, abdomen, skin, extremities, musculoskeletal and neurological system including mental status will be conducted at the screening visit ~~(days -28 to -3)~~, prior to study immunization (day 1) and at the day 30 follow-up visit. A symptom-directed physical exam may be performed at any other time, if necessary. The screening physical examination also includes evaluation of significant, ongoing medical conditions. Any changes between screening and randomization will be captured in the medical/surgical history.

12 Appendices *12.5 Laboratory Assessment*
WAS:

Laboratory Test	Visit	Parameters to be Analyzed
Virology	Screening (Visit 1) or Dosing (Visit 2)	• Anti-HAV (IgM) • HBsAg • Anti-HBc • Anti-HCV (reflex testing/HCV-RNA) • Anti-HIV-1 and 2

IS AMENDED TO:

Laboratory Test	Visit	Parameters to be Analyzed
Virology	Screening (Visit 1) or Dosing (Visit 2)	• Anti-HAV (IgM) • HBsAg • ~~Anti-HBc~~ • Anti-HCV (reflex testing/HCV-RNA) • Anti-HIV-1 and 2

14 COORDINATING INVESTIGATOR'S SIGNATURE

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

Version 2.1 Incorporating Nonsubstantial Amendment 2

02 Dec 2019

I have read all pages of this clinical study protocol for which Astellas is the sponsor. I agree that it contains all the information required to conduct this study.

Coordinating Investigator:

Signature: _____ _____

<Insert name, department/affiliation, name of institution> Date (DD Mmm YYYY)

Printed Name: _____

Address: _____

_____

15 SPONSOR SIGNATURES

ELECTRONIC SIGNATURE PAGE

Document Type : Clinical Study Protocol

Document Control Number : MGC1901015

Amendment Number : N/A

International Study Number : 3772-CL-1001

Departmental Study Number : N/A

Actual Version Number : Reissue v2.1, 02Dec2019

Document Version : 2.0

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

Date (UTC)	Signed by	Sign Off Meaning
12/03/2019 20:55:42	PPD	Study Statistician Approval
Full Name / Legal Name	PPD	
12/04/2019 23:14:52	PPD	(For Disclosure) Responsible Officer
Full Name / Legal Name	PPD	
Full Name / Legal Name		
Full Name / Legal Name		
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*UTC: Coordinated Universal Time