STATISTICAL ANALYSIS PLAN

Final Version 4.00, dated 13-JAN 2022

A Phase 2 Proof of Concept, Double-blind, Randomized, Placebo-controlled Study to Evaluate the Efficacy of ASP1128 (MA-0217) in Subjects at Risk for Acute Kidney Injury following Coronary Artery Bypass Graft (CABG) and/or Valve Surgery

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Astellas Pharma Inc.

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I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
AE	adverse event
AKI	acute kidney injury
AKI-KDIGO	acute kidney injury based on all criteria from the KDIGO guideline
AKI-SCr	acute kidney injury based on serum creatinine KDIGO criteria
AKI-UO	acute kidney injury based on urinary output
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APACHE-II	Acute Physiology and Chronic Health Evaluation II
ARMCD	Planned Arm Code
AST	aspartate aminotransferase
AUCSCr	Area under the curve of SCr
AUCSCysC	Area under the curve of S-cystatin-C through
BQL	below the quantification limit
CABG	coronary artery bypass graft
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMH	Cochran-Mantel-Haenszel
CPB	cardiopulmonary bypass pump
CV	coefficient of variation
DMC	Data Monitoring Committee
DoD	day of discharge from hospital
ECMO	Extracorporeal Membrane Oxygenation
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EOI	end of infusion
EoS	end of study
EQ-5D-5L	5-level European Quality of Life 5 Dimensions Questionnaire
FAS	full analysis set
GFR	glomerular filtration rate
ICH	International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	intensive-care unit
IP	investigational product
IRT	interactive response technology
KDIGO	kidney disease: improving global outcomes
KIM1	kidney injury molecule-1
MAKE	major adverse kidney events
MAKE30	MAKE within 30 days after day of surgery
MAKE90	MAKE within 90 days after day of surgery

Abbreviations	Description of abbreviations
MAP	mean arterial pressure
MCMC	Monte Carlo Markov Chain
MH	Mantel-Haenszel
MI	Multiple Imputation
NC	NephroCheck®
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
PDAS	pharmacodynamic analysis set
PFAS	prognostic factor analysis set
PGx	pharmacogenomics
PK	pharmacokinetics
PKAS	pharmacokinetic analysis set
PPS	per protocol set
PT	preferred term
RRT	renal replacement therapy
SAEs	serious adverse events
SAF	safety analysis set
SAP	statistical analysis plan
SCr	serum creatinine
SCysC	serum cystatin C
SD	standard deviation
SOC	system organ class
T0	time point 0
TEAE	treatment-emergent adverse event
TLF	Tables, Listings and Figures
ULN	upper limit of normal
UO	urinary output
VAD	ventricular assist device
VAS	visual analogue scale

List of Key Terms

Terms	Definition of Terms
Baseline	Assessments of subjects as they enter a study, before they receive any treatment.
End of Study	The last visit for this study.
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.
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.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a study.
Screening	A process of active consideration of potential subjects for enrollment 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.
Subject	An individual who participates in a clinical study, either as a recipient of the investigational product(s) or as a control.
T0	End of surgery for this study protocol is referred to as Time point 0 (T0), which is defined as the time point when the subject comes off the cardiopulmonary bypass pump (CPB) (i.e., the final separation from CPB) and normal circulation is restored.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

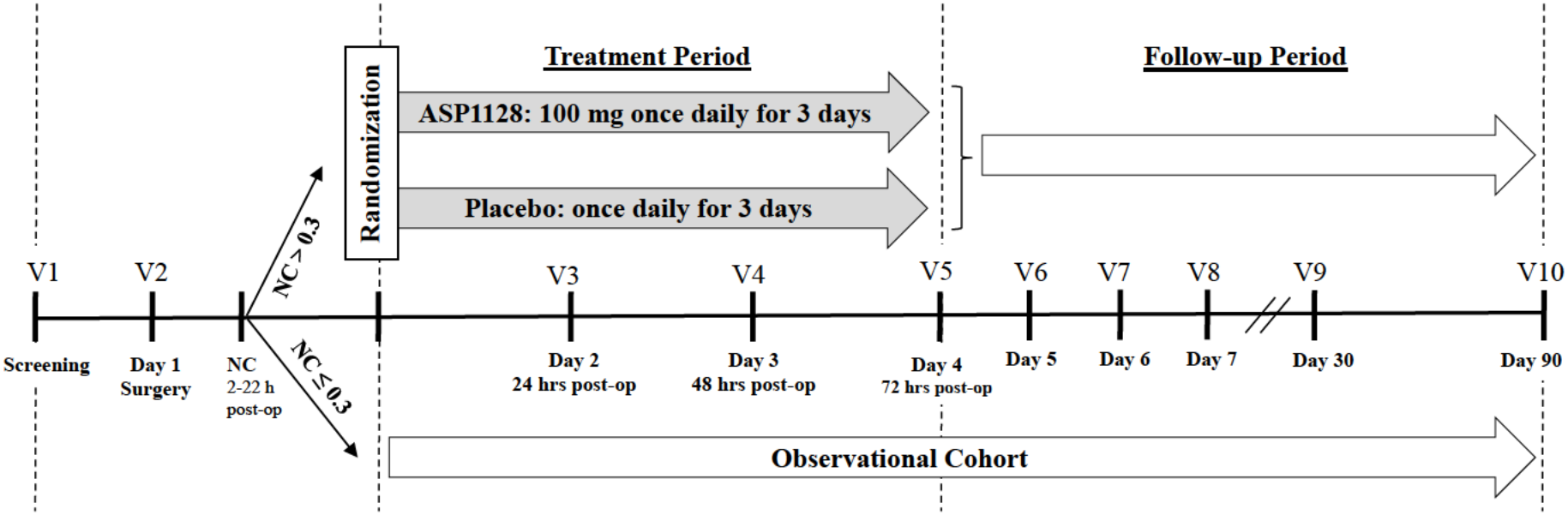
This Statistical Analysis Plan (SAP) contains technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes procedures for executing the statistical analysis to fulfil the objectives of the study.

The final SAP will be approved prior to database lock and unblinding the subject treatment assignments.

Changes from the planned analyses in the final SAP that impact the statistical analyses will be documented in the Clinical Study Report.

2 FLOW CHART AND VISIT SCHEDULE

Figure 1 Flow Chart



NC: NephroCheck® AKIRisk® score

Table 1 Schedule of Assessments — Randomized Subjects

Study Period	Screening	Surgery			Treatment Period			Follow-up Period					Unscheduled
Visit Number	1	2[1]			3	4	5	6	7	8	9	10/EoS	
Day	-28	pre	peri[2]	post	2	3	4	5	6	7/DoD[3]	30	90	
Visit Window	≥ -28 days	-	-	-	T0 + 24 hours	T0 + 48 hours	T0 + 72 hours	-	-	4 to 7 days	23 to 37 days	83 to 97 days	
Assessment													
Informed Consent	X												
Inclusion/exclusion criteria	X												
Medical history, demographics	X												
Medication use	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X[19]												
Body weight, height	X										X[4]	X[4]	
Subject randomization				X[5]									
Vital signs	X	X			X	X	X			X	X	X	O
Hematology, biochemistry[6, 7]	X[17]	X			X	X	X	X	X	X	X	X	X
Urinary output[8]				X	X	X	X	X	X	X			O
Urinalysis[9]	X[17]	X			X	X	X	X	X	X	X	X	O
Pregnancy test	X[17]	X[18]									X	X	
NephroCheck®				X[5]	X[5]	X[5]	X[5]						
Dosing investigational product[10]				X		X	X						
Biomarkers[11]		X		X	X	X	X			X			
Assessment of AEs[12]	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess follow-up parameters[13]				X	X	X	X	X	X	X	X	X	X
APACHE-II[14]		X		X	X	X	X						
EQ-5D-5L	X									X	X	X	
Blood samples for pharmacokinetics[15]				X		X	X						
Blood samples for pharmacodynamics[16]				X			X						
Biobanking sample PGx		X											

Footnotes appear on next page

AE: adverse event; APACHE-II: acute physiology and chronic health evaluation II; DoD: day of discharge from hospital; EoS: end of study; EQ-5D-5L: 5-level European Quality of Life 5 Dimensions Questionnaire; ICU: intensive care unit; O: if possible; PGx: pharmacogenomics; T0: time point 0; UO: urine output.

1. The preoperative period is defined as the part of the day of surgery before the incision. The peri-operative period is defined as the period between incision and T0 (i.e., the time point when the subject comes off the cardiopulmonary bypass pump [CPB]). The postoperative period is the period after T0.
2. During surgery, the following data have to be assessed in the electronic case report form (eCRF): Procedures (coronary artery bypass graft surgery and/or valve surgery, thoracic aortic surgery, extracorporeal membrane oxygenation, use of ventricular assist device, redo cardiac surgery) and duration of CPB; lowest intraoperative mean arterial pressure; perioperative fluid balance and medication (fluid intake, UO, blood products, drain output, blood loss, inotropics/vasopressors and furosemide); time of incision and closing of skin; T0 and perioperative complications.
3. If the subject is discharged from hospital before day 7, visit 8 will be done on the day of discharge and no site visits are required after discharge up to day 7. If discharged after day 7, no additional assessments are required after day 7 up to discharge except for the recording serum creatinine (SCr) from local laboratory testing.
4. At days 30 and 90, only body weight needs to be measured. A full physical examination is not required.
5. Subjects will only be randomized if postoperative NephroCheck® (NC) AKIRisk® score (assessed between 2 to 22 hours after T0) is > 0.3 $(ng/mL)^2/1000$. If the AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$, the subject cannot be randomized and will be followed up in the observational arm, see Schedule of Assessments [Table 2].
   NC measurements at visits 3, 4 and 5 do not have to be performed at sites: urine samples at visits 3, 4 and 5 will be taken, but frozen until later assessment at central lab.
6. Safety central laboratory tests including serum creatinine (SCr) and cystatin C.
7. If the subject is discharged from hospital after day 7, the last result of SCr before discharge from local laboratory testing will be collected.
8. UO assessment: from T0 (visit 2) to T72 (visit 5) when the subject is in the ICU, UO will be recorded every 12 hours and fluid intake will be recorded every 24 hours. The eCRF should indicate when UO data have to be recorded from the subject ICU record. During the first 72 hours following surgery (i.e., visits 3 to 5), the investigator will be asked to record information on AKI-UO stages 1 to 3 in the eCRF to assess their occurrence as per investigator discretion. When the subject is discharged from the ICU, but is still in the hospital UO and fluid intake every 24 hours will be recorded up to day 7 (visit 8).
9. Include albuminuria dipstick.
10. The first dose of investigational product (IP)/study drug to be administered as soon as possible after the NC AKIRisk score is assessed between 2 and 22 hours after T0 is > 0.3 $(ng/mL)^2/1000$ to allow completion of first treatment within 24 hours after T0. If the first dose of IP administration is completed within 8 hours after T0, the second dose of IP will be administered at 24 hours after T0 and the third dose of IP will be administered at 48 hours after T0. If the first dose of IP administration is completed past 8 hours after T0, the second dose of IP will be administered at 16 hours after the start of the first dose, and the third dose of IP will be administered at 40 hours after the start of the first dose of IP.
    Second and third dosing of the IP: a 2-hour treatment window before or after designated administration time is allowed.
11. Additional urine samples will be taken and stored to assess various clinical and compound related biomarkers.
12. AE will be collected from informed consent until visit 9 or until the subject is determined to be a screen failure. If the subject is discontinued from the study prior to visit 9, AEs will be collected until the EoS visit has been completed. After the end of all the procedures of visit 9 until visit 10, only serious AEs will be collected.
13. Assess data on renal replacement therapy, mortality, ICU stay, total hospital stay and duration of mechanical ventilation (time ventilation weaning, i.e., time from T0 to extubation).

Footnotes continued on next page

14. APACHE-II score is recorded on visit 2 preoperatively, postoperatively and during the time of drug administration at visits 2, 3 and 4 and at day 4 (visit 5) if the subject is still in the ICU. Results from local laboratory testing can be used, and the result closest to the indicated time points should be recorded.
15. Blood samples for pharmacokinetics will be collected at predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose of first administration, at end of infusion of second administration, and at predose and at end of infusion of third administration.
16. Blood samples for gene expression measurements will be collected at predose and 2 to 4 hours postdose of first and third administrations.
17. Visit 1 can be close to the day of surgery. If the results from the central laboratory testing of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and estimated glomerular filtration rate (eGFR) are not yet available at time of randomization, results from local laboratory can be used for verifying randomization requirements if assessed within 7 days before the day of surgery up to initiation of surgery. When the surgery is scheduled within 2 days after screening visit 1 and it is not feasible to perform blood and/or urine samplings at visit 1, the sampling at visit 1 can be by-passed if the results from local laboratory are available for verifying randomization requirements.
18. Presurgery pregnancy test at the day of surgery will be assessed locally with urine dipstick test.
19. When an assessment of a full physical examination is available and performed within 30 days before surgery, the assessment can be used even if it was performed prior to the informed consent.

Table 2 Schedule of Assessments – Subjects in the Observational Cohort

Study Period	**Screening**	**Surgery**			**Follow-up Period**						**Unscheduled**
Visit Number	**1**	**2**[1]			**3**	**4**	**5**	**8**	**9**[13]	**10/EoS**[13]	
Day	**-28**	**pre**	**peri**[2]	**post**	**2**	**3**	**4**	**7/DoD**	**30**	**90**	
Visit Window	**≥ -28 days**	**-**	**-**	**-**	**T0 + 24 hours**	**T0 + 48 hours**	**T0 + 72 hours**	**4 to 7 days**	**23 to 37 days**	**83 to 97 days**	
Assessment											
Informed Consent	X										
Inclusion/exclusion criteria	X										
Medical history, demographics	X										
Medication use	X	X	X	X	X[8]						
Physical examination	X[14]										
Body weight, height	X										
Subject randomization											
Vital signs	X	X									
Hematology, biochemistry	X[3, 6]	X[3]			X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]
Urinary output[10]				X	X	X	X	X			O
Urinalysis	X[3, 6]	X[3]						X[9]	X[9]	X[9]	X[9]
Pregnancy test	X[3, 6]	X[7]									
NephroCheck®				X[11]	X[12]	X[12]	X[12]				
Biomarkers[4]		X		X	X[12]	X[12]	X[12]				
Assessment of AEs	X	X	X	X	X[8]						
Assess follow-up parameters											
Assess MAKE parameters					X	X	X	X	X	X	X
APACHE-II[5]		X		X							
EQ-5D-5L	X										
Blood samples for pharmacokinetics											
Blood samples for pharmacodynamics											
Biobanking sample PGx		X									

AE: adverse event; APACHE-II: acute physiology and chronic health evaluation II; DoD: day of discharge from hospital; EoS: end of study; EQ-5D-5L: 5-level European Quality of Life 5 Dimensions Questionnaire; MAKE: major adverse kidney events; O: if possible; PGx: pharmacogenomics; T0: time point 0; UO: urine output.

1. The preoperative period is defined as the part of the day of surgery before the incision. The peri-operative period is defined as the period between incision and T0 (i.e., the time point when the subject comes off the cardiopulmonary bypass pump [CPB]). The postoperative period is the period after T0.
2. During surgery, the following data have to be assessed in the electronic case report form (eCRF): Procedures (coronary artery bypass graft surgery and/or valve surgery, thoracic aortic surgery, extracorporeal membrane oxygenation, use of ventricular assist device, redo cardiac surgery) and duration of CPB; lowest intraoperative mean arterial pressure; perioperative fluid balance and medication (fluid intake, UO, blood products, drain output, blood loss, inotropics/vasopressors and furosemide); time of incision and closing of skin; T0 and perioperative complications.
3. Central laboratory tests
4. Additional urine samples will be taken and stored to assess various clinical and compound related biomarkers.
5. APACHE-II score is recorded on visit 2 preoperatively, postoperatively. Results from local laboratory testing can be used, and the result closest to the indicated time points should be recorded.
6. Visit 1 can be close to the day of surgery. If the results from the central laboratory testing of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and eGFR are not yet available at time of randomization, results from local laboratory can be used for verifying randomization requirements if assessed within 7 days before the day of surgery up to initiation of surgery. When the surgery is scheduled within 2 days after screening visit 1 and it is not feasible to perform blood and/or urine samplings at visit 1, the sampling at visit 1 can be by-passed if the results from local laboratory are available for verifying randomization requirements.
7. Presurgery pregnancy test at the day of surgery will be assessed locally with urine dipstick test.
8. AEs and medication use will be collected until 24 hours after T0. However, AEs related to MAKE parameters will be collected until the EoS visit. AEs will be followed up until resolved including medication use for the AE.
9. Local laboratory test results will be collected from medical records, including serum creatinine and serum cystatin C (if available).
10. 24-hour urinary output data will be collected from medical records (if available).
11. Subjects will only be randomized if postoperative NephroCheck® (NC) AKIRisk® score (assessed between 2 to 22 hours after T0) is > 0.3 $(ng/mL)^2/1000$. If the AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$, the subject cannot be randomized and will be followed up in the observational arm.
12. Urine sampling only for NC and biomarker assessments if subjects are still in the intensive care unit and urine can be sampled from the catheter. Urine samples will be taken, but frozen until later assessment at the central laboratory.
13. Visit 9 and 10 may be conducted by phone if the subject is unable to visit the site and will require contact for MAKE parameters collection.
14. When an assessment of a full physical examination is available and performed within 30 days before surgery, the assessment can be used even if it was performed prior to the informed consent.

3 STUDY OBJECTIVE(S) AND DESIGN

3.1 Study Objective(s)

3.1.1 Primary Objective

- To evaluate the efficacy of postsurgery treatment with ASP1128 in subjects at risk for acute kidney injury (AKI) following CABG and/or valve surgery

3.1.2 Secondary Objectives

- To investigate the safety and tolerability of postsurgery treatment with ASP1128 in subjects at risk for AKI following CABG and/or valve surgery
- To investigate the pharmacokinetic characteristics of ASP1128 in subjects at risk for AKI following CABG and/or valve surgery

3.1.3 Exploratory Objective

- To investigate the pharmacodynamic characteristics of ASP1128 in subjects at risk for AKI following CABG and/or valve surgery
- To evaluate subject characteristics and biomarkers (including the NC device) for identifying subjects at risk for AKI following CABG and/or valve surgery

3.2 Study Design

The study is a double-blind, placebo-controlled, randomized study with 1 ASP1128 and 1 placebo treatment arm. Randomization will occur in a 1:1 manner. There will be a cap on the proportion of subjects randomized with an estimated glomerular filtration rate (eGFR) < 45 mL/min per 1.73 m^2 (assessed at visit 1 as per the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation) of 20% of the total randomized. Randomization will be stratified for eGFR at visit 1 < 45 mL/min per 1.73 m^2.

The eGFR will be calculated using the glomerular filtration rate (GFR) estimation equation in Kidney Disease Improving Global Outcomes (KDIGO) [KDIGO, 2013].

Gender	Serum Creatinine	Equation for Estimating GFR
Female	≤ 0.7 mg/dl (≤ 62 µmol/l)	$144 \times (SCr/0.7)^{-0.329} \times 0.993^{Age}$ [× 1.159 if black]
Female	> 0.7 mg/dl (> 62 µmol/l)	$144 \times (SCr/0.7)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
Male	≤ 0.9 mg/dl (≤ 80 µmol/l)	$141 \times (SCr/0.9)^{-0.411} \times 0.993^{Age}$ [× 1.159 if black]
Male	> 0.9 mg/dl (> 80 µmol/l)	$141 \times (SCr/0.9)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]

GFR: glomerular filtration rate; SCr: serum creatinine
Source: [KDIGO, 2013]

The subject's age at visit 1 will be used for calculation of eGFR in this study.

Subjects are eligible for screening if they are scheduled for non-emergent (i.e., non-acute or directly life threatening) CABG and/or valve surgery within 4 weeks after screening. Subjects cannot be rescreened once screening has failed.

Study visits include safety and efficacy assessments: chemistry, hematology and urinalysis laboratory assessments, UO, pregnancy test, physical examination, vital signs, blood pressure and assessment of AEs. In addition, blood and urine will be sampled for pharmacokinetics, pharmacodynamics, pharmacogenomics (PGx), metabolomics and biomarker assessments.

3.3 Randomization

Subjects who have a NC AKIRisk score of > 0.3 $(ng/ml)^2/1000$ will be randomized in a 1:1 ratio to a treatment arm according to the randomization schedules through interactive response technology (IRT). There will be a cap on the proportion of subjects randomized with a eGFR < 45 mL/min per 1.73 m^2 (assessed at visit 1 as per CKD-EPI equation) of 20% of total randomized. Randomization will also be stratified for eGFR < 45 and ≥ 45 mL/min per 1.73 m^2 to allocate subjects equally between study groups within each stratification. The site personal will dispense the treatment according to the IRT system's assignment. Specific procedures for randomization through the IRT are contained in the study procedures manual.

Subjects with NC AKIRisk score of 0.3 or less will be enrolled in the observational cohort.

4 SAMPLE SIZE

Sample size of this study is 220 randomized subjects (110 subjects in the ASP1128 group and 110 subjects in the placebo group).

The above sample size provides 80% power to detect the difference between ASP1128 and placebo on the primary endpoint of AKI proportion in the case of not considering an interim analysis, assuming the followings:

- AKI proportion in the placebo group is 60%
- Relative risk of ASP1128 against placebo is 0.7 (30% reduction)
- 1-sided significance level is 0.05
- Drop-out rate is around 10%

In addition, an interim analysis at 60% of enrollment as described below is planned by East® 6 software. Considering the interim analysis, the power is 78%.

- Futility stop criteria: conditional power to detect the relative difference between ASP1128 and placebo on the primary endpoint of AKI proportion is less than 10%, that is, 1-sided p-value is larger than 0.259, when approximately 60% of the planned acute kidney injury based on serum creatinine within 72 hours after Time point 0 (T0) (AKI-SCr72h) data have been collected in the study.
- There will be no provision to stop the study for efficacy in the interim analysis, so no efficacy stop criteria are defined.

Approximately 1000 subjects will be screened to enroll 600 subjects who meet the presurgery selection criteria. It is expected that 1 out of 3 subjects enrolled will meet the postsurgery criterion of having a NC AKIRisk score > 0.3 $(ng/mL)^2/1000$ and can be randomized: the

number of subjects to be randomized is 220. The subjects that have an AKIRisk score of ≤ 0.3 at the postsurgery 2- to 6-hour assessment will be enrolled in the observational cohort.

5 ANALYSIS SETS

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

Detailed criteria for analysis sets will be laid out in Classification Specifications and the allocation of subjects to analysis sets will be determined prior to database lock.

5.1 Full Analysis Set (FAS)

The Full Analysis Set (FAS) will consist of all randomized subjects who receives at least 1 dose of study treatment (ASP1128 or placebo), and the analysis is based on the randomized treatment. This will be the primary analysis set for efficacy analyses.

5.2 Per Protocol Set (PPS)

The Per-Protocol Set (PPS) includes all subjects of the FAS who do not meet criteria for exclusion from PPS listed in [Section 5.2.1] of this SAP.

The PPS will be a secondary analysis set for efficacy analyses. Select demographic and baseline characteristics may also be summarized for the PPS.

5.2.1 Reasons for Exclusion From PPS

The following reasons may lead to subject's exclusion from PPS:

1. Not complete scheduled three times administrations of study drugs
2. Administration of excluded concomitant treatment as described in the protocol
3. Violation of the any inclusion or exclusion criteria

5.3 Safety Analysis Set (SAF)

The Safety Analysis Set (SAF) consists of all subjects who receives at least 1 dose of study treatment (ASP1128 or placebo), and will be used for safety analyses.

For the statistical summary of the safety data, the SAF will be used.

5.4 Pharmacokinetics Analysis Set (PKAS)

The Pharmacokinetic Analysis Set (PKAS) consists of the subset of SAF for which at least 1 concentration available. Additional subjects may be excluded from the PKAS at the discretion of the pharmacokineticist.

The PKAS will be used for all summaries and analyses of the pharmacokinetic data.

5.5 Pharmacodynamic Analysis Set (PDAS)

The Pharmacodynamic Analysis Set (PDAS) will include the subjects from the administered population for whom sufficient pharmacodynamic measurements were collected The PDAS will be used for all analyses of pharmacodynamic data.

The PDAS will be used for all summaries and analyses of pharmacodynamic data.

5.6 Prognostic Factor Analysis Set (PFAS)

The Prognostic Factor Analysis Set (PFAS) consists of NC positive subjects, who are randomized to placebo group, and the NC negative subjects, who are in the observational cohort.

The PFAS will be used for exploring the prognostic factors and comparison with to NC test.

6 ANALYSIS VARIABLES

The value at presurgery will be used as the baseline unless otherwise specified for each endpoint. In case of missing value at presurgery, the last measurement before surgery will be used as the baseline.

The eGFR(SCr) will be calculated using the GFR estimation equation (2009 CKD-EPI creatinine equation) [KDIGO, 2013]. The subject's age at visit 1 should be used for calculation in this study.

Gender	Serum Creatinine	Equation for Estimating GFR
Female	≤ 0.7 mg/dl (≤ 62 μmol/l)	$144 \times (SCr/0.7)^{-0.329} \times 0.993^{Age}$ [× 1.159 if black]
Female	> 0.7 mg/dl (> 62 μmol/l)	$144 \times (SCr/0.7)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
Male	≤ 0.9 mg/dl (≤ 80 μmol/l)	$141 \times (SCr/0.9)^{-0.411} \times 0.993^{Age}$ [× 1.159 if black]
Male	> 0.9 mg/dl (> 80 μmol/l)	$141 \times (SCr/0.9)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]

GFR: glomerular filtration rate; SCr: serum creatinine

Reference: KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Official Journal of the International Society of Nephrology: Kidney International Supplements. 2013;3(1):1-150.

The eGFR(serum cystatin C [SCysC]) will be calculated using the GFR estimation equation (2012 CKD-EPI cystatin C equation) [KDIGO, 2013]. The subject's age at visit 1 should be used for calculation in this study.

Gender	Serum Creatinine	Equation for Estimating GFR
Female	≤ 0.8 mg/dl	$133 \times (SCysC/0.8)^{-0.499} \times 0.996^{Age} \times 0.932$
Female	> 0.8 mg/dl	$133 \times (SCysC/0.8)^{-1.328} \times 0.996^{Age} \times 0.932$
Male	≤ 0.8 mg/dl	$133 \times (SCysC/0.8)^{-0.499} \times 0.996^{Age}$
Male	> 0.8 mg/dl	$133 \times (SCysC/0.8)^{-1.328} \times 0.996^{Age}$

GFR: glomerular filtration rate; SCysC: serum cystatin C

Reference: KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Official Journal of the International Society of Nephrology: Kidney International Supplements. 2013;3(1):1-150.

6.1 Efficacy Endpoints

6.1.1 Primary Efficacy Endpoint

Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 72 hours after T0 (AKI-SCr72h).

[SCr evaluation visit: baseline, Visit 3, 4 and 5 (24, 48, 72 hours after T0)]

- If the subject meets at least one of the following conditions, then the subject will be treated as developing AKI-SCr72h.
  - Increasing in SCr ≥ 0.3 mg/dL within any 48 hours until Visit 5; and/or,
  - Increasing in SCr ≥ 1.5 times baseline until Visit 5.

6.1.2 Secondary Efficacy Endpoints

1. Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 7 days after T0 (i.e., until Visit 8) (AKI-SCr7d).
   [SCr evaluation visit: baseline, Visit 3, 4, 5, 6, 7 and 8 (24 hours, 48 hours, 72 hours after T0, and Day 5, 6 and 7/DoD). If the hospital discharge is earlier than Day 7, then the visits prior to DoD and Visit 8 (DoD) will be used.]
   - If the subject meets at least one of the following conditions, then the subject will be treated as developing AKI-SCr7d.
     - Increasing in SCr ≥ 0.3 mg/dL within any 48 hours until Visit 8; and/or,
     - Increasing in SCr ≥ 1.5 times baseline until Visit 8.
2. Proportion of subjects developing AKI based on all captured criteria from the KDIGO guideline within 72 hours after T0 (AKI-KDIGO72h).
   [SCr evaluation visit: baseline, Visit 3, 4 and 5 (24, 48, 72 hours after T0)]
   [UO evaluation visit: Visit 3, 4, and 5 (0-12 hours, 12-24 hours, 0-24 hours, ..., and 48-60 hours, 60-72 hours and 48-72 hours after T0)]

- If the subject meets at least one of the following conditions, then the subject will be treated as developing AKI-KDIGO72h.
  - Increasing in SCr ≥ 0.3 mg/dL within any 48 hours until Visit 5;
  - Increasing in SCr ≥ 1.5 times baseline until Visit 5; and/or,
  - Urine volume < 0.5 ml/kg per hour for any one of 12 consecutive hours until Visit 5 (i.e., 0-12 hours after T0, 12-24 hours after T0, ..., or 60-72 hours after T0).

3. Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 7 days after T0 (AKI-KDIGO7d).
   [SCr evaluation visit: baseline, Visit 3, 4, 5, 6, 7 and 8(24 hours, 48 hours, 72 hours after T0, and Day 5, 6 and 7/DoD). If the hospital discharge is earlier than Day 7, then the visits prior to DoD and Visit 8 (DoD) will be used.]
   [UO evaluation visit: Visit 3, 4, 5, 6, 7 and 8 (0-12 hours, 12-24 hours, 0-24 hours, ..., and 120-132 hours, 132-144 hours and 120-144 hours after T0). If the hospital discharge is earlier than Day 7, then the visits prior to DoD and Visit 8 (DoD) will be used.]
   - If the subject meets at least one of the following conditions, then the subject will be treated as developing AKI-KDIGO7d.
     - Increasing in SCr ≥ 0.3 mg/dL within any 48 hours until Visit 8;
     - Increasing in SCr ≥ 1.5 times baseline until Visit 8; and/or,
     - Urine volume < 0.5 ml/kg per hour for any one of 12 consecutive hours until Visit 8 (i.e., 0-12 hours after T0, 12-24 hours after T0, ..., or 132-144 hours after T0).
4. Proportion of subjects with MAKE defined as all-cause mortality, renal replacement therapy (RRT) and/or ≥ 25% sustained reduction in eGFR based on SCr within 30 days after day of surgery (MAKE30).
   - If the subject meets at least one of the following conditions by day 30 (Visit 9), then the subject will be treated as with MAKE30.
     - All-cause mortality;
     - Initiation of RRT; and/or,
     - eGFR based on SCr at day 30 (Visit 9) equal to or less than 75% of the baseline value.
5. Proportion of subjects with MAKE defined as all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR based on SCr within 90 days after day of surgery (MAKE90).
   - If the subject meets at least one of the following conditions by day 90 (Visit 10), then the subject will be treated as with MAKE90.
     - All-cause mortality;
     - Initiation of RRT; and/or,
     - eGFR based on SCr at day 90 (Visit 10) equal to or less than 75% of the baseline value.

6.1.3 Exploratory Efficacy Endpoints

For the exploratory efficacy endpoints of proportion of events, subjects whose status of events are unknown due to, e.g., missing data will not be counted as the events, unless specifically stated otherwise.

1. All-cause mortality at day 30 (Visit 9)
2. All-cause mortality at day 90 (Visit 10)
3. Number of subjects needing RRT at day 30 (Visit 9)
4. Number of subjects needing RRT at day 90 (Visit 10)
5. Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 30 (Visit 9)
6. Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 90 (Visit 10)
7. Proportion of subjects with MAKE defined as all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR based on cystatin-C within 30 days after day of surgery (MAKE30 with eGFR based on cystatin-C).
   - If the subject meets at least one of the following conditions by day 30 (Visit 9), then the subject will be treated as with MAKE30 with eGFR based on cystatin-C.
     - All-cause mortality;
     - Initiation of RRT; and/or,
     - eGFR based on cystatin-C at day 30 (Visit 9) equal to or less than 75% of the baseline value.
8. Proportion of subjects with MAKE defined as all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR based on cystatin-C within 90 days after day of surgery (MAKE90 with eGFR based on cystatin-C).
   - If the subject meets at least one of the following conditions by day 90 (Visit 10), then the subject will be treated as with MAKE90 with eGFR based on cystatin-C.
     - All-cause mortality;
     - Initiation of RRT; and/or,
     - eGFR based on cystatin-C at day 90 (Visit 10) equal to or less than 75% of the baseline value.
9. Number of subjects with ≥ 25% sustained reduction in eGFR based on cystatin-C at day 30 (Visit 9)
10. Number of subjects with ≥ 25% sustained reduction in eGFR based on cystatin-C at day 90 (Visit 10)
11. Number of hospital days during initial hospitalization
    - The number of hospital days per subject during initial hospitalization will be calculated as: Date of discharge – Date of admission + 1 (for the initial hospitalization including ICU).
      - Initial hospitalization is a hospitalization which includes the day of surgery
      - If the date of admission of a hospitalization record is the same as the date of discharge of the previous record, for example because two records are created to

illustrate that the subject is moved from one hospital to another hospital or from a standard care to ICU, then the two records will be combined into one record.
- If the hospitalization is ongoing at the end of study, the date of discharge is replaced by the last date of completed or discontinued recorded in eCRF period status forms.

12. Number of ICU days during initial hospitalization
    - The number of ICU days per subject during initial hospitalization will be calculated as: Date of discharge – Date of admission + 1 (for the initial ICU hospitalization).
    - Initial hospitalization is a hospitalization which includes the day of surgery
    - If the hospitalization is ongoing at the end of study, the date of discharge is replaced by the last date of completed or discontinued recorded in eCRF period status forms.
13. Total number of hospital days up to day 90
    - The number of hospital days per subject up to day 90 will be calculated as: Sum of the durations of all hospitalizations including ICU in days (Date of discharge – Date of admission + 1).
    - Initial hospitalization and all subsequently hospitalizations are included. Initial hospitalization is a hospitalization which includes the day of surgery
    - If the date of admission of a hospitalization record is the same as the date of discharge of the previous record, then the two records will be combined in one record.
    - If the hospitalization is ongoing at the end of study, the date of discharge is replaced by the last date of completed or discontinued recorded in eCRF period status forms.
14. Total number of ICU days up to day 90
    - The number of hospital days per subject up to day 90 will be calculated as: Sum of the durations of all ICU in days (Date of discharge – Date of admission + 1).
    - Initial hospitalization and all subsequently hospitalizations are included. Initial hospitalization is a hospitalization which includes the day of surgery
    - If the hospitalization is ongoing at the end of study, the date of discharge is replaced by the last date of completed or discontinued recorded in eCRF period status forms.
15. Alternative derivations of proportion of subjects developing AKI using different definitions [Table 3] within 72 hours after T0.
    - All stages AKI-UO (AKI-UO stage 2 or stage 3)
    - AKI-UO stage 3
    - AKI-SCr stage 2 or 3
    - AKI-SCr stage 2 or 3 and/or AKI-UO stage 3
    - AKI based on S-cystatin-C, defined as an increase of ≥ 10% of baseline serum cystatin-C [KDIGO, 2012]
    - AKI based on S-cystatin-C, defined as an increase of ≥ 25% of baseline serum cystatin-C

Table 3 Definitions of Stages

Stage	AKI-SCr	AKI-UO
1	≥ 1.5 to < 2 times baseline, or Increasing ≥ 0.3 mg/dL within any 48 hours	(Not used for this study)
2	≥ 2 to < 3 times baseline	< 0.5 mL/kg per hour for over 12 hours
3	≥ 3 times baseline, Increase in serum creatinine to ≥ 4.0 mg/dL, or Initiation of RRT	< 0.3 mL/kg per hour for over 24 hours, or Anuria for over 12 hours

AKI-SCr: acute kidney injury based on serum creatinine KDIGO criteria; AKI-UO: acute kidney injury based on urinary output; RRT: renal replacement therapy

KDIGO Clinical Practice Guideline for Acute Kidney Injury. Official Journal of the International Society of Nephrology: Kidney International Supplements. 2012;2(1):1-138.

16. Alternative derivations of proportion of subjects developing AKI using different definitions [Table 3] within 7 days after T0.
17. Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 5 days after T0 (i.e., until Visit 6) (AKI-SCr5d).
    [SCr evaluation visit: baseline, Visit 3, 4, 5 and 6 (24 hours, 48 hours, 72 hours after T0, and Day 5). If the hospital discharge is earlier than Day 5, then the visits prior to DoD and Visit 8 (DoD) will be used.]
    - If the subject meets at least one of the following conditions, then the subject will be treated as developing AKI-SCr5d.
      - Increasing in SCr ≥ 0.3 mg/dL within any 48 hours until Visit 6; and/or,
      - Increasing in SCr ≥ 1.5 times baseline until Visit 6.
18. Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 5 days after T0 (AKI-KDIGO5d).
    [SCr evaluation visit: baseline, Visit 3, 4, 5 and 6 (24 hours, 48 hours, 72 hours after T0, and Day 5). If the hospital discharge is earlier than Day 5, then the visits prior to DoD and Visit 8 (DoD) will be used.]
    [UO evaluation visit: Visit 3, 4, 5 and 6 (0-12 hours, 12-24 hours, 0-24 hours, ..., and 72-84 hours, 84-96 hours and 72-96 hours after T0). If the hospital discharge is earlier than Day 5, then the visits prior to DoD and Visit 8 (DoD) will be used.]
    - If the subject meets at least one of the following conditions, then the subject will be treated as developing AKI-KDIGO5d.
      - Increasing in SCr ≥ 0.3 mg/dL within any 48 hours until Visit 6;
      - Increasing in SCr ≥ 1.5 times baseline until Visit 6; and/or,
      - Urine volume < 0.5 ml/kg per hour for any one of 12 consecutive hours until Visit 6 (i.e., 0-12 hours after T0, 12-24 hours after T0, ..., or 84-96 hours after T0).
19. Severity of AKI within 72 hours after T0
    - Severity of AKI based on AKI-SCr stages (Stage 1, 2, 3 or no AKI. No AKI means subject not developing AKI-SCr72h.)

- Severity of AKI based on AKI-UO stages (Stage 2, 3, or no AKI. No AKI means subject not developing AKI-UO)
- Severity of AKI based on most severe of AKI-SCr or AKI-UO stages (Stage 1, 2, 3 or no AKI)

20. Severity of AKI (based on AKI stage using SCr and/or UO) within 7 days after T0
21. Duration of AKI based on AKI stage using SCr
    - Duration of AKI-SCr72h: time from moment of first meeting criteria for AKI-SCr72h until moment of first not meeting the criteria for AKI-SCr72h, death or hospital discharge. If the duration of AKI-SCr72h exceeds day 7 the time of ending of AKI will be assessed retrospectively on day 30 (visit 9) based on local SCr laboratory results. The date and value when respective criteria of AKI-SCRr72h were resolved is recorded in the eCRF as an unscheduled event with check of "First NOT meeting the criteria for AKI-SCr72h/7d (SCr >=1.5 times baseline) before hospital discharge, if the duration of AKI-SCr72h/7d exceeds Visit 8 (Day 7).".
    - The duration will be calculated as follows;

      (Date of first not meeting the criteria, death or hospital discharge – Date of first meeting the criteria) in days.

      For subjects who don't occur AKI-SCr72h, the duration of AKI will be treated as 0.
    - Duration of AKI-SCr7d (definition as above)
22. Time to AKI-SCr72h (i.e., the time from T0 to the time when criteria for AKI-SCr72h are met). The time to AKI-SCr72h will be calculated as: (Date of first meeting the criteria – Date of T0 + 1) in days.
23. Time to AKI-SCr7d (definition as above)
24. Proportion of subjects with AKI as defined by a 50% or higher reduction in eGFR based on the cystatin-C equation within 72 hours after T0.
    - If the subject meet the following condition, then the subject will be treated as with AKI for this endpoint.
      - At least one of eGFR based on cystatin-C equation within 72 hours after T0 ≤ 50% of the baseline value.
25. Proportion of subjects with AKI as defined by a 50% or higher reduction in eGFR based on the cystatin-C equation within 7 days after T0.
    - If the subject meet the following condition, then the subject will be treated as with AKI for this endpoint.
      - At least one of eGFR based on cystatin-C equation within 7 days after T0 ≤ 50% of the baseline value.
26. Proportion of subjects that have a reduction of NC AKIRisk score 24 hours after T0 (visit 3) to or below 0.3 $(ng/ml)^2/1000$
27. Proportion of subjects with renal recovery, defined as a SCr value at hospital discharge equal to or lower than that at baseline
    - SCr value at hospital discharge is defined as follows;

- If the hospital discharge is at or before day 7, the value at Visit 8 will be used.
- If the hospital discharge is after day 7, the value recorded as unscheduled event with check of "Last SCr value before hospital discharge." will be used.

28. Time to ventilator weaning, defined as the time from T0 to extubation (i.e., removal of the endotracheal ventilation tube)
    - Time to ventilator weaning is defined as: (Date and time of Extubation for the first record in "Mechanical Ventilation" form in eCRF – Date and time of T0) in hours.
29. Acute physiology and chronic health evaluation II (APACHE-II) up to day 4 (if the subject is on the ICU)
30. Number of readmissions to ICU
31. Number of readmissions to hospital
    - All readmissions after initial hospitalization are included. Initial hospitalization is a hospitalization which includes the day of surgery
    - If the date of admission of a hospitalization record is the same as the date of discharge of the previous record, then the two records will be combined into one record.
32. Changes from baseline in SCr, S-cystatin-C, eGFR based on SCr and Cystatin-C, and other biomarkers in blood and urine through 72 hours after T0
33. Area under the curve of SCr and S-cystatin-C through 72 hours after T0 (AUCSCr72h and AUCSCysC72h)
    - AUCSCr72h is defined by Linear Trapezoidal Method as below;
      AUCSCr72h (mg/dL*day) = sum of $(SCr_i + SCr_{i+1}) \times (Time_{i+1} - Time_i) / 2$
      where i=Visit 2 to 4
    - AUCSCysC72h is defined in the same way as above.
    - In the case of missing at 72 hours after T0, the missing value will be imputed from the last available value using Last Observation Carried Forward.
34. Area under the curve of SCr and S-cystatin-C through day 7 after T0(AUCSCr7d and AUCSCysC7d)
    - AUCSCr7d and AUCSCysC7d are defined based on all available values through day 7 after T0 in the same way as AUCSCr72h and AUCSCysC72h
35. 5-level European Quality of Life 5 Dimensions Questionnaire (EQ-5D-5L) on baseline, visit 8, day 30 and day 90
    - EQ-5D 5L includes two main components: (1) a Visual Analogue Scale (VAS) scale rating perception of overall health and (2) 5 qualitative domains: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression.

6.2 Safety Variables

Safety will be assessed by evaluation of the following variables:

- Treatment-emergent adverse events (TEAEs; frequency, severity, seriousness, and relationship to study drug).

- Clinical laboratory variables (hematology, biochemistry including liver enzymes and total bilirubin, and urinalysis)
- Vital signs (systolic and diastolic blood pressure and pulse rate)

TEAE is defined as an AE observed after starting administration of the study drug and by visit 9 (day 30). If the adverse event occurs on Day 1 and the onset check box is marked "Onset after first study drug taken" or the onset check box is left blank, then the adverse event will be considered treatment emergent. If the adverse event occurs on Day 1 and the onset check box is marked "Onset before first study drug taken", then the adverse event will not be considered treatment emergent. If a subject experiences an event both during the pre-investigational period and during the investigational period, the event will be considered as TEAE only if it has worsened in severity (i.e., it is reported with a new start date).

A study drug-related TEAE is defined as any TEAE with a causal relationship of YES by the investigator or with missing assessment of the causal relationship.

6.3 Pharmacokinetic Variables

- ASP1128 plasma concentrations

6.4 Pharmacodynamic Variables

- Target gene expression

6.5 Other Variables

- Randomization Arms

[Table 4] presents the groups to which subjects are randomized. The Randomization Arms will be used for analysis of efficacy, safety, pharmacokinetics and pharmacodynamics.

Table 4 Randomization Arm

Treatment Group Code (ARMCD)	Randomization Arm (Labels in TLFs)	
A	ASP1128	Total (Randomization)
P	Placebo	

ARMCD: Planned Arm Code; TLFs: Tables, Listings and Figures

- Prognostic Factor Analysis Arms

[Table 5] presents the groups to which subjects are in PFAS. The Prognostic Factor Analysis Arms will be used for analysis of demographic and other baseline characteristics for Observational Cohort.

Table 5 Prognostic Factor Analysis Arm

Treatment Group Code (ARMCD)	Prognostic Factor Analysis Arm (Labels in TLFs)	
P	NC positive	Total (PFAS)
O	NC negative	

ARMCD: Planned Arm Code; TLFs: Tables, Listings and Figures; NC: NephroCheck; PFAS: Prognostic Factor Analysis Set

- Duration of CPB

Duration of CPB in hours is defined as: 'Time of T0' - 'Time of Onset CPB'.

- Duration of Aortic Cross-Clamp

Duration of Aortic Cross-Clamp in hours is defined as: 'End Time of Aortic Cross-Clamp' - 'Start Time of Aortic Cross-Clamp'.

- Duration from Incision to Closing of Skin

Duration from Incision to Closing of Skin in hours is defined as: 'Time of Closing of Skin' - 'Time of Incision of Skin'.

- Duration of Study

Duration of Study in days is defined as: 'Date of the last date of completed or discontinued recorded in eCRF period status forms' - 'Date of Surgery' + 1.

- Previous and Concomitant Treatment (Medication and Non-Medication Therapy)

Previous medication is defined as medication with at least one dose taken before the date of the screening.

Concomitant medication is defined as medication with at least one dose taken between the screening (inclusive) and surgery, and following surgery to EoS.

7 STATISTICAL METHODOLOGY

7.1 General Considerations

- For continuous variables, descriptive statistics will include the number of subjects (n), mean, standard deviation (SD), median, minimum and maximum. The number of digits to be used for the mean, standard deviation and median will be added one more digit to raw data with rounding off. The minimum and maximum will be presented with the same number of digit(s) as raw data. In addition, for pharmacokinetics (PK) variables, the coefficient of variation (CV) and the geometric mean will also be calculated.

Frequencies and percentages will be displayed for categorical data. Percentages by categories will be based on the number of subjects with no missing data, i.e. will add up to 100%. Frequencies and percentages will be rounded to one decimal place. For time-to-event variables, the number and percentage of subjects with the event using Kaplan-Meier method, the cumulative event rate will be estimated and a plot will be constructed.

- Summaries based on FAS and PPS (e.g. disposition, baseline and efficacy data) will be presented by planned randomization arm, unless specifically stated otherwise. Safety analysis and other summaries based on SAF will be presented by actual treatment received. Analysis of Observational Cohort based on PFAS will be presented by prognostic factor analysis arm.
- All statistical comparisons will be made using two sided tests at the α=0.10 significance level unless specifically stated otherwise. All null hypotheses will be of no treatment difference, all alternative hypotheses will be two-sided, unless specifically stated otherwise.
- All data processing, summarization, and analyses will be performed using SAS® Version 9.4 or higher on Red Hat Enterprise Linux. Specifications for Table, Figures, and Listings can be found in the TLF specifications for this study.
- For continuous variables that are recorded as "<X", "<=X", ">X", or ">=X", the value of "X" will be used in the calculation of summary statistics if otherwise noted. The original values will be used for the listings.
- MedDRA will be used as the coding dictionary for adverse event and medical history.
- WHODrug Global B3 will be used as the coding dictionary for previous and concomitant medication.
- For the definition of subgroups of interest please refer to [Section 7.8].

7.2 Study Population

7.2.1 Disposition of Subjects

The following subject data will be presented:

[Analysis population: All subjects with informed consent, Analysis group: Overall]

- Number of subjects with informed consent, discontinued before surgery period, entered surgery period, discontinued before registration, registered, enrolled in the observational cohort, randomized (overall only); and
- Number and percentage of subjects discontinued before surgery period, by primary reason for study discontinuation.

[Analysis population: All subjects with surgery period, Analysis group: Overall]

- Number and percentage of subjects discontinued before registration, by primary reason for study discontinuation.

[Analysis population: All randomized subjects, Analysis group: Randomization Arm and Total]

- Number and percentage of subjects randomized in each analysis set except PFAS;
- Number and percentage of subjects completed and discontinued the treatment period, by primary reason for treatment period discontinuation;
- Number and percentage of subjects completed and discontinued the follow-up period, by primary reason for follow-up period discontinuation; and
- Number and percentage of subjects excluded from each analysis set except PFAS by reason for exclusion defined in [Section 5.1; 5.2; 5.3; 5.4 and 5.5].

[Analysis population: subjects enrolled in the observational cohort and randomized to placebo group, Analysis group: Prognostic Factor Analysis Arm and Total]

- Number and percentage of subjects in PFAS.

[Analysis population: subjects enrolled in the observational cohort, Analysis group: NC negative]

- Number and percentage of subjects completed and discontinued the follow-up period, by primary reason for follow-up period discontinuation.

7.2.2 Protocol Deviations

Protocol deviations as defined in the study protocol (Section 8.3 Major Protocol Deviations) will be assessed for all registered subjects. The number and percentage of subjects meeting any criteria will be summarized for each criterion and overall, by randomization arm and NC negative group as well as by study site. Subjects deviating from a criterion more than once will be counted once for the corresponding criterion. Any subjects who have more than one protocol deviation will be counted once in the overall summary. A data listing will be provided by site and subject.

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

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

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

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

7.2.3 Demographic and Other Baseline Characteristics

[Analysis population: SAF, FAS, PPS, PKAS and PDAS, Analysis group: Randomization Arm and Total]

[Analysis population: PFAS, Analysis group: Prognostic Factor Analysis Arm and Total]

- Demographic and other baseline characteristics will be summarized by descriptive statistics and frequency tabulations [Table 6].
- Surgery parameters will be summarized by descriptive statistics and frequency tabulations [Table 7].
- AKI Risk Factors will be summarized by descriptive statistics and frequency tabulations [Table 8].

Table 6 Demographic and Baseline Characteristics

Item	Classification
Sex	1: Male 2: Female
Age (years) [Informed Consent]	Measurement value
	1: < 65 2: >= 65
	1: < 70 2: >= 70
Ethnicity	1: Not Hispanic or Latino 2: Hispanic or Latino
Race	1: White 2: Black or African American 3: Asian 4: American Indian or Alaska Native 5: Native Hawaiian or Other Pacific Islander 6: Other
Height (cm) [Screening]	Measurement value
Weight (kg) [Screening]	Measurement value
	1: < 60 2: >= 60
Tobacco History	0: Never 1: Current 2: Former
Alcohol History	0: Never 1: Current 2: Former
Drug Abuse	0: Never 1: Current 2: Former
Left Ventricular Ejection Fraction (%)	Measurement value
	1: < 40 2: >= 40
AKIRisk Score ((ng/mL)^2/1000) [Postsurgery] [1]	Measurement value
	1: <=0.3 2: > 0.3 to <= 0.7 3: > 0.7
eGFR for Randomization (mL/min/1.73 m^2)	Measurement value
	1: < 45 2: >= 45
	1: < 60 2: >= 60
Peripheral Arterial Disease [2]	0: No 1: Yes
Use of Intravenous Radio-contrast [3]	0: No 1: Yes
Protocol Version Number at Enrollment	1: Before Version 2.0 2: Version 2.0 or Later

AKI: acute kidney injury; eGFR: estimated glomerular filtration rate

1: If AKIRisk Score at post surgery is recorded several times in eCRF, the last score will be used.

2: The record of "Are there any peripheral arterial diseases?" in Demographics eCRF will be used.

3: If subjects have at least one record in the Concomitant Medication eCRF with the flag selected "YES" for of use of Intravenous Radio-contrast between the date of screening and T0 of the date of surgery, then "Yes"; otherwise "No".

Table 7 Surgery parameters

Item	Classification
Procedure of Surgery: CABG	0: No 1: Yes
Procedure of Surgery: Valve Surgery [Aortic]	0: No 1: Yes
Procedure of Surgery: Valve Surgery [Mitral]	0: No 1: Yes
Procedure of Surgery: Valve Surgery [Tricuspid]	0: No 1: Yes
Procedure of Surgery: CABG + Valve Surgery [Any]	0: No 1: Yes
Procedure of Surgery: Thoracic Aortic Surgery	0: No 1: Yes
Procedure of Surgery: ECMO	0: No 1: Yes
Procedure of Surgery: Use of VAD	0: No 1: Yes
Surgery Type [1]	1: Lower Risk Surgery 2: Higher Risk Surgery
Duration of CPB (hours)	Measurement value
	1: < Median 2: >= Median
Duration of Aortic Cross-Clamp (hours)	Measurement value
	1: < 0.5 2: >= 0.5 to < 1.0 3: >= 1.0
Lowest Intraoperative MAP (mmHg)	Measurement value
Duration from Incision to Closing of Skin (hours)	Measurement value
Urine Output During Surgery (mL/kg/h)	Measurement value
Total Fluid Intake During Surgery (mL)	Measurement value
Volume of Blood Products During Surgery (mL)	Measurement value
Use of Furosemide During Surgery	0: No 1: Yes
Dose of Furosemide During Surgery (mg)	Measurement value
Drain Output During Surgery (mL)	Measurement value
Volume of Blood Loss During Surgery (mL)	Measurement value
Redoing Surgery	0: No 1: Yes

CABG: coronary artery bypass graft; ECMO: Extracorporeal Membrane Oxygenation; VAD: ventricular assist device; CPB: cardiopulmonary bypass pump; MAP: mean arterial pressure

1: If the procedure of surgery is only one of CABG, Valve Surgery [Aortic], Valve Surgery [Mitral] or Valve Surgery [Tricuspid] and not Thoracic Aortic Surgery then Surgery type is “Lower Risk Surgery”; otherwise “Higher Risk Surgery”.

Table 8 AKI Risk Factors

Item	Classification
Circulatory Shock	0: No 1: Yes
Cardiovascular Surgery (Especially With CPB)	0: No 1: Yes
Nephrotoxic Drugs	0: No 1: Yes
Radiocontrast Agents	0: No 1: Yes
Poisonous Plants and Animals	0: No 1: Yes
Dehydration or Volume Depletion	0: No 1: Yes
Advanced Age (>= 70 years)	0: No 1: Yes
Female Gender	0: No 1: Yes
Black Race	0: No 1: Yes
CKD	0: No 1: Yes
Chronic Diseases (Heart, Lung, Liver)	0: No 1: Yes
Diabetes Mellitus	0: No 1: Yes
Anemia	0: No 1: Yes
Prior AKI	0: No 1: Yes

AKI: acute kidney injury; CPB: cardiopulmonary bypass pump; CKD: chronic kidney disease

[Analysis population: FAS and PPS, Analysis group: Randomization Arm and Total]

[Analysis population: PFAS, Analysis group: Prognostic Factor Analysis Arm and Total]

Baseline values of efficacy variables will be summarized by descriptive statistics and frequency tabulations [Table 9].

Table 9 Baseline values of efficacy variables

Item	Classification
Serum Creatinine (mg/dL)	Measurement value
Cystatin-C (mg/L)	Measurement value
eGFR based on Serum Creatinine (mL/min/1.73 m^2)	Measurement value
	1: < 45 2: >= 45
	1: < 60 2: >= 60
eGFR based on Cystatin-C (mL/min/1.73 m^2)	Measurement value
	1: < 45 2: >= 45
	1: < 60 2: >= 60

eGFR: estimated glomerular filtration rate

7.2.4 Previous and Concomitant Medications

All previous and concomitant medications will be presented in a listing.

7.2.5 Duration of Study

[Analysis population: SAF, Analysis group: Randomization Arm and Total]

[Analysis population: PFAS, Analysis group: Prognostic Factor Analysis Arm and Total]

The duration of study for each subjects will be summarized by descriptive statistics.

7.3 Study Drugs

7.3.1 Exposure and Treatment Compliance

[Analysis population: SAF, Analysis group: Randomization Arm and Total]

The number of doses (1 time, 2 times or 3 times) and time of first study drug taken (within T0 + 8 hours, or after T0 + 8 hours) will be summarized in the frequency table. The time of first study drug taken will be summarized by protocol version number at enrollment (Before Version 2.0, Version 2.0 or Later).

7.4 Analysis of Efficacy

7.4.1 Analysis of Primary Endpoint

7.4.1.1 Primary Analysis

[Analysis population: FAS, Analysis group: Randomization Arm]

- The primary efficacy endpoint of AKI (AKI-SCr72h) proportion will be analyzed using the Cochran-Mantel-Haenszel (CMH) test with strata to control for baseline eGFR based

on Serum Creatinine (< 45 mL/min per 1.73 m^2, ≥ 45 mL/min per 1.73 m^2) on the FAS. The hypothesis testing on the primary analysis will be performed at 2-sided 0.10 significance level to test the null hypothesis that the AKI-SCr72h proportion is equal between the 2 treatment arms vs the alternative hypothesis that AKI-SCr72h proportion is different between the ASP1128 arm vs the placebo arm.

- The Mantel-Haenszel (MH) estimate of the adjusted common risk ratio and 2-sided 90% confidence interval will be calculated.

If the proportion of subjects with baseline eGFR based on Serum Creatinine < 45 mL/min per 1.73 m^2 for FAS is less than 10%, the above stratified analyses of CMH test and MH estimate of adjusted common risk ratio will be replaced with unstratified analyses of chi-square test and unadjusted risk ratio. It will also apply to the sensitivity analyses 2, 3 and 4 listed in [Table 10].

7.4.1.2 Sensitivity Analysis

- As a sensitivity analysis for the primary endpoint of AKI (AKI-SCr72h) proportion, the chi-square test will be performed to evaluate the appropriateness of CMH test with strata to control for baseline eGFR based on Serum Creatinine (< 45 mL/min per 1.73 m^2, ≥ 45 mL/min per 1.73 m^2). The estimate of the unadjusted risk ratio and 2-sided 90% confidence interval will be calculated. This analysis will be conducted using the FAS.
- Additional sensitivity analyses using other handlings of missing data will be performed to evaluate the impact on the analysis of AKI-SCr72h proportions due to any missing data/assessments and any loss to follow-up. If there is a missing value at any evaluation visit and the subject don't have the data meeting the criteria of the endpoint, then Multiple imputation (MI) will be conducted for each missing component data firstly, and AKI-SCr72h proportion will be derived based on the imputed data.
- In additional, different definition of handling of missing data will be used. The subjects whose status of AKI-SCr72h is unknown due to, say, the missing data of SCr will be counted as AKI-SCr72h event. That is, if there is a missing value at any evaluation visit, then the subject will be counted as AKI/MAKE event. The same analysis as primary analysis (CMH test) by using this handling of missing data will be performed.
- As a supplementary analysis, the same analysis of the primary endpoint as described in [Section 7.4.1.1 Primary Analysis] will be conducted using PPS.

[Table 10] summarizes all sensitivity analyses to be performed with the primary endpoint.

Table 10 Primary and Sensitivity Analyses for Primary Endpoint

Code	Set	Test	Handling of missing data
Primary	FAS	CMH test	Refer to 6.1
Sensitivity 1	FAS	Chi-square test	Same as Primary
Sensitivity 2	PPS	CMH test	Same as Primary
Sensitivity 3	FAS	CMH test	MI
Sensitivity 4	FAS	CMH test	Different Definition: missing data of SCr will be counted as AKI-SCr72h event

FAS: full analysis set; CMH: Cochran-Mantel-Haenszel; PPS: per protocol set; MI: multiple imputation; SCr: serum creatinine; AKI-SCr: acute kidney injury based on serum creatinine KDIGO criteria

CMH test with MI

The MI CMH model will be used to compare ASP1128 and placebo group in a fixed sequence procedure:

1. Generate 1000 datasets, using seed 346194, where intermittent missing SCr value will be imputed for each treatment relying on non-missing data from all subjects within each treatment group using the Monte Carlo Markov Chain (MCMC) imputation model with treatment and the available non-missing SCr value for each scheduled Visit. The MCMC statement in the SAS PROC MI procedure with monotone option will be used. As a result, each dataset will only have missing ending data, or a monotone missing data pattern.
2. For each dataset from step 1, missing ending data (SCr value up through 72 hours after T0) will be imputed using seed 478034. As a result, 1000 imputed complete datasets will be generated.
   - Missing data at Visit 3 (T0 + 24 hours) will be imputed using the regression imputation model with the stratification factors, baseline and SCr value from Visit 3, using the SAS PROC MI procedure with the REGRESSION option in the MONOTONE statement.
   - The SAS PROC MI procedure will use data separately from each treatment subjects to impute the missing data for a specific Visit (i.e., only those that need the imputation for the Visit). Since subjects from the different treatment groups for that Visit are excluded from the step, they will not contribute to the imputation for the Visit.
   - Repeat for all other scheduled Visit sequentially (Visit 4 to 5). Subjects whose missing data were imputed for previous Visit will contribute to the imputation for the current Visit.

   The regression imputation model includes an intercept and the slopes of the SCr value from previous Visit and the stratification factors.
3. The MH estimate of the adjusted common risk ratio, standard error and 2-sided 90% confidence interval for AKI-SCr72h proportion will be calculated using the observed or imputed SCr value.

4. Combine estimates from the results of each of the 1000 runs using SAS PROC MIANALYZE.

7.4.2 Analysis of Secondary Endpoints

[Analysis population: FAS, Analysis group: Randomization Arm]

- The secondary efficacy endpoints proportions (AKI-SCr7d, AKI-KDIGO72h, AKI-KDIGO7d, MAKE30 and MAKE90) will be analyzed using the stratified CMH test with strata to control for baseline eGFR based on Serum Creatinine (< 45 mL/min per 1.73 m^2, ≥ 45 mL/min per 1.73 m^2). The hypothesis testing will be performed at 2-sided 0.10 significance level. The MH estimate of the adjusted common risk ratio and 2-sided 90% confidence intervals will be calculated.
- As sensitivity analysis, chi-square test will be performed to evaluate the appropriateness of CMH test with strata to control for baseline eGFR based on Serum Creatinine (< 45 mL/min per 1.73 m^2, ≥ 45 mL/min per 1.73 m^2). The estimates of the risk ratio and 2-sided 90% confidence intervals will be calculated.
- Additional sensitivity analysis will be performed to evaluate the impact on the analysis of AKI/MAKE proportions due to any missing data/assessments and any loss to follow-up. If there is a missing value at any evaluation visit and the subject don't have the data meeting the criteria of the endpoint, then MI will be conducted for each missing component data firstly, then AKI/MAKE endpoint will be derived based on the imputed data.
- In additional, different definition of handling of missing data will be used. The subjects whose status of AKI/MAKE endpoint is unknown due to, say, the missing data of SCr will be counted as AKI/MAKE event. That is, if there is a missing value at any evaluation visit, then the subject will be counted as AKI/MAKE event. The same analysis as primary analysis (CMH test) by using this handling of missing data will be performed.
- As exploratory analyses, the association between MAKE30/MAKE90 (and also each individual outcomes, of all-cause mortality, initiation of RRT and eGFR based on SCr at day 30 (Visit 9)/day 90 (Visit 10) equal to or less than 75% of the baseline value) and primary, secondary and exploratory endpoint of AKI proportion is evaluated by a contingency table. The following endpoints of AKI proportion will be used;
  - AKI-SCr72h (primary endpoint),
  - AKI-SCr7d (secondary endpoint),
  - AKI-KDIGO72h (secondary endpoint),
  - AKI-KDIGO7d (secondary endpoint),
  - All stages AKI-UO (within 72 hours after T0)
  - All stages AKI-UO (within 7 days after T0),
  - AKI-UO stage 3 (within 72 hours after T0),
  - AKI-UO stage 3 (within 7 days after T0),

- AKI-SCr stage 2 or 3 (within 72 hours after T0),
- AKI-SCr stage 2 or 3 (within 7 days after T0),
- AKI-SCr stage 2 or 3 and/or AKI-UO stage 3 (within 72 hours after T0),
- AKI-SCr stage 2 or 3 and/or AKI-UO stage 3 (within 7 days after T0),
- AKI based on Cystatin-C, defined as an increase of ≥10% of baseline Cystatin-C (within 72 hours after T0),
- AKI based on Cystatin-C, defined as an increase of ≥10% of baseline Cystatin-C (within 7 days after T0),
- AKI-SCr5d,
- AKI-KDIGO5d,
- AKI as defined by a 50% or higher reduction in eGFR based on the Cystatin-C equation within 72 hours after T0, and
- AKI as defined by a 50% or higher reduction in eGFR based on the Cystatin-C equation within 7 days after T0.

If the proportion of subjects with baseline eGFR based on Serum Creatinine < 45 mL/min per 1.73 m^2 for FAS is less than 10%, the above stratified analyses of CMH test and MH estimate of adjusted common risk ratio will be replaced with unstratified analyses of chi-square test and unadjusted risk ratio.

7.4.3 Subgroup Analysis

[Analysis population: FAS, Analysis group: Randomization Arm]

- The estimate of the unadjusted risk ratio and 2-sided 90% confidence interval for the primary efficacy endpoint of AKI (AKI-SCr72h) proportion and secondary endpoints will be calculated by subgroups defined in [Section 7.8].
- In addition, the forest plot will be provided.
- For the continuous type subgroup factors, logistic regression model will be fitted for each continuous type subgroup factor 1 by 1. For each logistic regression model, response variable is primary or secondary endpoints (AKI-SCr72h, AKI-SCr7d, AKI-KDIGO72h, AKI-KDIGO7d, MAKE30 and MAKE90) and explanatory variables are treatment group, the subgroup factor and the interaction term of treatment group and the subgroup factor. The p-value of interaction will be calculated. The explanatory variables that will be used are listed as follows;
  - Left Ventricular Ejection Fraction (%),
  - AKIRisk Score $((ng/mL)^2/1000)$ [Postsurgery],
  - Duration of CPB (hours),
  - Duration of Aortic Cross-Clamp (hours),
  - eGFR based on Serum Creatinine (mL/min/1.73 m^2) [Baseline]

7.4.4 Analysis of Exploratory Endpoints

[Analysis population: FAS, Analysis group: Randomization Arm]

The following analysis will be conducted for endpoint group 1.

- The proportion of subjects who meet the criteria will be analyzed using the CMH test with strata to control for baseline eGFR (< 45, ≥ 45).
- The MH estimate of the adjusted common risk ratio and 2-sided 90% confidence interval will be calculated.
- If the proportion of subjects with baseline eGFR based on Serum Creatinine < 45 mL/min per 1.73 m^2 for FAS is less than 10%, the above stratified analyses of CMH test and MH estimate of adjusted common risk ratio will be replaced with unstratified analyses of chi-square test and unadjusted risk ratio.

Group 1:

- All-cause mortality at day 30 (Visit 9)
- All-cause mortality at day 90 (Visit 10)
- Number of subjects needing RRT at day 30 (Visit 9)
- Number of subjects needing RRT at day 90 (Visit 10)
- Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 30 (Visit 9)
- Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 90 (Visit 10)
- MAKE30 with eGFR based on cystatin-C
- MAKE90 with eGFR based on cystatin-C
- Number of subjects with ≥ 25% sustained reduction in eGFR based on cystatin-C at day 30 (Visit 9)
- Number of subjects with ≥ 25% sustained reduction in eGFR based on cystatin-C at day 90 (Visit 10)
- Alternative derivations of proportion of subjects developing AKI using different definitions [Table 3] within 72 hours after T0. The stages will be used for subject developing AKI-SCr72h.
  - All stages AKI-UO (AKI-UO stage 2 or stage 3)
  - AKI-UO stage 3
  - AKI-SCr stage 2 or 3
  - AKI-SCr stage 2 or 3 and/or AKI-UO stage 3
  - AKI based on Cystatin-C, defined as an increase of ≥ 10% of baseline serum cystatin-C [KDIGO, 2012]

- Alternative derivations of proportion of subjects developing AKI using different definitions within 7 days after T0.
- AKI-SCr5d
- AKI-KDIGO5d
- Proportion of subjects with AKI as defined by a 50% or higher reduction in eGFR based on the Cystatin-C equation within 72 hours after T0.
- Proportion of subjects with AKI as defined by a 50% or higher reduction in eGFR based on the Cystatin-C equation within 7 days after T0.
- Proportion of subjects that have a reduction of NC AKIRisk score 24 hours after T0 (visit 3) to or below 0.3 $(ng/ml)^2/1000$
- Proportion of subjects with renal recovery, defined as a SCr value at hospital discharge equal to or lower than that at baseline

The following analysis will be conducted for endpoint group 2.

- Descriptive statistics will be presented.
- The endpoint will be analyzed using the Wilcoxon-test.

Group 2:

- Number of hospital days during initial hospitalization
- Number of ICU days during initial hospitalization
- Total number of hospital days up to day 90
- Total number of ICU days up to day 90
- Duration of AKI based on AKI stage using SCr (Duration of AKI-SCr72h and Duration of AKI-SCr7d)

The following analysis will be conducted for endpoint group 3.

- The number and percentage of category will be presented.
- The endpoints will be analyzed using the Wilcoxon-test.

Group 3:

- Severity of AKI within 72 hours after T0
- Severity of AKI within 7 days after T0
- Number of readmissions to ICU (use “0 Times”, “1 Time”, “2 Times” and “3 Times or More” as category)
- Number of readmissions to hospital (use “0 Times”, “1 Time”, “2 Times” and “3 Times or More” as category)

The following analysis will be conducted for endpoint group 4.

- Time to event will be analyzed using Kaplan-Meier method. Kaplan-Meier estimator (1st Quartile, Median, 3rd Quartile, their 90% confidence intervals [CIs] and Range) will be presented. For AKI-SCr72h, subjects without developing AKI-SCr72h will be censored at the date of Visit 5 (Day 4, 72 hours after T0) or at the date of discontinuation if the subjects discontinue before Visit 5. For AKI-SCr7d, subjects without developing AKI-SCr7d will be censored at the date of Visit 8 (Day 7/DoD), at Day 7 if the Visit 8 (Day 7/DoD) assessment was not performed but still in the study or at the date of discontinuation if the subjects discontinue before Visit 8. For ventilator weaning, subjects who discontinued the study without ventilator weaning will be censored at the date of discontinuation.
- Time to event will be analyzed using the log-rank test.
- Hazard ratio and its 90% CI will be presented Based on Cox proportional hazards model with treatment as the only explanatory variable.

Group 4:

- Time to AKI-SCr72h
- Time to AKI-SCr7d
- Time to ventilator weaning, defined as the time from T0 to extubation (i.e., removal of the endotracheal ventilation tube)

The following analysis will be conducted for endpoint group 5.

- Descriptive statistics for the variables and changes from baseline at each visit will be presented.
- Mean difference between treatment groups for change from baseline and its 90% CI will be calculated at each visit and t-test will be conducted.
- Mean (+/- SD) plot for the variables and change from baseline will be produced.

Group 5:

- APACHE-II
- SCr, Cystatin-C, eGFR based on SCr and Cystatin-C, kidney injury molecule-1 (KIM1) and AKIRisk Score (For AKIRisk Score, postsurgery value will be used as baseline. If AKIRisk Score at postsurgery is recorded several times in eCRF, the last score will be used as the value at postsurgery.)

The following analysis will be conducted for endpoint group 6.

- Descriptive statistics for the variables will be presented.
- Mean difference between treatment groups for the variables and its 90% CI will be calculated and t-test will be conducted.

Group 6:

- AUCSCr72h
- AUCSCysC72h
- AUCSCr7d
- AUCSCysC7d

The following analysis will be conducted for other endpoints.

- EQ-5D-5L
  - Descriptive statistics for the EQ-5D-5L VAS score and changes from baseline at each visit will be presented for each treatment group. In addition, the descriptive statistics will be presented by subgroups of AKI-SCr72h (Yes/No) and AKI-SCr72h stage 2 or 3 (Yes/No)
  - Mean difference between treatment groups for the EQ-5D-5L VAS and its 90% CI will be calculated and t-test will be conducted.
  - For the EQ-5D-5L qualitative domains, the number and percentage of subjects in each response level value will be reported by visit.
  - Mean (+/- SD) plot for EQ-5D-5L VAS score and change from baseline will be produced.

7.5 Analysis of Safety

[Analysis population: SAF, Analysis group: Randomization Arm and Total]

7.5.1 Adverse Events

Summaries and listings of serious adverse events (SAEs) and Serious TEAEs include SAEs upgraded by the sponsor based on review of the Sponsor's list of Always Serious terms if any upgrade was done.

The coding dictionary for this study will be MedDRA. It will be used to summarize AEs by system organ class (SOC) and preferred term (PT).

An overview table will include the following details:

- Number of TEAEs,
- Number and percentage of subjects with TEAEs,
- Number of drug related TEAEs,

- Number and percentage of subjects with drug related TEAEs,
- Number of serious TEAEs,
- Number and percentage of subjects with serious TEAEs,
- Number of serious drug related TEAEs,
- Number and percentage of subjects with serious drug related TEAEs,
- Number of TEAEs leading to death,
- Number and percentage of subjects with TEAEs leading to death,
- Number of TEAEs leading to withdrawal of treatment,
- Number and percentage of subjects with TEAEs leading to withdrawal of treatment,
- Number of drug related TEAEs leading to withdrawal of treatment,
- Number and percentage of subjects with drug related TEAEs leading to withdrawal of treatment, and
- Number of deaths.

The number and percentage of subjects with TEAEs, as classified by SOC and PT will be summarized for each treatment group. Summaries will be provided for:

- TEAEs
- Drug related TEAEs,
- serious TEAEs,
- Drug related serious TEAEs,
- TEAEs leading to death,
- Drug related TEAEs leading to death,
- TEAEs leading to withdrawal of treatment,
- Drug related TEAEs leading to withdrawal of treatment,
- TEAEs excluding serious adverse events that equal to or exceed a threshold of 5.0% in any treatment group,
- Frequently reported TEAEs that equal to or exceed a threshold of 5.0% in any treatment group, and
- Frequently reported drug related TEAEs that equal to or exceed a threshold of 5.0% in any treatment group.

The number and percentage of participants with TEAEs, as classified by PT only, will be summarized for each treatment group.

The number of TEAEs and the number and percentage of subjects with TEAEs, as classified by SOC and PT will also be summarized by severity using National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE). In the subject count, if a subject has multiple TEAEs with the same SOC or PT, but with differing NCI-CTCAE Grade, then the subject will be counted only once with the worst NCI-CTCAE Grade, however, if any of the NCI-CTCAE Grade values are missing then the subject will be counted only once with missing NCI-CTCAE Grade. In the adverse event count, the adverse events will be presented in each category they were classified to. Drug related TEAEs will be presented in a similar way by NCI-CTCAE Grade.

The number and percentage of subjects with TEAEs, as classified by SOC and PT will be summarized for each treatment group by time interval. For each adverse event in a particular interval, a subject will be counted if there is an onset of a treatment-emergent adverse event regardless of onset in other intervals.

Time intervals will be categorized according to the following categories:

- >= 0 hours to <= 24 hours after T0
- > 24 hours to <= 48 hours after T0
- > 48 hours to <= 72 hours after T0
- > 72 hours after T0 to <= Day 7 or DoD
- > Day 7 or DoD to <= Day 30

The number and percentage of subjects with TEAEs, as classified by SOC and PT will be summarized for each treatment group by subgroups defined in [Section 7.8].

7.5.2 Clinical Laboratory Evaluation

The baseline visit is the presurgery measurement. In case of missing value at presurgery, the last measurement before surgery will be used as the baseline.

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

Frequency tabulations of qualitative clinical laboratory variables (urinalysis) will be presented for each treatment group at each visit.

For hematology and biochemistry shift table will be presented for each treatment group:

- Summary shifts from of reference range changes from baseline to each visit (shift from normal or high to low, shift from normal or low to high, categorized increase [shift from low to normal or from normal to high], categorized no change [value stays in the same reference range], categorized decrease [shift from high to normal or from normal to low]).

The following data will be presented graphically by treatment group:

- Quantitative Laboratory test results using box plot at each visit.

7.5.2.1 Liver Enzymes and Total Bilirubin

The following potentially clinically significant criteria for liver tests – defined as Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), total bilirubin, Aspartate Aminotransferase (AST) and their combination are defined. The subject's highest value after the date of first dosing will be used.

Parameter	Criteria
ALT	> 3x upper limit of normal (ULN)
	> 5xULN
	> 10xULN
	> 20xULN
AST	> 3xULN
	> 5xULN
	> 10xULN
	> 20xULN
ALT or AST	> 3xULN
Total Bilirubin	> 2xULN
ALP	> 1.5xULN
ALT and/or AST AND Total Bilirubin(*)	(ALT and/or AST > 3xULN) and total bilirubin > 2xULN

(*) Combination of values measured within same sample

The number and percentage of subjects with potentially clinically significant values in liver enzyme and total bilirubin tests during the investigational period will be presented by treatment group.

The following data will be presented graphically by treatment group:

- Matrix scatter plot of maximum liver tests values during the study.

7.5.3 Vital Signs

The baseline visit is the presurgery measurement. In case of missing value at presurgery, the last measurement before surgery will be used as the baseline.

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

Following data will be presented graphically by treatment group:

- Vital sign results using box plot at each visit.

7.6 Analysis of PK

[Analysis population: PKAS, Analysis group: ASP1128]

If more than and equal to 50% of values are below the quantification limit (BQL) at a given time point, SD and %CV will not be calculated.

7.6.1 Plasma Concentrations

Descriptive statistics will be presented for plasma concentration. Individual and mean concentrations of ASP1128 versus time will be plotted. Plasma concentration and sample times data will be listed. Allowable time windows for inclusion in summary statistics are defined as follows.

Table 11 Blood Sampling for PK for Inclusion in Summary Statistics

	Sampling Time	Window
Visit 2	Pre-dose	-90 min to 0 min
	EOI	±5 min
	2 to 4 hours	±20 min
	4 to 12 hours	±60 min
Visit 3	EOI	±5 min
Visit 4	Pre-dose	-60 min to 0 min
	EOI	±5 min

PK: pharmacokinetics; EOI: end of infusion

7.7 Analysis of PD

[Analysis population: PDAS, Analysis group: Randomization Arm and Total]

The natural logarithm of gene expression data and its change from baseline will be summarized using mean, standard deviation, coefficient of variation, minimum, maximum and median by treatment group and time points. The data at predose on Visit 2 will be used as baseline.

Following data will be presented graphically by treatment group:

- The natural logarithm of gene expression data using mean (+/- SD) plot.

Additionally, the gene expression data and its fold change from baseline will be summarized using mean, standard deviation, minimum, Q1, median, Q3 and maximum by treatment group and time points. The data at predose on Visit 2 will be used as baseline.

Following data will be presented graphically by treatment group:

- The fold change from baseline of gene expression data using box plot at each visit.

7.8 Subgroups of Interest

Primary efficacy endpoint, secondary efficacy endpoints and selected safety variables (treatment emergent adverse events) will be summarized by the treatment group for the subgroups defined on the basis of the categorized variables listed in [Table 12].

Table 12 Subgroups of Interest

Item	Classification	Endpoints to be assessed
Sex	1: Male 2: Female	Primary efficacy endpoint, Secondary efficacy endpoints, TEAEs
Age (years) [Informed Consent]	1: < 70 2: >= 70	Primary efficacy endpoint, Secondary efficacy endpoints, TEAEs
Left Ventricular Ejection Fraction (%)	1: < 40 2: >= 40	Primary efficacy endpoint, Secondary efficacy endpoints
AKIRisk Score ((ng/mL)^2/1000) [Postsurgery]	1: > 0.3 to <= 0.7 2: > 0.7	Primary efficacy endpoint, Secondary efficacy endpoints
Diabetes Mellitus	1: No 2: Yes	Primary efficacy endpoint, Secondary efficacy endpoints
Peripheral Arterial Disease	0: No 1: Yes	Primary efficacy endpoint, Secondary efficacy endpoints
Use of Intravenous Radio-contrast	0: No 1: Yes	Primary efficacy endpoint, Secondary efficacy endpoints
Surgery Type [1]	1: Lower Risk Surgery 2: Higher Risk Surgery	Primary efficacy endpoint, Secondary efficacy endpoints
Duration of CPB (hours)	1: < Median 2: >= Median	Primary efficacy endpoint, Secondary efficacy endpoints
Duration of Aortic Cross-Clamp (hours)	1: < 0.5 2: >= 0.5 to < 1.0 3: >= 1.0	Primary efficacy endpoint, Secondary efficacy endpoints
eGFR based on Serum Creatinine (mL/min/1.73 m^2) [Baseline]	1: < 45 2: >= 45	Primary efficacy endpoint, Secondary efficacy endpoints, TEAEs
	1: < 60 2: >= 60	Primary efficacy endpoint, Secondary efficacy endpoints, TEAEs
Protocol Version Number at Enrollment	1: Before Version 2.0 2: Version 2.0 or Later	Primary efficacy endpoint, Secondary efficacy endpoints
Time of First Study Drug Taken	1: Within T0 + 8 hours 2: After T0 + 8 hours	Primary efficacy endpoint, Secondary efficacy endpoints
Protocol Version Number at Enrollment and Time of First Study Drug Taken	1: [Before Version 2.0] and [Within T0 + 8 hours] 2: [Before Version 2.0] and [After T0 + 8 hours] 3: [Version 2.0 or Later] and [Within T0 + 8 hours] 4: [Version 2.0 or Later] and [After T0 + 8 hours]	Primary efficacy endpoint, Secondary efficacy endpoints

TEAE: treatment-emergent adverse event; CPB: cardiopulmonary bypass pump; eGFR: estimated glomerular filtration rate

1: If the procedure of surgery is only one of CABG, Valve Surgery [Aortic], Valve Surgery [Mitral] or Valve Surgery [Tricuspid] and not Thoracic Aortic Surgery then Surgery type is “Lower Risk Surgery”; otherwise “Higher Risk Surgery”.

For more details refer to [Section 7.4.3 and 7.5.1].

7.9 Other Analyses

7.9.1 Analysis for Observational Cohort

[Analysis population: PFAS, Analysis group: Weighted Total (PFAS)]

Potential risk factors (demographics, subject characteristics, biomarkers) for development of AKI and MAKE (AKI-SCr72h, AKI-SCr7d, MAKE30, and MAKE90) will be explored. Population standardization is used in this analysis. Weighted Total (PFAS) is the set of NC negative group and NC positive group weighted by (the number of subjects assigned to Placebo group + the number of subjects assigned to ASP1128 group)/ (the number of subjects in Placebo group). The estimate of the unadjusted risk ratio, 2-sided 90% confidence interval, true positive rate, and true negative rate for AKI and MAKE (AKI-SCr72h, AKI-SCr7d, MAKE30, and MAKE90) proportion will be calculated by risk factors listed in [Table 13]. In Analysis for Observational Cohort, subjects whose status of AKI and MAKE is unknown due to, say, the missing data of SCr will not be counted as AKI/MAKE event. This treatment for missing data is different from it for primary and secondary endpoints.

Table 13 Potential Risk Factors for Development of AKI and MAKE

Risk Factor	Classification
Circulatory Shock	0: No 1: Yes
Nephrotoxic Drugs	0: No 1: Yes
Radiocontrast Agents	0: No 1: Yes
Poisonous Plants and Animals	0: No 1: Yes
Dehydration or Volume Depletion	0: No 1: Yes
Advanced Age (>=70 years)	0: No 1: Yes
Female Gender	0: No 1: Yes
Black Race	0: No 1: Yes
CKD	0: No 1: Yes
Chronic Diseases (Heart, Lung, Liver)	0: No 1: Yes
Diabetes Mellitus	0: No 1: Yes
Anemia	0: No 1: Yes
Prior AKI	0: No 1: Yes
Left Ventricular Ejection Fraction (<40 %)	0: No 1: Yes
AKIRisk Score (>0.3 $(ng/mL)^2/1000$) [Postsurgery]	0: No 1: Yes
AKIRisk Score (>0.7 $(ng/mL)^2/1000$) [Postsurgery]	0: No 1: Yes
Peripheral Arterial Disease	0: No 1: Yes
Use of Intravenous Radio-contrast	0: No 1: Yes
Higher Risk Surgery	0: No 1: Yes
Duration of CPB (>=Median)	0: No 1: Yes
Duration of Aortic Cross-Clamp 1 (>=0.5 hours)	0: No 1: Yes
Duration of Aortic Cross-Clamp 2 (>=1.0 hours)	0: No 1: Yes
eGFR Based on Serum Creatinine (<45 mL/min/1.73 m^2) [Baseline]	0: No 1: Yes

CKD: chronic kidney disease; AKI: acute kidney injury; CPB: cardiopulmonary bypass pump; eGFR: estimated glomerular filtration rate

In addition to the above, the following analyses will be conducted for Observational Cohort.

- Primary efficacy endpoint and secondary efficacy endpoints will be summarized for the subjects with NC positive within T0 + 24 hours and NC negative within T0 + 24 hours and all subjects in Observational Cohort. In the table, each stage of AKI-SCr72h and AKI-SCr7d and each individual outcomes of MAKE30/90 will also be summarized.
- For Observational Cohort, the association between MAKE30/MAKE90 (and also each individual outcomes, of all-cause mortality, initiation of RRT and eGFR based on SCr at day 30 (Visit 9)/day 90 (Visit 10) equal to or less than 75% of the baseline value) and primary, secondary and exploratory endpoint of AKI proportion is evaluated by a contingency table. The following endpoints of AKI proportion will be used;
  - AKI-SCr72h (primary endpoint) and each stage,
  - AKI-SCr7d (secondary endpoint) and each stage,
  - AKI-SCr stage 2 or 3 (within 72 hours after T0), and
  - AKI-SCr stage 2 or 3 (within 7 days after T0)

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

A formal interim analysis for futility is planned when approximately 60% of the planned AKI-SCr72h data have been collected in the study to evaluate whether ASP1128 has particularly poor efficacy compared to placebo while the study is ongoing. When conditional power to detect the difference between ASP1128 and placebo on the primary endpoint of AKI-SCr72h proportion is < 10%, that is, 2-sided p-value is > 0.518 and/or MH estimate of the common risk ratio (ASP1128 / placebo) is > 1, the study may stop. No interim stop for efficacy is planned, so the overall 2-sided 0.10 significance level is controlled.

A Data Monitoring Committee (DMC) will be instated to perform the interim analysis, which may recommend (nonbinding) terminating the study for unfavorable results at the interim analysis as described above.

Details for the interim analysis, primary endpoint of AKI-SCr72h proportion will be contained in the interim analysis plan and DMC Charter. Recommendations regarding study conduct will be made by the DMC based on their assessment of this result. If the study is stopped at the interim analysis, a final analysis will be conducted after the database will be locked.

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

7.11.1 Missing Data

See [Section 6.1] for missing value handling of primary and secondary endpoints. Plasma concentrations of ASP1128 below the quantification limit (BQL) will be presented as "<LLOQ" in listings while treated as zero in the analyses. Target gene expression below the

quantification limit will be presented as "<LLOQ" in listings while treated as half of the LLOQ in the pharmacodynamic analyses.

7.11.2 Outliers

All values will be included in the analyses.

7.11.3 Visit Windows

All analyses will be performed based on CRF visit with the following exceptions:

- For the baseline, the value at presurgery will be used as the baseline. However in case of missing value at presurgery, the last measurement before surgery will be used as the baseline.
- For the pharmacodynamic analysis, the data for 2-4 hours postdose at visit 2 which is taken within 1 hour after the administration of study drug will be excluded for analysis.

8 DOCUMENT REVISION HISTORY

Version	Date	Changes	Comment/rationale for change
1.00	29-MAY-2019	NA	Document finalized
2.00	26-NOV-2019	• Adding the analysis for the duration of Aortic Cross-Clamp, MAKE30/90 with eGFR Based on Cystatin-C and AKIN-AKI-SCr5d • Correction of mistakes in [Section 6].	The start and end time of Aortic Cross-Clamp were added in eCRF.
3.00	9-JUL-2021	• Change the definition of the baseline	To handle missing data at presurgery
		• Change the handling of missing data in the primary and secondary efficacy analysis for AKI and MAKE	To update the handling of missing data to be consistent with medical/clinical point of view.
		• Correct the definition of the AKI	To align with the KDIGO guideline
		• Clarify the definition of the initial hospitalization in the number of hospital/ICU days	For clarification
		• Add new exploratory endpoint of AKI-KDIGO5d	To assess the endpoint
		• Add frequency table of protocol version number at enrollment, the time of first study drug taken and those subgroups • Add new analysis of observational cohort to summarize the subjects with NC positive within T0 + 24 hours in observational cohort	To assess the impact of the protocol amendment at Ver.2.0
		• Add new subgroup category of eGFR based on Serum Creatinine	To align with the demographic analysis

Version	**Date**	**Changes**	**Comment/rationale for change**
		• Add unstratified analyses with the case that one of the strata is too small to implement the stratified analyses in the primary efficacy analysis and some secondary/exploratory analyses	To deal with the case that one of the strata is too small to implement the stratified analyses
4.00	13-JAN-2022	• Clarify the definition of AKI-SCr stage 2 or 3 and/or AKI-UO stage 3	For clarification
		• Add the analyses of eGFR based on SCr and Cystatin-C	To assess the endpoint
		• Add the analyses of area under the curve of SCr and S-cystatin-C through 72 hours after T0 (AUCSCr72h and AUCSCysC72h)	To assess the endpoint
		• Clarify the definition of surgery type (lower risk surgery and higher risk surgery)	For clarification
		• Add frequency table of protocol version number at enrollment vs the time of first study drug taken and those subgroups	To assess the impact of the protocol amendment at Ver.2.0
		• Add the analyses of each individual outcomes of MAKE30/90	To assess the endpoint
		• Add the definition of censored subjects for time to AKI-SCr	To deal with censored subjects
		• Add the analyses of mean difference with 90% CI and t-test for APACHE-II, SCr, Cystatin-C, eGFR based on SCr and Cystatin-C, kidney injury molecule-1 (KIM1) and AKIRisk Score	For detailed summarization
		• Add the subgroup analysis of EQ-5D-5L • Add the analysis of mean difference with 90% CI and t-test for EQ-5D-5L	For detailed summarization

Version	Date	Changes	Comment/rationale for change
		• Add the analysis of TEAE table by PT only	For summarization
		• Add summary statistics of coefficient of variation, Q1 and Q3 for the pharmacodynamic variables	For detailed summarization
		• Add the following analyses for Observational Cohort: Each stage of AKI-SCr72h and AKI-SCr7d, Each individual outcomes of MAKE30/90, and Association between MAKE30/90 vs AKI-SCr72h, AKI-SCr7d, and AKI-SCr stage 2 or 3	For detailed summarization
		• Add the data handling of the visit windows for the pharmacodynamic variables	To exclude the values out of the visit window from the analysis

9 REFERENCES

ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

ICH Harmonized Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Official Journal of the International Society of Nephrology: Kidney International Supplements. 2013;3(1):1-150.

KDIGO Clinical Practice Guideline for Acute Kidney Injury. Official Journal of the International Society of Nephrology: Kidney International Supplements. 2012;2(1):1-138.

10 APPENDICES

10.1 Appendix 1: Key Contributors and Approvers

List of Key Contributors and Approvers

Key Contributors

The following contributed to or reviewed this Statistical Analysis Plan as relevant to their indicated discipline or role.

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