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

ISN/Protocol 1128-CL-0201

Version 2.0

Incorporating Substantial Amendment 1 [see Section 13]

08 Oct 2020

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Sponsor:

Astellas Pharma Inc. (API)

2-5-1, Nihonbashi-Honcho, Chuo-ku,
Tokyo 103-8411 Japan

Protocol History:
Version 1.0 [21Nov2018]
Version 1.1 [13Jan2019] Incorporating Nonsubstantial Amendment 1
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Version 1.3 [23Apr2019] Incorporating Nonsubstantial Amendment 3
Version 1.4 [20May2019] Incorporating Nonsubstantial Amendment 4
Version 1.5 [21Jan2020] Incorporating Nonsubstantial Amendment 5

Table of Contents

I. SIGNATURES

1. SPONSOR'S SIGNATURES

Required signatures (e.g., Protocol authors and contributors, etc.) are located in [Section 14 Sponsor's Signatures].

2. INVESTIGATOR'S SIGNATURE

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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08 Oct 2020

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

Principal Investigator:

Signature: ---------- Date (DD Mmm YYYY)

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

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

II. CONTACT DETAILS OF KEY SPONSOR'S PERSONNEL

24h-Contact for Serious Adverse Events (SAEs) See [Section 5.5.5 Reporting of Serious Adverse Events] for SAE Fax Number and Email	**Please fax or email the SAE Worksheet to:** **Astellas Pharma Global Development, Inc. (APGD) Pharmacovigilance** **Fax number: +1 888-396-3750** **Alternate Fax number: +1 847-317-1241** **Email: Safety-US@astellas.com**
Medical Monitor/Study Physician:	PPD Medical & Development Astellas Pharma Inc. 2-5-1, Nihonbashi-Honcho, Chuo-ku Tokyo 103-8411, Japan PPD
Clinical Research Contacts:	PPD Astellas Pharma Global Development, Inc., Clinical Science 1 Astellas Way, Northbrook, IL 60062, USA PPD

III. LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
ACE	angiotensin-converting enzyme
AE	adverse event
AKI	acute kidney injury
AKI-SCr	acute kidney injury based on serum creatinine KDIGO criteria
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APACHE-II	Acute Physiology and Chronic Health Evaluation II
API	Astellas Pharma Inc.
APS	Acute Physiology Score
AST	aspartate aminotransferase
AUC_{24}	area under the concentration-time curve at 24 hours
CA	Competent Authorities
CABG	coronary artery bypass graft
cEC	concerned Ethics Committee
CK	creatine kinase
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	maximum concentration
CMH	Cochran-Mantel-Haenszel
CPB	cardiopulmonary bypass pump
CRO	contract research organization
CYP	cytochrome P450
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
DMC	Data Monitoring Committee
DoD	Day of Discharge
ECG	electrocardiogram
ECMO	Extracorporeal Membrane Oxygenation
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EoS	end of study
EQ-5D-5L	5-level European Quality of Life 5 Dimensions Questionnaire
FAS	full analysis set
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice

Abbreviations	Description of abbreviations
GCS	Glasgow Coma Scale
GFR	glomerular filtration rate
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hERG	human ether à go go related gene
HIPAA	Health Insurance Portability and Accountability Act
HRT	hormone replacement therapy
IABP	intra-aortic balloon pump
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	intensive-care unit
IEC	Independent Ethics Committee
IGFBP7	insulin-like growth factor binding protein-7
INR	international normalized ratio
IRB	Institutional Review Board
IRT	interactive response technology
KDIGO	kidney disease: improving global outcomes
KIM1	kidney injury molecule-1
LA-CRF	liver abnormality case report form
LFT	liver function tests
LVAD	left ventricular assist device
MAKE	major adverse kidney events
MAKE30	MAKE within 30 days after day of surgery
MAKE90	MAKE within 90 days after day of surgery
MAP	mean arterial pressure
MDRD	Modification of Diet in Renal Disease Study
MH	Mantel-Haenszel
NC	NephroCheck®
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NOAEL	no observed adverse effect level
NSAID	nonsteroidal anti-inflammatory drug
PDAS	pharmacodynamic analysis set
PFAS	prognostic factor analysis set
PGx	pharmacogenomics
PKAS	pharmacokinetic analysis set

Abbreviations	Description of abbreviations
PPAR	peroxisome proliferator activated receptors
PPARδ	peroxisome proliferator activated receptor δ
PPS	per protocol set
PTM	placebo to match
RRT	renal replacement therapy
(S)AE	serious adverse event or adverse event
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SCr	serum creatinine
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reactions
T0	time point 0
$t_{1/2}$	terminal elimination half-life
TBL	total bilirubin
TEAE	treatment-emergent adverse event
TIMP2	tissue Inhibitor of metalloproteinase-2
ULN	upper limit of normal
UO	urinary output
USM	urgent safety measure
VAD	ventricular assist device

Definition of Key Study Terms

Terms	Definition of Terms
Baseline	Assessments of subjects as they enter a study, before they receive any treatment.
Child-Pugh B	Assessment scale used in clinical staging of cirrhosis.
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.
End of Study	The last visit for this study.
Intervention	The drug, device, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety and pharmacogenomics).
Randomization	The process of assigning study subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential subjects for enrollment in a study.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a study.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Subject	An individual who participates in a clinical study, either as a recipient of the investigational product(s) or as a control.
Time point 0 (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.

IV. SYNOPSIS

Date and Version No of Protocol:	08 Oct 2020, Version 2.0
Sponsor Astellas Pharma Inc. (API)	**Protocol Number:** 1128-CL-0201
Name of Study Drug: ASP1128 (MA-0217)	**Phase of Development:** 2a

Title of Study:

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

Planned Study Period:

From 2Q2019 to 1Q2022

Objectives:

Primary objective:

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

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

Exploratory objectives:

- 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 NephroCheck® [NC] device) for identifying subjects at risk for AKI following CABG and/or valve surgery

Planned Total Number of Study Centers and Location(s):

Approximately 50 centers in North America

Study Population:

Subjects undergoing CABG and/or valve surgery who have a moderate or high risk for developing AKI based on serum creatinine (SCr) Kidney Disease Improving Global Outcomes (KDIGO) criteria (AKI-SCr) postsurgery.

Number of Subjects to be Enrolled/Randomized:

Subjects who meet the presurgery selection criteria will be enrolled. Subjects who meet the postsurgery criterion of having a NC AKIRisk® score > 0.3 $(ng/mL)^2/1000$ between 2 and 22 hours after surgery can be randomized: the number of subjects to be randomized is 220. The subjects that have an AKIRisk score of ≤ 0.3 $(ng/mL)^2/1000$ at the postsurgery assessment will be enrolled in the observational cohort. The observational cohort will enroll a maximum of 440 subjects.

Study Design Overview:

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 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; eGFR obtained from central laboratory testing will be used. If the eGFR result from the central laboratory testing is not yet available at randomization, an eGFR result from local laboratory can be used if assessed within 7 days before the day of surgery up to initiation of surgery.

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, urinary output (UO), pregnancy test, physical examination, vital signs, blood pressure and assessment of adverse events (AEs). In addition, blood and urine will be sampled for pharmacokinetics, pharmacodynamics, pharmacogenomics (PGx), metabolomics and biomarker assessments.

Visit 1/Screening

The screening visit is the visit in which the indication for cardiovascular surgery is set, and the surgery will take place within 28 days from this visit. If the subject agrees to take part in the study, informed consent will be obtained at this visit before performing any study related procedures. Subjects will be assessed for presence of inclusion criteria and absence of exclusion criteria before surgery. Medical history and medication use (up to 28 days before screening) will be recorded. Subjects will be clearly instructed that randomization and treatment with investigational compound or placebo will occur after end of surgery and only if the subject qualifies for the NC AKIRisk score criterion as detailed below.

Double-blind Treatment Period (Visits 2, 3, 4 and 5)

Visit 2/Day of Surgery

Baseline assessments are performed before surgery on the day of surgery, including medication use between screening and day of surgery, safety laboratory tests, kidney function (eGFR, SCr and serum cystatin C), biomarkers (e.g., kidney injury molecule-1, NC), vital signs and cardiac function (previous ejection fraction data from recent echocardiogram).

During surgery, various parameters will be recorded (e.g., incision time, cardiopulmonary bypass pump [CPB] time, volume and type of blood products and infused fluids, UO and total blood loss). 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 CPB (i.e., the final separation from CPB and normal circulation is restored). This time point is recorded by the perfusionist in the subject's chart.

Four hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0), a urine sample will be collected and the concentration of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor binding protein-7 will be assessed utilizing the NC device as per the package insert instructions. The urine sample should be fresh (i.e., not from accumulated urine in the bag, so the bag should be emptied before the sample is taken); it can be obtained in the operating room/theater, recovery room or in the intensive care unit (ICU), as long as it is within the range of 2 to 6 hours post T0. The device will calculate the AKIRisk score. If NC assessment is positive (AKIRisk score > 0.3 $ng/mL^2/1000$), the subject will be randomized and will start treatment within 8 hours after T0 as per the randomization schedule.

If the first result is of the AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$, the NC assessment should be redone once only if within the 2- to 6-hour window after T0 (i.e., the urine sample for NC should be taken within the 2 to 6 hours post T0 range).

If the NC assessment is negative (AKIRisk score ≤ 0.3 $(ng/mL)^2/1000$ [at first and second measurements, if applicable]) or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated for up to 4 times within 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour. If all the NC assessments are negative up to 22 hours after T0, the subject cannot be randomized and will be followed up in the observational cohort (see below).

Randomized subjects will receive 3 subsequent treatments with ASP1128 or matching placebo, which will be administered intravenously through a peripheral/central catheter. The first administration of study drug will occur after randomization, as soon as possible after the positive NC. If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP should be completed within 8 hours after T0. If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but within 24 hours after T0.

In case of reoperation on the day of surgery, the same time range for NC and drug administration applies in relation to T0 (i.e., NC between 2 and 22 hours and first investigational drug administration within 24 hours). A reoperation is no reason for not enrolling a subject as long as the NC can be performed at the described time points.

After surgery, UO will be monitored, safety assessments will be done and laboratory tests (including NC, SCr, cystatin C and other biomarkers) will be performed as per the Schedule of Assessments [Table 1].

Visits 3, 4 and 5

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 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 IP. A window of 2 hours before and after the designated time is allowed. Applicable assessments will be done as per the Schedule of Assessments [Table 1] up to 72 hours after T0 (visit 5) to assess the study endpoints, including AKI (based on SCr and UO). When the subject is in the ICU, UO per 12 hours and total fluid intake per 24 hours (including blood products and intravenous fluid input) must be recorded. When the subjects are in the ICU, the time to ventilator weaning will be recorded.

Follow-up

It is assumed that the subjects will be in the hospital up to at least 1 week after the surgery. On days 5, 6 and 7 after T0 (visits 6, 7 and 8, respectively) applicable assessments will be performed to assess study endpoints, including AKI (based on SCr). After end of all the procedures of visit 5, subjects can be discharged at the discretion of the investigator. If the subject is discharged 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 the subject is discharged after day 7, visit 8 will be done on day 7 and no additional assessments are required after day 7 up to discharge, but the last result of SCr before discharge from local laboratory testing will be collected. Following day 7 (visit 8) or discharge, subjects will be followed up on day 30 (visit 9) and 90 (visit 10) following surgery. On visits 9 and 10, a composite endpoint of major adverse kidney events (MAKE) (all-cause mortality, renal replacement therapy [RRT] and/or ≥ 25% sustained reduction in eGFR) will be assessed.

Total number of hospital days and ICU days during initial hospitalization will be recorded. If the subjects are readmitted to hospital for any reason during the 90 follow-up days, the total number of hospitalizations and number of days hospitalized through day 30 and 90 will be recorded.

Observational Cohort

If at randomization NC is negative (AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$) at all assessments between 2 and 22 hours after T0, the subject cannot be randomized. These subjects will be included in the observational cohort. The rationale for following the subjects in the observational cohort is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. The observational cohort will enroll a maximum of 440 subjects.

The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (AKI-SCr using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Hospital and ICU stay will also be recorded. AEs and medication use will be recorded until 24 hours after T0. Urine samples will be collected for NC and biomarker assessments. If available, local laboratory test results and 24-hour UO data will be collected from medical records. No additional blood samples will be taken for study purposes after visit 2. See Schedule of Assessments below [Table 2].

Inclusion and Exclusion Criteria:

Inclusion:

Subject is eligible for the study if all of the following apply:

1. Institutional Review Board/Independent Ethics Committee-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication).
2. Subject agrees not to participate in another interventional study after signing the informed consent form and until the end of study (EoS) visit has been completed.
3. Subject is ≥ 35 years of age at the time of screening (visit 1).
4. Subject undergoing non-emergent open chest cardiovascular surgery with the use of CPB (i.e., CABG and/or valve surgery [including aortic root and ascending aorta surgery without circulatory arrest]) within 4 weeks of screening (visit 1).
5. Subject is at risk of developing AKI following cardiovascular surgery, i.e., has 1 or more of the following AKI risk factors:
    - Age at screening of ≥ 70 years
    - Documented history of eGFR < 60 mL/min per 1.73 m^2 as per Modification of Diet in Renal Disease Study (MDRD) or CKD-EPI equation (or documented measured glomerular filtration rate assessment)
        - History needs to be present for at least 90 days prior to screening. Both SCr and eGFR need to be documented in the chart, and
        - eGFR at screening or baseline needs to be < 60 mL/min per 1.73 m^2, as well as per CKD-EPI equation.
    - Documented history of congestive heart failure requiring hospitalization. This condition should exist for ≥ 90 days prior to screening.
    - Documented history of diabetes mellitus (type 1 or 2) of ≥ 90 days prior to screening, and subject is on active antidiabetic medication treatment for ≥ 90 days.

- Documented history of proteinuria/albuminuria at any time point before screening
  - Urinary dipstick result of ≥ 2+,
  - Documented urinary albumin creatinine ratio measurement of ≥ 300 mg/g, or
  - Documented total quantity of protein in a 24-hour urine collection test ≥ 0.3 g/day.

6. Subject must have the ability, in the opinion of the investigator, and willingness to return for all scheduled visits and perform all assessments.
7. A female subject is eligible to participate if she is not pregnant [see Appendix 12.3 Contraception Requirements] and at least 1 of the following conditions applies:
   - Not a woman of childbearing potential (WOCBP) as defined in [Appendix 12.3 Contraception Requirements]

     OR
   - WOCBP who agrees to follow the contraceptive guidance as defined in [Appendix 12.3 Contraception Requirements] throughout the treatment period and for at least 30 days after the final study drug administration.
8. Female subject must agree not to breastfeed starting at screening and throughout the study period, and for 30 days after the final study drug administration.
9. Female subject must not donate ova starting at screening and throughout the study period, and for 30 days after the final study drug administration.
10. A male subject with female partner(s) of childbearing potential must agree to use contraception as detailed in [Appendix 12.3 Contraception Requirements] during the treatment period and for at least 30 days after the final study drug administration.
11. A male subject must not donate sperm during the treatment period and for at least 30 days after the final study drug administration.
12. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding throughout the study period and for 30 days after the final study drug administration.

Exclusion

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

At Screening:

1. Subject has received investigational drug within 30 days or 5 half-lives, whichever is longer, prior to screening.
2. Subject has received RRT within 30 days prior to screening.
3. Subject has CKD stage 4 or 5, or stage 3 (i.e., eGFR 30-59 mL/min per 1.73 m^2) with a known history of eGFR < 30 mL/min per 1.73 m^2 as per CKD-EPI or MDRD equation within 6 months prior to screening.
4. Subject has a prior kidney transplantation.
5. Subject has a known or suspected glomerulonephritis (other than Diabetic Kidney Disease).
6. Subject has confirmed or treated endocarditis or other current active infection requiring antibiotic treatment within 30 days prior to screening.
7. Subject is using prohibited medications as specified in the concomitant medication section of the protocol [Section 5.1.3.1 Prohibited and Restricted Treatment].
8. Subject has a prior history of intravenous drug abuse within 1 year prior to screening.
9. Subject has a known chronic liver disorder with Child-Pugh B or C classification.

10. Subject has any of the following abnormal liver or kidney function parameters as assessed at screening. (If the results from the central laboratory are not yet available at time of randomization, results from local laboratory within 7 days before the surgery can be used for verifying this criterion. The subject will not be excluded from the study if central laboratory results from screening are exclusionary and are available only after randomization.):
    - Alanine aminotransferase (ALT), aspartate aminotransferase (AST) > 2 times the upper limit of normal (ULN) or bilirubin increased to > 1.5 times the ULN (not due to previously diagnosed Gilbert's syndrome).
    - eGFR < 30 mL/min per 1.73 m^2 as per CKD-EPI equation.
11. Subject has use of left ventricular assist device, intra-aortic balloon pump or other cardiac devices, or catecholamines within 7 days prior to screening.
12. Subject has surgery scheduled to be performed without CPB (i.e., "Off-Pump" surgery).
13. Subject has surgery scheduled to be performed under conditions of circulatory arrest, including planned deep hypothermic circulatory arrest.
14. Subject has surgery scheduled for aortic dissection.
15. Subject has surgery for a condition that is immediately life-threatening as per the discretion of the investigator.
16. Subject has surgery scheduled to correct major congenital heart defect.
17. Subject has current or previous malignant disease. Subjects with a history of cancer are considered eligible if the subject has undergone therapy and the subject has been considered disease free or progression free for at least 5 years. Subject with completely excised basal cell carcinoma or squamous cell carcinoma of the skin and completely excised cervical cancer in situ are also considered eligible.

Preoperatively on the Day of Surgery:

18. Exclusion criteria 1 to 17 are applicable at this time.
19. Subject has AKI (any stage) present at presurgery baseline at the discretion of the investigator.
20. Subject has known or suspected infection/sepsis at time of presurgery baseline at the discretion of investigator.

Perioperative Exclusion Criteria:

21. Subject requires Extracorporeal Membrane Oxygenation during or after completion of surgery.
22. Subject requires ventricular assist device during or after completion of surgery.
23. Subject has surgery performed "Off-Pump" at any time during surgery.

General:

24. Subject has other condition, which, in the investigator's opinion, makes the subject unsuitable for study participation.
25. Female subject who is pregnant or lactating or has a positive pregnancy test within 72 hours prior to screening and/or randomization, has been pregnant within 6 months before screening assessment or breastfeeding within 3 months before screening or who is planning to become pregnant within the total study period.
26. Subject has a known or suspected hypersensitivity to ASP1128 or any components of the formulation used.
27. Subject is an employee of the Astellas Group or the contract research organization involved in the study.

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

Investigational Product(s):

ASP1128 solution for intravenous administration in 20 mL vial (10 mL of 5 mg/mL ASP1128)

Dose(s):

Dose 100 mg once daily for 3 days

Mode of Administration:

Intravenous through a peripheral/central venous catheter

Comparative Drug(s):

Placebo to match solution for intravenous administration in 20 mL vial (10 mL of matching placebo)

Dose(s):

Not applicable

Mode of Administration:

Intravenous through a peripheral/central venous catheter

Concomitant Medication Restrictions or Requirements:

Medications that may significantly affect plasma creatinine levels without influencing kidney function, such as cimetidine, trimethoprim, pyrimidine analogues or derivatives, phenacemide and calcitriol or alfacalcidol are prohibited. These medications should be stopped more than 28 days before screening until EoS.

Following surgery up to 72 hours postsurgery, the use of nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme inhibitors and angiotensin receptor blockers is prohibited because of their potentially confounding effect on kidney function.

Other medications are not prohibited or restricted when clinically indicated, but will be recorded in the electronic case report form (eCRF).

Duration of Treatment:

Study treatment will be administered 3 times as follows:

ASP1128/placebo will be administered as soon as possible after the NC AKIRisk score is assessed between 2 and 22 hours after T0 is > 0.3 $(ng/mL)^2/1000$. If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP should be completed within 8 hours after T0. If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but 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 at 24 hours after T0 and the third dose of IP will be 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 at 16 hours after the start of the first dose, and the third dose of IP will be at 40 hours after the start of the first dose IP.

For the second and third dose, a 2-hour treatment window before or after designated administration time is allowed.

Endpoints for Evaluation:

Primary:

Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline (i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] within any 48 hours, or increase in SCr to ≥ 1.5 times baseline) within 72 hours after T0 (AKI-SCr72h).

Secondary:

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 7 days after T0 (AKI-SCr7d)
- Proportion of subjects developing AKI based on all captured criteria from the KDIGO guideline (i.e., AKI-SCr stage 1 to 3: increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] within any 48 hours, increase in SCr to ≥ 1.5 times baseline, and/or AKI-UO stage 2 and 3: urine volume < 0.5 mL/kg per hour for 12 consecutive hours) within 72 hours after T0 (AKI-KDIGO72h)
- Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 7 days after T0 (AKI-KDIGO7d)
- Proportion of subjects with MAKE defined as all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR based on SCr within 30 days after day of surgery (MAKE30). Sustained loss of renal function is defined as a reduction of kidney function (i.e., a reduction of eGFR of 25% or more compared to the baseline presurgery sample at visit 2) at the time of assessment, i.e., at day 30
- 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)

Exploratory:

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 5 days after T0 (AKI-SCr5d)
- Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 5 days after T0 (AKI-KDIGO5d)
- All-cause mortality at day 30
- All-cause mortality at day 90
- Number of subjects needing RRT at day 30
- Number of subjects needing RRT at day 90
- Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 30
- Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 90
- Number of subjects with ≥ 25% sustained reduction in eGFR based on Cystatin-C at day 30
- Number of subjects with ≥ 25% sustained reduction in eGFR based on Cystatin-C at day 90
- 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
- Alternative derivations of proportion of subjects developing AKI using different definitions within 72 hours after T0:
    - All stages AKI-UO
    - AKI-UO stage 3
    - AKI-SCr stage 2 or 3
    - AKI-SCr stage 2 or 3 and AKI-UO stage 3
    - AKI based on S-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
- Severity of AKI within 72 hours after T0
    - severity of AKI based on AKI-SCr stages
    - severity of AKI based on AKI-UO stages
    - severity of AKI based on most severe of AKI-SCr or AKI-UO stages

- Severity of AKI (based on AKI stage using SCr and/or UO) within 7 days after T0
- 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 investigator or staff should enter the date when respective criteria of AKI-SCRr72h were resolved in the eCRF.
    - Duration of AKI-SCr7d (definition as above)
- Time to AKI-SCr72h (i.e., the time from T0 to the time when criteria for AKI-SCr72h are met)
- Time to AKI-SCr7d
- 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) ≤ 0.3 $(ng/ml)^2/1000$
- Proportion of subjects with renal recovery, defined as the last SCr value before hospital discharge equal to or lower than that at baseline.
- Time to ventilator weaning, defined as the time from T0 to extubation (i.e., removal of the endotracheal ventilation tube)
- Acute physiology and chronic health evaluation-II up to day 4 (if the subject is on the ICU)
- Number of readmissions to ICU
- Number of readmissions to hospital
- Changes from presurgery baseline in SCr, S-cystatin-C and other biomarkers in blood and urine through 72 hours after T0.
- 5-level European Quality of Life 5 Dimensions Questionnaire (EQ-5D-5L) on presurgery baseline, visit 8, day 30 and day 90.

Safety:

- Nature, frequency and severity of AEs
- Vital signs
- Safety laboratory tests: biochemistry, hematology and urinalysis

Pharmacokinetics:

- Expression ASP1128 plasma concentrations

Pharmacodynamics:

- Target gene expression

Statistical Methods

Sample Size Justification:

Sample Size: 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 following:

- 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 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.

Primary Efficacy Analysis:

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 (< 45, ≥ 45) on the Full Analysis Set (FAS). The FAS is defined as all randomized subjects who receives at least 1 dose of study treatment (ASP1128 or placebo), and the analysis is based on the randomized treatment. The Mantel-Haenszel (MH) estimate of the common risk ratio and 2-sided 90% confidence interval will be calculated.

The hypothesis testing on the primary analysis will be performed at 2-sided 0.10 significance level to test the null hypothesis that AKI-SCr72h proportion is equal between the 2 treatment arms versus the alternative hypothesis that AKI-SCr72h proportion is different between the ASP1128 arm vs the placebo arm.

The supplementary analysis for the primary efficacy endpoint will be performed on the Per Protocol Set, which includes all subjects in the FAS and do not have any major protocol deviations.

As sensitivity analysis, chi-square test will be performed to evaluate the appropriateness of CMH test with strata to control for baseline eGFR (< 45, ≥ 45). The estimate of the unadjusted risk ratio and 2-sided 90% confidence interval will be calculated.

Additional sensitivity analysis 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.

Secondary Efficacy Analysis:

The secondary efficacy endpoints proportions (AKI-SCr7d, AKI-KDIGO72h, AKI-KDIGO7d, MAKE30, MAKE90) will be analyzed using the stratified CMH test with strata to control for baseline eGFR (< 45, ≥ 45) on the FAS. The hypothesis testing will be performed at 2-sided 0.10 significance level. The MH estimate of the 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 (< 45, ≥ 45). 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 MAKE proportions due to any missing data/assessments and any loss to follow-up.

Safety Analyses:

The Safety Analysis Set (SAF) is defined as all subjects who receive at least 1 dose of study treatment (ASP1128 or placebo).

The safety evaluation will be based mainly on AEs, clinical laboratory and vital signs. Descriptive statistics will be used to summarize safety data. All safety data will be summarized by treatment.

All summaries of AEs will include only treatment-emergent events unless otherwise stated. AEs will be categorized by system organ class and preferred term using MedDRA.

Pharmacokinetics Analyses:

Summary statistics (number of subjects, mean, standard deviation, coefficient of variation [CV] and median, minimum, maximum, geometric mean and geometric CV) will be tabulated using the data on plasma concentrations of ASP1128. Standard graph will be produced. Plasma pharmacokinetic parameters for ASP1128 will be summarized using descriptive statistics.

Pharmacodynamics Analyses:

Descriptive statistics will be used to summarize gene expression data in the pharmacodynamics analysis set. These endpoints will be summarized graphically.

Exploratory Analyses:

Descriptive statistics will be used to summarize exploratory efficacy endpoint on the FAS.

The statistical analyses on other exploratory endpoints include:

- CMH test on exploratory endpoints of all-cause mortality, RRT, eGFR reduction proportions, AKI proportions
- Wilcoxon-test for AKI severity, AKI duration, number of hospital days, number of ICU days, number of hospitalization and number of readmissions
- Kaplan-Meier estimator and log-rank test for time to AKI-SCr72h, time to AKI-SCr7d and time to ventilator weaning.

Other Analyses:

The Prognostic Factor Analysis Set (PFAS) is defined as the NC positive subjects, who are randomized to the placebo group, and the NC negative subjects, who are in the observational cohort. The prognostic factors for the followings will be explored and they will be compared to NC test in the PFAS.

- AKI-SCr72h
- AKI-SCr7d
- MAKE30
- MAKE90

Interim Analysis:

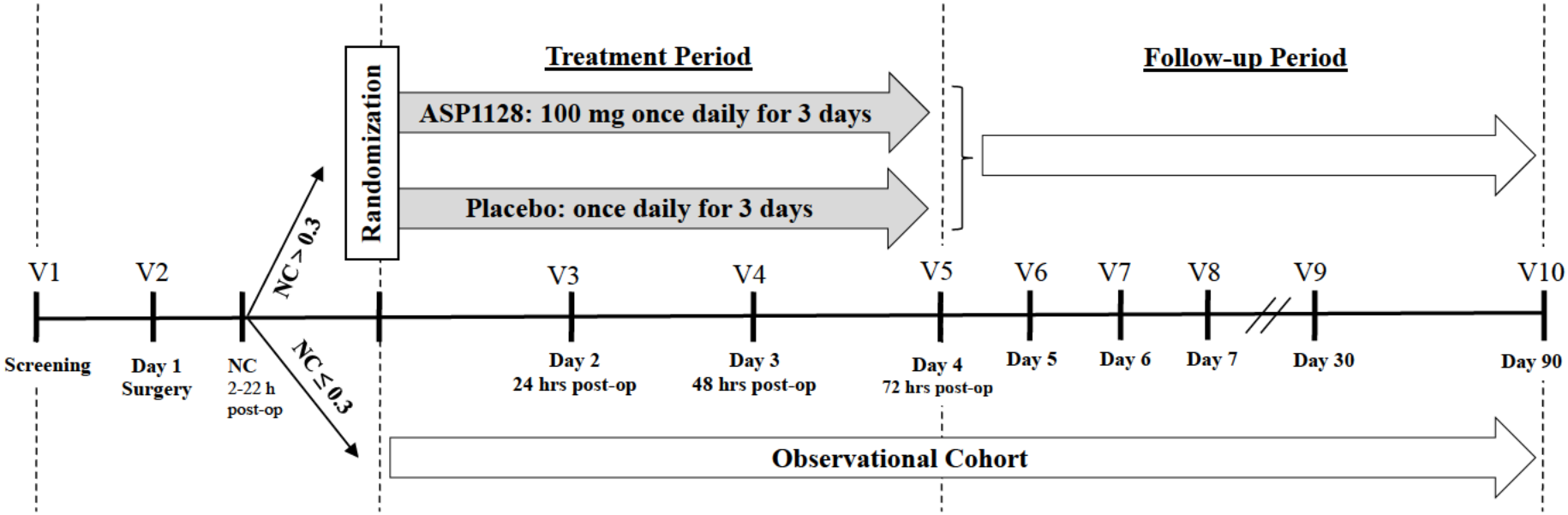
A formal interim analysis for futility is planned when approximately 60% of the planned AKI-SCr72h data have been collected in the study to evaluate whether ASP1128 has particularly poor efficacy compared to placebo while the study is ongoing. When conditional power to detect the difference between ASP1128 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.

V. FLOW CHART AND SCHEDULE OF ASSESSMENTS

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 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 AST, 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.
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									

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; 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 AST, 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.

1 INTRODUCTION

1.1 Background

Acute kidney injury (AKI) is a clinical syndrome characterized by rapid loss of renal function and is associated with increased morbidity and short and long term mortality. AKI occurs as a result of various etiologies, such as sepsis, cardiac surgery, exogenous and endogenous nephrotoxins and others [Bellomo et al, 2012]. Renal ischemia and reperfusion injury is 1 of the most common and significant causes of developing AKI [Bonventre & Yang, 2011]. AKI is associated with tubular dysfunction and as tubular cells rely on oxidative phosphorylation to provide energy, mitochondrial dysfunction has been recognized as a key factor in progression of tubular damage leading to AKI [Ishimoto & Inagi, 2016]. Persistent mitochondrial dysfunction occurs within damaged proximal tubular cells after AKI and may contribute to the sustained injury observed in these renal regions [Hall & Schuh, 2016; Ishimoto & Inagi, 2016]. In nonclinical studies, peroxisome proliferator-activated receptor δ (PPARδ) modulators have been demonstrated to increase gene expression of mitochondrial associated genes resulting in an increase in mitochondrial function through increased fatty acid oxidation, as well as decreases in inflammation and fibrosis [Feng et al, 2014; Sahebkar et al, 2014]. The non-clinical pharmacology data generated with ASP1128 show that it prevents or reduces the impact of surgery-induced AKI by increasing expression of PPARδ target including mitochondrial function-related genes in the kidney. As has been shown in the rat AKI model, these effects are associated with improved renal injury and function parameters. Therefore, PPARδ modulators may show beneficial effects on AKI.

Various definitions of AKI are used in the literature, but the most widely used are described in the 2012 Kidney Disease Improving Global Outcomes (KDIGO) guidelines [KDIGO, 2012] [see also Section 2.3 Endpoints]. Approximately 2 million major cardiovascular operations are performed globally per year, and AKI occurs often in this subject population. AKI stages 1 to 3 occur in 22.3% and an estimated 1.6% to 5.8% develop AKI that requires dialysis and has an overall mortality of 30% [Hu, 2016]. A large cohort study of 4466 subjects undergoing coronary artery bypass grafting (CABG) and/or valve surgery showed a similar occurrence of AKI: of all AKI 20.1%, and in those with chronic kidney failure (CKD) at baseline, 34.1% were reported to have AKI after surgery [Billings et al, 2016].

Cardiovascular surgery is associated with significant systemic ischemia and reperfusion because of the nature or the surgical processes: clamping of the aorta and the use of cardio-pulmonary bypass pump (CPB) and general low systemic blood flow will cause systemic visceral ischemia, while the restoration of the circulation at the end of the surgery (i.e., when the subject is taken off of the CPB) will induce reperfusion of the ischemic viscera. Various factors contribute to the development of AKI following cardiovascular surgery. These include the use of nephrotoxic agents (e.g., aminoglycoside antibiotics, radio-contrast), renal hypoxia (e.g., catecholamine release, emboli), hemo-dilution, toxins released after blood cell injury (e.g., heme pigments, catalytic iron), oxidative stress, and inflammation [Fuhrman & Kellum, 2017; Thiele et al, 2015].

So far, no specific medical treatment for AKI has been established. Primary treatment of AKI consists of management of blood pressure and cardiac output, which require careful titration of fluids and vasoactive medication. Vasopressors can further reduce blood flow to the tissues if there is insufficient circulating blood volume. Conversely, subjects with AKI are also at increased risk for fluid overload and continued fluid resuscitation despite increased intravascular volume can cause harm. Fluids and vasoactive medications should be managed carefully and in concert with hemodynamic monitoring. Available therapies to manage hypotension include fluids, vasopressors and protocols, which integrate these therapies with hemodynamic goals [KDIGO, 2012].

Renal replacement therapy (RRT) might play a key role in the treatment of critically ill subjects with AKI. Several clinical issues associated with RRT for AKI are yet to be solved. There is some consensus regarding the most appropriate RRT modality (intermittent or continuous RRT) and the optimal RRT dose for AKI. No definitive conclusions have been reached concerning the optimal timing of the initiation and discontinuation of RRT, the use of anticoagulants during RRT, and the most suitable dialysis membranes for AKI. Further investigations are needed to improve the morbidity and mortality rate of subjects with AKI [Negi et al, 2016].

ASP1128 (also known as MA-0217) is a new molecular entity and a potent and highly selective PPARδ modulator.

Peroxisome proliferator activated receptors (PPARs) act as transcription factors when activated and modulate (increase or decrease) gene transcription [Lee & Kim, 2015]. Activated PPAR protein complexes bind to a variety of transcription factors in combinations that regulate expression of specific sets of genes. In this way, many functions are regulated, such as fatty acid uptake and oxidation, cell proliferation and differentiation, lipid metabolism and inflammation [Takahashi et al, 2007]. Of the 3 PPAR isoforms (PPARα, PPARδ and PPARγ), PPARδ is most ubiquitously expressed, and is detected in a variety of tissues/cells in many systems including cardiovascular, urinary, respiratory, digestive, endocrine, nervous, hematopoietic, immune, musculoskeletal, sensory and reproductive organ systems [Higashiyama et al, 2007].

PPARδ is naturally activated by fatty acid ligands such as long-chain fatty acids, prostacyclin, lipoprotein lipase and very low-density lipoprotein [Takahashi et al, 2007]. As such, PPARδ expression levels are highest in metabolically active tissues such as skeletal muscle, liver and adipose tissue [Kleiner et al, 2009].

ASP1128 is being developed to prevent AKI or reduce its severity in subjects who are at increased risk of developing moderate to severe AKI after CABG and/or valve surgery. ASP1128 is believed to have protective effects on kidney cells that are under cellular stress as a result of ischemia, inflammation and oxidative stress following CABG and/or valve surgery. In addition, ASP1128 will reduce inflammatory responses and increased oxidative stress systemically, which is expected to reduce the immediate consequences of stress responses following CABG and/or valve surgery. Because injury occurs at a discrete point in time (i.e., after CPB is ended) in these subjects the timing of interventional treatment can be

planned and monitored accurately. This way treatment with ASP1128 can be timed optimally to provide efficacy in this subject population.

The phase 1 combined single and multiple ascending intravenous dose study was conducted to evaluate the safety, tolerability, pharmacokinetic and pharmacodynamics of intravenously administered ASP1128 in healthy adult and healthy elderly subjects [Study 1128-CL-0101]. Additionally, the drug was shown to be safe and well tolerated.

The present clinical study is a phase 2a proof of concept, double-blind, randomized, placebo-controlled study to evaluate the efficacy of ASP1128 in subjects at risk for AKI following CABG and/or valve surgery.

1.2 Nonclinical and Clinical Data

1.2.1 Nonclinical Data

Key nonclinical data are summarized in this section. A detailed summary of nonclinical data is found in the Investigator's Brochure (IB).

Unless stated otherwise in this summary of nonclinical data, all administrations in in vivo studies were intravenous.

ASP1128 is a potent and selective PPARδ modulator with a half-maximal effective concentration of 5.9 and 158 nmol/L against human and rat PPARδ, respectively. For human, selectivity for PPARδ over PPARα and PPARγ was more than 20000-fold. Selectivity for the rat has not been determined. Potency and selectivity in monkey could not be determined due to limitations of species-specific tools available.

ASP1128 (10 µmol/L, 4.61 µg/mL) was tested for affinity against a panel of receptors, ion channels, cytochrome P450 (CYP) enzymes and transporters. ASP1128 did not result in more than 50% inhibition in any of the targets tested.

The efficacy of ASP1128 in AKI was tested in a rat ischemia reperfusion injury model with intravenous treatment starting 4 hours post reperfusion. ASP1128 was effective in inhibiting ischemia reperfusion injury effects on renal function and kidney injury endpoints at a dose of 0.3 mg/kg.

Gene expression of PPARδ responsive genes in both blood and kidney tissue has been used to determine target engagement of ASP1128 in vivo. ASP1128 administered intravenously to normal rats at doses of 0.3, 3 and 10 mg/kg induced PPARδ target genes in blood and kidney tissue between 1 and 12 hours following administration at all dose levels.

Plasma protein binding of ASP1128 at 10 µmol/L was 96.1%, 98.1%, 97.3% and 99.4% in mice, rats, monkeys and humans, respectively.

No human specific ASP1128 metabolites were formed by human hepatocytes. The main metabolites were estimated to be a glucuronide of ASP1128 and a metabolite formed via ring opening of the methylimidazole followed by acetylation and deimination.

ASP1128 did not show inhibitory potential for CYP metabolizing enzymes in vitro. Preliminary data suggest a weak CYP1A2 induction potential.

Two Good Laboratory Practice (GLP) 2-week toxicity studies were conducted: 1 in monkeys and the other in rats.

In the GLP 2-week toxicity study in monkeys, ASP1128 was administered by intravenous bolus injection at doses of 0, 1, 10 and 60 mg/kg per day. At a dose of 60 mg/kg (1.5 mL/kg body weight at 40 mg/mL), several clinical signs including decreased activity, abnormal posture, ataxia (once in 1 animal), tremor, twitching, rapid breathing and salivation were observed shortly after dose administration and had resolved within 1 hour postdose. During the second study week of the 2-week toxicity study in monkeys, clinical signs including excessive struggling, vocalizing and salivation were observed during dose administration of 60 mg/kg and had resolved within 1 hour postdose. Despite trying different veins for dose administration, and decreasing the formulation concentrations (down to 20 mg/mL), these clinical signs returned upon each dose administration. For ethical reasons, it was decided to stop dosing the animals on days 9 or 10. Subsequent histopathological and clinical chemistry assessments did not reveal abnormalities in any organ, including the central nervous system. At doses of 1 and 10 mg/kg, no effects were observed. The no observed adverse effect level (NOAEL) was 10 mg/kg. In the GLP 2-week toxicity study in rats, ASP1128 was administered at doses of 0, 3, 10 and 30 mg/kg per day. No adverse finding was observed in any examination and the NOAEL was 30 mg/kg.

A cardiovascular safety pharmacology study was conducted in monkeys at doses of 0, 10, 30 and 60 mg/kg. At doses from 10 to 60 mg/kg, rapid and dose related increases in arterial blood pressure ranging from 22% to 35% were observed and had resolved within 1 hour postdose. At a dose of 60 mg/kg, an increase in heart rate of 29% was observed and had also resolved within 1 hour postdose. No effect of ASP1128 on QT or corrected QT interval was observed.

In human ether à go go related gene (hERG) transfected HEK293 cells, the hERG current was inhibited up to 22.3% and 21.0% at 100 and 300 µM, respectively.

ASP1128 did not have any effect in neurobehavioral tests conducted as part of the 2-week repeated dose toxicity study in rats up to the highest tested dose of 30 mg/kg per day.

A safety pharmacology study on the respiratory system in monkeys did not reveal any effect of ASP1128 on respiratory parameters up to doses of 60 mg/kg administered by intravenous bolus injections.

ASP1128 did not reveal any genotoxic potential in the AMES test up to 5000 µg/plate. In the in vitro chromosomal aberration test in human peripheral blood lymphocytes, ASP1128 was positive for the induction of structural chromosomal aberrations in the presence of an exogenous metabolic activation, and ASP1128 was negative for the induction of structural chromosomal aberrations in the absence of the exogenous metabolic activation system and negative for the induction of numerical chromosomal aberrations under all test conditions. In an in vivo peripheral blood micronucleus assay in rats, ASP1128 at intravenous doses up to

60 mg/kg per day was considered to be negative for clastogenic activity and/or disruption of the mitotic apparatus. In the in vivo comet assay in rats, ASP1128 at intravenous doses up to 60 mg/kg was considered to be negative for induction of DNA damage in the liver. Overall and in conclusion, even though a positive result was noted in 1 in vitro study (chromosomal aberration test), the negative results in 2 appropriate in vivo studies (micronucleus assay and comet assay) are considered sufficient to demonstrate absence of genotoxic risk of ASP1128 in accordance with the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline S2 (R1).

The results of the in vitro phototoxicity test with cultured mammalian cells (Balb/c 3T3 cells) indicate absence of phototoxicity of ASP1128. ASP1128 did not cause hemolysis or flocculation in human peripheral blood up to 1000 μg/mL.

In a preliminary non-GLP 5-day toxicity study in rats, ASP1128 was administered as intravenous bolus injections at a dose of 100 mg/kg. Dosing was stopped after the second dose because of injection site reactions (i.e., minimal to moderate congestion and vasodilatation, mild edema, mild to moderate hemorrhage and minimal to moderate inflammation). These injection site reactions suggest that ASP1128 has the potential to cause local irritation. No local irritation was observed in any other toxicity study.

1.2.2 Clinical Data

Study 1128-CL-0101 is a first-in-human phase 1 randomized, placebo-controlled, combined single and multiple ascending intravenous dose study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of ASP1128 in healthy adult subjects and healthy elderly subjects. Preliminary results of 1128-CL-0101 study are summarized in this section. For detailed information on the study, please refer to the IB.

Part 1 Single Ascending Dose:

A total of 49 healthy adult subjects have been enrolled into the 6 dose cohorts ranging from 0.3 to 100 mg. ASP1128 was well tolerated in healthy adult subjects after a single intravenous administration. All of treatment- emergent adverse events (TEAEs) were mild. The most commonly reported TEAEs regarded to be related to study drug was feeling hot.

Increase in dose from 0.3 to 100 mg seemed dose proportional for C_{max}, and a little more than dose proportional for AUC_{24} with a low to moderate between-subject variability. The $t_{1/2}$ for 3 mg and higher dose groups ranged from 8.92 to 16.7 hours.

ASP1128 at dose levels of 10 mg or higher showed consistent, treatment- and dose-dependent up-regulation of the PPARδ target genes in the whole blood cell. The gene set test revealed statistical significant result for 10, 30 and 100 mg dose levels.

Part 2 Multiple Ascending Dose:

A total of 48 healthy adult subjects have been enrolled into the 4 dose cohorts ranging from 3 to 100 mg and 5 healthy elderly subjects have been enrolled into the 1 dose cohort (100 mg). ASP1128 was well tolerated in healthy adult subjects and healthy elderly subjects

after a once daily intravenous administration for 7 days. All of TEAEs were mild. The most commonly reported TEAEs regarded to be related to study drug was infusion site reaction.

Increase in dose from 3 to 100 mg seemed dose proportional for C_{max}, and AUC_{tau} with a low between-subject variability. The $t_{1/2}$ ranged from 8.75 to 17.4 hours. R_{ac} (AUC)s and PTRs were consistent across the dose range of 3 to 100 mg ASP1128. C_{max} and AUC_{tau} were slightly higher in elderly relative to nonelderly subjects.

ASP1128 at dose levels of 10 mg or higher showed consistent, treatment- and dose-dependent up-regulation of the PPARδ target genes in the whole blood cell. The gene set test revealed statistical significant result for 10, 30 and 100 mg dose levels.

1.3 Summary of Key Safety Information for Study Drugs

ASP1128 showed proof of principle in animal studies for the applicability of the modulation of the PPARδ effect (i.e., improved mitochondrial function, inflammation and fibrosis) to ameliorate and prevent AKI.

There was no evidence for the formation of human-specific metabolites and no other critical absorption, distribution, metabolism and elimination or excretion issues that would prevent conducting clinical studies.

The main toxicity findings from the safety pharmacology and toxicity studies of ASP1128 were hemodynamic effects (short-lived increases in blood pressure and heart rate), clinical signs at high dose (60 mg/kg per day) and potential local irritation. The clinical signs observed at 60 mg/kg in the monkey 14-day toxicity study included excessive struggling, vocalizing, salivation, decreased activity, abnormal posture, rapid breathing, ataxia, tremors and twitching. They occurred shortly after infusion and are thought to be related to local irritation of the high concentration drug substance during infusion. The exposure margins for the findings are large. These findings were all transient, but will be monitored in the phase 2 clinical study.

Based on historical safety concerns with PPARδ and γ modulators, effects on cardiac and skeletal muscle tissue were assessed in exploratory toxicity studies. At high exposures, minimal degeneration in cardiac and skeletal muscle was observed. These findings were not confirmed in follow up 14-day GLP-toxicity studies. In addition, no changes were observed in markers for cardiac or muscle toxicity (cardiac troponins and creatine kinase [CK]). Although a direct toxic effect of ASP1128 on cardiac and skeletal muscle is considered unlikely, monitoring of vital signs, troponins, CK and CK-isoenzymes were be included in the phase 1 clinical study. These did not show clinically significant changes. Similar monitoring will be continued in the current study.

In the clinical phase 1 study (1128-CL-0101), there were no deaths or SAEs during the study. Drug treatment was discontinued on day 7 in 1 subject who received 3 mg in the multiple dosing cohort. On day 6 the subject reported a TEAE of infusion site erythema (mild of severity and recovering on day 7). The most commonly reported TEAEs regarded to be related to study drug in the single dose part were “feeling hot” (3 subjects 100 mg ASP1128),

and in the multiple dosing part 7 “infusion site reactions” (2 in 3 mg, 1 in 30 mg and 4 in 100 mg ASP1128). Drug-related TEAEs were considered to be mild and monitorable in the clinic, and have proven to be reversible upon stopping of treatment. No TEAEs from other preclinical toxicological targets were observed, except for local irritation (i.e., infusion site reactions, swelling and erythema). However, they were considered mild in severity and occurred on average after 5 days of multiple infusion. It was therefore concluded that peripheral intravenous administration is feasible in the current study. There were no clinically relevant findings in any of the laboratory parameters, vital signs or ECG assessments. No minimally intolerable dose or maximally tolerable dose was identified, because the tolerability of ASP1128 was overall good up to the highest dose (100 mg once daily up to 7 days). In phase 1, there was no need to dose higher than 100 mg, because upregulation of expression of genes related to the mode of action of ASP1128 seemed to stabilize below and around a dose of 100 mg and plasma concentrations of doses higher than 100 mg would approach the exposure limit.

1.4 Risk Benefit Assessment

Based on preclinical data there is a realistic probability that ASP1128 may be efficacious in the treatment of AKI. Therefore, subjects at risk of AKI, for which there currently is no approved drug treatment available, may benefit from treatment with ASP1128. Furthermore, a time and dose-dependent pharmacodynamic effect on expression of genes involved in the mechanism of action of ASP1128 was observed from doses of 10 mg onwards and was maintained during multiple dosing of 7 days. This effect indicates pharmacological effect of ASP1128, which may translate into clinical efficacy in AKI.

For ASP1128, the toxicological target organs and other AEs that were identified at high doses in the nonclinical toxicology studies in animals include hemodynamic effects, clinical signs of distress at high dose and potential local irritation.

Preliminary results of the phase 1 single ascending dose and multiple ascending dose study indicate that doses up to 100 mg once daily (single and up to 7 days multiple dosing) are safe and well tolerated in non-elderly and elderly healthy volunteers. However, in the current study subjects might experience AEs that may be related to the study drug or to procedural complications (e.g., infusion site reactions or inconvenience from blood draws). There are no severe side effects expected from the study treatment. Furthermore, for AEs reported in the first in man study, effects were regarded to be monitorable and reversible upon stopping of treatment.

Subjects randomized to the placebo arm are unlikely to benefit from the study while subjects in the non-placebo treatment arm of the study may benefit from reduction of severity of AKI during the study. At randomization subjects have 50% chance of being randomized to either of the arms, but it has to be emphasized that subjects randomized into the placebo group will not be denied any treatment for AKI, related or non-related conditions.

Additionally, enrollment in the study may be seen as an opportunity to contribute to the discovery of an effective drug for this unmet medical need. In general, the subject is free to

withdraw from the study treatment and/or study at any time, which can be done if they experience side effects or inconvenience from participation in the study.

The sponsor considers that the overall benefit-risk ratio to treat patients at risk for AKI is acceptable with a confirmed positive signal on targeted pharmacological effect in phase 1 healthy volunteers and a low risk to subjects participating in current study.

2 STUDY OBJECTIVE(S), DESIGN AND ENDPOINTS

2.1 Study Objective(s)

2.1.1 Primary Objective

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

2.1.2 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

2.1.3 Exploratory Objectives

- 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

2.2 Study Design and Dose Rationale

2.2.1 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.

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
Source: [KDIGO, 2013]

The subject's age at visit 1 should be used for calculation 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, urinary output (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.

Visit 1/Screening

The screening visit is the visit in which the indication for cardiovascular surgery is set, and the surgery will take place within 28 days from this visit. If the subject agrees to take part in the study, informed consent will be obtained at this visit before performing any study related procedures. Subjects will be assessed for presence of inclusion criteria and absence of exclusion criteria before surgery. Medical history and medication use (up to 28 days before screening) will be recorded. Subjects will be clearly instructed that randomization and treatment with investigational compound or placebo will occur after end of surgery and only if the subject qualifies for the NC AKIRisk® score criterion as detailed below.

Double-blind Treatment Period (Visits 2, 3, 4 and 5)

Visit 2/Day of Surgery

Baseline assessments are performed before surgery on the day of surgery, including medication use between screening and day of surgery, safety laboratory tests, kidney function (eGFR, SCr and serum cystatin-C), biomarkers (e.g., kidney injury molecule-1 [KIM1], NC [see Section 5.4.2 Laboratory Assessments, Table 7), vital signs and cardiac function (previous ejection fraction data from recent echocardiogram).

During surgery, various parameters will be recorded (e.g., incision time, CPB time, volume and type of blood products and infused fluids, UO and total blood loss). End of surgery for this study protocol is referred to as T0, which is defined as the time point when the subject comes off the CPB (i.e., the final separation from CPB and normal circulation is restored). This is thought to be the time point when the ischemia-reperfusion state starts that mediated the development of tubular damage and AKI. This time point is recorded by the perfusionist in the subject chart.

Four hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0) a urine sample will be collected and the concentration of tissue inhibitor of metalloproteinase-2 (TIMP2) and insulin-like growth factor binding protein-7 (IGFBP7) will be assessed utilizing the NC device as per the package insert instructions. The urine sample should be fresh (i.e., not from accumulated urine in the bag so the bag should be emptied before the sample is taken); it can be obtained in the operating room/theater, recovery room or in the intensive care unit (ICU), as long as it is within the range of 2 to 6 hours post T0. For urine [TIMP-2] *

[IGFBP7], a cutoff of 0.3 yielded good sensitivity and specificity at 4 hours after CPB [Meersch et al, 2014] and constituted an increased risk for developing AKI [Meersch et al, 2017]. The NC device will calculate the AKIRisk score. If NC is positive, i.e., the result of the AKIRisk score is > 0.3 $(ng/ml)^2/1000$, the subject will be randomized and start treatment within 8 hours after T0 as per randomization schedule.

If the first result of the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ the NC assessment should be redone once only if within the 2- to 6-hour window after T0 (i.e., the urine sample for NC should be taken within the 2 to 6 hours post T0 range).

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ (at first and second measurement, if applicable) or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated up to 4 times until 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour. If all the NC assessments are negative up to 22 hours after T0, the subject cannot be randomized and will be followed up in the observational cohort (see below).

Randomized subjects will receive 3 subsequent treatments with ASP1128 or matching placebo, which will be applied intravenously through a peripheral/central catheter. The first administration of study drug will occur after randomization, as soon as possible after the positive NC. If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP should be completed within 8 hours after T0. If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but within 24 hours after T0.

In case of reoperation on the day of surgery (e.g., for immediate surgical complications like ongoing hemorrhage), the same time range for NC and drug administration applies in relation to T0 (i.e., NC between 2 and 22 hours and first investigational drug administration within 24 hours). A reoperation is no reason for not enrolling a subject as long as the NC can be performed at the described time points. If for any reason the AKIRisk score cannot be assessed within the 2 to 22 hours from T0, the subject cannot be randomized and will be a screen failure. If the subject is randomized, but for any reason the first administration of the study drug cannot be done within 24 hours after T0, the subject should not be withdrawn from the study as an intention-to-treat principle. The indication of reoperation will be recorded as an adverse event (AE) in the electronic case report form (eCRF) as well as the (reason for) missed drug administration.

Study drug will need to be prepared for infusion by the study staff in the ICU. A detailed instruction is described in the pharmacy manual. The pH of the solution for injection is around 10 and there is a minor risk for irritation at the infusion site. Based on the results of the phase 1 study in healthy subjects it was determined that administration via a central line is allowed, but not required. The study drug can also be infused in a peripheral line on the forearm. The chosen peripheral line has to be reserved for study drug administration to avoid physicochemical interaction. If subjects have a central line this can be used for administration of study drug provided that 1 of the lumina is reserved for study drug in order to avoid physicochemical interactions.

After surgery, UO will be monitored, safety assessments will be done and laboratory tests (including NC, serum creatinine (SCr; cystatin-C and other biomarkers) will be performed as per the Schedule of Assessments [Table 1].

Visit 3, 4 and 5

It is expected that the subject will leave the ICU within 12 to 48 hours after surgery, but will remain in the hospital. Drug administration may be done via a central line in the ICU; however, this central line may have to be removed when the subject leaves the ICU. In this case, the study drug can also be infused in a peripheral line on the forearm. The chosen peripheral line has to be reserved for study drug administration to avoid physicochemical interaction. Please note that blood samples for pharmacokinetic measurements should be taken from a different location than the location of the peripheral intravenous device used for drug administration. A detailed instruction on drug preparation and administration is described in the pharmacy manual.

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. Applicable assessments will occur as per the Schedule of Assessments [Table 1] up to 72 hours after T0 (visit 5) to assess the study endpoints, including AKI (based on SCr and UO). When the subject is in the ICU, UO per 12 hours and total fluid intake per 24 hours (including blood products and intravenous fluid input) must be recorded.

In case of reoperation, or any other event that interferes with study drug administration, at the time of second or third administration of study drug, the subject will not be discontinued. A window of 2 hours before and after the designated time is allowed. The indication of reoperation will be recorded as an AE in the eCRF, as well as the (reason for) missed drug administration or changed drug administration time if applicable.

When the subjects are in the ICU the time to ventilator weaning (i.e., the time from T0 to extubation) will be recorded.

Follow up

It is assumed that the subjects will be in the hospital up to at least 1 week after the surgery. On days 5, 6 and 7 after T0 (visits 6, 7 and 8, respectively), applicable assessments will be performed to assess study endpoints, including AKI (based on SCr). After end of all the procedures of visit 5, subjects can be discharged at the discretion of the investigator. If the subject is discharged 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 the subject is discharged after day 7, visit 8 will be done on day 7 and no additional assessments are required after day 7 up to discharge, but the last result of SCr before discharge from local laboratory testing will be collected. Following day 7 (visit 8) or discharge, subjects will be followed up on day 30 (visit 9) and 90 (visit 10) following surgery. On visits 9 and 10 a composite endpoint of major adverse

kidney events (MAKE), including all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR will be assessed.

Total number of hospital days and ICU days during initial hospitalization will be recorded. If the subjects are readmitted to hospital for any reason during the 90 follow-up days, the total number of hospitalizations and number of days hospitalized through day 30 and 90 will be recorded.

Observational Cohort

If at randomization NC is negative (AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ at all assessments between 2 and 22 hours after T0), the subject cannot be randomized. These subjects will be included in the observational cohort. The observational cohort will enroll a maximum of 440 subjects. The aim of following up the subjects in the observational cohort is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. Clinical endpoints will be investigated in the subgroups defined by AKIRisk score at randomization and other baseline risk factors, in the population of the NC positive subjects, who are randomized to the active or the placebo group, and the NC negative subjects, who are in the observational cohort. The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (acute kidney injury based on serum creatinine KDIGO criteria [AKI-SCr] using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Hospital and ICU stay will also be recorded. AEs and medication use will be recorded until 24 hours after T0. Urine samples will be collected for NephroCheck® (NC) and biomarker assessments. If available, local laboratory test results and 24-hour UO data will be collected from medical records. No additional blood samples will be taken for study purposes after visit 2. See Schedule of Assessments [Table 2].

2.2.2 Dose Rationale

A dose of 100 mg once daily was selected for this proof-of-concept study. This dose was considered safe and well-tolerated in the phase 1 study 1128-CL-0101 with up to 7 days multiple dosing in healthy non-elderly and elderly subjects.

Pharmacokinetics of ASP1128 (C_{max} and AUC_{24}) increased dose proportionally ranging from 3 mg to 100 mg in multiple doses in healthy non-elderly subjects. Because of the limited exposure data in the elderly subjects (n = 3) an age difference (higher exposure in elderly) could not be ruled out. In both elderly and non-elderly plasma exposure of 100 mg was well-balanced vs exposure limit set on non-clinical adverse local irritation effects.

PPARδ target gene up-regulation demonstrated consistently and dose dependent increase. The gene set test reveals statistical significant result for 100 mg at all-time points in single and multiple dosing. Some genes showed saturated dose-responses at higher dose levels, i.e., no major increments from 30 mg to 100 mg in a single and/or multiple doses. However, some genes showed more extensive up-regulation at 100 mg. At 100 mg a more consistent effect was seen, also in “saturated” genes (i.e., earlier onset, more protracted effect).

Similar PPARδ target engagement patterns and saturation effects were observed non-clinically. In animal AKI efficacy models, dosing above the saturation point did not increase efficacy: 100 mg is suspected to be at this tipping point, so no higher dose proposed.

Study treatment will be administered 3 times as follows:

- As soon as possible after the NC AKIRisk score is assessed between 2 and 22 hours after T0 is > 0.3 $(ng/mL)^2/1000$. If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP should be completed within 8 hours after T0. If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but 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.
- For the second and third dose, a 2-hour treatment window before or after designated administration time is allowed.

2.3 Endpoints

The current study will focus on assessing the emergence of AKI. AKI can be defined in various different ways, but in this study AKI KDIGO stage determination criteria are utilized [KDIGO, 2012].

AKI is defined as any of the following:

- Increase in SCr by ≥ 0.3 mg/dl (≥ 26.5 μmol/L) within 48 hours
- Increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days or
- UO < 0.5 ml/kg per hour for 6 hours or more

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

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(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

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.

AKI severity is categorized in 3 stages based on either SCr or UO [KDIGO, 2012]:

Table 3 Staging of AKI

Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline OR ⩾0.3 mg/dl (⩾26.5 μmol/l) increase	<0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ⩾12 hours
3	3.0 times baseline OR Increase in serum creatinine to ⩾4.0 mg/dl (⩾353.6 μmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	<0.3 ml/kg/h for ⩾24 hours OR Anuria for ⩾12 hours

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

Various derivations of these definitions are used as efficacy outcome parameters in the current study.

A retrospective analysis of a large cohort database (HiDenIC15) inducing more than 120000 critically and 6610 subjects undergoing cardiovascular surgery was performed in cooperation with Pittsburgh University. One finding was that subjects who had isolated mild to moderate oliguria (no increase in creatinine) postoperatively were not likely to develop clinically significant related MAKE. Therefore, it was concluded that stage 1 AKI based on UO was not clinically relevant, and data on UO for 12 hours, not 6 hours, will be recorded in the current study to be able to analyze stage 2 and 3 AKI-UO.

2.3.1 Primary Endpoints

Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline (i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 μmol/L] within any 48 hours, or increase in SCr to ≥ 1.5 times baseline) within 72 hours after T0 (AKI-SCr72h).

2.3.2 Secondary Endpoints

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 7 days after T0 (AKI-SCr7d)
- Proportion of subjects developing AKI based on all captured criteria from the KDIGO guideline (i.e., AKI-SCr stage 1 to 3: increase in SCr ≥ 0.3 mg/dl [≥ 26.5 µmol/L] within any 48 hours, increase in SCr to ≥ 1.5 times baseline, and/or AKI-UO stage 2 and 3: urine volume < 0.5 ml/kg per hour for 12 consecutive hours) within 72 hours after T0 (AKI-KDIGO72h)
- Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 7 days after T0 (AKI-KDIGO7d)
- Proportion of subjects with MAKE defined as all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR based on SCr within 30 days after day of surgery (MAKE30). Sustained loss of renal function is defined as a reduction of kidney function (i.e., a reduction of eGFR of 25% or more compared to the baseline presurgery sample at visit 2) at the time of assessment (i.e., at day 30)
- 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)

2.3.3 Exploratory Endpoints

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline within 5 days after T0 (AKI-SCr5d)
- Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 5 days after T0 (AKI-KDIGO5d)
- All-cause mortality at day 30
- All-cause mortality at day 90
- Number of subjects needing RRT at day 30
- Number of subjects needing RRT at day 90
- Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 30
- Number of subjects with ≥ 25% sustained reduction in eGFR based on SCr at day 90
- Number of subjects with ≥ 25% sustained reduction in eGFR based on cystatin-C at day 30
- Number of subjects with ≥ 25% sustained reduction in eGFR based on cystatin-C at day 90
- 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
- Alternative derivations of proportion of subjects developing AKI using different definitions (see Table 3) within 72 hours after T0:
  - All stages AKI-UO

  - AKI-UO stage 3
  - AKI-SCr stage 2 or 3
  - AKI-SCr stage 2 or 3 and AKI-UO stage 3
  - AKI based on S-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
- Severity of AKI within 72 hours after T0
  - severity of AKI based on AKI-SCr stages
  - severity of AKI based on AKI-UO stages
  - severity of AKI based on most severe of AKI-SCr or AKI-UO stages
- Severity of AKI (based on AKI stage using SCr and/or UO) within 7 days after T0
- 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 investigator or staff should enter the date when respective criteria of AKI-SCRr72h were resolved in the eCRF.
  - Duration of AKI-SCr7d (definition as above)
- Time to AKI-SCr72h (i.e., the time from T0 to the time when criteria for AKI-SCr72h are met)
- Time to AKI-SCr7d
- 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) ≤ 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
- Time to ventilator weaning, defined as the time from T0 to extubation (i.e., removal of the endotracheal ventilation tube)
- Acute physiology and chronic health evaluation II (APACHE-II) up to day 4 (if the subject is on the ICU)
- Number of readmissions to ICU
- Number of readmissions to hospital

- Changes from presurgery baseline in SCr, S-cystatin-C and other biomarkers in blood and urine through 72 hours after T0
- 5-level European Quality of Life 5 Dimensions Questionnaire (EQ-5D-5L) on presurgery baseline, visit 8, day 30 and day 90

2.3.4 Safety

- Nature, frequency and severity of AEs
- Vital signs
- Safety laboratory tests: biochemistry, hematology and urinalysis

2.3.5 Pharmacokinetics

- ASP1128 plasma concentrations

2.3.6 Pharmacodynamics

- Target gene expression

3 STUDY POPULATION

3.1 Selection of Study Population

Subjects undergoing CABG and/or valve surgery who have a moderate or high risk for developing AKI-SCr postsurgery.

3.2 Inclusion Criteria

Subject is eligible for the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication).
2. Subject agrees not to participate in another interventional study after signing the informed consent form (ICF) and until the end of study (EoS) visit has been completed.
3. Subject is ≥ 35 years of age at time of screening (visit 1).
4. Subject undergoing non-emergent open chest cardiovascular surgery with use of CPB (i.e., CABG and/or valve surgery [including aortic root and ascending aorta surgery, without circulatory arrest]) within 4 weeks of screening (visit 1).
5. Subject is at risk of developing AKI following cardiovascular surgery, i.e., has 1 or more of the following AKI risk factors:
    - Age at screening of ≥ 70 years
    - Documented history of eGFR < 60 mL/min per 1.73 m^2 as per Modification of Diet in Renal Disease Study (MDRD) or CKD-EPI equation (or documented measured GFR assessment)

  - History needs to be present for at least 90 days prior to screening. Both SCr and eGFR need to be documented in the chart, and
  - eGFR at screening or baseline needs to be < 60 mL/min per 1.73 m^2, as well as per CKD-EPI equation.
- Documented history of congestive heart failure requiring hospitalization. This condition should exist for at least 90 days prior to screening.
- Documented history of diabetes mellitus (type 1 or 2) of ≥ 90 days prior to screening, and subject is on active antidiabetic medication treatment for ≥ 90 days.
- Documented history of proteinuria/albuminuria at any time point before screening
  - Urinary dipstick result of ≥ 2+, OR
  - Documented urinary albumin creatinine ratio measurement of ≥ 300 mg/g, or
  - Documented total quantity of protein in a 24-hour urine collection test ≥ 0.3 g/day.

6. Subject must have the ability, in the opinion of the investigator, and willingness to return for all scheduled visits and perform all assessments.
7. A female subject is eligible to participate if she is not pregnant see [Appendix 12.3 Contraception Requirements] and at least 1 of the following conditions applies:
   - Not a woman of childbearing potential (WOCBP) as defined in [Appendix 12.3 Contraception Requirements]

     OR
   - WOCBP who agrees to follow the contraceptive guidance as defined in [Appendix 12.3 Contraception Requirements] throughout the treatment period and for at least 30 days after the final study drug administration.
8. Female subject must agree not to breastfeed starting at screening and throughout the study period, and for 30 days after the final study drug administration.
9. Female subject must not donate ova starting at screening and throughout the study period, and for 30 days after the final study drug administration.
10. A male subject with female partner(s) of childbearing potential must agree to use contraception as detailed in [Appendix 12.3 Contraception Requirements] during the treatment period and for at least 30 days after the final study drug administration.
11. A male subject must not donate sperm during the treatment period and for at least 30 days after the final study drug administration.
12. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding throughout the study period and for 30 days after the final study drug administration.

3.3 Exclusion Criteria

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

At screening:

1. Subject has received investigational drug within 30 days or 5 half-lives, whichever is longer, prior to screening.
2. Subject has use of RRT within 30 days prior to screening.
3. Subject has CKD stage 4 or 5, or stage 3 (i.e., eGFR 30-59 mL/min per 1.73m^2) with a known history of eGFR < 30 mL/min per 1.73 m^2 as per CKD-EPI or MDRD equation within 6 months prior to screening.
4. Subject has a prior kidney transplantation.
5. Subject has a known or suspected glomerulonephritis (other than Diabetic Kidney Disease).
6. Subject has confirmed or treated endocarditis, or other current active infection requiring antibiotic treatment, within 30 days prior to screening.
7. Subject is using prohibited medications as specified in the concomitant medication section of the protocol [Section 5.1.3.1 Prohibited and Restricted Treatment].
8. Subject has a prior history of intravenous drug abuse within 1 year prior to screening.
9. Subject has a known chronic liver disorder with Child-Pugh B or C classification.
10. Subject has any of the following abnormal liver or kidney function parameters as assessed at screening. (If the results from the central laboratory are not yet available at time of randomization, results from local laboratory within 7 days before the surgery can be used for verifying this criterion. The subject will not be excluded from the study if central laboratory results from screening are exclusionary and are available only after randomization.):
    - Alanine aminotransferase (ALT), aspartate aminotransferase (AST) to > 2 times the upper limit of normal (ULN) or bilirubin increased to > 1.5 times the ULN (not due to previously diagnosed Gilbert's syndrome).
    - eGFR < 30 mL/min per 1.73 m^2 as per CKD-EPI equation.
11. Subject has use of left ventricular assist device (LVAD), intra-aortic balloon pump (IABP) or other cardiac devices, or catecholamines within 7 days prior to screening.
12. Subject has surgery scheduled to be performed without CPB (i.e., "Off-Pump" surgery).
13. Subject has surgery scheduled to be performed under conditions of circulatory arrest, including planned deep hypothermic circulatory arrest.
14. Subject has surgery scheduled for aortic dissection.
15. Subject has surgery for a condition that is immediately life-threatening as per the discretion of the investigator.
16. Subject has surgery scheduled to correct major congenital heart defect.

17. Subject has current or previous malignant disease. Subjects with a history of cancer are considered eligible if the subject has undergone therapy and the subject has been considered disease free or progression free for at least 5 years. Subject with completely excised basal cell carcinoma or squamous cell carcinoma of the skin and completely excised cervical cancer in situ are also considered eligible.

Preoperatively at the Day of Surgery:

18. Exclusion criteria 1 to 17 are applicable at this time.
19. Subject has AKI (any stage) present at presurgery baseline at the discretion of the investigator.
20. Subject has known or suspected infection/sepsis at time of presurgery baseline at the discretion of investigator.

Perioperative Exclusion Criteria:

21. Subject requires Extracorporeal Membrane Oxygenation (ECMO) during or after completion of surgery.
22. Subject requires ventricular assist device (VAD) during or after completion of surgery.
23. Subject has surgery performed "Off-Pump" at any time during surgery.

General:

24. Subject has other condition, which, in the investigator's opinion, makes the subject unsuitable for study participation.
25. Female subject who is pregnant or lactating or has a positive pregnancy test within 72 hours prior to screening and/or randomization, has been pregnant within 6 months before screening assessment or breastfeeding within 3 months before screening or who is planning to become pregnant within the total study period.
26. Subject has a known or suspected hypersensitivity to ASP1128 or any components of the formulation used.
27. Subject is an employee of the Astellas Group or the contract research organization (CRO) involved in the study.

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

4 TREATMENT(S)

4.1 Identification of Investigational Product(s)

4.1.1 Study Drug(s)

ASP1128 is a sterile, clear colorless liquid supplied in a single-use vial with a coated rubber stopper and an aluminum cap.

Each vial will contain 10.5 mL formulated solution, including an overfill sufficient to deliver 10 mL of the formulated solution (containing 50 mg ASP1128).

The preparation and administration of ASP1128 will be described in the pharmacy manual. The storage conditions will be described on the product label.

4.1.2 Comparative Drug(s)

Placebo to match (PTM) is a sterile, clear colorless liquid supplied in a single-use vial with a coated rubber stopper and an aluminum cap.

The preparation and administration of PTM will be described in the pharmacy manual. The storage conditions will be described on the product label.

4.2 Packaging and Labeling

All study drugs used in this study will be prepared, packaged and labeled under the responsibility of qualified staff at sponsor's designee in accordance with sponsor's designee Standard Operating Procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, ICH Good Clinical Practices (GCP) guidelines and applicable local laws/regulations.

Each vial will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as investigational drug.

4.3 Study Drug Handling

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

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

Study drug inventory and accountability records will be kept by the investigator or designee. Study drug accountability throughout the study must be documented and reconciled. The following guidelines are therefore pertinent:

- The investigator or designee agrees not to supply study drugs to any persons except the eligible subjects in this study in accordance with the protocol.
- The investigator or designee (i.e., study drug manager) will keep the study drugs in a pharmacy or other locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense these study drugs.
- A study drug inventory will be maintained by the investigator or designee (i.e., study drug manager). The inventory will include details of material received and a clear record of when they were dispensed and to which subject.
- At the conclusion or termination of this study, the investigator or designee (i.e., study drug manager) agrees to conduct a final drug supply inventory and to record the results of this inventory on the Drug Accountability Record. It must be possible to reconcile delivery records with those of used and/or returned study drug. Any discrepancies must

be accounted for and documented. Appropriate forms of deliveries and returns must be signed by the site staff delegated this responsibility.

- The site staff must return study drug to the sponsor or designee at the end of the study or upon expiration unless otherwise approved by the sponsor.

4.4 Blinding

4.4.1 Blinding Method

For the purpose of this study, the efficacy and safety of ASP1128 and placebo will be compared in a double-blind manner. ASP1128 and placebo will be indistinguishable in appearance. Packaging for each treatment group will also be indistinguishable in appearance. The randomization number will be assigned based on information obtained from the interactive response technology (IRT). The randomization code will remain confidential until completion of the study.

4.4.2 Confirmation of the Indistinguishability of the Study Drugs

The appearance and the form of both the drug and packaging of ASP1128 are identical to those of their matching placebo.

4.4.3 Retention of the Assignment Schedule and Procedures for Treatment Code Breaking

The randomization list and study drug blind will be maintained by the IRT system.

4.4.4 Breaking the Treatment Code for Emergency

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

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

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

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

4.4.5 Breaking the Treatment Code by the Sponsor

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

4.5 Assignment and Allocation

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 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 $(ng/mL)^2/1000$ or less will be enrolled in the observational cohort. The observational cohort will enroll a maximum of 440 subjects.

5 TREATMENTS AND EVALUATION

5.1 Dosing and Administration of Study Drug(s) and Other Medication(s)

5.1.1 Dose/Dose Regimen and Administration Period

5.1.1.1 Randomized Subjects

Randomized subjects will receive 3 subsequent treatments with 100 mg of ASP1128 or matching placebo. The study drugs will be supplied as solution in 20 mL vial, which contains 10 mL of 5 mg/mL ASP1128 or 10 mL of matching placebo. The study drugs will be applied intravenously through a peripheral/central catheter. The catheter should remain until the last administration ends. The first administration of study drug will occur after randomization, as soon as possible after positive NC. 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. For the second and third dosing of IP, a 2-hour treatment window before or after the designated administration time is allowed.

The preparation and administration procedures of study drug will be described in the pharmacy manual.

5.1.1.2 Subjects in the Observational Cohort

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ at all assessments between 2 and 22 hours after T0, the subject cannot be randomized. These subjects will be included in the observational cohort and receive no study drug administration.

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

Dose increases and decreases are not allowed.

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

The investigator should record the use of previous (up to 28 days before screening) and concomitant (any medication between screening and surgery, and following surgery to EoS) treatment in the eCRF, both medication and non-medication treatments. Subjects should be instructed not to start any new medication, both prescribed and over-the-counter (including herbals, alternative and homeopathic medicines) or non-medication treatments, without consulting the investigator, unless the new treatment is required for emergency use. In addition, non-medication treatments, including pacemakers, use of LVAD, IABP or other cardiac devices should be reported (also refer to exclusion criterion #11).

Medication Treatment

Prescribed, over-the-counter and all alternative medicines will be recorded. This includes medicines used on a chronic or as needed basis. Previous medicines taken within the 28 days prior to screening (visit 1) will be recorded.

To assess the possible contribution of nephrotoxic drugs to the occurrence of postsurgery AKI all medications taken from screening to day of surgery should be recorded. Special attention should be given to known neprotoxins (e.g., intravenous radio-contrast) and aminoglycosides. Nephrotoxic drugs should be avoided if possible, but will not be prohibited if clinically indicated. Subjects can only be enrolled providing the subject meets the criteria for enrollment, most specifically the subject should not have AKI (any stage) present at presurgery baseline (exclusion criterion #18). If the required clinical use of nephrotoxic medications results in conditions that exclude the subject from study enrollment the subject is not eligible for study participation.

Non-Medication Treatment

During the study non-medication treatment is not prohibited if clinically indicated. Non-medication treatments, including pacemakers, use of LVAD, IABP or other cardiac devices should be reported (also refer to exclusion criterion #11). Previous treatment taken within the 28 days prior to screening (visit 1) will be recorded.

5.1.3.1 Prohibited and Restricted Treatment

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

Prohibited Medications

Medications that may significantly affect plasma creatinine levels without influencing kidney function are prohibited. These medications should be stopped more than 28 days before screening until EoS. They include cimetidine, trimethoprim, pyrimidine analogues or derivatives, phenacemide and calcitriol or alfacalcidol [Andreev, 1999].

Following surgery up to 72 hours postsurgery the use of nonsteroidal anti-inflammatory drugs [NSAIDs], angiotensin-converting enzyme [ACE] inhibitors and angiotensin receptor blockers is prohibited because of their potentially confounding effect on kidney function.

After the subject is enrolled in the observational cohort, these medications are not prohibited if clinically indicated.

Restricted Medications

Restricted medication are medications, which are permitted to be used during the study provided that the subject has been taking the medication on a long-term basis, the subject remains on the medication at the same dose during the course of the double-blind treatment period and the follow-up period. Medication use is not restricted in the current study if clinically indicated. All medication used will be recorded in the eCRF.

Prohibited non-medication treatment (from screening until EoS)

During the study non-medication treatment is not prohibited if clinically indicated.

5.1.4 Treatment Compliance

The dose (prepared and actual) and schedule of the study drugs administered to each subject will be recorded on the appropriate form throughout the treatment period. Reasons for dose delay, reduction or omission will also be recorded.

5.1.5 Restrictions During the Study

Subjects ensure that the investigator will be informed about following events occurring outside the study sites:

- Number of hospitalizations and number of days hospitalized
- Start of RRT
- Death

5.2 Demographics and Baseline Characteristics

Baseline assessments are performed before surgery on the day of surgery, including safety laboratory tests, SCr and serum cystatin-C, biomarkers and vital signs, and will be used as baseline of each endpoint.

During surgery the following parameters will be recorded:

- Procedures (CABG surgery and/or valve surgery, thoracic aortic surgery, ECMO, use of VAD, redo thoracic surgery and indication of redo surgery).
- Duration of CPB (including aortic cross clamp time)

- Lowest intraoperative mean arterial pressure (MAP) (from the time of T0 until the time of leaving the operating room [i.e., entering the ICU])
- Perioperative fluid balance (fluid intake, UO, blood products, drain output and blood loss)
- Medication (including inotropics/vasopressors, furosemide)
- Time of incision and closing of skin
- T0 (i.e., end of CPB)
- Perioperative complications

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 CPB, i.e., the final separation from CPB and normal circulation is restored. This time point is recorded by the perfusionist in the subject chart.

5.2.1 Demographics

Demographic information will be collected for all subjects at screening (visit 1) as allowed per local regulation and will include age, sex, race, ethnicity, history of smoking and history of alcohol and recreational drug use. Ejection fraction value will be collected from the most recent local echocardiography, which was conducted within 90 days before the surgery.

5.2.2 Medical History

Medical history will include all significant medical conditions that have resolved prior to informed consent or are ongoing at the time of consent. Conditions that are ongoing at the time of consent will be collected as baseline conditions. Out of conditions that have resolved prior to informed consent, those can be a risk factor of AKI will be collected. Risk factors as per the KDIGO guideline [KDIGO, 2012] and the data source are described in Table 4. Several AKI risk factors will not be present at the start of the current study because they are exclusion criteria (e.g., sepsis, critical illness, cancer, major non-cardiac surgery), or will not be a significant factor because the study includes non-emergent surgery only (burns, trauma).

Details that will be collected include the onset date and recovery date for resolved conditions (including any prior AKI) and, if applicable for ongoing conditions.

Table 4 Causes of AKI: Exposures and Susceptibilities for Non-specific AKI [KDIGO, 2012] and Additional Risk Factors Identified. Data Source for Capture at Screening or Visit 2

Factors	Data Source
Exposures	
• Circulatory shock	Recorded perioperatively (lowest periop. MAP)
• Cardiovascular surgery (especially with CPB)	Inclusion criterion
• Nephrotoxic drugs	Recorded in previous medication
• Radiocontrast agents	Recorded in previous medication
Susceptibilities	
• Poisonous plants and animals	Recorded in previous medication if applicable
• Dehydration or volume depletion	Recorded perioperatively (fluid balance)
• Advanced age	Recorded in demographics
• Female gender	Recorded in demographics
• Black race	Recorded in demographics
• CKD	Assessed at screening
• Chronic diseases (heart, lung, liver)	Recorded in medical history
• Diabetes mellitus	Recorded in medical history
• Anemia	Assessed at screening
Additional Risk Factors	
• Prior AKI	Recorded in medical history

AKI: acute kidney injury; CKD, chronic kidney disease; CPB, cardiopulmonary bypass; MAP, mean arterial pressure; periop: perioperative.

5.2.3 Diagnosis of the Target Disease, Severity and Duration of Disease

Subjects are eligible for screening if they are scheduled for non-emergent (i.e., non-life threatening) open chest cardiovascular surgery. The surgery is required to include use of CPB (i.e., CABG and/or valve surgery [including aortic root and ascending aorta surgery without circulatory arrest]) within 4 weeks of screening (visit 1).

A risk of developing AKI following surgery will be assessed at screening (visit 1) according to inclusion criteria #5 based on AKI risk factors. Further risk assessment will be done after the surgery at visit 2: 4 hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0) urine will be collected for an examination by the NC to calculate the AKIRisk score, which indicates the risk of tubular damage that could lead to AKI. If the AKIRisk score is > 0.3 $(ng/ml)^2/1000$, the subject will be randomized and start treatment as per randomization schedule. If the AKIRisk score is 0.3 $(ng/mL)^2/1000$ or less, the NC assessment should be redone once only if within the 2- to 6-hour window after T0. If the NC is negative, i.e., the AKIRisk score is 0.3 $(ng/mL)^2/1000$ or less (at first and second measurement, if applicable) or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated up to 4 times until 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour. If all the NC assessments are negative up to 22 hours after T0, the subject cannot be randomized and will be followed up in the observational cohort.

Criteria for diagnosis and severity of AKI are described in [Section 2.3 Endpoints].

5.3 Efficacy, Pharmacokinetic and Pharmacodynamic Assessments

5.3.1 Efficacy Assessments

Laboratory tests and UO will be performed to assess occurrence and severity of AKI. An incidence of MAKE will be assessed until visit 10. MAKE is a composite endpoint including all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR. All-cause mortality over 30 and 90 days and the number of subjects needing RRT over 30 and 90 days will be also assessed. Sustained loss of renal function is defined as a reduction of kidney function (i.e., a reduction of eGFR of 25% or more compared to the baseline presurgery sample at visit 2) assumed to be present longer than 24 hours at the time of assessment, i.e., at day 30 and day 90 following surgery.

The numbers of hospital days and ICU days during initial hospitalization and up to day 30 and 90, respectively, will be also recorded, as well as the number of readmissions to the ICU and hospital.

5.3.1.1 Laboratory Assessments

Biochemistry tests utilized for AKI assessment, SCr and serum cystatin-C, will be done according to the Schedule of Assessments [Table 1] [see also Section 5.4.2 Laboratory Assessments]. SCr and S-cystatin-C data will also be used to calculate the number of subjects with ≥ 25% reduction in eGFR (based on SCr or S-cystatin-C) and proportion of subjects with renal recovery [see Section 2.3.3 Exploratory Endpoints]. If the subject is discharged from hospital after day 7, the last result of SCr before discharge from local laboratory testing will be collected.

5.3.1.2 Urinary Output

For randomized subjects, UO every 12 hours and total fluid intake every 24 hours will be recorded when the subject is in the ICU. During the first 72 hours following surgery (i.e., visit 3 to visit 5) the investigator will 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).

5.3.1.3 Follow-up Parameters

In addition to safety labs, vital signs, AEs and medication use for randomized subjects data on RRT, mortality, ICU stay, total hospital stay and duration of mechanical ventilation (time ventilation weaning, i.e., time from T0 to extubation) will be collected and assessed according to the Schedule of Assessments [Table 1]. Postoperative inotropic/vasopressor use should be registered on the ICU in the medication list. These include phosphodiesterase inhibitors (amrinone, milrinone), antidiuretic hormone analogues (vasopressin), pure alpha adrenergic agonists (phenylephrine) and both natural (epinephrine, dopamine) and synthetic (dobutamine, dopexamine) catecholamines [Williams et al, 2011].

5.3.1.4 Quality of Life

The EQ-5D-5L is an international standardized non-disease specific (i.e., generic) instrument for describing and valuing health status. It is a multidimensional measure of health-related quality of life, capable of being expressed as a single index value and specifically designed to complement other health status measures [EuroQol Group, 1990; Herdman et al, 2011].

The EQ-5D-5L has 5 dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort and Anxiety/Depression. Each dimension has 5 response levels (e.g., no problems, slight problems, moderate problems, severe problems and extreme problems/unable to perform the activity). In addition, it has a visual analogue scale that elicits a self-rating by the respondent of his/her health status.

The EQ-5D-5L will be completed at or after visit 1 up to visit 2 (presurgery), at visit 8 (day 7 or hospital discharge), at visit 9 (day 30) and at visit 10 (day 90).

5.3.1.5 APACHE-II Score

APACHE-II is a prognostic scoring system used to quantify severity-of-disease in adult ICU subjects [Knaus et al, 1985]. An integer score from 0 to 71 is computed based on several measurements; higher scores correspond to more severe disease and a higher risk of death.

To measure severity of disease 12 physiologic variables constitute the Acute Physiology Score (APS; Table 5): pulse rate, mean arterial blood pressure, temperature, respiratory rate or alveolar oxygen partial pressure, alveolar-arterial oxygen gradient, hematocrit, white blood cell count, SCr, serum sodium, serum potassium, pH arterial and Glasgow Coma Scale (GCS; Table 6). The weighted outcomes of these APS parameters are combined with information about previous health status (i.e., Chronic Health points based on recent surgery, history of severe organ insufficiency, immunocompromised state; see below) and baseline age demographics to calculate the overall score (= APS points + Age points + Chronic Health points).

Table 5 APACHE-II Score

Physiologic Variable	High Abnormal Range							Low Abnormal Range	
	+4	**+3**	**+2**	**+1**	**0**	**+1**	**+2**	**+3**	**+4**
Temperature (°C)	≥ 41.0	39.0-40.9		38.5-38.9	36.0-38.4	34.0-35.9	32.0-33.9	30.0-31.9	≤ 29.9
Mean Arterial Pressure (mmHg)	≥ 160	130-159	110-129		70-109		50-69		≤ 49
Heart Rate (ventricular response)	≥ 180	140-179	110-139		70-109		55-69	40-54	≤ 39
Respiratory Rate (non-ventilated or ventilated)	≥ 50	35-49		25-34	12-24	10-11	6-9		≤ 5
Oxygenation: A-a DO_2 or P_aO_2 (mmHg) $FiO_2 \geq 0.5$ record A-a DO_2 $FiO_2 < 0.5$ record only P_aO_2	≥ 500	350-499	200-349		< 200 $P_aO_2 > 70$	P_aO_2 61-70		P_aO_2 55-60	$P_aO_2 < 55$
Arterial pH (preferred) Serum HCO_3 (venous mMol/L) (not preferred, use if no ABGs)	≥ 7.70 ≥ 52	7.60-7.69 41.0-51.9		7.50-7.59 32.0-40.9	7.33-7.49 22.0-31.9		7.25-7.32 18.0-21.9	7.15-7.24 15.0-17.9	< 7.15 < 15.0
Serum sodium (mMol/L)	≥ 180	160-179	155-159	150-154	130-149		120-129	111-119	≤ 110
Serum potassium (mMol/L)	≥ 7.0	6.0-6.9		5.5-5.9	3.5-5.4	3.0-3.4	2.5-2.9		< 2.5
Serum creatinine (mg/100 mL) (Double point score for acute renal failure)	≥ 3.5	2.0-3.4	1.5-1.9		0.6-1.4		< 0.6		
Hematocrit (%)	≥ 60.0		50.0-59.9	46.0-49.9	30.0-45.9		20.0-29.9		< 20.0
White blood count (total/mm^3) (in 1000s)	≥ 40.0		20.0-39.9	15.0-19.9	3.0-14.9		1.0-2.9		< 1.0
Glasgow Coma Scale (GCS) Score = 15 minus actual GCS									
A. Total Acute Physiology Score (APS): Sum of the 12 individual variable points									
B. Age points (years) ≤ 44 = 0; 45-54 = 2; 55-64 = 3; 65-74 = 5; ≥ 75 = 6									
C. Chronic Health Points									
Total APACHE II Score (Sum of A. + B. + C.)									

Source: [Knaus et al, 1985]

The GCS is a neurological scale (ranging from 3 to 15) which aims to give a reliable and objective way of recording the conscious state of a subject for initial as well as subsequent assessment based on eye movement, verbal and motor function categories [Teasdale, 1974]. The GCS is the sum score of the Eye, Verbal and Movement scores [Table 6]. As lower scores indicate worse outcomes, the GCS subtracted from 15 is included in the APACHE-II score.

Table 6 The Glasgow Coma Scale

Category	Score					
	1	**2**	**3**	**4**	**5**	**6**
Eye	Does not open eyes	Opens eyes in response to pain	Opens eyes in response to voice	Opens eyes spontaneously	N/A	N/A
Verbal	Makes no sounds	Makes sounds	Words	Confused, disoriented	Oriented, converses normally	N/A
Motor	Makes no movements	Extension to painful stimuli (decerebrate response)	Abnormal flexion to painful stimuli (decorticate response)	Flexion/ withdrawal to painful stimuli	Localizes to painful stimuli	Obeys commands

N/A: not applicable
Source: [Teasdale & Jennett, 1974].

Chronic Health Points should be added to the total score when the subject has a history of severe organ system insufficiency or is immunocompromised as defined below. Assign points as follows: 5 points for non-operative or emergency postoperative subjects and 2 points for elective postoperative subjects.

Organ insufficiency or immunocompromised state must have been evident prior to this hospital admission and are defined to be present when 1 of the following conditions apply conform to the following criteria:

- Liver biopsy proven cirrhosis and documented portal hypertension; episodes of past upper GI bleeding attributed to portal hypertension; or prior episodes of hepatic failure/encephalopathy/coma.
- Cardiovascular: New York Heart Association Class IV.
- Respiratory: Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction (i.e., unable to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (> 40 mmHg), or respirator dependency.
- Renal: receiving chronic dialysis.
- Immunocompromised: the subject has received therapy that suppresses resistance to infection (e.g., immunosuppression, chemotherapy, radiation, long term or recent high dose steroids) or has a disease that is sufficiently advanced to suppress resistance to infection (e.g., leukemia, lymphoma or acquired immunodeficiency syndrome).

The APACHE-II score will be measured on visit 2 preoperatively and postoperatively during the time of drug administration at visits 2, 3 and 4, and at day 4 (visit 5) if the subject is still on the ICU. Results from local laboratory testing can be used and the results closest to the indicated time points should be recorded in the eCRF.

5.3.2 Pharmacokinetic Assessments

Blood samples will be collected for pharmacokinetic analysis of ASP1128 as indicated in the Schedules of Assessments [Table 1]. Each sample should be collected before a meal as much as possible and fed or fasted state will be recorded in the eCRF. Definition of fasting is at least 10 hours passed after subject last ate. The sample time schedule is as the followings:

- First administration: predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose
- Second administration: at end of infusion
- Third administration: predose and at end of infusion

The actual date and time of pharmacokinetic blood sample collection will be documented in the source. Blood sample collection, handling and storage will be described in the laboratory manual. Blood samples may not be collected from the same arm as where the intravenous infusion will be administered.

5.3.3 Pharmacodynamic Assessments

Blood samples will be collected for the analysis of expression of genes indicating target engagement as indicated in the Schedules of Assessments [Table 1]. Key genes are those that are considered to be pivotal for the efficacy mechanism of action of ASP1128, i.e., genes involved in the regulation of fatty acid oxidation (carnitine palmitoyltransferase 1α, acetyl-coenzyme A acyltransferase 2, pyruvate dehydrogenase lipoamide kinase isozyme 4, ATP binding cassette subfamily A member 1, catalase, acyl-CoA dehydrogenase very long chain and solute carrier family 25 member 20). The sample time schedule is as follows:

- First administration: predose and 2 to 4 hours after study drug administration
- Third administration: predose and 2 to 4 hours after study drug administration

The actual date and time of pharmacodynamic blood and urine sample collection will be documented in the source. Blood and urine sample collection, handling and storage will be described in the laboratory manual.

5.4 Safety Assessment

5.4.1 Vital Signs

Vital signs, including systolic and diastolic blood pressure (mmHg), pulse (beats/minute) and body temperature will be obtained according to the Schedule of Assessments [Table 1] and recorded. All vital sign measures will be obtained with the subject in the sitting or supine position prior to the administration in case on the day of administration of study drug.

5.4.2 Laboratory Assessments

The laboratory tests that will be performed during the conduct of the study are provided in Table 7 Laboratory tests will be performed according to the Schedule of Assessments [Table 1] and sent to a central laboratory for analysis except for the presurgery pregnancy test. Presurgery pregnancy test at the day of surgery will be assessed locally with urine dipstick test.

If the results from the central laboratory testing of AST, ALT, total bilirubin and eGFR at visit 1 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.

If the subject is discharged from hospital after day 7, the last result of SCr before discharge from local laboratory testing will be collected.

Table 7 Laboratory Tests (Efficacy and Safety Laboratory, Biomarkers, Pharmacokinetics, Pharmacodynamics and Pharmacogenomics) in Randomized Subjects

Visit	Type	Test
Visits 1 to 10/EoS	Hematology – Blood	Hemoglobin Hematocrit Red blood cell Reticulocytes Platelet count Leukocytes WBC differential: • % Neutrophils • % Immature granulocytes • % Lymphocytes • % Monocytes • % Eosinophils • % Basophils
Visits 1 to 10/EoS	Biochemistry – Blood	Sodium Potassium Calcium Chloride Glucose C-reactive protein Creatinine eGFR (CKD-EPI equation) eGFR based on cystatin-C Cystatin-C Blood urea nitrogen Alkaline phosphatase AST ALT Gamma-glutamyltransferase Total bilirubin Total protein Albumin LDH CK CK-MB Troponin T Total Cholesterol Triglycerides INR
Visits 1, 9 and 10/EoS	Biochemistry - Serum pregnancy test	β-HCG
Visit 2	Urine pregnancy test	Urine dipstick (locally)
Table continued on next page		

Visit	Type	Test
Visits 1 to 10/EoS	Urinalysis - Urine	Protein Albumin Glucose pH Blood Leukocytes Urobilinogen Bilirubin Ketones Nitrite
Visits 2, 3, 4 and 5	NephroCheck® - Urine	AKIRisk® score will be calculated at sites on visit 2 only. Urine samples on visits 3, 4 and 5 will be stored until assessment at central lab.
Visits 2 to 5, and 8	Biomarkers - Urine	KIM1
Visits 2 and 4	Biomarkers (Target Gene Expression) - Blood	ABCA1 ACAA2 SLC25A20 ACADVL CPT1a PDK4 CAT
Visits 2, 3 and 4	Pharmacokinetic sample - Blood	
Visit 2	Pharmacogenomic sample - Blood	

ACAA2: acetyl-coenzyme A acyltransferase 2; ABCA1: ATP binding cassette subfamily A member 1; ACADVL: acyl-CoA dehydrogenase very long chain ; ALT: alanine aminotransferase; AST: aspartate aminotransferase; β-HCG: β-human chorionic gonadotropin; CAT: catalase; eGRF: estimated glomerular filtration rate; CK: creatine kinase; CKD-EPI: chronic kidney disease epidemiology collaboration; CK-MB: creatine kinase-muscle/brain; INR: international normalized ratio; KIM1: kidney injury molecule-1; LDH: lactate dehydrogenase; PDK4: pyruvate dehydrogenase lipoamide kinase isozyme 4; SLC25A20: solute carrier family 25 member 20; WBC: white blood cell

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

5.4.3 Physical Examination

A full physical examination will be performed at screening to assess general appearance, skin, eyes, ears, nose, throat, neck, cardiovascular, chest and lungs, abdomen, musculoskeletal, neurologic status, mental status and lymphatic systems. Significant medical conditions will be recorded in appropriate eCRF (Medical History or AEs). 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.

At screening, body weight and height are measured and recorded. If body weight and height are available within 30 days before surgery, they still need to be measured at the screening visit. At visit 9 and visit 10, only body weight needs to be measured and recorded.

5.5 Adverse Events and Other Safety Aspects

5.5.1 Definition of Adverse Events

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

In order to identify any events that may be associated with study procedures and could lead to a change in the conduct of the study, Astellas collects AEs even if the subject has not received study drug treatment. AE collection begins after the signing of the ICF.

For screen failures, AEs will be collected until the subject is determined to be a screen failure.

For randomized subjects, all AEs will be collected until visit 9. If the subject is discontinued from the study prior to visit 9, AEs will be collected until the EoS visit has been completed. After end of all the procedures of visit 9 until visit 10, only SAEs will be collected.

For subjects in the observational cohort, AEs will be collected until 24 hours after T0. However, AEs related to MAKE parameters will be collected until the EoS visit. This includes events resulting in death or in RRT or relating to reduction in renal function.

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

5.5.1.1 Abnormal Laboratory Findings

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

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

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

5.5.1.2 Potential Cases of Drug-Induced Liver Injury

Refer to [Appendix 12.5 Liver Safety Monitoring and Assessment] for detailed instructions on Drug Induced Liver Injury. Abnormal values in AST and/or ALT concurrent or with abnormal elevations in total bilirubin that meet the criteria outlined in [Appendix 12.5 Liver

Safety Monitoring and Assessment], in the absence of other causes of liver injury, are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and are always to be considered important medical events and reported per [Section 5.5.5 Reporting of Serious Adverse Events].

5.5.1.3 Disease Progression and Study Endpoints

Under this protocol, the following event will not be considered as an(S)AE:

- Preplanned and elective hospitalizations or procedures for diagnostic, therapeutic, or surgical procedures for a preexisting condition that did not worsen during the course of the clinical study. These procedures are collected per the eCRFs Completion Guidelines.

5.5.1.4 Infusion Site Reactions

When infusion site reactions at a site of administration of study drug occur, detailed information including site and extent of reaction and duration of event will be collected in AE eCRF When the infusion site reactions lead catheter relocation, relocated place of catheter will be recorded in appropriate eCRF as a treatment for infusion site reactions. Infusion site reactions at any other sites than administration site will be handled in the same way as other AEs.

5.5.2 Definition of Serious Adverse Events (SAEs)

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

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

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

5.5.2.1 Always Serious Adverse Events

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

5.5.3 Criteria for Causal Relationship to Study Drug

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

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

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

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

- Plausible temporal relationship between exposure to the study drug and (S)AE onset and/or resolution. Has the subject actually received the study drug? Did the (S)AE occur in a reasonable temporal relationship to the administration of the study drug?
- Plausibility; i.e., could the event been caused by the study drug? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and clinical study data, etc.
- Dechallenge/Dose reduction/Rechallenge:
    - Did the (S)AE resolve or improve after stopping or reducing the dose of the suspect drug? Also consider the impact of treatment for the event when evaluating a dechallenge experience.
    - Did the (S)AE reoccur if the suspected drug was reintroduced after having been stopped?
- Laboratory or other test results; a specific lab investigation supports the assessment of the relationship between the (S)AE and the study drug (e.g., based on values pre, during and posttreatment)
- Available alternative explanations independent of study drug exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc. and strength of the alternative explanation

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

5.5.4 Criteria for Defining the Severity of an Adverse Event

AEs, including abnormal clinical laboratory values, will be graded using the National Cancer Institute-Common Terminology Criteria for Adverse Event (NCI-CTCAE) guidelines (Version 5.0). The items that are not stipulated in the NCI-CTCAE Version 5.0 will be assessed according to the criteria below and entered into the eCRF.

Grade	Assessment Standard
1-Mild	Asymptomatic or mild symptoms, clinical or diagnostic observations noted; intervention not indicated.
2-Moderate	Local or noninvasive intervention indicated.
3-Severe	Medically significant, but not immediately life threatening, hospitalization or prolonged hospitalization.
4-Life Threatening	Life threatening consequences, urgent intervention indicated
5-Death	Death related to AE

5.5.5 Reporting of Serious Adverse Events (SAEs)

The collection of AEs and the expedited reporting of SAEs will start following receipt of the ICF.

For screen failures, AEs will be collected until the subject is determined to be a screen failure.

For randomized subjects, all AEs will be collected until visit 9. If the subject is discontinued from the study prior to visit 9, AEs will be collected until the EoS visit has been completed. After end of all the procedures of visit 9 until visit 10, only SAEs will be collected.

For subjects in the observational cohort, AEs will be collected until 24 hours after T0. However, AEs related to MAKE parameters will be collected until the EoS visit. This includes events resulting in death or in RRT or relating to reduction in renal function.

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

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

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

If the SAE is associated with emergency unblinding as outlined in [Section 4.4.4 Breaking the Blind for Emergency] this is to be recorded on the SAE worksheet. Within the SAE worksheet, the investigator is to include when unblinding took place in association with the SAE.

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

Astellas Pharma Global Development – United States
Pharmacovigilance
Email: Safety-US@astellas.com
Fax number +1 888-396-3750

If there are any questions, or if clarification is needed regarding the SAE, please contact the sponsor's Medical Monitor/Study Physician or his/her designee [Section II Contact Details of Key Sponsor’s Personnel].

Follow-up information for the event should be sent promptly (within 7 days of the initial notification).

Full details of the SAE should be recorded on the medical records, SAE/Special Situation Worksheet and on the eCRF.

The following minimum information is required:

- International Study Number/Study number,
- Subject number, sex and age,
- The date of report,
- A description of the SAE (event, seriousness criteria),
- Causal relationship to the study drug (including reason) and
- Date(s) and time(s) of study drug administration

The sponsor or sponsor’s designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality and expectedness of the events (e.g., SUSAR reporting) according to current local/regional regulatory requirements in participating countries. The sponsor or sponsor's designee will submit expedited safety reports (e.g., IND Safety Reports, SUSAR, Council for International Organizations of Medical Sciences [CIOMS]-I) to Competent Authorities (CA) and concerned Ethics Committee (cEC) per current local regulations, and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports

as required by their IRB/IEC within timelines set by regional regulations (e.g., EMA, FDA) where required. Documentation of the submission to and receipt by the IRB/ local IEC of expedited safety reports should be retained by the site.

The sponsor/delegated CRO will notify all investigators responsible for ongoing clinical studies with the study drug of all SUSARs which require submission per local requirements IRB/local IEC.

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

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

5.5.6 Follow-up of Adverse Events

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

If after the protocol defined AE collection period [see Section 5.5.1 Definition of Adverse Event], an AE progresses to a SAE, or the investigator learns of any (S)AE including death, where he/she considers there is reasonable possibility it is related to the study drug treatment or study participation, the investigator must promptly notify the sponsor.

5.5.7 Monitoring of Common Serious Adverse Events

Common SAEs are SAEs commonly anticipated to occur in the study population independent of drug exposure. SAEs classified as “common” are provided in [Appendix 12.6 Common Serious Adverse Events] for reference. The list does NOT change the investigator’s reporting obligations, nor his obligations to perform a causality assessment, or prevent the need to report an AE meeting the definition of an SAE as detailed above. The purpose of this list is to alert the investigator that some events reported as SAEs may not require expedited reporting to the regulatory authorities based on the classification of “common SAEs” as specified in [Appendix 12.6 Common Serious Adverse Events]. The sponsor will monitor these events throughout the course of the study for any change in frequency. Any changes to this list will be communicated to the participating investigational sites. Investigators must report individual occurrences of these events as stated in [Section 5.5.5 Reporting of Serious Adverse Events].

5.5.8 Special Situations

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

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

The Special Situations are:

- Pregnancy
- Medication Error, Overdose and "Off-label use"
- Misuse/abuse
- Occupational exposure
- (Suspicion of) Transmission of infectious agent
- Suspected Drug-Drug interaction

5.5.8.1 Pregnancy

If a female subject becomes pregnant during the study period or within 30 days from the discontinuation of dosing, the investigator is to report the information to the sponsor according to the timelines in **[**Section 5.5.5 Reporting of Serious Adverse Events] using the Pregnancy Reporting Form and in the eCRF.

The investigator will attempt to collect pregnancy information on any female partner of a male subject who becomes pregnant during the study period or within 30 days from the discontinuation of dosing and report the information to sponsor according to the timelines in [Section 5.5.5 Reporting of Serious Adverse Event**s**] using the Pregnancy Reporting Form.

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

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female study subject as an AE in the eCRF or SAE per [Section 5.5.5 Reporting of Serious Adverse Events]. For (S)AEs experienced by a female partner of a male subject, (S)AEs are to be reported via the Pregnancy Reporting Form.

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

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

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

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

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

In the event of suspected ASP1128 overdose, the subject should receive supportive care and monitoring. The medical monitor/expert should be contacted as applicable.

5.5.8.3 Misuse/Abuse

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

5.5.8.4 Occupational Exposure

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

5.5.8.5 (Suspicion of) Transmission of Infectious Agent

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

5.5.8.6 Suspected Drug-Drug Interaction

If a suspected drug-drug interaction associated with the study drug(s) is suspected, the investigator must forward the Special Situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in

[Section 5.5.5 Reporting of Serious Adverse Events] together with details of the suspected drug-drug interaction.

5.5.9 Supply of New Information Affecting the Conduct of the Study

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

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

5.5.10 Urgent Safety Measures

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

5.5.11 Reporting Urgent Safety Measures

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

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

5.6 Test Drug Concentration

Blood samples for the analysis of ASP1128 in plasma will be collected as indicated in the Schedules of Assessments [Table 1] for evaluation of clinical pharmacokinetics. Each sample should be collected before a meal. The sample time schedule is as the followings:

- First administration: predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose
- Second administration: at end of infusion
- Third administration: predose and at end of infusion

The actual date and time of pharmacokinetic blood sample collection will be documented in the source. Blood sample collection, handling and storage will be described in the laboratory manual. Blood samples will be collected via a peripherally placed intravenous cannula or by

direct venipuncture in a suitable forearm vein. Blood samples may not be collected from the same arm as where the intravenous infusion will be administered.

When deemed appropriate at a later date, plasma samples remaining after the pharmacokinetic analysis may be used for exploratory metabolite profiling or exploratory biomarker analysis after the study. These tests will be described in a separate report and will not be incorporated in the integrated clinical study report.

5.7 Other Measurements, Assessments or Methods

5.7.1 NephroCheck

Urine samples will be collected as indicated in the Schedules of Assessments [Table 1] and [Table 2] and the concentration of TIMP2 and IGFBP7 will be assessed utilizing the NC as per the package insert instructions of the NC device. The device automatically multiplies the concentrations of the 2 biomarkers together and divides this product by 1,000 to report a single numeric test result with units of $(ng/mL)^2/1{,}000$ (the units for all TIMP2•IGFBP7 values in this protocol) referred to as the AKIRisk score. The urine sample should be fresh (i.e., not from accumulated urine in the bag), so the bag should be emptied before the sample is taken; on the day of surgery, it can be obtained in the operating room/theater, recovery room or in the ICU, as long as it is within the range of 2 to 6 hours post T0. A second urine sample for the assessment of the AKIRisk score can be collected within the 2 to 6 hour post T0 range and should be done if the first result was 0.3 $(ng/mL)^2/1000$ or less. If the NC is negative, i.e., the AKIRisk score is $\leq$ 0.3 $(ng/ml)^2/1000$ (at first and second measurement, if applicable) or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated for up to 4 times until 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour [see Section 2.2.1]. The 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. In the observational cohort the NC will be measured if the subject is still in the ICU and urine can be sampled from the catheter bag.

5.7.2 Biomarkers

Urine samples will be collected for the assessment of KIM1, a primary biomarker for tubular damage [KDIGO, 2012], and stored for potential future analysis of other clinical and/or compound related biomarkers on visits 2, 3, 4, 5 and 8 as indicated in the Schedules of Assessments [Table 1] and [Table 2] [see also Section 5.4.2 Laboratory Assessments and Section 5.7.4 Subjects in the Observational Cohort].

5.7.3 Blood Sample for Banked PGx Sample Analysis

A PGx research may be conducted in the future to analyze or determine genes of relevance to clinical response, pharmacokinetics and toxicity/safety issues. At a presurgery assessment on visit 2 (see schedule of assessments [Table 1]), a 6 mL sample of whole blood for possible retrospective PGx analysis will be collected. Samples will be shipped to a sponsor designated banking CRO.

Details on sample collection, labeling, storage and shipment procedures will be provided in a separate laboratory manual.

See [Appendix 12.7, Retrospective Pharmacogenomic Sub-study] for further details on the banking procedures.

5.7.4 Subjects in the Observational Cohort

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ at all assessments between 2 and 22 hours after T0, the subject cannot be randomized. These subjects will be included in the observational cohort. The aim of following up the subjects in the observational cohort is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (AKI-SCr using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Days 30 and 90 may be conducted by phone if the subject is unable to visit the site.

For subjects in the observational cohort, local laboratory test results of the parameters mentioned in Table 8 and 24-hours UO data will be collected, if available at visits according to the Schedule of Assessments [Table 2]. Urine sampling for NC and biomarkers will be performed separately according to the Schedule of Assessments [Table 2] for posthoc analysis of study outcomes, and only if the subject has a urinary catheter to facilitate sampling. MAKE parameters, i.e., all-cause mortality, RRT and/or ≥ 25% sustained reduction in eGFR based on SCr or S-Cystatin-C will be assessed according to the Schedule of Assessments [Table 2]. AEs and medication use will be recorded until 24 hours after T0.

Table 8 Laboratory Test Data in the Subjects of the Observational Cohort

Visit	Type	Test
Visits 3-10/EoS	Hematology	Hemoglobin Hematocrit Red blood cell count Platelet count Leukocytes
Visits 3-10/EoS	Biochemistry	C-reactive protein Creatinine eGFR (any equation) Cystatin-C Alkaline phosphatase AST ALT Gamma-glutamyltransferase Total bilirubin Total protein Albumin CK CK-MB
Visits 8, 9, 10/EoS	Urinalysis	Protein Albumin
Visits 3 to 5	Biomarkers/NephroCheck® - Urine	AKIRisk® score KIM1(Urine samples on visits 3, 4 and 5 will be stored until assessment at central lab)

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; CK-MB: creatine kinase-muscle/brain; EoS: end of study; KIM1: kidney injury molecule-1.

5.8 Total Amount of Blood

The total amount of blood to be taken over the entire study period per subject is approximately 163 mL. Extra blood sampling should only be performed according to clinical need, or when follow-up of abnormal clinical laboratory values makes it necessary.

- Hematology (10 x 3 mL) 30 mL
- Biochemistry (including pregnancy test) (10 x 10.5 mL) 105 mL
- Pharmacokinetics (7 x 2 mL) 14 mL
- Target gene expression (4 x 2 mL) 8 mL
- PGx (1 x 6 mL) 6 mL

6 DISCONTINUATION

6.1 Discontinuation of Individual Subject(s) From Study Treatment

A discontinuation from treatment is a subject who enrolled in the study and for whom study treatment is permanently discontinued for any reason. Because study treatment will start after randomization discontinuation applies to randomized subjects only. The reason for discontinuation from study treatment must be documented in the subject's medical records.

The subject is free to withdraw from the study treatment and/or study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to discontinue the subject from study treatment or to terminate a subject's involvement in the study at any time if the subject's clinical condition warrants it. Even though a subject discontinues study-drug administration, follow-up assessment will be conducted to the extent possible.

If a subject is discontinued from the study with an ongoing AE or an unresolved laboratory result that is significantly outside of the reference range, the investigator will attempt to provide follow-up until the condition stabilizes or no longer is clinically significant.

The investigator should consider to discontinue administration of the study drug to a subject in any of the following cases:

- When the study drug cannot be safely administered due to AEs as reported by the subject, or as per the investigator's discretion, or when the sponsor requests discontinuation of the study drug due to safety issues related to the subject.
- When the subject withdraws consent for further treatment.
- When a female subject becomes pregnant
- Other cases in which the investigator judges it inappropriate to continue administration of the study drug.

The reason for discontinuation, medical findings, and other relevant data should be recorded in the eCRF.

6.1.1 Lost to Follow Up

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

6.2 Discontinuation of the Site

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

6.3 Discontinuation of the Study

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

the sponsor terminates the study for safety reasons, the sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

7 STATISTICAL METHODOLOGY

A Statistical Analysis Plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database lock at the latest. Any changes from the analyses planned in SAP will be justified in the Clinical Study Report.

In general, continuous data will be summarized with descriptive statistics (number of subjects, mean, SD, minimum, median and maximum) and frequency and percentage for categorical data.

7.1 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 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.

Subjects who meet the presurgery selection criteria will be enrolled. Subjects who meet the postsurgery criterion of having a NC AKIRisk score > 0.3 $(ng/mL)^2/1000$ between 2 and 22 hours after surgery can be randomized: the number of subjects to be randomized is 220. The subjects that have an AKIRisk score of ≤ 0.3 $(ng/mL)^2/1000$ at the postsurgery assessment will be enrolled in the observational cohort. The observational cohort will enroll a maximum of 440 subjects.

7.2 Analysis Sets

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 hard-lock.

For each treatment group, the number and percentage of subjects will be characterized for all randomized subjects and by each analysis set.

7.2.1 Full Analysis Set

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.

7.2.2 Per Protocol Set

The Per Protocol Set (PPS) will consist of the subset of the FAS who do not meet criteria for PPS exclusion. These criteria are to capture relevant non-adherence to the protocol and will be defined in the SAP. Further criteria may be defined in the SAP. The PPS will be a secondary analysis set for efficacy analyses. Select demographic and baseline characteristics may also be summarized for the PPS.

7.2.3 Safety Analysis Set

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.

7.2.4 Pharmacokinetic Analysis Set

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.

7.2.5 Pharmacodynamic Analysis Set

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.

7.2.6 Prognostic Factor Analysis Set

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.

7.3 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized by treatment group as well as for all treatment groups combined.

7.3.1 Subject Disposition

The number and percentage of subjects who completed and discontinued treatment and reasons for treatment discontinuation will be presented for all randomized subjects and for subjects in the SAF by treatment group and overall. Similar tables for screening disposition, investigational period disposition and follow-up disposition will also be presented for all randomized subjects by treatment group and overall. All disposition details and dates of first and last evaluations for each subject will be listed.

7.3.2 Previous and Concomitant Medications

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

7.3.3 Medical History

Medical history for each subject will be presented in a listing.

7.4 Analysis of Efficacy

Efficacy analysis will be conducted on the FAS and PPS. The interpretation of results from statistical tests will be based on the FAS. The PPS will be used to assess the robustness of the results from the statistical tests based on the FAS.

7.4.1 Analysis of Primary Endpoint

7.4.1.1 Primary Analysis

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 (< 45, ≥ 45) 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 common risk ratio and 2-sided 90% confidence interval will be calculated.

Subjects whose status of AKI-SCr72h is unknown due to, say, the missing data of SCr will be counted as AKI event.

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 (< 45, ≥ 45). 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 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. The details of the additional sensitivity analyses will be described in the SAP.

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.

7.4.2 Analysis of Secondary Endpoints

The secondary efficacy endpoints proportions (AKI-SCr7d, AKI-KDIGO72h, AKI-KDIGO7d, MAKE30, MAKE90) will be analyzed using the stratified CMH test with strata to control for baseline eGFR (< 45, ≥ 45) on the FAS. The hypothesis testing will be performed at 2-sided 0.10 significance level. The MH estimate of the 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 (< 45, ≥ 45). The estimates of the risk ratio and 2-sided 90% confidence intervals will be calculated.

Subjects whose status of AKI and MAKE is unknown due to, say, the missing data of SCr will be counted as AKI/MAKE event. 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.

As exploratory analyses, the association between MAKE30/MAKE90 and primary, secondary and exploratory endpoint of AKI proportion is evaluated by a contingency table.

7.4.3 Subgroup Analysis

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 for the following factors;

- Diabetics: diabetes mellitus type 1 or 2, yes vs no
- Gender
- Kidney failure: eGFR at presurgery baseline < 45 vs ≥ 45, < 60 vs ≥ 60
- Surgery type: lower risk surgery (i.e., only CABG or single valve surgery, excluding Aortic root and ascending Aorta surgery) vs higher risk surgery (i.e., combined CABG and valve surgery, surgery for more than 1 cardiac valve, surgery for Aortic root, or ascending Aorta without circulatory arrest)
- NC AKIRisk score at randomization > 0.7 vs > 0.3 to 0.7 $(ng/ml)^2/1000$
- IV radio-contrast between screening and surgery, yes vs no
- Peripheral arterial disease, yes vs no
- Cardiac function: ejection fraction% < 40 vs ≥ 40
- Duration of CPB <median vs ≥ median

In addition, the forest plot will be provided. This analysis will be conducted using the FAS.

For the continuous type subgroup factors, including eGFR at baseline, AKIRisk score at randomization, cardiac function (ejection fraction %) and duration of CPB, 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 and explanatory

variables are treatment group, the subgroup factor and the interaction term of treatment group and the subgroup factor. The details of model specification will be described in the SAP.

7.4.4 Analysis of Exploratory Endpoints

Descriptive statistics will be used to summarize exploratory efficacy endpoint on the FAS. These endpoints will be summarized graphically.

The statistical analyses on other exploratory endpoints include:

- CMH test on exploratory endpoints of all-cause mortality, RRT, eGFR reduction proportions, AKI proportions
- Wilcoxon-test for AKI severity, AKI duration, number of hospital days, number of ICU days, number of hospitalization and number of readmissions
- Kaplan-Meier estimator and log-rank test for time to AKI-SCr72h, time to AKI-SCr7d and time to ventilator weaning.

7.5 Analysis of Safety

7.5.1 Adverse Events

AEs will be coded using MedDRA.

TEAE is defined as an AE observed after starting administration of the study drug and by visit 9 (day 30).

The number and percentage of subjects with TEAEs, treatment emergent SAEs, TEAEs leading to withdrawal of treatment and TEAEs related to study drug will be summarized by system organ class, preferred term and treatment group. The number and percentage of TEAEs by severity will also be summarized. All AEs will be listed.

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

7.5.2 Laboratory Assessments

For quantitative laboratory measurements descriptive statistics will be used to summarize results and change from baseline for subjects in the SAF by treatment group and visit.

Shifts relative to normal ranges from baseline to each time point during treatment period in lab tests will also be tabulated. Laboratory data will be displayed in listings.

7.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline for subjects in the SAF by treatment group and visit. Vital signs data will be displayed in listings.

7.6 Analysis of Pharmacokinetics

All pharmacokinetic summaries will be provided by time points. Descriptive statistics will include n, mean, minimum, median, maximum, SD, coefficient of variation (CV), geometric mean and geometric CV.

7.6.1 Plasma Concentrations

Descriptive statistics will be presented for plasma concentration. Standard graphics including scatter plots will be produced. Plasma concentration and sample times data will be listed.

7.7 Analysis of Pharmacodynamics

Descriptive statistics will be used to summarize gene expression data in the PDAS. These endpoints will be summarized graphically.

7.8 Major Protocol Deviations and Other Analyses

7.8.1 Major Protocol Deviations

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

The major protocol deviation criteria will be uniquely identified in the summary table and listing.

7.8.2 Analysis for Observational Cohort

Potential risk factors (demographics, subject characteristics, biomarkers) for development of AKI and MAKE (listed below) will be explored:

- AKI-SCr72h
- AKI-SCr7d
- MAKE30
- MAKE90

Risk factor for AKI and MAKE will be compared to NC test in the PFAS. Details of the analysis method for PFAS will be described in the SAP.

7.9 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.

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

For the primary and secondary endpoints of proportion of AKI and MAKE, subjects whose status of AKI and MAKE is unknown due to, say, the missing data of SCr will be counted as AKI/MAKE event.

Because AKI/MAKE is a composite variable based on SCr, urine volume or eGFR, it is sometimes possible that part of such component data is available (e.g., SCr up to visit 4 is available, but that of visit 5 is missing). In such a case, the following analysis will also be conducted; the multiple imputation will be conducted for each missing component data firstly, then AKI/MAKE endpoint will be derived based on the imputed data. The derived AKI/MAKE endpoint will be used for the same analysis of the primary endpoint as described in [Section 7.4.1.1 Primary Analysis].

See the SAP for details of the multiple imputation and the definition for windows to be used for analyses by visit.

8 OPERATIONAL CONSIDERATIONS

8.1 Data Collection

The investigator or site designee will enter data collected using an electronic data capture system. In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including laboratory values, if applicable) in the eCRF within 5 days after the subject's visit.

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

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

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

8.2 Screen Failures

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

8.3 Major Protocol Deviations

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

A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited.

The major protocol deviation criteria are 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.

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

If a major deviation impacts the safety of a subject, the investigator must contact the sponsor immediately.

The investigator will also assure that deviations meeting IRB/IEC and applicable regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and applicable regulatory authorities will be provided to the sponsor and maintained within the trial master file.

9 END OF STUDY

The end of the study is defined as the last visit or scheduled procedure shown in the Schedule of Assessments [Table 1 and Table 2] for the last study participant in the study.

10 STUDY ORGANIZATION

10.1 Data-Monitoring Committee

A DMC will be instated to perform the interim analysis, which may recommend (nonbinding) terminating the study for unfavorable results at the interim analysis. Recommendations regarding study conduct will be made by the DMC based on their assessment of this result. DMC will consist of an Astellas medical doctor and an Astellas biostatistician who are independent from the study team. A separate charter will outline the activities of this committee.

10.2 Other Study Organization

Not applicable.

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12 APPENDICES

12.1 Ethical, Regulatory and Study Oversight Considerations

12.1.1 Ethical Conduct of the Study

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

12.1.2 Institutional Review Board/Independent Ethics Committee

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

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

The investigator will be responsible for the following:

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

12.1.3 Protocol Amendment and/or Revision

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments: substantial amendments and/or non-substantial amendments. Depending on the nature of the amendment, either IRB/IEC, CA approval or notification may be required. The changes will become effective only after the approval of the sponsor, the investigator, the regulatory authority and the IRB/IEC (if applicable).

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

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

12.1.4 Financial Disclosure

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

12.1.5 Informed Consent of Subjects

12.1.5.1 Subject Information and Consent

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

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

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

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

12.1.6 Source Documents

Source data must be available at the site to document the existence of the study subjects and to substantiate the integrity of study data collected. Source data must include the original

documents relating to the study, as well as the medical treatment and medical history of the subject.

The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are hand-written on paper or entered electronically. If source data are created (first entered), modified, maintained, achieved, retrieved or transmitted electronically via computerized systems (and/or other kind of electric devices) as part of regulated clinical study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol related assessments, AE tracking and/or drug accountability.

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

12.1.7 Record Retention

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

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

12.1.8 Subject Confidentiality and Privacy

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

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

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

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

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

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

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

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

12.1.10 Insurance of Subjects and Others (UNIQUE to JP/Studies enrolling subjects in EU)

Not Applicable

12.1.11 Signatory Investigator for Clinical Study Report

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

12.2 Procedure for Clinical Study Quality Control

12.2.1 Clinical Study Monitoring

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

12.2.2 Direct Access to Source Data/Documents

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

12.2.3 Data Management

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

12.2.4 Quality Assurance

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

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

12.3 Contraception Requirements

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

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

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile.

Women in the following categories are not considered WOCBP

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

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

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

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

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

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILDBEARING POTENTIAL

One of the highly effective methods of contraception listed below is required at the time of informed consent and until the end of relevant systemic exposure, defined as 30 days after the final study drug administration.[a]

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

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

- oral
- intravaginal
- transdermal

Progestogen-only hormonal contraception associated with inhibition of ovulation

- oral
- injectable
- implantable

Hormonal methods of contraception containing a combination of estrogen and progesterone, vaginal ring, injectables, implants and intrauterine hormone-releasing system

- intrauterine device
- bilateral tubal occlusion

Vasectomized partner

Sexual abstinence

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

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

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

Male participants with female partners of childbearing potential are eligible to participate if they agree to the following during treatment and until the end of relevant systemic exposure defined as 30 days after final drug administration.[a]

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator.
- Male participants are required to use a condom during treatment and until end of relevant systemic exposure defined as 30 days after final drug administration.
- Female partners of male participants who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception until the end of relevant systemic exposure, defined as 30 days after final drug administration.

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

12.4 List of Excluded Concomitant Medications

Please note that this is not an exhaustive list. Investigators should verify the subject's concomitant medications for all prohibited and restricted drug classes.

Prohibited Medications

From 28 days before screening until EoS:

Class	Drug
Drugs may affect plasma creatinine levels without influencing kidney function	cimetidine
	trimethoprim
	pyrimidine analogues (e.g., flucytosine)
	pyrimidine derivates (e.g., pyrimethamine)
	phenacemide
	calcitriol/alfacalcidol

Following surgery up to 72 hours post-surgery:

Class	Drug
NSAIDs	sodium salicylate
	aspirin > 325 mg/day
	mefenamic acid
	flufenamate aluminum
	diclofenac sodium
	amefenac sodium hydrate
	indometacin
	acemetacin
	indometacin farnesil
	proglumetacin maleate
	sulindac
	mofezolac
	etodolac
	nabumetone
	ibuprofen
	flurbiprofen
	flubiprofen axetil
	ketoprofen
	naproxen
	pranoprofen

Table continued on next page

Class	Drug
NSAIDS (continued)	tiaprofenic acid
	oxaprozin
	loxoprofen sodium hydrate
	zaltoprofen
	piroxicam
	ampiroxicam
	lornoxicam
	meloxicam
	celecox
	tiaramide hydrochloride
ACE Inhibitors	captopril
	enalapril maleate
	alacepril
	delapril hydrochloride
	cilazapril hydrate
	lisinopril hydrate
	benazepril hydrochloride
	imidapril hydrochloride
	temocapril hedrochloride
	quinapril hydrochloride
	trandolapril
	perindopril erbumine
Angiotensin Receptor Blockers	losartan potassium
	candesartan cilexetil
	valsartan
	telmisartan
	olmesartan medoxomil
	irbesartan
	azilsartan

ACE: angiotensin-converting enzyme; NSAID: nonsteroidal anti-inflammatory drug

12.5 Liver Safety Monitoring and Assessment

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

Definition of Liver Abnormalities

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

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

The subject should be considered to have severe hepatic abnormalities for any of the following:

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

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

Follow-up Procedures

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

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

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a SAE. The sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to study drug are observed.

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

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

Study Treatment Discontinuation

In the absence of an explanation for increased LFT's, such as viral hepatitis, preexisting or acute liver disease, or exposure to other agents associated with liver injury, the subject may be discontinued from study treatment. The investigator may determine that it is not in the subject's best interest to continue study treatment. Discontinuation of study treatment should be considered if:

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

If close monitoring for a subject with moderate or severe hepatic laboratory tests is not possible, study treatment should be discontinued.

*Hy's Law Definition [Temple, 2006]: Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10 to 50% mortality (or transplant).

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

1. Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher 3 × ULN ("2 × ULN elevations are too common in treated and untreated subjects to be discriminating").
2. Cases of increased bilirubin (at least 2 × ULN) with concurrent transaminase elevations at least 3 × ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert's syndrome [Temple, 2006].

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

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (nonhepatotoxic) control drug or placebo.
2. Among study subjects showing such AT elevations, often with ATs much greater than 3 × ULN, 1 or more also show elevation of serum TBL to > 2 × ULN, without initial findings of cholestasis (elevated serum ALP).
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.

References

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

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

12.6 Common Serious Adverse Events

No serious adverse reactions are currently considered expected based upon the assessments made for the AKI study population.

12.7 Pharmacogenomic Analysis With Banked Sample (Optional)

INTRODUCTION

PGx research aims to provide information regarding how naturally occurring differences in a subject's gene and/or expression of genes based on genetic variation may impact what treatment options are best suited for the subject. Through investigation of PGx by technologies such as genotyping, gene sequencing, statistical genetics and Genome-Wide Association Studies, the relationship between gene profiles and a drug's kinetics, efficacy or toxicity may be better understood. As many diseases may be influenced by 1 or more genetic variations, PGx research may identify which genes are involved in determining the way a subject may or may not respond to a drug.

OBJECTIVES

The PGx research that may be conducted in the future with acquired blood samples is exploratory. The objective of this research will be to analyze or determine genes of relevance to clinical response, pharmacokinetics and/or toxicity/safety issues.

By analyzing genetic variations, it may be possible to predict an individual subject's response to treatment in terms of efficacy and/or toxicity.

SUBJECT PARTICIPATION

Subjects who have consented to participate in this study may participate in this PGx sub-study. Subjects must provide written consent prior to providing any blood samples that may be used at a later time for PGx analysis.

SAMPLE COLLECTION AND STORAGE

Subjects who consent to participate in this sub-study will provide 1 tube of whole blood of approximately 4 to 6 mL per Astellas' instructions. Each sample will be identified by the unique subject number. Samples will be shipped to a designated banking CRO as directed by Astellas.

PGx ANALYSIS

Details on the potential PGx analysis cannot be established yet. Astellas may initiate the PGx analysis if evidence suggests that genetic variants may be influencing the drug's kinetics, efficacy and/or safety.

DISPOSAL OF PGx SAMPLES / DATA

All PGx samples collected will be stored for a period of up to 15 years following study database hardlock. If there is no requirement for analysis, the whole blood sample will be destroyed after the planned storage period. The subject has the right to withdraw consent at any time. When a subject's withdraw notification is received, the PGx sample will be destroyed. The results of any PGx analysis conducted on a sample prior to its withdrawal will be retained at Astellas indefinitely unless otherwise specified by local regulation.

INFORMATION DISCLOSURE TO THE SUBJECTS

Exploratory PGx analysis may be conducted following the conclusion of the clinical study, if applicable. The results of the PGx analysis will not be provided to any investigators or subjects, nor can the results be requested at a later date. Any information that is obtained from the PGx analysis will be the property of Astellas.

12.8 Clinical Study Continuity

INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).

BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated Schedule of Assessments/Table 1 unless the site PI discusses the need with the Astellas Medical Monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed as described in Section 5 due to a crisis.

INFORMED CONSENT

Participants who need to follow any or all of the alternate measures outlined in this Appendix will be required to provide informed consent which explicitly informs them of the nature of, and rationale for these changes, and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the PI or designee will obtain oral agreement from the subject followed by written documentation as soon as is feasible. A separate addendum to the study informed consent will be provided to document the participant's consent of the changes.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if one or more of the following conditions are met due to the crisis:

- Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible
- Site facilities have been closed for clinical study conduct
- Site has been restricted to treating patients with conditions outside of the scope of the study
- Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel
- Participant(s) have temporarily relocated from the current study site to an alternate study site avoid placing a burden on the participant with respect to travel

- Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel
- Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety

Adherence to the original protocol as reflected in the Schedule of Assessment Table 1 is expected, where plausible, in the case of a crisis. The alternate measures as noted in the table below are only permissible in the event of a crisis, and after discussing the need with the Astellas Medical Monitor and/or designee to implement the alternate measures. This is to allow for maintaining critical safety and efficacy assessments for patients participating in the study at a time of crisis.

If one or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding time point(s).

Table 9 Alternative Schedule of Assessments in Response to a Crisis — Randomized Subjects

Study Period	**Screening**	**Surgery**			**Treatment Period**			**Follow-up Period**					**Unscheduled**
Visit Number	**1**	**2[1]**			**3**	**4**	**5**	**6**	**7**	**8**	**9[20]**	**10/EoS[20]**	
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[21]	X[21]	X
Physical examination	X[19]												
Body weight, height	X										X[4, 22]	X[4, 22]	
Subject randomization				X[5]									
Vital signs	X	X			X	X	X			X	X[22]	X[22]	O
Hematology, biochemistry[6, 7]	X[17]	X			X	X	X	X	X	X	X[23]	X[23]	X
Urinary output[8]				X	X	X	X	X	X	X			O
Urinalysis[9]	X[17]	X			X	X	X	X	X	X	X[23]	X[23]	O
Pregnancy test	X[17]	X[18]									X[23]	X[23]	
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[21]	X[21]	X
Assess follow-up parameters[13]				X	X	X	X	X	X	X	X[21]	X[21]	X
APACHE-II[14]		X		X	X	X	X						
EQ-5D-5L	X									X	X[21]	X[21]	
Blood samples for pharmacokinetics[15]				X		X	X						

Table continued on next page

Study Period	Screening	Surgery			Treatment Period			Follow-up Period					Unscheduled
Visit Number	1	2[1]			3	4	5	6	7	8	9[20]	10/EoS[20]	
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	
Blood samples for pharmacodynamics[16]				X			X						
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; 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 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.

Footnotes continued on next page

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).
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 AST, 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.
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.
20. In the event of crisis, the alternate measures may be implemented for study procedures and assessments only for visits 9 and 10/EoS after discussion with the Astellas Medical Monitor and/or designee.
21. Assess remotely. Telephone script can be available for EQ-5D-5L.
22. Collect local data, if available.
23. Alternative lab collection at a local clinic and results submitted to principal investigator.

DATA COLLECTION REQUIREMENTS

Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.

- Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window, or other modifications) due to the crisis.

13 ATTACHMENT 1: SUBSTANTIAL AMENDMENT 1

I. The purpose of this amendment is:

Substantial Changes
1. Changes in Enrollment Numbers
DESCRIPTION OF CHANGE:
The number of subjects to be enrolled in the observational cohort is changed to be capped at 440.
RATIONALE:
The current number of subjects in the observational cohort compared to the other cohort is higher than anticipated. The change is to cap the enrollment at the previously planned number. A higher number is not needed for the objectives of this cohort.
2. Changes in NephroCheck® (NC) Assessment
DESCRIPTION OF CHANGE:
The time window for post-surgical (coronary artery bypass graft and/or valve surgery) NC assessment is extended.
RATIONALE:
Review of the emerging data from the observation cohort showed a significant number of subjects had NC value > 0.3 after the current assessment window (i.e., 2 to 6 hours post-surgery). Based on available scientific literature and after confirmation with Key Opinion Leaders, NC increase within 24 hours after surgery is most likely due to an insult incited during surgery, and NC is approved to assess the acute kidney injury (AKI) risk in patients who have had acute cardiovascular compromise within the prior 24 hours. Extending the NC assessment window will identify subjects who have moderate or high risk for developing AKI, and therefore potentially be eligible for study treatment randomization.
3. Addition of Details for Duration of Treatment
DESCRIPTION OF CHANGE:
Parameters related to dosing of the investigational product are updated according to the extension of the NC assessment window.
RATIONALE:
The change is made to accommodate the extension of the NC assessment window. It is also expected that the wider treatment window after the surgery is supported to be effective based on preclinical data.

4. Endpoint Clarifications
DESCRIPTION OF CHANGE:
The timing of primary and secondary endpoint assessments is clarified.
RATIONALE:
The change is to add a clarification to ensure that those endpoints will be assessed according to the Kidney Disease Improving Global Outcomes (KDIGO) guideline.
5. Changes in Exploratory Endpoints
DESCRIPTION OF CHANGE:
The exploratory endpoint related to the proportion of subjects developing AKI based on serum creatinine criteria from the Acute Kidney Injury Network (AKIN) guideline is replaced with 2 new exploratory endpoints. These endpoints are related to the proportion of subjects developing AKI during treatment, both based on Kidney Disease Improving Global Outcomes (KDIGO) criteria.
RATIONALE:
The replacement is made to add additional endpoints that are comparable to the competitor's endpoint in order to compare results between studies, and also to evaluate the best timing of AKI assessment for later studies.
6. **Changes to Schedule of Central Laboratory Sampling**
DESCRIPTION OF CHANGE:
The schedule of central laboratory sampling is updated in the Schedule of Assessments/Evaluations and Section 5.4.2. The sampling for blood and/or urine at screening visit 1 may be by-passed when the surgery is scheduled within 2 days and if the results from local laboratory are available.
RATIONALE:
The requirement for the sampling is relaxed due to the challenge of frequent samplings before the surgery. The subject eligibility can be verified using local laboratory results per the protocol and the change makes the study more feasible without an impact on the safety or scientific value of the clinical study.
7. Remove Analysis of Pharmacokinetic Parameters
DESCRIPTION OF CHANGE:
Section 7.6.2, *Pharmacokinetic Parameters*, is removed from the protocol.
RATIONALE:
The analysis plan is changed to not calculate pharmacokinetic parameters.

Nonsubstantial Changes
1. Update Planned Study Period
DESCRIPTION OF CHANGE:
The study completion date is extended from the fourth quarter of 2020 to the 1Q 2022.
RATIONALE:
To update the study period based on the actual enrollment.
2. Change to Number of Study Centers
DESCRIPTION OF CHANGE:
The number of study centers in North America is increased from 40 to 50.
RATIONALE:
To update the number of study centers based on the actual enrollment.
3. Clarification to Blood Sampling Times
DESCRIPTION OF CHANGE:
The timing of blood sampling for pharmacokinetic and genetic testing is clarified in the Schedule of Events.
RATIONALE:
To clarify the timing according to the change in the schedule of study drug administration.
4. Clarification to Visit Windows for the Observational Cohort
DESCRIPTION OF CHANGE:
Visit windows for the follow-up period for the observational cohort are updated to provide clarification.
RATIONALE:
To provide clarifications to the protocol and to ensure complete understanding of study procedures.
5. Details for Adverse Events (AEs) Collection and Follow-up in Observational Cohort
DESCRIPTION OF CHANGE:
Collection of AEs related to major adverse kidney events and follow-up of the events in the observational cohort is clarified.
RATIONALE:
To provide clarifications to the protocol and to ensure complete understanding of study

procedures.
6. Add Clinical Study Continuity Plan
DESCRIPTION OF CHANGE:
Clinical study continuity procedures are added in Section 12.8.
RATIONALE:
To outline procedures to be prioritized and alternate methods of assessing safety and efficacy in the event the study is interrupted.
7. Minor Administrative-type Changes
DESCRIPTION OF CHANGE:
Include minor administrative-type changes (e.g., typos, format, numbering and consistency throughout the protocol) and update list of abbreviations.
RATIONALE:
To provide clarifications to the protocol and to ensure complete understanding of study procedures.

II. Amendment Summary of Changes:

IIa. Substantial Changes

IV Synopsis, Number of Subjects to be Enrolled/Randomized and 7 Statistical Methodology *7.1 Sample Size*
WAS:
Approximately 660 subjects who meet the presurgery selection criteria will be enrolled. 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.
IS AMENDED TO:
~~Approximately 660 subjects~~ **Subjects** who meet the presurgery selection criteria will be enrolled. ~~It is expected that 1 out of 3 subjects enrolled will~~ **Subjects who** meet the postsurgery criterion of having a NC AKIRisk® score > 0.3 $(ng/mL)^2/1000$ ~~and~~ **between 2 and 22 hours after surgery** can be randomized: the number of subjects to be randomized is 220. The subjects that have an AKIRisk score of ≤ 0.3 **$(ng/mL)^2/1000$** at the postsurgery ~~2- to 6-hour~~ assessment will be enrolled in the observational cohort. **The observational cohort will enroll a maximum of 440 subjects.**

IV Synopsis, Study Design Overview
WAS:
Visit 2/Day of Surgery Four hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0), a urine sample will be collected and the concentration of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor binding protein-7 will be assessed utilizing the NC device as per the package insert instructions. The urine sample should be fresh (i.e., not from accumulated urine in the bag, so the bag should be emptied before the sample is taken); it can be obtained in the operating room/theater, recovery room or in the intensive care unit (ICU), as long as it is within the range of 2 to 6 hours post T0. The device will calculate the AKIRisk score. If NC assessment is positive (AKIRisk score > 0.3 $ng/mL^2/1000$), the subject will be randomized and will start treatment per the randomization schedule. If the result is of the AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$, the NC assessment should be redone once only if within the 2- to 6-hour window after T0 (i.e., the urine sample for NC should be taken within the 2 to 6 hours post T0 range). If the NC assessment is negative (AKIRisk score ≤ 0.3 [at 1st and 2nd measurement, if applicable]), the subject cannot be randomized and will be followed up in the observational cohort (see below). Randomized subjects will receive 3 subsequent treatments with ASP1128 or matching placebo, which will be administered intravenously through a peripheral/central catheter. The first administration of study drug will occur after randomization, as soon as possible after the positive NC. Administration should be completed within 8 hours after T0. In case of reoperation on the day of surgery, the same time range for NC and drug administration applies in relation to T0. A reoperation is no reason for not enrolling a subject as long as the NC can be performed at the described time points. *Visits 3, 4 and 5* The second administration of study drug will occur the day after surgery: 24 hours after T0 (visit 3). The third administration will occur at 48 hours after T0 (visit 4). A margin of 2 hours before and after the designated time is allowed. Applicable assessments will be done as per the Schedule of Assessments [Table 1] up to 72 hours after T0 (visit 5) to assess the study endpoints, including AKI (based on SCr and UO). When the subject is in the ICU, UO per 12 hours and total fluid intake per 24 hours (including blood products and intravenous fluid input) must be recorded. When the subjects are in the ICU, the time to ventilator weaning will be recorded. *Observational Cohort* If at randomization NC is negative (AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ at t = 4 hours [range 2 to 6 hours; at 1st and 2nd measurement, if applicable]), the subject cannot be randomized. These subjects will be included in the observational cohort. The rationale for following the randomization failures is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE.

IS AMENDED TO:

Visit 2/Day of Surgery

Four hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0), a urine sample will be collected and the concentration of tissue inhibitor of metalloproteinase-2 and insulin-like growth factor binding protein-7 will be assessed utilizing the NC device as per the package insert instructions. The urine sample should be fresh (i.e., not from accumulated urine in the bag, so the bag should be emptied before the sample is taken); it can be obtained in the operating room/theater, recovery room or in the intensive care unit (ICU), as long as it is within the range of 2 to 6 hours post T0. The device will calculate the AKIRisk score. If NC assessment is positive (AKIRisk score > 0.3 ng/mL^2/1000), the subject will be randomized and will start treatment **within 8 hours after T0 as** per the randomization schedule.

If the result is of the AKIRisk score is ≤ 0.3 (ng/mL)^2/1000, the NC assessment should be redone once only if within the 2- to 6-hour window after T0 (i.e., the urine sample for NC should be taken within the 2 to 6 hours post T0 range).

If the NC assessment is negative (AKIRisk score ≤ 0.3 **(ng/mL)^2/1000** [at ~~1st~~ **first** and ~~2nd measurement~~ **second measurements**, if applicable~~]),~~**]) or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated for up to 4 times within 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour. If all the NC assessments are negative up to 22 hours after T0,** the subject cannot be randomized and will be followed up in the observational cohort (see below).

Randomized subjects will receive 3 subsequent treatments with ASP1128 or matching placebo, which will be administered intravenously through a peripheral/central catheter. The first administration of study drug will occur after randomization, as soon as possible after the positive NC. ~~Administration~~ **If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP** should be completed within 8 hours after T0. **If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but within 24 hours after T0.**

In case of reoperation on the day of surgery, the same time range for NC and drug administration applies in relation to T0 **(i.e., NC between 2 and 22 hours and first investigational drug administration within 24 hours).** A reoperation is no reason for not enrolling a subject as long as the NC can be performed at the described time points.

Visits 3, 4 and 5

~~The second~~ **If the first dose of IP** administration ~~of study drug~~ **is completed within 8 hours after T0, the second dose of IP** will ~~occur the day after surgery:~~ **be administered at** 24 hours after T0 ~~(visit 3). The~~ **and the** third ~~administration~~ **dose of IP** will ~~occur~~ **be** at 48 hours after T0 ~~(visit 4)~~**. 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 IP.** A ~~margin~~ **window** of 2 hours before and after the designated time is allowed. Applicable

assessments will be done as per the Schedule of Assessments [Table 1] up to 72 hours after T0 (visit 5) to assess the study endpoints, including AKI (based on SCr and UO). When the subject is in the ICU, UO per 12 hours and total fluid intake per 24 hours (including blood products and intravenous fluid input) must be recorded. When the subjects are in the ICU, the time to ventilator weaning will be recorded.

Observational Cohort

If at randomization NC is negative (AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ ~~at t = 4 hours [range 2 to 6 hours; at 1st and 2nd measurement, if applicable]~~) **at all assessments between 2 and 22 hours after T0**, the subject cannot be randomized. These subjects will be included in the observational cohort. The rationale for following the ~~randomization failures~~ **subjects in the observational cohort** is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. **The observational cohort will enroll a maximum of 440 subjects.**

IV Synopsis, Duration of Treatment

WAS:

ASP1128/placebo will be administered on day 1 (day of surgery; within 8 hours after T0), day 2 (24 hours after T0) and day 3 (48 hours after T0).

IS AMENDED TO:

ASP1128/placebo will be administered ~~on day 1 (day~~ **as soon as possible after the NC AKIRisk score is assessed between 2 and 22 hours after T0 is > 0.3 $(ng/mL)^2/1000$. If NC is positive in the initial 2 to 6 hours post T0 period, the first dose** of ~~surgery;~~ **IP should be completed** within 8 hours after T0~~), day 2 (~~**. If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but 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 at 24 hours after T0 and ~~day 3 (~~ **the third dose of IP will be 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 at 16 hours after the start of the first dose, and the third dose of IP will be at 40 hours after the start of the first dose IP.

For the second and third dose, a 2-hour treatment window before or after designated administration time is allowed.

IV Synopsis, Endpoints for Evaluation and 2 Study Objective(s), Design and Endpoints *2.3.1 Primary Endpoints, 2.3.2 Secondary Endpoints and 2.3.3 Exploratory Endpoints*

WAS:

Primary:

Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline (i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] from baseline within 48 hours after T0, or increase in SCr to ≥ 1.5 times baseline within 72 hours after T0) (AKI-SCr72h)

Secondary:

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline (i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] within any 48 hours, or increase in SCr to ≥ 1.5 times baseline) within 7 days after T0 (AKI-SCr7d)
- Proportion of subjects developing AKI based on all captured criteria from the KDIGO guideline (i.e., AKI-SCr stage 1 to 3: increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] from baseline within 48 hours after T0, increase in SCr to ≥ 1.5 times baseline within 72 hours after T0, and/or AKI-UO stage 2 and 3: urine volume < 0.5 mL/kg per hour for 12 consecutive hours within 72 hours after T0) (AKI-KDIGO72h)

Exploratory:

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the Acute Kidney Injury Network (AKIN) guideline (i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] from baseline, or increase in SCr to ≥ 1.5 times baseline) within 5 days after T0 (AKIN AKI-SCr5d)

IS AMENDED TO:

Primary:

Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline (i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] ~~from baseline~~ within **any** 48 hours ~~after T0~~, or increase in SCr to ≥ 1.5 times baseline**)** within 72 hours after T0~~)~~ (AKI-SCr72h).

Secondary:

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the KDIGO guideline ~~(i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] within any 48 hours, or increase in SCr to ≥ 1.5 times baseline)~~ within 7 days after T0 (AKI-SCr7d)
- Proportion of subjects developing AKI based on all captured criteria from the KDIGO guideline (i.e., AKI-SCr stage 1 to 3: increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] ~~from baseline~~ within **any** 48 hours ~~after T0~~, increase in SCr to ≥ 1.5 times baseline ~~within 72 hours after T0~~, and/or AKI-UO stage 2 and 3: urine volume < 0.5 mL/kg per hour for 12 consecutive hours**)** within 72 hours after T0~~)~~ (AKI-KDIGO72h)

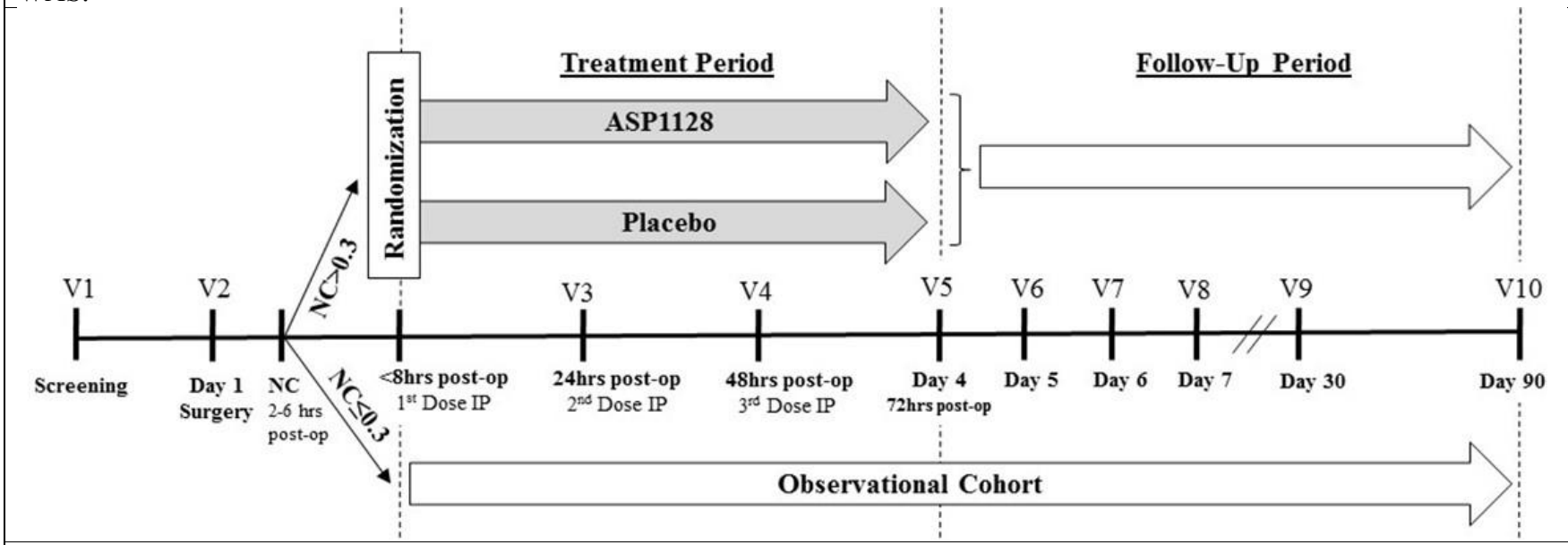
Exploratory:

- Proportion of subjects developing AKI (any stage) based on SCr criteria from the ~~Acute Kidney Injury Network (AKIN)~~ **KDIGO** guideline ~~(i.e., increase in SCr ≥ 0.3 mg/dL [≥ 26.5 µmol/L] from baseline, or increase in SCr to ≥ 1.5 times baseline)~~ within 5 days after T0 (~~AKIN~~ AKI-SCr5d)
- **Proportion of subjects developing AKI based on all criteria from the KDIGO guideline within 5 days after T0 (AKI-KDIGO5d)**

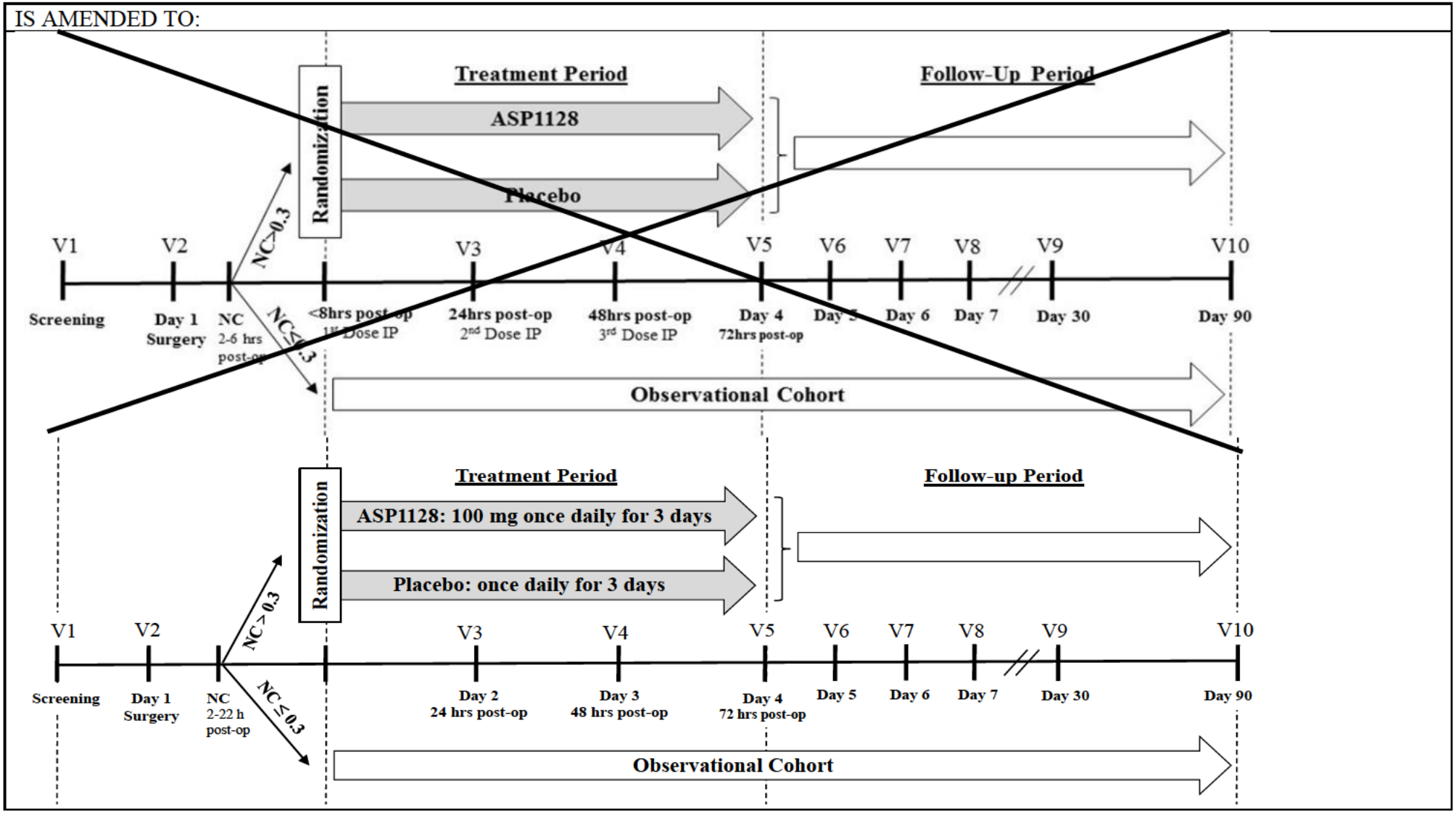
V. Flow Chart and Schedule of Assessments

Figure 1 Flow Chart

WAS:



IS AMENDED TO:



V. Flow Chart and Schedule of Assessments

Table 1 Schedule of Assessments — Randomized Subjects

WAS:

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	X			X	X	X	X	X	X	X	X	O
Pregnancy test	X	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
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											

5. Subjects will only be randomized if postoperative NephroCheck® (NC) AKIRisk® score (measured 2 to 6 hours after T0) is > 0.3. If the AKIRisk score is ≤ 0.3 (up to 2 times during the 2- to 6-hour time point postsurgery), 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.
10. Dosing of the first dose of investigational product/study drug to be administered as soon as possible after NC AKIRisk score is > 0.3 and up to 8 hours after T0. Dosing of the 2nd and 3rd investigational product: a 2-hour treatment window before or after designated administration time is allowed.
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 on visit 2, at end of infusion on visit 3, and at predose and at end of infusion on visit 4.
16. Blood samples for gene expression measurements will be collected at predose and 2 to 4 hours after study drug administration on visits 2 and 4.
17. Visit 1 can be close to the day of surgery. If the results from the central laboratory testing of AST, 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.

IS AMENDED TO:

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~~ **X**[17]	X			X	X	X	X	X	X	X	X	O
Pregnancy test	~~X~~ **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	X
Assess follow-up parameters[13]				X	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												

5. Subjects will only be randomized if postoperative NephroCheck® (NC) AKIRisk® score (~~measured~~ **assessed between** 2 to ~~6~~ **22** hours after T0) is > 0.3 **(ng/mL)²/1000.** If the AKIRisk score is ≤ 0.3 ~~(up to 2 times during the 2- to 6-hour time point postsurgery),~~ **(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.
10. ~~Dosing of the~~ **The** first dose of investigational product **(IP)/**study drug to be administered as soon as possible after NC AKIRisk score **assessed between 2 and 22 hours after T0** is > 0.3 ~~and up~~ **(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~~. Dosing of,~~ the ~~2nd~~ **second dose of IP will be administered at 24 hours after T0** and ~~3rd investigational product~~ **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.
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 ~~on visit 2~~ **of first administration**, at end of infusion ~~on visit 3~~ **of second administration**, and at predose and at end of infusion ~~on visit 4~~.**of third administration.**
16. Blood samples for gene expression measurements will be collected at predose and 2 to 4 hours ~~after study drug administration on visits 2 and 4.~~**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 AST, 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.**

V. Flow Chart and Schedule of Assessments

Table 2 Schedule of Assessments – Subjects in the Observational Cohort

WAS:

Study Period	Screening	Surgery			Follow-up Period					Unscheduled
Visit Number	1	2[1]		3	4	5	8	9[12]	10/EoS[12]	
Day	-28	pre	peri[2]	post	2	3	4	7	30	90	
Visit Window	≥ -28 days	-	-	-				-	23 to 37	83 to 97	
Assessment											
Informed Consent	X										
Inclusion/exclusion criteria	X										
Medical history, demographics	X										
Medication use	X	X	X	X	X[8]						
Physical examination	X[13]										
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]	X[3]						X[9]	X[9]	X[9]	X[9]
Pregnancy test	X[3]	X[7]									
NephroCheck®				X	X[11]	X[11]	X[11]				
Biomarkers[4]		X		X	X[11]	X[11]	X[11]				
Assessment of AEs		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; 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 AST, 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.
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.
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. Urine sampling only for NephroCheck® 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.
12. 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.
13. 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.

IS AMENDED TO:

Study Period	Screening	Surgery			Follow-up Period						Unscheduled
Visit Number	1	2[1]			3	4	5	8	~~9[12]~~ **13**	10/EoS~~[12]~~ **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~~[13]~~ **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~~[11]~~ **12**	X~~[11]~~ **12**	X~~[11]~~ **12**				
Biomarkers[4]		X		X	X~~[11]~~ **12**	X~~[11]~~ **12**	X~~[11]~~ **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 AST, 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.**

~~11~~ **12**. Urine sampling only for ~~NephroCheck®~~ **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.

~~12~~ **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.

~~13~~ **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.

2 Study Objective(s), Design and Endpoints
2.2.1 Study Design

WAS:

Visit 2/Day of Surgery

Four hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0) a urine sample will be collected and the concentration of tissue inhibitor of metalloproteinase-2 (TIMP2) and insulin-like growth factor binding protein-7 (IGFBP7) will be assessed utilizing the NC device as per the package insert instructions. The urine sample should be fresh (i.e., not from accumulated urine in the bag so the bag should be emptied before the sample is taken); it can be obtained in the operating room/theater, recovery room or in the intensive care unit (ICU), as long as it is within the range of 2 to 6 hours post T0. For urine [TIMP-2] * [IGFBP7], a cutoff of 0.3 yielded good sensitivity and specificity at 4 hours after CPB [Meersch et al, 2014] and constituted an increased risk for developing AKI [Meersch et al, 2017]. The NC device will calculate the AKIRisk score. If NC is positive, i.e., the result of the AKIRisk score is > 0.3 $(ng/ml)^2/1000$, the subject will be randomized and start treatment as per randomization schedule.

If the result of the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ the NC assessment should be redone once only if within the 2- to 6-hour window after T0 (i.e., the urine sample for NC should be taken within the 2 to 6 hours post T0 range).

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ (at 1st and 2nd measurement, if applicable) , the subject cannot be randomized and will be followed up in the observational cohort (see below).

Randomized subjects will receive 3 subsequent treatments with ASP1128 or matching placebo, which will be applied intravenously through a peripheral/central catheter. The first administration of study drug will occur after randomization, as soon as possible after the positive NC. Administration should be completed within 8 hours after T0. The limit of 8 hours is set to provide the best window of induction of ASP1128 effect and development of tubular cell damage and AKI.

In case of reoperation on the day of surgery (e.g., for immediate surgical complications like ongoing hemorrhage), the same time range for NC and drug administration applies in relation to T0 (i.e., end of CPB of initial operation). A reoperation is no reason for not enrolling a subject as long as the NC can be performed at the described time points. If for any reason the AKIRisk score cannot be assessed with the 2 to 6 hours from T0 the subject cannot be randomized and will be a screen failure. If the subject is randomized, but for any reason the first administration of the study drug cannot be done within 8 hours after T0, the subject should not be withdrawn from the study as an intention-to-treat principle. The indication of reoperation will be recorded as an adverse event (AE) in the electronic case report form (eCRF) as well as the (reason for) missed drug administration.

The second administration of study drug will occur the day after surgery: 24 hours after T0 (visit 3). The third administration will be given at 48 hours after T0 (visit 4). Applicable

assessments will occur as per the Schedule of Assessments [Table 1] up to 72 hours after T0 (visit 5) to assess the study endpoints, including AKI (based on SCr and UO). When the subject is in the ICU, UO per 12 hours and total fluid intake per 24 hours (including blood products and intravenous fluid input) must be recorded.

In case of reoperation, or any other event that interferes with study drug administration, at the time of second or third administration of study drug, the subject will not be discontinued. A margin of 2 hours before and after the designated time is allowed. The indication of reoperation will be recorded as an AE in the eCRF, as well as the (reason for) missed drug administration or changed drug administration time if applicable.

Observational Cohort

If at randomization NC is negative (AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ at t = 4 hours [range 2 to 6 hours; at 1st and 2nd measurement, if applicable]), the subject cannot be randomized. These subjects will be included in the observational cohort. The aim of following up the randomization failures is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. Clinical endpoints will be investigated in the subgroups defined by AKIRisk score at randomization and other baseline risk factors, in the population of the NC positive subjects, who are randomized to the active or the placebo group, and the NC negative subjects, who are in the observational cohort. The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (acute kidney injury based on serum creatinine KDIGO criteria [AKI-SCr] using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Hospital and ICU stay will also be recorded. AEs and medication use will be recorded until 24 hours after T0. Urine samples will be collected for NephroCheck® (NC) and biomarker assessments. If available, local laboratory test results and 24-hour UO data will be collected from medical records. No additional blood samples will be taken for study purposes after visit 2. See Schedule of Assessments [Table 2].

IS AMENDED TO:

Visit 2/Day of Surgery

Four hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0) a urine sample will be collected and the concentration of tissue inhibitor of metalloproteinase-2 (TIMP2) and insulin-like growth factor binding protein-7 (IGFBP7) will be assessed utilizing the NC device as per the package insert instructions. The urine sample should be fresh (i.e., not from accumulated urine in the bag so the bag should be emptied before the sample is taken); it can be obtained in the operating room/theater, recovery room or in the intensive care unit (ICU), as long as it is within the range of 2 to 6 hours post T0. For urine [TIMP-2] * [IGFBP7], a cutoff of 0.3 yielded good sensitivity and specificity at 4 hours after CPB [Meersch et al, 2014] and constituted an increased risk for developing AKI [Meersch et al, 2017]. The NC device will calculate the AKIRisk score. If NC is positive, i.e., the result of the AKIRisk score is > 0.3 $(ng/ml)^2/1000$, the subject will be randomized and start treatment

within 8 hours after T0 as per randomization schedule.

If the **first** result of the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ the NC assessment should be redone once only if within the 2- to 6-hour window after T0 (i.e., the urine sample for NC should be taken within the 2 to 6 hours post T0 range).

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ (at ~~1st~~ **first** and ~~2nd~~ **second** measurement, if applicable) **or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated up to 4 times until 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour. If all the NC assessments are negative up to 22 hours after T0,** the subject cannot be randomized and will be followed up in the observational cohort (see below).

Randomized subjects will receive 3 subsequent treatments with ASP1128 or matching placebo, which will be applied intravenously through a peripheral/central catheter. The first administration of study drug will occur after randomization, as soon as possible after the positive NC. ~~Administration~~ **If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP** should be completed within 8 hours after T0. ~~The limit of 8 hours is set to provide the best window of induction of ASP1128 effect and development of tubular cell damage and AKI.~~ **If NC is positive in any of the additional NC measurements within 22 hours after T0, the first dose should be completed as soon as possible but within 24 hours after T0.**

In case of reoperation on the day of surgery (e.g., for immediate surgical complications like ongoing hemorrhage), the same time range for NC and drug administration applies in relation to T0 (i.e., ~~end of CPB of initial operation~~ **NC between 2 and 22 hours and first investigational drug administration within 24 hours**). A reoperation is no reason for not enrolling a subject as long as the NC can be performed at the described time points. If for any reason the AKIRisk score cannot be assessed ~~with~~ **within** the 2 to ~~6~~ **22** hours from T0 the subject cannot be randomized and will be a screen failure. If the subject is randomized, but for any reason the first administration of the study drug cannot be done within ~~8~~ **24** hours after T0, the subject should not be withdrawn from the study as an intention-to-treat principle. The indication of reoperation will be recorded as an adverse event (AE) in the electronic case report form (eCRF) as well as the (reason for) missed drug administration.

~~The second administration of study drug will occur the day after surgery: 24 hours after T0 (visit 3). The third administration will be given at 48 hours after T0 (visit 4). Applicable~~ **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.** Applicable assessments will occur as per the Schedule of Assessments [Table 1] up to 72 hours after T0 (visit 5) to assess the study endpoints, including AKI (based on SCr and UO). When the subject is in the ICU, UO per 12 hours and total fluid intake per 24 hours (including blood products and intravenous fluid input) must be

recorded.

In case of reoperation, or any other event that interferes with study drug administration, at the time of second or third administration of study drug, the subject will not be discontinued. A ~~margin~~ **window** of 2 hours before and after the designated time is allowed. The indication of reoperation will be recorded as an AE in the eCRF, as well as the (reason for) missed drug administration or changed drug administration time if applicable.

Observational Cohort

If at randomization NC is negative (AKIRisk score is ≤ 0.3 $(ng/mL)^2/1000$ at ~~t = 4 hours [range~~ **all assessments between** 2 ~~to 6 hours; at 1st~~ and ~~2nd measurement, if applicable]~~), **22 hours after T0),** the subject cannot be randomized. These subjects will be included in the observational cohort. **The observational cohort will enroll a maximum of 440 subjects.** The aim of following up the ~~randomization failures~~ **subjects in the observational cohort** is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. Clinical endpoints will be investigated in the subgroups defined by AKIRisk score at randomization and other baseline risk factors, in the population of the NC positive subjects, who are randomized to the active or the placebo group, and the NC negative subjects, who are in the observational cohort. The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (acute kidney injury based on serum creatinine KDIGO criteria [AKI-SCr] using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Hospital and ICU stay will also be recorded. AEs and medication use will be recorded until 24 hours after T0. Urine samples will be collected for NephroCheck® (NC) and biomarker assessments. If available, local laboratory test results and 24-hour UO data will be collected from medical records. No additional blood samples will be taken for study purposes after visit 2. See Schedule of Assessments [Table 2].

2 Study Objective(s), Design and Endpoints

2.2.2 Dose Rationale

WAS:

Study treatment will be administered 3 times on day 1 (day of surgery; within 8 hours after T0), day 2 (24 hours after T0) and day 3 (48 hours after T0).

IS AMENDED TO:

Study treatment will be administered 3 times ~~on day 1 (day of surgery; within 8 hours after T0), day 2 (24 hours after T0) and day 3 (48 hours after T0).~~ **as follows:**

- **As soon as possible after NC AKIRisk score assessed between 2 and 22 hours after T0 is > 0.3 $(ng/mL)^2/1000$. If NC is positive in the initial 2 to 6 hours post T0 period, the first dose of IP should be completed within 8 hours after T0. If NC is positive in any of the additional NC measurements within 22 hours after T0,**

the first dose should be completed as soon as possible but 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.**
- **For the second and third dose, a 2-hour treatment window before or after designated administration time is allowed.**

4 Treatment(s)
4.5 Assignment and Allocation

WAS:

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

IS AMENDED TO:

Subjects with NC AKIRisk score of 0.3 **$(ng/mL)^2/1000$** or less will be enrolled in the observational cohort. **The observational cohort will enroll a maximum of 440 subjects.**

5 Treatments and Evaluation
5.1.1.1 Randomized Subjects

WAS:

Assessments required on the day of administration of study drug except for blood sampling for pharmacokinetics (i.e., vital signs, blood and urine sampling, UO and NC) will be finished prior to the administration.

Randomized subjects will receive 3 subsequent treatments with 100 mg of ASP1128 or matching placebo. The study drugs will be supplied as solution in 20 mL vial, which contains 10 mL of 5 mg/mL ASP1128 or 10 mL of matching placebo. The study drugs will be applied intravenously through a peripheral/central catheter. The catheter should remain until the last administration ends. The first administration of study drug will occur after randomization, as soon as possible after positive NC and ultimately within 8 hours after T0. The second administration of study drug will be given at 24 hours with a range of 2 hours before and after (i.e., 22 to 26 hours after T0 [visit 3]). The third administration will be given at 48 hours with a range of 2 hours before and after (i.e., 46 to 50 hours after T0 [visit 4]).

IS AMENDED TO:

~~Assessments required on the day of administration of study drug except for blood sampling for pharmacokinetics (i.e., vital signs, blood and urine sampling, UO and NC) will be~~

~~finished prior to the administration.~~

Randomized subjects will receive 3 subsequent treatments with 100 mg of ASP1128 or matching placebo. The study drugs will be supplied as solution in 20 mL vial, which contains 10 mL of 5 mg/mL ASP1128 or 10 mL of matching placebo. The study drugs will be applied intravenously through a peripheral/central catheter. The catheter should remain until the last administration ends. The first administration of study drug will occur after randomization, as soon as possible after positive NC ~~and ultimately within 8 hours after T0~~. ~~The second~~ **If the first dose of IP** administration ~~of study drug~~ **is completed within 8 hours after T0, the second dose of IP** will be ~~given~~ **administered** at 24 hours ~~with a range of 2 hours before~~ **after T0** and ~~after (i.e., 22 to 26 hours after T0 [visit 3]). The~~ **the** third **dose of IP will be administered at 48 hours after T0. If the first dose of IP** administration ~~will be given at 48 hours with a range of 2 hours before and after (i.e., 46 to 50~~ **is completed past 8** hours after T0 ~~[visit 4]).~~**, 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. For the second and third dosing of IP, a 2-hour treatment window before or after the designated administration time is allowed.**

5 Treatments and Evaluation
5.1.1.2 Subjects in the Observational Cohort

WAS:

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 at t = 4 hours (range 2 to 6 hours; at 1st and 2nd measurement, if applicable), the subject cannot be randomized. These subjects will be included in the observational cohort and receive no study drug administration.

IS AMENDED TO:

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 ~~at t = 4~~ **(ng/mL)²/1000 at all assessments between 2 and 22** hours ~~(range 2 to 6 hours; at 1st and 2nd measurement, if applicable),~~ **after T0,** the subject cannot be randomized. These subjects will be included in the observational cohort and receive no study drug administration.

5 Treatments and Evaluation
5.1.3.1 Prohibited and Restricted Treatment

WAS:

Following surgery up to 72 hours postsurgery the use of nonsteroidal anti-inflammatory drugs [NSAIDs], angiotensin-converting enzyme [ACE] inhibitors and angiotensin receptor blockers is prohibited because of their potentially confounding effect on kidney function.

IS AMENDED TO:

Following surgery up to 72 hours postsurgery the use of nonsteroidal anti-inflammatory

drugs [NSAIDs], angiotensin-converting enzyme [ACE] inhibitors and angiotensin receptor blockers is prohibited because of their potentially confounding effect on kidney function**.**

After the subject is enrolled in the observational cohort, these medications are not prohibited if clinically indicated.

5 Treatments and Evaluation
5.2.3 Diagnosis of the Target Disease, Severity and Duration of Disease

WAS:

A risk of developing AKI following surgery will be assessed at screening (visit 1) according to inclusion criteria #5 based on AKI risk factors. Further risk assessment will be done after the surgery at visit 2: 4 hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0) urine will be collected for an examination by the NC to calculate the AKIRisk score, which indicates the risk of tubular damage that could lead to AKI. If the AKIRisk score is > 0.3 $(ng/ml)^2/1000$, the subject will be randomized and start treatment as per randomization schedule. If the AKIRisk score is 0.3 or less, the NC assessment should be redone once only if within the 2- to 6-hour window after T0. If the NC is negative, i.e., the AKIRisk score is 0.3 or less (at 1st and 2nd measurement, if applicable) the subject cannot be randomized and will be followed up in the observational cohort.

IS AMENDED TO:

A risk of developing AKI following surgery will be assessed at screening (visit 1) according to inclusion criteria #5 based on AKI risk factors. Further risk assessment will be done after the surgery at visit 2: 4 hours (with a range of 2 hours before and after) postsurgery (i.e., 2 to 6 hours after T0) urine will be collected for an examination by the NC to calculate the AKIRisk score, which indicates the risk of tubular damage that could lead to AKI. If the AKIRisk score is > 0.3 $(ng/ml)^2/1000$, the subject will be randomized and start treatment as per randomization schedule. If the AKIRisk score is 0.3 **$(ng/mL)^2/1000$** or less, the NC assessment should be redone once only if within the 2- to 6-hour window after T0. If the NC is negative, i.e., the AKIRisk score is 0.3 **$(ng/mL)^2/1000$** or less (at ~~1st~~ **first** and ~~2nd~~ **second** measurement, if applicable) **or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated up to 4 times until 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour. If all the NC assessments are negative up to 22 hours after T0,** the subject cannot be randomized and will be followed up in the observational cohort.

5 Treatments and Evaluation
5.3.2 Pharmacokinetic Assessments

WAS:

Blood samples will be collected for pharmacokinetic analysis of ASP1128 as indicated in the Schedules of Assessments [Table 1]. Each sample should be collected before a meal. The

sample time schedule is as the followings: ● Visit 2: predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose ● Visit 3: at end of infusion ● Visit 4: predose and at end of infusion
IS AMENDED TO:
Blood samples will be collected for pharmacokinetic analysis of ASP1128 as indicated in the Schedules of Assessments [Table 1]. Each sample should be collected before a meal~~.~~ **as much as possible and fed or fasted state will be recorded in the eCRF. Definition of fasting is at least 10 hours passed after subject last ate.** The sample time schedule is as the followings: ● ~~Visit 2~~ **First administration**: predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose ● ~~Visit 3~~ **Second administration**: at end of infusion ● ~~Visit 4~~ **Third administration**: predose and at end of infusion

5 Treatments and Evaluation *5.3.3 Pharmacodynamic Assessments*
WAS:
The sample time schedule is as follows: ● Visit 2: predose and 2 to 4 hours after study drug administration ● Visit 4: predose and 2 to 4 hours after study drug administration
IS AMENDED TO:
The sample time schedule is as follows: ● ~~Visit 2~~**First administration**: predose and 2 to 4 hours after study drug administration ● ~~Visit 4~~**Third administration**: predose and 2 to 4 hours after study drug administration

5 Treatments and Evaluation *5.4.2 Laboratory Assessments*
WAS:
If the results from the central laboratory testing of AST, ALT, total bilirubin and eGFR at visit 1 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.
IS AMENDED TO:
If the results from the central laboratory testing of AST, ALT, total bilirubin and eGFR at

visit 1 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.

5 Treatments and Evaluation
5.6 Test Drug Concentration

WAS:

The sample time schedule is as the followings:

- Visit 2: predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose
- Visit 3: at end of infusion
- Visit 4: predose and at end of infusion

IS AMENDED TO:

The sample time schedule is as the followings:

- ~~Visit 2~~ **First administration**: predose, at end of infusion and twice after study drug administration at 2 to 4 hours postdose and at 4 to 12 hours postdose
- ~~Visit 3~~ **Second administration**: at end of infusion
- ~~Visit 4~~ **Third administration**: predose and at end of infusion

5 Treatments and Evaluation
5.7.1 NephroCheck

WAS:

Urine samples will be collected as indicated in the Schedules of Assessments [Table 1] and [Table 2] and the concentration of TIMP2 and IGFBP7 will be assessed utilizing the NC as per the package insert instructions of the NC device. The device automatically multiplies the concentrations of the 2 biomarkers together and divides this product by 1,000 to report a single numeric test result with units of $(ng/mL)^2/1,000$ (the units for all TIMP2•IGFBP7 values in this protocol) referred to as the AKIRisk score. The urine sample should be fresh (i.e., not from accumulated urine in the bag), so the bag should be emptied before the sample is taken; on the day of surgery, it can be obtained in the operating room/theater, recovery room or in the ICU, as long as it is within the range or 2 to 6 hours post T0. A second urine sample for the assessment of the AKIRisk score can be collected within the 2 to 6 hour post T0 range and should be done if the first result was 0.3 or less [see Section 2.2.1]. The 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. In the observational

cohort the NC will be measured if the subject is still in the ICU and urine can be sampled from the catheter bag.

IS AMENDED TO:

Urine samples will be collected as indicated in the Schedules of Assessments [Table 1] and [Table 2] and the concentration of TIMP2 and IGFBP7 will be assessed utilizing the NC as per the package insert instructions of the NC device. The device automatically multiplies the concentrations of the 2 biomarkers together and divides this product by 1,000 to report a single numeric test result with units of $(ng/mL)^2/1,000$ (the units for all TIMP2•IGFBP7 values in this protocol) referred to as the AKIRisk score. The urine sample should be fresh (i.e., not from accumulated urine in the bag), so the bag should be emptied before the sample is taken; on the day of surgery, it can be obtained in the operating room/theater, recovery room or in the ICU, as long as it is within the range ~~or~~ **of** 2 to 6 hours post T0. A second urine sample for the assessment of the AKIRisk score can be collected within the 2 to 6 hour post T0 range and should be done if the first result was 0.3 **$(ng/mL)^2/1000$** or less**. If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 $(ng/ml)^2/1000$ (at first and second measurement, if applicable) or not assessable within the 2- to 6-hour window after T0, NC assessment may be repeated for up to 4 times until 22 hours after T0. Urine sampling should be done at intervals of at least 1 hour** [see Section 2.2.1]. The 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. In the observational cohort the NC will be measured if the subject is still in the ICU and urine can be sampled from the catheter bag.

5 Treatments and Evaluation
5.7.4 Subjects in the Observational Cohort

WAS:

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 at t = 4 hours (range 2 to 6 hours; at 1st and 2nd measurement, if applicable), the subject cannot be randomized. These subjects will be included in the observational cohort. The aim of following up the randomization failures is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (AKI-SCr using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Days 30 and 90 may be conducted by phone if the subject is unable to visit the site.

IS AMENDED TO:

If the NC is negative, i.e., the AKIRisk score is ≤ 0.3 ~~at t = 4~~ **$(ng/mL)^2/1000$ at all assessments between 2 and 22** hours ~~(range 2 to 6 hours; at 1st and 2nd measurement, if~~

~~applicable)~~ **after T0**, the subject cannot be randomized. These subjects will be included in the observational cohort. The aim of following up the ~~randomization failures~~ **subjects in the observational cohort** is to further evaluate the AKIRisk score, as well as other risk factors (including clinical characteristics and biomarkers) for AKI and MAKE. The subjects in this cohort can be discharged at the discretion of the investigator and will be followed up after surgery for assessment of AKI up to 7 days (AKI-SCr using SCr values from local laboratory testing and 24-hour UO to assess stage 3 AKI-UO) and for assessment of MAKE event at day 30 and day 90. Days 30 and 90 may be conducted by phone if the subject is unable to visit the site.

7 Statistical Methodology
7.6.2 Pharmacokinetic Parameters

DELETED:

~~7.6.2 Pharmacokinetic Parameters~~

~~Plasma pharmacokinetic parameters for ASP1128 will be summarized using descriptive statistics. Plasma concentration collected at end of infusion will be treated as C_{max}. Further details about the pharmacokinetic parameters will be provided in the SAP.~~

IIb. Nonsubstantial Changes

II Contact Details of Key Sponsor's Personnel

WAS:

Medical Monitor/Study Physician:	PPD Medical & Development Astellas Pharma Inc. 2-5-1, Nihonbashi-Honcho, Chuo-ku Tokyo 103-8411, Japan PPD

IS AMENDED TO:

Medical Monitor/Study Physician:	PPD Medical & Development Astellas Pharma Inc. 2-5-1, Nihonbashi-Honcho, Chuo-ku Tokyo 103-8411, Japan

	PPD

II List of Abbreviations and Definition of Key Terms *List of Abbreviations*	
WAS:	
AKIN	Acute Kidney Injury Network
IS AMENDED TO:	
~~AKIN~~	~~Acute Kidney Injury Network~~
DoD	**Day of Discharge**

IV Synopsis, Planned Study Period
WAS:
From 2Q2019 to 4Q2020
IS AMENDED TO:
From 2Q2019 to ~~4Q2020~~ **1Q2022**

IV Synopsis, Planned Total Number of Study Centers and Location(s)
WAS:
Approximately 40 centers in North America
IS AMENDED TO:
Approximately ~~40~~ **50** centers in North America

12. Appendices *12.8 Clinical Study Continuity*
ADDED:
12.8 Clinical Study Continuity **INTRODUCTION** **The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).** **BENEFIT-RISK RATIONALE**

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated Schedule of Assessments/Table 1 unless the site principal investigator discusses the need with the Astellas Medical Monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed as described in Section 5 due to a crisis.

INFORMED CONSENT

Participants who need to follow any or all of the alternate measures outlined in this Appendix will be required to provide informed consent which explicitly informs them of the nature of, and rationale for these changes, and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the principal investigator or designee will obtain oral agreement from the subject followed by written documentation as soon as is feasible. A separate addendum to the study informed consent will be provided to document the participant's consent of the changes.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if one or more of the following conditions are met due to the crisis:

- **Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible**
- **Site facilities have been closed for clinical study conduct**
- **Site has been restricted to treating patients with conditions outside of the scope of the study**
- **Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel**
- **Participant(s) have temporarily relocated from the current study site to an alternate study site avoid placing a burden on the participant with respect to travel**
- **Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel**
- **Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety**

Adherence to the original protocol as reflected in the Schedule of Assessment Table 1 is expected, where plausible, in the case of a crisis. The alternate measures as noted in the table below are only permissible in the event of a crisis, and after discussing the need

with the Astellas Medical Monitor and/or designee to implement the alternate measures. This is to allow for maintaining critical safety and efficacy assessments for patients participating in the study at a time of crisis.

If one or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding time point(s).

12. Appendices

12.8 Clinical Study Continuity

ADDED:

Table 9 Alternative Schedule of Assessments in Response to a Crisis — Randomized Subjects

Study Period	Screening	Surgery			Treatment Period			Follow-up Period					Unscheduled
Visit Number	1	2[1]			3	4	5	6	7	8	9[20]	10/EoS[20]	
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[21]	X[21]	X
Physical examination	X[19]												
Body weight, height	X										X[4, 22]	X[4, 22]	
Subject randomization				X[5]									
Vital signs	X	X			X	X	X			X	X[22]	X[22]	O
Hematology, biochemistry[6, 7]	X[17]	X			X	X	X	X	X	X	X[23]	X[23]	X
Urinary output[8]				X	X	X	X	X	X	X			O
Urinalysis[9]	X[17]	X			X	X	X	X	X	X	X[23]	X[23]	O
Pregnancy test	X[17]	X[18]									X[23]	X[23]	
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[21]	X[21]	X
Assess follow-up parameters[13]				X	X	X	X	X	X	X	X[21]	X[21]	X
APACHE-II[14]		X		X	X	X	X						
EQ-5D-5L	X									X	X[21]	X[21]	

Blood samples for pharmacokinetics[15]				**X**		**X**		**X**					
Blood samples for pharmacodynamics[16]				**X**				**X**					
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; 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 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).**
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 AST, 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.**
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.**
20. **In the event of crisis, the alternate measures may be implemented for study procedures and assessments only for visits 9 and 10/EoS after discussion with the Astellas Medical Monitor and/or designee.**
21. **Assess remotely. Telephone script can be available for EQ-5D-5L.**
22. **Collect local data, if available.**
23. **Alternative lab collection at a local clinic and results submitted to principal investigator.**

12. Appendices *12.8 Clinical Study Continuity*
ADDED:
DATA COLLECTION REQUIREMENTS **Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.** • **Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window, or other modifications) due to the crisis.**

14 SPONSOR'S SIGNATURES