Cover Page – CTP

Title of the trial: A randomized double-blind placebo-controlled phase 1 study on the safety, tolerability and pharmacokinetics/-dynamics of escalating single intravenous doses of AK1967 (Procizumab) in healthy male volunteers

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- CTP Version 5.0

Research protocol

AK1967 (Procizumab) phase 1 study

A randomized double-blind placebo-controlled phase 1 study on the safety, tolerability and pharmacokinetics/-dynamics of escalating single intravenous doses of AK1967 (Procizumab) in healthy male volunteers.

Version 5.0, 22nd of May 2024

Radboudumc

4TEEN4 Pharmaceuticals

CONFIDENTIAL

Title: *'A randomized double-blind placebo-controlled phase 1 study on the safety, tolerability and pharmacokinetics/-dynamics of escalating single intravenous doses of AK1967 (Procizumab) in healthy male volunteers.'*

Protocol ID	***AK1967 (Procizumab) phase 1 study***
Short title	Phase 1 safety and tolerability study of AK1967 (Procizumab) in healthy male volunteers
EU trial number	*2023-507035-37-00*
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Protocol date	*22-05-2024*
Sponsor (in Dutch: verrichter/opdrachtgever)	4TEEN4 Pharmaceuticals GmbH Neuendorfstr. 15a 16761 Hennigsdorf, Germany
Legal representative(s) of the sponsor:	Dr. Andreas Bergmann, CEO 4TEEN4 Pharmaceuticals GmbH Neuendorfstr. 15a, 16761 Hennigsdorf, Germany
Principal Investigator (in Dutch: hoofdonderzoeker/uitvoerder)	Prof. Dr. P. Pickkers, Internist-intensivist Radboud university medical center Department of Intensive Care Medicine, PO box 9101, 6525 GA Nijmegen, The Netherlands
Coordinating investigator/project leader	Drs. D.P.T. van Lier, MD Radboud university medical center Department of Intensive Care Medicine, PO box 9101, 6525 GA Nijmegen, The Netherlands
Associate investigator	Dr. M. Kox, Assistant Professor Radboud university medical center Department of Intensive Care Medicine, PO box 9101, 6525 GA Nijmegen, The Netherlands
Funding party	4TEEN4 Pharmaceuticals GmbH Neuendorfstr. 15a 16761 Hennigsdorf, Germany
Laboratory sites	Department of Clinical Chemistry Radboud university medical center Postbus 9101, 6500 HB Nijmegen, The Netherlands
Pharmacy	Department of Clinical Pharmacy Radboud university medical center Postbus 9101, 6500 HB Nijmegen, The Netherlands

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or Legal representative: Dr. A. Bergmann, PhD CEO, 4TEEN4 Pharmaceuticals GmbH	DocuSigned by: Andreas Bergmann Name des Unterzeichners: Andreas Bergmann Signiergrund: Ich genehmige dieses Dokument Signierzeit: 6/11/2024 | 1:35:37 AM PDT	6/11/2024
Head of Department: Prof. dr. L. Heunks, MD Department of Intensive Care Medicine	DocuSigned by: 85BBA4A5EC754915B940459E6FC56908 Leo Heunks Naam ondertekenaar: Leo Heunks Reden voor ondertekening: Ik keur dit document goed Ondertekentijd: 6/11/2024 | 1:33:06 AM PDT	6/11/2024
Principal Investigator: Prof. Dr. P. Pickkers, MD Department of Intensive Care Medicine	DocuSigned by: EF06CF9FE2384B778F89520A6A2C6197 Peter Pickkers Naam ondertekenaar: Peter Pickkers Reden voor ondertekening: Ik keur dit document goed Ondertekentijd: 6/11/2024 | 10:33:49 AM CEST	6/11/2024
Coordinating Investigator: D.P.T. van Lier, MD PhD candidate, department of Intensive Care Medicine	DocuSigned by: DF3F9B42DA4750A9E41741FA130D29 Dirk van Lier Naam ondertekenaar: Dirk van Lier Reden voor ondertekening: Ik keur dit document goed Ondertekentijd: 6/21/2024 | 3:42:50 AM PDT DD636F46C69F49FF84DFFD6ABC03D92D	6/21/2024

DOCUMENT HISTORY

Document	Date of version	Summary of Changes
Fourth revision	**22-05-2024**	Non-substantial modifications: - correction of typographic error: dose in the text as 1 mg/kg instead of the approved starting dose of 3 mg/kg; - correction of typographic error: (ECG-timepoint mentioned in text as 10h, not the intended 9h (= 540 min after start of IMP administration); - correction of typographic errors in table 8: clarification of pharmacokinetic and DPP3 timepoints (as stated in laboratory manual) and clarification on the RAAS assessment; - Correction of typographic error in section 10.1: minimum of 48h instead of 1 day for reflection between screening and the treatment day.
Third revision	**03-04-2024**	Non-substantial modification: correction of typographic error: (ECG-timepoint mentioned in text as 10h, not the intended 9h (= 540 min after start of IMP administration)
Second revision	**15-02-2024**	- Overnight in-hospital monitoring for up until 24 hours after IMP dosing included in the protocol - Starting dose of AK1967 changed from 1 to 3 mg/kg
First revision	**30-01-2024**	- NFU-risk category amended to moderate - Added physical follow-up 48 hours after IMP dosing - Section on permission for use of data and bodily materials beyond scope of current research updated
Original protocol	**17-11-2023**	Not applicable

CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

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Declaration by the Principal Investigator

I have read this protocol and agree that it contains all the necessary details for carrying out this trial. I agree to personally conduct or supervise the trial as described herein and will complete the trial within the time designated. I verify that I am suitably qualified by my education, scientific medical training and experience to conduct the trial. Documentation of my qualifications and professional affiliations are contained in my signed and dated current Curriculum Vitae.

I will provide the supplied copies of the protocol and all information relating to pre-clinical and prior clinical experience (e.g., Investigator's Brochure; IB) to all staff in my unit who participate in this trial. I will discuss this material with them to ensure that they are fully conversant with the medical treatment in, and the conduct of, the trial, and that they will handle the data and information generated in the trial confidentially.

I agree not to start screening subjects until a duly appointed Ethics Committee (EC) has issued a favourable opinion and the Competent Authorities (CA) have approved the trial.

I have read the IB, including the potential risks and side effects of the investigational medicinal product and I agree to report adverse events which occur during the trial.

Where applicable, the information contained in the electronic Case Report Forms (eCRFs) will be transcribed from my records, reports and manuscripts. The eCRF may be the original source document for certain items. Either I, or an appointed person, will attest to the authenticity of the data and accuracy and completeness of the transcriptions by signing the eCRF.

I agree to the audit and monitoring procedures described in the protocol which involve verification of trial records against the original records. I will make available additional background data from my records at the request of government regulatory agencies, if allowed by the hospital or institution where the trial was conducted.

I certify that any laboratory appointed by my unit/site for the trial in which laboratory parameters will be determined is subject to regular external quality control.

I understand that I am obliged to provide to the sponsor for the sponsor's unrestricted use the complete results and all data generated during the trial, and that all information concerning the investigational medicinal products and sponsor's activities, such as patents, formulae, manufacturing procedures and basic, unpublished scientific data and information supplied by the sponsor are confidential and are the exclusive property of the sponsor.

I undertake only to use this information to conduct the trial and not to use it for any other purpose without the written agreement of the sponsor.

Prof. dr. Peter Pickkers
Principal Investigator

DocuSigned by:
Peter Pickkers
Naam ondertekenaar: Peter Pickkers
Reden voor ondertekening: Ik keur dit document goed
Ondertekentijd: 6/11/2024 | 10:33:54 AM CEST
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Signature

6/11/2024
Date

ABBREVIATIONS

AE	Adverse event
ALT	Alanine transaminase
ANOVA	Analysis of variance
AUC	Area under the curve
AST	Aspartate aminotransferase
ATC	Anatomic therapeutic chemical classification system
AUC	Area under the curve
AUC(0-∞)	Area under the plasma concentration-time curve from time zero to infinity
BMI	Body mass index
BP	Blood pressure
CL	Total body clearance
CLP	Cecal ligation and puncture
C_{max}	Maximum concentration
eCRF	(electronic) Case report form
CTA	Clinical trial authorization
DPP3	Dipeptidyl Peptidase 3
cDPP3	Circulating Dipeptidyl Peptidase 3
DRF	Dose-range finding
DSMB	Data safety monitoring board
EC	Ethics committee
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EU	European union
EudraCT	European union drug regulating authorities clinical trials
FDA	Food and drug administration
g	Gram
GCP	Good clinical practice
GFR	Glomerular filtration rate
GMP	Good manufacturing practice
h	Hour
hDPP3	Human DPP3
HED	Human equivalent dose
HR	Heart rate
RBF	Renal blood flow
IB	Investigator's brochure
IFN	Interferon
IL	Interleukin
IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
i.v.	Intravenous
KD	Dissociation constant
kDa	Kilo Dalton

LPS	Lipopolysaccharide
µmol	Micromol
MAP	Mean Arterial Blood Pressure
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
mmHg	Millimeter mercury
MRSD	Maximum recommended starting dose
MTD	Maximum tolerated dose
NFU	Netherlands Federation of Universities
NHP	Non-human primate
nM	Nanomolar
NOAEL	No observable adverse effect level
PD	Pharmacodynamic
pH	Negative log of hydrogen ion concentration
PI	Principal Investigator
PK	Pharmacokinetic(s)
PQ (PR)	PQ interval of ECG
QRS	QRS complex of ECG
QT	QT interval of ECG
QTc	QT interval corrected for heart rate
QTcB	QTc interval corrected for heart rate using Bazett's formula
SAE	Serious adverse event
S(m)PC	Summary of (medicinal) Product Characteristics
SUSAR	Suspected unexpected serious adverse reaction
TEAEs	Treatment-Emergent Adverse Events
Tmax	Time at maximum concentration
TNF	Tumor necrosis factor
t1/2	Terminal elimination half-life
ULN	Upper limit of normal
VD	Volume of distribution
WBC	White blood cell
WHO	World health organization
WMO	Medical Research Involving Human Subjects Act (Wet Medisch-wetenschappelijk onderzoek met Mensen)

1. SYNOPSIS

Title: *'A randomized double-blind placebo-controlled phase 1 study on the safety, tolerability and pharmacokinetics/-dynamics of escalating single intravenous doses of AK1967 (Procizumab) in healthy male volunteers.'*

EU trial number: 2023-507035-37-00

Rationale

Cardiogenic shock is defined as a persistent low cardiac output leading to development of multi-organ failure, often necessitating Intensive Care Unit admission for invasive cardiopulmonary supportive therapies. Although knowledge on the molecular mechanisms associated with development of cardiogenic shock have vastly increased, its mortality rate on the Intensive Care remains in excess of 50%. Thus, there is an unmet need for novel cardiogenic shock therapies.

Dipeptidyl peptidase 3 (DPP3) is a protease involved in the degradation of several cardiovascular mediators. During cardiogenic shock, upregulation of the vasoconstrictive molecule angiotensin II is a physiologic and potentially life-saving response aimed at maintaining adequate tissue perfusion. As circulating (c)DPP3 is able to effectively cleave angiotensin II, it may represent a novel factor contributing to hemodynamic instability during cardiogenic shock.

Recently, a cDPP3-antagonizing antibody called AK1967 (commonly referred to as Procizumab) has been developed. In animal models of cardiogenic- and septic shock, inhibition of cDPP3 by AK1967 resulted in improved cardiac function and survival. Furthermore, AK1967 has shown an excellent safety record in different preclinical studies. In the current study, we intend to investigate the safety, tolerability and pharmacokinetics/-dynamics of AK1967 in a 'first-in-human study' in healthy male subjects.

Objective

Primary Objective:
1. To assess the safety and tolerability of single escalating doses of AK1967 in healthy male volunteers.

Secondary Objectives:
2. To determine the pharmacokinetics of single escalating doses of AK1967 in healthy male volunteers.
3. To determine the effects of AK1967 on cDPP3 concentrations and activity (pharmacodynamics) in healthy male volunteers.
4. To determine the effects of AK1967 on circulating angiotensin metabolite concentrations (pharmacodynamics) in healthy male volunteers.
5. To determine the effects of AK1967 on circulating adrenergic metabolite concentrations (pharmacodynamics) in healthy male volunteers.
6. To determine the effects of AK1967 on inflammatory cytokines, including, but not limited to TNF, IL-6, IL-8, IL-10.

Main trial endpoints

The main trial endpoint is safety and tolerability of AK1967, which is defined as differences between the placebo group and different AK1967 dosage groups in:

- Reported number of adverse events from baseline (start of IMP administration) up until the last follow-up visit 28 days after IMP administration.
- Vital signs during the first 24 hours after start of IMP administration, as well as during the six follow-up visits. Vital signs included are: heart rate, blood pressure, peripheral oxygen saturation and body temperature.
- Local tolerability at site of i.v. infusion of the IMP.
- Safety laboratory parameters from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration. Laboratory parameters included are: Hb, Ht, leukocytes, thrombocytes, leukocyte differential blood count, sodium, potassium, creatinine, urea, alkaline phosphatase, ALT, AST, bilirubin, GGT, CK, CRP, PT, APTT, fibrinogen.
- 12-lead electrocardiogram (ECG) at baseline (screening), compared to ECG's performed 2 hours, 9 hours and 7 days after start of IMP administration.

Secondary trial endpoints

Secondary study endpoints include different pharmacokinetic/dynamic effects of AK1967, which are defined as differences between different AK1967 dosage groups and/or the placebo group in;

- Pharmacokinetics of AK1967 (including AUC, C_{max}, Terminal $T_{1/2}$, Cl, VD) from baseline (start of IMP administration) up until the last follow-up visit 28 days after IMP administration.
- Pharmacodynamics (blood plasma levels and enzyme-activity of DPP3) from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration.
- Pharmacodynamics (blood plasma levels of angiotensin metabolites) at baseline (just prior to start of IMP administration) and at 2 and 10 hours after start of IMP administration.
- Pharmacodynamics (blood plasma levels of adrenergic metabolites) at baseline (just prior to start of IMP administration) and at 2 and 10 hours after start of IMP administration
- Plasma levels of inflammatory mediators (including but not limited to TNF, IL-6, IL-8, IL-10), measured from baseline (just prior to start of IMP administration) up until 24 hours after start of IMP administration.

Trial design

A randomized, double-blind, placebo-controlled phase 1 safety study in 24 healthy male volunteers. All subjects will receive one course of treatment with study medication, being randomized to either 3 escalating doses of AK1967 (3 groups of 6, total n=18) or placebo (n=6). In each dose group, 6 volunteers will receive AK1967 and 2 placebo. Study medication will be administered as an intravenous infusion over a 2-hour period.

Trial population

24 male volunteers of 18-35 years who did not participate in other medical trials during the last 3 months. Subjects have to be healthy, defined as no clinically relevant findings during a medical screening, which includes medical history, physical examination, vital signs, 12-lead electrocardiogram and clinical laboratory parameters.

Interventions

Subjects will visit the Intensive Care department on eight occasions (screening, the treatment day of 24 hours, and on days 2 (± 1 hour), 3 (± 3 hours), 7 (± 1 day), 14 (± 2 days), 21 (± 3 days) and 28 (± 3

days)) post-treatment. After the informed consent procedure, a medical screening will be performed. This screening includes an assessment of medical history, a physical examination, checking of vital signs, a 12-lead electrocardiogram and a blood draw for laboratory evaluations.

On the treatment day, subjects will be hospitalized at the Research Intensive Care Unit for approximately 26 hours (taking preparation time prior to administration of the IMP into account). There will be continuous monitoring of symptoms, (intra-arterial) blood pressure, SpO_2 (%) and heart rate (ECG). Subjects will receive one venous catheter in the forearm. An arterial cannula will be placed in the radial artery, following local anesthesia using a lidocaine 1% solution for injection. Subjects will receive a single dose of study medication (AK1967 3, 6, or 12 mg/kg, or placebo), administered as an intravenous infusion over a 2-hour period. During the treatment day, serial blood samples of relevant pharmacokinetic and pharmacodynamic outcome parameters will be drawn from the arterial cannula. A 12-lead electrocardiogram will be performed 2 and 9 hours after start of IMP administration. Approximately 12 hours after initiation of drug administration, cannulas will be removed, and a pressure bandage will be applied to the site of arterial cannulation. Subjects stay overnight at the research Intensive Care Unit for continuous monitoring of vital signs. In the morning, a venous blood withdrawal is performed approximately 24 hours after start of IMP administration. Afterwards, the pressure bandage will be removed and subjects are released from the research unit. The subjects will return an additional 6 times for follow-up visits over the next 28 days (subjects will be checked for adverse events and blood samples will be obtained via venipuncture).

Ethical considerations relating to the clinical trial including the expected benefit to the individual subject or group of patients represented by the trial subjects as well as the nature and extent of burden and risks

Total time burden for the study is approximately 30 hours: 1 hour for screening, 26 hours for the admission/treatment day, and 6 follow-up visits of approximately 30 minutes. Volunteers will be recruited and are subject to a medical examination (including interview, medical history, blood withdrawal and physical examination). Cumulative blood withdrawal during the study is restricted to a smaller volume (<450 mL) than is withdrawn during routine phlebotomy at the blood bank, and is not associated with relevant risks. Venipunctures and vascular access at the several study visits carries the risk of hematoma at the puncture sites, which will resolve spontaneously, should they occur. Blood loss from puncture sites after removal of cannulas will be stopped by applying pressure. A pressure bandage will be applied to the site of arterial cannulation. Also, vasovagal reactions may occur during a puncture procedure, which can be adequately treated.

AK1967 is a recombinant monoclonal antibody against DPP3, that inhibits the enzymatic activity of DPP3 as part of its mode of action. AK1967 binds to a conserved, surface exposed loop in proximity to DPP3's active site, which is conserved in all mammals. The humanized version of the antibody was implemented in all preclinical experiments, effectively demonstrating that AK1967's primary mechanism (binding and inactivation of circulating DPP3) was closely matched between all investigated species.

The administration of AK1967 to rodents and non-human primates (NHP) has been tolerated very well. Single dose administration up to 150 mg/kg AK1967 to mice and 400 mg/kg to NHP have not shown any clinical adverse effects and no abnormal histopathological findings. Also the repeated administrations of up to 350 mg/kg in NHPs (administration at days 1, 2, 13 & 14) within the

regulatory pre-clinical toxicity and safety study have not led to any clinical adverse effects or abnormal histopathological findings.

Subjects will not directly benefit from participation to the study. The total risks to the subjects in this study is classified as a 'moderate risk (low risk of severe harms). A subject fee is provided.

2. INTRODUCTION AND RATIONALE

2.1 Therapeutic condition and current treatment status

Cardiogenic shock (CS) is defined as a state of end-organ hypoperfusion due to cardiac failure (1, 2). During cardiogenic shock, persistent low cardiac output may lead to multi-organ failure, often necessitating Intensive Care Unit (ICU) admission for invasive cardiopulmonary supportive therapies (3). Refractory CS, the most severe form of CS, may appear in the first hours to days of ICU admission (4). Although there is no consensual definition, refractory CS is generally described as tissue hypoperfusion not responding to increasing doses of inotrope and/or vasopressor therapy, despite adequate fluid resuscitation (5). Its pathophysiology includes an insufficient cardiac output combined with a blunted response to endogenous vasopressors as a consequence of profound systemic inflammatory responses (6). Although knowledge on the molecular mechanisms underlying CS have increased, this has not translated in relevant improvements in prognosis. Its mortality rate on the ICU remains in excess of 50% (7, 8). Consequently, a global research-effort is directed at improving the outcome of patients suffering from this lethal syndrome (9, 10).

2.2 Clinical trial rationale

Dipeptidyl peptidase 3 (DPP3) is an ubiquitous catalytic cytosolic enzyme only present at low plasma concentration in healthy subjects. There it is involved in the degradation of several peptides that are important regulators of vascular tone (11, 12). Recently, specific assays for the detection of circulating (c)DPP3 concentrations in plasma have been developed (13). High cDPP3 concentrations were found to be strongly associated with impaired outcome in cardiogenic- and septic shock (12, 14-16). Interestingly, a *decrease* of cDPP3 following organ-supportive treatment is associated with less requirement for organ support and a lower mortality (14, 15). Based on these clinical associations, combined with the known short half-life and primary cytosolic localization of DPP3 (13, 17), it was hypothesized that high cDPP3 levels in critical illness represent a state of ongoing cell death (necrosis), leading to the release of cytosolic DPP3 into the circulation (12). Correspondingly, if cDPP3 levels remain high despite adequate supportive treatment, this indicates that tissue damage perpetuates and explains the observed association with unfavorable clinical outcomes.

DPP3 may also directly contribute to impaired outcomes through its activity. During shock, upregulation of the vasoconstrictive molecule angiotensin II is a physiologic and potentially life-saving compensatory response aimed at maintaining adequate tissue perfusion (18, 19). Since DPP3 released into the circulation is able to effectively cleave angiotensin II, DPP3 might well represent a novel factor contributing to hemodynamic instability in different shock conditions (12, 15).

The biological activity of DPP3 on angiotensin II and the vasculature has been exemplified in different preclinical animal studies. In healthy mice, intravenous administration of the DPP3 enzyme itself provoked a rapid deterioration of left ventricular function, as well as impaired kidney hemodynamics (16). Following cessation of DPP3 administration, both left ventricular function and cDPP3 levels returned to pre-infusion levels within approximately 120 minutes (16). In another murine study, DPP3 infusion nullified the blood pressure-potentiating effects of angiotensin II (20). Based on the results of these preclinical studies, antagonizing DPP3 shows promise as a novel therapeutic for the treatment of circulatory shock.

2.3 Mechanism of action, Drug class

Following the results of preclinical studies on DPP3's biological effects, DPP3-antagonizing antibodies were generated. One of these antibodies, AK1967, which effectively and selectively inhibited DPP3's enzymatic activity *in vitro*, was subsequently humanized and named Procizumab (16).

AK1967 has already demonstrated promising results in animal models of cardiogenic- and septic shock (see IB section 4.1, primary pharmacodynamics). For instance, in a murine isoproterenol-induced model of heart failure, AK1967 administration effectively normalized left ventricular function, an effect which was sustained after 24 hours and up to 14 days (16). Furthermore, administration of AK1967 in a murine cecal ligation and puncture (CLP) sepsis model attenuated sepsis-induced cardiac dysfunction and cardiac oxidative stress, and improved survival (21). Lastly, in an experimental pig model of cardiovascular dysfunction induced by sepsis, AK1967 treatment resulted in reduced catecholamine requirement, an improved fluid balance and an increase in circulating Ang II concentrations (see IB study 07-07-02).

AK1967 has shown an excellent safety record (explained in detail in section 3 of the protocol, also refer to IB section 4.3 Toxicology and the Investigational Medicinal Product Dossier (IMPD) Safety & Efficacy). In this study, we intend to investigate the safety, tolerability and pharmacokinetics/-dynamics of AK1967 in a 'first-in-man study' in healthy male subjects.

3. STRUCTURED RISK ANALYSIS

3.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Multiple studies have already demonstrated the rapid angiotensin-scavenging properties of DPP3 based on *in vitro* experiments (11, 16, 20). Angiotensin II, III, IV, 1-5 and 1-7 were all found to be effectively hydrolyzed by DPP3 (11, 22, 23), with Angiotensin IV (six amino-acids in length) being hydrolyzed ten times faster than Angiotensin II (eight amino-acids in length) (20).

To exemplify; in an *in vitro*-setup, human heparinized plasma was spiked with purified native human DPP3 in concentrations of 100, 500 and 1000 ng/mL. Afterwards, plasma samples were incubated at 37° C for 30 minutes. The concentration of angiotensin peptides was determined via RAS equilibrium quantification (24). From **table 1**, it can be appreciated that DPP3 caused a dose-dependent decrease of Angiotensin II, as well as the other metabolically active downstream metabolites angiotensin III and IV. In contrast, angiotensin I, the non-metabolically active precursor of angiotensin II, was unaffected.

Angiotensin II (pmol/L)	Angiotensin I (pmol/L)	Angiotensin III (pmol/L)	Angiotensin IV (pmol/L)	
190.4	102.5	9.7	18.1	**Human plasma (control – 5 ng/mL hDPP3)**
111.3	81.2	<4	5.1	**Human plasma + 100 ng/mL hDPP3**
49.0	95.4	<4	<2	**Human plasma + 500 ng/mL hDPP3**
27.2	96.0	<4	<2	**Human plasma + 1000 ng/mL hDPP3**

Table 1. *Measurement of angiotensin peptides in human plasma spiked with different hDPP3 (human DPP3) concentrations.*

AK1967 (Procizumab) is a humanized full-length IgG1κ antibody recombinantly produced with a CHO DG44 cell line (**figure 1**). The antibody binds to its target molecule DPP3, inhibiting its enzymatic activity as part of its mode of action. AK1967 is directed against a linear epitope ((I)NPETGEQIQ) within the DPP3 sequence, a conserved, surface exposed loop in proximity to DPP3's active site, which is conserved in all mammals (13).

In vitro experiments using native serum of mice, pigs, non-human primates (NHPs), and humans demonstrated that the inhibitory potential of AK1967 on cDPP3 was comparable in all investigated species, supporting the selection of these species for subsequent *in vivo* testing (**table 2**, also see IB section 4.1.1.2).

Healthy species serum	Mean ± SD IC_{50} [µg/mL]	Mean ± SD I_{max} [%]
Human	5.8 ± 1.7	85.2 ± 9.1
Mouse	2.3 ± 0.5	83.2 ± 1.0
Pig	4.0 ± 0.2	80.8 ± 1.7
NHP	10.1 ± 2.5	57.2 ± 3.4

Table 2. *DPP3 inhibition by AK1967 (Procizumab) in native serum of different species compared to humans. Imax - maximal inhibition; IC50 - half-maximum inhibitory concentration; NHP - non-human primate; SD - Standard deviation.*

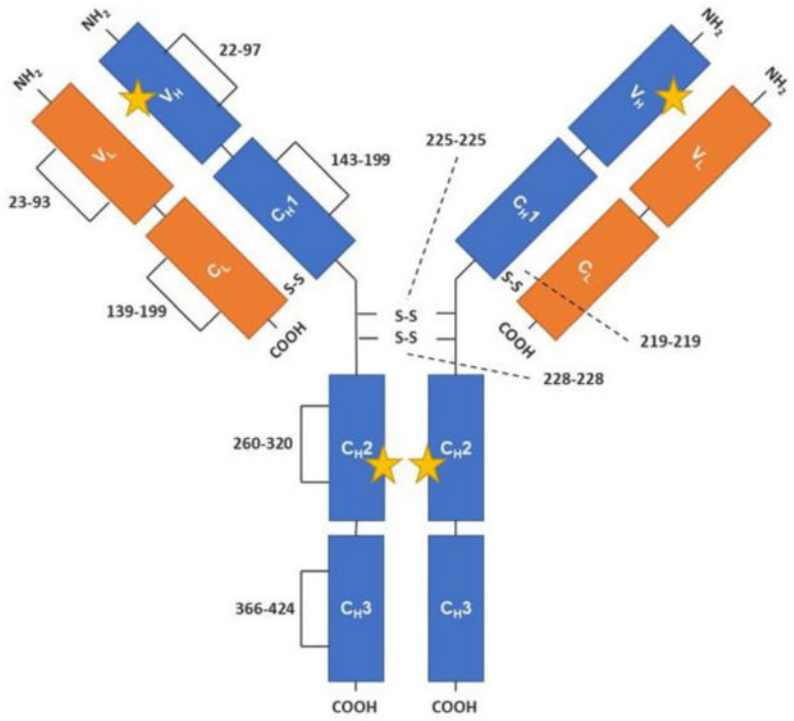

Figure 1. *Structure of AK1967 including glycosylation sites (yellow stars) and location of disulfide bridges (S-S). The heavy chain domains are depicted in blue and light chain domains in orange. Inter- and intra-molecule disulfide bridges are marked with the respective position in the sequence.*

These *in vitro* pharmacodynamic properties of DPP3 and AK1967 were also reproduced *in vivo* in a murine study (IB study 07-02-02, see section 4.1.1.3). Administration of the enzyme DPP3 in healthy mice was associated with a significant decrease in levels of angiotensin II, III and IV, while angiotensin I levels were increased (25). Conversely, injection of AK1967 in these animals resulted in significantly increased levels of angiotensin II compared to placebo (25).

Interestingly, in comparison with placebo-injected mice, DPP3 administration caused a progressive release of catecholamines during the experiment, while AK1967-injected mice exhibited opposite effects. These endogenous adrenergic responses may represent a counter-effective mechanism aimed to mitigate the hemodynamic effects of changes in circulating angiotensin metabolites, explaining why no differences in blood pressure were observed in these initial experiments. To confirm this hypothesis, another group of mice was pre-treated with the alpha- and beta-adrenergic receptor antagonist Labetalol prior to injection of DPP3. As expected, an abrupt decrease in blood pressure was observed upon DPP3-injection in labetalol pretreated mice, confirming the presence of compensatory catecholamine release responses to compensate for the hemodynamic changes induced by DPP3 in the healthy state (IB study 07-02-02, see section 4.1.1.3).

Of note, DPP3 does not represent the sole enzyme responsible for degradation of systemic angiotensin metabolites, as angiotensin converting enzyme 2 (ACE2) also represents a key regulator of RAAS, able to effectively degrade angiotensin II. Putatively, higher levels of angiotensin metabolites caused by AK1967's inhibition of cDPP3 should reach a plateau phase, as higher angiotensin-II levels are shunted away through the ACE2 associated pathway until a new equilibrium is reached. The presence of this alternative pathway represents a self-limiting effect of AK1967's maximum pharmacodynamic potential, meaning that the expected therapeutic window until side effects like hypertension become apparent in the healthy state is large. Correspondingly,

hypertension has not been observed as an adverse event in any of the preclinical studies with AK1967 in healthy animals.

b. Previous exposure of human beings

There is no information about any adverse events of AK1967 in humans, because this study intends to investigate its first use in man. Based on the preclinical data however, adverse events are not expected.

c. Induction of the mechanism in animals and/or *ex-vivo*

Preclinical studies with AK1967 administration showed improved clinical outcomes in different animal disease models. Administration of AK1967 in a murine sepsis model attenuated sepsis-induced cardiac dysfunction, cardiac oxidative stress and improved overall survival (21), study 07-01-02 in the IB. In a murine isoproterenol-induced model of heart failure, AK1967 administration effectively normalized left ventricular function, an effect which was sustained after 24 hours and 14 days (16), study 07-01-01 in the IB. Lastly, in an experimental pig model of cardiovascular dysfunction during sepsis, AK1967 treatment resulted in reduced catecholamine requirement, an improved fluid balance, and an increase in circulating angiotensin-II concentrations (IB study 07-07-02).

AK1967 binds to a conserved, surface-exposed loop in proximity to the active site, which is conserved in all mammals (13, 16). Due to cDPP3's high inter-species amino acid sequence homology, the binding epitope is 100% conserved in all investigated species (mice, pigs, non-human primates and humans) (26). As the humanized version of the antibody was implemented for all preclinical experiments, the pharmacodynamic results of these studies provide additional evidence that AK1967's primary mechanism (binding and inactivation of circulating DPP3) is closely matched between all investigated species.

d. Selectivity of the mechanism

Western Blot analyses of human and murine blood cell lysates, hybridized with AK1967 as primary antibody showed only a single band between 70 and 100 kDa, indicating that AK1967 specifically binds DPP3 (~83 kDa; **figure 2**). The large molecular weight of the AK1967 antibody precludes its free diffusion, meaning it is confined to the circulation. Thus, while cytosolic DPP3 is ubiquitously expressed in a range of cell-types and tissues including erythrocytes, leukocytes, lung, heart, kidney, intestines, skeletal muscle, skin, brain, liver and spleen (17, 27-34), AK1967 will only able to exert relevant effects on DPP3 present in the circulation.

Correspondingly, standard tissue cross-reactivity (TCR) studies were not conducted with AK1967 as the target, DPP3, is constitutively expressed in all human tissues, which would result in reactivity to most tissues in standard TCR studies. To gain relevant information on potential cross-reactivity, a human cell microarray assay developed at Retrogenix (Charles River Discovery Research Services UK Limited) was implemented (see IB section 4.3.7.1 for details). To summarize, five potential off-targets of AK1967 were identified (EPB4IL3, KLRG2, PCDH1, PON1 and PCDHGB2). Due to the scarce published research on the identified off-targets, the potential consequences of *in vivo* binding of AK1967 to these proteins is uncertain. Sequence homology with non-human primates was high for all off-targets, indicating that the absence of adverse effects in the NHP studies is reassuring for the planned first-in-man study.

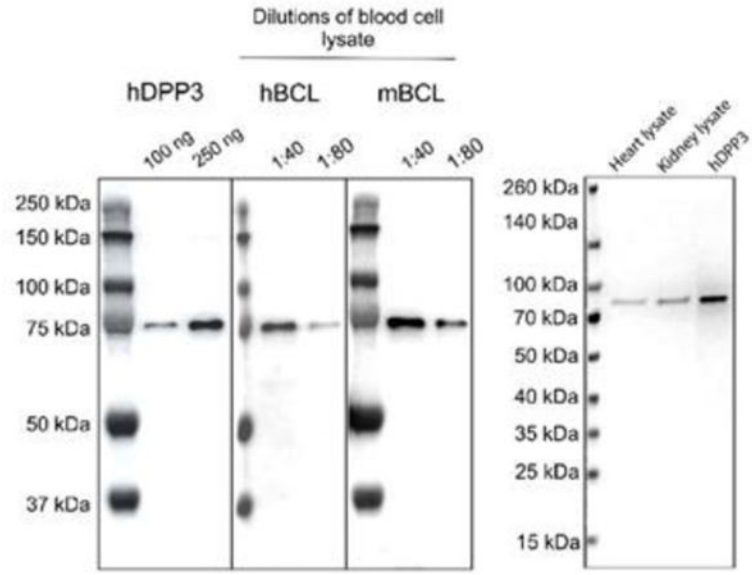

Figure 2. *Western blot specificity analysis of PCZ in human (hBCL) and murine blood cell lysates, heart and kidney homogenates (mBCL). PCZ identifies a single band between 75 and 80 kDa in western blot. As control, human DPP3 natively purified from blood cell lysate was used (hDPP3).*

e. Analysis of potential effect

Mice and cynomolgus monkeys were identified as relevant species for pre-clinical safety and toxicity testing. This is based on the fact that mice and cynomolgus monkeys all express the circulating DPP3 enzyme, with AK1967's binding site being conserved between all species.

The administration of AK1967 to mice and non-human primates (NHP) has been tolerated very well. Single dose administration up to 150 mg/kg AK1967 to mice and 400 mg/kg to NHP have not shown any clinical adverse effects and no abnormal histopathological findings (Study number 8485680 and 8485682, IB section 4.3). Also the repeated administrations of up to 150 mg/kg in mice and 350 mg/kg in NHPs (administration at days 1,2,13 & 14) within the regulatory pre-clinical toxicity and safety study have not shown any clinical or histopathological findings (Study number 8485681 and 8485683, IB section 4.3).

Based on the GLP studies the NOAEL (none-observed adverse level) in mice is 150 mg/kg (max. tested feasible dose) and the NOAEL in NHPs is 350 mg/kg (max. tested). Considering the recommendations provided in the *EMA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07 Rev. 1)*, these NOAELs provide a 50- and 116-fold safety margin to the intended starting dose of 3 mg/kg (**table 3**).

Species	NOAEL (mg/kg)	Safety Margin to starting dose (3 mg/kg)	Safety Margin to max. clinical dose (12 mg/kg)
Mouse	150	50	13
NHP	350	116	29

Table 3. *NOAELs of the mouse and NHP studies, and associated safety margins compared to the minimal and maximal intended doses in the phase 1 study.*

Based on the aforementioned safety margins, the starting dose for this phase 1 study will be 3 mg/kg AK1967, administered as a single infusion over 2 hour. This dose should be increased up to a maximum of 12 mg/kg, to provide a sufficient safety margin for the intended therapeutic dose of 5-10 mg/kg. As the actual body weight might be difficult to acquire in critically ill patients, and a slightly higher dose for a later treatment may turn out to be more beneficial, 12 mg/kg provides a sufficient safety window. The intended maximum dose of 12 mg/kg still provides a 13- and 29-fold safety margin to the observed NOAELs of the GLP-toxicity studies (**table 3**).

f. Pharmacokinetic considerations

From preclinical animal studies, it is known that AK1967 has a short half-life compared to other IgG1 antibodies (see GLP-toxicity studies, section 4.3 of the IB). This shorter half-life also results in a reduced time-range of relevant pharmacodynamic effects compared to other antibodies, with animal disease models demonstrating therapeutic effects in a 24-48 hour time-window (see relevant studies in IB section 4.1.1). The administration of the AK1967 antibody is not associated with the occurrence of any toxic or active metabolites.

g. Predictability of effect

Prior to performing preclinical experiments with AK1967, luminometric immunoassays (LIA) for cDPP3 concentration quantification and fluorescence assays for cDPP3 activity quantification were already developed (13) (see IB section 4.2.1 for reference assays 080-06000/03, 02-16-03 and 02-16-02). These assays allow for the accurate quantification of cDPP3 enzyme activity, both under baseline circumstances as well as during different critical illness etiologies. The availability of enzyme activity assays allows for an additional method to monitor AK1967's pharmacokinetic (and -dynamic) effects, because the dose-dependency of its enzyme-inhibitory potential can be quantified with relative ease. Thus, these assays will serve as additional (secondary) measures of AK1967's pharmacokinetic properties.

Regarding the pharmacodynamic effects of AK1967, assessment of both systemic angiotensin responses and systemic adrenergic responses will be performed at key timepoints just prior to and-after AK1967 administration, serving as additional (secondary) measures of the main (molecular) pharmacodynamic properties. To allow for the accurate quantification of these metabolites, assays implementing liquid chromatography mass spectrometry (LC-MS) will be used (24, 35).

h. Interaction with other products

Not applicable, as no comedication is allowed for this phase-1 safety study.

i. Managing of effects

In order to minimize risks and to make sure that an eventual adverse effect is managed rapidly and effectively, there will a physician in the experimental room at all times during the treatment day. Furthermore, subjects will be monitored closely with continuous measurements of peripheral saturation, ECG, heart rate and blood pressure. There is emergency medication and oxygen on the research unit. The experiments take place at the research unit situated on the Medium Care ward of the Intensive Care department, meaning experienced intensivists are also present in the ward. In case of a dangerous adverse event that is not easily corrected, a quick transfer to the medium or intensive care ward is possible. After discharge from the research-unit, subjects return for daily physical follow-up visits with monitoring of vital signs and relevant safety lab parameters for the first 72 hours after IMP administration, meaning development of early adverse events continue to be

closely monitored during this time-interval. Lastly, the first 4 subjects of each dosage group will be tested on an individual basis (only 1 subject per treatment day), with a minimum interval of 48 hours between dosing of these subjects. This means that no more than one subject will have been exposed to study medication in the unlikely case that an unexpected serious adverse events may develop within the first 48 hours after study medication administration.

j. Study population

The study population consists of healthy male volunteers who undergo an extensive medical screening prior to study drug administration. The condition of subjects who participate in this study can therefore be considered as stable.

3.2 Overall synthesis of the direct risks for the research subjects

The risks for participation in this study are estimated to be moderate, we have made every effort to minimize risks or counteract potential adverse reactions. The method of administration of AK1967 (infusion in 2 hours) enables us to stop the treatment at any time during administration in case an adverse effect is suspected. Hospitalization of the subjects for 24 hours after start of AK1967 administration will enable us to react immediately and adequately in case unexpected adverse events would occur later on. The high level of humanization of AK1967 and the safety results from preclinical studies are reassuring. Therefore, we feel that the remaining risks are acceptable and do not outweigh the scientific and medical relevance of this study.

According to the criteria of the *NFU Guideline quality assurance of research involving human subjects*, the risks of participation in this study are judged to be moderate (small chance at severe damage, see **table 4**). Accordingly, monitoring will occur according to the 'moderate' risk level. This means, monitoring of 100% of informed consent forms, check in- and exclusion criteria for the first 3 subjects and 25% thereafter, check 100% of subjects for missed SAEs, check reception, issuing, storage, expiration dates of investigative drugs and check if there are emergency envelopes for unblinding in emergencies.

Possibility/ Extent of damage	Slight damage	Moderate damage	Severe damage
Small chance	Negligible risk	Negligible risk	Moderate risk
Moderate chance	Negligible risk	Moderate risk	High risk
Large chance	Moderate risk	High risk	High risk

Table 4. *NFU risk classification table.*

4. OBJECTIVES AND ENDPOINTS

Objective(s)	**Endpoint(s)**
Primary objective(s)	**Endpoint for the primary objective(s)**
• Investigate the safety and tolerability of escalating dosages of AK1967 in healthy male volunteers	• Reported number of adverse events from baseline (start of IMP administration) up until the last follow-up visit 28 days after IMP administration. • Vital signs during the first 24 hours after start of IMP administration, as well as during the six follow-up visits. Vital signs included are; o Heart rate o Blood pressure o Oxygen saturation o Temperature • Local tolerability at site of i.v. infusion of the IMP. • Safety laboratory parameters from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration. Laboratory parameters included are; Hb, Ht, leukocytes, thrombocytes, leukocyte differential blood count, sodium, potassium, creatinine, urea, alkaline phosphatase, ALT, AST, bilirubin, GGT, CK, LDH, CRP, PT, APTT, fibrinogen and albumin. • 12-lead electrocardiogram (ECG) at baseline (screening), compared to ECG's performed 2 hours, 9 hours and 7 days after start of IMP administration.
Secondary objective(s)	**Endpoint(s) for secondary objective(s)**
• Investigate the pharmacokinetics of escalating dosages of AK1967 in healthy male volunteers • Investigate the pharmacodynamics of escalating dosages of AK1967 in healthy male volunteers	• Pharmacokinetics of AK1967 (including AUC, C_{max}, Terminal $T_{1/2}$, Cl, VD). • Blood plasma levels and enzyme activity of cDPP3) from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration. • Blood plasma levels of angiotensin metabolites at baseline (just prior to start of IMP administration) and at 2 and 10 hours after start of IMP administration.

	• Blood plasma levels of adrenergic metabolites at baseline (just prior to start of IMP administration) and at 2 and 10 hours after start of IMP administration. • Blood plasma levels of inflammatory mediators (including but not limited to TNFα, IL-6, IL-8, IL-10), measured from baseline (just prior to start of IMP administration) up until 24 hours after start of IMP administration.

5. STUDY PLAN AND DESIGN

5.1 Trial Design

A randomized, double-blind, placebo-controlled, escalating single-dose phase 1 study in healthy male subjects. The double-blind, placebo-controlled, single-dose design is used to best address the trial objectives. Double-blind investigational medicinal product (IMP) administration is used to reduce expectancy and bias in all study procedures. A placebo control will be used to establish the frequency and magnitude of changes in study endpoints that may have occurred in the absence of active treatment.

5.2 Number of Patients

A total of 24 healthy male subjects will be recruited and divided over 3 groups of 8 subjects each. Subjects will be randomly assigned to either AK1967 (n=6 per group) or placebo treatment (n=2 per group). A single (escalating per dose-group) dose of study drug will be administered to healthy volunteers by intravenous infusion over a 2-hour period.

5.3 Overall study duration and follow-up

The expected duration of participation for individual subjects is 6-8 weeks. This includes up to 2 weeks of expected waiting time between informed consent and medical screening, until planning of the study day. After the study day, subjects will be followed up for a total period of 4 weeks.

5.4 Patient participation

Not applicable.

6. STUDY POPULATION

6.1 Population

The study population consists of healthy young male volunteers. Before inclusion, subjects must meet all inclusion criteria and none of the exclusion criteria. Recruitment of healthy volunteers will be done by poster advertising on locations on the campus of the Radboud University Nijmegen. Interested people will be emailed the subject information document and the *"Algemene brochure medisch-wetenschappelijk onderzoek met mensen"* (which translates to *"General brochure for medical-scientific research involving people/humans"*. Subjects will be given a minimal period of 1 day to consider their decision before they can participate in the study.

6.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Written informed consent to participate in this trial prior to any study-mandated procedure.
2. Male subjects aged 18 to 35 years inclusive.
3. Subjects have to agree to use a reliable way of contraception with their partners from study entry until one month after study drug administration.
4. BMI between 18 and 30 kg/m², with a lower limit of body weight of 50 kg and an upper limit of 100 kg.
5. Healthy as determined by medical history, physical examination, vital signs, 12-lead electrocardiogram, and clinical laboratory parameters.

6.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation:

1. Unwillingness to abstain from any medication, including recreational drugs or vitamin supplements during the course of the study and within two days prior to the treatment day.
2. Unwillingness to abstain from alcohol within one day prior to the treatment day until one day after the treatment day.
3. Surgery or trauma with significant blood loss or blood donation within one month prior to the treatment day.
4. History, signs or symptoms of cardiovascular disease, in particular:
    a. History of frequent vasovagal collapse or of orthostatic hypotension
    b. Resting pulse rate ≤45 or ≥100 beats/min
    c. Hypertension (RR systolic >160 or RR diastolic >90 mmHg)
    d. Hypotension (RR systolic <100 or RR diastolic <50 mmHg)
    e. Conduction abnormalities on the ECG consisting of a 1st degree atrioventricular block or a complex bundle branch block
    f. Any chronic cardiac arrhythmias (except PAC's, PVC's)

5. Renal impairment: plasma creatinine >120 µmol/L
6. Liver function tests (alkaline phosphatase, AST, ALT and/or γ-GT) above 2x the upper limit of normal.
7. History of asthma
8. Atopic constitution
9. CRP above 2x the upper limit of normal, or clinically significant acute illness, including infections, within two weeks prior to the treatment day.
10. Treatment with investigational drugs or participation in any other clinical trial within 30 days prior to the treatment day.
11. Known or suspected of not being able to comply with the trial protocol.
12. Known hypersensitivity or allergic reactions to drug compounds, (i.e. previous adverse drug reactions).
13. Inability to personally provide written informed consent (e.g. for linguistic or mental reasons) and/or take part in the study.

6.4 Vulnerable populations and clinical trials in emergency situations

This trial will not be conducted in a population that is defined as vulnerable.

7. STUDY TREATMENTS

7.1 Investigational Medicinal Product(s) (IMP(s))

7.1.1 Name and description of the IMP

AK1967 (Procizumab). The investigational medicinal product is a solution for intravenous application presented in Ph.Eur. type I glass vials with fluorpolymer coated bromobutyl rubber stoppers and tear-off plain aluminum over seals. 20R vials (25 mL total container volume) are aseptically filled with 20 mL Procizumab to allow for an extractable volume of 18.5 mL. Procizumab composition is listed in **table 5** below.

Name of Ingredient	Function	Reference	Quantity (per mL)	Quantity (per vial)
AK1967 protein	Active	IMPD	20 mg	400mg
Trehalose Dihydrate	Stabilizer	Ph.Eur/USP	90.7 mg	1.8154 g
L-Methionine	Antioxidant	Ph.Eur/USP	2.86 mg	57.2 mg
L-Histidine	Buffer	Ph.Eur/USP-NF	1.11 mg	22.2 mg
L-Histidine HCl * H2O	pH adjustment	Ph.Eur	0.51 mg	10.2 mg
Water for Injection	Solvent	Ph.Eur/USP/JP	Ad 1 ml	Ad 20 ml

Table 5. *Composition of the Procizumab drug product. Manufacturer: BioConnection, Oss, the Netherlands*

Any clinical trial medication has to be diluted from the Drug Product stock solution with sterile NaCl solution 0.9% to the required dosing (3.0 mg/kg, 6.0 mg/kg or 12.0 mg/kg) with an infusion volume of 1.0-1.2 mL/kg depending on the required dose.

7.1.2 Status of development of the IMP

As this is a phase 1 first-in-human clinical trial, there is no information on AK1967 available from previous human clinical studies.

AK1967 has proven to be tolerable and safe in healthy animals up to the maximum tested doses (regulatory toxicity & safety: 150 mg/kg in mice, 350 mg/kg in NHPs). Moreover, AK1967 demonstrated beneficial effects on left ventricular ejection fraction, cardiac oxidative stress, renal blood flow parameters and vasopressor requirement in different preclinical disease models. For detailed information please refer to the Investigators Brochure, sections 4.1 (non-clinical pharmacology) and 4.3 (toxicology).

AK1967 is a humanized full-length IgG1κ antibody recombinantly produced with a CHO DG44 cell line. The antibody binds to its target molecule DPP3, inhibiting its enzymatic activity as part of its mode of action. AK1967 is directed against a linear epitope ((I)NPETGEQIQ) within the DPP3 sequence. AK1967 was generated from an IgG2 antibody produced in mice, which were injected with a linear immunization peptide originated from human DPP3. After humanization and codon optimization, the light and heavy chain sequences were inserted into an expression vector for stable production in CHO cells.

The manufacturing process for AK1967 follows all relevant GMP guidelines and requirements.
For more detailed description of physical, chemical, and pharmaceutical parameters of AK1967 please refer to the IMPD Quality.

7.1.3 Description and justification of dosage and route of administration

Based on the preclinical data (see above), as well as the high-grade of humanization of the AK1967 antibody, study drug-related adverse events are not expected. The starting dose of 3 mg/kg was chosen based on the preclinical data. The drug AK1967 will be administered via intravenous infusion over a 2-hour period, enabling us to stop infusion in case of any observed significant clinical reaction. Due to the intravenous route of administration, local irritation and hematoma (specific to intravenous injection but not to the test article) at the site of injection cannot be ruled out.

7.2 Comparator IMP(s)

Not applicable.

7.3 Placebo

The placebo is a solution for intravenous application presented in Ph.Eur. type I glass vials with fluorpolymer coated bromobutyl rubber stoppers and tear-off plain aluminum over seals. 20R vials (25 mL total container volume) are aseptically filled with 20 mL placebo to allow for an extractable volume of 18.5 mL. Placebo composition is listed in **table 6** below.

Name of Ingredient	Function	Reference	Quantity (per mL)	Quantity (per vial)
Trehalose Dihydrate	Stabilizer	Ph.Eur/USP	90.7 mg	1.8154 g
L-Methionine	Antioxidant	Ph.Eur/USP	2.86 mg	57.2 mg
L-Histidine	Buffer	Ph.Eur/USP-NF	1.11 mg	22.2 mg
L-Histidine HCl * H2O	pH adjustment	Ph.Eur	0.51 mg	10.2 mg
Water for Injection	Solvent	Ph.Eur/USP/JP	Ad 1 ml	Ad 20 ml

Table 6. *Composition of the placebo drug product. Manufacturer: BioConnection, Oss, the Netherlands*

7.4 Auxiliary Medicinal Products (AxMPs)

7.4.1 Name and description of the AxMPs

Lidocaine 1%

Prior to cannulation of the radial artery (for blood withdrawal and continuous monitoring of blood pressure), local anaesthesia will be attained by subcutaneous injection of lidocaine 1%. For further information on lidocaine 1%, see the summary of product characteristics (SmPC) (*document H1 SmPC lidocaine injection*).

Hydration fluids (NaCl 0.9%)

To keep the intravenous catheter patent during the experimental procedure, an infusion of 5 mL/hours of NaCl 0.9% will be administered through the intravenous catheter for the entire experimental procedure. For further information on NaCl 0.9%, see the SmPC (*document H2 SmPC NaCl 0.9%).*

7.4.2 Statement on authorisation and justification unauthorised AxMP

Both previously described AxMP's (Lidocaïne 1% and NaCl 0.9%) will be used in the clinical study in accordance with the terms of their marketing authorisations.

7.4.3 Description and justification of dosage and route of administration

Not applicable.

7.5 Additional considerations for trials involving a medical device

Not applicable.

7.6 Additional considerations for trials involving an in-vitro diagnostic or companion diagnostic

Not applicable.

7.7 Preparation and labelling of the study treatment

AK1967- and placebo-containing vials will be supplied by the sponsor and labeled according to the requirements of EC Guide on GMP, Annex 13. The boxes containing AK1967 and placebo will be shipped under controlled and monitored temperature conditions at 2-8°C. The receiving site has to confirm the receipt and the condition of the delivery in writing on provided forms. Should the temperature monitoring device indicate that the specified shipment conditions were not maintained during the transport, the AK1967 and placebo must be quarantined such that they cannot be used unintentionally and the sponsor must be immediately contacted.

The label of AK1967 will look as follows (in Dutch, English template also submitted) (**figure 3)**:

Vials:

EU CT Nr. 2023-507035-37-00
AK1967 Studie Protocol Nr. CT-P1-001
AK1967 20mL/ampul, 20mg/mL
Batch ID C2309 / AK1967-DS01-FF01 ampul nr. 00X
Voor intraveneuze toediening
Alleen voor gebruik in klinisch onderzoek
PI: Prof. Peter Pickkers Tel: +31 6 525 929 82
Hertest datum: 10/2024

Box:

EU CT Nr. 2023-507035-37-00
AK1967 STUDIE PROTOCOL NR. CT-P1-001
AK 1967 20mL/ampul, 20mg/mL in Formulatie Buffer – 6 ampullen
Batch ID C2309 / AK1967-DS01-FF01, ampul # 00X – 00X
Voor intraveneuze toediening
Na verdunning volgens onderzoeksprotocol
Alleen voor gebruik in klinisch onderzoek
Bewaar bij 2-8°C
PI: Prof. Peter Pickkers Tel: +31 6 525 929 82
4TEEN4 Pharmaceuticals GmbH
Neuendorfstr. 15A, 16762 Hennigsdorf, Germany
Hertest datum 10/2024

The label for placebo looks as follows (in Dutch, English template also submitted) (**figure 4**):

Vials:

EU CT Nr. 2023-507035-37-00
AK1967 Studie Protocol Nr. CT-P1-001
PLACEBO-AK1967 20mL/ampul
Batch ID C2310 / AK1967-PL01-FF01 ampul nr. 00X
Voor intraveneuze toediening
Alleen voor gebruik in klinisch onderzoek
PI: Prof. Peter Pickkers Tel: +31 6 525 929 82
Hertest datum: 10/2024

Box:

EU CT Nr. 2023-507035-37-00
AK1967 STUDIE PROTOCOL NR. CT-P1-001
Placebo-AK 1967 20mL/ampul – 6 ampullen
Batch ID C2310 / AK1967-PL01-FF01, ampul # 00X – 00X
Voor intraveneuze toediening
Na verdunning volgens onderzoeksprotocol
Alleen voor gebruik in klinisch onderzoek
Bewaar bij 2-8°C
PI: Prof. Peter Pickkers Tel: +31 6 525 929 82
4TEEN4 Pharmaceuticals GmbH
Neuendorfstr. 15A, 16762 Hennigsdorf, Germany
Hertest datum 10/2024

Study medication must be stored at 2-8°C and protected from light under temperature-controlled and restricted access conditions. The individual dose for each subject will be prepared by unblinded qualified site staff otherwise not involved in the conduct of the trial. Details of the AK1967 and placebo dose preparation will be provided in an assembly manual. The study medication vials will be warmed to room temperature within 1 hour prior to use.

The intended dose will be administered using an infusion pump, over a 2-hour period. The solution for injection is visually inspected prior to use. Only clear solutions without particles will be used. Should the study medication fail to meet these criteria, the corresponding vials have to be separated and the sponsor has to be contacted immediately.

Aseptic techniques will be strictly observed throughout handling of study medication, since they contain no preservative. If any precipitate is visible or the solution is not clear, it will not be used. Standard laboratory care will be used during handling and preparation of study medication; i.e. use of gloves and other protective clothing to prevent skin contact is recommended. The intravenous dose will be prepared for administration according to the Procizumab and placebo preparation manuals provided by the sponsor, and will be administered via the venous cannula of the subject.

8. OTHER TREATMENTS AND RESTRICTIONS

8.1 Concomitant therapy

8.1.1 Permitted medications

No medications will be allowed during the course of the study, starting at 2 days prior to the treatment day up until the last check-up 28 days after the treatment day.

8.1.2 Prohibited medications

As this is a phase 1 safety study, Subjects have to abstain from all medication during the course of the study, starting at 2 days prior to the treatment day up until the last check-up 28 days after the treatment day. If subjects are considering the use of medication (e.g. paracetamol) for newly developed complaints during the study protocol, they are asked to first consult the coordinating researcher. Any use of medication which could not be avoided, either because it was deemed necessary after consultation with the coordinating investigator, or prescribed by a doctor taking care of the subject, will be registered in the eCRF along with the AE of the accompanying symptoms.

8.2 Lifestyle restrictions

8.2.1 Contraception measures

All subjects have to agree to use a reliable way of contraception with their partners from study entry until 1 month after study drug administration.

8.2.2 Other requirements

Subjects have to abstain from any medication, including recreational drugs or vitamin supplements during the course of the study, starting at 2 days prior to the treatment day up until the last check-up 28 days after the treatment day.
Subjects have to abstain from alcohol, starting at 1 day prior to the treatment day up until 1 day after the treatment day.

9. TRACEABLILITY, STORAGE, ACCOUNTABLILITY AND COMPLIANCE

9.1 Traceability and storage of the study treatment

AK1967- and placebo-containing vials will be supplied by the sponsor and labeled according to the requirements of EC Guide on GMP, Annex 13. The boxes containing study medication will be shipped at 2-8°C under controlled and monitored temperature conditions. The receiving site has to confirm the receipt and the condition of the delivery in writing on provided forms. Should the temperature monitoring device indicate that the specified shipment conditions were not maintained during the transport, the study medication must be quarantined such that they cannot be used unintentionally and the sponsor must be immediately contacted.

The Department of Clinical Pharmacy of the Radboud university medical center will obtain the AK1967 and placebo used in this trial from the sponsor. An incoming goods check must be performed and a drug receipt log filled out and signed by the person accepting the shipment. The products will be stored at the department of Clinical Pharmacy under GMP conditions. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be immediately reported to the sponsor and documented in the study files.

Study medication reconciliation will be conducted after the last subject received the last dose. This reconciliation will be documented appropriately and signed and dated by the Principal Investigator. Any discrepancies noted will be investigated, resolved, and documented prior to return of unused study drug. Unused study medication shall be returned to the sponsor under temperature-controlled and monitored conditions. Used AK1967 and placebo containers will be kept at the site for monitoring / reconciliation. They will be destroyed on site only after approval by the sponsor.

9.2 Accountability of the study treatment and compliance

After randomization of an individual subject, study medication will be ordered by a research nurse of the unblinded team. Study medication (prescribed on an individual basis to the subject's identifier code) will be retrieved by research nurse at the department of Clinical Pharmacy. The medication will be prepared by research nurses, who will sign off the drug accountability form. This form will list what drug is used for which subjects on what date. Used syringes/vials will be stored at the research unit until completion of the trial (including data analysis) and will be destroyed afterwards. The investigator is responsible for maintaining a study treatment stock sheet including up-to-date information on date and time of study treatment administration to the subject. The unblinded research staff will maintain a drug accountability form which lists the date of administration, study identification code of the subject and the drug administered. All doses of the study treatment in this study will be administered in the clinical unit by the Principal Investigator or his representative, and will be documented in the eCRF with date and time of administration.

The unblinded research staff will make sealed envelopes which contain the treatment allocated to each randomization number for emergency unblinding situations. That will reduce the impact on the trial data to a minimum if emergency unblinding becomes inevitable. The code break envelopes will be kept in the subject's file containing all source data. It is the responsibility of the study team to

keep the information accessible in case of an emergency, and to access it only in the case of an emergency.

Double-blind conditions have to be maintained for all subjects and study personnel. AK1967 infusion syringes and solution will be identical to placebo infusion syringes and solution in appearance and texture (syringe label displayed in **figure 5**). It is the responsibility of all unblinded staff, including the unblinded person preparing the study medication, to ensure the blinded condition at any time except in case of immediate medical emergency in line with ICH-GCP and local regulatory requirements. The unblinded staff must keep all documentation of manufacturing records and sealed envelopes and must not disclose any treatment information to blinded staff.

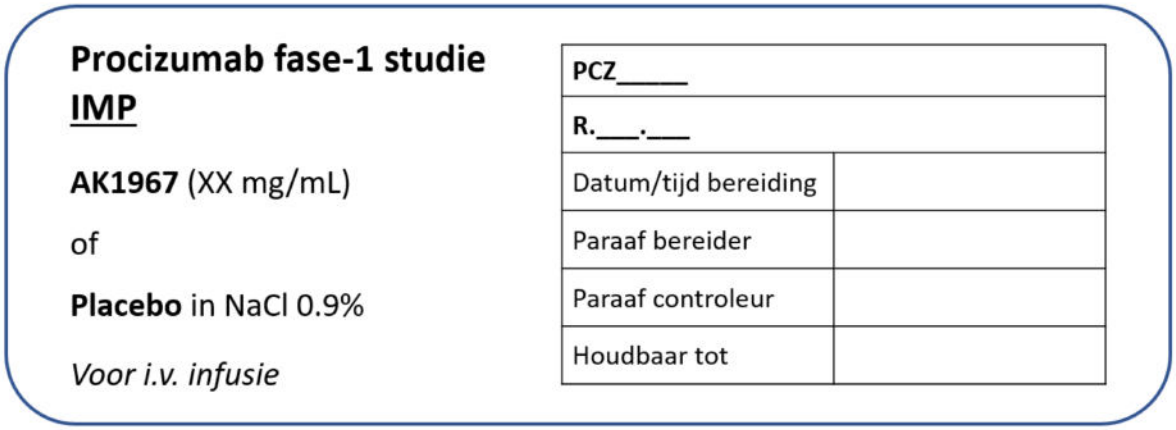

Figure 5. *Intended label for the blinded study medication, which will be present on syringes during the experiment day. Dutch text provided, an English template is also submitted together with the study protocol.*

10. STUDY ASSESSMENTS AND PROCEDURES

10.1 Screening procedure

The screening will take place one to 28 days before the treatment day. The subject should have read the information for subjects before the screening visit. Upon planning of the screening visit, subjects are assigned a screening number (PCZ.XX). The screening visit will consist of a detailed explanation of the study background and study procedures. If the subject still wants to participate after the detailed explanation, written informed consent will be asked. After informed consent has been provided, questions will be asked about the medical history of the subject, especially in relation to the in- and exclusion criteria. Then, a general physical examination will be performed, an ECG will be made, and blood will be drawn for laboratory evaluation. If based on the results of the screening visit, the subject is found to be eligible for the study, the subject is planned to participate in the experiment. If time between screening and the treatment day will be more than four weeks, a re-screening check will be performed to ensure the subject is still eligible for testing. Additional laboratory evaluation will be performed on indication, as assessed by the medical staff. There will be a minimum of 48h for reflection for the subject between screening and the treatment day.

10.2 Randomisation, blinding and treatment allocation

The randomization procedure is stratified into the three groups, consisting of 8 subjects each, with sequential groups being administered increasing doses of Procizumab (**table 7**). Six subjects of each group will be treated with active medication and two subjects with placebo. These groups are further divided into two randomization blocks consisting of four subjects each (one placebo treatment and three active medication treatment). This is done as an additional safety measure, to ensure an even spread of placebo-treated subjects over all groups (using this approach, the study can never start with two placebo-treated subjects).

Group 1		**Group 2**		**Group 3**	
Randomization block 1 (R1.1 to R.1.4)	Randomization block 2 (R.2.1 R.2.4)	Randomization block 3 (R3.1 to R.3.4)	Randomization block 4 (R4.1 to R.4.4)	Randomization block 5 (R5.1 to R.5.4)	Randomization block 6 (R6.1 to R.6.4)
1x placebo	1x placebo	1x placebo	1x placebo	1x placebo	1x placebo
3x 3.0 mg/kg AK1967	3x 3.0 mg/kg AK1967	3x 6.0 mg/kg AK1967	3x 6.0 mg/kg AK1967	3x 12.0 mg/kg AK1967	3x 12.0 mg/kg AK1967

Table 7. *Block-randomization groups of different study groups*

A randomization list will be created by unblinded research staff prior to the treatment day. This list contains the randomization numbers, consisting of a two-digit number preceded by capital 'R' to identify this number as being the randomization number. The first digit corresponds with the randomization block (1 to 6). The second digit corresponds with the randomization number within the randomization block, starting with 1 (for example R.1.1 for randomization block 1, subject 1, followed by R.1.2, R.1.3, onwards to R.6.4). The unblinded research staff will assign a treatment group (active medication or placebo) at random to each number on the list. They will start with randomizing block 1 and will continue until they reach randomization block 6.

This randomization list will only be in the possession of dedicated unblinded research staff at the study site, which is responsible for the preparation of the study medication and drug accountability. The randomization list must be kept secret for the study team at all times until the study has been finalized and the database containing all primary endpoint data is locked. A spare set of randomization numbers will be available with ascending numbers from Rep.01 onwards (Rep.01, Rep.02, Rep.03 etc.) for assignment to subjects replacing subjects who were randomized but discontinued prior to administration of the study medication. The subjects to which these spare randomization numbers will be assigned receive the treatment that was originally assigned to the subject they replace. If replacing subjects also discontinue the study prior to administration of the study medication, another subject will again replace this subject according to the same procedure. A maximum of 5 subjects will be replaced. The unblinded personnel will safeguard the procedure of adequate treatment assignment to replacing subjects by keeping an up-to-date randomization log. Subjects who discontinue the study prematurely after administration of study medication will not be replaced.

10.3 Study procedures and assessments

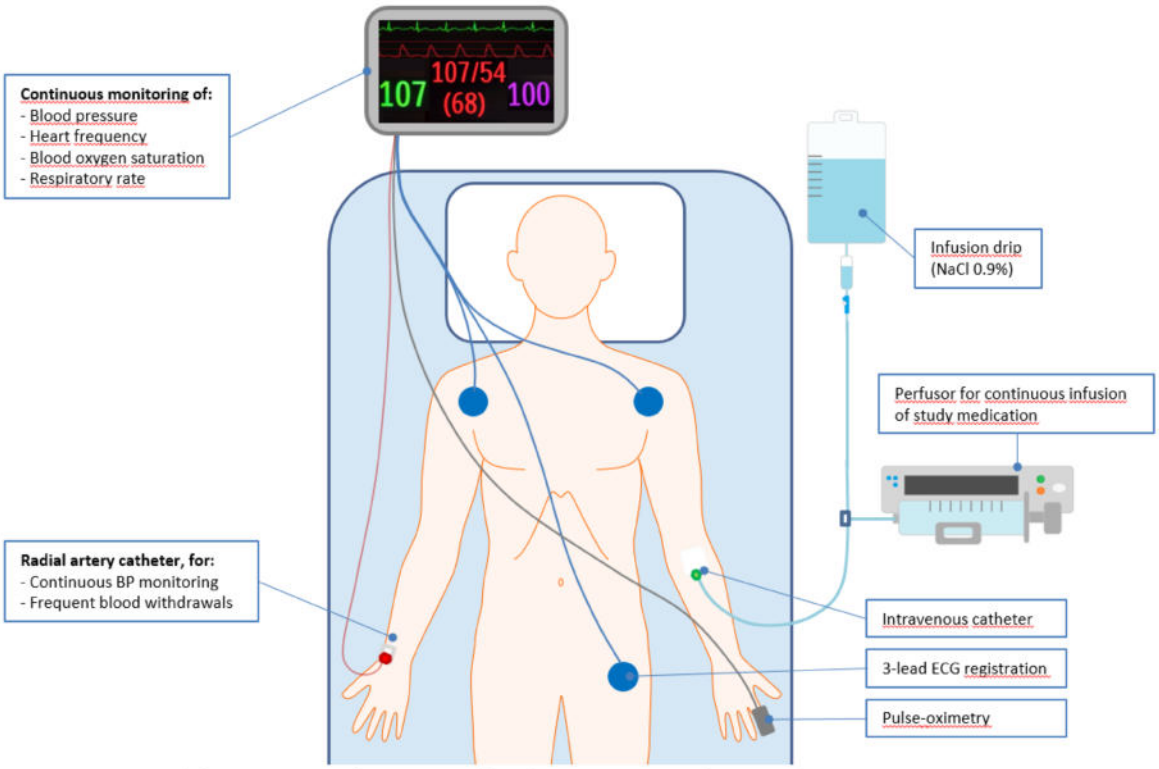

Figure 6. *Overview of the experimental setup. BP = blood pressure, ECG = electrocardiogram.*

On the morning of the treatment day, subjects will arrive at the Intensive Care Research Unit at 07:00. After the subject has arrived, in- and exclusion criteria, concomitant medication, body weight, blood pressure and heart rate will be re-checked. If the subject still is eligible, one venous catheter will be placed in a vein of the forearm. An arterial cannula is placed in the radial artery. Placement of the arterial cannula will be locally anesthetized using lidocaine 1% solution for injection. Placement of the arterial line will provide the possibility of easy blood sampling and at the same time enable the continuous measurement of blood pressure. An overview of instrumentation of the subjects enrolled in this study is provided in **figure 6**.

A person of the unblinded team (not involved in any other study procedures) will prepare the study medication according to the procedures described in the study medication preparation manual. After cannulation, blood will be drawn for baseline determinations just prior to start of study medication administration (which takes place at T = 0). After the start of study medication administration, all subsequent time points for sampling are fixed with a margin for error of ± two minutes for the blood withdrawal on the treatment day (15 min, 30 min, 60 min, 90 min, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 8 hrs, 10 hours, 24 hours), ± 1 hour for blood withdrawal on the 48 hour follow-up day and ± 3 hours for blood withdrawal after 72 hours. On day 7 (± 1 day), 14 (± 2 days), 21 (± 3 days) and 28 (± 3 days), it is not specified at which time point of the day the sampling is performed. An overview of all study-related procedures on the treatment day is provided in **figure 7**.

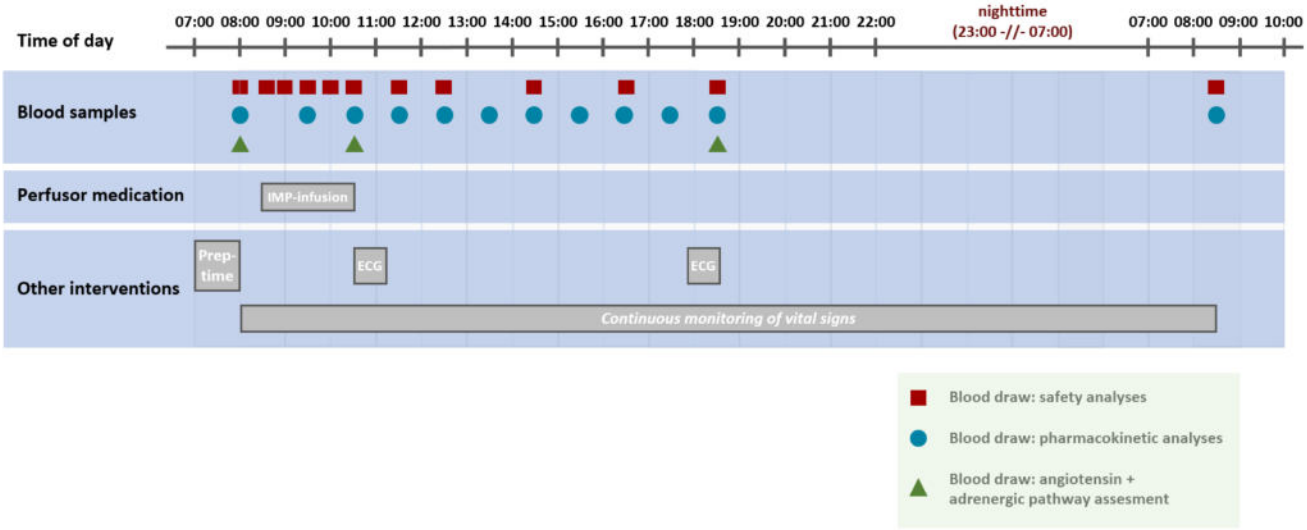

Figure 7. *Overview of interventions performed on the treatment day, including the in-hospital (overnight) observation period up until 24 hours after start of IMP administration. IMP-infusion = investigation medicinal product infusion, prep-time = preparation time needed for placement of venous and arterial catheter, applying equipment necessary for patient monitoring. ECG = electrocardiogram.*

Apart from continuous ECG monitoring, a 12-lead ECG will be made two and nine hours after start of study medication administration. Throughout the day, standard meals and drinks will be provided to the subjects. Ten hours after start of study drug administration, cannulas will be removed and a pressure bandage will be applied to the site of arterial cannulation. Subjects stay overnight at the research Intensive Care Unit for continuous monitoring of vital signs. In the morning, a venous blood withdrawal is performed approximately 24 hours after start of IMP administration. Afterwards, the pressure bandage will be removed and subjects are released from the research unit. The subjects will return an additional 6 times for follow-up visits over the next 28 days (subjects will be checked for adverse events and blood samples will be obtained via venipuncture).

At the end of the treatment day, a form is filled in together with the subjects through which a subject fee of €400,- will be transferred digitally. The remainder of the subject compensation fee (also €400,) will be transferred following the last visit on day 28.

The first four subjects in each dose-group are tested **individually** (only one subject per day), while for the last four subjects of each dose-group, two subjects are tested simultaneously. Furthermore,

treatment of the first 4 consecutive subjects of each dose-group will have a minimal interval of 48 hours, meaning no more than 1 subject will have been exposed to study medication if serious adverse events develop during this interval. After the last subjects of a dose-group have received study medication, an interim report will be written containing all the relevant safety data including (serious) adverse events ((S)AEs), vital signs, clinical laboratory values and ECGs per dose-group. Based on the information in this report, the data safety monitoring board (DSMB, see section 12.10 of this protocol for details) will decide whether continuation of the trial to a next treatment group (with a higher IMP dosage) is feasible, maintaining the safety of all subjects as the highest priority. This approach to dosing is similar to the one implemented for two previous phase-I studies conducted at our department (Arnhem-Nijmegen Regional Ethics Committee numbers 2016-2283 and 2016-2704, respectively).

10.3.1 Efficacy assessments

- Pharmacokinetics of AK1967 (including AUC, C_{max}, Terminal $T_{1/2}$, Cl, V) will be assessed from baseline (start of IMP administration) up until the last follow-up visit 28 days after IMP administration. During the study day, a total of 11 drug concentration measurements are performed to allow for accurate determination of AUC, C_{max}, Terminal $T_{1/2}$, Cl, V. Drug concentration measurements will also be performed during each of the 6 follow-up visits.
- Pharmacodynamics (blood plasma levels and enzyme-activity of cDPP3) will be assessed from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration. cDPP3 measurements will also be performed during each of the 6 follow-up visits.
- Blood plasma levels of angiotensin metabolites will be assessed at key timepoints before and after start of IMP administration, to allow for accurate determination of systemic angiotensin responses related to the study medication.
- Blood plasma levels of adrenergic metabolites will be assessed at key timepoints before and after start of IMP administration, to allow for accurate determination of systemic adrenergic responses related to the study medication.
- Plasma levels of inflammatory mediators (including but not limited to TNF, IL-6, IL-8, IL-10) will be measured from baseline (just prior to start of IMP administration) up until 24 hours after start of IMP administration. These measurements will be performed a total of 11 times during the study day, to capture any inflammatory changes that might be related to the study medication in detail.

10.3.2 Safety assessments

Safety parameters assessed in the study include;

- Reported number of adverse events, serious adverse events and suspected unexpected serious adverse reactions. These will be assessed from baseline (start of IMP administration) up until the last follow-up visit 28 days after IMP administration. During the study day, adverse events are monitored continuously up until twenty-four hours after start of IMP administration, as subjects are continuously monitored by a research-physician. During the six follow-up visits, adverse events will be recorded for the time-period preceding the visit.
- Vital signs will be registered continuously during the first 24 hours after start of IMP administration. Afterwards, they will be assessed during each of the six follow-up visits.

The vital signs assessed are; heart rate, blood pressure, oxygen saturation and body temperature.

- Local tolerability at the site of i.v. infusion of the IMP. This symptom will be monitored continuously during the study day up until ten hours after start of IMP administration. During each of the 6 follow-up visits, local tolerability will also be assessed.
- Safety laboratory parameters from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration. Laboratory parameters included are; Hb, Ht, leukocytes, thrombocytes, leukocyte differential blood count, sodium, potassium, creatinine, urea, alkaline phosphatase, ALT, AST, bilirubin, GGT, CK, LDH, CRP, PT, APTT, fibrinogen and albumin. All laboratory safety measurements will also be performed during each of the follow-up visits. A full overview of the specific timepoints on which all specific lab measurements are performed is provided below in **table 8**.
- 12-lead electrocardiogram (ECG) at baseline (screening), compared to ECG's performed 2 hours, 9 hours and 7 days after start of IMP administration.

If any adverse reaction (not deemed a serious adverse event) occurs, subjects will be asked to report to the coordinating investigator when symptoms fully abated, so this can be registered in the eCRF. The status of a newly developed symptom will also be monitored during each of the study's follow-up visit, and recorded in the eCRF.

Study visit	Screening	Treatment day															Follow up visits					
Days relative to dosing of IMP	-1 to -28 days																2	3	7	14	21	28
Time relative to dosing of IMP		Baseline	15 min	30 min	60 min	90 min	120 min	180 min	240 min	300 min	360 min	420 min	480 min	540 min	600 min	24 h	48 h	3 days	7 days	14 days	21 days	28 days
Hematology[1]	x	x		x	x		x		x		x				x	x	x	x	x	x	x	x
Clotting[2]	x	x			x		x		x		x				x	x	x	x	x	x	x	x
Biochemistry[3]	x	x		x	x		x		x		x				x	x	x	x	x	x	x	x
Pharmacokinetics[4]		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
RAAS-pathways assessment[5]		x					x								x							
Adrenergic-pathway assessment[6]		x					x								x							
DPP3 activity/ concentration		x		x	x	x	x	x	x		x		x		x	x	x	x	x	x	x	x
Cytokines[7]		x	x	x	x	x	x	x	x		x		x		x	x	x					

[1] Hematology: Hb, Ht, Leucocytes, Thrombocytes, Leucocyte differential
[2] Clotting: PT, APTT, fibrinogen
[3] Biochemistry: sodium, potassion, creatinine, urea, alkaline phosphatase, ALT, AST, LDH, bilirubin, γGT, CK, albumin, CRP
[4] Pharmacokinetics: Procizumab (including AUC, Cmax, Terminal $T_{1/2}$, Cl, V)
[5] Assessed RAAS pathway include: angiotensins 1, 2, 1-5, 1-7, aldosterone and renin
[6] Assessed adrenergic metabolites include: epinephrine, norepinephrine (potentially dopamine)
[7] Assessed cytokines: TNF, IL-6, IL-8, IL-10, MCP-1, MIP-1A, MIP-1B, IL-1RA, G-CSF

Table 8. *Overview of all blood sampling performed during the course of the study protocol. Different rows represent different sample-mediums, all analytes corresponding with a sample-medium are displayed in the lower part of the table.*

11. STUDY DISCONTINUATION AND COMPLETION

11.1 Definition End of Trial

The trial will end either after the last subject has successfully completed the 28-day follow-up visit, or after the last subject that developed symptoms deemed an adverse event possibly related to the study drug reports that symptoms have fully abated.

11.2 Criteria for temporary halt and early termination of the clinical trial

Temporary halt

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited medical Ethics Committee (EC) without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited EC. The investigator will take care that all subjects are kept informed.

Early termination

As primary condition, in case of SAE that will result in death, is life threatening, requires hospitalization or prolongation of existing inpatients hospitalization or results in persistent or significant disability or incapacity, premature termination will take place.

Further conditions for premature termination include the following clauses:

- If the approval by the Ethics Committee (EC) in charge of the Clinical Trial is irrevocably revoked;
- If it can be reasonably assumed that the Clinical Trial must be terminated in the interests of the health of the Clinical Trial Subjects;
- If it becomes apparent, following confirmation of the EC, that continuation of the Clinical Trial cannot serve a scientific purpose, and this is notified to the EC;
- If the Sponsor and/or the Institution become or are declared insolvent or a petition in bankruptcy has been filed against it or if one of them is dissolved;
- If circumstances beyond a party's control occur that render continuation of the Clinical Trial unreasonable;
- If one of the parties fails to comply with the obligations arising from the Agreement and, if capable of remedy, is not remedied within 30 days after receipt of notice from the other Party specifying the non-compliance and requiring its remedy, unless failure to comply is not in reasonable proportion to the premature termination of the Clinical Trial.

In all circumstances causing the early termination the study, the Principal Investigator shall confer with the sponsor and use their best endeavors to minimize any inconvenience or harm to clinical trial subjects caused by the premature termination.

11.3 Discontinuation/withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

11.4 Arrangements for subjects after their participation in the clinical trial ended

Not applicable.

12. SAFETY REPORTING

12.1 Definitions

12.1.1 Adverse events (AEs)

Adverse events are defined as any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

12.1.2 Serious adverse events (SAEs)

Serious adverse event is any untoward medical occurrence in a patient or trial subject that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect.

12.1.3 Suspected unexpected serious adverse reactions (SUSARs)

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. The event must be serious;
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the reference safety information (RSI).

12.2 Recording of AEs/SAEs/SUSARS

All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. There are no pre-defined exceptions. Given the nature of this study, any serious adverse event occurring after treatment administration will immediately be labeled as a SUSAR. If however, after close examination, there is a 100% certainty that the cause of damage is unrelated to the drug administration, there should be the possibility to reverse this labeling and to correctly label it as a SAE.

12.3 Reporting of AEs and SAEs

12.3.1 Reporting of SAEs by the investigator to the sponsor

All SAEs will be reported by the investigators to the sponsor and sponsor representative for pharmacovigilance monitoring within 24 hours from the onset of the event. The SAEs will also be recorded in the eCRF.

12.3.2 List of SAEs which do not require immediate reporting and procedure for reporting

Not applicable, since all SAEs will require reporting to the sponsor and sponsor representative within 24 hours from the onset of the event.

12.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

12.5 Reporting of SUSARs by the sponsor to the EudraVigilance database

The sponsor will keep detailed records of all AEs which are reported to him/her by the investigator or investigators.

The sponsor will report electronically and without delay to EudraVigilance database all relevant information about any SUSAR.

The period for the reporting of SUSARs by the sponsor to the EMA will take account of the seriousness of the reaction and will be as follows:

- In the case of fatal or life-threatening SUSARs, as soon as possible and in any event not later than **seven days** after the sponsor became aware of the reaction;
- In the case of non-fatal or non-life-threatening SUSARs, not later than **15 days** after the sponsor became aware of the reaction;
- In the case of a SUSARs which was initially considered to be non-fatal or nonlife threatening but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than **seven days** after the sponsor became aware of the reaction being fatal or life-threatening.

Where necessary to ensure timely reporting, the sponsor may, in accordance with section 2.4 of Annex III, submit an initial incomplete report followed up by a complete report.

12.6 Annual safety report

Regarding investigational medicinal products other than placebo, the sponsor shall submit annually through CTIS to all Member States concerned a report on the safety of each investigational medicinal product used in a clinical trial.

12.7 Unblinding procedures for safety reporting

The investigator will only unblind the treatment allocation of a subject in the course of a clinical trial if unblinding is relevant to the safety of the subject.

When reporting a SUSAR to the EMA, the sponsor will only unblind the treatment allocation of the affected subject to whom the SUSAR relates.

In case emergency unblinding is necessary, a member of the unblinded team (i.e. the research nurses) will share the unblinded information to the Principal Investigator, so that in this case the coordinating investigator and other persons involved in gathering and analyzing data will remain blinded.

Unblinded information will be accessible only to persons who need to be involved in the safety reporting to the EMA, to Data Safety Monitoring Boards (DSMB), or to persons performing ongoing safety evaluations during the clinical trial.

12.8 Temporary halt for reasons of subject safety

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will submit the notification through CTIS without undue delay of a temporary halt but not later than in 15 days of the date of the temporary halt. It shall include the reasons for such action and specify follow-up measures. The study will be suspended pending a further positive decision by the concerned member state. The investigator will take care that all subjects are kept informed.

12.9 Urgent safety measures and other relevant safety reporting

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator will take appropriate urgent safety measures to protect the subjects. In addition, the sponsor will notify the Member States concerned, through CTIS, of the event and the measures taken. That notification will be made without undue delay but no later than **seven days** from the date the measures have been taken.

12.10 Data Safety Monitoring Board (DSMB)/Data Monitoring Committee (DMC)

A DSMB is established for this study to perform ongoing safety surveillance and to perform interim analyses on the safety data generated during the study as well as after the study completion. The DSMB is an independent committee, composed of the following persons:

- **Chair:** Dr. Rob ter Heine (clinical pharmacologist)
- Dr. Saskia Houterman (statistician and epidemiologist)
- Dr. Quirijn de Mast (internal medicine and infectious disease specialist)

The DSMB will perform two interim analyses, each one after completion of each study group (escalating dosages). An advice will be given whether to continue the study or make adjustments (to the protocol). The DSMB will focus on the following issues:

- Adverse events: type, severity, duration, action taken and attributability to the investigation treatment (AK1967).
- Safety data: vital signs, laboratory safety data (hematology, clinical chemistry etc.)
- Procedures and methodology.
- Decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated either for everyone or for some treatments groups.

The following criteria are defined on which basis the DSMB may decide to terminate the trial prematurely: any serious adverse event.

It may be necessary to (partially) deblind members of the DSMB for an adequate safety assessment. Therefore (partial) deblinding of the DSMB by someone of the unblinded research staff may be requested by the members of the DSMB. The advice of the DSMB will be sent to the sponsors and investigators of the study. In case of disagreement, a meeting of the DSMB and investigators can be held. In case of ongoing disagreement, the medical Ethics Committee will decide.

For a more detailed description on DSMB responsibilities and workflow, please see the DSMB charter submitted with this study protocol (*document D3, DSMB charter*).

13. STATISTICAL ANALYSIS

13.1 Description of statistical methods

Parameters will be presented as mean ± SEM or median and interquartile ranges, depending on their distribution. Categorical data will be presented as counts and percentages. A two sided p-value <0.05 is considered significant. Statistical analyses will be performed with R (*http://www.r-project.org*). No interim analyses will be performed during the study protocol.

13.2 Analysis sets

All subjects that successfully received study medication will be included in the analyses. Subjects that completed the medical screening, but fail to attend the study day (and thus not receive study medication) will not be included in the analyses.

13.3 Participant demographics and other baseline characteristics

Demographic and other baseline characteristics (including baseline blood sample measurements) will be summarized for the placebo and Procizumab dosage groups.

13.4 Randomisation and blinding

Randomization procedures were already described in detail in section 10.2 Randomization, blinding and treatment allocation. To summarize; the randomization procedure is stratified into the three groups, consisting of eight subjects each, with sequential increased administrated doses of Procizumab. Six subjects of each group who will be treated with active medication and two subjects with placebo treatment. These groups are further divided into two randomization blocks consisting of four subjects each (1 placebo treatment and 3 active medication treatment). This is done as an extra safety measure to ensure an even spread of placebo treated subjects over all groups (so that there can never be two placebo's at the start of the trial).

A randomization list will be created by unblinded research staff prior to the treatment day. This list contains the randomization numbers, consisting of a two- digit number preceded by capital 'R' to identify this number as being the randomization number. The first digit corresponds with the randomization block (1 to 6). The second digit corresponds with the randomization number within the randomization block, starting with one (for example R.1.1 for randomization block one, subject one, followed by R.1.2, R.1.1, onwards to R.6.4). The unblinded research staff will assign a treatment group (active medication treatment or placebo treatment) at random to each number on the list. They will begin with randomization block 1 and will continue until they reach randomization block 6. A spare set of randomization numbers will be available with ascending numbers from Rep.01 onward (Rep.01, Rep.02, Rep.03 etc.) for assignment to subjects replacing subjects who were randomized but discontinued prior to administration of the study medication.

13.5 Sample size, trial power and level of significance used

As the primary endpoint is safety and no comparative statistical analyses will be performed on this endpoint, a sample size calculation is not warranted. A generally accepted group size (for dose escalating phase 1 studies) of 6 subjects per dosing group will be included (apart from the 2 placebo-treated subjects per group). A sample size of 6 subjects per treatment group is regarded as being large enough to justify continuation of the trial with the next dosing group in the absence of adverse events related to the study medication. Likewise, one or more severe or serious adverse events

considered to be related to the study medication could be enough to discontinue the study without a statistical difference in AE's between the groups.

13.6 Planned analysis

13.6.1 Analysis primary endpoint

Safety data will be listed by subject number and summarized descriptively by group and by dose. Only subjects that were dosed on the study day will be included in the analyses, as safety parameters cannot be assessed in subjects that did not receive study medication.

13.6.2 Analysis secondary endpoint(s)

Pharmacokinetics of Procizumab (including AUC, C_{max}, terminal $t_{1/2}$, Cl, V) will be determined for each dose and overall. Changes over time in blood plasma levels and activity of cDPP3 and other variables (vital signs, laboratory parameters, ECG, angiotensin metabolites, cytokines) within each treatment group will be described and plotted. They may be analyzed exploratory (within or between groups) by repeated measures one- or two-way analysis of variance. Data will be log-transformed if necessary.

Differences between subjects will be analyzed using Chi^2 tests or Fisher exact tests for counts (e.g. adverse events). T-tests, Mann-Whitney-U tests, one- or two-way analyses of variance tests or Kruskal-Wallis tests will be implemented for continuous variables, with the choice of test depending on the number of groups compared, as well as the data's distribution.

13.6.3 Analysis other study parameters/endpoints

Not applicable.

13.7 Interim analysis

Not applicable.

13.8 (Statistical) criteria for termination of the trial

There are no pre-defined statistical criteria for discontinuing parts of the clinical trial, as the coordinating investigator responsible for primary data analysis will remain blinded during the study protocol.

We also refer to section 12.10. The DSMB will perform interim analyses after completion of each study group (escalating dosages). An advice will be given whether to continue the study or make adjustments (to the protocol). The DSMB will focus on the following issues:

- Adverse events: type, severity, duration, action taken and attributability to the investigation treatment (Procizumab).
- Safety data: vital signs, laboratory safety data (hematology, clinical chemistry etc.)
- Procedures and methodology.
- Decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated either for everyone or for some treatments groups.

13.9 Procedure for accounting for missing, unused and spurious data

Due to the small sample size spurious missing data will be imputed, if necessary, instead of deletion of the subject from the dataset. For continuous data, a missing value of a specific timepoint will be imputed by the mean of the value of the previous and next timepoint. If the last timepoint is missing,

the last observation is carried forward. If a subject drops out of the study prior to receiving study medication, this subject will be replaced, with a maximum of five replaced subjects. A newly recruited subject replacing a subject that dropped out will receive the treatment that was originally allocated for the subject they replace.

13.10 Procedure for reporting any deviation(s) from the original statistical plan

In case deviations from the original statistical plan are necessary, this will be documented in a note to file.

14. ETHICAL CONSIDERATIONS

14.1 Declaration of Helsinki

The sponsor will ensure that this study is conducted in accordance with the ethical principles that have their origins in the declaration of Helsinki.

14.2 Recruitment and informed consent procedures

Informed consent will be obtained prior to any study related procedures being undertaken at screening. Informed consent will be written, dated and signed by the investigator performing the interview, and by the subject. The investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. In the interview it will be verified that the subject has understood the information. The subject will be provided with a copy of the document (or the record) by which informed consent has been given. The informed consent will be documented. Adequate time will be given for the subject to consider his decision to participate in the clinical trial, with a minimum decision time of 48 hours.

Healthy subjects will be recruited by posters at several faculties on the campus of the Radboud University Nijmegen and online. Interested people will be emailed the subject information document. Subjects will be given at least one day to consider their decision. On screening day, subjects will be informed about the study by the coordinating investigator (research-physician) and their remaining questions will be answered. Finally, they can sign and date the informed consent in the presence of the investigator. Two identical informed consent forms will be signed and dated, one for the subject and one for the investigator.

14.3 Benefits and risks assessment, group relatedness

The subjects will not benefit directly from participation to the study. A subject fee is provided.

Total time burden for the study is approx. 30 hours: 1 hour for screening, 26 hours for the day of admission and 6 follow-up visits of approximately 30 minutes.

Blood withdrawal during the study is restricted to a smaller volume (<450 mL) than is withdrawn during routine phlebotomy at the blood bank, and is not associated with relevant risks.

Venipunctures, vascular access at the several study visits carries the risk of hematoma at the puncture sites, which will spontaneously resolve, should they occur. Blood loss from puncture sites after removal of cannulas will be stopped by applying pressure. A pressure bandage will be applied to the site of arterial cannulation. Also, vasovagal reactions can occur during a puncture procedure, which can be adequately treated.

Procizumab is a recombinant monoclonal antibody against DPP3, inhibiting its enzymatic activity as part of its mode of action. Procizumab binds to a conserved, surface exposed loop in proximity to DPP3's active site, which is conserved in all mammals. The humanized version of the antibody was implemented in all preclinical experiments, effectively demonstrating that Procizumab's primary mechanism (binding and inactivation of circulating DPP3) was closely matched between all investigated species.

The administration of Procizumab to rodents and non-human primates (NHP) has been tolerated very well. Single dose administration up to 150 mg/kg Procizumab to mice and 400 mg/kg to NHP have not shown any clinical adverse effects and no histopathological findings. Even the repeated administrations of up to 350 mg/kg in NHPs (administration at days 1, 2, 13 & 14) within the regulatory pre-clinical toxicity and safety study have not shown any clinical or abnormal histopathological findings.

In conclusion, we feel that the risk to, and burden for the subjects are in proportion to the potential value of the research.

14.4 Compensation for injury

Both the sponsor and Radboud university medical center have a liability insurance which is in accordance with article 7 of the WMO. Also, the required subject insurance has been acquired by Radboud university medical center. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

This insurance provides cover for damage to research subjects through injury or death caused by the study.

- € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
- € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

14.5 Compensation for subjects

At the end of the treatment day, a form is filled in together with the subjects through which a subject fee of €400,- will be transferred digitally. The remainder of the subject compensation fee (also €400,- will be transferred following the last visit on day 28.

14.6 Compensation for investigators

Not applicable.

14.7 Other ethical considerations

There are no other ethical considerations that were not already described in section 3.2.

15. ADMINISTRATIVE ASPECTS, MONITORING AND CONFIDENTIALITY

All experiments and handling of samples will be performed according to the latest version of the declaration of Helsinki, the Medical Research Involving Human Subjects Act (WMO) and Good Clinical Practice (GCP).

15.1 Approval initial application and substantial modifications

The trial protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other documents required by the Regulation will be submitted for the regulatory approval before the clinical trial is started via CTIS.

The sponsor will also submit and obtain approval for substantial modifications to the original approved documents via CTIS.

A 'substantial modification' is defined in the CTR as any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 and which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial.

15.2 Monitoring

Monitoring will be carried out by a BROK-certified internal monitor of the Radboud university medical center during the study. The stored data in the eCRFs, all informed consents, SAE reports and the Investigator Site File (also called study site TMF) will be monitored. Monitoring will take place according to the working method, responsibilities and specific requirements described in the monitoring plan.

15.3 Recording, handling and storage of information

15.3.1 Handling of data and data protection

Data will be handled confidentially and coded. A subject identification code list will be used to link the data to the subject. The code is not based on the patient initials and/or birth-date. The key to the code will be safeguarded by the Principal Investigator and coordinating investigator. The handling of subject data in this study complies with the *Dutch Personal Data Protection Act (in Dutch: Algemene Verordening Gegevensbescherming, AVG)* and the *General Data Protection Regulation (EU) 2016/679 (GDPR)*.

Data will be collected in an electronic CRF from a GCP compliant and validated data management system (Castor EDC). Study documents and study agreements will be kept in the Investigator Site File.

15.3.2 Source documents and case report forms (CRF)

Source documents for this study will include anonymised hospital records (i.e. a unique research dummy patient is made in the electronic health system (EPIC) of the Radboud University Medical Center for each subject) and procedure reports and data collection forms. These documents will be used to enter data on the CRFs. Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained. Data will be

collected in an electronic CRF from a validated data management system (Castor EDC). Study documents and study agreements will be kept in the Investigator Site File.

All documents will be stored safely in confidential conditions. On all study-specific documents other than the signed consent, the subject will be referred to by the study subject identification code. Source data will be archived in the personal medical file of each participant. This medical file will not leave the Radboud university medical center. This source document can only be viewed by trial monitors and investigators involved.

15.3.3 Clinical trial master file and data archiving

The sponsor and the investigator shall keep a clinical trial master file (referred to as TMF for the sponsor, as Investigator Site File or ISF for the investigator). The clinical TMF and ISF shall at all times contain the essential documents relating to the clinical trial which allow verification of the conduct of a clinical trial and the quality of the data generated.

The sponsor and the investigator shall archive the content of the clinical TMF and the ISF for at least 25 years after the end of the clinical trial, unless other EU law requires archiving for a longer period. The medical files of subjects shall be archived in accordance with national law.

The contents of the clinical TMF and ISF shall be archived in a way that ensures that it is readily available and accessible, upon request.

15.3.4 Collection and storage of biological samples

During the experiment, all blood samples will be collected in tubes identified by the unique subject code and a code for the specific time point. Blood samples will be stored for a maximum of five years after conclusion of the current trial. During this time period, samples can be used for additional analyses directly related to the current research objectives. When these additional analyses carry a risk for incidental medical findings, the METC that gave a favorable opinion for the study has to be notified to obtain permission beforehand.

Prior to the study, subjects will be asked if they agree to having their left-over materials (blood) stored for up to a maximum of five years. This material will be kept for the purpose of possible future investigations related to the original research question. This material will be safely stored in a freezer, behind a locked door, located in the research department of the Intensive Care. Access to this freezer is limited to selected research personnel from the Department of Intensive Care from the Radboud university medical center only. The material will be recognizable only by the screening number and abbreviated name of the study.

The Sponsor, the Principal Investigator and coordinating investigator of the present study will be allowed to use the material for scientific research within the original research question. For use of the materials outside of the original research question, permission for use will always have to be acquired from the Ethics Committee and also from the subject (unless the material is processed anonymously and they have agreed to this on beforehand). The principal and coordinating investigators will manage the materials. Any third-party requesting use of the material will have to ask permission through the principal and coordinating investigators of the study. METC approval is required if human materials are transferred to the sponsor or third parties for research purposes other than described in this study protocol.

15.4 Audits and inspections and direct access to source data/documents

This trial may be subject to internal or external monitoring, auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents including direct access to source data will be given at that time.

15.5 Reporting of serious breaches

The sponsor will notify the Member States concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than **7 days** of becoming aware of that breach.

15.6 Notification of the start and the end of the recruitment

The sponsor will notify within 15 days each Member State concerned of the start of a clinical trial in relation to that Member State through CTIS.

The sponsor will notify within 15 days each Member State concerned of the first visit of the first subject in relation to that Member State through CTIS.

The sponsor will notify within 15 days each Member State concerned of the end of the recruitment of subjects for a clinical trial in that Member State through the EU.

15.7 Temporary halt/(early) termination

The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in relation to that Member State through CTIS.

The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in all Member States concerned and in all third countries in which the clinical trial has been conducted through CTIS.

15.7.1 Temporary halt/early termination for reasons not affecting the benefit-risk balance

The sponsor will notify with 15 days each Member State concerned of a temporary halt of a clinical trial in all Member States concerned for reasons not affecting the benefit-risk balance through CTIS.

When a temporarily halted clinical trial for reasons not affecting the benefit-risk balance is resumed the sponsor will notify each Member State concerned through CTIS.

The sponsor will notify to the EU portal CTIS of early termination of the clinical trial for reasons not affecting the benefit-risk balance through CTIS. The reasons for such action and, when appropriate, follow-up measures for the subjects will be provided as well.

15.7.2 Temporary halt/early termination for reasons of subject safety

In accordance to article 38 of the CTR, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The temporary halt or early termination of a clinical trial for reasons of a change of the benefit-risk balance will be notified to the

Member States concerned through the EU portal CTIS without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures. The restart of the clinical trial following a temporary halt as referred to in paragraph 1 shall be deemed to be a substantial modification subject to the authorisation procedure laid down in Chapter III of the CTR (CTR: Article 38).

15.8 Summary of the results

Within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU database CTIS a summary of the results of the clinical trial. The content of the summary of the results is set out in CTR Annex IV. It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of the summary is set out in CTR Annex V (CTR: Article 37(4)).

15.9 Public disclosure and publication policy

The Principal Investigator and the sub-investigators will write a manuscript which is to be published in a peer-reviewed scientific medical journal after completion of the trial. The study will be published regardless of the results of the trial. Authors have no conflict of interest to declare.

16. REFERENCES

1. Hollenberg SM, Kavinsky CJ, Parrillo JE. Cardiogenic shock. Annals of internal medicine. 1999;131(1):47-59.
2. Reynolds HR, Hochman JS. Cardiogenic shock: current concepts and improving outcomes. Circulation. 2008;117(5):686-97.
3. Puymirat E, Fagon JY, Aegerter P, Diehl JL, Monnier A, Hauw-Berlemont C, et al. Cardiogenic shock in intensive care units: evolution of prevalence, patient profile, management and outcomes, 1997-2012. Eur J Heart Fail. 2017;19(2):192-200.
4. Champion S. Extracorporeal membrane oxygenation in the therapy of cardiogenic shock (ECMO-CS): the need for a better definition of refractory cardiogenic shock. Eur J Heart Fail. 2018;20(2):197-8.
5. Reyentovich A, Barghash MH, Hochman JS. Management of refractory cardiogenic shock. Nat Rev Cardiol. 2016;13(8):481-92.
6. Hochman JS. Cardiogenic shock complicating acute myocardial infarction: expanding the paradigm. Circulation. 2003;107(24):2998-3002.
7. Shah M, Patnaik S, Patel B, Ram P, Garg L, Agarwal M, et al. Trends in mechanical circulatory support use and hospital mortality among patients with acute myocardial infarction and non-infarction related cardiogenic shock in the United States. Clin Res Cardiol. 2018;107(4):287-303.
8. Vahdatpour C, Collins D, Goldberg S. Cardiogenic Shock. J Am Heart Assoc. 2019;8(8):e011991.
9. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, et al. Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama. 2016;315(8):775-87.
10. Cooper HA, Panza JA. Cardiogenic shock. Cardiol Clin. 2013;31(4):567-80, viii.
11. Prajapati SC, Chauhan SS. Dipeptidyl peptidase III: a multifaceted oligopeptide N-end cutter. FEBS J. 2011;278(18):3256-76.
12. van Lier D, Kox M, Pickkers P. Promotion of vascular integrity in sepsis through modulation of bioactive adrenomedullin and dipeptidyl peptidase 3. J Intern Med. 2020.
13. Rehfeld L, Funk E, Jha S, Macheroux P, Melander O, Bergmann A. Novel Methods for the Quantification of Dipeptidyl Peptidase 3 (DPP3) Concentration and Activity in Human Blood Samples. J Appl Lab Med. 2019;3(6):943-53.
14. Blet A, Deniau B, Santos K, van Lier DPT, Azibani F, Wittebole X, et al. Monitoring circulating dipeptidyl peptidase 3 (DPP3) predicts improvement of organ failure and survival in sepsis: a prospective observational multinational study. Critical care. 2021;25(1):61.
15. Takagi K, Blet A, Levy B, Deniau B, Azibani F, Feliot E, et al. Circulating dipeptidyl peptidase 3 and alteration in haemodynamics in cardiogenic shock: results from the OptimaCC trial. Eur J Heart Fail. 2019.
16. Deniau B, Rehfeld L, Santos K, Dienelt A, Azibani F, Sadoune M, et al. Circulating dipeptidyl peptidase 3 is a myocardial depressant factor: dipeptidyl peptidase 3 inhibition rapidly and sustainably improves haemodynamics. Eur J Heart Fail. 2019.
17. Abramic M, Zubanovic M, Vitale L. Dipeptidyl peptidase III from human erythrocytes. Biol Chem Hoppe Seyler. 1988;369(1):29-38.
18. Bitker L, Burrell LM. Classic and Nonclassic Renin-Angiotensin Systems in the Critically Ill. Crit Care Clin. 2019;35(2):213-27.
19. Antonucci E, Gleeson PJ, Annoni F, Agosta S, Orlando S, Taccone FS, et al. Angiotensin II in Refractory Septic Shock. Shock. 2017;47(5):560-6.
20. Pang X, Shimizu A, Kurita S, Zankov DP, Takeuchi K, Yasuda-Yamahara M, et al. Novel Therapeutic Role for Dipeptidyl Peptidase III in the Treatment of Hypertension. Hypertension. 2016;68(3):630-41.
21. Deniau B, Blet A, Santos K, Vaittinada Ayar P, Genest M, Kastorf M, et al. Inhibition of circulating dipeptidyl-peptidase 3 restores cardiac function in a sepsis-induced model in rats: A proof of concept study. PLoS One. 2020;15(8):e0238039.

22. Cruz-Diaz N, Wilson BA, Pirro NT, Brosnihan KB, Marshall AC, Chappell MC. Identification of dipeptidyl peptidase 3 as the Angiotensin-(1-7) degrading peptidase in human HK-2 renal epithelial cells. Peptides. 2016;83:29-37.
23. Jha S, Taschler U, Domenig O, Poglitsch M, Bourgeois B, Pollheimer M, et al. Dipeptidyl peptidase 3 modulates the renin-angiotensin system in mice. J Biol Chem. 2020.
24. Poglitsch M, Sturrock ED, Danser AH. Letter to the editor: Angiotensin quantification by mass spectrometry. Am J Physiol Heart Circ Physiol. 2016;310(3):H452-3.
25. Picod A, Genest M, Assad N, Polidano E, Placier S, Gaudry S, et al. Circulating dipeptidyl peptidase 3 modulates hemodynamics and the renin-angiotensin-aldosterone system in mice. Archives of Cardiovascular Diseases Supplements. 2022;14(2):185.
26. Baral PK, Jajcanin-Jozic N, Deller S, Macheroux P, Abramic M, Gruber K. The first structure of dipeptidyl-peptidase III provides insight into the catalytic mechanism and mode of substrate binding. J Biol Chem. 2008;283(32):22316-24.
27. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.
28. Abramic M, Schleuder D, Dolovcak L, Schroder W, Strupat K, Sagi D, et al. Human and rat dipeptidyl peptidase III: biochemical and mass spectrometric arguments for similarities and differences. Biol Chem. 2000;381(12):1233-43.
29. Hashimoto J, Yamamoto Y, Kurosawa H, Nishimura K, Hazato T. Identification of dipeptidyl peptidase III in human neutrophils. Biochem Biophys Res Commun. 2000;273(2):393-7.
30. Grdisa M, Vitale L. Types and localization of aminopeptidases in different human blood cells. Int J Biochem. 1991;23(3):339-45.
31. Kar NC, Pearson CM. Dipeptidyl peptidases in human muscle disease. Clin Chim Acta. 1978;82(1-2):185-92.
32. Hopsu-Havu VK, Jansen CT. Peptidases in the skin. II. Demonstration and partial separation of several specific dipeptide naphthylamidases in the rat and human skin. Arch Klin Exp Dermatol. 1969;235(1):53-62.
33. Alba F, Arenas JC, Lopez MA. Properties of rat brain dipeptidyl aminopeptidases in the presence of detergents. Peptides. 1995;16(2):325-9.
34. Lynn KR. The isolation and some properties of dipeptidyl peptidases II and III from porcine spleen. Int J Biochem. 1991;23(1):47-50.
35. van Lier D, Kox M, Pickkers P. Commentary: Plasma angiotensin II is increased in critical coronavirus disease 2019. Front Cardiovasc Med. 2022;9:1012452.

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