A Phase IIb Randomised, Double-blind, Placebo-controlled, Multi-centre, Dose-ranging Study of AZD3427 in Participants with Heart Failure and Pulmonary Hypertension due to Left Heart Disease (WHO Group 2)

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Clinical Study Protocol	
Study Intervention	AZD3427
Study Code	D8330C00003
Version	4.0
Date	20 November 2024

A Phase IIb Randomised, Double-blind, Placebo-controlled, Multi-centre, Dose-ranging Study of AZD3427 in Participants with Heart Failure and Pulmonary Hypertension due to Left Heart Disease (WHO Group 2)

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This protocol has been subject to a peer review according to AstraZeneca standard procedures. The protocol is publicly registered and the results are disclosed and/or published according to the AstraZeneca Global Standard – Bioethics and in compliance with prevailing laws and regulations.

Version Scope: Global

Brief Title: A Phase IIb Study of AZD3427 in Participants with Heart Failure and Pulmonary Hypertension Group 2

Study Phase: II

Acronym: Re-PHIRE (**Re**laxin in **P**ulmonary **H**ypertens**I**on due to left hea**R**t diseas**E**)

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SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
CSP Version 4.0	20 November 2024
CSP Version 3.0	09 January 2024
CSP Version 2.0	01 December 2022
CSP Version 1.0	24 November 2022

CSP Version 4.0

This modification is considered to be substantial based on the criteria set forth in the EU Clinical Trial Regulation No 536/2014 Article 2(2)(13).

Overall Rationale for the Modification:

This amendment has been made to move NAbs (Neutralising antibodies) from secondary endpoint to exploratory endpoint.

Summary of Changes

Section Number and Name	Description of Change	Brief Rationale
Title Page	Update Study Clinical Lead	Change in Study Clinical Lead.
Section 1.1 Synopsis Section 3.2 Objectives and Endpoints Section 9.4.4.1 Immunogenicity Assessments	Neutralising antibodies (NAbs) was removed from the secondary endpoints and added to the exploratory endpoints.	Neutralising antibodies will only be assessed as an exploratory endpoint
Section 5.2 Exclusion Criteria Section 6.11 Prior and Concomitant Therapy	Exclusion criterion number 17 and Section 6.11 was updated to clarify that the use of inhibitors of cGMP specific phosphodiesterase type 5 (PDE5-I if taken for erectile dysfunction or other occasional, non-continuous uses) are prohibited for 48 hours before echocardiography and RHC (Screening Visits 1 and 2 and Visit 15).	Clarification
Section 9.4.3.2 Vital Signs	Vital signs updated to include pulse rate.	Consistency
Section 8.3.3 Electrocardiograms	Text written more clearly in the wording referring to parameters being measured and calculated, and wording added to confirm when ECG machine is not able to calculate automatically manual calculation is acceptable	Clarification

Section Number and Name	Description of Change	Brief Rationale
Appendix A1 Regulatory and Ethical Considerations	Regulatory Reporting Requirements for SAEs wording updated. Regulatory Reporting Requirements for Serious Breaches of Protocol or GCP wording simplified.	Template update
Appendix E6 Laboratory Tests, Hy's Law Lab Kit for Central Laboratories	Updated wording related HSV testing for Hy's Law	Update due to change in testing laboratory (except China)
Throughout the CSP	Minor editorial updates through the CSP for consistency and clarity.	Consistency

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation/Term	Explanation
6MWD	6-minute walking distance
6MWT	6-minute walking test
ADA	Anti-drug antibody
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase/transaminase
ANCOVA	Analysis of covariance
anti-HBc	Total hepatitis B core antibody
AST	Aspartate aminotransferase/transaminase
ATS	American Thoracic Society
BP	Blood pressure
CFR	Code of Federal Regulations
CKP-EPI	Chronic Kidney Disease Epidemiology Collaboration
COVID-19	Coronavirus disease of 2019
CpC	Combined pre- and post-capillary
CRO	Contract Research Organisation
CRT-P	Cardiac resynchronisation therapy with a pacemaker
CSP	Clinical study protocol
CSR	Clinical study report
CV	Cardiovascular
DAE	Discontinuation due to AE
DILI	Drug induced liver injury
DNA	Deoxyribonucleic acid
E/e’	Early mitral filling velocity/early diastolic mitral annular velocity (ratio)
ECG	Electrocardiogram
Echo	Echocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESC/ERS	European Society of Cardiology/European Respiratory Society
ET	Early termination
EU	European Union
EU CT	EU Clinical Trial

Abbreviation/Term	Explanation
FC	Functional Class
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in the first second
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBS	Human biological sample(s)
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HF	Heart failure
HFmrEF	Heart failure with midrange ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Heart rate
hsCRP	High-sensitive C-reactive protein
hs-TnT	High-sensitive troponin T
IA	Interim analysis
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IgG1	Immunoglobulin G1
IND	Investigational New Drug
IpC	Isolated post-capillary
IRB	Institutional Review Board
IRT	Interactive Response Technology
IV	Intravenous
IVC	Inferior vena cava
KCCQ	Kansas City Cardiomyopathy Questionnaire
KIM-1	Kidney injury molecule 1
LH	Luteinising hormone
LVGLS	Left ventricular global longitudinal strain
MAD	Multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
mPAP	Mean pulmonary arterial pressure

Abbreviation/Term	Explanation
NA	Not applicable
NAbs	Neutralising antibodies
NHP	Non-human primates
NIMP	Non-investigational medicinal product
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
NYHA	New York Heart Association
PAP	Pulmonary arterial pressure
PASP	Pulmonary arterial systolic pressure
PAWP	Pulmonary artery wedge pressure
PD	Pharmacodynamic(s)
PDE5-I	Phosphodiesterase type 5 inhibitor
PH	Pulmonary hypertension
PH-LHD	Pulmonary hypertension due to left heart disease
PIIINP	Amino-terminal propeptide of type III procollagen
PK	Pharmacokinetic(s)
PRD	Patient rating of dyspnoea
PRO	Patient reported outcomes
PRO-C6	P204 endotrophin
PVR	Pulmonary vascular resistance
Q1	First quartile
Q2W	Twice weekly
Q3	Third quartile
QTcF	QT interval corrected for heart rate using Fridericia's formula
QTL	Quality tolerance limit
RAAS	Renin-angiotensin-aldosterone system
Re-PHIRE	**Re**laxin in **P**ulmonary **H**ypertens**I**on due to left hea**R**t diseas**E**
RHC	Right heart catheterisation
RNA	Ribonucleic acid
RTSM	Randomisation and Trial Supply Management
RV/LV	Right ventricle/left ventricle
RVOT AT	Right ventricular outflow tract acceleration time
RXFP1	Relaxin/insulin-like family peptide receptor-1
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous

Abbreviation/Term	Explanation
SD	Standard deviation
SoA	Schedule of activities
SOC	System Organ Class
SV	Stroke volume
SVR	Systemic vascular resistance
TAPSE	Tricuspid annular plane systolic excursion
TBL	Total bilirubin
TGF-β	Transforming growth factor beta
TNF-α	Tumour necrosis factor alpha
TR Vmax	Maximum tricuspid regurgitation velocity
TRV	Tricuspid regurgitation velocity
TSS	Total symptom score
uACR	Urine albumin-creatinine ratio
ULN	Upper limit of normal
US	United States
VC	Vital capacity
WHO	World Health Organisation

1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title:

A Phase IIb Randomised, Double-blind, Placebo-controlled, Multi-centre, Dose-ranging Study of AZD3427 in Participants with Heart Failure and Pulmonary Hypertension due to Left Heart Disease (WHO Group 2)

Brief Title:

A Phase IIb Study of AZD3427 in Participants with Heart Failure and Pulmonary Hypertension Group 2

Regulatory Agency Identifier Number(s):

Registry	ID
IND Number:	151646
EU CT Number:	2022-502382-25-00

Rationale:

Pulmonary hypertension Group 2 results from dysfunction of the left heart and is the most common cause of pulmonary hypertension (PH). Left-sided dysfunction causes pulmonary pressure to rise, leading to lung oedema, damage of pulmonary vessels, and ultimately right ventricle failure. Pulmonary hypertension Group 2 is primarily comprised of heart failure (HF) New York Heart Association (NYHA) Functional Class II to IV patients, with 40% to 75% of HF patients with reduced ejection fraction (HFrEF) and 36% to 83% of HF patients with preserved ejection fraction (HFpEF) also having PH.

Pulmonary hypertension Group 2 is associated with impaired exercise capacity and reduced survival. These patients live with significantly limited physical activity and quality of life despite optimal guideline-directed management. Currently, there is no dedicated treatment for this group of patients.

The primary objective of this study is to assess the ability of AZD3427 to reduce pulmonary vascular resistance (PVR) after 24 weeks of treatment. Additional safety, function and symptoms, hemodynamic, and biomarker assessments will also be performed, with key secondary endpoints assessed at baseline and after 12 and 24 weeks of treatment. Right heart catheterisation (RHC) and echocardiograms will be performed at baseline and after 24 weeks of treatment.

Objectives, Endpoints, and Estimands:

The following information relates to the objectives of the study and the rationale behind their selection:

Objectives	Endpoints
Primary	
To evaluate the effect of AZD3427 on PVR after 24 weeks of treatment in participants with HF and PH Group 2	Change in PVR from baseline to Week 25 compared with placebo, as measured by RHC.
Secondary	
To evaluate the effect of AZD3427 on additional hemodynamic markers of cardiac function after 24 weeks of treatment in participants with HF and PH Group 2	Change from baseline to Week 25 in RHC parameters compared with placebo. Parameters include: • mPAP and PAWP. Change from baseline to Week 25 in echocardiographic parameters compared with placebo. Parameters include: • Cardiac output, SV, EF, LVGLS, PASP, RV/LV ratio, RVOT AT, TRV, TAPSE/PASP. • Systemic vascular resistance.
To evaluate the effect of AZD3427 on function and symptoms after 24 weeks of treatment in participants with HF and PH Group 2	Change in 6MWD and KCCQ TSS from baseline to Week 25 compared with placebo. Change in NYHA FC from baseline to Week 25 compared with placebo.
To evaluate the effect of AZD3427 on biomarkers of cardiac and renal function after 12 and 24 weeks of treatment in participants with HF and PH Group 2	Change in serum creatinine, NT-proBNP, cystatin C, and eGFR from baseline to Week 13 and Week 25 compared with placebo.
To evaluate the PK of AZD3427 after repeat Q2W SC dosing in participants with HF and PH Group 2	AZD3427 serum PK concentrations.
To evaluate the immunogenicity of AZD3427	Presence of ADAs and evaluation of ADA titres.
Safety	
To evaluate the safety and tolerability of AZD3427 as compared to placebo in participants with HF and PH Group 2	Adverse events/SAEs, vital signs (BP, HR, respiratory rate, body temperature, and pulse rate), body weight, ECGs, clinical laboratory assessments, physical examinations, and injection site reactions.

6MWD = 6-minute walking distance; ADA = anti-drug antibodies; BP = blood pressure; EF = ejection fraction; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; HF = heart failure; HR = heart rate; IVC = inferior vena cava; KCCQ = Kansas City Cardiomyopathy Questionnaire; LVGLS = left ventricular global longitudinal strain; NYHA FC = New York Heart Association Functional Class; NT-proBNP = N-terminal prohormone of brain natriuretic peptide; PAP = pulmonary arterial pressure; PASP = pulmonary arterial systolic pressure; PAWP = pulmonary artery wedge pressure; PH = pulmonary hypertension; PK = pharmacokinetic(s); PVR = pulmonary vascular resistance; Q2W = twice weekly; RHC = right heart catheterisation; RV/LV = right ventricle/left ventricle; RVOT AT = right ventricular outflow tract acceleration time; SAE = serious adverse event; SC = subcutaneous; SV = stroke volume; TAPSE = tricuspid annular plane systolic excursion; TR = tricuspid regurgitation velocity; TSS = total symptom score.

Overall Design Synopsis:

This is a Phase IIb randomised, double-blind, placebo-controlled, multi-centre, dose-ranging study to evaluate the efficacy, safety, and pharmacokinetics (PK) of AZD3427 in addition to standard of care in participants with HF and PH due to left heart disease (World Health Organisation [WHO] Group 2). This study will be agnostic to ejection fraction (EF) and agnostic to PVR (includes both isolated post-capillary PH and combined pre- and post-capillary PH participants).

Approximately 220 participants will be randomised to 4 treatment groups (in a 1:1:1:1 ratio) to receive a SC injection of AZD3427 or placebo every 2 weeks for 24 weeks. All participants will be centrally assigned to randomised study intervention. Randomisation to study intervention will be performed to ensure approximate balance between the treatment groups. This study will evaluate 3 dose levels of AZD3427: 1.1, 5.4, and 30.0 mg. Each treatment group will comprise of approximately 55 participants.

The study will be conducted in approximately 60 study centres across an estimated 15 countries.

The study will include approximately 16 study visits: 2 visits during the Screening Period, 13 visits during the Treatment Period, and one visit during the Follow-up Period. The expected total duration of the study is 32 to 37 weeks, depending on the length of the Screening Period.

Participants will remain at the study centre for 1 hour after the first 3 doses, and then for 30 minutes after each subsequent dose, or longer at the judgement of the investigator considering the risk of anaphylaxis and hypotension for the individual participant.

The primary objective of this study is to assess the ability of AZD3427 to reduce PVR after 24 weeks of treatment. Additional safety, function and symptoms, hemodynamic, and biomarker assessments will also be performed. Key secondary endpoints will be assessed at baseline and after 12 and 24 weeks of treatment. Right heart catheterisation and echocardiograms will be performed at baseline and after 24 weeks of treatment.

Participants will receive an echocardiogram at Screening Visit 1. Only participants who fulfil all eligibility criteria after Visit 1 will receive a RHC at Screening Visit 2. Echocardiographic parameters from Screening Visit 1 will be assessed locally to determine eligibility for progression to Screening Visit 2.

Randomisation of participants with mPAP values of 20 to 24.9 mmHg (inclusive) is capped at no more than 15% of the total study population. If this cap is reached, future participants must have mPAP values ≥ 25 mmHg to be randomised into the study. There is no cap on participants with mPAP values ≥ 25 mmHg.

An interim analysis (IA) will be performed by an Unblinded Review Committee after approximately 50% of participants complete Visit 15 with an option to trigger early initiation of Phase III activities.

Brief Summary:

The purpose of this study is to evaluate the efficacy, safety, and PK of AZD3427 in addition to standard of care in participants with HF and PH due to left heart disease (WHO Group 2).

Study details include:

- The study duration will be up to 37 weeks.
- The treatment duration will be up to 24 weeks.
- The visit frequency will be every 2 weeks during treatment.

Disclosure Statement:

This is a randomised, placebo-controlled, dose-ranging study with 4 treatment groups that is double blinded.

Number of Participants:

Approximately 220 participants will be randomised to study intervention such that approximately 188 evaluable participants complete the study.

Note: 'Screened' means a participant's agreement to participate in a clinical study following completion of the informed consent process. Potential participants who are screened for the purpose of determining eligibility for the study, but are not randomised/assigned in the study, are considered 'screen failures', unless otherwise specified by the protocol.

Study Groups and Duration:

Eligible participants will be randomised equally to 1 of 4 treatment groups. The total study duration for each participant will be up to 37 weeks (from screening to follow-up). The Treatment Period will be 24 weeks, during which participants will receive AZD3427 or placebo by SC injection every 2 weeks (Q2W). A Follow-up Visit will take place 8 weeks after the last dose.

Participants will be administered AZD3427 (1.1, 5.4, or 30.0 mg) or placebo every 2 weeks by SC injection during the Treatment Period. Study intervention will be labelled as required per country requirement.

Dose modification is not applicable for this study.

Data Monitoring/Other Committee:

An independent Unblinded Review Committee will be set up for this study to assess the IA data to accelerate internal resourcing of the Phase III study. The IA will be performed after approximately 50% of participants complete Visit 15.

Statistical Methods

All efficacy and safety variables will be summarised by treatment group using descriptive statistics (n, mean, standard deviation [SD], median, minimum and maximum for continuous data that is expected to be normally distributed; n, geometric mean, coefficient of variation, median, minimum and maximum for continuous data that is expected to be lognormal; and n, frequencies and percentages for categorical data). Data will be summarised by time point/visit, as applicable.

Outputs will also include a total pooled AZD3427 group (comprising of 1.1, 5.4, and 30.0 mg treatment groups) and a high pooled AZD3427 group (comprising of 5.4 and 30.0 mg treatment groups).

P-values will be presented as 2-sided without multiplicity adjustments. Confidence intervals will be presented as 95% 2-sided, without multiplicity adjustments.

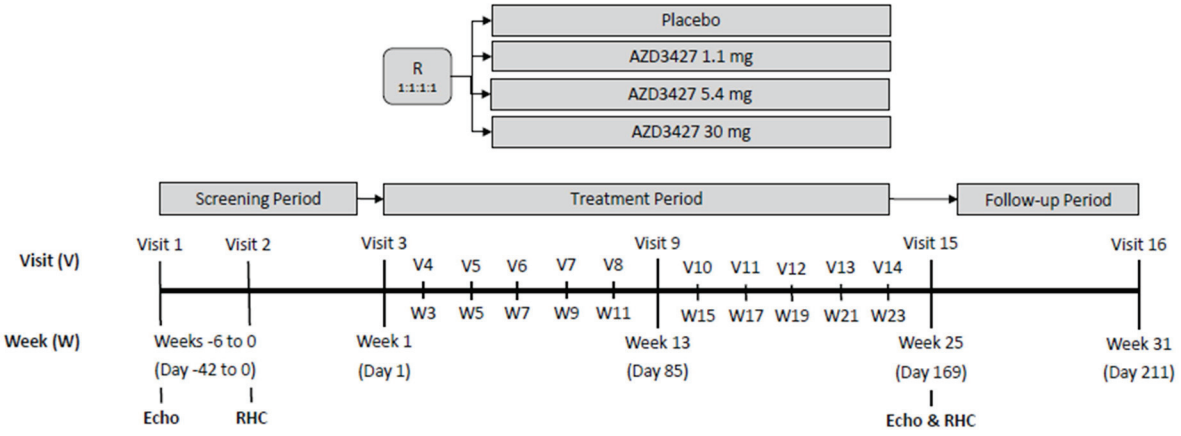
For the primary endpoint, 47 evaluable participants per group will provide approximately 80% power to detect a 20% reduction in PVR at Week 25 compared to placebo, assuming a 5% 2-sided significance level and a SD on the loge scale of 0.38. A total of approximately 220 participants (55 participants per treatment group) will be randomised to account for incomplete data and participant drop-out.

The primary efficacy endpoint is the change in PVR from baseline to Week 25. Pulmonary vascular resistance will be log-transformed for statistical analysis purposes. The mean log changes in PVR at Week 25 for each of the 3 AZD3427 doses and placebo will be estimated using an ANCOVA model, with treatment as fixed effect, stratification factors fitted as categorical covariates and baseline PVR fitted as a continuous covariate.

Secondary endpoints which are assessed post-baseline at Week 25 only, such as change from baseline in mean pulmonary arterial pressure (mPAP) and pulmonary artery wedge pressure (PAWP), will be analysed using an analysis of covariance (ANCOVA) model as described for the primary efficacy endpoint.

1.2 Schema

Figure 1 Study Schema



Echo = echocardiogram; R = randomisation; RHC = right heart catheterisation.

1.3 Schedule of Activities

Table 1 Schedule of Activities

Study Period	Screening		Treatment Period													FU
Visit	**V1** [a]	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15/ ET** [b]	**V16**
Study Week	**W-6 to W-1**		**W1**	**W3**	**W5**	**W7**	**W9**	**W11**	**W13**	**W15**	**W17**	**W19**	**W21**	**W23**	**W25**	**W31**
Study Day	**D-42 to D-1**		**D1**	**D15**	**D29**	**D43**	**D57**	**D71**	**D85**	**D99**	**D113**	**D127**	**D141**	**D155**	**D169**	**D211**
Visit window (days)				**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**-7/+ 3**	**± 3**
General procedures:																
Informed consent	X															
Eligibility	X	X [c]	X													
Demography	X															
Medical history	X	X [d]	X [d]													
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X															
Body weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination [e]	Full		Full	Brief	Brief	Brief	Brief	Brief	Brief	Brief	Brief	Brief	Brief	Brief	Full	Full
Pregnancy test [f]		X														
LH/FSH (females only)	X															
Drug screen [g]	X															
NYHA Functional Classification	X		X												X	
Blood sample for Genomics Initiative (optional) [h]			X													
Randomisation			X													
Study intervention administration			X	X	X	X	X	X	X	X	X	X	X	X		
Safety and tolerability:																
Assessment of AEs and SAEs	Only SAEs	Only SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 1 Schedule of Activities

Study Period	Screening		Treatment Period													FU
Visit	**V1** [a]	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15/ ET** [b]	**V16**
Study Week	**W-6 to W-1**		**W1**	**W3**	**W5**	**W7**	**W9**	**W11**	**W13**	**W15**	**W17**	**W19**	**W21**	**W23**	**W25**	**W31**
Study Day	**D-42 to D-1**		**D1**	**D15**	**D29**	**D43**	**D57**	**D71**	**D85**	**D99**	**D113**	**D127**	**D141**	**D155**	**D169**	**D211**
Visit window (days)				**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**-7/+ 3**	**± 3**
Assess injection site reaction			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (BP, HR, respiratory rate, body temperature, and pulse rate) [i]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Haematology, clinical chemistry, urinalysis, and coagulation (including eGFR)	X		X	X	X	X [j]			X			X			X	X
12-lead ECG [k]	X		X		X	X			X						X	
Pharmacokinetics and Immunogenicity:																
Serum for AZD3427 PK				X	X				X			X			X	X
Serum for AZD3427 ADAs/NAbs [l]			X	X	X				X						X	X
Pharmacodynamics:																
RHC [m]		X													X [n]	
Echocardiography [o]	X														X	
6MWD [p]			X												X	
KCCQ [q]			X												X	
Serum creatinine	X		X	X	X	X			X			X			X	X
NT-proBNP			X			X			X			X			X	X
Cystatin C			X	X	X	X			X			X			X	X
Exploratory:																
Assessment of CV events				X	X	X	X	X	X	X	X	X	X	X	X	

Table 1 Schedule of Activities

Study Period	Screening		Treatment Period													FU
Visit	**V1** [a]	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15/ ET** [b]	**V16**
Study Week	**W-6 to W-1**		**W1**	**W3**	**W5**	**W7**	**W9**	**W11**	**W13**	**W15**	**W17**	**W19**	**W21**	**W23**	**W25**	**W31**
Study Day	**D-42 to D-1**		**D1**	**D15**	**D29**	**D43**	**D57**	**D71**	**D85**	**D99**	**D113**	**D127**	**D141**	**D155**	**D169**	**D211**
Visit window (days)				**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**-7/+ 3**	**± 3**
Urine, serum, and plasma for biomarkers of inflammation, fibrosis, RAAS, tissue remodelling, fluid/electrolyte balance, and RXFP1 signalling and plasma for multiplex biomarker analysis			X						X						X	
hs-TnT			X	X	X	X			X			X			X	
uACR [r]			X						X						X	X
Plasma for endogenous relaxin			X													
Plasma, serum, and urine samples for Future Use (optional) [h]			X						X						X	

Note: all samples and assessments performed on dosing days should be performed pre-dose unless otherwise specified.

[a] Screening Visit 1 activities may be split across more than one day if required.

[b] If participation in the study is terminated early, the participant should return for an ET Visit as soon as possible. Early termination participants should still be requested to do a safety Follow-up Visit 8 weeks after the last dose. For scheduling purposes Visit 15 activities can be performed across multiple days but must all be completed within the visit window.

[c] Check all results from Screening Visit 1 and any changes before performing RHC.

[d] Any updates since Visit 1.

[e] Special attention should be paid to symptoms of fluid balance as a sign of worsening of HF during the Treatment Period.

[f] Pregnancy testing at the Screening Visit 2 to be performed with urine and will only be performed if LH and FSH levels are not in the postmenopausal range.

[g] Drug screening will be completed for all participants locally with laboratory kits provided by the central laboratory.

[h] Only if optional additional consent is obtained.

[i] Vital sign measurements will be measured with the participant in seated position after 5-minute rest. Vital signs will be measured pre-dose, before any blood draws, at all visits. Seated BP measurements (20, 40, and 60 minutes post-dose), will be recorded for the 2 first doses (Visit 3 and Visit 4) of study intervention. Seated BP measurements will be recorded post-dose at the remaining dosing visits, prior to leaving the study centre.

[j] Only full blood count and urinalysis.

[k] The ECGs will be collected with the participant in supine position after 10-minute supine rest and performed before any blood draws for overall evaluation (normal/abnormal with description if abnormal).

[l] If a participant's end-of-study sample (early termination or follow-up visit whichever is the latest) is confirmed ADA-positive and their baseline sample was ADA-negative, the participant will be asked to return to provide another sample after approximately 3 months to evaluate whether or not ADAs persist. If the sample taken after 3 months is ADA-positive, the participant will be asked to return to provide a sample after another 3 months (ie, 6 months after the end-of-study visit). If the sample after 6 months is ADA-positive, the investigator and the Medical Monitor will discuss any further action.

[m] Participants must have a combination of echocardiographic parameters at Screening Visit 1 that show intermediate or high probability of PH as per 2022 ESC/ERS guidelines (as specified in Appendix I). Findings on echocardiography will be assessed locally to determine eligibility for progression to Screening Visit 2.

[n] For the ET Visit, RHC will only be performed for participants that were randomised to the study at least 12 weeks prior to the ET Visit.

[o] The screening echocardiogram can be performed on a different day if it is not possible to be done at Screening Visit 1 due to scheduling purposes but must be completed as soon as possible after Screening Visit 1. This must be completed prior to the RHC in order to have results available to assess if participant meets the criteria to undergo the RHC.

[p] The 6MWT consists of a suite of assessments that are carried out before and after the 6MWT itself: seated pulse rate, blood pressure, oxygen saturation, and PRD-acute version.

[q] In order to limit bias in participant responses, KCCQ will be completed prior to any other study procedures at that visit.

[r] Urine will be collected at the study centre for testing at central laboratory.

6MWD = 6-minute walking distance; 6MWT = 6-minute walking test; ADA = anti-drug antibodies; AE = adverse event; BP = blood pressure; CV = cardiovascular; D = day; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; ESC/ERS = European Society of Cardiology/European Respiratory Society; ET = early termination; FSH = follicle stimulating hormone; FU = follow-up; HF = heart failure; HR = heart rate; hs-TnT = high-sensitive troponin T; FSH = follicle stimulating hormone; KCCQ = Kansas City Cardiomyopathy Questionnaire; LH = luteinising hormone; NAbs = neutralising antibodies; NT-proBNP = N-terminal prohormone of brain natriuretic peptide; NYHA = New York Heart Association; PH-LHD = pulmonary hypertension due to left heart disease; PK = pharmacokinetic(s); PRD = patient rating of dyspnoea; RAAS = renin-angiotensin-aldosterone system; RHC = right heart catheterisation; RXFP1 = relaxin/insulin-like family peptide receptor-1; SAE = serious adverse event; TRV = tricuspid regurgitation velocity; uACR = urine albumin-creatinine ratio; V = visit; W = week.

2 INTRODUCTION

AZD3427 (previously known as MEDI6998) is a human recombinant fusion protein, consisting of the fragment crystallisable portion of human IgG1 connected to the sequence derived from human relaxin. The relaxin portion of the fusion protein is almost identical in amino acid sequence to the naturally-occurring human hormone, which consists of chain A (24 amino acids) and chain B (29 amino acids), except that in AZD3427, the first amino acid (aspartic acid) was removed from chain B (resulting in 28 amino acids), with chain A remaining intact. The relaxin portion of AZD3427 is a functionally selective agonist of its cognate G protein-coupled receptor, RXFP1. AZD3427 is being developed as a treatment for patients with HF and PH due to left heart disease (WHO Group 2).

The relaxin portion of AZD3427 is a functionally selective agonist of the relaxin receptor RXFP1, with PK properties suitable for SC Q2W dosing. We hypothesise that activation of RXFP1 via AZD3427 may induce favourable CV changes, such as reduced systemic and pulmonary vascular resistance, improved cardiac remodelling, and improved renal perfusion. These actions may restore exercise capacity, improve quality of life, and reduce morbidity and mortality in participants with HF and PH Group 2.

2.1 Study Rationale

Pulmonary hypertension Group 2 results from dysfunction of the left heart and is the most common cause of PH. Left-sided dysfunction causes pulmonary pressure to rise, leading to lung oedema, damage of pulmonary vessels, and ultimately right ventricle failure. Pulmonary hypertension Group 2 is primarily comprised of HF NYHA FC II to IV patients, with 40% to 75% of HFrEF patients and 36% to 83% of HFpEF patients also having PH.

Pulmonary hypertension Group 2 is associated with impaired exercise capacity and reduced survival. These patients live with significantly limited physical activity and quality of life despite optimal guideline-directed management. Currently, there is no dedicated treatment for this group of patients.

The pathophysiology of PH Group 2 is a continuum. Following left heart disease, elevated left-sided filling pressure initially increases pressure in the pulmonary circulation (IpC PH). This is followed by compensatory pulmonary vasoconstriction and vascular remodelling including thickening, hypertrophy and fibrosis of vessels, which further increases PAP and PVR and decreases pulmonary vasculature compliance (CpC PH). Elevated PAP and PVR are associated with higher all-cause mortality in these patients regardless of PAWP, and reductions in PAP and PVR are associated with improved survival in HF patients regardless of EF.

The postulated mechanisms of action of AZD3427 includes vasodilatory, anti-inflammatory and anti-fibrotic effects that are expected to improve left ventricle function and stop and/or reverse remodelling of the diseased heart and pulmonary vasculature, decrease PAP and PVR and improve CV outcomes.

2.2 Background

The HF clinical syndrome is a complex pathophysiological state where an abnormality in cardiac function results in the heart failing to meet the body's metabolic demands. Most commonly, this is a consequence of reduced cardiac output caused by impaired ventricular filling or contraction (Ponikowski et al 2016). The disease manifests in the form of symptoms such as shortness of breath, fatigue, and oedema, and by signs of pulmonary crackles, a rapid HR, and fluid overload (Ponikowski et al 2016).

Depending on the EF of the failing heart, HF is classified as HFrEF, HFmrEF, or HFpEF (Ponikowski et al 2016, Yancy et al 2013, McDonagh et al 2021). Differentiating between these forms of HF is important because, although all exhibit similar signs and symptoms, each has a distinct underlying pathophysiological process. HFrEF is mostly caused by reduced contractile function, whereas HFpEF is caused by impaired filling of the heart (Simmonds et al 2020).

Pulmonary hypertension refers to high BP in the arteries of the lungs. As pulmonary pressure increases the right side of the heart must work harder to pump blood through the lungs, which over time can weaken and damage the right side of the heart. The most common form of PH is due to left heart disease (PH Group 2), which accounts for 65% to 80% of PH cases (Rosenkranz et al 2016).

In PH Group 2, dysfunction of the left heart causes pulmonary pressure to rise leading to lung oedema, damage of pulmonary vessels, and right ventricle failure. Treatment of PH Group 2 traditionally focuses on treatment of the underlying causes of left heart dysfunction. An estimated 40% to 75% of HFrEF patients and 36% to 83% of HFpEF patients also have PH (Rosenkranz et al 2016).

In HF deteriorating systolic and diastolic function of the left ventricle initiates a cascade of compensatory mechanisms (Tanai and Frantz 2015). However, although these adaptations, on a neurohormonal, cellular, structural, and functional level, may initially improve cardiac output, over time they become detrimental 'maladaptations', compounding the problem by placing excessive strain on the already failing heart (ATS 2002, Azevedo et al 2016, Bertero and Maack 2018).

In contrast, pregnancy is a normal physiological state in which adaptations are successful in supporting the heart in meeting the increased metabolic demands of gestation without causing cardiac damage. Here, relaxin seems to play a role via reducing both SVR and renal resistance

concomitantly (Conrad 2011). If relaxin were to replicate these haemodynamic adaptations in the setting of HF and PH Group 2, it could reduce afterload, filling pressure, myocardial demand, and improve end-organ perfusion.

Relaxin's candidacy as a potential therapeutic agent in HF and PH Group 2 is further strengthened by its reported ability to attenuate, halt, or reverse several key processes in the disease mechanism of HF, such as endothelial dysfunction, myocyte apoptosis, myocardial inflammation, fibrosis, and hypertrophy (Devarakonda and Salloum 2018). In addition to enhancing atrial function through rhythm stabilisation and increased contractility, relaxin also promotes cell healing and angiogenesis and inhibits proliferation (Sarwar et al 2017). This is supported by data in a population of acute HF patients, where admission serum relaxin levels were associated with clinical and echocardiographic markers of PH, right ventricular dysfunction, and overload, thus suggesting that relaxin may be upregulated to help compensate for adverse cardiopulmonary pathophysiology (Pintalhao et al 2017).

A detailed description of the chemistry, and nonclinical pharmacology, efficacy, and safety of AZD3427 is provided in the IB (IB 2022).

2.3 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected AEs of AZD3427 may be found in the IB (IB 2022).

2.3.1 Risk Assessment

There are no identified safety risks with AZD3427. Potential risks are related to the mechanism of action of relaxin, the method of administration and immune reactivity to AZD3427. For the risks associated with higher exposure (eg, higher single doses or multiple doses), please refer to IB (IB 2022).

Table 2 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
Study intervention AZD3427		
Potential Risks Based on Mechanism of Action		
Hypotension	Relaxin is a potent dilator of the systemic and renal vasculature. The administration of a recombinant human relaxin (Serelaxin) to healthy participants at concentrations comparable to those in early pregnancy had no effect on BP, (Smith et al 2006). However, in participants with HF, there were greater reductions in BP and	The risk of potential hypotension with AZD3427 will be mitigated by the selected inclusion/exclusion criteria (see Section 5), safety assessments (see Section 8.3.2) and the selected criteria for discontinuation of study intervention (see Section 7.1).

Table 2 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
	higher numbers of episodes of hypotension (Teerlink et al 2009). AZD3427 administration during a 1-month toxicity study increased BP in rats but did not affect BP in cynomolgus monkeys in either a 1-month or 6-month toxicity study. This species-specific observation is not predictive for humans. In Study D8330C00001, 8 AEs of hypotension were reported in 6 HF participants treated with AZD3427 and all were mild in intensity. See IB Section 5.5.1.1.	
Heart rate increase	Relaxin and RXFP1 agonists have positive chronotropic effects eliciting an increased HR in rats (Debrah et al 2005) but not in cynomolgus monkeys, likely due to the RXFP1 expression pattern and biology. The administration of a recombinant human relaxin (Serelaxin) had no effect on HR in participants with HF (Liu et al 2016). AZD3427 administration during 1-month toxicity studies increased HR in rats but not in cynomolgus monkeys. This species-specific observation is not predictive for humans. No pattern of heart rate changes were observed in either healthy participants or participants with HF in the D8330C00001 study. See IB Section 5.5.1.2.	The risk of potential HR increase with AZD3427 will be mitigated by the selected inclusion/exclusion criteria (see Section 5) and safety assessments (see Section 8.3.2).
Effects on female reproduction	Relaxin is expressed in several different reproductive tissues and is involved in multiple and diverse physiologic functions, including extracellular matrix remodelling, neoangiogenesis, and vasodilation (Bani 1997, Bathgate et al 2003, Ivell and Bathgate 2006, Schwabe and Bullesbach 2007, Sherwood 2004) in reproductive and non-reproductive tissues (Parry and Vodstrcil 2007). Adverse events reported by women during studies with a recombinant human relaxin	The risk of potential effects on female reproduction with AZD3427 will be mitigated by the selected inclusion/exclusion criteria (see Section 5).

Table 2 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
	include menorrhagia and metrorrhagia (heavier-than-usual or irregular menstrual bleeding) (Khanna et al 2009). AZD3427 administration during 1-month toxicity study in rats, and 1-month and 6-month toxicity studies in cynomolgus monkeys did not affect histological structure of female reproductive organs. Reproductive and developmental toxicity studies have not yet been conducted. See IB Section 5.5.1.3.	
Effects on male reproduction	Relaxin is expressed in the testes and in the prostate and may have a role in the development of the male reproductive tract, spermatogenesis, sperm motility, and the induction of the post-mating uterine response initiated by exposure to relaxin-containing seminal fluid (Ivell et al 2017, Samuel et al 2003). AZD3427 administration during 1-month toxicity study in rats did not affect histological structure of male reproductive organs nor in a 1-month and 6-month toxicity study in cynomolgus monkeys. See IB Section 5.5.1.4.	The risk of potential effects on male reproduction with AZD3427 will be mitigated by the selected inclusion/exclusion criteria (see Section 5).
Hypertrophic/ hyperplastic effects	Available data support that many extracellular matrix remodelling cascades initiated by relaxin overlap with common tumour promoting pathways (Thanasupawat et al 2019). Relaxin is known to exert a trophic effect on female reproductive tissues (Ohleth et al 1999). However, persistent exposure to relaxin is not physiological, and the long-term consequences of persistent exposure may theoretically include increased cancer risk. No malignancies were observed in the 1-month repeat-dose toxicity study with AZD3427 in rat and 6-month repeat dose toxicity study in cynomolgus monkeys. See IB Section 5.5.1.5.	The risk of potential effects on pre-existing tumour cells with AZD3427 will be mitigated by the selected inclusion/exclusion criteria (see Section 5).

Table 2 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
Potential Risks Based on Clinical Data		
Haemoglobin and/or haematocrit decrease	Reduction of haemoglobin and haematocrit is commonly reported with vasodilators, but the mechanism is not fully understood. Minor decreases in haemoglobin and haematocrit were observed after administration of a recombinant human relaxin (Serelaxin) AHF and scleroderma patients (Metra et al 2019). There were no AZD3427 related reduction of haemoglobin and haematocrit levels in rats (1-month GLP toxicity study) and cynomolgus monkeys (1-month and 6-month GLP toxicity studies). In Part B of the D8330C00001 study, on average there was a mild reduction of haemoglobin and haematocrit levels relative to placebo, with the 45 mg AZD3427 group generally showing the strongest reduction. The drop was transient and corrected during the washout period. See IB Section 5.5.2.1.	The risk of haemoglobin and/or haematocrit decrease will be mitigated by the selected inclusion/exclusion criteria (see Section 5) and safety assessments (see Section 8.3.4).
Anti-drug antibodies/neutralising antibodies	Administration of protein-based therapeutics, including peptides, carries the potential risk of induction of ADAs. Anti-drug antibodies have the potential to affect the PK, PD, efficacy, and safety of AZD3427. AZD3427 administration has induced positive ADA responses in both rats and cynomolgus monkeys. These species-specific observations are not predictive for humans. No treatment-induced ADAs were detected in participants in the D8330C00001 study. See IB Section 5.5.5.1.	Anti-drug antibodies will be monitored (see Section 8.7) and the risk will be mitigated by the selected inclusion/exclusion criteria (see Section 5).
Hypersensitivity reactions	Administration of protein-based therapeutics, including peptides may lead to hypersensitivity reactions, including anaphylactic reactions and immune-complex disease.	The risk of hypersensitivity reactions with AZD3427 will be mitigated by the selected inclusion/exclusion criteria (see Section 5), safety assessments (see Section 8.3.5), and the selected

Table 2 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
	No AZD3427-related hypersensitivity reactions were observed in cynomolgus monkeys or rats. No severe hypersensitivity reactions were detected in participants in the D8330C00001 study. See IB Section 5.5.5.2.	criteria for discontinuation of study intervention (see Section 7.1). Severe hypersensitivity reactions should be managed according to standard clinical practice, and medical equipment and staff trained to treat acute anaphylactic reactions must be available at all study centres.
Study procedures		
Injection site reactions	Injection site reactions may occur following SC injections of protein-based therapeutics, including peptides. Local tolerance of AZD3427 at IV and SC injection sites was evaluated as part of the non-GLP, single-dose study in cynomolgus monkeys and the 1-month, repeat-dose, SC/IV, toxicity studies in rats and cynomolgus monkeys. No AZD3427-related injection site reactions were observed. In the D8330C00001 study, there were 4 AEs related to injection site reactions (1 injection site pruritus, 1 injection site bruising, 1 injection site reaction, and 1 injection site pain) reported in the AZD3427-treated group and none in the placebo group. All 4 events were reported to be mild in intensity and resolved. See IB Section 5.5.4.1.	Will be mitigated by monitoring of safety data (see Section 8.3) during the study.
Right heart catheterisation	Risks are associated with the procedure (including but not limited to bruising and excessive bleeding of the site where the catheter is inserted, ventricular arrhythmias, and right bundle-branch block, which are generally self-limiting, and pulmonary-artery perforation).	Will be mitigated by adequate preparation for the procedure and pre- and post-procedure medication may reduce potential side effects. Risk of participants not being able to tolerate RHC will be mitigated by the selected inclusion/exclusion criteria (see Section 5).

Table 2 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
Other		
COVID-19	Potential infection of participants with COVID-19.	Considerations related to COVID-19, including risks to participants, risk mitigation measures, and vaccine guidance, are addressed in Appendix J.

ADA = anti-drug antibody; AE = adverse event; BP = blood pressure; COVID-19 = coronavirus disease of 2019; GLP = Good Laboratory Practice; HF = heart failure; HR = heart rate; IB = Investigator's Brochure; IV = intravenous; PD = pharmacodynamic(s); PK = pharmacokinetic(s); RHC = right heart catheterisation; SC =subcutaneous.

2.3.2 Benefit Assessment

It is expected that participants randomised to active treatment will benefit from the postulated mechanisms of action of AZD3427 which includes vasodilatory, anti-inflammatory and anti-fibrotic effects that are expected to improve left ventricle function and stop and/or reverse remodelling of the diseased heart and pulmonary vasculature, which in turn should decrease PAP and PVR and improve CV outcomes. All participants will have the benefit of a detailed baseline medical assessments, and safety and monitoring throughout the study period. Data collected during the study may also help other patients in the future by improving the understanding of the safety and PK of AZD3427 in participants with HF and PH.

2.3.3 Overall Benefit/Risk Conclusion

Taking into account the measures taken to minimise risk to participants participating in this study, the potential risks identified in association with AZD3427 are justified by the anticipated benefits that may be afforded to participants with HF and PH due to left heart disease (WHO Group 2).

3 OBJECTIVES, ENDPOINTS, AND ESTIMANDS

3.1 Hypothesis

Primary Hypothesis:

- AZD3427 will reduce PVR compared with placebo after 24 weeks of treatment, on top of current standard of care.

Key Secondary Hypotheses:

- AZD3427 will reduce mPAP compared with placebo after 24 weeks of treatment.
- AZD3427 will improve function and symptoms compared with placebo after 24 weeks of treatment.
- AZD3427 will have an acceptable safety profile in participants with HF and PH Group 2.

3.2 Objectives and Endpoints

Table 3 Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the effect of AZD3427 on PVR after 24 weeks of treatment in participants with HF and PH Group 2	Change in PVR from baseline to Week 25 compared with placebo, as measured by RHC.
Secondary	
To evaluate the effect of AZD3427 on additional hemodynamic markers of cardiac function after 24 weeks of treatment in participants with HF and PH Group 2	Change from baseline to Week 25 in RHC parameters compared with placebo. Parameters include: • mPAP and PAWP. Change from baseline to Week 25 in echocardiographic parameters compared with placebo. Parameters include: • Cardiac output, SV, EF, LVGLS, PASP, RV/LV ratio, RVOT AT, TRV, TAPSE/PASP. • Systemic vascular resistance.
To evaluate the effect of AZD3427 on function and symptoms after 24 weeks of treatment in participants with HF and PH Group 2	Change in 6MWD and KCCQ TSS from baseline to Week 25 compared with placebo. Change from baseline in NYHA FC at Week 25 compared with placebo.
To evaluate the effect of AZD3427 on biomarkers of cardiac and renal function after 12 and 24 weeks of treatment in participants with HF and PH Group 2	Change in serum creatinine, NT-proBNP, cystatin C, and eGFR from baseline to Week 13 and Week 25 compared with placebo.
To evaluate the PK of AZD3427 after repeat Q2W SC dosing in participants with HF and PH Group 2	AZD3427 serum PK concentrations.
To evaluate the immunogenicity of AZD3427	Presence of ADAs and evaluation of ADA titres.

Table 3 Objectives and Endpoints

Objectives	Endpoints
Safety	
To evaluate the safety and tolerability of AZD3427 as compared to placebo in participants with HF and PH Group 2	Adverse events/SAEs, vital signs (BP, HR, respiratory rate, body temperature, and pulse rate), body weight, ECGs, clinical laboratory assessments, physical examinations, and injection site reactions.
Exploratory	
To evaluate the effect of AZD3427 on the plasma proteome, and urine, serum and/or plasma biomarkers of inflammation, fibrosis, RAAS, tissue remodelling, fluid/electrolyte balance, and RXFP1 signalling after 12 and 24 weeks of treatment in participants with HF and PH Group 2 [a].	Change in plasma and/or serum biomarkers from baseline to Week 13 and Week 25 compared with placebo: • Renin, aldosterone, sodium, potassium, copeptin, serum osmolality, and erythropoietin. • hs-TnT, PIIINP, hsCRP, and PRO-C6. • TNF-α, TGF-β. • Plasma proteome through multiplex protein panel. • Endogenous plasma relaxin levels at baseline. Change in urine biomarkers from baseline to Week 13 and Week 25 compared with placebo: • Sodium, potassium, and albumin-to-creatinine ratio. • Creatinine and KIM-1.
To evaluate the diagnostic accuracy of echocardiographic parameters to diagnose PH Group 2 based on RHC in the study population	To evaluate the capability of echocardiographic parameters to diagnose of PH Group 2 based on RHC at baseline.
To evaluate the effect of AZD3427 on CV outcomes	Time to CV death, HF hospitalisation, or outpatient visit due to HF deterioration from randomisation to Week 25.
To evaluate the effect of AZD3427 on clinical outcomes	• Clinical benefit at Week 25, consisting of a composite of all-cause death, HF hospitalisations, outpatient visit due to HF deterioration and change from baseline at Week 25 in KCCQ TSS, assessed using a 'win-ratio' approach. • Clinical benefit at Week 25, consisting of a composite of all-cause death, HF hospitalisations, outpatient visit due to HF deterioration and change from baseline at Week 25 in 6MWD, assessed using a 'win-ratio' approach. • Change in KCCQ CSS and OSS from baseline to Week 25 compared with placebo.
To assess the incidence of neutralizing antibodies (NAbs) in participants [a]	• Presence of NAbs

[a] Results of each of these exploratory biomarker analyses and NAbs may or may not be included in the CSR of this study.
6MWD = 6-minute walking distance; ADA = anti-drug antibodies; BP = blood pressure; CSR = clinical study report; CSS = clinical summary score; CV = cardiovascular; EF = ejection fraction; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; HF = heart failure; HR = heart rate; hsCRP = high-sensitive C-reactive protein; hs-TnT = high-sensitivity troponin T; IVC = inferior vena cava; KCCQ = Kansas City Cardiomyopathy Questionnaire; KIM-1 = kidney injury molecule 1; LVGLS = left ventricular global longitudinal strain; NAbs = neutralising antibodies; NT-proBNP = N-terminal prohormone of brain natriuretic peptide; NYHA FC = New York Heart Association Functional Class; OSS = overall summary score; PAP = pulmonary arterial pressure; PASP = pulmonary arterial systolic pressure; PIIINP = amino-terminal propeptide of type III procollagen; PAWP = pulmonary artery wedge pressure; PH = pulmonary hypertension; PK = pharmacokinetic(s); PRO-C6 = P204 endotrophin; PVR = pulmonary vascular resistance; Q2W = twice weekly; RAAS = renin-angiotensin-aldosterone system; RHC = right heart catheterisation; RV/LV = right ventricle/left ventricle; RVOT AT = right ventricular outflow tract acceleration time; RXFP1 = relaxin/ insulin-like family peptide receptor-1; SAE = serious adverse event; SC = subcutaneous; SV = stroke volume; SVR = systemic vascular resistance

TAPSE = tricuspid annular plane systolic excursion; TGF-β = transforming growth factor beta; TNF-α = tumour necrosis factor alpha; TRV = tricuspid regurgitation velocity; TSS = total symptom score.

4 STUDY DESIGN

4.1 Overall Design

This is a Phase IIb randomised, double-blind, placebo-controlled, multi-centre, dose-ranging study to evaluate the efficacy, safety, and PK of AZD3427 in addition to standard of care in participants with HF and PH due to left heart disease (WHO Group 2). This study will be agnostic to EF and agnostic to PVR (includes both IpC PH and CpC PH participants).

The study will be conducted in approximately 60 study centres across an estimated 15 countries.

Approximately 220 participants will be randomised to 4 treatment groups (in a 1:1:1:1 ratio) to receive a SC injection of AZD3427 or placebo every 2 weeks for 24 weeks. This study will evaluate 3 dose levels of AZD3427: 1.1, 5.4, and 30.0 mg. Each treatment group will comprise of approximately 55 participants.

The study will include approximately 16 study visits: 2 visits during the Screening Period, 13 visits during the Treatment Period, and one visit during the Follow-up Period. The expected total duration of the study is 32 to 37 weeks, depending on the length of the Screening Period.

The study will include 3 periods:

- Screening Period of up to 42 days (6 weeks).
- Treatment Period of 24 weeks, during which participants will receive AZD3427 or placebo by SC injection Q2W.
- Follow-up Period of 56 days (8 weeks) after the last dose ([redacted])

Participants will remain at the study centre for 1 hour after the first 3 doses, and then for 30 minutes after each subsequent dose, or longer at the judgement of the investigator considering the risk of anaphylaxis and hypotension for the individual participant.

The primary objective of this study is to assess the ability of AZD3427 to reduce PVR after 24 weeks of treatment. Elevated PVR and mPAP are associated with higher all-cause mortality in patients with PH Group 2, and reductions in PVR and mPAP are associated with improved survival and patient reported outcomes in patients with PH Group 1 and PH Group 2.

Additional safety, function and symptoms, hemodynamic, and biomarker assessments will also be performed. Key secondary endpoints will be assessed at baseline and after 12 and 24 weeks of treatment. Right heart catheterisation and echocardiograms will be performed at baseline and after 24 weeks of treatment.

Participants will receive an echocardiogram at Screening Visit 1. Only participants who fulfil all eligibility criteria after Screening Visit 1 will receive a RHC at Screening Visit 2. Echocardiographic parameters from Screening Visit 1 will be assessed locally to determine eligibility for progression to Screening Visit 2. Based on the RHC measurements, participants will be randomised into the study according to the following capping:

- 20 to 24.9 mmHg: is capped at no more than 15%.
- ≥ 25 mmHg: no capping.

An IA will be performed after approximately 50% of participants complete Visit 15 with an option to trigger early initiation of Phase III activities. The IA will assess, but not be limited to PVR, mPAP, 6MWD, KCCQ, NT-proBNP, renin, AZD3427 serum concentrations, and safety. In order to maintain the study blind, the study staff who perform the IA will be separate from those in the study team. No details that could compromise the integrity of the study blind will be passed to the study team.

If a participant's end-of-study sample is confirmed ADA-positive and their baseline sample was ADA-negative, the participant will be asked to take part in an extended Follow-up Period and provide another sample after approximately 3 months to evaluate whether or not ADAs persist. If the sample taken after 3 months is ADA-positive, the participant will be asked to return to provide a sample after another 3 months (ie, 6 months after the end-of-study visit). If the sample after 6 months is ADA-positive, the investigator and the Medical Monitor will discuss any further action.

4.2 Scientific Rationale for Study Design

A double-blind, placebo-controlled approach will be applied for the study. This design will minimise inadvertent biases such as placebo effect and participant bias, as well as provide reference data (ie, data from placebo-treated participants) which will aid in the interpretation of results. Selection bias will be limited by strict adherence to the inclusion and exclusion criteria.

An IA is a reliable rational approach to clinical studies that incorporate what is learned during the course of a clinical study and how it is completed, without compromising the validity or integrity of the study. The IA is designed to allow an option to trigger early initiation of Phase III activities for internal program acceleration.

To meet classic criteria of WHO PH Group 2, HF participants need to undergo RHC, which allows evaluation of PAWP (> 15 mmHg) and stratification of participants into either isolated post-capillary or combined pre- and post-capillary PH based on PVR levels.

Alternatively, echocardiographic examination provides an indirect measurement of pulmonary pressure and can be conducted in a larger population of HF participants. However, precise evaluation of hemodynamic parameters including PAWP, mPAP, and PVR using echocardiography is not possible.

It has been established that elevated PVR and mPAP are associated with higher all-cause mortality in patients with PH Group 2, and reductions in PVR and mPAP are associated with improved survival and patient reported outcomes in patients with PH Group 1 and PH Group 2. Therefore, the primary and secondary endpoints in this study include change from baseline in PVR and mPAP.

The safety assessments for the study are accepted measures for ensuring safety of participants during a clinical study. The PK sampling schedule is considered appropriate given the information available from previous studies with AZD3427.

4.3 Justification for Dose

In this study, 1.1, 5.4, and 30.0 mg Q2W doses of AZD3427 will be evaluated. The dose selection of AZD3427 is based on PK, PD, and safety data from a previous study in healthy participants and HFrEF/HFpEF participants (SAD/MAD Study D8330C00001) and from a study in metabolic syndrome NHPs with HFrEF (Study KBI-0514202007-34). The average concentration at steady state (Cave,ss) at these dose levels are predicted to correspond to 0.1, 0.5, and 3.2-fold of relaxin pregnancy levels (preliminary predictions assuming relaxin concentrations in pregnancy of 1 ng/mL and correcting for differences in molecular weight and in vitro potency; Hayes 2004).

Using concentration-response relationship for EF, SV, and SVR from the NHP HFrEF model and PK data from the SAD/MAD study (Study D8330C00001), it is predicted that the dose range selected in the current study will enable characterisation of the dose response for these parameters. Dose dependent increases of renin were observed both in the NHP and the SAD/MAD study, the renin response is likely a compensatory response to vasodilation. CCI [redacted]

The maximum exposure to AZD3427 in the current study is predicted to be about CCI [redacted] of the maximum exposure to AZD3427 in the MAD part of Study D8330C00001. CCI [redacted]

4.4 End-of-study Definition

For the purpose of clinical trial transparency, the definition of the end of the study differs under FDA and EU regulatory requirements:

European Union requirements define study completion as the last visit of the last participant for any protocol related activity.

Food and Drug Administration requirements defines 2 completion dates:

- Primary Completion Date – the date that the final participant is examined or receives an intervention for the purposes of final collection of data for the primary outcome measure, whether the clinical study concluded according to the pre-specified protocol or was terminated. In the case of clinical studies with more than one primary outcome measure with different completion dates, this term refers to the date on which data collection is completed for all of the primary outcomes.
- Study Completion Date – the date the final participant is examined or receives an intervention for purposes of final collection of data for the primary and secondary outcome measures and AEs (for example, last participant's last visit), whether the clinical study concludes according to the pre-specified protocol or is terminated.

A participant is considered to have completed the study if they have completed all phases of the study including the last Follow-up Visit or the last scheduled procedure shown in the SoA (Section 1.3).

5 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1 Participant must be ≥ 18 years of age inclusive (or meet the minimum age of maturity according to local regulations in their country), at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2 Participants must have a pre-existing diagnosis of HF, NYHA FC II to IV, and a pre-existing diagnosis of PH-LHD or **likely or intermediate probability** of PH-LHD as

per 2022 ESC/ERS guidelines (Appendix I). Participants must be on stable HF standard of care medication, including diuretics, for at least 4 weeks prior to Screening Visit 1.

3 For progression to Screening Visit 2, participants must have a combination of echocardiographic parameters at Screening Visit 1 that show **intermediate or high** probability of PH as per 2022 ESC/ERS guidelines (as specified in Appendix I).

4 Participants must have an on-study elevated pulmonary artery pressure from RHC performed as per RHC manual provided by the Sponsor, at Screening Visit 2:

(a) PAWP ≥ 15 mmHg

(b) mPAP ≥ 20 mmHg

Weight

5 Minimum body weight of 45 kg (inclusive).

Sex and Contraceptive/Barrier Requirements

6 For female participants, the participant must not be pregnant or lactating and must be of non-childbearing potential, confirmed at Screening Visit 1 by one of the following:

(a) Postmenopausal, defined as amenorrhea for ≥ 12 months following cessation of all exogenous hormonal treatments, and with LH and FSH levels in the postmenopausal range.

(b) Documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy, or bilateral salpingectomy. Tubal ligation is not considered as irreversible surgical sterilisation.

7 Non-sterilised male study participants should be advised to use a condom for all sexual intercourse with a female partner of childbearing potential from first dose until 3 months after last dose. All male participants should refrain from fathering a child or donating sperm for 3 months after last dose.

Informed Consent

8 Capable and willing of giving signed informed consent as described in Appendix A which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

9 Provision of signed and dated written Optional Genetic Research Information informed consent prior to collection of samples for optional genetic research that supports the Genomic Initiative (see Appendix D 2).

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1 Diagnosis of PH in WHO Group 1, WHO Group 3, WHO Group 4, or WHO Group 5.

2 Historical or current evidence of a clinically significant disease or disorder including, but not limited to:

   (a) Myocardial infarction, stroke, transient ischaemic attack, coronary artery bypass grafting, percutaneous coronary intervention, implantable cardioverter defibrillator implantation (implanted standard pacemaker or CRT-P are not exclusionary), within 12 weeks prior to Screening Visit 1.

   (b) Sarcoidosis, restrictive cardiomyopathy, active myocarditis, constrictive pericarditis, hypertrophic (obstructive) cardiomyopathy, complex congenital heart disease. Greater than moderate mitral or aortic valve regurgitation or greater than mild aortic or mitral stenosis. Severe tricuspid regurgitation due to primary valvular disease, eg, from endocarditis or mechanical destruction.

   (c) Any history of pulmonary embolism or deep vein thrombosis in the last 12 months.

   (d) Known coagulation disorders.

3 Decompensated HF or hospitalisation due to decompensated HF within 4 weeks prior to Screening Visit 1.

4 Any contraindications to RHC.

5 History of hypersensitivity to SC injections or devices.

6 History of hypersensitivity to drugs with a similar chemical structure or class to AZD3427 or any component of AZD3427 drug product, or ongoing clinically important allergy/hypersensitivity.

7 History of active malignancy within 2 years, with the exception of fully excised or treated basal cell carcinoma, or ≤ 2 squamous cell carcinomas of the skin.
Participants who are under investigation for breast or cervical cancer, including participants with a pap smear of ≥ 3; all investigations must be resolved as negative for breast and cervical cancer at least 12 weeks before Screening Visit 1.

8 Current diagnosis of active hepatitis.

9 Known lung disease with FEV_1 < 30% of predicted.

10 Known history of drug abuse within 24 months of Screening Visit 1.

11 Congenital long QT syndrome.

12 Cardiac ventricular arrhythmia which requires treatment. Participants with atrial fibrillation or flutter and controlled ventricular rate are permitted.

13 History of or anticipated heart transplant or ventricular assist device implantation.

14 Any known planned (scheduled) highly invasive CV procedure (eg, coronary revascularisation, ablation of atrial fibrillation/flutter, valve repair/replacement, aortic aneurysm surgery, etc).

15 As judged by the investigator, any evidence of clinically important disease or disorder which in the investigator's opinion makes it undesirable for the participant to participate in the study.

Prior/Concomitant Therapy

16 Plasma donation within 1 month prior to Screening Visit 1 or any blood donation/blood loss > 500 mL during the 3 months prior to Screening Visit 1.

17 Inhibitors of cGMP-specific phosphodiesterase type 5 (PDE5-I if taken for erectile dysfunction or other occasional, non-continuous uses) such as, but not limited to, Sildenafil and Tadalafil are prohibited for 48 hours before echocardiography and RHC (Screening Visits 1 and 2 and Visit 15).

Prior/Concurrent Clinical Study Experience

18 Participants who have previously received AZD3427.

19 Participation in another clinical study with a study intervention administered in the last 6 months or 5 half-lives prior to Screening Visit 1 (whichever is longer), or planned participation in such study prior to end of the Follow-up Period.
Note: Participants consented and screened, but not entered in this study or a previous study, are not excluded.

20 Known history of ADAs to relaxin or relaxin analogues.

Diagnostic Assessments

21 Any laboratory values with the following deviations:
   (a) Estimated GFR < 30 mL/min/1.73 m^2 at Screening Visit 1 assessed by the CKD-EPI equation.
   (b) Haemoglobin < 10 g/dL at Screening Visit 1.
   (c) Persistent electrolytes abnormalities not corrected before Screening Visit 1.

22 Abnormal vital signs defined as any of the following at Screening Visit 1:
   (a) Sitting SBP > 160 mmHg or sitting DBP > 110 mmHg after a period of rest.
   (b) Sitting SBP < 100 mmHg or sitting DBP < 50 mmHg.
   (c) Resting HR of < 50 bpm or > 115 bpm.

Other Exclusions

23 Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study centre).

24 Judgement by the investigator that the participant should not participate in the study if the participant is unlikely to comply with study procedures, restrictions, and requirements.

25 Previous enrolment or randomisation in the present study.

26 For females only - currently pregnant (confirmed with positive pregnancy test) or breast-feeding.

5.3 Lifestyle Considerations

The following restrictions apply for the specified times during the study period.

5.3.1 Caffeine and Alcohol

- Participants will abstain from ingesting extreme caffeine- containing products (eg, energy drinks) on each day of dosing until after the study visit.
- Participants will abstain from alcohol from midnight on each day of dosing until after the study visit.

5.3.2 Activity

- There are no specific restrictions on exercise, participants should follow their normal daily activities.

5.3.3 Other Restrictions

- Participants will be required to abstain from blood or plasma donation until 3 months after the final study Follow-up Visit.
- For RHC, participants should follow their local preparation and post-visit restrictions.

5.4 Screen Failures

A screen failure occurs when a participant who has consented to participate in the clinical study is not subsequently assigned to study intervention/entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria including RHC and echocardiogram if performed, and any SAE.

Individuals who miss the inclusion/exclusion criteria (for example eGFR, haemoglobin, or

vital signs), may be retested once during the Screening Period and the retest results must be available before the RHC.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened up to 2 times at a later date if, in the opinion of the investigator, there is a reason to believe they may be eligible/fulfil the inclusion/exclusion criteria. If the reason for screen failure was due to administrative or personal events that result in scheduling issues with the Screening Visit(s) then one further additional rescreening is allowed in the study. Rescreened participants should be assigned the same participant number as for the initial screening.

There is no time limit for rescreening. All enrolment assessments and procedures, including signing of the ICF, should be performed again.

However, if a participant fails on the RHC, this should not be performed again, the participant should be a screen failure and not be rescreened.

5.5 Participant Enrolment and Randomisation

The investigator or designee should keep a record (the participant's screening log) of participants who entered pre-study screening. The investigator or designee will:

- Obtain informed consent from the potential participant before any study specific procedures are performed.
- Assign potential participant a unique enrolment number.
- Determine participant eligibility (see Sections 5.1 and Section 5.2). More than one attempt to meet the randomisation criteria is allowed.
- Assign eligible participant a unique randomisation code.
- If a participant withdraws from the study, then his/her enrolment/randomisation code cannot be reused.
- Withdrawn participants will not be replaced.

Randomisation codes will be assigned strictly sequentially as participants become eligible for randomisation (see Section 6.3).

5.6 Criteria for Temporarily Delaying Enrolment, Randomisation, or Administration of Study Intervention

Not applicable to this study.

6 STUDY INTERVENTION AND CONCOMITANT THERAPY

Study interventions are defined as all pre-specified, IMPs and NIMPs, medical devices, and other interventions (eg, surgical and behavioural) intended to be administered to the study participants during the study conduct.

6.1 Study Intervention(s) Administered

6.1.1 Investigational Products

Details on the identity of the study intervention are presented in Table 4.

Table 4 Study Intervention

Group name	AZD3427	Placebo	AZD3427 CCI
Intervention name	AZD3427	Placebo	CCI
Type	Biologic	Placebo	CCI
Dose formulation	CCI	CCI	CCI
Unit dose strength(s)	CCI mg/CCI	NA	NA
Dosage level(s)	1.1, 5.4, and 30.0 mg	1.1, 5.4, and 30.0 mg will be placebo controlled	1.1 and 5.4 mg
Route of administration	SC injection	SC injection	SC injection
Regimen	Single dose every 2 weeks for 24 weeks	Single dose every 2 weeks for 24 weeks	Single dose every 2 weeks for 24 weeks
Use	Experimental	Placebo	CCI
Study intervention or NIMP/AxMP	Study intervention	Study intervention	Study intervention
Sourcing	Provided centrally by AstraZeneca (or subsidiary or designee).	Provided centrally by AstraZeneca (or subsidiary, or designee).	Provided centrally by AstraZeneca (or subsidiary or designee).
Packaging and labelling	Study intervention will be provided in CCI in a carton. Each CCI and carton will be labelled in accordance with country regulatory requirements.	Study intervention will be provided in CCI in a carton. Each CCI and carton will be labelled in accordance with country regulatory requirements.	Study intervention will be provided in CCI in a carton. Each CCI and carton will be labelled in accordance with country regulatory requirements.

AxMP = auxiliary medicinal product; NA = not applicable; NIMP = non-investigational medicinal product; SC = subcutaneous.

6.2 Preparation/Handling/Storage/Accountability of Interventions

The investigator or designee CCI must confirm appropriate conditions (eg, temperature) have been maintained during transit for all study intervention received at the study centre and throughout the entire study until authorisation is provided for on-site destruction or removal of the study intervention, reflecting completion of the study. In the event of a temperature excursion detected at any time during the study, study centres will follow the reporting procedures for notifying AstraZeneca (or designated party); release of study intervention for clinical use can only occur once the event has been reviewed and approval is provided by AstraZeneca (or designated party).

Only participants enrolled in the study may receive study intervention, and only authorised study centre staff may supply, prepare, or administer study intervention. All study intervention must be stored in a secure, environmentally-controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorised study centre staff.

The investigator, institution, the head of the medical institution (where applicable), or authorised study centre staff is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Further guidance and information for preparation of study intervention and final disposition of unused study interventions are provided in the Pharmacy Manual.

6.2.1 Identity of Study Intervention(s)

AZD3427 will be supplied by AstraZeneca CCI
CCI
CCI
CCI

Placebo will be supplied as a CCI
CCI
CCI

AZD3427 diluent will be supplied as a CCI
CCI
CCI

6.2.2 Study Intervention Inspection

Each CCI selected for dose preparation should be inspected. If any defects are noted with the investigational product(s), the investigator and study centre monitor should be notified

immediately.

6.2.3 Storage of Study Intervention(s)

Study intervention AZD3427, placebo, CCI [redacted] are stored at CCI [redacted] CCI [redacted] and must not be frozen. The investigator, or an approved representative (eg, pharmacist), will ensure that all study intervention is stored in a secured area, in refrigerated at appropriate storage temperatures and in accordance with applicable regulatory requirements.

A temperature log will be used to record the temperature of the storage area. Temperature excursions outside the permissible range listed in the clinical supply packaging are to be reported to the monitor upon detection. A calibrated temperature-monitoring device will be used to record the temperature conditions in the drug storage facility. Storage conditions stated in the IB may be superseded by the label storage.

Study intervention must be kept in original packaging until time of preparation to prevent prolonged light exposure.

6.2.4 AZD3427 Preparation and Administration

6.2.4.1 Preparation

The dose of AZD3427 for administration must be prepared by the investigator's or study centre's designated CCI [redacted]

CCI [redacted]

CCI [redacted]

- CCI [redacted]
- CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

6.2.4.2 Administration

A vial should be used only once to prepare a single dose.

[redacted]

The SC injection may be administered at the skin surface of the anterolateral thigh, upper arm or abdomen (avoid a 2-inch [5 cm]) radius around the umbilicus). Slow injection of study intervention using gentle pressure (at least 5 seconds duration is recommended) and rotation of injection sites is advised. The anatomical location of each injection site should be documented in the eCRF.

Participants will remain at the study centre for 1 hour after the first 3 doses, and then for 30 minutes after each subsequent dose, or longer at the judgement of the investigator considering the risk of anaphylaxis and hypotension for the individual participant.

6.3 Assignment to Study Intervention

All participants will be centrally assigned to randomised study intervention using an IRT/RTSM. Randomisation to study intervention will be performed to ensure approximate balance between the treatment groups (1:1:1:1). The Sponsor or delegate is responsible for generating the randomisation scheme for this study using a validated system. Before the study is initiated, the telephone number and call-in directions for the IRT and/or the log in information and directions for the RTSM will be provided to each study centre.

There will be country level stratification for China and Japan to ensure their randomisation is balanced across treatment groups. Study intervention will be dispensed at the study visits summarised in the SoA (Section 1.3).

Unused study intervention should not be re-dispensed to the participants.

6.4 Methods for Ensuring Blinding

The blinding of study intervention is ensured by having [redacted], using a double-blind technique of matching placebo and [redacted] The IRT/RTSM will provide blinded notifications to the investigator(s) and [redacted] of the kit identification number to be allocated to the participant at the dispensing visits as summarised in the SoA (for the visits where the study intervention is administered see Section 1.3). Routines for this will be described in the IRT/RTSM user manual that will be provided to each study centre.

No member of the extended Sponsor study team, staff at study centres, AstraZeneca study team, or the Sponsor's delegate handling study data will have access to the randomisation scheme during the study. The Sponsor or delegate generating the randomisation scheme and

the Supply Chain Study Management may be able to access the randomisation scheme as appropriate. Neither the participant nor any of the investigator's staff/designee who are involved in the treatment or clinical evaluation and monitoring of the participant will be aware of the treatment received. In the event that the treatment allocation for a participant becomes known to the investigator or other study staff involved in the management of study participants, the investigator must notify the Sponsor immediately.

Investigators will remain blinded to each participant's assigned study intervention throughout the course of the study. CCI

CCI

CCI

CCI

CCI

CCI

CCI

CCI

6.5 Methods for Unblinding

Individual treatment codes, indicating the treatment randomisation for each randomised participant, will be available to the investigator(s) and pharmacists at the study centre from the IRT. Instructions for code breaking/unblinding are provided to each study centre.

AstraZeneca retains the right to break the code for SAEs that are unexpected and are suspected to be causally related to a study intervention and that potentially require expedited reporting to regulatory authorities. Randomisation codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual participant have been made and documented.

The IRT/RTSM will be programmed with blind-breaking instructions. In case of an emergency, in which the knowledge of the specific blinded study intervention will affect the immediate management of the participant's condition (eg, antidote available), the investigator has the sole responsibility for determining if unblinding of a participant's intervention assignment is warranted. Participant safety must always be the first consideration in making such a determination. If a participant's intervention assignment is unblinded, AstraZeneca must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation. The investigator documents and reports the action to AstraZeneca, without revealing the treatment given to the participant to the AstraZeneca staff.

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participant's intervention assignment is warranted. Participant safety must

always be the first consideration in making such a double-blind determination. If the investigator decides that unblinding is warranted, the investigator may, at the investigator's discretion, contact AstraZeneca to discuss the situation prior to unblinding a participant's intervention assignment unless this could delay emergency treatment for the participant. If a participant's intervention assignment is unblinded, AstraZeneca must be notified within 24 hours of this occurrence.

In the event of a Quality Assurance audit, the auditor(s) will be allowed access to unblinded study intervention records at the study centre(s) to verify that randomisation/dispensing has been done accurately.

Sponsor safety staff may unblind the study intervention assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's intervention assignment, may be sent to investigators in accordance with local regulations and/or Sponsor policy.

Pharmacokinetic samples will be analysed by the bioanalytical laboratory performing the bioanalyses only for participants on active study intervention, as referenced in Section 8.5.1. To allow for the appropriate selection of samples, the bioanalytical laboratory will, therefore, have access to the treatment codes but will not share the codes with the Sponsor or others involved in the study until the blinding is broken for the study.

6.6 Unblinding for Unblinded Review Committee

An independent Unblinded Review Committee will be set up for this study to assess the IA data to accelerate internal resourcing of the Phase III study. Details on the staff and methods for unblinding will be captured in a separate Unblinded Review Committee Charter which is to be finalised before the IA data cut-off.

6.7 Study Intervention Compliance

When participants are dosed at the study centre, they will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of dose administered in the study centre will be recorded in the source documents and recorded in the eCRF. The administration of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study centre staff other than the person administering the study intervention.

6.8 Dose Modification

Dose modification is not applicable for this study.

6.9 Continued Access to Study Intervention After the End of the Study

Study intervention will not be provided after the end of the study.

6.10 Treatment of Overdose

For this study, any dose of AZD3427 greater than the maximum specified dose in this protocol will be considered an overdose.

Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator/treating physician should:

- Evaluate the participant to determine, in consultation with the Study Clinical Lead, if possible, whether study intervention should be interrupted or stopped.
- Closely monitor the participant for any AE/SAE and laboratory abnormalities as medically appropriate and at least until the next scheduled visit. Refer to Section 8.4.10 for details of AE/SAE reporting related to overdose.
- Obtain a serum sample for PK analysis if requested by the Study Clinical Lead (determined on a case-by-case basis).
- Document the quantity of the excess dose as well as the duration of the overdose.

6.11 Prior and Concomitant Therapy

Any medication or vaccine (including over the counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements) or other specific categories of interest that the participant (except for screen failures) is receiving at the time of enrolment or receives during the study must be recorded along with:

- Reason for use.
- Dates of administration including start and end dates.
- Dosage information including dose, frequency and route.

Participants should abstain from using non-steroidal anti-inflammatory drugs during the study unless it is already part of the participant's current standard of care.

The use of inhibitors of cGMP-specific phosphodiesterase type 5 (PDE5-I if taken for erectile dysfunction or other occasional, non-continuous uses) such as, but not limited to, Sildenafil and Tadalafil are prohibited for 48 hours before echocardiography and RHC (Screening Visits 1 and 2 and Visit 15).

There are no other specifically prohibited concomitant medications.

It is preferable that any background medical therapy will not be changed during study, ie, between Screening Visit 1 and the last dose of study intervention. However, if this is necessary, the participant does not need to be withdrawn from study intervention.

The Study Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

7 DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

Discontinuation of specific study centres or of the study as a whole are handled as part of Appendix A.

7.1 Discontinuation of Study Intervention

Dosing for any participant must be stopped if the participant experiences any of the following:

- Worsening of HF that requires use of continuous infusion of IV diuretics, inotropes, or vasodilators.
- Systolic BP < 90 mmHg (confirmed by repeat measurement within 30 minutes) with symptoms of hypotension (eg, dizziness and/or light headedness).
- Asymptomatic systolic BP < 80 mmHg (confirmed by repeated measurement within 30 minutes).
- Hypotension due to conditions requiring intervention (eg, need for supportive care and/or treatment of dehydration), over-treatment with other BP lowering medications requiring dose modification, ongoing infection, or arrhythmia).
- Anaphylactic reaction or severe hypersensitivity reaction suspected to be related to study intervention.
- An SAE that is assessed as possibly related to the study intervention by the Investigator.

Note that discontinuation from study intervention is not the same thing as a discontinuation or withdrawal from the study (see Section 7.2).

Depending on the clinical situation and the likely underlying causes, the investigator will decide whether to discontinue the study intervention temporarily or permanently.

If study intervention is permanently discontinued, the participant should, if at all possible, return for an ET Visit, including RHC and echocardiogram, and attend a Follow-up Visit 8 weeks after their last dose. See the SoA (Section 1.3) for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need

to be completed.

7.1.1 Liver Chemistry Stopping Criteria

Discontinuation of study intervention for abnormal liver tests will be considered by the investigator when a participant meets one of the conditions outlined or in the presence of abnormal liver chemistries not meeting protocol-specified stopping rules, if the investigator believes that it is in best interest of the participant.

Liver function abnormalities include:

- ALT or AST > 8 × ULN.
- ALT or AST > 5 × ULN for more than 2 weeks.
- ALT or AST > 3 × ULN and TBL > 2 × ULN or INR > 1.5 (see Appendix E for additional details regarding reporting of participants with ALT or AST > 3 × ULN and TBL > 2 × ULN with unknown aetiology [ie, Hy's Law cases]).
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

7.1.2 QTc Stopping Criteria

If a clinically significant finding is identified (including, but not limited to, changes from baseline in QT interval corrected using Fridericia's formula [QTcF]) after enrolment, the investigator or qualified designee will determine if the participant can continue on the study intervention and if any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

7.2 Participant Discontinuation/Withdrawal from the Study

Discontinuation of the participant from the study by the investigator:

- A participant may be discontinued from the study at any time at the discretion of the investigator for behavioural or compliance reasons.
- At the time of discontinuing from the study, if the participant has not been discontinued from the study intervention, see Section 7.1 for discontinuation from the study intervention. If study intervention is permanently discontinued, the participant should, if at all possible, return for an ET Visit, including RHC and echocardiogram, and attend a Follow-up Visit 8 weeks after their last dose. See the SoA for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

Voluntary withdrawal from the study by the participant:

- A participant may withdraw from the study at any time at the participant's own request for any reason (or without providing any reason).
- If the participant withdraws from the study, the participant should, if at all possible, return for an ET Visit, including RHC and echocardiogram, and a Follow-up Visit 8 weeks after their last dose. See the SoA for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.
- If the participant withdraws consent for disclosure of future information, AstraZeneca may retain and continue to use any data collected before such a withdrawal of consent.
- If the participant withdraws from the study, AstraZeneca may retain and continue to use any samples collected before such a withdrawal of consent for the purposes the participant originally consented unless the participant withdraws consent for use of samples already collected. Data from samples analysed before the participant withdrew may be used by AstraZeneca. If the participant specifically withdraws consent for any use of samples, it must be documented in the study centre's study records by the investigator and the investigator must inform the Local and Global Study Team. Destruction of any samples taken and not yet tested should be carried out in line with documented sample withdrawal wishes in conjunction with what was stated in the informed consent and local regulation.

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if the participant repeatedly fails to return for scheduled visits and is unable to be contacted by the study centre.

The following actions must be taken if a participant fails to return to the study centre for a required study visit:

- The study centre must attempt to contact the participant and reschedule the missed visit as soon as possible. The participant should be counselled on the importance of maintaining the assigned visit schedule. At this time ascertain whether the participant should or wishes to or continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls, texts, emails, and if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, the participant will be considered to have withdrawn from the study/been lost to follow-up.

- Study centre staff, or an independent third party, will attempt to collect the vital status of the participant within legal and ethical boundaries for all participants randomised, including those who did not get study intervention. Public sources may be searched for vital status information. If vital status is determined as deceased, this will be documented, and the participant will not be considered lost to follow-up. Sponsor staff will not be involved in any attempts to collect vital status information.

Discontinuation of specific study centres or of the study as a whole are handled as part of Appendix A.

8 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarised in the SoA (Section 1.3). Protocol waivers or exemptions are not allowed.
- Urgent safety concerns should be discussed with AstraZeneca immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Instructions for the collection and handling of HBS will be provided in the study specific Laboratory Manual. Samples should be stored in a secure storage space with adequate measures to protect confidentiality. For further details on handling of HBS see Appendix C.

In the event of a significant study-continuity issue (eg, caused by a pandemic), alternate strategies for participant visits, assessments, medication distribution and monitoring may be implemented by AstraZeneca or the investigator, as per local health authority/ethics requirements.

Safety, laboratory, and analyte results that could unblind the study will not be reported to study centres or other blinded staff until the study has been unblinded.

The maximum amount of blood collected from each participant, including any extra assessments that may be required, will not exceed 500 mL during the study. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples. Blood samples may also be taken as part of the RHC as per the requirements for the

procedure.

8.1 Administrative and General/Baseline Procedures

The following assessments will be performed at timepoints as specified in the SoA (Section 1.3).

8.1.1 Informed Consent

Participants will give informed consent to participate in the study as described in Appendix A.

8.1.2 Inclusion/Exclusion Criteria

Inclusion/exclusion criteria will be reviewed. Participants have to fulfil all of the inclusion criteria and none of the exclusion criteria (see Section 5) to be included in the study.

8.1.3 Demography and Medical/Surgical History

Demographic data (including age, gender, race, and ethnicity), and medical and surgical history will be collected.

8.1.4 Height and Weight

Height and weight will be recorded (and body mass index calculated) in ordinary indoor clothing with shoes removed.

8.2 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (Section 1.3).

8.2.1 Right Heart Catheterisation

Right heart catheterisation will be performed at baseline (Screening Visit 2) only after the participant has met all other eligibility criteria. Right heart catheterisation will also be performed at Visit 15. For the ET Visit, RHC will only be performed for patients that have been randomised to the study at least 12 weeks prior to the ET Visit. Further details regarding the RHC will be described in a separate manual. Right heart catheterisation results will be sent to a central core laboratory for exploratory analysis.

8.2.2 Echocardiographic Assessments

Echocardiogram will be performed at baseline (Screening Visit 1) and at Visit 15. The Screening Visit 1 echocardiogram images will be assessed locally for study eligibility. All echocardiogram images will undergo quality control checks by a core laboratory.

If the echocardiogram is not readable by the core laboratory for the study endpoints the echocardiogram may be repeated at the discretion of the investigator and Medical Monitor prior to the first dose of study intervention.

If the echocardiogram from Visit 15 is deemed not readable by the core laboratory, then the echocardiography may be repeated after discussion with the Medical Monitor.

Imaging staff and equipment will undergo certification by a separate, blinded, independent core laboratory. Echocardiographic assessments will be performed at times as specified in the SoA (Section 1.3). Evaluated echocardiographic parameters are listed in Appendix I and Echocardiography Imaging Manual.

Echocardiogram images will be assessed by a core laboratory for secondary and exploratory endpoints.

The Echocardiography Imaging Manual describes the imaging protocol, which will accommodate differences in imaging platforms across study centres.

The de-identified echocardiographic images may be transferred to the Sponsor at the end of the study or before for potential future scientific health-related research.

8.2.3 Patient Reported Outcomes

Participants will perform the PRO assessments on paper at the timepoints indicated in the SoA (Section 1.3) and entered by study centre staff into the eCRF. Each study centre must allocate the responsibility for the administration of the PROs to a specific study centre staff and, if possible, assign a back-up person to cover if that individual is absent.

If participants have any medical problems, they should discuss them with their doctor or research nurse separately from the PRO assessment.

The research nurse or appointed study centre staff member must remind participants there are no right or wrong answers and that the value and relevance of PRO data are to hear directly from participants, without interpretation from health care professionals or others, how they function and feel.

Study centre staff must administer questionnaires available in the language that the participant speaks and understands. Questions should not be read in an available language and translated into another language for the participant.

The following best practice guidelines should be followed for KCCQ:

- The PRO questionnaire must be completed before any other study procedures are conducted at the relevant visit, including being seen by the doctor.
- To avoid bias participants must not receive help from relatives, friends or study centre staff to answer or to clarify the PRO questionnaire.
- The PRO questionnaire must be completed by the participant in privacy.

- The participant should be given enough time to complete the PRO questionnaire at his/her own speed.

On completion of PRO questionnaires during study centre visits, the paper-based questionnaires should be handed back to the designated responsible study centre staff member, who should check that the questionnaire was completed. If the questionnaire was not completed the study centre staff must document the reason a participant could not complete assessments in the eCRF. As the questionnaire is being completed on paper the designated responsible centre staff member must also check for any questions that may have been missed by the participant. This must be checked immediately for any missed questions.

The appointed study centre staff member should also stress that the information is confidential. Therefore, if the participant has any medical problems, he/she should discuss them with the doctor or study nurse separately from the PRO assessment.

The following PROs are being used in this study:

- KCCQ
- PRD-acute version

8.2.3.1 Kansas City Cardiomyopathy Questionnaire

The KCCQ is a psychometrically validated questionnaire developed for patients with congestive HF (Green et al 2000). It is a 23-item, self-administered health status measure that quantifies physical limitations, symptoms, social interference, self-efficacy, and quality of life. Results for each domain are summarised and transformed to a score of 0 to 100 with higher scores indicating better health status. Total symptom score will be calculated to assess the frequency and burden of clinical symptoms. The KCCQ English US Master version is enclosed in Appendix F.

8.2.3.2 Patient Rating of Dyspnoea - Acute Version

The PRD-acute version (Appendix G) is a single question item asking the participant to rate his or her shortness of breath right now on a scale from 0 to 10, where 0 indicates ´No shortness of breath´ and 10 ‘As bad as you can imagine’.

This item will be collected as part of the 6MWT and recorded by study centre staff in the eCRF.

8.2.4 6 Minute Walk Test

The 6MWT will be conducted based on the ATS guidelines (ATS 2002) at the timepoints specified in SoA (Section 1.3). As far as possible, for each participant, the test should be performed in the same location, by the same study centre staff, and at the same time of day (in

the morning, if possible) in order to minimise within-participant variability.

Firstly, participant BP, pulse rate, and oxygen saturation in seated position will be recorded. Thereafter, immediately before starting the 6MWT, the participant will stand and using the PRD-acute version (Appendix G) assess his or her perceived level of dyspnoea. Assessment of fatigue is not required to be measured.

After completing the PRD-acute version, the participant will walk as far as he/she can in 6 minutes. The 6MWD will be assessed under standard test conditions, in accordance with the ATS guidelines.

Immediately after completing the 6MWT, the participant will use the PRD-acute version again to reassess his/her perceived level of dyspnoea following exertion. In the meantime, BP, pulse rate, and oxygen saturation in seated position will be recorded again (within 1 minute after completing the 6MWT). Assessment of fatigue is not required to be measured.

If the participant uses oxygen therapy for a HF condition, its use during the test should be recorded (including the volume/minute) and be kept consistent during each 6MWT. If the participant is wearing a face mask, eg, due to COVID-19, it should also be recorded and should be used consistently thereafter for each test.

8.2.5 New York Heart Association Functional Class

The NYHA FC provided in Appendix H will be assessed by the investigator at the timepoints specified in the SoA (Section 1.3) and recorded in the eCRF.

8.3 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.3.1 Physical Examinations

- A full physical examination will include assessments of the following: general appearance, respiratory, CV, abdomen, skin, head and neck (including ears, eyes, nose, and throat), lymph nodes, thyroid, musculoskeletal (including spine and extremities), and neurological systems, with specific attention paid to symptoms of fluid balance as a sign of worsening of HF.
- A brief physical examination will include, at a minimum, assessments of the skin, lungs, CV system, and abdomen (liver and spleen), with specific attention paid to symptoms of fluid balance as a sign of worsening of HF.

Physical examination will be performed at timepoints as specified in the SoA (Section 1.3).

Body weight will be measured at specified timepoints in the SoA (Section 1.3).

8.3.2 Vital Signs

Seated vital signs (BP, HR, respiratory rate, body temperature, and pulse rate) will be assessed after at least 5-minute rest, and before any blood draws scheduled at the same timepoint (eg pre-dose). If abnormalities beyond predefined discontinuation criteria (see Section 7.1) are observed, assessments should be repeated to confirm if the abnormality persists.

Vital signs will be performed at times as specified in the SoA (Section 1.3).

Seated BP measurements (20, 40, and 60 minutes post-dose), will be recorded for the 2 first doses (Visit 3 and Visit 4) of study intervention. Seated BP measurements will be recorded post-dose at the remaining visits, prior to leaving the study centre.

8.3.3 Electrocardiograms

A 12-lead ECG will be obtained after at least 10-minute supine rest, using an ECG machine that automatically measures the RR, PR, QRS, QT intervals, and calculates HR and QTcF values and to be confirmed by the investigator. If the ECG machine is not able to automatically calculate the required parameters manual calculation by qualified physician is acceptable. The investigator may perform additional resting 12-lead ECG safety assessments in case of abnormalities or if required for any other safety reason. These assessments will be recorded as an unscheduled assessment.

The investigator will judge the overall interpretation as normal or abnormal and this evaluation will be reported in the eCRF. If abnormal, the investigator will further document as to whether or not the abnormality is clinically significant. For all abnormalities (regardless of clinical significance) the specific type and nature of the abnormality will be documented in the eCRF. Clinically significant findings should also be documented on the AE page of the eCRF, if applicable. In case of technical problems, and if the investigator considers any result clinically relevant or doubtful, additional digital ECGs may be performed, using the same equipment, within a reasonable time. These assessments should be entered as an unscheduled assessment.

Results will be available on the ECG report printed from the ECG machine. The responsible investigator will need to print, date, and sign the printed ECG report.

12-lead ECG will be performed at timepoints as specified in the SoA (Section 1.3).

8.3.4 Clinical Safety Laboratory Tests

Blood and urine samples for determination of clinical chemistry, haematology, coagulation, and urinalysis will be taken at the visits indicated in the SoA (Section 1.3).

Additional safety samples may be collected if clinically indicated at the discretion of the

investigator. The date, time of collection and results (values, units, and reference ranges) will be recorded on the appropriate eCRF.

The clinical chemistry and haematology will be performed at a central laboratory. Sample tubes and sample sizes will be described in the Laboratory Manual. Dipstick urinalysis will be performed locally.

The following laboratory variables will be measured (see Table 5).

Table 5 Laboratory Safety Variables

Haematology/Haemostasis (whole blood)	**Clinical Chemistry (serum or plasma)**
B-Haemoglobin (Hb)	S/P-Creatinine
B-Leukocyte count	S/P-Bilirubin, total
B-Leukocyte differential count (absolute count) including neutrophils, lymphocytes, basophils, eosinophils and monocytes	S/P-Alkaline phosphatise (ALP)
B-Platelet count	S/P-Aspartate transaminase (AST)
B-Red blood cell count	S/P-Alanine transaminase (ALT)
B-Haematocrit	S/P-Albumin
B-Prothrombin time	S/P-Potassium
B-Activated partial thromboplastin time	S/P-Calcium, total
Reticulocytes	S/P-Sodium
Indices (MCV, MCH, MCHC)	S/P-Creatine kinase (CK)
	S/P-Phosphate
Urinalysis (dipstick)	S/P-Chloride
U-Hb/Erythrocytes/Blood	S/P-Bicarbonate
U-Protein/Albumin	S/P-Magnesium
U-Glucose	S/P-Glucose
U-Drug screen (Screening only)	**Other Tests**
	eGFR [b]
Females Only	
LH [a]	
FSH [a]	
Pregnancy test [c]	

CKP-EPI = Chronic Kidney Disease Epidemiology Collaboration; eGFR = glomerular filtration rate; FSH = follicle stimulating hormone; LH = luteinising hormone; MCHC = mean corpuscular haemoglobin concentration; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; U = urine; S/P = serum/plasma.

[a] Screening Visit 1 only.

[b] Estimated by the CKD-EPI formula (Levey et al 2009).

[c] Pregnancy testing at Screening Visit 2 only, completed with urine pregnancy test.

In case a participant shows an AST or ALT ≥ 3 × ULN together with TBL ≥ 2 × ULN please refer to Appendix E, for further instructions.

8.3.5 Other Safety Assessments

Participants will be monitored for hypersensitivity reactions, including for signs and symptoms of immune complex disease. Severe hypersensitivity reactions should be managed according to standard clinical practice. Medical equipment and staff trained to treat acute anaphylactic reactions must be available at all study centres.

8.3.5.1 Injection Site Reactions

Study centre staff will assess the study intervention injection site for injection site reactions during study visits. All injection site reactions will be recorded as AEs and further characterised. Injection site reactions may include (but are not limited to) pain, itching, swelling, or redness around the site of injection.

8.4 Adverse Events, Serious Adverse Events, and Other Safety Reporting

The principal investigator is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

The definitions of an AE or SAE is available in Appendix B.

Participants (or, when appropriate, a caregiver, surrogate, or the participant's legally authorised representative) will notify the investigator or designees of symptoms. These must then be assessed by the investigator and if considered an AE it will be reported by the investigator.

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE.

Adverse Event Variables

The following variables will be collected for each AE:

- AE (verbatim).
- The date and time when the AE started and stopped.
- Maximum intensity.
- Whether the AE is serious or not.
- Investigator causality rating against the study intervention (yes or no).
- Action taken with regard to study intervention.
- AE caused participant's withdrawal from the study (yes or no).
- Outcome.

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for SAE
- Date investigator became aware of SAE
- AE description
- AE is serious due to
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Causality assessment to other medication

8.4.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

Adverse Events will be collected from randomisation throughout the Treatment Period and including the Follow-up Visit.

Serious adverse events will be recorded from the time of signing of the ICF.

If the investigator becomes aware of an SAE with a suspected causal relationship to the study intervention that occurs after the end of the clinical study in a treated participant, the investigator shall, without undue delay, report the SAE to AstraZeneca.

8.4.2 Follow-up of Adverse Events and Serious Adverse Events

Any AEs that are unresolved at the participant's last AE assessment or other assessment/visit as appropriate in the study are followed up by the investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca retains the right to request additional information for any participant with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

8.4.3 Causality Collection

The investigator should assess causal relationship between the study intervention and each AE and/or incident, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the study intervention?'

For SAEs and serious incidents, causal relationship should also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is available in Appendix B.

8.4.4 Adverse Events Based on Examinations and Tests

Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should only be reported as AEs if they meet any of the following:

- Fulfil any of the SAE criteria.
- Are the reason for discontinuation of the study intervention.
- Are clinically relevant as judged by the investigator (which may include but is not limited to consideration as to whether intervention or non-planned visits were required or other action was taken with the study intervention, eg, dose adjustment or drug interruption).

If deterioration in a laboratory value or vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE unless unequivocally related to the disease under study.

The results from the protocol-mandated laboratory tests and vital signs will be summarised in the CSR.

8.4.5 Adverse Events Based on Signs and Symptoms

All signs or symptoms spontaneously reported by the participant or reported in response to the open question from the study centre staff: 'Have you had any health problems since the previous visit/you were last asked?' or revealed by observation will be collected and recorded in the eCRF.

When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

8.4.6 Hy's Law

Cases where a participant shows elevations in liver biochemistry may require further evaluation, and occurrences of AST or ALT ≥ 3 × ULN together with TBL ≥ 2 × ULN may need to be reported as SAEs. Please refer to Appendix E for further instruction on cases of increases in liver biochemistry and evaluation of Hy's Law.

8.4.7 Reporting of Serious Adverse Events

All SAEs must be reported whether or not considered causally related to the study intervention. All SAEs will be recorded in the eCRF.

If any SAE occurs during the study, investigators or other study centre staff will inform the appropriate AstraZeneca representatives within one day, ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative will work with the investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within one calendar day** of initial receipt for fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up will be undertaken immediately. Investigators or other study centre staff will inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day, ie, immediately but **no later than 24** hours of when he or she becomes aware of it.

Once the investigators or other study centre staff indicate an AE is serious in the electronic data capture (EDC) system, an automated email alert is sent to the designated AstraZeneca representative.

If the EDC system is not available, then the investigator or other study centre staff reports the SAE via secure method to the appropriate AstraZeneca representative.

When the EDC is temporarily not accessible, the AstraZeneca Study Representative should confirm that the investigator/study centre staff enters the SAE in the AstraZeneca EDC when access resumes.

For further guidance on the definition of an SAE, see Appendix B.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca study intervention.

8.4.8 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to AstraZeneca except for:

- If the pregnancy is discovered before the study participant has received any study intervention.

8.4.8.1 Maternal Exposure

Females of childbearing potential are not allowed to be included in this study. Should a pregnancy still occur, the study intervention should be discontinued immediately, and the pregnancy reported to AstraZeneca.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study intervention may have interfered with the effectiveness of a contraceptive medication. Congenital anomalies/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital anomaly/birth defect) should be followed up and documented even if the participant was discontinued from the study.

If any pregnancy occurs during the study, then the investigator or other study centre staff informs the appropriate AstraZeneca representatives immediately but no later than **24 hours** after he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site **within one or 5 calendar days** for pregnancies associated with SAEs (see Section 8.4.7) and **within 30 days** for all other pregnancies.

The same timelines apply when outcome information is available.

The PREGREP module in the eCRF is used to report the pregnancy and the paper-based PREGOUT module is used to report the outcome of the pregnancy.

8.4.8.2 Paternal Exposure

All male participants should refrain from fathering a child or donating sperm during the study and for 3 months following the last dose.

Pregnancy of the participant’s partners is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal

birth or congenital anomaly), occurring from the date of the first dose until 3 months after the last dose and as indicated by previous studies (nonclinical and clinical) should, if possible, be followed up and documented in the Pregnancy Report Form. Consent from the pregnant partner must be obtained before the Pregnancy Report Form is completed.

8.4.9 Medication Error, Drug Abuse, and Drug Misuse

8.4.9.1 Timelines

If an event of medication error, drug abuse, **or** drug misuse occurs during the study, then the investigator or other study centre staff informs the appropriate AstraZeneca representatives within **one calendar day**, ie, immediately but **no later than 24 hours** of when they become aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is completed within **one** (initial fatal/life-threatening or follow-up fatal/life-threatening) **or 5** (other serious initial and follow-up) **calendar days** if there is an SAE associated with the event of medication error, drug abuse, or misuse (see Section 8.4.7) and **within 30 days** for all other events.

8.4.9.2 Medication Error

For the purposes of this clinical study a medication error is an **unintended** failure or mistake in the treatment process for a study intervention or AstraZeneca NIMP that either causes harm to the participant or has the potential to cause harm to the participant.

The full definition and examples of medication error can be found in Appendix B 4.

8.4.9.3 Drug Abuse

Drug abuse is the persistent or sporadic **intentional**, non-therapeutic excessive use of study intervention or AstraZeneca NIMP for a perceived reward or desired non-therapeutic effect.

The full definition and examples of drug abuse can be found in Appendix B 4.

8.4.9.4 Drug Misuse

Drug misuse is the **intentional** and inappropriate use (by a study participant) of study intervention or AstraZeneca NIMP for medicinal purposes outside of the authorised product information, or for unauthorised study interventions or AstraZeneca NIMPs, outside the intended use as specified in the protocol and includes deliberate administration of the product by the wrong route.

The full definition and examples of drug misuse can be found in Appendix B 4.

8.4.10 Reporting of Overdose

Refer to Section 6.10 for definition and treatment of overdose.

- An overdose with associated AEs is recorded as the AE diagnoses/symptoms on the relevant AE modules in the eCRF and on the Overdose eCRF module.
- An overdose without associated symptoms is only reported on the Overdose eCRF module.

If an overdose on a study intervention or AstraZeneca NIMP occurs in the course of the study, the investigator or other study centre staff inform appropriate AstraZeneca representatives immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site **within one or 5 calendar days** for overdoses associated with an SAE (see Section 8.4.7) and **within 30 days** for all other overdoses.

8.5 Pharmacokinetics

Blood samples for the determination of serum concentrations of AZD3427 will be collected at the time points specified in the SoA (Section 1.3).

- Samples may be collected at additional time points during the study if warranted and agreed upon between the investigator and AstraZeneca, eg, for urgent safety reasons, and this may be reflected as a protocol deviation.
- The timing of sampling may be altered during the study based on newly available data (eg, to obtain data closer to the time of peak or trough matrix concentrations) to ensure appropriate monitoring.
- The samples will be used to analyse the PK of AZD3427. The samples collected for analyses of AZD3427 concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.
- Samples will be collected, labelled, stored, and shipped as detailed in the Laboratory Manual.
- For storage, re-use, and destruction of samples for PK see Appendix C.
- Pharmacokinetics samples will be disposed of after the Bioanalytical Report finalisation or 6 months after issuance of the draft Bioanalytical Report (whichever is earlier).
- Additional analyses may be conducted on the anonymised, pooled, or individual PK samples to further evaluate and validate the analytical method. Any results from such analyses may be reported separately from the CSR.

- Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. 10% of the total number of samples received with quantifiable results will be included in the ISR.

8.5.1 Determination of Drug Concentration

Samples for determination of AZD3427 concentration in serum will be assayed by bioanalytical test sites operated by or on behalf of AstraZeneca, using an appropriately validated bioanalytical method. Full details of the analytical method used will be described in a separate Bioanalytical Report.

AZD3427 concentration information that may unblind the study will not be reported to investigative study centres or blinded staff until the study has been unblinded.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. The results from the evaluation, if performed, will be reported in a separate Bioanalytical Report.

Placebo samples will not be analysed unless there is a need to confirm that correct treatment has been administered to study participants.

8.6 Biomarkers

8.6.1 Mandatory Biomarker Sample Collection

By consenting to participate in the study the participant consents to the mandatory research components of the study. Samples for biomarker research are required and will be collected from all participants in this study as specified in the SoA (Section 1.3). Mandatory biomarker collection include:

- Blood and urine samples to assess PD effects of AZD3427 on cardiac and renal parameters by evaluating change in serum creatinine, NT-proBNP, cystatin C, and eGFR.
- Blood and urine samples for exploratory evaluation of the effect of AZD3427 on biomarkers of inflammation, fibrosis, the RAAS pathway, tissue remodelling, fluid/electrolyte balance, and RXFP1 signalling, including renin, aldosterone, sodium, potassium, copeptin, serum osmolality, PIIINP, PRO-C6, erythropoietin, TNF-α, TGF-β, KIM-1, hsCRP, uACR, hs-TnT, and endogenous relaxin. Tests also include assessment of plasma proteome by multiplex protein panel (eg, Olink® Explore 3072). The results of each of these exploratory biomarker analyses may or may not be included in the CSR of this study.

Samples for mandatory biomarker testing are collected according to local regulatory approval. Samples collected from participants in China will be destroyed or repatriated within 1 year of

CSR.

8.6.2 Optional Biomarker Sample Collection

Collection of optional samples for biomarker research is also part of this study as specified in the SoA and is subject to agreement to optional consent. Samples for optional biomarker are collected according to local regulatory approval.

Plasma, serum, and urine samples will be collected for future research projects. These future scientific research projects are beyond the scope of the clinical study and use of samples may occur while the study is ongoing or after the study has finished. Biosamples may only be used for scientific health-related research within the cardiovascular disease area to find new ways to detect, treat, prevent or cure health problems. For storage, re-use, and destruction of biomarker samples see Appendix C.

8.7 Immunogenicity Assessments

- Blood samples for determination of ADA, ADA titres, and NAbs in serum will be assayed by bioanalytical test sites operated by or on behalf of AstraZeneca, using an appropriately validated bioanalytical method. A tiered testing scheme will be employed, with the first step being screening. Samples found positive in the screening step will be tested in the confirmatory step. Samples confirmed positive for ADA in the confirmatory step will undergo endpoint titre determination. Full details of the methods used will be described in a separate report.
- Anti-drug antibody samples may also be further tested for characterisation of the ADA response.
- Participants with ADA-positive titres (titre ≥ 100) at ET or Follow-up Visit (whichever is latest) will be asked to return to provide another sample at approximately 3 months after the ET/Follow-up Visit. If that sample is ADA-positive (titre ≥ 100), the participant will be asked to return to provide a sample every third month until a sample tests negative for ADA or returns to within one titre dilution step of baseline. If participants still reach the criteria for further follow-up at 12 months after the last study intervention dose, designated study Sponsor staff will provide recommendations to the investigator on the need for continued ADA monitoring. Data from these additional samples and visits may be reported in the CSR or in a CSR addendum, if applicable.
- Samples will be collected, labelled, stored, and shipped as detailed in the Laboratory Manual.
- For storage, re-use, and destruction of ADA and NAbs samples see and Appendix C.
- Remaining ADA sample aliquots will be retained at AstraZeneca or its designee for a maximum of 15 years from last participant last visit. Additional use includes but is not limited to further characterisation of any ADAs, confirmation and/or requalification of the

assay as well as additional assay development work. The results from future analysis will not be reported in the CSR.

Samples for ADA/NAbs testing from participants in China will be destroyed or repatriated within 1 year of CSR.

8.7.1 Collection of Samples for Immunogenicity Assessments

Blood samples will be collected for measurement of ADA, ADA titres, and NAbs at timepoints specified in the SoA (Section 1.3).

8.8 Pharmacodynamics

Pharmacodynamic assessments will include RHC, echocardiography, 6MWD and KCCQ, as well as change in serum creatinine, NT-proBNP, cystatin C, and eGFR. Planned time points for all PD assessments are provided in the SoA (Section 1.3).

8.8.1 Collection of Samples for Pharmacodynamics

Blood samples will be collected for measurement of serum creatinine, NT-proBNP, cystatin C, and eGFR.

Samples will be collected, labelled, stored, and shipped as detailed in the Laboratory Manual. For storage, re-use, and destruction of samples for PD see Appendix C.

8.8.2 Samples for Exploratory Pharmacodynamics

Samples will be collected for exploratory measurement of hs-TnT and uACR.

Blood samples for hs-TnT and urine samples for uACR will be collected at timepoints specified in the SoA (Section 1.3).

8.9 Optional Genomics Initiative

Collection of optional samples for Genomics Initiative research is also part of this study as specified in the SoA and is subject to participant agreement in the optional genetic research information ICF.

Blood sample for DNA isolation will be collected from participants who have consented to participate in the genetic analysis component of the study. Participation is optional. Participants who do not wish to participate in the genetic research may still participate in the study.

See Appendix D for information regarding the Genomics Initiative genetic sample. Details on processes for collection and shipment and destruction of these samples can be found either in the appendices of this protocol or in the Laboratory Manual.

8.10 Medical Resource Utilisation and Health Economics

Health economics/Medical resource utilisation and health economics parameters are not evaluated in this study.

8.11 Study Participant Feedback Questionnaire

A study participant feedback questionnaire will not be completed for this study.

9 STATISTICAL CONSIDERATIONS

A comprehensive SAP will be prepared and finalised within 90 days of first participant in, the first dry run, or before any unblinding (whichever occurs first) and will include a more technical and detailed description of the statistical analyses described in this section. Any subsequent amendments to the SAP will be documented, with final amendments completed before unblinding of the data for the analysis. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.1 Statistical Hypotheses

The primary hypothesis for this study is that AZD3427 will reduce PVR compared with placebo after 24 weeks of treatment, on top of current standard of care. Dose response may be evaluated outside of the CSR and will be performed by AstraZeneca.

There are 3 key secondary hypotheses, as follows:

- AZD3427 will reduce mPAP compared with placebo after 24 weeks of treatment.
- AZD3427 will improve function and symptoms compared with placebo after 24 weeks of treatment.
- AZD3427 will have an acceptable safety profile in participants with HF and PH Group 2.

9.2 Sample Size Determination

Approximately 220 participants will be randomised to study intervention such that approximately 188 evaluable participants complete the study.

For the primary endpoint, 47 evaluable participants per group will provide approximately 80% power to detect a 20% reduction in PVR at Week 25 compared to placebo, assuming a 5% 2-sided significance level and a SD on the loge scale of 0.38. A total of approximately 220 participants (55 participants per treatment group) will be randomised to account for incomplete data and participant drop-out.

9.3 Populations for Analyses

The populations for analyses are defined in Table 6.

Table 6 Populations for Analysis

Population/analysis set	Description
Enrolled	All participants who sign the ICF.
Full Analysis Set	The Full Analysis Set consists of all participants who are randomised and have received at least one dose of study intervention. Participants are evaluated according to the treatment assigned at randomisation.
Safety Analysis Set	The Safety Analysis Set consists of all participants who have received at least one dose of study intervention. Erroneously treated participants (eg, those randomised to one treatment group but actually administered the study intervention from another treatment group) are accounted for in the treatment group of the study intervention they actually received. A participant who has received any dose of AZD3427 will be classified as in the appropriate AZD3427 dose treatment group.
PK Analysis Set	The PK Analysis Set consists of all participants in the Full Analysis Set who have at least one detectable AZD3427 serum concentration measurement post-treatment.

ICF = Informed Consent Form; PK = pharmacokinetic(s).

9.4 Statistical Analyses

9.4.1 General Considerations

Relevant demographic and baseline characteristics will be summarised descriptively for each treatment group and overall.

The study will include 3 periods:

- Screening Period of up to 42 days (6 weeks) before dosing.
- Treatment Period of 24 weeks (baseline to Week 25 Visit).
- Follow-up Period of 56 days (8 weeks) after last dose CCI [redacted]

All efficacy and safety variables will be summarised by treatment group using descriptive statistics (n, mean, SD, median, minimum and maximum for continuous data that is expected to be normally distributed; n, geometric mean, coefficient of variation, median, minimum and maximum for continuous data that is expected to be lognormal; and n, frequencies and percentages for categorical data). Data will be summarised by time point/visit, as applicable.

Outputs will also include a total pooled AZD3427 group (comprising of 1.1, 5.4, and 30.0 mg treatment groups) and a high pooled AZD3427 group (comprising of 5.4 and 30.0 mg treatment groups).

For analyses with treatment comparisons, comparisons will be done for each AZD3427 group versus placebo as well as the total pooled AZD3427 group (comprising of 1.1, 5.4, and 30.0 mg treatment groups) versus placebo, the high pooled AZD3427 group (comprising of 5.4 and 30.0 mg treatment groups) versus placebo. The pooled AZD3427 groups versus placebo comparisons are included to provide results under assumptions that all, or the 2 highest doses, are similar with respect to efficacy and or safety.

P-values will be presented as 2-sided without multiplicity adjustments. Confidence intervals will be presented as 95% 2-sided, without multiplicity adjustments.

In general, the baseline value for statistical analysis is the last non-missing value prior to or on the same date as administration of the first dose of study intervention. The statistical methods and presentation of the variables, as well as the potential impact of missing data and methods, will be described in the SAP.

9.4.2 Efficacy

9.4.2.1 Primary Endpoint

The primary efficacy endpoint is the change in PVR from baseline to Week 25.

Pulmonary vascular resistance will be log-transformed for statistical analysis purposes. The mean log changes in PVR at Week 25 for each of the 3 AZD3427 doses and placebo will be estimated using an ANCOVA model, with treatment as fixed effect, stratification factors fitted as categorical covariates and baseline PVR fitted as a continuous covariate. The values will be back transformed onto the original scale to give the geometric mean relative change from baseline at Week 25. The comparison of each dose of AZD3427 versus placebo will be performed using the geometric mean ratio.

The primary estimand is a treatment policy estimand such that the treatment effect is quantified using all participants' data, regardless of the occurrence of any intercurrent events. The population of interest is the full analysis set. The endpoint being assessed is the change in log-transformed PVR from baseline to Week 25. The summary measure being evaluated is the geometric mean reduction of PVR from baseline to Week 25.

9.4.2.2 Secondary Endpoints

Secondary endpoints which are assessed post-baseline at Week 25 only, such as change from baseline in mPAP and PAWP, will be analysed using an ANCOVA model as described for the primary efficacy endpoint. Secondary endpoints which are assessed at more than one post-baseline visit will be analysed using a mixed model for repeated measures.

Secondary endpoints, aside from KCCQ and 6MWD, will follow a treatment policy estimand as described for the primary estimand. The KCCQ and 6MWD will follow a while-alive estimand, whereby death is considered an intercurrent event.

Pharmacokinetic Endpoints

Serum concentration of AZD3427 will be summarised by descriptive statistics timepoints and dose level.

If PK data permit a population PK model will be developed, possibly with the support of PK data from other studies, using nonlinear mixed effects modelling in NONMEM. Furthermore, if data allows, the population PK may be coupled with separate PD models.

All PK/PD modelling will be described in a separate data analysis plan. The results of any such modelling will be provided in a separate PK and population PK/PD report (as an appendix to the CSR or as stand-alone report).

9.4.2.3 Exploratory Endpoints

The statistical methods and presentation of the variables will be detailed in the SAP.

9.4.3 Safety

Safety analyses will be performed using the Safety Analysis Set. Safety data will be presented using descriptive statistics unless otherwise specified.

9.4.3.1 Adverse Events

Adverse events will be coded using the most recent version of MedDRA that will have been released for execution at AstraZeneca or its designee.

Adverse events will be presented for each treatment group by MedDRA SOC and preferred term covering the number and percentage of participants reporting at least one event and number of events where appropriate.

Adverse events occurring prior to administration of study intervention, treatment-emergent adverse events and post-treatment AEs will be presented separately.

An overview of AEs will be presented for each treatment group including the number and percentage of participants with any AE, AEs with outcome of death, SAEs, and DAE of study intervention, AEs leading to withdrawal from the study, as well as the number of individual occurrences in those categories.

Separate AE tables will be provided taken into consideration relationship as assessed by the investigator, maximum intensity, seriousness, death, and AEs leading to discontinuation of study intervention, as well as other action taken in relation to the study intervention, AESI, other significant AEs, and timing of events.

An additional table will present the number and percentage of participants with most common AEs. Most common (eg, frequency of > x%, ≥ x%) will be defined in the SAP.

In accordance with the requirements of the regulatory authority, a separate table will present non-serious AEs occurring in more than 5% of participants in any treatment group.

Key participant information will be presented for participants with AEs with outcome of death, SAEs, and DAE of study intervention.

An AE listing for the Safety Analysis Set will include details for each individual AE.

Full details of AE analyses will be provided in the SAP.

9.4.3.2 Vital Signs

Vital sign parameters (BP, HR, respiratory rate, body temperature, and pulse rate) will be presented for each treatment group. Summary statistics for continuous variables will cover n, mean, SD, minimum, Q1, median, Q3, and maximum.

For each scheduled post-baseline visit, descriptive statistics for all vital sign parameters will be presented for observed values and change from baseline.

Supportive vital sign listings will include observed values and changes from baseline, as well as abnormalities.

Details of vital sign analyses including definition of abnormality criteria (eg, definition of low, normal, high, and clinically significant) and project-specific predefined criteria for treatment-emergent changes in vital sign parameters will be provided in the SAP.

9.4.3.3 Laboratory Assessments

Laboratory parameters will be presented for each treatment group. Summary statistics for continuous variables include n, mean, SD, minimum, Q1, median, Q3, and maximum. Frequency tables include number and percentage of participants in the respective category for categorical laboratory parameters.

For each scheduled post-baseline visit, descriptive statistics for all clinical chemistry, haematology parameters, and for coagulation parameters will be presented for observed values and change from baseline.

Elevation in liver parameters for assessment of Hy's Law will be assessed and reported appropriately if potential cases have been identified during the course of the study.

Key participant information will be presented for participants with treatment-emergent changes in laboratory parameters outside of predefined criteria.

For urinalysis parameters, a frequency table will be presented with the number of participants reporting at least one treatment-emergent increase from baseline category, and/or a shift table

will be presented with the baseline assessment against the maximum on treatment category.

Supportive laboratory listings will include observed values and changes from baseline for each individual participant as well as abnormalities.

Details of laboratory analyses including definition of abnormality criteria (eg, definition of low, normal, high, and clinically significant) and project-specific predefined criteria for treatment-emergent changes in laboratory parameters will be provided in the SAP.

9.4.3.4 Electrocardiograms

For the interpretation of the ECG findings, a frequency table will include the number and percentage of participants in the respective overall evaluation category: normal, abnormal not clinically significant, and abnormal clinically significant, and ECG parameters at baseline, and for each scheduled post-baseline visit. Change from baseline to each post-baseline time point will also be summarised, where appropriate.

9.4.4 Other Analyses

9.4.4.1 Immunogenicity Assessments

The number and percentage of participants with ADAs will be summarised along with descriptive statistics of ADA titre at each visit by treatment group.

The number and percentage of participants with NAbs will also be summarised in an addendum, if applicable.

9.4.4.2 Subgroup Analyses

Details of the planned subgroup analyses will be provided in the SAP.

9.5 Interim Analyses

An IA will be performed after approximately 50% of participants complete Visit 15 with an option to trigger early initiation of Phase III activities. The IA will assess, but not be limited to PVR, mPAP, 6MWD, KCCQ, NT-proBNP, renin, AZD3427 serum concentration, and safety.

In order to maintain the study blind, the study staff who perform the IA will be separate from those in the study team. No details that could compromise the integrity of the study blind will be passed to the study team.

The planned IA will be described in detail in the SAP.

9.6 Unblinded Review Committee

For details on Unblinded Review Committee, refer to Appendix A 5.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

Appendix A Regulatory, Ethical, and Study Oversight Considerations

A 1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki as amended at 64th WMA General Assembly, Fortaleza, Brazil, October 2013 and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH GCP Guidelines
  - Applicable laws and regulations
- The protocol, revised protocol, ICF, IB, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any revised protocol will require IRB/IEC and applicable Regulatory Authority approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- AstraZeneca will be responsible for obtaining the required authorisations to conduct the study from the concerned Regulatory Authority. This responsibility may be delegated to a CRO, but the accountability remains with AstraZeneca.
- The investigator will be responsible for providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR 312.120, ICH guidelines, the IRB/IEC, European Regulation 536/2014 for clinical studies (if applicable), European Medical Device Regulation 2017/745 for clinical device research, ISO-14155 for clinical device (if applicable), and all other applicable local regulations.

Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to AstraZeneca of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- AstraZeneca has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. AstraZeneca will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and investigators.
- In the European Union, the Sponsor will comply with safety reporting requirements and procedures as described in the European Clinical Trials Regulation (EU) No 536/2014.

All Suspected Unexpected Serious Adverse Reactions (SUSARs) to investigational medicinal product will be reported to the Eudra Vigilance database within the required regulatory timelines.

- For all studies except those utilising medical devices, investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to investigators, as necessary.
- Adherence to European Medical Device Regulation 2017/745 for clinical device research (if applicable), and all other applicable local regulations.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from AstraZeneca will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

Regulatory Reporting Requirements for Serious Breaches of Protocol or GCP

A 'Serious Breach' means any deviation of the approved version of the protocol or Good Clinical Practice (GCP) that is likely to affect the safety, rights of trial participants and/or data reliability and robustness to a significant degree in a clinical trial.

All parties (Sponsor, Service Provider, Investigator Site Staff) involved in the conduct of the clinical trial:

- are responsible for promptly identifying and documenting any actual or potential serious breach.
- should have a process in place to ensure that they are able to identify the occurrence of a (actual/potential) serious breach.
- should report to AstraZeneca (or delegated party) without delay, through the contacts (email address or telephone number) provided by AstraZeneca.

A 2 Financial Disclosure

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

A 3 Informed Consent Process

- The investigator or their representative will explain the nature of the study to the participant or their legally authorised representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary, and they are free to refuse to participate and may withdraw their consent at any time and for any reason during the study. Participants or their legally authorised representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, privacy and data protection requirements, where applicable, and the IRB/IEC or study centre.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorised person obtaining the informed consent must also sign the ICF.
- If new information requires changes to the ICF, consider if participants must be re-consented and if so, this must be to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorised representative.

Participants who are rescreened are required to sign a new ICF.

The ICF will contain a separate section that addresses and documents the collection and use of any mandatory and/or optional HBS. The investigator or authorised designee will explain to each participant the objectives of the analysis to be done on the samples and any potential future use. Participants will be told that they are free to refuse to participate in any optional samples or the future use and may withdraw their consent at any time and for any reason during the retention period.

A 4 Data Protection

- Participants will be assigned a unique identifier by AstraZeneca. Any participant records or datasets that are transferred to AstraZeneca will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that their personal study-related data will be used by AstraZeneca in accordance with local data protection law. The level of disclosure and use of their data must also be explained to the participant in the informed consent.

- The participant must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorised staff appointed by AstraZeneca, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- The participant must be informed that data will be collected only for the business needs. We will only collect and use the minimum amount of personal data to support our business activities and will not make personal data available to anyone (including internal staff) who is not authorised or does not have a business need to know the information.
- The participant must be informed that in some cases their data may be pseudonymised. The General Data Protection Regulation (GDPR) defines pseudonymisation as the processing of personal data in such a way that the personal data can no longer be attributed to a specific individual without the use of additional information, provided that such additional information is kept separately and protected by technical and organisational measures to ensure that the personal data are not attributed to an identified or identifiable natural person.

Personal Data Breaches

A 'personal data breach' means a breach of security leading to the accidental or unlawful destruction, loss, alteration, unauthorised disclosure of, or access to, personal data transmitted, stored or otherwise processed.

- In compliance with applicable laws, the Data Controller [a] for the processing activity where the personal data breach occurred (AstraZeneca or respectively the site), will notify the data protection authorities without undue delay within the legal terms provided for such notification and within the prescribed form and content.
- Whilst AstraZeneca has processes in place to deal with personal data breaches it is important that investigators that work with AstraZeneca have controls in place to protect patient data privacy.

The Investigator should have a process in place to ensure that:

- allow site staff or service providers delegated by the investigator/institution to identify the occurrence of a (potential) personal data breaches.
- Any (potential) personal data breach is promptly reported to AstraZeneca or delegated party, through the contacts (email address or telephone number) provided by AstraZeneca.

[a] The **data controller** determines the **purposes** for which and the **means** by which personal data are processed, as defined by the European Commission.

AstraZeneca and the site must demonstrate that they:

- have taken all necessary steps to avoid personal data breaches and
- have undertaken measures to prevent such breaches from occurring in the first place and to mitigate the impact of occurred data breaches (eg, applying encryption, maintaining and keeping systems and IT security measures up-to-date, regular reviews and testing, regular training of employees, and developed security policies and standards).
- where possible, have developed an internal data breach reporting and investigation process and internal protocols with guidance on how to respond swiftly and diligently to the occurrence of a personal data breach.
- where it has not been possible to develop an internal data breach reporting and investigation process, the site follows AstraZeneca's instructions.

Notification of personal Data Breach to participants:

- notification to participants is done by the site for the data breaches that occurred within the processing activities for which the site is the Data Controller and for data breaches occurred within the processing activities of AstraZeneca as the Data Controller, the notification is done in collaboration with the site and is performed by the site and/or Principal Investigator, acting on behalf of AstraZeneca, so that AstraZeneca has no access to the identifying personal information of the participants. The site and/or Principal Investigator shall conduct the notification by contacting the participants using the information that they gave for communication purposes in clinical research.
- If a personal data breach occurs in a processor's systems, engaged by AstraZeneca, the processor under contractual obligations with AstraZeneca promptly and in due course after discovering the breach notifies AstraZeneca and provides full cooperation with the investigation. In these cases, to the extent AstraZeneca is the Data Controller for the processing activity where the breach occurred, it will be responsible for the notification to data protection authorities and, if applicable, to participants. If the personal data breach needs to be notified to the participants, the notification to participants is done in collaboration with the site and is performed by the site and/or Principal Investigator, acting on behalf of the Sponsor, so that AstraZeneca has no access to the identifying personal information of the participants.
- If a personal data breach involving an AstraZeneca's representative device (i.e Study Monitor laptop), AstraZeneca representative will provide AstraZeneca with all of the information needed for notification of the breach, without disclosing data that allows AstraZeneca directly or indirectly to identify the participants. The notification will be done by AstraZeneca solely with the information provided by the Study Monitor and in no event with access to information that could entail a risk of re-identification of the participants. If the data breach must be notified to the data subjects, the notification will

be done directly by the Study Monitor in collaboration with the site and/or Principal Investigator, acting on behalf of the Sponsor, so that AstraZeneca has no access to the identifying personal information of the participants. The contract between AstraZeneca and the Study Monitor shall expressly specify these conditions.

- The contract between the site and AstraZeneca for performing the clinical research includes the provisions and rules regarding who is responsible for coordinating and directing the actions in relation to the breaches and performing the mandatory notifications to authorities and participants, where applicable.

A 5 Committee Structure

An independent Unblinded Review Committee will be set up for this study to assess the IA data to accelerate internal resourcing of the Phase III study. Additional details about the Unblinded Review Committee will be contained in the Unblinded Review Committee Charter.

A 6 Dissemination of Clinical Study Data

Any results both technical and lay summaries for this trial, will be submitted to EU CTIS within a year from global End of Trial Date in all participating countries, due to scientific reasons, as otherwise statistical analysis is not relevant. The content of the summaries will align to the requirements set out in EU Clinical Trials Regulation 536/2014 Annex IV and Annex V.

A description of this clinical study will be available on www.astrazenecaclinicaltrials.com, http://www.clinicaltrials.gov and https://euclinicaltrials.eu/ as will the summary of the study results when they are available. The clinical study and/or summary of study results may also be available on other websites according to the regulations of the countries in which the study is conducted.

A 7 Data Quality Assurance

- All participant data relating to the study will be recorded on eCRF unless transmitted to AstraZeneca or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Quality tolerance limits (QTLs) will be predefined to identify systematic issues that can impact participant safety and/or reliability of study results. These predefined parameters

will be monitored during the study, and important deviations from the QTLs and remedial actions taken will be summarised in the CSR.

- Monitoring details describing strategy, including definition of study-critical data items and processes (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are included in the Monitoring Plan.
- AstraZeneca or designee is responsible for medical oversight throughout the conduct of the study which includes clinical reviews of study data in accordance with the currently approved protocol. Monitoring details describing clinical reviews of study data from a medical perspective are included in more detail in the Monitoring Plan.
- AstraZeneca or designee is responsible for the data management of this study including quality checking of the data.
- AstraZeneca assumes accountability for actions delegated to other individuals (eg, CROs).
- Study monitors will perform ongoing source data verification as per the Monitoring Plan(s) to confirm that data entered into the eCRF by authorised study centre staff are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for a minimum of 25 years after study archiving or as required by local regulations, according to the AstraZeneca Global retention and Disposal (GRAD) Schedule. No records may be destroyed during the retention period without the written approval of AstraZeneca. No records may be transferred to another location or party without written notification to AstraZeneca.

A 8 Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator’s study centre.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

- Definition of what constitutes source data and its origin can be found in the source document declaration for each study centre.

A 9 Study and Study Centre Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the first participant screened and will be the study start date.

AstraZeneca designee reserves the right to close the study centre or terminate the study at any time for any reason at the sole discretion of AstraZeneca. Study centres will be closed upon study completion. A study centre is considered closed when all required documents and study supplies have been collected and a study centre closure visit has been performed.

The investigator may initiate study centre closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study centre by AstraZeneca or the investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, AstraZeneca's procedures, or GCP guidelines.
- Inadequate recruitment of participants by the investigator.
- Discontinuation of further study intervention development.

If the study is prematurely terminated or suspended, AstraZeneca shall promptly inform the investigators, the IRBs/IECs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

Participants from terminated study centres will have the opportunity to be transferred to another study centre to continue the study.

A 10 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to AstraZeneca before submission. This allows AstraZeneca to protect proprietary information and to provide comments.

- AstraZeneca will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, AstraZeneca will generally support publication of multi-centre studies only in their entirety and not as individual study centre data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

A 11 Clinical Outcome Assessment/Participant Reported Outcome Questionnaire

A study participant feedback questionnaire will not be completed for this study.

Appendix B Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

B 1 Definition of Adverse Events

An AE is the development of any untoward medical occurrence in a participant or clinical study participant administered a medicinal product, and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (eg, an abnormal laboratory finding), symptom (for example nausea, chest pain), or disease temporally associated with the use of a medicinal product, whether it is considered related to the medicinal product.

The term AE is used to include both serious and non-serious AEs and can include a deterioration of a pre-existing medical occurrence. An AE may occur at any time, including run-in or washout periods, even if no study intervention has been administered.

B 2 Definition of Serious Adverse Events

An SAE is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death.
- Is immediately life-threatening.
- Requires in-patient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect.
- Is an important medical event that may jeopardise the participant or may require medical treatment to prevent one of the outcomes listed above.

Adverse Events for **malignant tumours** reported during a study should generally be assessed as **SAEs**. If no other seriousness criteria apply, the ‘important medical event’ criterion should be used. In certain situations, however, medical judgement on an individual event basis should be applied to clarify that the malignant tumour event should be assessed and reported as a **non-SAE**. For example, if the tumour is included as medical history and progression occurs during the study, but the progression does not change treatment and/or prognosis of the malignant tumour, the AE may not fulfil the attributes for being assessed as serious, although reporting of the progression of the malignant tumour as an AE is valid and should occur. Also, some types of malignant tumours, which do not spread remotely after a routine treatment that does not require hospitalisation, may be assessed as non-serious; examples in adults include Stage 1 basal cell carcinoma and Stage 1A1 cervical cancer removed via cone biopsy.

Life-threatening

'Life-threatening' means that the participant was at immediate risk of death from the AE as it occurred, or it is suspected that use or continued use of the medicinal product would result in the participant's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a SAE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the participant was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important Medical Event or Medical Treatment

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardise the participant or may require medical treatment to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

Intensity Rating Scale:

- Mild (awareness of sign or symptom, but easily tolerated).
- Moderate (discomfort sufficient to cause interference with normal activities).
- Severe (incapacitating, with inability to perform normal activities).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Appendix B 2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE unless it meets the criteria shown in Appendix B 2. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE when it satisfies the criteria shown in Appendix B 2.

B 3 A Guide to Interpreting the Causality Question

When assessing causality consider the following factors when deciding if there is a 'reasonable possibility' that an AE may have been caused by the medicinal product.

- Time Course. Exposure to suspect drug. Has the participant received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?
- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? Or could the AE be anticipated from its pharmacological properties?
- De-challenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host, or environmental factors.
- Re-challenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a re-challenge.
- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?
- Is there a known mechanism?

Causality of 'related' is made if following a review of the relevant data, there is evidence for a 'reasonable possibility' of a causal relationship for the individual case. The expression 'reasonable possibility' of a causal relationship is meant to convey, in general, that there are facts (evidence) or arguments to suggest a causal relationship.

The causality assessment is performed based on the available data including enough information to make an informed judgement. With no available facts or arguments to suggest a causal relationship, the event(s) will be assessed as 'not related'.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as 'no reasonable possibility'.

B 4 Medication Error, Drug Abuse, and Drug Misuse

Medication Error

For the purposes of this clinical study a medication error is an unintended failure or mistake in the treatment process for a study intervention or AstraZeneca NIMP that either causes harm to the participant or has the potential to cause harm to the participant.

A medication error is not lack of efficacy of the drug, but rather a human or process related failure while the drug is in control of the study centre staff or participant.

Medication error includes situations where an error:

- Occurred.
- **Was identified and** intercepted before the participant received the drug.
- Did not occur, but circumstances were recognised that could have led to an error.

Examples of events to be reported in clinical studies as medication errors:

- Drug name confusion.
- Dispensing error, eg, medication prepared incorrectly, even if it was not actually given to the participant.
- Drug not administered as indicated, eg, wrong route or wrong site of administration.
- Drug not taken as indicated, eg, tablet dissolved in water when it should be taken as a solid tablet.
- Drug not stored as instructed, eg, kept in the refrigerator when it should be at room temperature.
- Wrong participant received the medication (excluding IRT/RTSM errors).
- Wrong drug administered to participant (excluding IRT/RTSM errors).

Examples of events that **do not** require reporting as medication errors in clinical studies:

- Errors related to or resulting from IRT/RTSM - including those which led to one of the above listed events that would otherwise have been a medication error.
- Participant accidentally missed drug dose(s), eg, forgot to take medication.
- Accidental overdose (will be captured as an overdose).
- Participant failed to return unused medication or empty packaging.

Medication errors are not regarded as AEs but AEs may occur as a consequence of the medication error.

Drug Abuse

For the purpose of this study, drug abuse is defined as the persistent or sporadic intentional, non-therapeutic excessive use of study intervention or AstraZeneca NIMP for a perceived reward or desired non-therapeutic effect.

Any events of drug abuse, with or without associated AEs, are to be captured and forwarded to the data entry site (DES) using the Drug Abuse Report Form. This form should be used both if the drug abuse happened in a study participant or if the drug abuse involves a person not enrolled in the study (such as a relative of the study participant).

Examples of drug abuse include but are not limited to:

- The drug is used with the intent of getting a perceived reward (by the study participant or a person not enrolled in the study).
- The drug in the form of a tablet is crushed and injected or snorted with the intent of getting high.

Drug Misuse

Drug misuse is the intentional and inappropriate use (by a study participant) of study intervention or AstraZeneca NIMP for medicinal purposes outside of the authorised product information, or for unauthorised study interventions or AstraZeneca NIMPs, outside the intended use as specified in the protocol and includes deliberate administration of the product by the wrong route.

Events of drug misuse, with or without associated AEs, are to be captured and forwarded to the DES using the Drug Misuse Report Form. This form should be used both if the drug misuse happened in a study participant or if the drug misuse regards a person not enrolled in the study (such as a relative of the study participant).

Examples of drug misuse include but are not limited to:

- The drug is used with the intention to cause an effect in another person.
- The drug is sold to other people for recreational purposes.
- The drug is used to facilitate assault in another person.
- The drug is deliberately administered by the wrong route.
- The drug is split in half because it is easier to swallow, when it is stated in the protocol that it must be swallowed whole.
- Only half the dose is taken because the study participant feels that he/she is feeling better when not taking the whole dose.

- Someone who is not enrolled in the study intentionally takes the drug.

Appendix C Handling of Human Biological Samples

C 1 Chain of Custody

A full chain of custody is maintained for all samples throughout their lifecycle.

The investigator at each study centre keeps full traceability of collected biological samples from the participants while in storage at the study centre until shipment or disposal (where appropriate) and records relevant processing information related to the samples while at the study centre.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps record of receipt of arrival and onward shipment or disposal.

AstraZeneca or delegated representatives will keep oversight of the entire life cycle through internal procedures, monitoring of study centres, auditing or process checks, and contractual requirements of external laboratory providers.

Samples retained for further use will be stored in the AstraZeneca-assigned biobanks or other sample archive facilities and will be tracked by the appropriate AstraZeneca team for the remainder of the sample life cycle.

All appropriately consented samples will be retained for maximum 15 years from last participant last visit. Samples collected from participants in China will be destroyed or repatriated within one year of CSR.

If required, AstraZeneca will ensure that remaining biological samples are returned to the study centre according to local regulations or at the end of the retention period, whichever is the sooner.

C 2 Withdrawal of Informed Consent for Donated Biological Samples

AstraZeneca ensures that biological samples are returned to the source or destroyed at the end of a specified period as described in the informed consent.

If a participant withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed/repatriated, and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

Following withdrawal of consent for biological samples, further study participation should be considered in relation to the withdrawal processes outlined in the informed consent.

The investigator:

- Ensures the participant's withdrawal of informed consent to the use of donated samples is highlighted immediately to AstraZeneca or delegate.
- Ensures that relevant human biological samples from that participant, if stored at the study centre, are immediately identified, disposed of as appropriate, and the action documented.
- Ensures that the participant and AstraZeneca are informed about the sample disposal.

AstraZeneca ensures the organisation(s) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of or repatriated as appropriate, and the action is documented, and study centre is notified.

C 3 International Air Transport Association Guidance Document 62nd Edition

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

The International Air Transport Association (IATA) (https://www.iata.org/whatwedo/cargo/dgr/Pages/download.aspx) classifies infectious substances into 3 categories: Category A, Category B, or Exempt

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.

Category A Pathogens are, eg, Ebola, Lassa fever virus. Infectious substances meeting these criteria which cause disease in humans or both in humans and animals must be assigned to UN 2814. Infectious substances which cause disease only in animals must be assigned to UN 2900.

Category B Infectious Substances are infectious substances that do not meet the criteria for inclusion in Category A. Category B pathogens are, eg, Hepatitis A, C, D, and E viruses. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B.
- Are to be packed in accordance with UN 3373 and IATA 650.

Exempt Substances are substances which do not contain infectious substances, or substances which are unlikely to cause disease in humans or animals, are not subject to these regulations unless they meet the criteria for inclusion in another class.

- Clinical study samples will fall into Category B or exempt under IATA regulations.
- Clinical study samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging (https://www.iata.org/contentassets/b08040a138dc4442a4f066e6fb99fe2a/dgr-62-en-pi650.pdf).
- Biological samples transported in dry-ice require additional dangerous goods specification for the dry-ice content.

Appendix D Optional Genomics Initiative Sample

D 1 Use/Analysis of Deoxyribonucleic Acid

- AstraZeneca intends to collect and store DNA for genetic research to explore how genetic variations may affect clinical parameters, risk and prognosis of diseases, better diagnosis of diseases or other improvements in health care and to the discovery of new diagnostics, treatments and or medications. Therefore, where local regulations and IRB/IEC allow, a blood sample will be collected for DNA analysis from consenting patients.
- This optional genetic research may consist of the analysis of the structure of the participant's DNA, ie, the entire genome.
- The results of genetic analyses may be reported in a separate study summary.
- AstraZeneca will store the DNA in a secure storage space with adequate measures to protect confidentiality.

D 2 Genetic Research Plan and Procedures

Selection of Genetic Research Population

All participants will be asked to participate in this genetic analysis. Participation is voluntary and if a participant declines to participate there will be no penalty or loss of benefit. The participant will not be excluded from any aspect of the main study.

Inclusion Criteria

For inclusion in this genetic research, participants must fulfil all of the inclusion criteria described in the main body of the protocol and: Provide informed consent for the Genomics Initiative sampling and analyses.

Exclusion Criteria

Exclusion from this genetic research may be for any of the exclusion criteria specified in the main study or any of the following:

- Previous allogeneic bone marrow transplant.
- Non-leukocyte depleted whole blood transfusion in 120 days of genetic sample collection.

Withdrawal of Consent for Genetic Research

- Participants may withdraw from this genetic research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary withdrawal will not prejudice further treatment. Procedures for withdrawal are outlined in Section 7.2 of the main protocol.

Collection of Samples for Genetic Research

- The blood sample for this genetic research will be obtained from the participants at Visit 3 at or after randomisation. Although DNA is stable, early sample collection is preferred to avoid introducing bias through excluding participants who may withdraw due to an AE. If for any reason the sample is not drawn at Visit 3, it may be taken at any visit until the last study visit. Only one sample should be collected per participant for genetics research during the study.

Coding and Storage of DNA Samples

- The processes adopted for the coding and storage of samples for genetic analysis are important to maintain participant confidentiality. Samples will be stored for a maximum of 15 years from the date of last participant last visit, after which they will be destroyed. DNA is a finite resource that will be used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.
- An additional second code will be assigned to the samples either before or at the time of sample processing, replacing the information on the sample tube. Thereafter, the sample will be identifiable only by the second, unique number. This number is used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated organisation. No personal details identifying the individual will be available to any person (AstraZeneca employee or designated organisations working with the DNA).
- The link between the participant enrolment/randomisation code and the second number will be maintained and stored in a secure environment, with restricted access at AstraZeneca or designated organisations. The link will be used to identify the relevant samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit, and permit tracing of samples for destruction in the case of withdrawal of consent.

Ethical and Regulatory Requirements

- The principles for ethical and regulatory requirements for the study, including this genetic research component, are outlined in Appendix A.

Informed Consent

- The genetic component of this study is optional and the participant may participate in other components of the main study without participating in this genetic component. To participate in the genetic component of the study the participant must sign and date both the consent form for the main study and the optional genetic research information ICF. Copies of both signed and dated consent forms must be given to the participant and the originals filed at the study centre. The principal investigator(s) is responsible for ensuring

that consent is given freely, and that the participant understands that they may freely withdraw from the genetic aspect of the study at any time.

Participant Data Protection

- AstraZeneca will not provide individual genotype results to participants, any insurance company, any employer, their family members, or general physician unless required to do so by law.
- Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the participant. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a participant. For example, in the case of a medical emergency, an AstraZeneca Physician or an investigator might know a participant's identity and also have access to his or her genomic data. Regulatory authorities may require access to the relevant files, though the participant's medical information and the genetic files would remain physically separate.

Data Management

- Any genetic data generated in this study will be stored at a secure system at AstraZeneca and/or designated organisations to analyse the samples.
- AstraZeneca and its designated organisations may share summary results (such as genetic differences from groups of individuals with a disease) from this genetic research with other researchers, such as hospitals, academic organisations, or drug- or health-related companies. This can be done by placing the results in scientific databases, where they can be combined with the results of similar studies to learn even more about health and disease. The researchers can only use this information for health-related research purposes. Researchers may see summary results, but they will not be able to see individual participant data or any personal identifiers.
- Some or all of the clinical datasets from the main study may be merged with the genetic data in a suitable secure environment separate from the clinical database.

Appendix E Actions Required in Cases of Increases in Liver Biochemistry and Evaluation of Hy's Law

E 1 Introduction

This appendix describes the process to be followed in order to identify and appropriately report potential Hy's Law cases and Hy's Law cases. It is not intended to be a comprehensive guide to the management of elevated liver biochemistries.

During the study, the investigator will remain vigilant for increases in liver biochemistry. The investigator is responsible for determining whether a participant meets potential Hy's Law criteria at any point during the study.

All sources of laboratory data are appropriate for the determination of potential Hy's Law and Hy's Law events; this includes samples taken at scheduled study visits and other visits including central and all local laboratory evaluations even if collected outside of the study visits; for example, potential Hy's Law criteria could be met by an elevated ALT from a central laboratory **and/or** elevated TBL from a local laboratory.

The investigator will also review AE data (for example, for AEs that may indicate elevations in liver biochemistry) for possible potential Hy's Law events.

The investigator participates, together with AstraZeneca clinical project representatives, in review and assessment of cases meeting potential Hy's Law criteria to agree whether Hy's Law criteria are met. Hy's Law criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than a drug-induced liver injury (DILI) caused by the study intervention.

The investigator is responsible for recording data pertaining to potential Hy's Law/Hy's Law cases and for reporting SAEs and AEs according to the outcome of the review and assessment in line with standard safety reporting processes.

E 2 Definitions

Potential Hy's Law

Aspartate aminotransferase or ALT ≥ 3 × ULN **together with** TBL ≥ 2 × ULN at any point during the study following the start of study medication irrespective of an increase in alkaline phosphatase (ALP).

Hy's Law

Aspartate aminotransferase or ALT ≥ 3 × ULN **together with** TBL ≥ 2 × ULN, where no other reason, other than the study intervention, can be found to explain the combination of increases, eg, elevated ALP indicating cholestasis, viral hepatitis, another drug.

For potential Hy's Law and Hy's Law the elevation in transaminases must precede or be coincident with (ie, on the same day) the elevation in TBL, but there is no specified timeframe within which the elevations in transaminases and TBL must occur.

E 3 Identification of Potential Hy's Law Cases

In order to identify cases of potential Hy's Law it is important to perform a comprehensive review of laboratory data for any participant who meets any of the following identification criteria in isolation or in combination:

- ALT ≥ 3 × ULN
- AST ≥ 3 × ULN
- TBL ≥ 2 × ULN

Central Laboratories Being Used:

When a participant meets any of the potential Hy's Law identification criteria, in isolation or in combination, the central laboratory will immediately send an alert to the investigator (also sent to AstraZeneca representative).

The investigator will also remain vigilant for any local laboratory reports where the potential Hy's Law identification criteria are met, where this is the case, the investigator will:

- Notify the AstraZeneca representative.
- Request a repeat of the test (new blood draw) by the central laboratory without delay.
- Complete the appropriate unscheduled laboratory eCRF module(s) with the original local laboratory test result.

When the identification criteria are met from central or local laboratory results the investigator will without delay:

- Determine whether the participant meets potential Hy's Law criteria (see Appendix E 2 for definition) by reviewing laboratory reports from all previous visits (including both central and local laboratory results).

E 4 Follow-up

E 4.1 Potential Hy's Law Criteria not met

If the participant does not meet potential Hy's Law criteria the investigator will:

- Inform the AstraZeneca representative that the participant has not met potential Hy's Law criteria.
- Perform follow-up on subsequent laboratory results according to the guidance provided in the protocol.

E 4.2 Potential Hy's Law Criteria met

If the participant does meet potential Hy's Law criteria the investigator will:

- Notify the AstraZeneca representative who will then inform the central Study Team.
- Within one day of potential Hy's Law criteria being met, the investigator will report the case as an SAE of Potential Hy's Law; serious criteria 'important medical event' and causality assessment 'yes/related' according to the protocol process for SAE reporting.
- For participants who met potential Hy's Law criteria prior to starting study intervention, the investigator is not required to submit a potential Hy's Law SAE unless there is a significant change[#] in the participant's condition.
- The Study Clinical Lead will contact the investigator, to provide guidance, discuss, and agree an approach for the study participant's follow-up (including any further laboratory testing) and the continuous review of data.
- Subsequent to this contact the investigator will:
  - Monitor the participant until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated. Complete follow-up SAE Form as required.
  - Investigate the aetiology of the event and perform diagnostic investigations as discussed with the Study Clinical Lead. This includes deciding which the tests available in the Hy's Law laboratory kit should be used.
  - Complete the 3 Liver eCRF Modules as information becomes available.

A **'significant' change** in the participant's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST, or TBL) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the investigator, this may be in consultation with the Study Clinical Lead if there is any uncertainty.

E 5 Review and Assessment of Potential Hy's Law Cases

The instructions in this appendix should be followed for all cases where potential Hy's Law criteria are met.

As soon as possible after the biochemistry abnormality is initially detected, the Study Clinical Lead will contact the investigator in order to review available data and agree on whether there is an alternative explanation for meeting potential Hy's Law criteria other than DILI caused by the study intervention, to ensure timely analysis and reporting to health authorities within 15 calendar days from date potential Hy's Law criteria was met. The AstraZeneca Global Clinical Lead or equivalent and Global Safety Physician will also be involved in this review together with other subject matter experts as appropriate.

According to the outcome of the review and assessment, the investigator will follow the instructions below.

Where there is an agreed alternative explanation for the ALT or AST and TBL elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for an SAE:

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate eCRF
- If the alternative explanation is an AE/SAE: Update the previously submitted Potential Hy's Law SAE and AE eCRFs accordingly with the new information (reassessing event term, causality and seriousness criteria) following the AstraZeneca standard processes.

If it is agreed that there is **no** explanation that would explain the ALT or AST and TBL elevations other than the study intervention:

- Send updated SAE (report term 'Hy's Law') according to AstraZeneca standard processes.
  - The 'Medically Important' serious criterion should be used if no other serious criteria apply.
  - As there is no alternative explanation for the Hy's Law case, a causality assessment of 'related' should be assigned.

If, there is an unavoidable delay, of over 15 calendar days in obtaining the information necessary to assess whether the case meets the criteria for Hy's Law, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Provide any further update to the previously submitted SAE of Potential Hy's Law, (report term now 'Hy's Law case') ensuring causality assessment is related to study intervention and seriousness criteria is medically important, according to protocol process for SAE reporting.
- Continue follow-up and review according to agreed plan. Once the necessary supplementary information is obtained, repeat the review and assessment to determine whether Hy's Law criteria are still met. Update the previously submitted potential Hy's Law SAE report following protocol process for SAE reporting, according to the outcome of the review and amending the reported term if an alternative explanation for the liver biochemistry elevations is determined.

E 6 Laboratory Tests

Hy's Law Laboratory Kit for Central Laboratories

Additional standard chemistry and coagulation tests	GGT
	LDH
	Prothrombin time
	INR
Viral hepatitis	IgM anti-HAV
	HBsAg IgM and IgG anti-HBc
	HBV DNA [a]
	IgG anti-HCV
	HCV RNA [b]
	IgM anti-HEV
	HEV RNA
Other viral infections	IgM & IgG [d] anti-CMV
	IgG anti-HSV, and HSV 1 /2 IgM testing or HSV PCR[d]
	IgM & IgG[d] anti-EBV
Alcoholic hepatitis	Carbohydrate deficient transferrin (CD-transferrin) [c]
Autoimmune hepatitis	Antinuclear antibody (ANA)
	Anti-Liver/Kidney Microsomal Ab (Anti-LKM)

	Anti-Smooth Muscle Ab (ASMA)
Metabolic diseases	alpha-1-antitrypsin
	Ceruloplasmin
	Iron
	Ferritin
	Transferrin [c]
	Transferrin saturation

[a] HBV DNA is only recommended when IgG anti-HBc is positive.
[b] HCV RNA is only recommended when IgG anti-HCV is positive or inconclusive.
[c] CD-transferrin and transferrin are not available in China.
[d] HSV 1 /2 IgM testing or HSV 1 /2 PCR testing depending on region.
Anti-HBc = total hepatitis B core antibody; anti-HCV = HCV antibody; CD = carbohydrate deficient; CMV = cytomegalovirus; DNA = deoxyribonucleic acid; EBV = Epstein-Barr virus; GGT = gamma-glutamyl transferase; HAV = hepatitis A virus; HBc = hepatitis B core antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HEV = hepatitis E virus; HSV = herpes simplex virus; IgG = immunoglobulin G; IgM = immunoglobulin M; INR = international normalised ratio; LDH = lactate dehydrogenase; RNA = ribonucleic acid.

E 7 References

Aithal et al, 2011
Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, Hunt CM, et al. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther. 2011; 89(6):806-15.

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Patient Reported Kansas City Cardiomyopathy Questionnaire under copyrights.

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Appendix H New York Heart Association Functional Classification

NYHA Functional Classification	
Class	Patient symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnoea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnoea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

Appendix I Detailed Description of Inclusion Criterion 2 and Criterion 3 According to ESC/ERS Guidelines

EXTRACT FROM:

2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension.

Marc Humbert, Gabor Kovacs, Marius M. Hoeper, Roberto Badagliacca, Rolf M.F. Berger, Margarita Brida, Jørn Carlsen, Andrew J.S. Coats, Pilar Escribano-Subias, Pisana Ferrari, Diogenes S. Ferreira, Hossein Ardeschir Ghofrani, George Giannakoulas, David G. Kiely, Eckhard Mayer, Gergely Meszaros, Blin Nagavci, Karen M. Olsson, Joanna Pepke-Zaba, Jennifer K. Quint, Göran Rådegran, Gerald Simonneau, Olivier Sitbon, Thomy Tonia, Mark Toshner, Jean-Luc Vachiery, Anton Vonk Noordegraaf, Marion Delcroix, Stephan Rosenkranz, the ESC/ERS Scientific Document Group. European Respiratory Journal 2022; DOI: 10.1183/13993003.00879-2022.

REFERENCE FOR INCLUSION CRITERION 2:

8.2.2. Evaluation of pulmonary hypertension and patient phenotyping

Patients with LHD and suspected PH should be evaluated following the diagnostic strategy for PH (see Section 5). This requires identifying clinical features and a multimodal approach using non-invasive diagnostic tests such as echocardiography, ECG, and BNP/NT-proBNP levels. In the presence of mild PH and predominant LHD, no further testing may be necessary. Otherwise, CTEPH and significant lung disease should be ruled out by V/Q scan and PFTs, and additional cardiac imaging including cMRI may be considered in selected cases. For phenotyping, a combination of variables may help to determine the likelihood of LHD, and HFpEF in particular, versus other causes of PH (Table 23). Pulmonary hypertension associated with left heart disease is likely in the presence of known cardiac disease, multiple cardiovascular comorbidities/risk factors, atrial fibrillation at diagnosis, and specific imaging findings (LV hypertrophy, increased LA size, and reduced LA strain). Although exercise echocardiography has been proposed to uncover HFpEF, it is unable to diagnose or classify PH in this context. A combination of clinical findings and phenotyping is required to decide about the need for further invasive assessment.

TABLE 23: Patient phenotyping and likelihood for left heart disease as cause of pulmonary hypertension

Feature	PH-LHD unlikely	Intermediate probability	PH-LHD likely
Age	< 60 years	60–70 years	> 70 years
Obesity, hypertension, dyslipidaemia, glucose intolerance/diabetes	No factors	1–2 factors	> 2 factors
Presence of known LHD	No	Yes	Yes
Previous cardiac intervention	No	No	Yes
Atrial fibrillation	No	Paroxysmal	Permanent/persistent
Structural LHD	No	No	Present
ECG	Normal or signs of RV strain	Mild LVH	LBBB or LVH
Echocardiography	No LA dilation E/e′ < 13	No LA dilation Grade < 2 mitral flow	LA dilation (LAVI > 34 mL/m^2) LVH Grade > 2 mitral flow
CPET	High VE/VCO2 slope No EOV	Elevated VE/VCO2 slope EOV	Mildly elevated VE/VCO2 slope EOV
cMRI	No left-heart abnormalities		LVH LA dilation (strain or LA/RA > 1)

cMRI, cardiac magnetic resonance imaging; CPET, cardiopulmonary exercise testing; E/e′, ratio between early mitral inflow velocity and mitral annular early diastolic velocity; ECG, electrocardiogram; EOV, exercise oscillatory ventilation; LA, left atrial; LAVI, left atrial volume index; LBBB, left bundle branch block; LHD, left heart disease; LVH, left ventricular hypertrophy; PH, pulmonary hypertension; PH-LHD, left heart disease associated with pulmonary hypertension; RA, right atrium; RV, right ventricle; VE/VECO2, ventilatory equivalents for carbon dioxide. Assigning the likelihood of LHD as a cause of PH. This assessment may help to decide which patients should undergo a full work-up, including invasive haemodynamic assessment (see Figure 11 and Figure S2).

REFERENCE FOR INCLUSION CRITERION 3: PROGRESSION TO SCREENING VISIT 2, ECHOCARDIOGRAPHIC PARAMETERS:

5. Pulmonary hypertension diagnosis

5.1. Diagnosis

The diagnostic approach to PH is mainly focused on two tasks. The primary goal is to raise early suspicion of PH and ensure fast-track referral to PH centres in patients with a high likelihood of PAH, CTEPH, or other forms of severe PH. The second objective is to identify underlying diseases, especially LHD (group 2 PH) and lung disease (group 3 PH), as well as comorbidities, to ensure proper classification, risk assessment, and treatment.

5.1.5. Echocardiography

Independent of the underlying aetiology, PH leads to RV pressure overload and dysfunction, which can be detected by echocardiography [82–84]. When performed accurately, echocardiography provides comprehensive information on right and left heart morphology, RV and LV function, and valvular abnormalities, and gives estimates of haemodynamic parameters. Echocardiography is also a valuable tool with which to detect the cause of suspected or confirmed PH, particularly with respect to PH associated with LHD or CHD. Yet, echocardiography alone is insufficient to confirm a diagnosis of PH, which requires RHC.

Given the heterogeneous nature of PH and the peculiar geometry of the RV, there is no single echocardiographic parameter that reliably informs about PH status and underlying aetiology. Therefore, a comprehensive echocardiographic evaluation for suspected PH includes estimating the systolic pulmonary arterial pressure (sPAP) and detecting additional signs suggestive of PH, aiming at assigning an echocardiographic level of probability of PH. Echocardiographic findings of PH, including estimating pressure and signs of RV overload and/or dysfunction, are summarized in Figure 4.

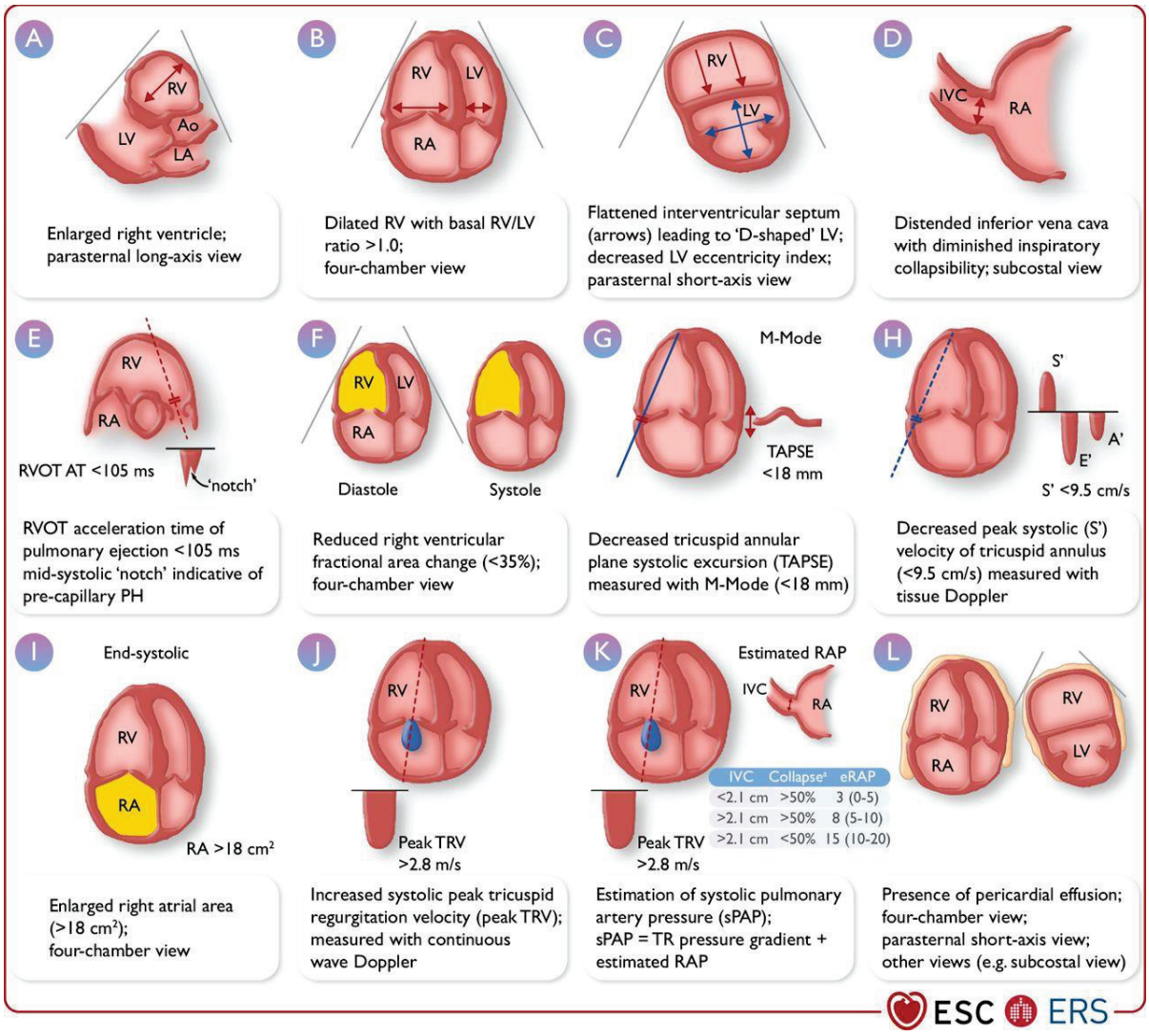


FIGURE 4: Transthoracic echocardiographic parameters in the assessment of pulmonary hypertension.

Ao, aorta; IVC, inferior vena cava; LA, left atrium; LV, left ventricle; PH, pulmonary hypertension; RA, right atrium; RAP, right atrial pressure; RV, right ventricle; RVOT AT, right ventricular outflow tract acceleration time; sPAP, systolic pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation; TRV, tricuspid regurgitation velocity.
[a] Refers to collapse on inspiration.

Estimates of sPAP are based on the peak tricuspid regurgitation velocity (TRV) and the TRV-derived tricuspid regurgitation pressure gradient (TRPG)—after excluding pulmonary stenosis—taking into account non-invasive estimates of RA pressure (RAP). Considering the inaccuracies in estimating RAP and the amplification of measurement errors by using derived variables [85–87], these guidelines recommend using the peak TRV (and not the estimated sPAP) as the key variable for assigning the echocardiographic probability of PH. A peak TRV > 2.8 m/s may suggest PH; however, the presence or absence of PH cannot be reliably

determined by TRV alone [88]. Lowering the TRV threshold in view of the revised haemodynamic definition of PH is not supported by available data (key narrative question 2, Supplementary Data, Section 5.1) [89–92]. Tricuspid regurgitation (TR) velocity may underestimate (e.g. in patients with severe TR) [28] or overestimate (e.g. in patients with high CO in liver disease or sickle cell disease [SCD] [93, 94], misinterpretation of tricuspid valve closure artefact for the TR jet, or incorrect assignment of a peak TRV in the case of maximum velocity boundary artefacts) pressure gradients. Hence, additional variables related to RV morphology and function are used to define the echocardiographic probability of PH (Table 10) [82–84, 95], which may then be determined as low, intermediate, or high. When interpreted in a clinical context, this probability can be used to decide the need for further investigation, including cardiac catheterization in individual patients (Figure 5).

TABLE 10: Additional echocardiographic signs suggestive of pulmonary hypertension [a]

A: The ventricles	**B: Pulmonary artery**	**C: Inferior vena cava and RA**
RV/LV basal diameter/area ratio > 1.0	RVOT AT < 105 ms and/or mid-systolic notching	IVC diameter > 21 mm with decreased inspiratory collapse (< 50% with a sniff or < 20% with quiet inspiration)
Flattening of the interventricular septum (LVEI > 1.1 in systole and/or diastole)	Early diastolic pulmonary regurgitation velocity > 2.2 m/s	RA area (end-systole) > 18 cm^2
TAPSE/sPAP ratio < 0.55 mm/mmHg	PA diameter >AR diameter PA diameter > 25 mm	

AR, aortic root; IVC, inferior vena cava; LV, left ventricle; LVEI, left ventricle eccentricity index; PA, pulmonary artery; RA, right atrium; RV, right ventricle; RVOT AT, right ventricular outflow tract acceleration time; sPAP, systolic pulmonary arterial pressure; TAPSE, tricuspid annular plane systolic excursion; TRV, tricuspid regurgitation velocity.

[a] Signs contributing to assessing the probability of PH in addition to TRV (see Figure 5).

Signs from at least two categories (A/B/C) must be present to alter the level of echocardiographic probability of PH.

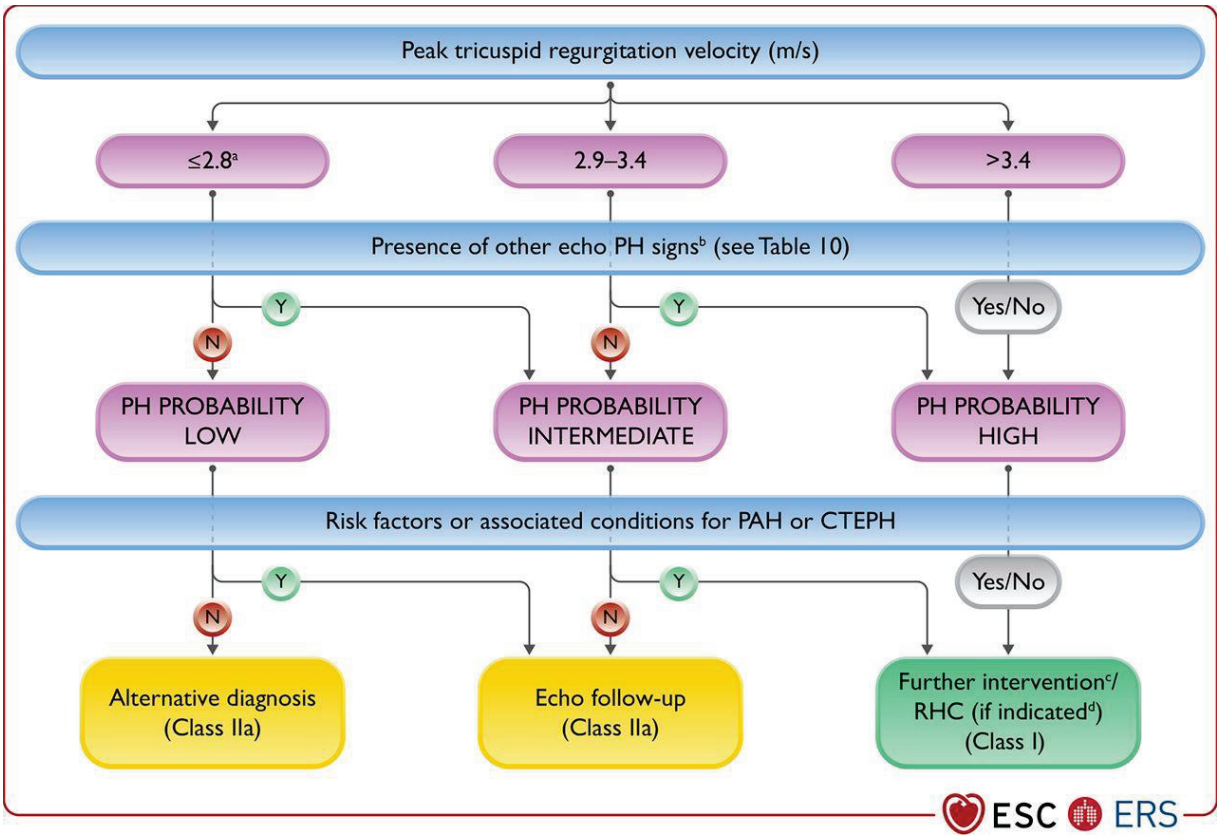


FIGURE 5: Echocardiographic probability of pulmonary hypertension and recommendations for further assessment

CPET, cardiopulmonary exercise testing; CTEPH, chronic thrombo-embolic pulmonary hypertension; echo, echocardiography; LHD, left heart disease; N, no; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; RHC, right heart catheterization; TRV, tricuspid regurgitation velocity; Y, yes.

[a] Or unmeasurable. The TRV threshold of 2.8 m/s was not changed according to the updated haemodynamic definition of PH.

[b] Signs from at least two categories in Table 10 (A/B/C) must be present to alter the level of echocardiographic probability of PH.

[c] Further testing may be necessary (e.g. imaging, CPET).

[d] RHC should be performed if useful information/a therapeutic consequence is anticipated (e.g. suspected PAH or CTEPH), and may not be indicated in patients without risk factors or associated conditions for PAH or CTEPH (e.g. when mild PH and predominant LHD or lung disease are present).

Echocardiographic measures of RV function include the tricuspid annular plane systolic excursion (TAPSE), RV fractional area change (RV-FAC), RV free-wall strain, and tricuspid annulus velocity (S′ wave) derived from tissue Doppler imaging, and potentially RV ejection fraction (RVEF) derived from 3D echocardiography. Furthermore, the TAPSE/sPAP ratio-representing a non-invasive measure of RV-PA coupling [96] - may aid in diagnosing PH [90, 97, 98]. The pattern of RV outflow tract (RVOT) blood flow (mid-systolic ‘notching’) may suggest pre-capillary PH [99, 100].

To separate between group 2 PH and other forms of PH, and to assess the likelihood of left ventricle (LV) diastolic dysfunction, LA size and signs of LV hypertrophy should always be measured, and Doppler echocardiographic signs (e.g. E/A ratio, E/E′) should be assessed even if the reliability of the latter is considered low [16]. To identify CHD, 2D Doppler and contrast examinations are helpful, but transoesophageal contrast echocardiography or other imaging techniques (e.g. computer tomography [CT] angiography, cMRI) are needed in some cases to detect or exclude sinus venosus atrial septal defects, patent ductus arteriosus, and/or anomalous pulmonary venous return [101]. The clinical value of exercise Doppler echocardiography in identifying exercise PH remains uncertain because of the lack of validated criteria and prospective confirmatory data. In most cases, increases in sPAP during exercise are caused by diastolic LV dysfunction [16].

Appendix J Considerations Related to COVID-19

J 1 Risks

Through its mechanism of action, AZD3427 is believed not to impact the immune system or cause immune suppression.

Therefore, the risk of the participants to be exposed to SARS-CoV-2 or to suffer from coronavirus disease 2019 (COVID-19) will be similar to the general population. However, the risk of exposure to infected people cannot be completely excluded as the participants might need to expose themselves to public areas (eg, commute to the study centre) and have additional human contact (eg, with study centre staff and other participants of the clinical study).

Measures to mitigate the additional risks caused by COVID-19 are:

- Current national laws and local recommendations for prevention of pandemic will be strictly adhered to.
- This study is going to start enrolling only when the Sponsor and CRO in collaboration deem it is safe to start the study. In addition, the study will not start until the local confinement measures or other safety restrictions linked to COVID-19 are compatible with study start-up.
- Participants will be closely monitored for any signs and symptoms of COVID-19, including fever, dry cough, dyspnoea, sore throat, and fatigue throughout the study. Once clinical signs of infection are reported by participants, the investigator needs to determine whether samples can be collected, and safety data can be recorded on-site. If not, AEs, and concomitant medications will be obtained via phone calls.
- Confirmation of COVID-19 infection by optional laboratory assessment will be conducted based on availability (test capacity and turnaround time) of approved tests and on investigator's discretion.
- The probability of virus transmission will be controlled as much as possible by:
  - Advice for participant to adhere to local requirements for reduction of the public exposure while ambulatory.
  - Physical distancing and person-to-person contact restrictions according to local recommendations will be applied during visits.
  - Where physical distancing is not possible personal protective equipment will be used by participants (surgical face mask, gloves) and staff (for example, but not limited to masks, gloves, protectors, medical suits) if deemed appropriate by the investigator and study centre staff and guided by local requirements.

- Logistical improvements of the study centre and structural measures of the study centre building will be implemented to further improve physical distancing.

J 2 Data Quality Assurance Related to COVID-19

Monitoring visits at study centres will be limited to a minimum required as deemed appropriate during COVID-19 pandemic, per local regulations.

J 3 Vaccines

For AstraZeneca, the safety and well-being of study participants are of primary importance, including vaccination.

Inactive non-replicating COVID-19 vaccines have been approved across the globe as emergency use products. AstraZeneca has no data on the co-administration of AZD3427 and respective COVID-19 vaccines. Potential AZD3427-vaccine interactions affecting participant safety or study intervention and vaccine efficacy are therefore unclear. In addition, vaccine-related AEs may confound the AE profile of the study intervention.

COVID-19 vaccination of clinical study participants should take into consideration current local guidelines, vaccine availability, and benefit-risk assessment. Ultimately, the decision to vaccinate will be based on the judgement of the treating physician, taking into account the participant's best interest.

Participants enrolled in studies with AZD3427 can be vaccinated against COVID-19, particularly because many participants belong to one of the risk groups targeted for vaccination.

J 3.1 Considerations to Facilitate COVID-19 Vaccination in Clinical Studies

COVID-19 vaccination may be administered prior to or after dosing with AZD3427, or as a concomitant medication during the clinical studies.

J 3.1.1 COVID-19 Vaccination Prior to Entering Clinical Study or Randomisation

For study participants with flexibility as to when to start the AZD3427 AstraZeneca-sponsored study and for whom COVID-19 vaccination is planned, vaccination (all doses) prior to first study intervention dose may be advisable. If possible, the first dose of AZD3427 should be given at least 14 days after the last dose of vaccine.

J 3.1.2 COVID-19 Vaccination During the Ongoing Study (Dosing Period)

For study participants already receiving treatment in AstraZeneca-sponsored studies when COVID-19 vaccines become available, COVID-19 vaccination should be acceptable given the benefit-risk assessment does not indicate otherwise.

To facilitate understanding if a potential reaction is caused by administration of a vaccine or by AZD3427, consider not administering both study intervention and vaccine injections on the same day (consider a time window of ± 48 hours). Deviation from this time window should be discussed on a case-by-case basis.

J 3.1.3 End-of-study

For study participants who are approaching the end of the study, COVID-19 vaccination should be delayed until after the last dose of study intervention is given, if possible.

J 3.2 Adverse Event Monitoring

Vaccinated study participants should be monitored according to local guidelines and AZD3427 study protocol. If an AEs occurs and are in proximity to a COVID-19 vaccination, the investigator should adhere to the standard practice and judge whether there is a reasonable possibility that the AE is considered related to AZD3427 or not.

Appendix K Protocol Version History

The Summary of Changes Table for the current revision is located directly before the Table of Contents.

CSP Version 3.0

This modification is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and in the EU Clinical Trial Regulation Article 2, 2 (13).

Overall Rationale for the Modification:

This amendment has been made to include in the global protocol the recommendations received during the EU-CTR procedure, and therefore this CSP Version 3.0 includes the changes incorporated in EU Region 1 amendment 04 July 2023. This CSP Version 3.0 has also been made to include recommendations received from the FDA, and in addition, clarifications and corrections to some inconsistencies have been made.

Summary of Changes:

List of Substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Section 5.1 Inclusion Criteria	Inclusion criterion number 5 was updated to change the minimum body weight from 50 kg to 45 kg (inclusive).	Updated to assist with recruitment among small body weight, elderly, and Asian populations.
Section 5.2 Exclusion Criteria	Exclusion criterion number 7 was updated to remove cervical carcinoma in situ from the sentence on exceptions and also added wording that for participants who are under investigation for breast or cervical cancer, including participants with a pap smear of ≥ 3, and to require that all investigations must be resolved as negative for breast and cervical cancer at least 12 weeks before Screening.	Text updated in line with recommendation from FDA, as a precaution, based on non-clinical information from other programs available to the Agency. This change is not related to data from AZD3427 studies.

List of Non-substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Title Page	Local Sponsor: AstraZeneca K.K Japan added as Japan is one of the countries the study is conducted in.	Correction.
List of Abbreviations and Definitions of Terms	Corrected the definition of TNF-α = Tumour necrosis factor alpha	Clarification.
Section 1.1 Synopsis and Section 3.2 Objectives and Endpoints	The endpoint for the secondary objective 'To evaluate the effect of AZD3427 on additional hemodynamic markers of cardiac function after 24 weeks of treatment in participants with HF and PH Group 2' was corrected to remove RVS/PASP, IVC diameter with inspiratory collapse from the echocardiographic parameters.	Correction
Section 1.3 Schedule of Activities	Table 1 Schedule of Activities Footnote I was updated to clarify that the pre-dose vital signs will be measured before any blood draws, at all visits.	Clarification
Section 1.3 Schedule of Activities	Table 1 Schedule of Activities Footnote J related to Visit 6, was updated to clarify that in addition to full blood count, urinalysis is performed. This is in the Table 1 but made clearer in the related footnote.	Clarification
Section 1.3 Schedule of Activities	Table 1 Schedule of Activities New footnote 'n' was added to clarify that at the Early Termination Visit, RHC only needs to be performed if participant has been randomized at least 12 weeks prior to the Early Termination Visit.	Clarification
Section 3.2 Objectives and Endpoints	Table 3 Objectives and Endpoints A footnote was added to the exploratory objective 'To evaluate the effect of AZD3427 on the plasma proteome, and urine, serum and/or plasma biomarkers of inflammation, fibrosis, RAAS, tissue remodelling, fluid/electrolyte balance, and RXFP1 signalling after 12 and 24 weeks of treatment in participants with HF and PH Group 2' To clarify that the results of each of the exploratory biomarker analyses may or may not be included in the CSR of this study.	Clarification
Section 3.2 Objectives and Endpoints	Table 3 Objectives and Endpoints The exploratory endpoint 'Change in plasma and/or serum biomarkers from baseline to Week 13 and Week 25 compared with placebo' Bullet containing plasma osmolality corrected to serum osmolality.	Correction

Section Number and Name	Description of Change	Brief Rationale
Section 3.2 Objectives and Endpoints	Table 3 Objectives and Endpoints The following endpoint was added to the exploratory objective 'To evaluate the effect of AZD3427 on clinical outcomes' Change in KCCQ CSS and OSS from baseline to Week 25 compared with placebo.	Clarification
Section 5.1 Inclusion Criteria and Appendix I	Inclusion criterion number 2 and the header of Appendix I was updated to correct a typographical error, 'ESR was changed to ERS'.	Correction
Section 5.2 Exclusion Criteria	Exclusion criterion number 2b was updated: Added complex congenital heart disease. Removed 'History of' in the sentence 'History of greater than moderate mitral or aortic regurgitation…' Clarified 'aortic valve regurgitation' rather than 'aortic regurgitation valvular disease'.	Clarification
Section 5.2 Exclusion Criteria	Exclusion criterion number 3 was updated: Clarified 'hospitalisation due to decompensated HF' rather than 'any hospitalisation'.	Clarification
Section 5.2 Exclusion Criteria	Exclusion criterion number 9 was updated to remove 'VC' the exclusion criterion only applies to FEV_1 and added 'of predicted' after %..	Correction
Section 6.1.1 Investigational Products, Table 4, Study Interventions	Text clarified for Placebo to remove wording regarding corresponding and add controlled for placebo.	Correction.
Section 7.1 Discontinuation of Study Intervention	Clarified that dosing for any participant must be stopped if the participant experiences worsening of HF that requires use of continuous infusion of IV diuretics, inotropes, **or** vasodilators ('or' vasodilators rather than 'and' vasodilators, and replaced by or).	Clarification
Section 7.1 Discontinuation of Study Intervention	Text was added to clarify that depending on the clinical situation and the likely underlying causes, the investigator will decide whether to discontinue the study intervention temporarily or permanently.	Clarification
Section 8.2.1 Right Heart Catheterisation	Text was updated to clarify that at the Early Termination visit RHC only needs to be performed if the patients have been randomized at least 12 weeks prior to the Early Termination Visit. And to state that right heart catheterisation results **will be** sent to a central core laboratory for exploratory analysis(will be sent rather than may be sent, 'may' replaced by 'will').	Clarification

Section Number and Name	Description of Change	Brief Rationale
Section 8.3.2 Vital Signs	Text was updated to clarify that vital signs will be measured before any blood draws scheduled at the same timepoint (eg, pre-dose).	Clarification
Section 8.3.4 Clinical Safety Laboratory Tests	Table 5 Laboratory Safety Variables was updated to clarify urinalysis by dipstick. Line corrected to show urine drug screen will only be at screening.	Clarification
Section 8.4 Adverse Events, Serious Adverse Events, and Other Safety Reporting	The variables collected for AEs was updated for the third bullet point to remove changes in intensity, only maximum intensity will be collected.	Clarification
Section 8.5 Pharmacokinetics	Text was updated to remove 'unless consented to future analyses'. Pharmacokinetics samples will be disposed of after the Bioanalytical Report finalisation or 6 months after issuance of the draft Bioanalytical Report (whichever is earlier).	Clarification
Section 8.6.1 Mandatory Biomarker Sample Collection	Text was updated to clarify that the results of exploratory biomarker analyses may or may not be included in the CSR of this study.	Clarification
Section 8.7 Immunogenicity Assessments	Reference to 'treatment-emergent' was removed from the sentence 'Participants with treatment emergent ADA-positive…' Follow-up will occur for all participants with ADA-positive titres not just those that are treatment emergent. Text added on reporting of data from additional samples.	Clarification
Section 8.9 Optional Genomics Initiative and Appendix D Optional Genomics Initiative Sample	Text was updated to remove references to multi omics research, as multi omics are not being done.	Clarification
Appendix A Regulatory, Ethical, and Study Oversight Considerations	Appendix A 4 Data Protection, was updated to include text related to personal data breaches. Appendix A 6 Dissemination of Clinical Study Data, was updated to change the website from to http://astrazenecagrouptrials.pharmacm.com to www.astrazenecaclinicaltrials.com and to add and https://euclinicaltrials.eu/	Change in standard template text
Appendix J Considerations Related to COVID-19	The numbering of the sub-headings in Appendix J was corrected	Correction

Section Number and Name	Description of Change	Brief Rationale
The changes below were incorporated in the EU Region 1 amendment 04 July 2023 and have now been included in this global protocol version 3.0. These changes include the recommendations received during the EU-CTR procedure.		
List of Abbreviations and Definitions of Terms	Added definition of the following abbreviations: GLP = Good Laboratory Practice	Clarification.
Section 1 Protocol Summary, Section 6.6 Unblinding for Unblinded Review Committee, Section 9.6 Unblinded Review Committee, Appendix A 5 Committee Structure	The description of the Unblinded Review Committee was updated to clarify that the committee will be independent.	Clarification.
Section 1.3 Schedule of Activities	Footnote "l" was added to provide clarification on repeat blood sampling in the event of positive ADA-positive results at the end-of-study.	Clarification.
Section 2.3.1 Risk Assessment	Table 2 was updated to include the details provided in the IB.	Clarification.
Section 5.1 Inclusion Criteria	Inclusion Criterion number 8 was updated to clarify that participants must be capable and **willing** to provide informed consent. Addition of the word 'willing'.	Clarification.
Section 5.1 Inclusion Criteria	Incorrect numbering of inclusion criterion number 23 was corrected. Inclusion number 23 was previously missing numbering was 22 and then 24.	Correction at the time of amendment not due to any recommendation by EU-CTR procedure.
Section 6.11 Prior and Concomitant Therapy	Added information regarding prohibited medication	Clarification
Section 7.1 Discontinuation of Study Intervention	Clarified that dosing for any participant must be stopped if the participant experiences an SAE that is assessed as possibly related to the study intervention by the Investigator.	Clarification
Appendix A 6 Dissemination of Clinical Study Data	Clarified that the content of the summaries will align to requirements set out in the EU Clinical Trials Regulation 536/2014 Annex IV and Annex V.	Clarification

Version 2.0, 01 December 2022

This modification is considered to be non-substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and in the EU Clinical Trial Regulation Article 2, 2 (13).

Summary of Changes:

List of Non-Substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Title page and headers for all pages	Study intervention code corrected on title page and in headers of all pages	Typing error corrected

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