Bial - Portela & C[a], S.A

CLINICAL STUDY PROTOCOL

An open-label, multicentre study to evaluate pharmacokinetics, safety and efficacy of zamicastat as adjunctive therapy in pulmonary arterial hypertension (PAH)

Protocol Short Title	Pharmacokinetics, safety and efficacy of BIA 5-1058 in PAH
Protocol Number	BIA-51058-201
EudraCT Number	2018-002448-10
Phase	II
Version	Final Version 3.0
Date	11-MAY-2020
Product Name	Zamicastat (BIA 5-1058)
Indication	Pulmonary arterial hypertension
Sponsor	Bial - Portela & C[a], S.A. À Av. da Siderurgia Nacional 4745-457 Coronado (S. Romão e S. Mamede), Portugal Phone: +351 229866100 Fax: +351 229866192 http://www.bial.com
24/7 Medical Contact	SCOPE International Medical Monitoring Phone: +370 52 360 336

Protocol Version History

This protocol, Final version 3.0, 11-MAY-2020 supersedes the following protocol versions:

Protocol version	Countries
Final Version 2.0, 18-JUN-2019	Austria, Italy, Portugal, Spain, Ukraine
Final Version 2.0_UK, 08-JUL-2019	United Kingdom
Final Version 2.0_DE, 23-SEP-2019	Germany

Summary of Changes from Final Protocol Version 2.0, 18-JUN-2019

This protocol Final version 3.0 was prepared to increase the age limit provided in inclusion criterion no. 1, to potentially reduce the number of right heart catheterisations (RHCs) and to implement minor updates.

Section*	Change
5	The drug safety manager changed.
6.2	This section was updated according to Investigator's Brochure edition no 6.2.
9.1, 9.2	Inclusion criterion no. 1 was amended to increase the age limit from 65 to 75 years, inclusive.
9.2, 11.4.4, 11.6.2	Right heart catheterisation results which were measured at the study site from within 90 days before V1 will be accepted as baseline value (new inclusion criterion no. 4).
9.2	Inclusion criterion 7 was introduced; women must agree not to donate ova from the time of informed consent until 30 days after the last IMP intake und men must agree not to donate sperm from the time of informed consent until 90 days after the last IMP intake.
9.3	Relevant previous spirometry data must be available in the medical history for checking exclusion criteria no. 6 and 7.
9.3	Exclusion criterion no 13 was updated; women of childbearing potential have to use highly effective contraceptive measures in combination with a barrier method.

Please note that minor clarifications and corrections of typing errors are not listed above.
*In addition, all changes have also been implemented in the protocol synopsis (Section 2).

1. SIGNATURE PAGES

1.1 Sponsor Signatures

PROTOCOL TITLE: An open-label, multicentre study to evaluate pharmacokinetics, safety and efficacy of zamicastat as adjunctive therapy in pulmonary arterial hypertension (PAH)

PROTOCOL NUMBER: BIA-51058-201

PROTOCOL VERSION: Final Version 3.0

PROTOCOL DATE: 11-MAY-2020

Protocol approved by:

SIGNATURE: DATE:

Patrício Soares-da-Silva, MD, PhD

Research and Development General Manager
Research & Development Area
Bial - Portela & C[a], S.A.
À Av. da Siderurgia Nacional
4745-457 Coronado (S. Romão e S. Mamede), Portugal
Phone: +351 229866100
Fax: +351 229866192
psoares.silva@bial.com

1.2 Coordinating Investigator's Declaration

PROTOCOL TITLE: An open-label, multicentre study to evaluate pharmacokinetics, safety and efficacy of zamicastat as adjunctive therapy in pulmonary arterial hypertension (PAH)

PROTOCOL NUMBER: BIA-51058-201

PROTOCOL VERSION: Final Version 3.0

PROTOCOL DATE: 11-MAY-2020

I have read and understand the protocol and agree to conduct the study in compliance with this protocol, Good Clinical Practice, designated Standard Operating Procedures, applicable regulatory requirements and within the principles of the Declaration of Helsinki (adopted Version, Fortaleza, Brazil, 2013).

SIGNATURE: DATE:

Prof. Dr. med. Marius Hoeper

Medizinische Hochschule Hannover
Klinik für Pneumologie
Carl-Neuberg-Straße 1
30623 Hannover, Germany

1.3 Principal Investigator's Declaration

PROTOCOL TITLE: An open-label, multicentre study to evaluate pharmacokinetics, safety and efficacy of zamicastat as adjunctive therapy in pulmonary arterial hypertension (PAH)

PROTOCOL NUMBER: BIA-51058-201

PROTOCOL VERSION: Final Version 3.0

PROTOCOL DATE: 11-MAY-2020

I have read and understand the protocol and agree to conduct the clinical study in compliance with this protocol, Good Clinical Practice, designated Standard Operating Procedures, applicable regulatory requirements and within the principles of the Declaration of Helsinki (adopted Version, Fortaleza, Brazil, 2013).

SIGNATURE: DATE:

Print principal investigator name and address

2. PROTOCOL SYNOPSIS

Name of sponsor/company: Bial - Portela & C[a], S.A., À Av. da Siderurgia Nacional, 4745-457 Coronado (S. Romão e S. Mamede), Portugal

Name of active ingredient: Zamicastat (BIA 5-1058)

Title of study: An open-label, multicentre study to evaluate pharmacokinetics, safety and efficacy of zamicastat as adjunctive therapy in pulmonary arterial hypertension (PAH)

Study number: BIA-51058-201

EudraCT number: 2018-002448-10

Coordinating investigator: Prof. Dr. med. Marius Hoeper, Medizinische Hochschule Hannover, Klinik für Pneumologie, Carl-Neuberg-Straße 1, 30623 Hannover, Germany

Study centre(s): Approximately 15 sites in 9 countries. Other sites and countries may be added as needed.

Planned duration of the study:	**Phase of development:** II
First patient first visit: May 2019 Last patient last visit: Nov 2020	

Objectives:

Primary:

To evaluate pharmacokinetic (PK) profile of different zamicastat doses in PAH patients to find the most promising therapeutic dosage range for the treatment of PAH disease

Secondary:

1. To assess further PK parameters including apparent total clearance, terminal half-life and apparent volume of distribution
2. To assess the safety and tolerability of different zamicastat doses
3. To investigate the effect of different zamicastat doses on plasma dopamine β hydroxylase (DβH) activity
4. To investigate the effect of different zamicastat doses on urinary catecholamine levels (norepinephrine and dopamine)
5. To investigate the change in pulmonary haemodynamic parameters
6. To investigate the change in the 6-minute walk test (6-MWT)
7. To investigate the change in biomarkers
8. To investigate the change in cardiac structure and contractibility, assessed by echocardiography
9. To investigate the change in quality of life, assessed by SF-36v2

Methods / study design:

This is an open-label, multi-centre study in patients with PAH who are currently on stable treatment with at least one PAH medication. It is planned to evaluate the PK profile (24-hour profile and trough levels) and the safety, tolerability and efficacy of four different zamicastat doses. Each patient will start treatment with the lowest dose (50 mg zamicastat once daily) and the dose will be up-titrated to the individual highest tolerated dose (HTD) i.e. up to 200 mg zamicastat once daily.

A data safety monitoring board (DSMB) will periodically review the safety data and will issue a recommendation if the doses can be used as planned.

This study will consist of:

- A screening period, 5 to 12 days: visit V1
- Up to four dose finding periods, 14 days each:
  - Dose A: visits A1, A2 and A3
  - Dose B: visits B2 and B3
  - Dose C: visits C2 and C3
  - Dose D: visits D2 and D3
- Maintenance period, 42 days: visits MPV1, MPV2 and MPV3
- Follow-up period, 14 to 28 days: visits FU (down-titration) and FU

After confirmed eligibility, patients will initiate treatment with Dose A (50 mg zamicastat once daily) at visit A1 and will be treated with this dose level for 14 days. If Dose A was considered safe by the investigator, it will be up-titrated to Dose B (100 mg zamicastat once daily) at visit A3 and the patient will continue with this dosage for 14 days. Patients not tolerating Dose A have to be withdrawn from the study.

The up-titration is based on safety criteria, including but not limited to:

- Absence of symptomatic and/or clinically relevant orthostatic hypotension
- Systolic blood pressure (SBP) > 95 mmHg or diastolic blood pressure (DBP) > 50 mmHg
- Absence of clinically significant changes in electrocardiogram (ECG) parameters (heart rate, P-R interval, QRS duration, QT and QTc interval)
- Absence of clinically significant hypersensitivity/skin allergic reactions

After a positive safety evaluation at visit B3, the dose will be up-titrated to Dose C (150 mg zamicastat once daily) and the patient will continue on this dose level for a further 14 days. If Dose C was tolerated by the patient, it will be up-titrated at visit C3 to Dose D (200 mg zamicastat once daily) where the patient will remain for 56 days (14 days dose finding period + 42 days maintenance period).

Patients not tolerating their next dose at any time during the 14-days dose finding period will come immediately to the site or call the investigator (unscheduled visit), unless intolerance was detected during a scheduled visit and the dose will be down-titrated to the patient's previous tolerated dose. Then they will continue this treatment for 42 days during the maintenance period.

The dose which will be taken during the maintenance period will be considered as an individual HTD.

At the end of the maintenance period, the patient will have the opportunity to continue treatment in an extension study (BIA-51058-202), which will be described in a separate protocol.

Patients not tolerating the individual HTD during the maintenance period have to be withdrawn from the study. An overview of the study design is provided in Figure 1.

Number of patients (planned):
It is planned to allocate approximately 32 patients to treatment to meet the objectives of the study.

Diagnosis and main criteria for inclusion and exclusion:

Diagnosis: Pulmonary arterial hypertension (PAH)

Inclusion criteria:

For inclusion in the study, patients must fulfil all of the following criteria:

1. Male or female patients aged 18 to 75 years, inclusive.
2. Able to comprehend and willing to sign an informed consent form.
3. Diagnosis of PAH (pulmonary arterial hypertension WHO Group 1), documented by right heart catheterisation with a mean pulmonary artery pressure (mPAP) ≥ 25 mmHg, a pulmonary artery wedge pressure (PAWP) ≤ 15 mmHg and a pulmonary vascular resistance (PVR) > 3 WU [Galie N, et. al 2015; Lau EMT, et. al. 2017]:
    a) Idiopathic, in non-vasoreactive patients
    b) Heritable: Bone morphogenetic protein receptor type II (BMPR2) mutation and other mutations, in non-vasoreactive patients
    c) Drugs and toxin induced, in non-vasoreactive patients
    d) Associated with connective tissue disease
    e) Associated with simple congenital defects (atrial septal defect and/or ventricular septal defect) if closed > 12 months before inclusion.
4. The patient's last right heart catheterisation results, which were measured at the study site, must not be older than 90 days before V1 (will be considered as baseline value). Otherwise, a right heart catheterisation has to be performed as part of the study at visit A1.
5. WHO functional class II or III as judged by the investigator.
6. Stable treatment with at least one of the following approved PAH therapies for at least 90 days prior to V1: Ambrisentan, Bosentan, Macitentan, Riociguat, Selexipag, Sildenafil, Tadalafil, Epoprostenol intravenous, Iloprost inhaled or Treprostinil intravenous or subcutaneous.
7. For women: Agree not to donate ova from the time of informed consent until 30 days after the last IMP intake.

   For men: Agree not to donate sperm from the time of informed consent until 90 days after the last IMP intake.

Exclusion criteria:

Patients having or being any of the following are to be excluded from the study:

1. Contraindication to zamicastat, i.e. known hypersensitivity to ingredients of zamicastat formulation.
2. Two or more consecutive measurements of SBP < 95 mmHg or DBP < 50 mmHg measured at V1.
3. Uncontrolled diabetes mellitus with HbA1c ≥ 8.5% within the last three months or at screening.
4. PAH WHO Group 1 due to portal hypertension, human immunodeficiency virus (HIV) infection and schistosomiasis.
5. Any disease known to cause pulmonary hypertension other than PAH WHO Group 1.
6. Obstructive lung disease: Forced Expiratory Volume in 1 second/Forced Vital Capacity (FEV1/FVC) < 60% and FEV1 < 60% of predicted value after bronchodilator administration, as demonstrated and documented by previous spirometry data which, in

the opinion of the investigator, represent the clinical state of the patient at the time of the screening visit.

7. Restrictive lung disease: Total Lung Capacity (TLC) < 70% of predicted value, as demonstrated and documented by previous spirometry data which, in the opinion of the investigator, represent the clinical state of the patient at the time of the screening visit.
8. History of moderate to severe hepatic impairment (Child-Pugh B and C).
9. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2 (measured at V1).
10. Use of the following prohibited medication or treatments during study participation: calcium channel blockers (CCBs) if used for the treatment of PAH in vasoreactive patients; drugs containing a catechol group that is metabolised by DβH e.g. rimiterole, isoprenaline, dopamine, dopexamine or dobutamide or α- and/or β-blockers.
11. Current or previous (within the past year) alcohol or substance abuse excluding caffeine or nicotine.
12. Presence of any other significant or progressive/unstable medical condition that, in the opinion of the investigator, would compromise evaluation of the study treatment or may jeopardise the patient's safety, compliance or adherence to protocol requirements.
13. For women: Pregnancy or breast-feeding. Women of childbearing potential unable or unwilling to undergo pregnancy tests and practice highly effective contraceptive measures in combination with a barrier method e.g. condom (without spermicidal foam/gel/film/cream/suppository or fat- or oil-containing lubricants), occlusive cap (diaphragm or cervical/vault caps) with spermicidal gel/film/cream/suppository from the time of informed consent until 30 days after last IMP intake. Highly effective methods for women are surgical intervention (e.g. bilateral tubal occlusion), non-hormonal implantable intrauterine device, true sexual abstinence (i.e. when this is in line with the preferred and usual lifestyle of the patient) and vasectomised partner (provided that the partner is the sole sexual partner of the patient and the partner has received medical assessment of the surgical success). Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), hormonal contraceptives and withdrawal are not acceptable methods of contraception.

    For men: Male patients who are sexually active with a partner of childbearing potential must use, with their partner, a condom plus an approved acceptable contraceptive measure from the time of informed consent until 90 days after the last IMP intake. The following methods are acceptable methods of contraception: partner's use of combined (oestrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal); partner's use of progestogen-only hormonal contraception (oral, injectable/implantable, intrauterine hormone-releasing system); partner's use of implantable intrauterine device; surgical sterilisation (for example, vasectomy or bilateral tubal occlusion).
14. Previous participation in any other drug investigational study within the past 30 days (or five half-lives of investigational medicinal product [IMP] whichever is longer) prior to V1.
15. Vulnerable patients according to Section 1.61 of the ICH guideline for Good Clinical Practice E6.

Duration of treatment for the individual patient: Maximum treatment duration will depend on the individual HTD. Patients tolerating Dose D (200 mg zamicastat) will take zamicastat for up to 124 days.

Test product, dose and mode of administration: Tablets for oral administration under fed conditions containing 100 mg of zamicastat (BIA 5-1058) each. It is planned to use the following doses:

Dose A	50 mg zamicastat once daily (half a tablet of 100 mg)
Dose B	100 mg zamicastat once daily (one tablet of 100 mg)
Dose C	150 mg zamicastat once daily (one and a half tablet of 100 mg)
Dose D	200 mg zamicastat once daily (two tablets of 100 mg)

Reference therapy, dose and mode of administration: NA

Criteria for evaluation:

Primary endpoint; pharmacokinetics:

The following PK parameters (24-hour profile) for zamicastat and its metabolites will be derived after a single dose of 50 mg zamicastat at visit A1 (Day 1):

1. Area under the curve 0-24h ($AUC_{0\text{-}24h}$)
2. Maximum plasma concentration (C_{max})
3. Time until C_{max} (t_{max})

The following PK parameters (24-hour profile) for zamicastat and its metabolites will be derived at steady-state at the individual HTD and will be taken at visit MPV3.

4. Area under the curve 0-24h ($AUC_{0\text{-}24h,SS}$)
5. Maximum plasma concentration ($C_{max,SS}$)
6. Time until $C_{max,SS}$ ($t_{max,SS}$)
7. Minimum plasma concentration at the end of the dosing interval ($C_{min,SS}$)

The following PK parameter (trough level) will be derived for each dose at steady-state.

8. Minimum plasma concentration at the end of the dosing interval ($C_{min,SS}$)

Additional PK parameters may also be calculated if considered appropriate.

Secondary endpoints:

Safety and tolerability endpoints:

1. Adverse events
2. Clinically relevant changes in laboratory parameters (haematology, biochemistry, coagulation, urinalysis, arterial blood gas analysis)
3. Clinically relevant changes in vital signs
4. Clinically relevant changes in ECG
5. Number of digital scars (only in patients with scleroderma)
6. Skin score (only in patients with scleroderma)

Efficacy endpoints:

Change from baseline to MPV3 in:

7. PVR, right atrial pressure (RAP), mPAP, cardiac index (CI) and mixed venous oxygen saturation (SvO2). Further haemodynamic parameters may also be calculated if considered appropriate.
8. 6-MWT, including Borg dyspnoea score
9. Biomarker (N-terminal pro brain natriuretic peptide [NT-proBNP])
10. Echocardiogram parameters:
    - Tricuspid regurgitation, classified as absent, mild, moderate or severe
    - Right ventricular contractility, measured via tricuspid annular plane systolic excursion (TAPSE)
    - Pericardial effusion, classified as absent, traces or present
    - Right atrial area (end-systolic), right ventricular end-diastolic area
11. WHO functional class
12. Quality of life

Pharmacokinetic endpoints:

13. Apparent total clearance (CL/f)

14. Terminal half-life ($t_{1/2}$)
15. Apparent volume of distribution (V_z/f)

Pharmacodynamic endpoints:

16. Percent inhibition in plasma DβH activity (i.e. % change from baseline of DβH activity) on visits A3/B3/C3/D3, MPV1 and MPV2 (trough samples) and to visits A1 and MPV3 (24-hour profiles)
17. Maximal and minimal observed effect on DβH activity and time to occurrence of E_{max} (T_{Emax}) and E_{min} (T_{Emin}) on visit A1 (Day 1) and MPV3
18. Area under the effect-time curve (AUEC24) for % DβH inhibition on visit A1 (Day 1) and MPV3
19. Change from baseline 24-hour urinary catecholamine levels (norepinephrine and dopamine) on visits A3/B3/C3/D3 and MPV3

Additional parameters may be calculated if considered appropriate.

Statistical methods:

Analysis populations:

The **enrolled set** (ES) includes all patients who provided informed consent.

The **full analysis set** (FAS) includes all patients with at least one IMP administration.

The **pharmacokinetic analysis set** (PKS) will include all patients with an evaluable 24-hour PK profile and no major protocol deviations with an impact on PK analysis.

The **safety set** (SS) includes all patients with at least one IMP administration. The FAS and the SS are identical.

Sample size:

The clinical study BIA-51058-201 was designed to explore for the first time the pharmacokinetics, safety and efficacy of zamicastat in PAH patients in its potential therapeutic dose range (50 mg to 200 mg). Since this is an exploratory and descriptive study and no powered comparative statistical analysis is planned, study sample size was not formally calculated. Thus, it was considered that 32 patients constitute an appropriate sample size to meet the objectives of the study, considering the low prevalence of the disease and the consequent recruitment constrains.

Pharmacokinetic analysis:

The primary analysis of this study will be the analysis of the PK parameters derived by non-compartmental analysis from the plasma drug concentration-time data. Actual sampling times will be used. The area under the plasma concentration-time curve from time zero to 24-hour post-dose will be calculated using the linear trapezoidal rule.

Summary statistics will be reported, as appropriate, using the geometric mean, arithmetic mean, standard deviation, coefficient of variation, median, minimum, and maximum.

PK analysis will be based on the PKS.

Pharmacodynamic analysis:

Maximum observed effect on plasma DβH activity (E_{max}), time to occurrence of E_{max} (TE_{max}), area under the effect-time curve (AUEC24) and urinary catecholamine levels (norepinephrine and dopamine) including changes from baseline will be described using summary statistics by each dose group. Pharmacodynamic analysis will be based on the PKS.

Safety analysis:

Treatment-emergent adverse events (TEAEs) will be summarised by MedDRA primary system organ class (SOC) and preferred term (PT). TEAEs will be summarised by severity

and drug relationship. TEAEs leading to discontinuation from the study, adverse events (AEs) of special interest and serious TEAEs will be summarised separately. All AEs will be listed.

Laboratory, vital signs and ECG parameters will be described using summary statistics.

The number of digital scars and skin score in patients with scleroderma will be analysed using standard summary statistics.

All safety analyses will be based on the SS.

Efficacy analysis:
Efficacy data will be analysed exploratively. Continuous measures will be summarised using summary statistics: mean, standard deviation, median, minimum and maximum, in addition for the changes from baseline a 95% confidence interval will be provided. Categorical variables will be summarised by using frequency counts and percentages.

The scores of each SF-36v2 subscale, as well as the physical and mental component summary scores (PCS and MCS), will be described using summary statistics.

Efficacy analyses will be based on the FAS.

Interim analyses:
No interim analyses are planned in this study.

Schedule of study procedures

	Screening	Dose finding period			42-days Maintenance period			Follow-up	
Visit no.	**V1**	**A1[2]**	**A2/B2/C2/D2**	**A3/B3/C3/D3[5]**	**MPV1[5]**	**MPV2[5]**	**MPV3[2,5]/ EDV[1]**	**Follow-up down-titration[7]**	**FU[17]**
	Day -12 to -5	**Day 1**	**8 days ±2 after A1/A3/B3/C3**	**14 days ±2 after A1/A3/B3/C3**	**13 days ±2 after D3/down-titration[19]**	**27 days ±2 after D3/down-titration[19]**	**41 days ±2 after D3/down-titration[19]**	**14 days ±2 after MPV3/EDV**	**14 days ±2 after last IMP intake**
on-site visit ⊗ / telephone contact ☎	⊗	⊗	☎	⊗	⊗	⊗	⊗	☎	⊗
Initiation procedures									
Informed consent	●								
Demographics	●								
Height	●								
Weight	●						●		
Medical history	●								
Prior medication	●								
Concomitant medication	●	●	●	●	●	●	●	●	●
WHO functional class	●						●		
Inclusion/exclusion criteria	●	●							
Medication									
First IMP administration		●							
Dose increase				●[3]					
Dispense IMP		●		●	●	●	●[7]		
IMP accountability				●	●	●	●		●[7]
Pharmacokinetics/ Pharmacodynamics									
Blood withdrawal (24h profile)		●[4]					●[4,8]		
Blood withdrawal (trough level)				●[14]	●[14]	●[14]			
Blood withdrawal (DβH activity)		●[4]		●[14]	●[14]	●[14]	●[4,8]		

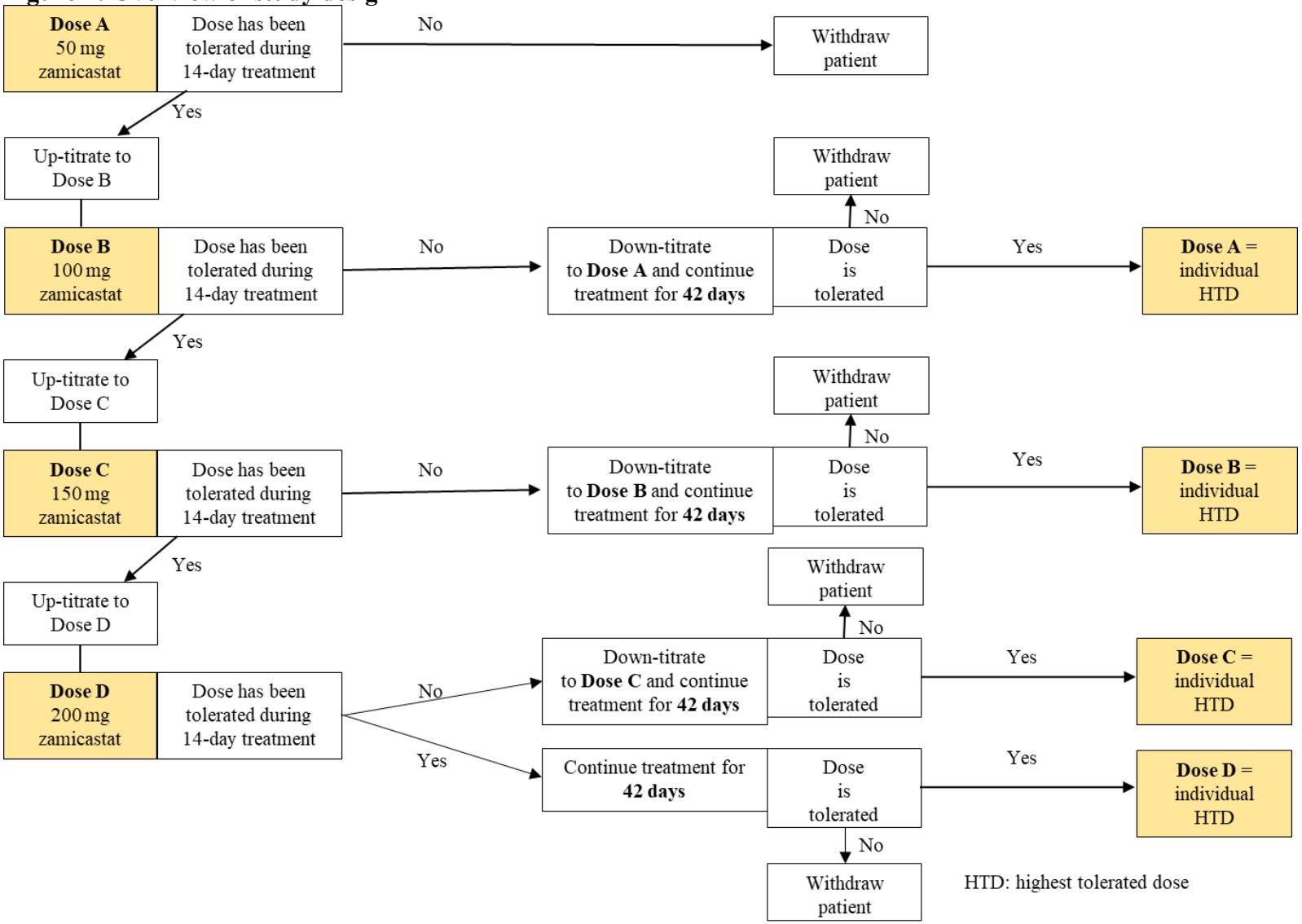
	Screening	Dose finding period			42-days Maintenance period			Follow-up	
Visit no.	**V1**	**A1[2]**	**A2/B2/C2/D2**	**A3/B3/C3/D3[5]**	**MPV1[5]**	**MPV2[5]**	**MPV3[2,5]/ EDV[1]**	**Follow-up down-titration[7]**	**FU[17]**
	Day -12 to -5	**Day 1**	**8 days ±2 after A1/A3/B3/C3**	**14 days ±2 after A1/A3/B3/C3**	**13 days ±2 after D3/down-titration[19]**	**27 days ±2 after D3/down-titration[19]**	**41 days ±2 after D3/down-titration[19]**	**14 days ±2 after MPV3/EDV**	**14 days ±2 after last IMP intake**
on-site visit ⊗ / telephone contact ☎	⊗	⊗	☎	⊗	⊗	⊗	⊗	☎	⊗
Urinalysis (dopamine and norepinephrine levels, 24h urine collection)[15]		●		●			●[8]		
Safety									
Adverse events	●	●	●	●	●	●	●	●	●
Vital signs[11] (blood pressure, heart rate, tympanic body temperature)	●	●[12]		●	●	●	●[12]		●
Blood withdrawal (haematology, biochemistry and coagulation)	●			●	●		●		●
Urinalysis	●			●	●		●		●
Physical examination	●			●	●	●	●		
12-lead electrocardiogram	●	●[18]		●	●	●	●[18]		●
Number of digital scars[10]	●			●	●	●	●		
Modified Rodnan Skin Score[10]	●						●		
Blood pregnancy test[6]	●								
Urine pregnancy test[6]		●		●	●	●	●		●
Arterial blood gas analysis[16,21]		●					●[8]		
Efficacy									
SF-36v2	●						●[8]		
6-min walk test/Borg dyspnoea score	●[13]						●[8,20]		
Echocardiography	●						●[8]		

	Screening	Dose finding period			42-days Maintenance period			Follow-up	
Visit no.	**V1**	**A1[2]**	**A2/B2/C2/D2**	**A3/B3/C3/D3[5]**	**MPV1[5]**	**MPV2[5]**	**MPV3[2,5]/ EDV[1]**	**Follow-up down-titration[7]**	**FU[17]**
	Day -12 to -5	**Day 1**	**8 days ±2 after A1/A3/B3/C3**	**14 days ±2 after A1/A3/B3/C3**	**13 days ±2 after D3/down-titration[19]**	**27 days ±2 after D3/down-titration[19]**	**41 days ±2 after D3/down-titration[19]**	**14 days ±2 after MPV3/EDV**	**14 days ±2 after last IMP intake**
on-site visit ⊗ / telephone contact ☎	⊗	⊗	☎	⊗	⊗	⊗	⊗	☎	⊗
Right heart catheterisation[16,22]		●					●[8]		
Blood withdrawal biomarker	●						●[8]		

1. EDV = early discontinuation visit. An EDV has to be performed within 10 days of early discontinuation. Additionally, patients who will not participate in the extension study will be contacted by phone at the time of last patient's last visit (LPLV) to document their vital status.
2. Patients will be hospitalised for 24 hours.
3. The dose will be increased if the previous dose was considered safe by the investigator according to safety criteria (at A3, B3 and C3).
4. Blood samples for PK and PD to be taken prior to IMP intake as well as 1, 2, 4, 8, 16 and 24 hours after IMP intake (but still prior to next IMP intake).
5. The patient has to be advised not to take the IMP at home on the day of A3, B3, C3, D3, MPV1, MPV2 and MPV3.
6. Only in women of childbearing potential.
7. Only applicable in patients taking 150 mg or 200 mg zamicastat and who will not participate in the extension study. In case a patient will discontinue the study early due to the occurrence of an AE, down-titration is not mandatory, but at the investigator's discretion.
8. Not applicable at EDV.
9. Visits during the Dose-finding period:
   Dose A (50 mg zamicastat): visits A1, A2 and A3
   Dose B (100 mg zamicastat): visits B2 and B3. These visits will only be performed if the patient was up-titrated to 100 mg zamicastat at A3.
   Dose C (150 mg zamicastat): C2 and C3. These visits will only be performed if the patient was up-titrated to 150 mg zamicastat at B3.
   Dose D (200 mg zamicastat): D2 and D3. These visits will only be performed if the patient was up-titrated to 200 mg zamicastat at C3.
   If the patient's next dose will not be tolerated, the patient will be asked to come to an unscheduled visit (UV) and he/she will be down-titrated to his/her previous dose.
10. In scleroderma patients only.
11. Blood pressure and heart rate will be measured in supine position (two measurements) and afterwards in standing position (one measurement).

12. At A1 and MPV3 blood pressure and heart rate (two measurements in supine and one measurement in standing position) will be measured before IMP intake as well as 4, 8 and 24 hours after IMP intake.
13. Two baseline tests, separated by an interval of at least 30 minutes.
14. Blood samples will be taken prior to IMP intake.
15. 24-hour urine collection will start on the day before the respective visit.
16. Right heart catheterisation and arterial blood gas analysis have to be performed before IMP intake.
17. Applicable in all patients who will not participate in the extension study as well as in patients who discontinued early.
18. At A1 and MPV3 triplicate ECGs recordings will be performed before IMP intake, 4 and 8 hours after IMP intake. At EDV only one ECG recording will be performed.
19. Down-titration can be performed at a scheduled visit or an unscheduled visit (on-site visit or telephone contact) during the dose finding period.
20. 6-MWT has to be performed after arterial blood gas analysis but before right heart catheterisation and IMP intake.
21. Arterial blood gas analysis has to be performed before right heart catheterisation.
22. Right heart catheterisation will only be performed if no results which were measured at the study site from within 90 days before V1 are available.

Figure 1: Overview of study design



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4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

4.1 List of Abbreviations

ABBREVIATION	EXPLANATION
6-MWT	6-minute walk test
AE	adverse event
AESI	adverse events of special interest
ADR	adverse drug reaction
ALP	alkaline phosphatase
ALT	alanine transaminase
AoR	acknowledgment of receipt form
APD90	action potential duration at 90% repolarisation
APT	action potential triangulation
AST	aspartate transaminase
AUC	area under the curve
AUEC	area under the effect-time curve
BMPR2	bone morphogenetic protein receptor type II
CCB	calcium channel blocker
CI	cardiac index
CL/f	apparent total clearance
CL_R	renal clearance
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
CNS	central nervous system
CO	cardiac output
CPK	creatine phosphokinase
CRA	clinical research associate
CRO	contract research organisation
DßH	dopamine ß-hydroxylase
DA	dopamine
DMP	Data Management Plan
DBP	diastolic blood pressure
DOPAC	3,4-dihydroxyphenylacetic acid
DPG	diastolic pressure gradient
DSMB	data safety monitoring board
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EDV	early discontinuation visit
eGFR	estimated glomerular filtration rate
E_{max}	maximum observed effect on DβH activity
ES	enrolled set
FAS	full analysis set

ABBREVIATION	EXPLANATION
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GMP	Good Manufacturing Practices
HbA1c	haemoglobin A1c
HCG	human chorionic gonadotropin
HEK	human embryonic kidney
hERG	human-ether-à-go-go related gene
HIV	human immunodeficiency virus
HTD	highest tolerated dose
HVA	homovanillic acid
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IMP	investigational medicinal product
INR	International normalised ratio
ISF	investigator site file
LDH	lactate dehydrogenase
LLOQ	lower limit of quantification
LPLV	last patient's last visit
MAD	multiple ascending dose
MCT	monocrotaline
MedDRA	Medical Dictionary for Regulatory Activities
mPAP	mean pulmonary artery pressure
mRSS	modified Rodnan skin score
NE	norepinephrine
NOAEL	no-observed adverse effect level
NT-proBNP	N-terminal pro brain natriuretic peptide
NYHA/WHO	New York Heart Association/Word Health Organization
PAC	pulmonary arterial compliance
PAH	pulmonary arterial hypertension
PAP	pulmonary artery pressure
PAWP	pulmonary artery wedge pressure
PCWP	pulmonary capillary wedge pressure
PD	pharmacodynamic
PK	pharmacokinetic

ABBREVIATION	EXPLANATION
PKS	pharmacokinetic analysis set
PT	preferred term
PVR	pulmonary vascular resistance
RAP	right atrial pressure
RBC	red blood cell count
RHC	right-heart catheterisation
RV	right ventricular
RVP	right ventricular pressure
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SAS	Statistical Analysis System
SBP	systolic blood pressure
S_{Cr}	standardised serum creatinine
SDV	source document verification
SF-36v2	Short Form – 36 version 2
SHR	spontaneously hypertensive rat
SNS	sympathetic nervous system
S_vO_2	mixed venous oxygen saturation
SOC	system organ class
SS	safety set
SUSAR	suspected unexpected serious adverse reaction
SVR	systemic vascular resistance
$t_{1/2}$	terminal half-life
TAPSE	tricuspid annular plane systolic excursion
TEAE	treatment emergent adverse event
TE_{max}	time to occurrence of E_{max}
TLC	Total Lung Capacity
TMF	Trial Master File
t_{max}	time until C_{max}
TPG	transpulmonary pressure gradient
UV	unscheduled visit
V	visit
V_z/f	apparent volume of distribution
WBC	white blood cell count
WHO	World Health Organization
WKY	Wistar-Kyoto rats
WU	Wood unit

4.2 Definition of Terms

Woman of childbearing potential	A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. [1]

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

This study will be conducted in approximately 15 sites in nine European countries. Other sites and other countries may be added as needed. A list of principal investigators and their affiliations will be kept in the Trial Master File (TMF).

The sponsor, Bial, will contract planning, conduct, data management, medical monitoring support, pharmacovigilance, statistical analysis (except pharmacokinetic [PK] and pharmacodynamic [PD] analysis) and medical writing to the contract research organisation (CRO) given below.

SPONSOR:

Bial - Portela & C[a], S.A. (further Bial)
À Av. da Siderurgia Nacional
4745-457 Coronado (S. Romão e S. Mamede), Portugal
Phone: +351 229866100
Fax: +351 229866192
www.bial.com

***Research and Development General Manager**:*
Patrício Soares-da-Silva, MD, PhD
Research & Development Area
psoares.silva@bial.com

***Project Managers**:*
Ana Santos, BSc, MBA
Department of Development
ana.santos@bial.com

Andreia Guimarães, BSc, PhD
Department of Development
andreia.guimaraes@bial.com

SPONSOR:

***Drug Safety Managers**:*
Helena Gama, MD, MSc
Head of Pharmacovigilance and Drug Safety, EU QPPV
Av. Da Siderurgia Nacional, 4745-457 Coronado (S. Romão e S. Mamede)
Phone: +351 229866100 (working hours)
Phone: +351 966633353 (nights, weekends and holidays)
Fax: +351 229866198
e-mail: helena.gama@bial.com

Mariana Vieira, PharmD, MSc
Senior Pharmacovigilance and Drug Safety Manager (Deputy EU QPPV)
Av. Da Siderurgia Nacional, 4745-457 Coronado (S. Romão e S. Mamede)
Phone: +351 22 9866100 (working hours)
Phone: +351 963469988 (nights, weekends and holidays)
Fax: +351 229866198
e-mail: mariana.vieira@bial.com

***Medical Monitors**:*
Luís Magalhães, MD, PhD, PharmD
Department of Development
luis.magalhaes@bial.com

Fábio Ikedo, MD, MSc (back-up)
Department of Development
fabio.ikedo@bial.com

CONTRACT RESEARCH ORGANISATION

Scope International AG (further SCOPE)
Konrad-Zuse-Ring 18
68163 Mannheim, Germany
Phone: +49 621 429 39 0
Fax: +49 621 429 39 40

COORDINATING PRINCIPAL INVESTIGATOR

Prof. Dr. med. Marius Hoeper
Medizinische Hochschule Hannover
Klinik für Pneumologie
Carl-Neuberg-Straße 1
30623 Hannover, Germany

DATA SAFETY MONITORING BOARD (DSMB)

The DSMB will independently safeguard the interests of patients in this study and will enhance the data integrity and credibility of this study. The primary responsibilities of the DSMB, its relationship with other study components, and the purpose and timing of its meetings are described in the DSMB Charter. The DSMB membership includes three specialists with experience in cardiology and in pulmonary hypertension, one expert with experience in dermatology as well as one statistician.

ELECTRONIC DATA CAPTURE (EDC) SYSTEM PROVIDERS

Electronic data capture (EDC) system provider

Oracle America, Inc.

500 Oracle Parkway
Redwood Shores, CA 94065, USA

Phone: +44 (0) 118 9249 766

Fax: +44 (0) 118 9249 766

http://www.oracle.com/us/products/applications/health-sciences/e-clinical/inform/index.html

Electronic case report form:

Oracle Health Sciences InForm

LABORATORY

Safety laboratory assessments (blood), blood pregnancy testing and biomarker (blood samples) assessments will be performed by:

LKF Laboratorium für Klinische Forschung GmbH

Lise-Meitner-Str. 25

D- 24223 Schwentinental, Germany

Phone: +49 4307 82760

Fax: +49 4307 827679

www.lkf-kiel.de

Octopamine level assessments will be performed by:

Anapharm

Encuny 22, 2nd floor

08038 Barcelona, Spain

Phone: +34 93 223 86 36

Email info@anapharmbioanalytics.com

www.anapharmbioanalytics.com

LABORATORY

PK assessments will be performed by:
SYNLAB Analytics & Services Switzerland AG
Sternenfeldstrasse 14 CH-4127 Birsfelden, Switzerland
Phone: +41 61 317 9010
Fax: +41 61 317 2310
Email: Anette.Orjuela@synlab.com
https://www.synlab.de/de/home/

Catecholamine (norepinephrine and dopamine) level assessment will be performed by:
The Doctors Laboratory Ltd
The Halo Building, 1 Mabledon Place
London WC1H 9AX, United Kingdom
Phone: + 44 (0)20 7307 7373
Fax: + 44 (0)20 7307 7374
Email: tdl@tdlpathology.com
https://tdlpathology.com/

CENTRAL CLINICAL STUDY SUPPLY MANAGEMENT

Bial - Portela & C[a], S.A.
À Av. da Siderurgia Nacional
4745-457 Coronado (S. Romão e S. Mamede), Portugal
Phone: +351 229866100
Fax: +351 229866192
www.bial.com

STATISTICAL ANALYSIS

Pharmacokinetic and pharmacodynamic analysis will be performed by:
Quotient Science
Mere Way, Ruddington
Nottingham, NG11 6JS, United Kingdom
https://www.quotientsciences.com

Project Manager:
Claire Swann, Senior Project Manager
Phone: +44 15 931 5505
Email: Claire.Swann@quotientsciences.com

Note: Labelling and shipping of the study drug might be subcontracted to a contract manufacturing organisation.

All other study personnel not included in this section is identified in a separate personnel list as appropriate. This list will be regularly updated as needed. Its most current version will be available in each centre's Investigator Site File (ISF) and/or in the TMF.

6. INTRODUCTION

6.1 Background

Pulmonary arterial hypertension (PAH) is a chronic and progressive disease which affects small pulmonary arteries. It is characterised by vascular obstruction leading to progressive increase in vascular resistance. This increases right ventricular (RV) afterload and consequently results in RV failure. Intima and media proliferation and its consequent pulmonary vascular obstruction are considered to be the key elements in the pathogenesis of PAH. Vasoconstriction, vascular remodelling and thrombosis are factors that increase pulmonary vascular resistance (PVR) in PAH. These processes involve a multitude of cellular and molecular elements. [2]

PAH is diagnosed by right-heart catheterisation (RHC) showing precapillary pulmonary hypertension with a mean pulmonary artery pressure (mPAP) of ≥ 25 mmHg and a normal pulmonary artery wedge pressure (PAWP) of ≤ 15 mmHg and increased pulmonary vascular resistance (PVR > 3 Wood units [WU]). [3]

6.2 Investigational Medicinal Product

Zamicastat (also known as BIA 5-1058; 1-[(3R)-6,8-difluoro-3,4-dihydro-2H-1-benzopyran-3-yl]-1,3- dihydro-5-[2-[(phenylmethyl)amino]ethyl]-2H-Imidazole-2-thione) is currently being developed by BIAL - Portela & C[a], S.A. for the treatment of cardiovascular diseases.

Zamicastat is a reversible dopamine ß-hydroxylase (DßH) inhibitor that prevents the conversion of dopamine (DA) to norepinephrine (NE) in sympathetically innervated tissues and reduces the drive of the sympathetic nervous system (SNS). Zamicastat is a peripherally-selective inhibitor as a result of its reduced ability to cross the blood-brain barrier. In contrast to what was found in peripheral tissues, zamicastat failed to affect brain levels of NE and had minimal impact on DA tissue levels in the brain, which is unique among DßH inhibitors previously tested for the treatment of cardiovascular disorders. The PD and PK profiles of zamicastat envisage a long duration of DßH inhibition, which may allow a once-daily administration. [4]

6.2.1 Non-clinical aspects

Zamicastat, BIA 5-453 - a metabolite of zamicastat and its N-acetylated metabolite BIA 5-961, produced concentration-dependent decreases in DβH activity in human neuroblastoma SK-N-SH cells. In mice, zamicastat produced significant decreases in NE levels and increases in DA levels in the heart but no marked effect on NE tissue levels in the parietal and frontal cortex of the brain. Zamicastat (30 mg/kg) reduced to a similar extent the urinary excretion of NE in normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), accompanied by a significant increase in the urinary excretion of DA, and metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The effects of zamicastat have been explored in various animal models of disease. In the SHR model, zamicastat reduced systolic and diastolic blood pressure with no changes in heart rate. In the monocrotaline (MCT) rat model of PAH, zamicastat treatment, initiated on day 12 post MCT, significantly improved the survival rate, decreased the frequency of arrhythmic beats and the number of arrhythmic episodes, and reduced the incidence of lung plexiform lesions. There was no effect on the deteriorated hemodynamics and cardiac contractility. Safety pharmacology studies demonstrated that zamicastat had no significant effects on the gross behavioural state and gastrointestinal transit in the rat. Some effects on renal function were observed at 100 or 300 mg/kg (p.o.) in the rat; small increases in urinary volume, pH and sodium excretion with a more marked effect on potassium excretion. In conscious dogs monitored by telemetry, single administration of zamicastat (20, 60 or 120 mg/kg) had no

substantial effect on arterial blood pressure, heart rate, and on the PR, QRS, QT and the QTc intervals as compared with the vehicle control animals.

Zamicastat had no or only slight effects on the action potential parameters in the dog Purkinje fiber at 0.222 μg/ml (measured concentration) and higher pro-arrhythmic potential related to lengthened action potential duration at 90% repolarization (APD90) and enhanced action potential triangulation (APT) at the measured concentration of 1.930 μg/ml. In a study that evaluated the effects of zamicastat on hERG potassium channel the IC_{50} was determined to be 113.84 ng/mL, and no difference from the vehicle group was observed up to 39.2 ng/mL of zamicastat. These concentrations (up to 39.2 ng/ml) exceed by 22 times the free maximum observed plasma concentration at steady state (C_{max}) of zamicastat in human plasma at zamicastat doses of 200 mg/day after repeated administration for 10 days under fed conditions and micronized API (BIA-51058-116). In a chronic toxicity study in non-rodents, zamicastat was administered by oral capsule to beagle dogs for 6 months at dose levels of 30, 60 or 90 mg/kg/day in Week 1 and 10, 30 or 60 mg/kg/day thereafter followed by a 7-week recovery period. Electrocardiogram measurements showed decreases in heart rate for treated groups only during Week 13 when compared with control and pretreatment. Increases in QT and QTc interval were observed during Weeks 13 and 26 for all treated groups. These alterations did not result in any clinically detectable evidence of cardiac malfunction in the dogs. There was less of an effect at end of recovery phase, indicating partial recovery, and as no heart lesions were seen during the histopathological examination, in the context of this study the changes were considered non-adverse. Zamicastat has undergone a comprehensive toxicological evaluation in studies of appropriate design consistent with ICH requirements in the mouse, rat and dog. Embryofetal development studies have been conducted in rats and rabbits and fertility studies and pre- and post-natal development toxicity have been conducted in rats. Administration of zamicastat was associated with dose-dependent increases in zamicastat plasma levels. Table 1 indicates the extent of exposure [area under the plasma concentration-time curve up to the last measurable concentration (AUC_{0-t})] of zamicastat and its metabolite BIA 5-453 corresponding to the no-observed adverse effect level (NOAEL) at the end of treatment with zamicastat in toxicity studies.

Table 1: Extent of exposure of zamicastat and its metabolites

Study	Study number	NOAEL (mg/kg/day)	AUC_{0-t} (ng.h/mL)			
			Zamicastat		BIA 5-453	
			Males	Females	Males	Females
4-week oral repeat administration in the mouse	C64635	100	15100	15000	1580	2240
3-month oral repeat administration in the mouse	C75007	80	11800	6000	1350	1130
4-week oral repeat administration in the rat	C64657	100	60200	112000	5120	7410
3-month oral repeat administration in the rat	C75018	20	4890	10100	50 4	786
6-month oral repeat administration in the rat	D35961	20	9940	8660	1170	313
4-week oral repeat administration in the dog	C77922	180	12400	29400	15800	25500
3-month oral repeat administration in the dog	S31096	80	7730	2510	3360	2640
6-month oral repeat administration in the dog	WJ02CV	60	6860	7890	4850	7630

Studies to evaluate the genotoxic potential of zamicastat included in vitro bacterial assay, in vitro chromosomal aberration assay and in vivo mouse bone marrow micronucleus assay. Overall, there was no indication of mutagenicity, clastogenicity or aneugenicity for zamicastat. Pharmacokinetic studies in male Wistar rats demonstrated that following a single oral (30 mg/kg) and intravenous (2.5 mg/kg) administration of [^{14}C]-zamicastat, C_{max} of total radioactivity were observed (t_{max}) at 6-8 hours and 0.083 hours post-dose, respectively. Following single oral administration of 30 mg/kg [^{14}C]- zamicastat to the male Beagle dog, C_{max} of total radioactivity were observed between 1-4 hours post-dose (t_{max}). Following intravenous administration, plasma concentrations of total radioactivity were quantifiable up to 72 hours post-dose. Following a single oral administration of [^{14}C]-zamicastat in male rats, radioactivity was quickly distributed in the tissues achieving the maximum concentration of radioactivity at the initial sampling point of 8 hours post-dose, and the majority of tissues were observed to have levels BLQ at the final sampling point of 168 hours post-dose. No evidence of concentration-dependent blood cell binding for [^{14}C]-zamicastat was observed. The zamicastat binding interaction studies with warfarin, diazepam, digoxin or tolbutamide indicated that the plasma protein binding site of zamicastat differs from that of the interactants. The intrinsic clearance (CL_{int}) values for zamicastat were estimated at 40.3, 32.6, 13.6, 44.2 and 6.29 µL/min/106 cells in mouse, rat, dog, monkey and human hepatocytes, respectively. In general, the metabolites observed in hepatocytes across species were formed as a result of multiple (2 or more) biotransformation processes such as potential oxidation which include potential hydroxylation, epoxidation or oxide formation and N-dealkylated. BIA 5-453, a zamicastat N-dealkylated metabolite was identified as an active metabolite. Zamicastat is metabolised by CYP1A2, CYP2C8, CYP2C19, CYP2D6 and CYP3A4 recombinant enzymes with CYP2D6 with the highest metabolic clearance followed by CYP1A2, CYP2C19, CYP2C8 and CYP3A4, respectively. In human hepatic microsomes, zamicastat inhibited CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP3A4 activity. Faecal excretion was the primary route of excretion of total radioactivity, following both oral and intravenous administration of [^{14}C]-zamicastat.

6.2.2 Experience in humans

Clinical experience with zamicastat in humans includes 11 completed phase I studies [4]:

- a combined rising single-dose and multiple-dose study, including food interaction (BIA-51058-101), to investigate the safety, tolerability, PK and PD profile of zamicastat, in healthy male young subjects;
- a food interaction study (BIA-51058-102), to investigate the effect of food (different meals and time of food intake) on the PK of zamicastat, in healthy male and female young subjects;
- an open-label study to assess the absorption, distribution, metabolism and excretion, including the mass balance, of [^{14}C]-labelled BIA 5-1058 and metabolites following a single oral dose administration in healthy male subjects (BIA-51058-104);
- a 10-day multiple-dose study (BIA-51058-105) to investigate the effect of age-gender on the PK and PD profiles of zamicastat, in healthy male and female young and elderly subjects.
- an open-label, three period, fixed sequence study to assess the effect of a single dose of BIA 5-1058 200 mg on the steady state pharmacokinetics of treprostinil in healthy subjects under fed conditions (BIA-51058-106);
- an open-label, three period, fixed sequence study to assess the effect of a single dose of BIA 5-1058 400 mg on the steady state pharmacokinetics of bosentan in healthy subjects under fasting conditions (BIA-51058-107);
- an open-label, three period, fixed sequence study to assess the effect of a single dose of BIA 5-1058 400 mg on the steady state pharmacokinetics of sildenafil in healthy subjects under fasting conditions (BIA-51058-108);
- an open-label, three period, fixed sequence study to assess the effect of a single dose of BIA 5-1058 400 mg on warfarin pharmacokinetics in healthy subjects under fasting conditions (BIA-51058-109);
- an open-label, three period, fixed sequence study to assess the pharmacokinetics, safety and tolerability of concurrent doses of BIA 5-1058 and furosemide in healthy subjects under fasting conditions (BIA-51058-111);
- a randomised, double-blind, placebo-controlled and open-label, active-controlled, 4-period crossover trial to evaluate the effect of BIA 5-1058 on cardiac repolarisation in healthy adult males and females under fed conditions (BIA-51058-115);
- a double-blind, randomised, placebo-controlled, parallel-group study to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of multiple doses of BIA 5-1058 in healthy volunteers (BIA-51058-116).

Clinical experience with zamicastat in humans also includes three ongoing phase I clinical studies:

- an open-label, sequential group, multiple-dose study to evaluate the PK, safety, and tolerability of zamicastat in subjects with hepatic impairment and normal hepatic function (BIA-51058-113);
- an open-label, sequential group, multiple-dose study to evaluate the pharmacokinetics, safety, and tolerability of zamicastat in subjects with renal impairment and normal renal function (BIA-51058-114);

- an open-label study to assess the absorption, distribution, metabolism and excretion, including the mass balance recovery, metabolite profiling and identification, of [^{14}C] labelled BIA 5-1058 following a single oral dose administration in healthy male subjects (BIA-51058-119).

Overall, 571 healthy subjects aged 18 to 73 years, have been enrolled in the completed phase I studies and 512 have been exposed to zamicastat. The highest doses of zamicastat administered in the clinical studies were 2400 mg under single-dose conditions (study BIA-51058-101) and 1200 mg under multiple-dose conditions (studies BIA-51058-101, and 105). On the other hand, the highest single and multiple-dose exposure to zamicastat were achieved with the 1200 mg, micronized API, under fed conditions (BIA-51058-102) and 1200 mg, micronized API, under fasted conditions (BIA-51058-105), respectively.

Pharmacokinetic properties

Systemic plasma exposure of zamicastat increases in a dose-dependent manner and pharmacokinetics indicates to be dose-linear over the dose range of 25 to 400 mg in fed and micronized conditions (BIA-51058-116), despite the moderate to high variability between subjects.

The AUC_{0-t} of total radioactivity in plasma was 91-fold higher than the cumulative concentration of zamicastat and its metabolites (BIA 5-453 and BIA 5-961) in plasma suggesting that other metabolites than BIA 5-453 and BIA 5-961 may be formed during metabolism of zamicastat which have not yet been identified (BIA-51058-104). Moreover, zamicastat and its metabolites demonstrated to be excreted both by faeces and urine, with faeces as the major route (65.3%). Overall, zamicastat plasma exposure is higher in multiple-dose than in single-dose conditions (time-dependent pharmacokinetics), with the mean accumulation ratios (C_{max} and AUC) ranging from 0.963 to 2.597 and 1.095 to 3.462. Zamicastat steady-state is reached between Day 3 and Day 5 following once-daily administration (BIA-51058-104).

Peak exposures (C_{max}) of zamicastat at steady state (Day 10) in fed conditions were 60% higher than those in the fasted state at 400 mg, but there were no other statistically significant differences in C_{max} or in overall exposure (AUC_{0-t}) at any dose level examined (50 mg, 100 mg, 200 mg, 400 mg), indicating the lack of a meaningful food effect for zamicastat following repeated dosing (BIA-5-1058-116).

Also, the micronization step performed on the API before the beginning of the BIA-51058-102 study was found to increase the plasma exposure of zamicastat at 400, 800 and 1200 mg (BIA-51058-102).

In turn, age appeared to have a positive effect on the PK of zamicastat, despite the reduced number of subjects included in the study (BIA-51058-105). Specifically, plasma exposure of zamicastat was found to be higher in elderly subjects compared to young subjects following multiple oral dose administration of 400 mg and 1200 mg of micronized zamicastat for 10 days (BIA-51058-105).

Pharmacodynamic properties

Zamicastat showed a dose-dependent and a long-lasting plasma DβH inhibition, with E_{max} ranging from 11% to 62% following once-daily doses. In line with the accumulation effect observed in the pharmacokinetics, the overall extent of DβH inhibition was found to be higher on Day 10 than Day 1, following multiple-dose administration of zamicastat.

Doses of zamicastat that achieved 30-40% inhibition of plasma DβH activity in phase I studies serve as the basis for establishing its likely therapeutic dose (BIA-51058-101, -105, 116).

Drug-drug interactions

The single-dose PK of zamicastat (400 mg) is affected by the co-administration of multiple oral doses of treprostinil (BIA-51058-106) and by multiple oral doses of bosentan (BIA-51058-107). Treprostinil increased the peak (C_{max}) and total exposure (AUC_{0-t}) of zamicastat, whilst co-administration of bosentan increased the peak exposure and decreased the total exposure of zamicastat. On the other hand, single-dose PK of zamicastat (400 mg) is not affected by the co-administration of multiple oral doses of sildenafil (BIA-51058-108), single-dose of warfarin (BIA-51058-109) or single-dose of furosemide (BIA-51058-111).

Zamicastat did not significantly affect the steady-state PK of bosentan and sildenafil, neither the single-dose PK and PD (International Normalized Ratio of prothrombin time) of warfarin (*S*- and *R*-warfarin), when co-administered with these drugs in single dose of 400 mg under fasting conditions (BIA-51058-107, BIA-510158-108 and BIA-51058-109, respectively). On the other hand, single-dose of zamicastat (200 mg) decreased the total exposure of treprostinil in steady-state conditions, whilst single-dose of zamicastat (400 mg) reduced the peak and total exposure of single-dose of furosemide.

Tolerability and safety

Overall, no serious or severe adverse events were reported. Eight out of 512 (1.6%) subjects exposed to zamicastat reported skin and subcutaneous tissue disorders assessed as at least possibly related to zamicastat (5 cases of moderate diffuse erythemato papulous cutaneous eruption from study BIA-51058-105, one case of mild rash erythematous from study BIA-51058-106, one case of mild papular rash from study BIA-51058-109 and one case of mild rash pruritic from study BIA-51058-111); these TEAEs were considered to be of special interest. All 8 cases were assessed as non-serious, were self-limited and subjects recovered completely. The potential maximum therapeutic exposure of zamicastat (400 mg, single-dose, fed conditions, micronized API) did not have a clinically significant effect on cardiac repolarization, whilst a supratherapeutic exposure (1200 mg, single-dose, fed conditions, micronized API) had a minimal effect. The 400 mg and 1200 mg doses of zamicastat did not have a clinically relevant effect on heart rate, PR interval, or QRS duration, and there were no new clinically significant ECG morphology events (BIA-51058-115). There was no clear trend or effect with respect to tolerability of zamicastat through dose escalation or administration with food and no clear indication that food status influenced a subject's dose tolerability (BIA-51058-116). Overall, zamicastat was well tolerated in all the completed clinical trials till date.

Further details are provided in the current Investigator's Brochure (IB). [4]

6.3 Rationale

The currently licensed therapies for PAH – prostacyclin-cAMP, nitric oxide-soluble guanylate cyclase-phosphodiesterase type 5 (cGMP-PDE5) and endothelin – target the three main vasoactive pathways. Despite improvement in patient symptoms and well-being with these agents, mortality rates remain high (~65% survival at 5 years) and there is a need for therapies targeting alternative pathways that can reverse pulmonary vascular remodelling, inhibit disease progression and improve survival. [3]

Experimental and clinical evidence supports a relation between PAH and the SNS, indicating that PAH can be mediated, at least partly, by SNS hyperactivation. A strategy for the modulation of sympathetic nerve function is to reduce the biosynthesis of NE DβH, the enzyme that catalyses the conversion of DA to NE in sympathetic nerves. In the monocrotaline (MCT)-induced pulmonary hypertension model in the rat oral treatment with the DβH inhibitor

zamicastat prevented the MCT-induced increase in mean right ventricular pressure (RVP) and decrease in pulse rate was significantly attenuated. [5]

Based on these promising experimental results and the need of new therapies with different mechanism of action for the treatment of PAH, the effect of zamicastat as adjunctive therapy will be investigated in PAH patients. Moreover, this study is particularly planned to evaluate the PK profile, safety and tolerability of different zamicastat doses in order to define the suitable dosage window for the treatment of PAH disease, considering that the PK profile of zamicastat may be different in PAH patients due to the known haemodynamic changes.

6.4 Benefit-Risk Considerations

Zamicastat is a newly developed reversible DßH inhibitor. Preclinical results suggest that PAH patients potentially benefit from zamicastat treatment.

Potential risks of study participation are experiencing an ADR to treatment with zamicastat. A detailed description of preclinical and clinical experience with zamicastat and of all treatment emergent adverse events (TEAEs) is provided in the IB. All reported related TEAEs were of mild or moderate intensity and resolved without sequelae. [4] No deaths and SAEs were reported. The safety of the patients will be monitored by a data safety monitoring board (DSMB).

In case of unusual symptoms or questions the patients can always contact the investigator and arrange an unscheduled visit (UV). The planned study procedures, standard examinations and questionnaires, will not pose a risk to the patients other than those associated with assessments in general common clinical practice.

This study will be performed in PAH specialised centres. The study will be conducted in compliance with this protocol, by study personnel who are qualified by education, training, and experience in their roles, with adherence to Good Clinical Practice (GCP), the applicable regulatory requirements, and ethical principles based on the Declaration of Helsinki.

7. STUDY OBJECTIVES

7.1 Primary Objective

The primary objective of this study is to evaluate the PK profile of different zamicastat doses in PAH patients to find the most promising therapeutic dosage range for the treatment of PAH disease.

7.2 Secondary Objectives

The secondary objectives of this study are:

1. To assess further PK parameters including apparent total clearance, terminal half-life and apparent volume of distribution
2. To assess the safety and tolerability of different zamicastat doses
3. To investigate the effect of different zamicastat doses on plasma DβH activity
4. To investigate the effect of different zamicastat doses on urinary catecholamine levels (norepinephrine and dopamine)
5. To investigate the change in pulmonary haemodynamic parameters
6. To investigate the change in the 6-minute walk test (6-MWT)
7. To investigate the change in biomarkers
8. To investigate the change in cardiac structure and contractibility, assessed by echocardiography
9. To investigate the change in quality of life, assessed by SF-36v2

8. STUDY DESIGN

8.1 Overall Study Design and Plan

This is an open-label, multi-centre study in patients with PAH who are currently on stable treatment with at least one PAH medication. It is planned to evaluate the PK profile (24-hour profile and trough levels) and the safety, tolerability and efficacy of four different zamicastat doses. Each patient will start treatment with the lowest dose (50 mg zamicastat once daily) and the dose will be up-titrated to the individual highest tolerated dose (HTD) i.e. up to 200 mg zamicastat once daily.

A DSMB will periodically review the safety data and will issue a recommendation if the doses can be used as planned.

This study will consist of:

- A screening period, 5 to 12 days: visit V1
- Up to four dose finding periods, 14 days each:
  - Dose A: visits A1, A2 and A3
  - Dose B: visits B2 and B3
  - Dose C: visits C2 and C3
  - Dose D: visits D2 and D3
- Maintenance period, 42 days: visits MPV1, MPV2 and MPV3
- Follow-up period:
  - Visit FU (down-titration): 14 days after MPV3 (only for patients taking 150 mg or 200 mg zamicastat who will not participate in the extension study)
  - Visit FU: 14 days after last IMP intake

After confirmed eligibility, patients will initiate treatment with Dose A (50 mg zamicastat once daily) at visit A1 and will be treated with this dose level for 14 days. If Dose A was considered safe by the investigator, it will be up-titrated to Dose B (100 mg zamicastat once daily) at visit A3 and the patient will continue with this dosage for 14 days. Patients not tolerating Dose A have to be withdrawn from the study.

The up-titration is based on safety criteria, including but not limited to:

- Absence of symptomatic and/or clinically relevant orthostatic hypotension
- Systolic blood pressure (SBP) > 95 mmHg or diastolic blood pressure (DBP) > 50 mmHg
- Absence of clinically significant changes in ECG parameters (heart rate, P-R interval, QRS duration, QT and QTc interval)
- Absence of clinically significant hypersensitivity/skin allergic reactions

After a positive safety evaluation at visit B3, the dose will be up-titrated to Dose C (150 mg zamicastat once daily) and the patient will continue on this dose level for a further 14 days. If Dose C was tolerated by the patient, it will be up-titrated at visit C3 to Dose D (200 mg zamicastat once daily) where the patient will remain for 56 days (14 days dose finding period + 42 days maintenance period).

Patients not tolerating their next dose at any time during the 14-days dose finding period will come immediately to the site or call the investigator (unscheduled visit), unless intolerance was detected during a scheduled visit and the dose will be down-titrated to the patient's previous

tolerated dose. Then they will continue this treatment for 42 days during the maintenance period.

The dose which will be taken during the maintenance period will be considered as an individual HTD.

At the end of the maintenance period, the patient will have the opportunity to continue treatment in an extension study (BIA-51058-202), which will be described in a separate protocol.

Patients not tolerating their individual HTD during the maintenance period have to be withdrawn from the study. An overview of the study design is provided in Figure 1.

8.2 Discussion of Study Design

Zamicastat is currently being developed for the treatment of cardiovascular disorders, including PAH. At this level, previous clinical experience with zamicastat includes phase I clinical studies in healthy volunteers in a wide doses range (5 mg to 2400 mg in single-dose conditions and 50 mg to 1200 mg in multiple-dose conditions), which were found to be well tolerated.

Nevertheless, patients with PAH frequently present an altered haemodynamic, as well as other individual factors, which may change the drug pharmacokinetics in relation to healthy volunteers.

Thus, in this study, PAH patients on stable treatment with at least one approved PAH therapy will be included and treated with the individual HTD zamicastat dose for 42 days during the maintenance period.

In a clinical study with healthy subjects, PK and PD analysis demonstrated that the dose range between 50 mg and 200 mg zamicastat once daily is the most promising therapeutic dose range, considering a DβH inhibition of 30-40% as the target pharmacodynamic effect. Moreover, it is expected that this dose range is considerably conservative to account for potential pharmacokinetic differences between PAH patients and healthy volunteers. Noteworthy, the maximum dose planned for PAH patients in the present clinical study (200 mg) is 6-fold lower than the maximum multiple-dose tested in healthy volunteers (1200 mg, study BIA-51058-105).

Bearing in mind the exploratory character of the study, the zamicastat dose will be increased in steps of 50 mg every 14 days up to the maximum dose of 200 mg zamicastat. Thereby, the dose increase is based on safety criteria to guaranty the safety of the patients. Additionally, the patient's safety will be supervised by a DSMB.

The treatment duration of 42 days at the individual HTD will be sufficient to investigate PK/PD parameters and safety aspects of zamicastat. Patients benefiting from treatment with zamicastat will be offered to participate in a subsequent extension study which will be described in a separate protocol.

8.3 Duration of the Study

First patient first visit (start date): May 2019

Last patient last visit (end of study): Nov 2020

9. STUDY POPULATION

9.1 Selection of Study Population and Diagnosis

This study will be performed in male and female PAH patients (WHO Group 1) aged 18 to 75 years, inclusive. The patients have to be on stable treatment with at least one approved PAH therapy within the 3 months before V1 (for details see Section 10.8.1). Additionally, patients have to be classified by the investigator as WHO functional class II or III according to ESC/ERS guidelines. [8] The sample size rationale is given in Section 13.3.

Patients will be enrolled in approximately 15 sites in 9 European countries. Other sites and other countries may be added as needed.

9.2 Inclusion Criteria

At **Visit 1 (Screening)**, patients must meet ALL of the following criteria:

1. Male or female patients aged 18 to 75 years, inclusive.
2. Able to comprehend and willing to sign an informed consent form (ICF).
3. Diagnosis of PAH (pulmonary arterial hypertension WHO Group 1) documented by RHC with a mPAP $\geq$ 25 mmHg, a PAWP $\leq$ 15 mmHg and a PVR > 3 WU [8,9]:
    a) Idiopathic, in non-vasoreactive patients
    b) Heritable: Bone morphogenetic protein receptor type II (BMPR2) mutation and other mutations, in non-vasoreactive patients
    c) Drugs and toxin induced, in non-vasoreactive patients
    d) Associated with connective tissue disease
    e) Associated with simple congenital defects (atrial septal defect and/or ventricular septal defect) if closed > 12 months before inclusion.
4. The patient's last right heart catheterisation results, which were measured at the study site, must not be older than 90 days before V1 (will be considered as baseline value). Otherwise a right heart catheterisation has to be performed as part of the study at visit A1.
5. WHO functional class II or III as judged by the investigator.
6. Stable treatment with at least one of the following approved PAH therapies for at least 90 days prior to V1: Ambrisentan, Bosentan, Macitentan, Riociguat, Selexipag, Sildenafil, Tadalafil, Epoprostenol intravenous, Iloprost inhaled or Treprostinil intravenous or subcutaneous.
7. For women: Agree not to donate ova from the time of informed consent until 30 days after the last IMP intake.

    For men: Agree not to donate sperm from the time of informed consent until 90 days after the last IMP intake.

9.3 Exclusion Criteria

Patients are to be excluded from the study for ANY ONE of the following reasons:

Criterion:	Rationale:
1. Contraindication to zamicastat, i.e. known hypersensitivity to ingredients of zamicastat formulation.	Lack of suitability
2. Two or more consecutive measurements of SBP < 95 mmHg or DBP < 50 mmHg measured at V1.	Safety

3. Uncontrolled diabetes mellitus with HbA1c ≥ 8.5% within the last three months or at screening.	Safety
4. PAH WHO Group 1 due to portal hypertension, human immunodeficiency virus (HIV) infection and schistosomiasis.	Lack of suitability
5. Any disease known to cause pulmonary hypertension other than PAH WHO Group 1.	Lack of suitability
6. Obstructive lung disease: Forced Expiratory Volume in 1 second/Forced Vital Capacity (FEV1/FVC) < 60% and FEV1 < 60% of predicted value after bronchodilator administration, as demonstrated and documented by previous spirometry data which, in the opinion of the investigator, represent the clinical state of the patient at the time of the screening visit.	Safety
7. Restrictive lung disease: Total Lung Capacity (TLC) < 70% of predicted value, as demonstrated and documented by previous spirometry data which, in the opinion of the investigator, represent the clinical state of the patient at the time of the screening visit.	Safety
8. History of moderate to severe hepatic impairment (Child-Pugh B and C).	Safety
9. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2 (measured at V1).	Safety
10. Use of the following prohibited medication or treatments during study participation: calcium channel blockers (CCBs) if used for the treatment of PAH in vasoreactive patients; drugs containing a catechol group that is metabolised by DβH e.g. rimiterole, isoprenaline, dopamine, dopexamine or dobutamide or α- and/or β-blockers.	Safety
11. Current or previous (within the past year) alcohol or substance abuse excluding caffeine or nicotine.	Lack of suitability
12. Presence of any other significant or progressive/unstable medical condition that, in the opinion of the investigator, would compromise evaluation of the study treatment or may jeopardise the patient's safety, compliance or adherence to protocol requirements.	Safety
13. For women: Pregnancy or breast-feeding. Women of childbearing potential (as defined in Section 4.2) unable or unwilling to undergo pregnancy tests and practice highly effective contraceptive measures in combination with a barrier method e.g. condom (without spermicidal foam/gel/film/cream/suppository or fat- or oil-containing lubricants), occlusive cap (diaphragm or cervical/vault caps) with spermicidal gel/film/cream/suppository from the time of informed consent until 30 days after last IMP intake. Highly effective methods for women are surgical intervention (e.g. bilateral tubal occlusion), non-hormonal implantable intrauterine device, true sexual abstinence (i.e. when this is in line with the preferred and usual lifestyle of the patient) and vasectomised partner (provided that the partner is the sole sexual partner of the patient and the partner has received medical assessment of the surgical success). Periodic abstinence (e.g.	Safety

calendar, ovulation, symptothermal, post-ovulation methods), hormonal contraceptives and withdrawal are not acceptable methods of contraception. For men: Male patients who are sexually active with a partner of childbearing potential must use, with their partner, a condom plus an approved acceptable contraceptive measure from the time of informed consent until 90 days after the last IMP intake. The following methods are acceptable methods of contraception: partner's use of combined (oestrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal); partner's use of progestogen-only hormonal contraception (oral, injectable/implantable, intrauterine hormone-releasing system); partner's use of implantable intrauterine device; surgical sterilisation (for example, vasectomy or bilateral tubal occlusion).	
14. Previous participation in any other drug investigational study within the past 30 days (or five half-lives of investigational medicinal product [IMP] whichever is longer) prior to V1.	Lack of suitability
15. Vulnerable patients according to Section 1.61 of the ICH guideline for Good Clinical Practice E6	Vulnerable patients according to GCP 1.61

9.4 Screening Failures and Withdrawals

9.4.1 Screening failures

Screening failures will be all patients who have been enrolled in the study (i.e. ICF signed), but discontinue the study before the first IMP intake at visit A1 due to whatever reason (withdrawn consent, do not fulfil criteria, by decision of investigator, etc.).

One additional attempt at screening may be permitted within 4 weeks.

In addition to their records of performed assessments, screening failures have to be documented on the termination page of the electronic case report form (eCRF).

9.4.2 Withdrawals

Patients who have been enrolled and, for whatever reason, discontinue the study after first IMP intake at visit A1 are classified as withdrawals. Patients may withdraw from the study at any time, either on their own request or at the discretion of the investigator.

Reasons for withdrawal must be documented carefully in the patient's medical records and in the eCRF. Investigators must attempt to contact patients who fail to attend scheduled visits by telephone, letter, visit, etc., to exclude the possibility of an AE being the cause. Should this be the case, the AE must be documented, reported and followed up as described in Section 12. Attempts to contact the patient must be documented in the patient's medical records. The project leader and the medical monitor of the study at Bial will be informed of each withdrawal and the reason for it. To minimize the loss to follow-up, the specified patient's family member/friend (if available) will be contacted. An early discontinuation visit (EDV) (see Section 11.6.7) should be performed within 10 days, if possible, on each withdrawal. However, in case of early discontinuation due to withdrawal of consent, the patient will have the right to refuse this additional visit and phone calls and no further data will be collected for this patient apart from the reason for discontinuation. The investigator should attempt to retrieve all IMPs from all patients.

Patients may withdraw or may be withdrawn from the study for the following reasons:

- At their own request (withdrawal of consent)
- If in the investigator's opinion, for reasons of safety or ethics, continuation in the study would be detrimental to the patient's well-being
- Pregnancy
- Ineligibility/development of an exclusion criterion
- In case of an intolerable adverse event(s)
- At the specific request of the sponsor
- Lost to follow up
- Other reasons

Patients who are withdrawn are not to be replaced.

9.5 Termination of Study

9.5.1 Regular termination of study

The end of this study is defined as the date of last patient's last visit (LPLV) undergoing this study. Within 90 days of the end of a clinical study SCOPE will notify Independent Ethics Committees (IECs) and regulatory authorities about the regular termination of the study as required according to national laws and regulations. If the study has to be terminated early, this period shall be reduced to 15 days and the reasons must be clearly explained.

9.5.2 Premature termination of study

The study may be terminated prematurely for any reason and at any time by Bial, IECs, or regulatory authorities. A decision to prematurely terminate the study is binding to all investigators of all study centres. IECs and regulatory authorities will be informed about reason and time of termination according to the applicable laws and regulations.

Once dosing has begun, the study will be completed as planned unless the following criteria are satisfied that require temporary suspension or early termination of the study.

- One SAE in one patient considered at least possibly related to the IMP administration will imply an urgent benefit-risk assessment and might result in a premature study termination in favour of patients' well-being.
- New information regarding the safety of the IMP becomes available, reviewed by the DSMB, and indicating a change in the risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for patients participating in the study. The DSMB will also periodically review the safety data and is empowered to recommend modifications of the protocol (dosage levels) or temporary suspension or early termination of the study, if major concerns arise about patient safety at any time during the course of the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises patient safety.

If the study is terminated prematurely, all investigators have to inform their patients in writing and take care of appropriate follow-up and further treatment of the patients.

9.5.3 Premature termination of the study in a study centre

The sponsor reserves the right to discontinue the study at a specific study site any time. The reasons will be discussed with the principal investigator. A study site may also be discontinued

by the sponsor for major deviations from the protocol or due to difficulties experienced in running the study at that centre.

Bial may terminate this study in one particular or several study centre(s) for one of the following reasons:

- Non-compliance with GCP and/or regulatory requirements
- Centre cannot recruit an adequate number of patients
- False documentation in the eCRF due to carelessness or deliberately
- Inadequate co-operation with Bial or its representatives
- The principal investigator requests closure of his/her study centre

If the study is prematurely terminated in one (or more) study centre(s), all investigators in this study centre (in these study centres) have to inform their patients and take care of appropriate follow-up and further treatment of the patients. IEC and regulatory authorities will be informed about reason and time of termination according to the applicable laws and regulations.

10. TREATMENTS

10.1 Identity of Investigational Medicinal Product(s)

Name(s):	Zamicastat (BIA 5-1058)
Dosage form:	Tablet
Active ingredient:	Zamicastat; 1-[(3R)-6,8-difluoro-3,4-dihydro-2H-1-benzopyran-3-yl]- 1,3-dihydro-5-[2-[(phenylmethyl)amino]ethyl]-2H- imidazole-2-thione
Strength:	100 mg of zamicastat
Excipients:	Microcrystalline cellulose (Avicel PH 101®), lactose 200M, povidone K-30, croscarmelose sodium, magnesium stearate, OPADRY® II 85F205017 blue and purified water (does not appear in the final product)
Presentation:	Blue oblong tablets, grooved on both sides, without further engravings
Pharmaceutical Manufacturer:	BIAL - Portela & C[a], S.A., À Av. da Siderurgia Nacional, 4745-457 Coronado (S. Romão e S. Mamede), Portugal

A complete record of batch numbers and expiry dates of all IMP will be maintained in the TMF.

This is an open-label study. IMP will be manufactured, packed and labelled according to current Good Manufacturing Practice (GMP) and GCP guidelines, and national legal requirements.

10.2 Identity of Non-Investigational/Auxiliary Medicinal Product(s)

Not applicable

10.3 Selection of Doses in the Study

10.3.1 Selection and timing of dose for each patient

Each patient will start treatment with 50 mg zamicastat once daily (Dose A) at visit A1 and will be up-titrated step by step to the individual HTD i.e. up to 200 mg zamicastat once daily. The 100 mg tablet has to be split at the break score in two equal halves using a pill splitter.

Table 2: Dosage regimen

	Daily dose
Dose A	50 mg (half a tablet of 100 mg)
Dose B	100 mg (one tablet of 100 mg)
Dose C	150 mg (one and a half tablet of 100 mg)
Dose D	200 mg (two tablets of 100 mg)

Each next dose will start after 14 days of treatment if the previous dose was considered safe by the investigator. The up-titration is based on the following safety criteria, including but not limited to:

- Absence of symptomatic and/or clinically relevant orthostatic hypotension
- SBP > 95 mmHg or DBP > 50 mmHg
- Absence of clinically significant changes in ECG parameters (heart rate, P-R interval, QRS duration, QT and QTc interval)
- Absence of clinically significant hypersensitivity/skin allergic reactions

Patients not fulfilling the criteria for up-titration after 14 days of treatment with 50 mg zamicastat have to be withdrawn from the trial.

If the patient's next dose will not be tolerated by the patient, the dose has to be down-titrated to his/her previous dose and this dose will be considered as the individual HTD, if tolerated by the patient. Treatment at the individual HTD will be for 42 days during the maintenance period.

Zamicastat has to be taken in the morning after breakfast. The patient must not take the tablet at home on visit days (A3/B3/C3/D3, MPV1, MPV2 and MPV3).

In case a patient missed to take the dose after breakfast, (s)he should take the dose after lunch. The next dose should be taken as scheduled. If the dose was missed after lunch too, the dose for this day has to be skipped.

Patients taking 150 mg or 200 mg of zamicastat and who will not participate in the extension study will take 100 mg zamicastat for 14 days.

In case a patient will discontinue the trial early due to the occurrence of an AE, down-titration is not mandatory, but at the investigator's discretion. In case down-titration is recommended by the investigator, it should follow the same procedure as described above.

10.3.2 Dose adjustment criteria

For dose adjustment please refer to Section 10.3.1.

10.4 Method of Assigning Patients to Treatment Groups

10.4.1 Randomisation

Not applicable to this study.

10.4.2 Blinding

Not applicable to this study.

10.5 Labelling and Packaging

Packaging of study medication will be performed by Bial - Portela & C[a], S.A., 4745-457 Coronado (S. Romão e S. Mamede), Portugal. Labelling and shipping of the study medication might be subcontracted to a contract manufacturing organisation.

Zamicastat (BIA 5-1058) tablets will be provided in HDPE bottles, labelled specifically for this clinical study. The labels for the IMP will be in accordance with the GMP, more specifically with the annex 13, and with local regulations i.e. label terminology may vary according to local regulations, and country-specific remarks will be added as needed. Adequate labels with the relevant information will be affixed to each bottle. Each bottle label will bear the following information: name, address and phone number of sponsor, name of principal investigator (information to be added/completed by the site), protocol code, patient number (information to be added/completed by the site), batch number, bottle number, open expiry

date, storage conditions, pharmaceutical dosage form and strength, route of administration, dosing instructions, number of tablets (content), and the following statements: "For clinical trial use only"," Do not store above 25°C" and "Keep out of the sight and reach of children". Sample labels will be filed in the TMF.

Each patient will be supplied with IMP sufficient for periods of 14 days, including additional medication for visit windows, taking also into account the highest daily dose of 200 mg zamicastat (i.e. two tablets of 100 mg daily).

10.6 Supply, Storage, Dispensing and Return

The IMP is to be used exclusively in the clinical study according to the instructions of this protocol.

The investigator must confirm the receipt by completing an acknowledgment of receipt form (AoR) of the IMP with his/her signature.

A USB temperature monitor will be included in the shipments. Once the shipment arrives at the site, the staff must download the temperature data immediately and print out the PDF report. This report should be filled together with the shipment documentation in the study file. In case of excursions, the Sponsor must be contacted to decide on the IMP final disposition.

A copy of all the documentation, including AoR must be kept by the investigator and another copy will be stored at SCOPE.

IMP must be stored in securely locked areas not generally accessible until dispensed to the patients.

The investigator/institution and/or a pharmacist or other appropriate individual, who is designated by the investigator/institution, will maintain records of the IMPs' delivery to the study site, the inventory at the site, the use by each patient, and the return to the sponsor or alternative disposition of unused study medication(s). These records will include dates, quantities, batch/serial numbers, expiration dates, and any unique code numbers assigned to the IMP(s) (kit number) and study patient (subject number). The investigator will maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all IMP received from the sponsor.

Zamicastat should be kept out of the reach and sight of children and at temperatures up to 25 ºC. Additionally, it should be kept in the original container. The storage temperature should be recorded continuously and documented, e.g. by system print-outs or by documenting minimum/maximum temperature on logs. Temperature logs should be completed once daily on each working day.

The investigator or an authorised delegate is responsible for the dispensing of medication according to the dosage scheme (see Section 10.3).

At the end of the study, or as directed during the course of the study, all IMP supplies (including partial and empty packaging) will be returned to the study medication supplier with the accountability sheets. However, before IMP will be returned to the study medication supplier, accountability has to be performed by the site.

10.7 Treatment Compliance and Drug Accountability

At each study visit, the patient will be reminded of the importance of taking the IMP exactly as prescribed. IMP is to be taken by study patients only, during the course of the study. Study staff will dispense the appropriate amount of IMP for each patient and for each treatment interval plus additional medication. At each on-site visit, patients must bring back the IMP

(including empty and partially empty containers) and drug accountability must be performed and documented.

10.8 Prior and Concomitant Therapy

10.8.1 Previous therapy

Patients must be on stable treatment with at least one of the following approved PAH therapies for at least 90 days prior to V1:

- Ambrisentan
- Bosentan
- Epoprostenol intravenous
- Iloprost inhaled
- Macitentan
- Riociguat
- Selexipag
- Sildenafil
- Tadalafil
- Treprostinil intravenous or subcutaneous

10.8.2 Concomitant medication

Treatment with the following medications and therapies is prohibited:

- CCBs, if taken for the treatment of PAH in vasoreactive patients.
- Drugs containing a catechol group that is metabolised by DβH e.g. rimiterole, isoprenaline, dopamine, dopexamine or dobutamide
- α- and/or β-blockers

The potential for drug interactions has not yet been assessed. Therefore, the use of concomitant medications should be cautiously evaluated and avoided whenever possible.

10.8.3 Other restrictions

There are no study restrictions with respect to caffeine and smoking. Grapefruits as well as foods or drinks containing grapefruit are not permitted during entire study participation (from V1 to FU).

Since zamicastat increases dopamine exposure, dopaminergic effects associated with dopaminergic therapy, such as hallucinations, nausea, dyskinesia, orthostatic hypotension and syncope, may be potentiated. Patients should be informed about the possible occurrence of such effects and cautioned against rising rapidly after sitting or lying down. Patients should be advised not to drive or operate dangerous machinery. Because of potential additive sedative effects, caution should be used when taking central nervous system (CNS) depressants.

Additional information regarding contraindications, precautions and warnings concerning zamicastat are provided in the current version of the IB. [4]

10.9 Further Treatment after the End of the Study

After the end of the study, patients will be offered to take part in the subsequent extension study which is described in a separate protocol.

11. ASSESSMENTS

The assessments and procedures will be performed at the timepoints indicated in the schedule of study procedures (page 14).

11.1 Baseline Characteristics

11.1.1 Demographic data, medical history and concomitant diseases

Demographic data (age, gender and race), relevant medical history for former 6 months (longer in case of PAH relevance) and concomitant diseases or interventions are to be documented.

Additionally, height and weight will be documented.

11.1.2 New York Heart Association/Word Health Organisation functional class (NYHA/WHO)

Classification of functional status of patients will be performed by the investigator by using the NYHA/WHO classification system. [7]

Table 3: Functional classification of pulmonary hypertension modified after the NYHA functional classification according to the WHO 1998

Class	Description
Class I	Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain or near syncope.
Class II	Patients with pulmonary hypertension resulting in a slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain or near syncope.
Class III	Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnoea or fatigue, chest pain or near syncope.
Class IV	Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity.

11.2 Pharmacokinetic and Pharmacodynamic Assessment Criteria

It has to be ensured that PK/PD blood sampling will be performed at the indicated time points.

11.2.1 Pharmacokinetics

<u>24-hour profile</u>

Blood sampling for PK assessments will be performed at the following time points, preferably using an in-dwelling catheter:

- Prior to IMP intake (0 hours)
- 1, 2, 4, 8 and 16 hours after IMP intake
- 24 hours after IMP intake, but still prior to next IMP intake (i.e. at the end of one dosing interval)

Blood samples of approximately 8-9 mL have to be taken at each sampling time point (includes the sample for PK and plasma DβH activity). Blood samples from central catheters or port

systems should be withdrawn according to respective clinic standards, safeguarding that blood samples will not be diluted and no residual amounts of other drugs are included.

Trough level assessment

A blood sample of approximately 8-9 mL will be taken immediately before the intake of the next zamicastat dose (includes the sample for PK and plasma DβH activity).

The site will instruct the patients not to take the tablet at home on the day of an on-site visit.

If the tablet was already taken by the patient on the day of the visit before blood withdrawal or if the tablet of the day before this visit was not taken by the patient, the blood sample should be taken on the next day.

A total blood volume of 180 mL at maximum will be collected together for PK and PD analysis (24-hour profile and trough level assessment). The time of the previous dose of IMP (not at visit A1) and time of sampling must be recorded in the eCRF. Plasma concentrations of zamicastat and its metabolites will be determined by a central laboratory for PK analyses (SYNLAB Analytics & Services Switzerland AG, Birsfelden, Switzerland). Details regarding the collection, storage and shipment of samples and reporting of results will be outlined in a laboratory manual, which will be supplied to all sites.

Plasma concentrations of zamicastat and its metabolites will be used for evaluation/calculation of PK parameters as outlined in detail in Section 13.4.1 and Section 13.4.4.

For diet restrictions please refer to Section 10.8.3.

11.2.2 Pharmacodynamics

The assessment of plasma DβH activity (octopamine levels) will be performed centrally by Anapharm, Barcelona, Spain. Blood sampling for the assessment of DβH activity will be performed together with the PK blood sampling (see Section 11.2.1).

Catecholamine levels (norepinephrine and dopamine) will also be assessed centrally by The Doctors Laboratory Ltd, London, United Kingdom. For the assessment of catecholamines, a 24-hour urine collection will start on the day before the respective visit.

Details regarding the collection, storage and shipment of samples and reporting of results will be outlined in a laboratory manual, which will be supplied to all sites.

Urine catecholamine levels (24-hour collection) and determination of plasma DβH activity by measuring octopamine levels will be used for the evaluation/calculation of PD parameters as outlined in detail in Section 13.4.5.

11.3 Safety Assessment Criteria

11.3.1 Adverse events

For adverse events see Section 12.

11.3.2 Clinical laboratory evaluations

A central laboratory (LKF Laboratorium für Klinische Forschung GmbH, Schwentinental, Germany) will be used for all safety laboratory analyses and the blood pregnancy test. Details regarding the collection, shipment of samples, reporting of results, and alerting of abnormal values will be outlined in a laboratory manual, which will be supplied to all sites. For these tests, approximately 12 mL of blood will be collected at each blood withdrawal, resulting in a total amount of 96 mL at maximum.

Urine safety analysis and urine pregnancy tests will be performed on-site. Microscopy and other appropriate tests (as needed) will be performed by the central laboratory if dipstick indicates any clinically relevant abnormality, as judged by the investigator.

All laboratory reports must be reviewed for clinical relevance, signed and dated by the investigator. A copy of all reports must be filed in patient's medical record (source document) for that visit. Urinalysis results will be recorded in patient's medical record (source document) for that visit.

Haematology: haemoglobin, haematocrit, red blood cell count (RBC), white blood cell count (WBC), differential - neutrophils, eosinophils, lymphocytes, monocytes and basophils, and platelet count

Coagulation: prothrombin time test (International normalised ratio [INR])

Biochemistry: sodium, potassium, chloride, calcium, phosphate, blood urea nitrogen, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine phosphokinase (CPK), creatinine, glucose, C-reactive protein, albumin, total protein, total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, triglycerides, total bilirubin (bilirubin will be fractionated direct/indirect if elevated) and haemoglobin A1c (HbA1c)

eGFR [13] will be estimated at V1 and MPV1 based on serum creatinine value using the according CKD-EPI formula using age, sex and race as follows:

eGFR [mL/min/1.73 m^2]

= 141 x Min (S_{Cr}/κ, 1)α x Max (S_{Cr} /κ, 1)-1.209 x 0.993Age x 1.018 [if female] x 1.159 [if Black]

Using the following abbreviations / units:

S_{Cr} (standardised serum creatinine) = [mg/dL]

κ = 0.7 (females) or 0.9 (males)

α = -0.329 (females) or -0.411 (males)

Min = indicates the minimum of S_{Cr}/κ or 1

Max = indicates the maximum of S_{Cr}/κ or 1

age = [years].

Arterial blood gas analysis: pH, oxygen partial pressure (pO_2), carbon dioxide partial pressure (pCO_2), bicarbonate, base excess (BE), arterial oxygen saturation (S_aO_2), electrolytes (Na^+ and K^+), and lactate.

The blood gas analysis should be performed from arterial or arterialised capillary blood; this analysis may be replaced by peripheral oxygen saturation in stable patients or if a blood gas analysis cannot be performed. Blood sampling has to be performed before right heart catheterisation and IMP intake. Blood gas analysis will be performed on-site.

Urinalysis: pH, specific gravity, protein, blood, glucose, ketones, bilirubin, urobilinogen, leucocytes and nitrites (local dipstick). Microscopy and other appropriate tests (as needed) will be performed if dipstick indicates any relevant abnormality, as judged by the investigator.

Blood pregnancy test: human chorionic gonadotropin (HCG), in females of childbearing potential

Urine pregnancy test: in females of childbearing potential.

11.3.3 Physical examinations

Physical examination will be performed for relevant body systems (appearance, skin, eyes, ears-nose-throat, lungs-chest, heart, abdomen, extremities, other).

11.3.4 Vital signs

Vital signs (systolic and diastolic blood pressure and heart rate) will be measured after the patient has rested for at least 5 minutes in supine position (first measurement) and at least one minute after the first measurement, again in supine position (second measurement). Afterwards, vital signs will be measured again in standing position after the patient has been standing for at least 3 minutes (third measurement).

At A1 and MPV3, blood pressure and heart rate will be measured before IMP intake as well as 4, 8 and 24 hours after IMP intake. Painful procedures, like drawing blood, should always be performed after vital signs measurements (not before).

Tympanic body temperature will be assessed once at all on-site visits.

11.3.5 Electrocardiogram

After each recording of a simultaneous 12-lead resting ECG, a copy will be printed and assessed by an investigator for at least the following parameters: heart rate, heart rhythm, P pulmonale, right axis deviation, RV hypertrophy, RV strain, right bundle branch block, P-R interval, QRS duration, QT interval and QTc interval. [8]

At visits A1 and MPV3 triplicate ECG recordings will be performed before IMP intake as well as 4 and 8 hours after IMP intake. Painful procedures, like drawing blood, should always be performed after ECG recordings (not before).

11.3.6 Number of digital scars

At screening the investigator will document the number of existing digital scars at the fingers and toes. At subsequent on-site visits the investigator will examine fingers and toes of the patient for digital scars and will document the number of digital scars. Additionally, the clinical examination will be supported by interviewing the patient. This assessment will be performed in scleroderma patients only.

11.3.7 Modified Rodnan skin score (mRSS)

The mRSS is a measure of skin thickness and is used as an outcome measure for systemic sclerosis (scleroderma).

This score consists of an evaluation of patient's skin thickness rated by clinical palpation using a scale from 0 to 3 (0=normal skin; 1=mild thickness; 2=moderate thickness; 3=severe thickness with inability to pinch the skin into a fold) for each of 17 surface anatomic areas of the body: face, anterior chest, abdomen, (right and left separately) fingers, forearms, upper arms, tights, lower legs, dorsum of hands and feet. These individual values are added and the sum is defined as the total skin score. [9]

11.4 Efficacy Assessment Criteria

11.4.1 36-item Short Form Survey version2 (SF-36v2)

The SF-36v2 asks 36 questions to measure functional health and well-being from the patient's point of view in eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions.

The questionnaire will be completed by the patient at the beginning of the visit.

11.4.2 Echocardiography

Transthoracic echocardiography is used to image the effects of PAH on the heart. The following parameters will be determined [6].:

- Tricuspid regurgitation, classified as absent, mild, moderate or severe
- Right ventricular contractility, measured via tricuspid annular plane systolic excursion ([TAPSE]; mm or cm)
- Pericardial effusion, classified as absent, traces or present
- Right atrial area (end-systolic), right ventricular end-diastolic area (cm^2)

A cardiologist will evaluate the echocardiography records.

11.4.3 6-min walk test (6-MWT)

The 6-MWT is a self-paced test of walking capacity used to assess aerobic capacity and endurance. It plays a key role in evaluation functional exercise capacity, assessing prognosis and evaluating response to treatment across a wide range of respiratory diseases. The distance covered over a time of 6 minutes is used as the outcome by which to compare changes in performance capacity. The 6-MWT will be performed according to the Technical Standard described by Holland et. al. [11].

At screening (V1) two tests must be completed, separated by at least 30 minutes. Additionally, heart rate and arterial oxygen saturation measured by pulse oximetry (SpO_2) must have returned to baseline prior to the second test. At visit MPV3 only one test will be performed after arterial blood gas analysis but before IMP intake.

11.4.4 Right heart catheterisation

Right heart catheterisation will be performed at visit A1, unless an RHC was performed within 90 days before visit V1 at the study site and the result of the following parameters is available: PVR, right atrial pressure (RAP), mPAP, cardiac index (CI) and mixed venous oxygen saturation ($SvO_{2\%}$). Additionally, an RHC will be performed at MPV3 to determine the change of haemodynamic parameters in the patients. The RHC has to be performed before IMP intake. The following haemodynamic parameters will be determined:

- Cardiac output (CO, L/min)
- Mixed venous oxygen saturation ($S_vO_{2,\%}$)
- Pulmonary artery pressure systolic, diastolic and mean (PAPs, PAPd and mPAP, mmHg)
- Pulmonary artery wedge pressure (PAWP, mmHg)
- Right atrial pressure (RAP, mmHg)
- Transpulmonary pressure gradient (TPG, mean PAP – mean PCWP)
- Diastolic pressure gradient (DPG, diastolic PAP – mean PCWP)
- Pulmonary vascular resistance (PVR; dyn x s xcm^{-5})
- Systemic vascular resistance (SVR, dyn · s/cm^5)
- Cardiac index (CI, $L/min/m^2$)
- Pulmonary arterial compliance (PAC; SV/PP, mL/mmHg)
- Heart rate (bpm)
- Stroke volume (mL/beat)
- Stroke volume index (mL/m^2)

RHC will be performed according to the standard procedures provided in the 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. [8]

If data from an RHC within 90 days before visit V1 are available, which were measured at the study site, the available data points will be transcribed to the eCRF and the procedure will not be repeated at visit A1.

11.4.5 Biomarker

For biomarker laboratory tests an additional amount of approximately 6 mL blood will be collected twice. The blood samples for biomarker assessment will be analysed by a central laboratory. Details regarding the collection, storage and shipment of samples and reporting of results will be outlined in a laboratory manual, which will be supplied to all sites.
The following biomarker will be analysed:

- N-terminal pro brain natriuretic peptide (NT-proBNP)

For possible further analyses of PAH biomarkers, remaining samples will be stored for a maximum of 10 years.

11.5 Appropriateness of Measurements

All measurements performed in this study are recognised standard methods. Therefore, no further details concerning reliability or relevance will be discussed here.

11.6 Description of Study Visits

For the schedule of study procedures, see Page 14.

11.6.1 Visit 1 (V1, Screening visit, Day -12 to -5)

The following assessments will be performed:

- Informed consent
- Demographics, including height and weight
- Medical history
- Prior and concomitant medication
- WHO functional class
- Inclusion/exclusion criteria
- SF-36v2
- AEs
- Vital signs* (blood pressure, heart rate and tympanic body temperature; blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position)
- 12-lead ECG*
- Blood withdrawal (haematology, coagulation and biochemistry, including hCG [in women of childbearing potential])
- Urinalysis
- Physical examination
- Number of digital scars, in scleroderma patients only
- mRSS, in scleroderma patients only
- 6-MWT, two baseline tests, separated by an interval of at least 30 minutes/Borg dyspnoea score

- Echocardiography
- Blood withdrawal (biomarker)

*Painful procedures should be performed only afterwards.

11.6.2 Visit A1 (Day 1)

The following assessments will be performed:

- Concomitant medication
- Vital signs* (blood pressure, heart rate and tympanic body temperature). Blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position before IMP intake as well as 4, 8 and 24 hours after IMP intake.
- Triplicate 12-lead ECG*, before IMP intake, 4 and 8 hours after IMP intake
- Urine pregnancy test, in women of childbearing potential
- Inclusion/exclusion criteria
- Arterial blood gas analysis (to be performed before first IMP intake and right heart catheterisation)
- Right heart catheterisation (unless RHC results which were measured at the study site from within 90 days before V1 are available), to be performed before first IMP intake
- Pharmacokinetic blood withdrawal (blood samples will be taken prior to IMP intake as well as 1, 2, 4, 8, 16 and 24 hours after IMP intake [but still prior to next IMP intake])
- Plasma DβH activity (blood samples will be taken prior to IMP intake as well as 1, 2, 4, 8, 16 and 24 hours after IMP intake [but still prior to next IMP intake])
- First IMP administration
- Urinalysis (dopamine and norepinephrine levels, 24-hour urine collection [from Day -1 to Day 1])
- Dispense IMP
- AEs

*Painful procedures should be performed only afterwards.

11.6.3 Visit A2/B2/C2/D2 (8 days ±2 after A1/A3/B3/C3)

This is a telephone visit. The following assessments will be performed:

- Concomitant medication
- AEs

11.6.4 Visit A3/B3/C3/D3 (14 days ±2 after A1/A3/B3/C3)

The following assessments will be performed:

- Concomitant medication
- Urine pregnancy test, in women of childbearing potential
- Vital signs* (blood pressure, heart rate and tympanic body temperature; blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position)
- 12-lead ECG*
- Pharmacokinetic blood withdrawal (trough level), blood sample will be taken prior to IMP intake

- Plasma DβH activity (trough level), blood sample will be taken prior to IMP intake
- Dose increase, if applicable
- Dispense IMP
- IMP accountability
- Urinalysis (dopamine and norepinephrine levels, 24-hour urine collection on the day before the visit)
- AEs
- Blood withdrawal (haematology, coagulation and biochemistry)
- Urinalysis
- Physical examination
- Number of digital scars, in scleroderma patients only

*Painful procedures should be performed only afterwards.

11.6.5 Visit MPV1 (13 days ±2 after D3/down-titration during the dose-finding period)

The following assessments will be performed:

- Concomitant medication
- Vital signs* (blood pressure, heart rate and tympanic body temperature; blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position)
- 12-lead ECG*
- Pharmacokinetic blood withdrawal (trough level), blood sample will be taken prior to IMP intake
- Plasma DβH activity (trough level), blood sample will be taken prior to IMP intake
- Urine pregnancy test, in women of childbearing potential
- Dispense IMP
- IMP accountability
- AEs
- Blood withdrawal (haematology, coagulation and biochemistry)
- Urinalysis
- Physical examination
- Number of digital scars, in scleroderma patients only

*Painful procedures should be performed only afterwards.

11.6.6 Visit MPV2 (27 days ±2 after D3/UV)

The following assessments will be performed:

- Concomitant medication
- Vital signs* (blood pressure, heart rate and tympanic body temperature; blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position)
- 12-lead ECG*
- Urine pregnancy test, in women of childbearing potential

- Pharmacokinetic blood withdrawal (trough level), blood sample will be taken prior to IMP intake
- Plasma DβH activity (trough level), blood sample will be taken prior to IMP intake
- Dispense IMP
- IMP accountability
- AEs
- Physical examination
- Number of digital scars, in scleroderma patients only

*Painful procedures should be performed only afterwards.

11.6.7 Visit MPV3 (41 days ±2 after D3/UV)/ EDV

In case of a patient's premature discontinuation, this visit should be performed as an EDV. The following assessments will be performed:

- Weight
- Concomitant medication
- WHO functional class
- Dispense IMP, only applicable in patients taking 150 mg or 200 mg zamicastat and who will not participate in the extension study
- IMP accountability
- Vital signs* (blood pressure, heart rate and tympanic body temperature). Blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position before IMP intake as well as 4, 8 and 24 hours after IMP intake.
- Triplicate 12-lead ECG*, before IMP intake, 4 and 8 hours after IMP intake. At EDV only one ECG recording will be performed.
- SF-36v2 (not applicable at EDV)
- Pharmacokinetic blood withdrawal (24-hour profile, not applicable at EDV), blood samples will be taken prior to IMP intake as well as 1, 2, 4, 8, 16 and 24 hours after IMP intake (but still prior to next IMP intake)
- Plasma DβH activity (24-hour profile, not applicable at EDV), blood sample will be taken prior to IMP intake as well as 1, 2, 4, 8, 16 and 24 hours after IMP intake (but still prior to next IMP intake
- Urinalysis (dopamine and norepinephrine levels, 24-hour urine collection on the day before the visit) (not applicable at EDV)
- AEs
- Blood withdrawal (haematology, coagulation and biochemistry)
- Urinalysis
- Physical examination
- Number of digital scars, in scleroderma patients only
- mRSS, in scleroderma patients only
- Urine pregnancy test, in women of childbearing potential
- 6-MWT, including Borg dyspnoea score (to be performed after arterial blood gas analysis but before right heart catheterisation and IMP intake [not applicable at EDV])

- Echocardiography (not applicable at EDV)
- Right heart catheterisation, to be performed before IMP intake (not applicable at EDV)
- Arterial blood gas analysis; blood sampling has to be performed before right heart catheterisation and IMP intake (not applicable at EDV)
- Blood withdrawal (biomarker), (not applicable at EDV)

*Painful procedures should be performed only afterwards.

11.6.8 Follow-up visit (FU [down-titration], 14 days ±2 after MPV3/EDV)

This is a telephone visit and will be performed in patients who will be down-titrated (see Section 10.3.1). The following assessments will be performed:

- Concomitant medication
- AEs

11.6.9 Follow-up visit (FU, 14 days ±2 after last IMP intake)

This visit will be performed in all patients who will not participate in the extension study as well as in patients who discontinued early. The following assessments will be performed:

- Concomitant medication
- IMP accountability, only applicable in patients who were down-titrated
- AEs
- Vital signs* (blood pressure, heart rate and tympanic body temperature; blood pressure and heart rate will be measured twice in supine position and afterwards once in standing position)
- 12-lead ECG*
- Urine pregnancy test, in women of childbearing potential
- Blood withdrawal (haematology, coagulation and biochemistry)
- Urinalysis

*Painful procedures should be performed only afterwards.

11.7 Unscheduled Visits

Unscheduled visits can be performed as on-site visit or as telephone contact at any time during the trial for any reason. If a visit outside of the protocol evaluation time points is performed, the eCRF form for unscheduled visits will be completed.

The following assessments will be at least performed:

- Concomitant medication
- AEs

Additional examinations and blood sampling may be performed as deemed necessary by the investigator.

If the patient's next dose will not be tolerated, the patient will be asked to come immediately to an on-site visit or call the investigator and the dose will be down-titrated to the patient's previous tolerated dose. The reason for down-titration has to be documented in the source data and eCRF.

Patients who will not continue in the extension study will be contacted by phone at the time of LPLV to document their vital status. If it is not possible to contact the patient, the specified

patient's family member/friend (if available) will be contacted. If a patient died the date of death has to be documented in the source data and eCRF.

12. ADVERSE EVENTS

12.1 Definitions

12.1.1 Adverse event

An AE is any untoward medical occurrence in a patient to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease occurring during the course of the study.

Laboratory/ECG/vital signs abnormalities should not be documented as AEs unless they are considered clinically significant, require treatment, fulfil any SAE criterion, or cause the patient to change the study schedule.

In the case of laboratory/ECG abnormalities that are a sign of a medical condition, the condition should be reported as an AE and not the sign.

Events occurring in patients in the course of a clinical study during treatment free periods are also to be considered AEs.

12.1.2 Adverse drug reaction

All untoward and unintended responses to an IMP related to any dose administered should be considered ADRs.

All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship ("possible" or "probable" or "definite") to a medicinal product qualify as an ADR.

12.1.3 Unexpected adverse drug reaction

An unexpected ADR is defined as an adverse reaction, the nature, severity, or outcome of which is not consistent with the applicable product information e.g, IB for an unapproved IMP or Summary of Product Characteristics for an approved drug. [4]

The term "expected" in pharmacovigilance, is not used to describe an event which might be anticipated from knowledge of the pharmacological properties of a substance. An event is also not to be described as "expected," merely because it was foreseeable due to the health status (e.g., age, medical history) of the study patient. It refers strictly to the event being mentioned or listed in the applicable product information.

In determining whether an AE is unexpected, consideration shall be given to whether the event adds significant information on the specificity, increase of occurrence, or severity of a known, already documented ADR.

12.1.4 Serious adverse event

An SAE is any untoward medical occurrence or effect that at any dose:

- results in death,
- is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- requires 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 requires intervention to prevent one of the above.

NOTE: Medical and scientific judgement should be exercised in deciding whether an event is "serious" in accordance with these criteria. Some medical events may jeopardise the patient or may require intervention to prevent one of the above characteristics/consequences. Such "important medical events" should also be considered as "serious" in accordance with the definition.

* "Hospitalisation" is to be considered only as an overnight admission.

EXCEPTION(S):

1) The hospitalisation or prolongation of hospitalisation needed for a procedure required by the protocol.
2) The hospitalisation or prolongation of hospitalisation as a part of a routine procedure followed by the centre (e.g. stent removal after surgery).
3) The hospitalisation for a survey visit, annual physicals, or social reasons.
4) Elective hospitalisations for pre-existing condition that had not worsened (e.g. elective hospitalisation for a total knee replacement due to a pre-existing condition of osteoarthritis of the knee that had not worsened during the course of the study).

12.1.5 Treatment emergent adverse event

An event that emerges during treatment having been absent prior to treatment or worsens relative to the pre-treatment state is defined as TEAE. In this clinical study, all AEs with onset or worsening after first intake of IMP until 14 days after last intake of IMP are defined as treatment emergent. For medical conditions diagnosed at screening also see Section 12.2.

12.1.6 Suspected unexpected serious adverse reaction

A suspected unexpected serious adverse reaction (SUSAR) is an adverse reaction that is both unexpected and serious.

12.1.7 Severity of adverse events

The maximum severity (intensity) of the AE will be categorised by the investigator as follows:

Mild: a type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: a type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but possess no significant or permanent risk of harm to the research participant.

Severe: a type of AE that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects clinical status. The event possesses a significant risk of harm to the research participant and hospitalisation may be required.

12.1.8 Drug relationship of adverse events

The relationship of an AE to the IMP is a clinical decision by the investigator based on all available information and is graded as follows:

Not related: A clinical event with no evidence of any causal relationship.

Unlikely: A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Possible: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Probable: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.

Definite: A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Mapping to the two categories "related" and "unrelated":

The categories "not related" and "unlikely" will be mapped to "unrelated", the categories "possible", "probable" and "definite" will be mapped to "related".

12.1.9 Adverse event outcome

The outcome will be classified as follows:

Recovered/Resolved: The patient has fully recovered from the event or the condition has returned to the level observed at baseline.

Recovering/Resolving: The patient is improving but is still not fully recovered.

Not recovered/Not resolved: As a final outcome, the condition is still present after appropriate follow-up. Improvement has not been observed and is no longer expected.

Recovered/Resolved with sequelae: As a result of the AE, the patient suffers persistent and significant disability/incapacity (e.g., became blind, deaf or paralyzed).

Fatal: The patient died due to the event.

Unknown: If outcome is not known or not reported.

12.1.10 Adverse events of special interest

Adverse events of special interest (AESI) are AEs that may constitute a hypersensitivity/allergic reaction (cutaneous and/or non-cutaneous presentation) assessed by the investigator as moderate or severe and at least possibly related to IMP.

The investigator will complete a Hypersensitivity/Allergic Reaction Adverse Event Report Form and (s)he will send this form to the SCOPE Safety Manager. Adverse events of special interest should be reported to the SCOPE Safety Manager following the SAE notification timelines (see Section 12.3.1).

Whenever available, photographs of the affected skin area(s) should be provided to the SCOPE Safety Manager for assessment of individual events by the DSMB. In case an AESI is ongoing during a patient visit at the site, the site should take photographs of the affected skin area(s). Patients will also be advised to take photographs of the affected skin area(s) when a

hypersensitivity/allergic reaction occurs while not being at the site and to send these photographs to the site.

Further details on procedure regarding AESI will be described in the Safety Management Plan (SMP).

12.2 Documentation and Treatment of Adverse Events by the Investigator

All AEs, including SAEs, occurring within the period of observation for the clinical study must be recorded. The period of observation for the collection of AEs extends from the time when the patient gives informed consent until the date of final visit.

Medical conditions that are diagnosed at the screening visit will only be documented as AEs, if they are known to have started or are suspected to have started after the ICF has been signed. All other medical findings at the medical examination at the screening visit will be documented as medical history. Medical judgement should be exercised to estimate if a condition is likely to have started between the signing of the informed consent and the date/time of the medical examination.

Any AE that is still ongoing after final visit will be left as ongoing in the eCRF. However, the investigator will continue to follow up ongoing SAEs and record information in the source documents and on the Follow-Up SAE Form, until resolution or no further improvement can be expected.

There is no time limit on collection of SAEs that are considered related to study drug. If the investigator detects a SAE of a study patients after the last visit, and considers the event possibly related to prior study treatment or procedures, he/she should contact one of the Bial safety managers to determine how the event should be documented and reported.

At each visit, the investigator should ask the study patient in a non-leading manner about the state of his/her health in order to elicit information on AEs which may have occurred since the last visit. Any clinically significant observations made during the visit itself also constitute AEs.

The AEs must be documented as soon and as completely as possible on the “Adverse Events” pages in the eCRF. Follow up information must be entered as soon as available.

The following will also be specified:

- Action taken with IMP: dose not changed, dose reduced, drug interrupted, drug withdrawn, not applicable, unknown.
- Other actions (none, medication required, tests required, hospitalisation required or prolonged, withdrawn from the trial, other – please specify).
- Outcome of event (fatal, not recovered/not resolved, recovered/resolved, recovered/resolved with sequelae, recovering/resolving, unknown) and date of outcome.
- Seriousness: yes or no (criteria for SAE see above).
- Severity.
- Causal relationship to IMP

Adverse events which occur during the study should be treated by established standards of care that will protect the life and health of the patient.

12.3 Reporting of Serious Adverse Events

12.3.1 Reporting of serious adverse events to the sponsor

See Section 12.1.4 for the definition of an SAE and also for study-specific exceptions.

As soon as the investigator learns of the occurrence of an SAE he/she must:

- Ensure appropriate medical treatment.
- Decide whether to withdraw the patient.
- Immediately notify the SCOPE Safety Manager for the study (see below) by telephone, e-mail or fax, of the event, providing the following information: suspect medicinal product, clinical study, reporter (study centre, investigator), study patient identification and AE with reason for seriousness and causal relationship. If the initial SAE notification was done by phone, the SCOPE Safety Manager must have received, within 24 hours, the respective form. The "Adverse Events" pages in the eCRF must also then be filled out as always.
- Follow up as for all AEs. Basic follow up information must also be documented on the respective form and in the eCRF. On a revised version of the *SAE Report Form*, the box "Follow Up" must be ticked and it must be sent to the recipients of the initial information.
- If requested, ensure that further information about the event is supplied to the SCOPE Safety Manager (see below) as soon as available (e.g., autopsy/biopsy reports, hospital admissions or discharge summaries, summaries of laboratory tests and other diagnostic examinations).

SCOPE International Pharmacovigilance

E-Mail: safety@scope-international.com

Tel: +370 52 360 349

Fax: +370 52 327 903

The SCOPE Safety Manager must report the SAEs to Bial Pharmacovigilance within one working day of receipt.

12.3.2 Reporting of serious adverse events and other relevant safety information to the health authorities, ethics committees, study investigators

Expedited and periodic reporting required by the functionaries and institutions mentioned above will be fulfilled according to current local laws and guidance. The detailed reporting duties and division of responsibilities between the sponsor and the CRO will be detailed in the SMP.

12.4 Pregnancy

12.4.1 Patient's pregnancy

Pregnancy discovered during the clinical study must lead immediately to exclusion (if at Screening) or withdrawal of the patient.

A pregnancy with a calculated conception date after first IMP intake until 30 days after last IMP intake, should be documented on the corresponding form. The case must be reported within 24 hours of knowledge to SCOPE Safety manager. The investigator must follow up on pregnancies discovered after IMP administration until the end of pregnancy to document the outcome on the corresponding form. The event fulfils the criterion for an SAE in case of a congenital anomaly (birth defect), foetus death or spontaneous abortion, or adverse events in the neonate that are classified as serious.

12.4.2 Pregnancy of male patient's partner

A pregnancy with a calculated conception date after first IMP intake until 90 days after last IMP intake in the partner of a patient should be documented on the corresponding form. The case must be reported within 24 hours of knowledge to SCOPE Safety manager.

12.5 Overdose

There is no human overdose experience. There is no known specific antidote. Symptomatic and supportive treatment should be administered as appropriate. Removal of the drug by gastric lavage and/or inactivation by administering activated charcoal should be considered.

13. STATISTICS

This section presents a summary of the planned statistical analyses. All details of the statistical analyses and methods will be contained in a separate *statistical analysis plan* (SAP).

13.1 Variables

13.1.1 Primary endpoint; pharmacokinetics

The following PK parameters (24-hour profile) for zamicastat and its metabolites will be derived after a single dose of 50 mg zamicastat at visit A1 (Day 1):

1. Area under the curve 0-24 h ($AUC_{0\text{-}24h}$)
2. Maximum plasma concentration (C_{max})
3. Time until C_{max} (t_{max})

The following PK parameter (24-hour profile) for zamicastat and its metabolites will be derived at steady-state at the individual HTD and will be taken at visit MPV3.

4. Area under the curve 0-24 h ($AUC_{0\text{-}24h,SS}$)
5. Maximum plasma concentration ($C_{max,SS}$)
6. Time until $C_{max,SS}$ ($t_{max,SS}$)
7. Minimum plasma concentration at the end of the dosing interval ($C_{min,SS}$)

The following PK parameter (trough level) will be derived for each dose at steady-state.

8. Minimum plasma concentration at the end of the dosing interval ($C_{min,SS}$)

Additional PK parameters may also be calculated if considered appropriate.

13.1.2 Secondary endpoints

13.1.2.1 Safety and tolerability endpoints

1. Adverse events
2. Clinically relevant changes in laboratory parameters (haematology, biochemistry, coagulation, urinalysis, arterial blood gas analysis)
3. Clinically relevant changes in vital signs
4. Clinically relevant changes in ECG
5. Number of digital scars (only in patients with scleroderma)
6. Skin score (only in patients with scleroderma)

13.1.2.2 Efficacy endpoints

Change from baseline to MPV3 in:

7. PVR, RAP, mPAP, CI and SvO_2. Further haemodynamic parameters may also be calculated if considered appropriate.
8. 6-MWT, including Borg dyspnoea score
9. Biomarker (NT-proBNP)
10. Echocardiogram parameters:
    - Tricuspid regurgitation, classified as absent, mild, moderate or severe
    - Right ventricular contractibility, measured via TAPSE
    - Pericardial effusion, classified as absent, traces or present
    - Right atrial area (end-systolic), right ventricular end-diastolic area
11. WHO functional class

12. Quality of life (SF-36v2)

13.1.2.3 Pharmacokinetic endpoints

13. Apparent total clearance (CL/f)
14. Terminal half-life ($t_{1/2}$)
15. Apparent volume of distribution (Vz/f)

13.1.2.4 Pharmacodynamic endpoints

16. Percent inhibition in plasma DβH activity (i.e. % change from baseline of DβH activity) on visits A3/B3/C3/D3, MPV1 and MPV2 (trough samples) and to visits A1 and MPV3 (24-hour profiles)
17. Maximal and minimal observed effect on DβH activity and time to occurrence of E_{max} (T_{Emax}) and E_{min} (T_{Emin}) on visit A1 (Day 1) and MPV3
18. Area under the effect-time curve (AUEC24) for % DβH inhibition on visit A1 (Day 1) and MPV3
20. Change from baseline 24-hour urinary catecholamine levels (norepinephrine and dopamine) on visits A3/B3/C3/D3 and MPV3.

Additional parameters may be calculated if considered appropriate.

13.2 Analysis Populations

Analysis will be based on the following populations:

13.2.1 Enrolled Set

The enrolled set (ES) includes all patients who provided informed consent.

13.2.2 Full Analysis Set

The full analysis set (FAS) includes all patients with at least one IMP administration.

13.2.3 Pharmacokinetic Analysis Set

The pharmacokinetic analysis set (PKS) will include all patients with an evaluable 24-hour PK profile and no major protocol deviations with an impact on PK analysis.

13.2.4 Safety Set

The safety set (SS) includes all patients with at least one IMP administration. The FAS and the SS are identical.

13.2.5 Procedures for accounting for missing, unused and spurious data

All data will be summarised descriptively using the observed cases; no data imputation other than imputation of missing dates is required.

Imputation rules for partially or completely missing dates will be specified in the SAP.

13.3 Determination of Sample Size

The clinical study BIA-51058-201 was designed to explore for the first time the pharmacokinetics, safety and efficacy of zamicastat in PAH patients in its potential therapeutic dose range (50 mg to 200 mg). Since this is an exploratory and descriptive study and no powered comparative statistical analysis is planned, study sample size was not formally calculated. Thus, it was considered that 32 patients constitute an appropriate sample to meet the objectives of the study, considering the low prevalence of the disease and the consequent recruitment constrains.

13.4 Statistical Methods

13.4.1 Analysis of primary pharmacokinetic endpoints

The primary analysis of this study will be the analysis of the PK parameters derived by non-compartmental analysis from the plasma drug concentration versus time using Phoenix WinNonlin v8.0 or a more recent version (Certara USA, Inc., USA). Actual sampling times will be used. The area under the plasma concentration-time curve from time zero to 24 hours post-dose will be calculated using the linear trapezoidal rule.

Summary statistics will be reported, as appropriate, using the geometric mean, arithmetic mean, standard deviation, coefficient of variation, median, minimum, and maximum.

Further details of the statistical analysis and reporting of the pharmacokinetic endpoints, such as, assessment of dose proportionality (e.g. C_{maxSS} and $AUC_{0\text{-}24hSS}$) and time to steady state using concentration trough levels, will be included in a separate SAP.

13.4.2 Analysis of secondary efficacy endpoints

Efficacy data will be analysed exploratively and will be based on the FAS. Continuous measures will be summarised using summary statistics: mean, standard deviation, median, minimum and maximum, in addition for the changes from baseline a 95% confidence interval will be provided. Categorical variables will be summarised by using frequency counts and percentages.

The scores of each SF-36v2 subscale, as well as the Physical and mental Component summary scores (PCS and MCS), will be described using summary statistics. The derivation of PCS and MCS will be defined in the SAP.

13.4.3 Analysis of secondary safety endpoints

All safety analyses will be based on the SS. Treatment-emergent AEs will be summarised by Medical Dictionary for Regulatory Activities (MedDRA) primary system organ class (SOC) and preferred term (PT).

In addition, TEAEs will be summarised by severity and drug relationship. TEAEs leading to discontinuation from the study, AEs of special interest and serious TEAEs will be summarised sin addition. All AEs will be listed.

Laboratory, vital signs and ECG parameters will be described using summary statistics. The incidence of clinically relevant abnormalities in laboratory and ECG results will be described using frequency counts and percentages.

The number of digital scars and skin score in patients with scleroderma will be analysed using summary statistics.

13.4.4 Analysis of secondary pharmacokinetic endpoints

Apparent total clearance (CL/f), terminal half-life ($t_{1/2}$) and apparent volume of distribution (Vz/f) will be described using summary statistics by each dose group. PK analysis will be based on the PKS.

In the PK analysis all concentrations below the limit of quantitation or missing data will be labelled as such in the concentration data listings.

13.4.5 Analysis of secondary pharmacodynamic endpoints

Maximum observed effect on plasma DβH activity (E_{max}), minimal observed effect on plasma DβH activity (E_{min}), time to occurrence of E_{max} (TE_{max}), time to occurrence of E_{min} (TE_{min}) and area under the effect-time curve (AUEC24) will be calculated using % inhibition of DβH

activity using Phoenix WinNonlin v8.0 or a more recent version (Certara USA, Inc., USA). Percent inhibition of DβH activity, PD parameter estimates and urinary catecholamine levels (norepinephrine and dopamine) including changes from baseline will be described using summary statistics by each dose group. Pharmacodynamic analysis will be based on the PKS.

Further details of the statistical analysis and reporting of the pharmacodynamic endpoints, such as, dose response modelling for % DβH inhibition and exposure response modelling to assess relationship between drug plasma concentrations and corresponding % DβH inhibition, will be included in a separate Statistical Analysis Plan.

13.4.6 Interim analyses and criteria for the termination of the study

No interim analyses are planned in this study.

The DSMB will periodically review the safety data and is empowered to recommend modifications of the protocol (dosage levels) or to recommend temporary suspension or early termination of the study, if major concerns arise about patient safety at any time during the course of the study.

13.5 Deviations from the Original Statistical Analysis Plan

Any deviation from the original SAP will be described and justified in the final report.

14. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator/institution will give the clinical research associate(s) (CRAs) direct access to primary patient data (i.e. source data) which support the data on the eCRFs for the study, i.e. general practice charts, hospital notes, appointment books, original laboratory records, electronic patient files etc. Other authorised persons such as auditors, IEC members and regulatory inspectors will be provided direct access to these source data, too.

14.1 Source Data

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies).

14.2 Source Documents

Source documents are defined as original documents, data and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, patient diaries or evaluation check lists, pharmacy dispensing records, recorded data from automated instruments, copies or manuscripts certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, records kept at pharmacy, at the laboratories and at medico technical departments involved in clinical study). Source documents comprise documents in paper and electronic format.

14.3 Direct Access

Direct access is defined as the permission to examine, analyse, verify and reproduce any records and reports that are important to evaluation of a clinical study. Any party (e.g. domestic and foreign regulatory authorities, Bial/SCOPE CRAs, IEC members and auditors) with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of patient identities and sponsor proprietary information.

15. DATA HANDLING AND RECORD KEEPING

15.1 Completion of Case Report Forms

Data reported on the eCRF that are derived from source documents should be consistent with the source documents.

All clinical information requested in this protocol will be recorded by the principal investigator or his/her authorised staff in the eCRF in accordance with the study-specific data entry rules. Entries in the eCRFs must be reviewed and verified for accuracy by the principal investigator and signed off with electronic signature.

Periodically, a CRA will review the accuracy, completeness and timeliness of all the data entered in the eCRF against source documents.

No direct entries on the eCRFs (i.e. entries with no prior written or electronic record of data) will be allowed.

15.2 Patient Diary

Not applicable in this study.

15.3 Monitoring

On site monitoring visits will be made before the study begins and at regular intervals during the study. Other forms of communication (by telephone, mail, fax, etc.) may be used as needed to supplement (but not substitute) visits. The following will be reviewed at these visits:

- Compliance with the protocol.
- Patient enrolment and consent procedure.
- Completeness, exactness and plausibility of data entered in the eCRF.
- Source document verification (SDV, see below).
- Occurrence of AEs and AE procedures.
- Storage and accountability of materials.

The purpose of SDV is to verify, so far as is possible, that the information in the eCRF reflects the data recorded in the patient's medical records. Source document verification will be performed with due regard for patient confidentiality. Source document verification will be undertaken on an ongoing basis as part of the monitoring visits. Direct access to the source documents will be required (see Section 14.3). The CRA will make a direct comparison with data entered in the eCRFs.

The principal investigator (or deputy) agrees to assist the CRA in resolving any problem that may be detected during the monitoring visit.

It is the responsibility of the CRA to ensure that the principal investigator (or deputy) has correctly documented the dispensing and return of IMP. The CRA will arrange the collection of unused IMP returned by the patient. The CRA will verify the drug accountability in the course of the clinical study and also at the close-out visit. All discrepancies must be accounted for and documented.

15.4 Data Processing

All data management procedures will be described in the *Data Management Plan* (DMP). The data will be captured in the eCRF and will be transferred from the eCRFs to SCOPE for processing (i.e. medical review, coding, statistical analysis, etc.) as defined in the DMP.

Automated checks will be implemented and manual checks will be done against the data to ensure completeness and consistency as defined in the *Data Validation Plan*. The database and check programming will be validated prior to implementation.

Data identified as erroneous or inconsistent, or key data that are missing, will be referred to the CRA and to the principal investigator for clarification. The principal investigator or his/her authorised staff will correct the data as required. All modifications to the data will be logged in the audit trail.

For data coding, the following thesauri will be used:

- Adverse Events, concomitant diseases and medical history: MedDRA, in the version as specified in the DMP.
- Concomitant medication: WHO Drug Dictionary, in the version as specified in the DMP.

Visual and computerised methods of data validation will be applied in order to ensure accurate, consistent and reliable data for the subsequent statistical analysis. These procedures aim to detect out of range values, contradictory data, abnormal evolutions over time, and possible protocol deviations (eligibility criteria, time and medication compliance, etc.).

The database will be closed in order to protect write access after the following preconditions are fulfilled:

- All data are entered in the database
- SAP is finalised
- Decisions have been made and agreed as to the identities of all protocol deviations
- Written authorisation to close the database from the project manager of the study at Bial is obtained.

Statistical analysis will be performed by SCOPE, using the SAS for Windows (SAS Institute Inc., Cary, NC, USA) in the actual available version as specified in the DMP.

15.5 Archiving

The principal investigator through his/her clinical study site shall retain and preserve one copy only of all data generated in the course of the clinical study for the period of 15 years after the end of the clinical study, or such longer period in accordance with relevant regulatory requirements.

15.6 Data protection

All clinical study information will be recorded, processed, handled, and stored without disclosing personal information of the patients so that it can be accurately reported, interpreted and verified while the confidentiality of records and the personal data of the patients remain protected in accordance with the applicable law on personal data protection.

Appropriate technical and organisational measures shall be implemented to protect processed information and personal data against unauthorised or unlawful access, disclosure, dissemination, alteration, or destruction or accidental loss, in particular where the processing involves the transmission over a network.

16. QUALITY ASSURANCE/AUDITS

This study may be subject to audit by the sponsor or their representatives. The audits will be undertaken to check compliance with the requirements of GCP.

In addition, the sponsor or their representatives may conduct additional audits on a selection of investigator sites, requiring access to patient notes, study documentation and facilities or laboratories used for the study.

The investigator's site, facilities and all data (including source data) and documentation will be made available for audit by an independent quality assurance unit and for IEC or regulatory authorities according to the ICH-GCP-guideline. The principal investigator agrees to cooperate with the auditor during his/her visit and will be available to give the auditor access to eCRFs or other files necessary to conduct that audit. Any findings will be strictly confidential.

In the event that a regulatory authority informs the principal investigator that it intends to conduct an inspection, Bial shall be notified immediately.

17. ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Patients, adopted by the General Assembly of the World Medical Association, Fortaleza, Brazil 2013, as well as with the valid national law(s) of the participating countries, with the International Council on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (E6), and with the Commission Directives 2001/20/EC and 2005/28/EC.

The CRO will submit the protocol to the responsible IEC(s) and their written unconditional approval must be obtained and submitted to the sponsor before the start of the study.

Verification of the IEC unconditional approval of the protocol will be transmitted to the sponsor prior to the start of the study. A copy of the written approval of the IEC must be available before dispensing any study medication to study patients.

The responsible IEC will be informed by the CRO of all subsequent protocol amendments and of unexpected SADRs occurring during the study that are likely to affect the safety of the patients or the conduct of the study.

Protocol amendments referring to logistical or administrative changes (non-substantial) may be implemented with notification of the IEC and competent authority only.

A list of the IEC members with names and qualifications plus a statement that it is organised according to GCP and the applicable laws should be provided to the investigator or Scope.

17.1 Informed Consent

Patients must give their informed consent prior to admission to a clinical study and before any protocol specified procedures are carried out. Patients must therefore declare their consent in written form even before the first study-related visit by personally dating and signing the ICF. The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations.

Each patient should be made aware by the investigator of the nature of the study (aims, methods and potential hazards and benefits) and the procedures involved, using the information on the consent form. Information should be given in both oral and written form whenever possible and deemed appropriate by the IEC. Patients must be given ample opportunity to inquire about details of the study.

Patient information and the consent form must be in a language fully comprehensible to the prospective patient. The written information must be handed over to the patient to give him/her sufficient time to understand the information provided and to prepare questions before being asked for his/her consent. The investigator must confirm that the text was understood by the patient. The patient will then sign and date the IEC approved consent form indicating that he/she has given his/her consent to participate in the study. The signature confirms the consent is based on information that has been understood. The form will also be signed by the investigator obtaining the consent and annotated with the study patient's name or individual patient number, as applicable. Each patient's signed ICF must be kept on file by the investigator for possible inspection by regulatory authorities, Bial and/or SCOPE personnel. It will be documented on the eCRF that informed consent has been obtained. A signed copy of the informed consent and any additional patient information must be given to each patient.

Should a protocol amendment become necessary, the ICF and patient information may need to be revised. Bial/SCOPE is responsible that an amended ICF has received approval from the respective IECs and competent authorities before being used.

Furthermore, the patient will be informed that if he/she wishes to withdraw (for definition see Section 9.4) at any time during the study, this will not have any negative consequences. Patients may be withdrawn by the investigator if any change related to safety or ethics precludes further participation in the study. Patients will be asked to agree to a final assessment in the event of an early termination of the study.

Patients will be informed that data from their case may be stored in a computer without inclusion of their name and such data will not be revealed to any unauthorised third party. Patients will also be informed that data will be reviewed by the CRA, an independent auditor and possibly by representatives of regulatory authorities and/or ethics committees. The terms of the local data protection legislation will be applied as appropriate.

18. FINANCING AND INSURANCE

Details on financing will be outlined in a separate agreement between the investigators and the sponsor/CRO.

Each investigator (including principal investigator and any sub-investigators) who is directly involved in the treatment or evaluation of patients has to provide a financial disclosure according to all applicable legal requirements.

The sponsor will take out insurance for each patient against any health impairment occurring as a result of participation in the study in accordance with the laws and regulations of each country where the study is performed. The investigator has to inform the patients about the procedures and conditions for compensation and will provide them with a copy of the study specific insurance certificate and the terms of insurance.

All relevant information regarding financial disclosure and insurance will be filed in the TMF and the ISF.

19. PUBLICATION POLICY

The information generated by this study is the property of Bial. It is agreed that the results of the study will not be submitted for presentation, abstract, poster exhibition or publication by the investigator until Bial has reviewed and commented on such a presentation or manuscript for publication.

The sponsor will publish the results of this study.

20. REFERENCE LIST

1. Clinical Trail Facilitation Group (CTFG), Recommendation related to contraception and pregnancy testing in clinical trials, Final Version – 2014-09-15
2. Montani D, et.al. Pulmonary arterial hypertension. Orphanet J Rare Dis. 2013 Jul 6;8:97. doi: 10.1186/1750-1172-8-97. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3750932/pdf/1750-1172-8-97.pdf
3. Ghataorhe P, Rhodes CJ, Harbaum L, Attard M, Wharton J, Wilkins MR. Pulmonary arterial hypertension - progress in understanding the disease and prioritizing strategies for drug development. J Intern Med. 2017 Aug;282(2):129-141. doi: 10.1111/joim.12623. Epub 2017 May 19. Available at: https://onlinelibrary.wiley.com/doi/epdf/10.1111/joim.12623
4. BIAL Zamicastat (development code BIA 5-1058) Investigator's Brochure. Edition No. 6.2, 2020.04.14
5. Igreja B, Pires N, Moser P, and Soares-da-Silva P. Effect of Chronic Treatment With Zamicastat on Right Ventricle Pressure Overload in the Rat Monocrotaline Lung Injury Model of Pulmonary Hypertension. FASEB, No. 1_supplenment April 2017, Abstract number 1070.7
6. Rudski LG et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. J Am Soc Echocardiogr. 2010 Jul;23(7):685-713; quiz 786-8. doi: 10.1016/j.echo.2010.05.010. http://www.onlinejase.com/article/S0894-7317(10)00434-7/pdf
7. Barst RJ et al. Diagnosis and differential assessment of pulmonary arterial hypertension. J Am Coll Cardiol. 2004 Jun 16;43(12 Suppl S):40S-47S. DOI: 0.1016/j.jacc.2004.02.032
8. Galiè N, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J. 2016 Jan 1;37(1):67-119. doi: 10.1093/eurheartj/ehv317. Epub 2015 Aug 29
9. Lau EMT et al, Epidemiology and treatment of pulmonary arterial hypertension. Nat Rev Cardiol. 2017 Oct;14(10):603-614. doi: 10.1038/nrcardio.2017.84. Epub 2017 Jun 8
10. Khanna D et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. J Scleroderma Relat Disord. 2017 Jan-Apr; 2(1): 11–18. doi: 10.5301/jsrd.5000231
11. Holland AE et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J. 2014 Dec;44(6):1428-46. doi: 10.1183/09031936.00150314. Epub 2014 Oct 30. Available on: http://erj.ersjournals.com/content/erj/44/6/1428.full.pdf
12. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377-81
13. Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrie H, Verma S, Goddard R., CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry. 1986 Dec;149:698-70