IRCCS POLICLINICO SAN DONATO

Protocol BESAFE

AN OPEN LABEL, SINGLE ARM, EXPLORATIVE TRIAL TO EVALUATE EFFICACY AND SAFETY OF BEROTRALSTAT IN PREVENTING ANGIOEDEMA ATTACKS IN SUBJECTS WITH ACQUIRED ANGIOEDEMA DUE TO C1 INHIBITOR DEFICIENCY (AAE)

Version 1.2 27 JULY 2026

EU CT No. 2025-524747-13-00

IRCCS Policlinico San Donato
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Protocol Number:	BESAFE
Study Title:	Open label, single arm, explorative trial to evaluate efficacy and safety of Berotralstat in preventing angioedema attacks in subjects with Acquired Angioedema due to C1 Inhibitor deficiency (AAE) BESAFE: Berotralstat Efficacy and Safety in Acquired Angioedema Explorative trial
EU CT No.	2025-524747-13-00
Investigational Product:	BEROTRALSTAT
Indication Studied:	Acquired angioedema due to C1 Inhibitor deficiency (AAE)
Sponsor:	IRCCS Policlinico San Donato Via Morandi 30 20097 San Donato Milanese (MI) Italy
Principal Investigator:	Prof. Andrea Zanichelli IRCCS Policlinico San Donato Via Morandi 30 20097 San Donato Milanese (MI) Italy
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines, International Council for Harmonization guidelines and all locally applicable regulations. Essential study documents are currently archived in accordance with applicable regulations
Final Protocol Date:	27 JULY 2026
Previous Version(s):	Version 1.1 07 JUL 2026 Version 1.0 26 JUN 2025

1.1. Protocol Approval Signature Page

Protocol No: BESAFE

Protocol Title: Open label, single arm, explorative trial to evaluate efficacy and safety of Berotralstat in preventing angioedema attacks in subjects with Acquired Angioedema due to C1 Inhibitor deficiency (AAE)

Date:

Reviewed and Approved by:

Andrea Zanichelli, MD PhD Date 27 JUL 2026

1.2. Clinical Study Protocol Agreement

Protocol No: BESAFE

Protocol Title: Open label, single arm, explorative trial to evaluate efficacy and safety of Berotralstat in preventing angioedema attacks in subjects with Acquired Angioedema due to C1 Inhibitor deficiency (AAE)

Date:

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Council for Harmonisation guidelines for Good Clinical Practices, and all applicable regulatory requirements.

_____

Investigator's Signature Date

_____

Name (Print)

2. SYNOPSIS

Name of Sponsor/Company: IRCCS Policlinico San Donato
Name of Investigational Product: Berotralstat
Name of Active Ingredient: (*R*)-1-(3-(aminomethyl)phenyl)-N-(5-((3-cyanophenyl)(cyclopropylmethylamino)methyl)-2-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide
Title of Study: Open label, single arm, explorative trial to evaluate efficacy and safety of Berotralstat in preventing angioedema attacks in subjects with Acquired Angioedema due to C1 Inhibitor deficiency (AAE)
Study centers: Multiple study centers in Italy
Principal Investigator: Andrea Zanichelli, MD PhD
Enrollment period (months): Approximately 9 months
Phase of development: 2
Primary Objective: The primary objective of the study is to explore the effectiveness of Berotralstat in preventing angioedema attacks in patients with AAE. **Secondary Objective**: The secondary objectives of this study are to further characterize the clinical efficacy of berotralstat in subjects with acquired angioedema due to C1 inhibitor deficiency (AAE).The **safety objective** is to evaluate the proportion of patients with adverse events (AEs) including treatment-emergent serious adverse events (TESAEs).
Efficacy Endpoint: The primary efficacy endpoint is the change in the monthly HAE attacks rate, from week 1 to week 24 of treatment compared to attack rate during the run-in period. Secondary Efficacy Endpoints: monthly HAE attacks rate over 6 months of treatment rate of attacks requiring rescue treatment attack free period the proportion of days without angioedema symptoms proportion of attack rated mile, moderate or severe Monthly investigator-confirmed angioedema attack rate during the stable-treatment period (Weeks 24)
Safety Endpoints: the number of the total adverse events and serious adverse events the number of TEAEs and TEASEs discontinuations due to lack of efficacy
Methodology: A multicentric phase II study, open label, single arm.

Individual percent change from baseline in attack rate will be summarized using descriptive statistics. A waterfall plot will show the percent change from baseline each individual.
The primary efficacy analysis will compare attack rates during the treatment period and the run-in period using a Mantel-Haenszel paired rate-ratio approach. A mixed-effects negative binomial regression model including subjects as a random effect and observation time as an offset term will be performed as a supportive sensitivity analysis to assess robustness of results in the presence of overdispersion and between-subject variability.
Patients will be included in the study and evaluated during the run-in period and will initiate the treatment after detection of more than 1 attack in 4 weeks period or >3 attacks in 8 weeks.
In patients included in the study the use of Berotralstat could be extended up to one year with a periodic follow up.

Main criteria for inclusion:

1. Males and non-pregnant, non-lactating females ≥ 18 years of age
2. Able to provide written, informed consent.
3. Subject weight of ≥ 40 kg.
4. A clinical diagnosis of AAE is defined from the following criteria:
    - Recurrent angioedema without wheals
    - No family history of angioedema and/or C1-INH deficiency
    - C1-inhibitor activity level of <50% of normal and **at least 1 of the following**:
        - Age at least 40 years at reported onset of first angioedema symptoms
        - Reduced C1q level (below the lower limit of normal), recognizing that normal C1q levels do not exclude the diagnosis of AAE.Absence of a pathogenic variant in the SERPING1 gene encoding for the C1-inhibitor protein.
        - Serologic confirmation of anti–C1-inhibitor antibodies.
5. Access to and ability to use one or more acute medications approved by the relevant competent authority for the treatment of acute attacks of HAE (icatibant, plasma-derived C1-INH).
6. The subject must have at least 2 AAE attacks which meet all the requirements below during the run-in period of 28 days. In case of not recurrence of 2 attacks in 28 days or attacks do not meet all the requirements below, the run-in period will be extended to a maximum of 56 days from the Screening visit:
    - The attacks are unique, which is defined as an attack that does not begin within 48 hours of the end of a previous attack.
    - The attacks must include symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may also include symptoms in the oropharyngeal or abdominal regions which are indicative of internal swelling.
    - The attacks are confirmed by the Investigator to be AAE attacks. Subjects will be contacted within 2 business days of the attack to discuss the attack, any queries on the entered data in the diary, as applicable.
7. In the opinion of the Investigator, the subject is expected to adequately comply with all required study procedures for the duration of the study. The subject must demonstrate adequate compliance with all study procedures required from the screening visit, including diary recording of HAE attacks beginning at the screening visit.

Women of childbearing potential and male participants with partners of childbearing potential must agree to use a highly effective method of contraception, throughout the study and for the protocol-defined period after the last dose of the investigational medicinal product (IMP). Acceptable highly effective methods include combined (estrogen- and progestogen-containing) hormonal contraception, progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine devices (IUDs), intrauterine hormone-releasing systems (IUSs), bilateral tubal occlusion, vasectomized partner (provided he is the sole sexual partner), and true sexual abstinence when this is the participant's usual and preferred lifesty

Main criteria for exclusion:

1. Any clinically significant medical or psychiatric condition or medical history that, in the opinion of the Investigator or Sponsor, would interfere with the subject's ability to participate in the study or increases the risk to the subject by participating in the study.
2. Concurrent diagnosis of any other type of recurrent angioedema.
3. Any abnormal clinical, laboratory or urinalysis parameter at screening that, in the opinion of the Investigator or any contraindication describe in berotralstat SmPC
4. Known hypersensitivity to the active substance of berotralstat or to any of its excipients
5. Current treatment with any long-term prophylactic therapy intended for the prevention of angioedema attacks, unless the required washout period has been completed before the start of the run-in period. Required washout periods are as follows:
    - plasma-derived or recombinant C1 inhibitor used for long-term prophylaxis: 14 days;
    - lanadelumab: 28 days;
    - berotralstat or any other oral plasma kallikrein inhibitor: 28 days;
    - attenuated androgens (e.g., danazol): 28 days;
    - tranexamic acid: 28 days;
    - any other investigational or approved long-term prophylactic treatment for angioedema: at least 28 days, or 5 half-lives, whichever is longer.

    Use of approved on-demand (rescue) therapies for the treatment of acute angioedema attacks (e.g., icatibant or plasma-derived C1 inhibitor) is permitted throughout the study, including during the run-in period and the treatment period, according to standard clinical practice.
6. Pregnant women
7. Patients with moderate or severe hepatic impairment (Child-Pugh Class B or C) in alignment with SmPC requirement

Investigational product, dosage and mode of administration:
Berotralstat, to be administered orally every day at 150 mg dosage.

Criteria for evaluation:
Efficacy:
Number of HAE attacks and related details (timing, duration of symptoms, anatomical location, treatment used, and emergency room visits and hospitalizations, number of days

with HAE symptoms, number of subjects who are attack-free, assessment of attack severity, and discontinuations due to lack of efficacy.

Safety:

AEs, laboratory analyses (clinical chemistry, hematology, coagulation, urinalysis, vital signs, ECGs, and physical examinations.

Health Outcomes:

AECT, AE-QoL and EQ-5D-5Lquestionnaire scores.

Statistical methods:

The sample size calculation is based on the methodology proposed by Sumi and Tango (stat. med. 2010) for inference on recurrent-event rate ratios in a matched-pairs design. In the BESAFE study, each subject serves as his/her own matched pair, with recurrent angioedema attacks observed during the run-in period being compared with those observed during the treatment period.

The primary efficacy analysis evaluates the rate ratio of investigator-confirmed angioedema attacks during Berotralstat treatment relative to the run-in period. The sample size was calculated assuming:

- Baseline attack rate during the run-in period: 24 attacks per patient-year.
- Treatment-period attack rate: 17 attacks per patient-year.
- Target rate ratio (treatment/run-in): aproximately 0.65.
- Two-sided significance level (α) of 0.05.
- Statistical power of 80%.
- Run-in period duration of 28 days.
- Treatment period duration of 168 days, corresponding to an observation-period ratio (k) of 6.

The expected number of attacks during the run-in period was derived from the assumed baseline attack rate and run-in duration. The sample size was then calculated using the Sumi and Tango matched-pair recurrent-event rate-ratio formula. Under these assumptions, approximately 16 evaluable subjects are required.

Duration of treatment:

Subjects will take capsules of Berotralstat for 24 weeks till the end of the study. In addition, commercial drug supply free of charge is required for all patients for 1 year treatment.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Table 1. Abbreviations and Specialist Terms

Abbreviation	Explanation
AAE	acquired angioedema
ABW	absolute body weight
AE	adverse event
AECT	Angioedema Control Test
AE-QoL	Angioedema Quality of Life Questionnaire
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC_{tau}	area under the plasma concentration versus time curve over the dosing interval (tau)
BK	bradykinin
BMI	body mass index
BUN	blood urea nitrogen
C1-INH	C1 esterase inhibitor
C3	complement 3
C4	complement 4
CBC	complete blood count
CK	creatine kinase
CK-MB	creatine kinase MB isoenzyme
CL_{CR}	creatinine clearance
C_{max}	maximum plasma concentration of the drug
CRF	case report form
CRA	clinical research associate
CSR	clinical study report
CYP	cytochrome P450
DMC	Data Monitoring Committee
DMID	Division of Microbiology and Infectious Disease
ECG	Electrocardiogram
eCRF	electronic Case Report Form
EOSI	event of special interest
EQ-5D-5L	EuroQoL 5-dimensional, 5-level questionnaire
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma-glutamyl transpeptidase
GI	Gastrointestinal
GMP	Good Manufacturing Practice
HAE	hereditary angioedema

Abbreviation	Explanation
HBV	hepatitis B virus
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HK	high-molecular weight kininogen
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonization
IEC	independent ethics committee
IMP	investigational medicinal product (study drug)
INR	international normalized ratio
IRB	institutional review board
ITT	intent to treat
IUD	intrauterine device
IV	Intravenous
LDH	Lactate dehydrogenase
LLN	lower limit of normal
LTP	Long term prophylaxis
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	mixed model of repeated measures
NHP	nonhuman primate
nM	nanomolar
NOAEL	no observed adverse effect level
P-gp	P-glycoprotein
PK	pharmacokinetic
PP	per protocol
PT	Prothrombin Time
QD	once daily
QoL	quality of life
RNA	Ribonucleic Acid
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SGART	Subject's Global Assessment of Response to Therapy
SmPC	Summary of Product Characteristics
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
ULN	upper limit of normal
US	United States

5. INTRODUCTION

5.1. Background

Acquired angioedema (AAE) is characterized by acquired deficiency of C1 inhibitor (C1-INH), hyperactivation of the classical pathway of human complement and angioedema symptoms mediated by bradykinin released by inappropriate activation of the contact-kinin system.

The symptomatology overlapping C1-INH hereditary angioedema (HAE), but the majority of the patients have a onset of the disease in late adulthood.

AAE recurs in association with various conditions and particularly with different forms of lymphoproliferative disorders and/or autoimmune diseases. Neutralizing autoantibodies to C1-INH are present in most patients.

In AAE no clinical trials with drugs for on demand and long term prophylaxis have been performed and no specific drugs have been approved for this rare form of angioedema nowadays. Since the edema formation is mediated by contact-kallikrein system as in HAE, the same drugs studied and approved in HAE for on demand treatment (i.e. Icatibant and plasma derived C1 INH) are used off-label in AAE. Because of presence of autoantibodies to C1-INH, some patients become resistant to C1-INH infusion. In these patients Icatibant is the election treatment for acute attacks. Regarding long term prophylaxis, androgens and tranexamic acid have been used although their efficacy has not been evaluated in clinical trials. More recently kallikrein inhibitor, i.e. Lanadelumab, has been used for prophylaxis. In literature a few cases are reported on the use of this drug in AAE.

Berotralstat is a potent, synthetic small molecule inhibitor of plasma kallikrein. In contrast to parenterally administered options commercially available for prophylaxis against HAE attacks, inhibition of kallikrein with an orally bioavailable small molecule such as Berotralstat offers the advantage of oral administration.

The aim of this phase 2 study is to explore the efficacy and safety of a new oral plasma Kallikrein inhibitor in reducing the rate of angioedema acute attack in AAE patient and improving the control of the disease. The efficacy and safety of this drug has been already demonstrated in phase III clinical trials for prophylaxis in HAE patients.

5.2. Nonclinical Findings for Berotralstat

The principal results of nonclinical pharmacology, pharmacokinetic (PK), and toxicology studies of Berotralstat are described briefly below; additional details can be found in the Berotralstat Investigator's Brochure (IB).

Safety pharmacology studies of Berotralstat indicated multiple cardiac ion channel effects, including the human ether-a-go-go related gene K^+ channel and the Na^+ and Ca^{2+} channels. However, studies in non-human primates (NHP) showed quantitatively small drug-related prolongation of the QT interval. There were no concerns for phototoxicity or genotoxicity.

Embryo-fetal developmental toxicity studies in the rat and rabbit showed no evidence of direct fetal toxicity of Berotralstat, and no effects were seen in male and female rats in fertility studies.

Chronic-dosing toxicology studies were conducted in the NHP (39 weeks) and rat (26 weeks). BEROTRALSTAT was well tolerated in both rats and monkeys at doses up 30 and 20 mg/kg/day, respectively, and these dose levels were the no observed adverse effect levels (NOAELs). In NHP, at the NOAEL dose of 30 mg/kg/day, mean maximal plasma concentration of the drug (C_{max}) and area under the concentration vs. time curve (AUC) from time 0 to 24 hours (AUC_{0-24}) on Day 270 (sexes combined) were 277 ng/mL and 3950 ng.h/mL, respectively. In the rat, at the NOAEL dose level of 20 mg/kg/day, C_{max} was 686 ng/mL, and AUC_{0-24} was 9710 ng.h/mL, on Day 182.

In the chronic toxicology study in rats the target organ was the liver. Minimal to mild bile duct hyperplasia and foamy/pigmented macrophages were observed at 20 mg/kg/day. Vacuolated epithelium of hepatic bile ducts was noted with minimal severity. A pathology peer-review of liver findings in the 26-week study concluded that the microscopic changes, including bile duct hyperplasia, were non-adverse.

In monkeys, the primary target organs were the liver and kidney. In one study that evaluated doses up to 20 mg/kg/day, the only effect noted was a mild increase in alanine aminotransferase (ALT), which partially resolved during the study despite continued dosing. In a subsequent study that evaluated doses of 30, 55, and 80 mg/kg/day, adverse findings in the liver and kidney were noted at 55 and 80 mg/kg/day. Increased liver weights and panlobular hepatocellular hypertrophy were observed together with increased ALT and aspartate aminotransferase (AST); and increased kidney weights, renal tubular epithelial cell degeneration, and renal tubular hyperplasia were also observed. Each of these effects were reversible during a 13-week drug-free recovery period.

In monkeys, pigmented and foamy macrophages were present in all regions of the small intestine, in mesenteric lymph nodes and in liver Kupffer cells at doses ≥ 30 mg/kg/day in females, and at doses ≥ 55 mg/kg/day in males. Dose-dependent increases in the urinary concentrations of the experimental biomarker for phospholipidosis (PLD), di-docosahexaenoyl (22:6)- Bis(mono) acylglycerol phosphate (BMP), were observed in both rats and monkeys. Electron microscopy evaluation of liver from rats in the 13-week toxicity studies demonstrated myelinosomes within the cytoplasm of Kupffer cells. These effects are consistent with PLD, a phenomenon noted in nonclinical toxicity studies with several approved drugs. PLD is considered an adaptive response to the presence of a drug, rather than a toxic manifestation.

In monkeys, spleen and thymus weights were increased at 55 and 80 mg/kg/day. The increased spleen weight correlated with lymphoid hyperplasia; there was no microscopic correlate for the increased thymus weights.

5.3. Clinical Findings for BEROTRALSTAT

A summary of the relevant clinical and non-clinical data supporting the use of Berotralstat may be found in the SmPC

5.4. Rationale for Study

At present, no prophylactic treatments have been approved for the prevention of angioedema attacks in AAE. A variety of LTP drugs for HAE are used off-label for AAE. Additionally, numerous case reports and series have documented the efficacy of plasma kallikrein inhibitors in preventing acute attacks of angioedema in AAE patients.

This study is designed to explore the efficacy of berotralstat in preventing attacks in subjects with AAE, as measured by the rate of investigator-confirmed angioedema attacks.
The primary efficacy analysis will compare the monthly attack rate during the entire 24-week treatment period (Day 1–168) with the monthly attack rate during the run-in period. Analyses focusing on Weeks 24 may be performed as supportive exploratory analyses.

Furthermore, the current study will evaluate the efficacy of berotralstat in an AAE population, which may exhibit a broader range of attack frequencies (a minimum of two attacks in four weeks is required for entry).

Berotralstat is a selective oral inhibitor of plasma kallikrein and is approved at a dose of 150 mg once daily for the prevention of hereditary angioedema (HAE) attacks. Although acquired angioedema due to C1 inhibitor deficiency (AAE) differs from HAE with respect to its underlying cause, both disorders share the same final pathophysiological mechanism leading to recurrent angioedema attacks.

In HAE, C1 inhibitor deficiency results from pathogenic variants in the SERPING1 gene, whereas in AAE the deficiency is acquired, most commonly due to increased C1 inhibitor consumption associated with lymphoproliferative disorders or to neutralizing autoantibodies directed against C1 inhibitor. Despite these differences in disease etiology, both conditions are characterized by inadequate regulation of the plasma kallikrein-kinin system, resulting in excessive generation of bradykinin, the principal mediator responsible for increased vascular permeability and angioedema attacks.

Plasma kallikrein occupies the same central position within the effector pathway in both hereditary and acquired C1 inhibitor deficiency. Consequently, inhibition of plasma kallikrein is expected to reduce bradykinin generation irrespective of the mechanism responsible for C1 inhibitor deficiency. This mechanistic rationale is further supported by the established efficacy of plasma-derived C1 inhibitor replacement and the bradykinin B2 receptor antagonist icatibant in both HAE and AAE, indicating that therapeutic interventions targeting the kallikrein-bradykinin pathway remain effective despite differences in disease etiology.

Berotralstat directly inhibits plasma kallikrein and its pharmacological activity is independent of the mechanism responsible for C1 inhibitor deficiency. Currently, there is no evidence suggesting that patients with AAE exhibit clinically relevant differences in plasma kallikrein pharmacology, berotralstat pharmacokinetics, or pharmacodynamic target engagement compared with patients with HAE that would warrant the evaluation of an alternative dosing regimen.

The selected dose of 150 mg once daily therefore corresponds to the approved therapeutic regimen demonstrated to provide sustained plasma kallikrein inhibition in HAE and represents the most scientifically justified dose for evaluation in AAE. Given the rarity of AAE, dedicated dose-finding studies have not been performed. Consequently, dose selection is based on the shared disease biology, the common bradykinin-mediated mechanism of angioedema attacks, the established pharmacokinetic and pharmacodynamic profile of berotralstat, and the available clinical experience supporting inhibition of the kallikrein-bradykinin pathway in both hereditary and acquired C1 inhibitor deficiency.

Although important clinical differences exist between HAE and AAE, including age at disease onset, the frequent association of AAE with lymphoproliferative or autoimmune disorders, and the presence of reduced C1q levels in many patients with AAE, these characteristics primarily reflect differences in disease origin rather than in the downstream mechanism responsible for angioedema attacks. Since plasma kallikrein activation and bradykinin overproduction represent the final common pathway in both disorders, these differences are not expected to alter the pharmacological activity of plasma kallikrein inhibition.

5.4.1. Rationale for BEROTRALSTAT Doses and Regimen

The dosage regimens of berotralstat selected for evaluation in this study are 150 mg of berotralstat administered once a day, which is the approved dose for patients with hereditary angioedema (HAE).

5.4.2. Study Population Rationale

The current study is limited to adults (≥ 18 years of age) of both sexes with confirmed diagnosis AAE.

The diagnosis of acquired angioedema (AAE) in this study is based on the combination of clinical presentation, complement testing, and exclusion of hereditary C1 inhibitor deficiency, in accordance with current international recommendations.

A reduced C1q level is included as a supportive diagnostic feature because it is frequently observed in patients with acquired C1 inhibitor deficiency and contributes to the differential diagnosis from hereditary angioedema due to C1 inhibitor deficiency. However, it is recognized that normal C1q levels may occur in a proportion of patients with confirmed AAE. Therefore, a reduced C1q level is **not** required as a mandatory eligibility criterion but represents one of several alternative diagnostic features that may support the diagnosis, together with disease onset at ≥40 years of age, absence of a pathogenic SERPING1 variant, or the presence of anti-C1 inhibitor autoantibodies. This approach is intended to ensure diagnostic accuracy while avoiding unnecessary exclusion of patients with clinically confirmed AAE.

Population PK modeling of PK data generated to date indicates that weight is a covariate on the bioavailability of berotralstat. Simulations of exposures by weight at clinically relevant doses indicated that a weight of < 40 kg is associated with exposures considered significantly higher (ie, > 20%) than that generated from an adult of 70 kg at doses to be studied in this protocol.
Therefore, participation in the trial will be restricted to subjects who weigh at least 40 kg.
Patients with a life expectancy of less than one year will be excluded from the trial due to the underlined disease.
Pregnant women will be excluded from participation in the current study. Additionally, any female subject who becomes pregnant on study will be required to immediately discontinue study drug and will be followed through the end of the pregnancy.
Women Of Child-Bearing Potential (WOCBP) defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or is not postmenopausal, will be test for pregnancy at screening with serum pregnancy test. Urinary pregnancy test will be assessed at all subsequent visits. A serum

pregnancy test will be immediately drawn and sent for analysis for any positive urine pregnancy test.
FSH will be measured at screening in women declaring themselves in postmenopausal status (defined as 12 months of amenorrhea in women over 45 years) to exclude childbearing status. FSH will not be measured in surgically sterile subjects with documented permanent infertility resulting from hysterectomy, bilateral salpingectomy or bilateral oophorectomy. Surgically sterile subjects are eligible for the study.

5.4.3. Patients with independent risk factors for QT prolongation should not to be excluded from the study. In particular, whether subjects presenting with electrolyte disturbances, known pre-existing QT prolongation (acquired or familial), advancing age, or concomitant use of medicinal products known to prolong the QT interval are eligible for inclusion but should be monitored with ECG at every visit Berotralstat Benefit-Risk Analysis

Given that berotralstat is a small molecule kallikrein inhibitor with safety data available from HAE studies performed and described in the SmPC, there is an acceptably low risk of severe or serious adverse reactions. Berotralstat has an established safety profile characterized primarily by gastrointestinal adverse events, mild to moderate elevations in liver function tests, and non-serious cutaneous reactions. Cardiac safety monitoring has shown only minimal effects on ventricular repolarization with a low concern for clinically meaningful QTc prolongation. Potential risks include pharmacokinetic interactions with P-gp and BCRP inhibitors or inducers and with CYP2D6/3A4 substrates.
Risk minimisation measures implemented in the study include scheduled monitoring of hepatic parameters, ECG assessments to detect QTc changes, active surveillance and symptomatic management of gastrointestinal and dermatologic events, strict review and control of concomitant medications with interaction potential, and protocol-defined exclusion and discontinuation criteria for participants with clinically significant hepatic, cardiac, or drug-interaction risks. Participants will be informed of relevant risks and instructed to promptly report any symptoms consistent with these known or potential adverse effects.

5.4.4. Benefits of Trial Participation

No commercial products are approved for LTP in patients affected by AAE and study subjects will receive regular medical care for the duration of the study. Subjects may experience a reduction in the number of attacks during the study.

5.4.5. Overall Benefit-Risk Assessment

The risks from daily oral administration of berotralstat seen to date in SmPC are monitorable, and reversible. Based on the utility of other kallikrein inhibitors such as lanadelumab and the pharmacology of Berotralstat, there is an expectation of benefit to the individual subject. The information obtained from this study will support the development of berotralstat for AAE, a serious, debilitating, and potentially life-threatening disease. The overall benefit-risk balance is therefore considered to be acceptable.

5.5 Current Standard of Care and Management of Patients Not Enrolled in the Study

At present, no medicinal products are specifically approved or reimbursed for the prophylactic prevention of angioedema attacks in patients with acquired C1-inhibitor deficiency (AAE). Clinical management therefore relies on off-label use of therapies approved for hereditary angioedema (HAE) and on treatment of the underlying lymphoproliferative or autoimmune disorder when present.

Patients who are screened but not enrolled in the study will continue to receive standard clinical care according to national practice in Italian angioedema referral centres:

- **Treatment of acute attacks (on-demand):** Off-label use of **icatibant** (subcutaneous) and/or **plasma-derived C1-inhibitor (pdC1-INH)** (intravenous) remains the standard of care for AAE attacks. Both agents have demonstrated effectiveness in observational cohorts of AAE patients and are recommended by expert consensus for acute management.
- **Long-term prophylaxis (LTP):** In patients with frequent or severe attacks, clinicians may consider off-label prophylaxis on an individual basis:
    - **Tranexamic acid**, which may reduce attack frequency in selected patients despite limited evidence;
    - **Attenuated androgens** (e.g., danazol), historically used with variable efficacy and requiring careful monitoring;
    - **pdC1-INH prophylaxis** (IV or SC) or **lanadelumab**, both of which have shown benefit in case series of AAE despite the absence of formal approval for this indication.
- **Treatment of the underlying disease:** Because AAE is frequently associated with B-cell lymphoproliferative disorders or autoimmune conditions, disease-directed therapy (e.g., **rituximab** or targeted agents such as BTK inhibitors) may lead to substantial improvement or remission of angioedema symptoms. This approach is considered part of standard management when an underlying disorder is identified.

Therefore, patients who are not enrolled in the present study will not be deprived of treatment. They will continue to receive evidence-based, off-label therapies for acute attacks and, when clinically indicated, long-term prophylaxis according to current Italian practice and international expert recommendations. The investigational study does not replace or suspend standard-of-care options; rather, it aims to systematically evaluate berotralstat as a potential oral prophylactic therapy in a population for whom no approved or reimbursed prophylactic treatments currently exist.

6. TRIAL OBJECTIVES

6.1. Objectives

6.1.1. Primary Efficacy Objective

- The primary objective of the study is to explore the effectiveness of Berotralstat in preventing angioedema attacks in patients with AAE.

6.1.2. Secondary Efficacy Objectives

The secondary objectives of this study are to further characterize the clinical efficacy of berotralstat in subjects with acquired angioedema due to C1 inhibitor deficiency (AAE) by evaluating:

I. the monthly rate of investigator-confirmed angioedema attacks throughout the 24-week treatment period;

II. the rate of angioedema attacks requiring rescue medication;

III. the duration of attack-free periods during treatment;

IV. the proportion of days free from angioedema symptoms;

V. the distribution of investigator-assessed attack severity (mild, moderate, or severe).

In addition, the study aims to explore the impact of berotralstat treatment on disease control, health-related quality of life, and patient-reported outcomes, as assessed by the Angioedema Control Test (AECT), the Angioedema Quality of Life questionnaire (AE-QoL), and the EQ-5D-5L questionnaire.

6.1.3. Safety Objectives

The safety objective is to evaluate the proportion of patients with adverse events (AEs) including treatment-emergent serious adverse events (TESAEs).

7. OVERALL STUDY DESIGN AND PLAN

This is Phase 2, open label study is designed to test the hypothesis that the HAE attack rate after 24 weeks of prophylactic Berotralstat will be less than that observed during the run-in phases. The primary efficacy endpoint will be assessed after the last subject completes the study.

Qualifying attacks during the run-in are characterized as follows:

- The attacks must occur during the run-in period, which is a minimum of 28 consecutive days and a maximum of 56 consecutive days, starting on the day of the screening visit.
- The attacks must include symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may also include symptoms in the oropharyngeal or abdominal regions which are indicative of internal swelling.

- The attacks are otherwise confirmed by the Investigator to be HAE attacks.

Once a subject records 2 such attacks, they be entered into the study drug beginning at Day 28 of the run-in period.

Beginning at screening and through the Week 24 visit, details of acute attacks of angioedema will be recorded in a diary. Attacks will be treated in accordance with the subject's normal standard of care. Within approximately 2 business days of the end of each attack that occurs from the screening visit through the Week 24 visit, subjects will be contacted by the Investigator (or appropriately trained designee) to discuss the clinical characteristics of the attack, any questions on the entered data or to gain additional attack details not included in the diary that the Investigator deems important to clinically evaluate the event, as applicable. This information, in conjunction with the diary record, will be used by the Investigator to verify or reject the event recorded in the diary as an angioedema attack. All Investigator-confirmed attacks must include symptoms of swelling; duration, severity and treatment used.

Study visits will occur at screening, baseline and Weeks 4, 12 and 24. The primary efficacy analysis will occur after the last subject completes their Week 24 visit and will include all data through Week 24.

No formal interim efficacy or safety analyses are planned. All efficacy and safety analyses will be performed after completion of the Week 24 visit by the last subject and database lock

7.1. Endpoints

7.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint of the study is as follows:

- the change in the monthly HAE attacks rate, from week 1 to week 24 (Day 1 to day 168) of treatment compared to attack rate during the run-in period (28-56 days before treatment initiation)

7.1.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints are as follows:

- monthly HAE attacks rate over 6 months of treatment
- rate of attacks requiring rescue treatment
- attack free period
- the proportion of days without angioedema symptoms
- proportion of attack rated mile, moderate or severe

7.1.3. Safety Endpoints

- the number of total adverse events and serious adverse events
- the number of TEAEs and TESAEs
- discontinuations due to lack of efficacy

7.1.4. Health Outcome Endpoints

- EuroQoL five-dimensional, 5-level questionnaire (EQ-5D-5L) scores
- Angioedema control test (AECT) scores
- Angioedema quality of life (AEQoL) scores

8. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. Number of Subjects

Approximately 16 subjects are planned to be enrolled in the study.

8.2. Subject Selection

8.2.1. Inclusion Criteria

Subjects must meet all the following inclusion criteria to be eligible for participation in this study:

1. Males and non-pregnant, non-lactating females ≥ 18 years of age
2. Able to provide written, informed consent.
3. Subject weight of ≥ 40 kg.
4. A clinical diagnosis of AAE is defined from the following criteria:
    - Recurrent angioedema without wheals
    - No family history of angioedema and/or C1-INH deficiency
    - C1-inhibitor activity level of <50% of normal) and **at least 1 of the following**:
        - Age at least 40 years at reported onset of first angioedema symptoms
        - Reduced C1q level (below the lower limit of normal), recognizing that normal C1q levels do not exclude the diagnosis of AAE.Absence of a pathogenic variant in the SERPING1 gene encoding for the C1-inhibitor protein.
        - Serologic confirmation of anti–C1-inhibitor antibodies.
5. Access to and ability to use one or more acute medications approved by the relevant competent authority for the treatment of acute attacks of HAE (icatibant, plasma-derived C1-INH).
6. The subject must have at least 2 AAE attacks which meet all the requirements below during the run-in period of 28 days. In case of not recurrence of 2 attacks in 28 days or attacks do not meet all the requirements below, the run-in period will be extended to a maximum of 56 days from the Screening visit:
    - The attacks are unique, which is defined as an attack that does not begin within 48 hours of the end of a previous attack.

- The attacks must include symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may also include symptoms in the oropharyngeal or abdominal regions which are indicative of internal swelling.
- The attacks are confirmed by the Investigator to be AAE attacks. Subjects will be contacted within 2 business days of the attack to discuss the attack, any queries on the entered data in the diary, as applicable.

7. In the opinion of the Investigator, the subject is expected to adequately comply with all required study procedures for the duration of the study. The subject must demonstrate adequate compliance with all study procedures required from the screening visit, including diary recording of HAE attacks beginning at the screening visit.
8. Women of childbearing potential and male participants with partners of childbearing potential must agree to use a highly effective method of contraception, throughout the study and for the protocol-defined period after the last dose of the investigational medicinal product (IMP). Acceptable highly effective methods include combined (estrogen- and progestogen-containing) hormonal contraception, progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine devices (IUDs), intrauterine hormone-releasing systems (IUSs), bilateral tubal occlusion, vasectomized partner (provided he is the sole sexual partner), and true sexual abstinence when this is the participant's usual and preferred lifestyle

8.2.2. Exclusion Criteria

Subjects must meet none of the numbered exclusion criteria below to be eligible for participation in this study. Medications prohibited for use during the study are addressed in specific sections.

1. Any clinically significant medical or psychiatric condition or medical history that, in the opinion of the Investigator or Sponsor, would interfere with the subject’s ability to participate in the study or increases the risk to the subject by participating in the study.
2. Concurrent diagnosis of any other type of recurrent angioedema.
3. Any abnormal clinical, laboratory or urinalysis parameter at screening that, in the opinion of the Investigator or any contraindication describe in berotralstat SmPC.
4. Known hypersensitivity to the active substance of berotralstat or to any of its excipients
5. Current treatment with any long-term prophylactic therapy intended for the prevention of angioedema attacks, unless the required washout period has been completed before the start of the run-in period. Required washout periods are as follows:
    - plasma-derived or recombinant C1 inhibitor used for long-term prophylaxis: 14 days;
    - lanadelumab: 28 days;
    - berotralstat or any other oral plasma kallikrein inhibitor: 28 days;
    - attenuated androgens (e.g., danazol): 28 days;

- tranexamic acid: 28 days;
- any other investigational or approved long-term prophylactic treatment for angioedema: at least 28 days, or 5 half-lives, whichever is longer.

Use of approved on-demand (rescue) therapies for the treatment of acute angioedema attacks (e.g., icatibant or plasma-derived C1 inhibitor) is permitted throughout the study, including during the run-in period and the treatment period, according to standard clinical practice.

6. Pregnant women
7. Patients with moderate or severe hepatic impairment (Child-Pugh Class B or C) in alignment with SmPC requirement.

8.3. Subject Withdrawal from the Study and from Study Drug

8.3.1. Subject Withdrawal from the Study

Participation in the study is strictly voluntary; a subject may withdraw consent to contribute additional study information at any point. A subject who withdraws consent will be requested to attend an early termination visit to complete all end-of-study evaluations. Although a subject may withdraw from the study at any time without specifying a reason for withdrawal, if known, the reason for withdrawal will be recorded in the subject's medical records (source documents) and also in the CRF. If the reason for the subject withdrawal is not known, the subject must be contacted to establish whether the reason was an AE, and if so, this must be reported in accordance with the procedures outlined AE section of the protocol. If at any point in the study the clinic is unable to contact the subject after appropriate attempts have been made, the subject will be considered lost to follow-up.

Once subjects have withdrawn from the study, the Sponsor will no longer provide treatment through the study. Following withdrawal from the study, a subject will be able to receive further treatment as recommended by their treating physician and according to the accepted standard of care.

8.3.2. Subject Discontinuation from Study Drug

A subject will be permanently discontinued from study drug for any of the following reasons, which will be recorded in the source documents and CRF.

- patient request
- investigator decision:
    - Emergence of any laboratory abnormality or AE that in the judgment of the Investigator compromises the ability of the subject to continue study-specific procedures or it is considered not to be in the subject's best interest due to an altered risk/benefit profile.
    - Subsequent determination that inclusion/exclusion criteria were not met

- Intercurrent illness or emergence of a new illness/medical condition that would, in the judgment of the Investigator, affect assessments of clinical status to a significant degree.
- Subject noncompliance with study drug or to the protocol.

8.4. If a subject discontinues from study drug subsequently withdraws consent to continue study visits as previously outlined.End of Study Definition

The end of study will be defined as when the last subject completes the last protocol-scheduled visit.

9. TREATMENT OF SUBJECTS

9.1. Description of Study Drug and Study Drug Product

Berotralstat is an oral small molecule inhibitor of plasma kallikrein. All subjects will receive berotralstat (150 mg) capsules for oral administration once daily for up to 24 weeks. The drug is a commercial drug (Orladeyo) rebelled in order to fulfill GMP requirements.

9.2. Description of Study Drug Packaging, Labeling, and Storage

Each container of study drug will be labeled with the information required by local law and may include: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, expiry date, and kit number. The identification code, batch number, expiration date, and the warning "experimental drug" will be present on each blister.

Subjects will be dispensed on a sufficient number of capsules to cover the dosing period until the next study visit.

Study drug must be stored between 15°C and 25°C (room temperature) and the drug must be stored in a designated area, separate from other medications.

Details on the study drug packaging, labeling, shipment, storage and dispensing will be provided in the investigational medicinal product (IMP) manual.

9.3. Study Drug Administration and Treatment Compliance

Subjects will be instructed to take Berotralstat capsules orally QD at approximately the same time each day through up to 24 weeks.
Subjects will be instructed to take study drug at approximately the same time each day, with whichever meal is typically the largest meal of the day, or up to 30 minutes after consuming that meal. It is recommended that the study drug be administered with food to help minimize GI effects. If GI-related symptoms are noted as an AE, the site should query the subject and record whether the drug is being taken as instructed (ie, with a meal).

Day 1 for the purposes of analysis is defined as the day that subjects take their first dose of study drug. If the first study of drug administration is different than the day of the baseline visit,

subsequent visits will be calculated from the day of first dose (Day 1), rather than the day of the baseline visit. Clinic administration of study drug during study visits is not required. Subjects will take study drug in beginning on Day 1 and will complete dosing on Study Day 168 (the day before the Week 24 visit).

Subjects will be instructed to maintain approximately the same daily dosing interval between study drug doses. If a subject forgets to take the study drug at the correct time, the dose may be taken later in the day; however, no more than 1 dose of Berotralstat should be taken on any calendar day. The subject should resume their regular dosing schedule on the next day. Dosing may not be split across a day.

Subjects will be instructed to record in their diary the time of day study drug was taken, and the number of capsules of study drug taken.

The subjects will be instructed to bring all drug kits (including both unused and used box and blister) and diaries with them for each study visit.
Accountability and adherence will be reviewed at these visits.

During the conduct of the study, responsibility for kit supply and resupply may be transferred to a specialty pharmacy selected by the Sponsor and subjects may receive study drug directly from the specialty pharmacy. Detailed instructions will be provided at the time of implementation.

If a specialty pharmacy is used, specialty pharmacy staff may contact subjects directly to better understand the subject experience with study drug during the subject's participation in the study.

If the patient use any off label drug for the treatment of acquired angioedema a washout must be done.

9.4. Study Drug Dose Modification

Dose reductions are not permitted.

9.5. Study Drug (Investigational Medicinal Product) Accountability

On Day 1, the patient will be provided with a quantity of capsules sufficient to cover the treatment until the next visit. A backup blister will also be given, which the patient should use only in case of loss of one or more capsules.

At each visit, the patient must return all empty blisters in their possession before receiving a new supply of the drug. These blisters must be stored and documented by the study staff and will later be returned to the study sponsor.

The Investigator/pharmacist must maintain accurate records of the disposition of all study drugs received from the Sponsor, issued to the subject (including date), and any drug accidentally destroyed. At the end of the study, information describing study drug supplies (eg, kit numbers) and disposition of supplies for each subject must be provided, signed by the Investigator or designee, and collected by the CRA. If any errors or irregularities in any shipment of study medication to the site are discovered at any time, the Sponsor (and or designee) must be contacted immediately.

At the end of the study or at other times as agreed by all involved parties, all study drug not dispensed or administered will either be collected under the supervision of the CRA and returned to the Sponsor or destroyed on site as dictated by the appropriate Standard Operating Procedure at the participating institution.

9.6. Concomitant Medications

All subjects in the study must refrain from taking prohibited concomitant medications as outlined in specific section.

Any regularly administered concomitant medication not listed as prohibited must be anticipated to be continued through the study and be of a stable dose and regimen up to end of the study.

Details of all prior medications (taken within 30 days of screening; prior contraceptive medications taken within 60 days of screening) and all current concomitant medication use (including herbal and supplements) through the follow-up/early termination visit, including all medications administered for the treatment of AEs, will be recorded in the source documentation/CRFs.

9.6.1. Prohibited Medications

Subjects must discontinue all long-term prophylactic therapies for angioedema before entering the run-in period and must not receive any prophylactic treatment throughout study participation. Only on-demand treatment for acute angioedema attacks is permitted during the run-in and treatment periods. Required washout periods before the start of the run-in are described below.

C1-INH for prophylaxis of HAE attacks is prohibited within the 14 days prior to the Screening visit or initiation during the study. However, use of a C1-INH therapy for treatment of attacks is not excluded at any time, nor is C1-INH for unplanned/unanticipated preprocedure prophylaxis.

Use of lanadelumab and other long-term prophylaxis for HAE attacks is prohibited

during the study. In addition, the following medications are excluded during the study:

- Angiotensin-converting enzyme inhibitors within 7 days of the baseline visit or planned initiation during the study (potential for exacerbation of HAE).
- Another investigational drug within 30 days of the Screening visit or initiation during the study.
- Initiation of an estrogen-containing hormonal contraceptive within 56 days of the screening visit or planned initiation during the study.
- Use of a medication that is transported by P-gp and has a narrow therapeutic range, within 7 days of the baseline visit or planned initiation during the study. For the purposes of this protocol, these are limited to: aliskiren, digoxin, posaconazole, and talinolol.
- Use of a concomitant medication that is metabolized by CYP2D6 and/or CYP3A4 and has a narrow therapeutic range, within 7 days of the baseline visit or planned initiation during the study. For the purposes of this protocol, these are limited to:

warfarin, phenytoin, s-mephenytoin, thioridazine, alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus and desogestrel. Note: Topical or ophthalmic tacrolimus or sirolimus is allowed.

- Use of a medication that is clinically known to prolong the QT interval and is metabolized by CYP2D6 and/or CYP3A4 7 days prior to the baseline visit or planned initiation during the study. For the purposes of this protocol, these are limited to: donepezil, thioridazine, haloperidol, methadone, procainamide and amitriptyline.
- All drugs approved for long term prophylaxis of HAE attacks within the 28 days prior to the Screening visit or initiation during the study. Androgens must not be used at all during the study. Note: Use of testosterone replacement therapy is allowed.

10. STUDY CONDUCT

10.1. Overview

This is an open label single arm study. A subject's participation in this study is expected to be up to 72 weeks (inclusive of the screening and follow-up periods), or until another mechanism is available to provide drug to the subject, or until the Sponsor discontinues development of the product for the prevention of angioedema attacks, whichever comes first.

Each eligible subject who consents to participate in the study will receive either 24 weeks (168 days) of Berotralstat (150 mg). All subjects will undergo a screening period (including a prospective HAE attack run- in period between 28 and 56 days). A follow-up visit of study drug will also be scheduled at the end of the study. A compassionate use programme will be available and will be submitted to the relevant competent authority for approval in a timely manner, in order to ensure treatment continuity as soon as the first patient reaches the end of the current trial
Upon overall study results, all subjects will be transitioned to compassionate use of Berotrastat for at least 1 year from the first assumption of the drug.
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10.2. Schedule of Assessments

The schedule of assessments for this study is presented in Table 1; study procedures are described in spefic section.

Table 1. Schedule of Assessments:

Assessment	**Screening Period**		**Baseline**	**Treatment Phase**		**End of Trial**
	Screening Visit[a,b] (up to Week -10)	Run-in Period[a]	Day 1[a]	Week 4 Day 29 ±2 days	Week 12 Day 85 ± 2 days	Week 24 Day 169[d]
Informed consent	X					
In-clinic evaluations	X		X	X[c]	X	X
Inclusion-exclusion criteria	X	X	X			
Medical history	X		X			
AAE medical and medication history	X		X			
Weight/height/BMI	X		X		X	X
Physical examination	X		X		X	X
Pregnancy test[j]	X		X		X	X
Vital signs[k]	X		X		X	X
Diagnosis of AAE established	X					
Attack qualification confirmation		X				
C4 and C1-INH antigenic level			X			
Urinalysis	X		X	X[c]	X	X
Blood sample	X		X	X	X	X
12-lead ECG	X		X			X
EQ-5D-5L[s]			X		X	X
AE-QoL, AECT, SGART			X		X	X
Concomitant medications	←					→
AEs	←					→
diaryinstruction/review/ set-up	X		X	X	X	X
Investigator confirmation of attacks	←					→
Telephone contact	←					→

10.3. Study Visits

10.3.1. Screening Visit

Written informed consent and assent (as applicable) must be obtained from each subject before initiation of any screening assessments or procedures. Each subject will receive a copy of the signed and dated study-specific informed consent form (ICF). Prospective subjects who have signed an ICF who are interested in participation in the study will then undergo assessments at a screening visit to determine eligibility. Signing of the ICF may occur prior to the screening visit, which is defined as the visit where site-conducted screening procedures, including diary dispensing, are performed.

The Investigator (or designee) will conduct the following assessments at the screening visit, including:

- Signing of informed consent form (if not done prior to the visit) and assent (as applicable)
- Review of inclusion and exclusion criteria
- Medical and medication history (including angioedema medical and medication history)
- Complete physical examination
- 12-lead ECG
- Height/weight/body mass index (BMI) estimation
- Vital signs (blood pressure, pulse rate, temperature, and respiratory rate)
- Serum pregnancy test for female subjects of child-bearing potential
- Blood collection for clinical chemistry, hematology, coagulation, AST, ALT, HBV/HIV/HCV serology, C1-INH function, C4 level, and FSH (for women who declare that they have been post-menopausal ≤ 2 years).
- C1 inhibitor level, C1q antigen and C1 inhibitor autoantibody (if available)
- Urine collection for urinalysis, drugs of abuse screen and possible reflex testing for abnormal results
- Recording of AEs and concomitant medications
- Angioedema attack diary provision and instruction

All subjects will receive a diary at the screening visit to establish eligibility during the run-in period (ie, a minimum of 14 days to a maximum of 56 days from the date of the screening visit) and also to provide a baseline attack rate to properly evaluate the disease severity. The subject will record daily attacks in the diary beginning at the screening visit.

In the case of time limitations for conduct of the screening visit, a site is permitted to perform screening assessments over more than one screening visit; however, the diary should be dispensed on the first screening visit day, initiating the run-in period.

10.3.2. Period Between Screening and Baseline

Procedures to be performed by the site and/or clinical trial participants between screening and baseline are outlined in Table 2.

A subject's eligibility based upon the number of angioedema attacks will be determined during the run-in period; the baseline attack rate of the subject will also be calculated during the period from the Screening visit through start of treatment for the purposes of properly evaluate the disease severity.

For all attacks that occur following the screening visit, subjects will be contacted within approximately 2 business days of the attack to discuss the clinical characteristics of the attack, any questions on the entered data or to gain additional attack details not included in the diary that the Investigator deems important to clinically evaluate the event, as applicable. This information, in conjunction with the diary record, will be used by the Investigator to verify or reject the record as an attack. The diary data review, subject contact summary, and Investigator verification of the attack will be documented in the source records; the Investigator attack verification (confirmed or rejected) will also be recorded.

Once a subject records 2 such attacks, they may be enroll to study drug beginning on or after Day 28 of the run-in period. The maximum run-in period duration is 56 days.

Under no circumstances should the run-in attack requirement for eligibility be disclosed to study subjects.

The Investigator (or designee) must call and talk to the subject at least weekly in between the screening and baseline visits; alternative forms of interactive communication such as returned email and cellular text correspondence are acceptable. During all calls, the Investigator (or designee) will assess the subject's overall wellbeing, discuss compliance (if applicable), proper recording of attack details (if applicable), or any usability issues with the diary. A weekly phone call may be omitted if the subject records an attack as the Investigator must call and confirm or reject the attack.

Rescreening of ineligible subjects, where there is a reasonable expectation that the subject will become eligible, will be approved or denied on a case-by-case basis by the Sponsor. Retesting of specific assessments within the screening period without entirely rescreening a subject may be permitted. Additionally, the Investigator must gain Sponsor approval to enroll subjects who are not randomized within 10 weeks of the screening visit; this may require screening labs to be redrawn. Subjects will not be permitted to rescreen if they did not meet the AAE attack requirements during the run-in period.

A screening failure CRF page will be completed for those subjects who do not proceed with study dosing, recording the reason for screen failure.

AEs and concomitant medications will be recorded if reported during this period.

10.3.3. Baseline Visit (Day 1)

Subjects who meet all study eligibility criteria, and who agree to participate will be asked to return for a scheduled Day 1 visit, to be held 70 days or less from the screening visit.

Before any study drug is administered the following assessments will be completed:

- Administration of EQ-5D-5L, AE-QoL, AECT questionnaires. Where possible, the questionnaires should be completed by the subject prior to other assessments to prevent influencing subject perceptions.
- Review of inclusion and exclusion criteria, medical and medication history (including AAE medical and medication history) and prohibited medications
- Subject weight
- Vital signs (blood pressure, temperature, respiratory rate, and pulse rate)
- 12-lead ECG
- Complete physical examination
- Blood collection for clinical chemistry, hematology, and coagulation, C1-INH antigenic level,
- Urine pregnancy test for female subjects of childbearing potential. A negative urine pregnancy result must be recorded for the subject to be dosed.
- Review of concomitant medications and AEs
- Diary instruction and review

After completion of the above bullet items, the first dose of study drug may be administered in the clinic or administered at home on the day of the visit. Day 1 of the study is defined as the day in which subjects take the first dose of study drug.

During the Part 1 dosing period, Investigators will contact subjects to confirm all HAE attacks recorded in the diary within approximately 2 business days of the end of the attack. Moreover, any noncompliance will warrant contact with the subject.

The Investigator (or designee) must call and talk to the subject at least weekly; alternative forms of interactive communication such as returned email and cellular text correspondence are acceptable. During all calls, the Investigator (or designee) will assess the subject's overall wellbeing, discuss compliance (if applicable), proper recording of attack details (if applicable), or any usability issues with the e-diary. A weekly phone call may be omitted if the subject records an attack as the Investigator must call and confirm or reject the attack.

10.3.3.2. Week 4 and 12 Visits

Subjects will return to the clinic during Week 4 (Day 29 ± 2 days) and Week 12 (Day 85 ± 2 days).

Subjects do not need to withhold any doses on clinic days or take a dose in the clinic, unless the clinic visit falls during the subject's normal time of dosing.

The following assessments will be performed:

- Administration of EQ-5D-5L, AE-QoL, AECT questionnaires. Where possible, the questionnaires should be completed by the subject prior to other assessments to prevent influencing subject perceptions.
- Subject weight
- Vital signs (blood pressure and pulse rate)
- 12-lead ECG

- Abbreviated physical examination (targeted to new signs and symptoms)
- Blood collection for clinical chemistry, hematology, and coagulation
- Urine collection for urinalysis
- Urine pregnancy test for female subjects of childbearing potential
- Review of concomitant medications and AEs
- Review of attack and dosing diary completion and study drug compliance
- Study drug accountability and dispensing

During dosing period, Investigators will contact subjects to confirm all HAE attacks recorded in the diary within approximately 2 business days of the end of the attack. Moreover, any noncompliance will warrant contact with the subject.

The Investigator (or designee) must call and talk to the subject at least weekly; alternative forms of interactive communication such as returned email and cellular text correspondence are acceptable. During all calls, the Investigator (or designee) will assess the subject's overall wellbeing, discuss compliance (if applicable), proper recording of attack details (if applicable), or any usability issues with the e-diary. A weekly phone call may be omitted if the subject records an attack as the Investigator must call and confirm or reject the attack.

10.3.3.3. Week 24 Visit

The Week 24 visit will be conducted on Day 169, the day after the last dose of study drug.

- Administration of EQ-5D-5L, AE-QoL, AECT questionnaires. Where possible, the questionnaires should be completed by the subject prior to other assessments to prevent influencing subject perceptions.
- Subject weight
- Vital signs (blood pressure, temperature, respiratory rate, and pulse rate)
- 12-lead ECG
- Complete physical examination
- Blood collection for clinical chemistry, hematology, and coagulation,
- Urine collection for urinalysis
- Urine pregnancy test for female subjects of child-bearing potential. A negative urine pregnancy result must be recorded for the subject to be dosed.
- Review of concomitant medications and AEs
- Study drug collection/accountability
- diary review

After completion of the above bullet items, complete the study and start the compassionate use of berotralstat.

11. ASSESSMENTS

The schedule of procedures and assessments to be conducted throughout the study are outlined in table with details on the conduct of the procedures/ assessments provided below.

11.1. Investigator-Completed Assessments

Demographic information, including year of birth, sex, race, and ethnicity will be captured for each subject participating in the study at the screening visit. Medical and medication history will be captured at the screening visit and updated at baseline. Subject participation in a prior study will be captured (eg, study, previous subject number).

Contraceptive methods enabling eligibility will be captured in source documentation at the screening visit. Contraceptive methods and/or lifestyle should be reviewed throughout the study to ensure they remain appropriate for the subject.

11.1.1. AAE Medical and Medication History

An AAE medical history questionnaire provided by the Sponsor will be completed at screening. All questions should be completed by the Investigator (or designee) from historical source documentation when available, with subject input as necessary to complete the remaining questions. The completed AAE Medical History Questionnaire will be considered a source document and must be entered in the CRF in full to enable study entry.

11.1.2. Physical Examination

A full physical examination will be conducted at Screening, Baseline, and at Week 24. All other physical examinations will be abbreviated (ie, targeted or symptom-directed) to include, at a minimum, evaluation of any new signs or symptoms.

Genitourinary and breast examinations may be omitted when not required by normal site practice.

11.1.3. Weight/Body Mass Index

For determination of height and weight, subjects should be clothed with shoes removed.

BMI should be calculated using the following formula:

$$\text{BMI} = \text{weight (kg)}/\text{height (m)}^2$$

BMI and height are only to be captured at the screening visit.

11.1.4. 12-lead Electrocardiograms

A standard bedside or routine 12-lead ECG machine that calculates heart rate and measures the PR, QRS, QT, RR, and QTc (QTcF) intervals will be utilized. Prior to obtaining an ECG, subjects should rest quietly in a supine position for at least 10 minutes.

Qualified site personnel should review the ECGs and automated findings in real-time for gross abnormalities and interval measurements of concern (absolute readings and for postbaseline ECGs, a change from baseline). For all ECGs, the clinical interpretation of the ECG and calculated QTcF (including adjudication of any automated measurements or diagnoses) should be recorded directly on a hard copy of the ECGs. Copies of the ECGs may be requested by the Sponsor. All subject identifiers will be masked prior to provision to the Sponsor.

An ECG should be repeated for a change from baseline in QTcF > 60 msec or a QTcF interval > 500 msec.

11.1.5. Vital Signs

Blood pressure (systolic and diastolic) and pulse rate should be taken after the subject has rested in the supine position for at least 5 minutes. Blood pressure measurements must be obtained with an appropriate cuff size and with the subject's arm supported at the level of the heart. It is acceptable to obtain a pulse rate from the blood pressure or ECG machine. Temperature and respiratory rate will be captured at Screening, Baseline, and Week 24 only.

11.1.6. Clinical Laboratory Evaluations

Blood and urine samples will be obtained per the schedule of events. Individual laboratory tests to be performed are provided in Table 2.

All laboratory samples will be perform locally. Additionally, urine pregnancy tests will be perform with urine stick.

Results from the laboratory values should be reviewed as received by the Investigator. Evidence of this review should be provided in the source records and may include printing of the laboratory reports with a signature attesting to a review. For out-of-range laboratory findings, the interpretation of clinically significant or not clinically significant should be denoted in the source records. Clinically significant laboratory findings in the opinion of the Investigator should be recorded as an AE and handled as described in the specific section.

Table 2. Clinical Laboratory Evaluations

Chemistry	**Hematology**
• Albumin • Alkaline phosphatase (ALP) • Alanine aminotransferase (ALT) • Aspartate aminotransferase (AST) • Bilirubin (total and direct) • Blood glucose • Blood urea nitrogen (BUN) • Electrolytes (calcium, sodium, potassium, chloride, bicarbonate [CO2], phosphorus) • Lipid panel (total cholesterol, triglycerides) • Creatine kinase • Creatinine and calculated CL_{CR} • Gamma-glutamyl transferase (GGT) • Lactate dehydrogenase (LDH) • Total serum protein • Uric acid • *If amylase is > 2 × ULN, reflex to* lipase	• Hemoglobin • Hematocrit • Erythrocytes • Mean corpuscular haemoglobin (MCH) • Mean corpuscular haemoglobin concentration (MCHC) • Mean corpuscular volume (MCV) • White blood cell count, with differential (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) • Platelets
Urinalysis	**Coagulation**
• Specific gravity • Blood • Bilirubin • Glucose • Leukocytes • Ketones • Nitrites • pH • Protein • Urobilinogen • Microalbumin to creatinine ratio • Reflex Microscopy if dipstick is abnormal	• Prothrombin time (PT) and international normalized ratio (INR) Activated partial thromboplastin time (aPTT)
	Pregnancy Test
	Serum (screening) and urine (other scheduled visits) βHCG for women of childbearing potential
	Additional Tests at screening
	• Hepatitis B surface antigen, hepatitis C antibody, HIV antibody; if HCV antibody positive, reflex to HCV RNA testing • C1-INH level and function • C1-INH antigenic level • C4 • C1q

CL_{CR} will be calculated using the Cockcroft-Gault formula and actual body weight (ABW):

$$CL_{CR}\ (mL/min) = \frac{(140 - \text{age in years}) \times ABW\ (kg)}{72 \times \text{serum creatinine (in mg/dL)}}\ (\times 0.85 \text{ for females})$$

11.1.7. Screening for Human Immunodeficiency Virus, Hepatitis B, and Hepatitis C Serology

Blood samples will be collected at screening for serologic testing for evidence of HIV, chronic hepatitis B, and chronic hepatitis C infection.

11.1.8. Pregnancy Testing

FSH will be measured at screening in women declaring themselves postmenopausal ≤ 2 years to establish childbearing status. At screening, a serum pregnancy test should also be drawn in the event a woman subject postmenopausal ≤ 2 years is found to be of childbearing potential.

For women of childbearing potential, a serum pregnancy test will be administered at screening. Urinary pregnancy tests will be assessed at all subsequent visits. A serum pregnancy test should immediately be drawn and sent for analysis for any positive urine pregnancy test.

11.1.9. AAE Diagnostic Confirmation

C1-INH functional level and C4 are to be drawn at the screening visit; it is recommended that samples not be drawn within 3 days of C1-INH administration

Confirmed diagnosis of AAE is defined as:

- Recurrent angioedema without wheals
- No family history of angioedema and/or C1-INH deficiency
- C1-inhibitor activity level of <50% of normal and **at least 1 of the following**:
  - Age at least 40 years at reported onset of first angioedema symptoms
  - C1q level below the lower limit of normal.
  - Absence of a pathogenic variant in the SERPING1 gene encoding for the C1-inhibitor protein.
  - Serologic confirmation of anti–C1-inhibitor antibodies.

11.1.10. Biobank Sample

Not applicable.

11.1.11. Adverse Events

AEs will be assessed and recorded from the time that the ICF is signed through the last follow-up visit or until the AE is resolved or the subject is in a clinically stable condition with regards to the AE. Full details of recording and reporting AEs are provided in specific sections .

11.2. Patient-Reported Outcomes

The AE-QoL, EQ-5D-5L and AECT will each be administered once at Baseline (predose) and at Weeks 4, 8, 12, 18, 24.

Subjects will also be queried about their long-term experience on study beginning at Week 96 via brief long-term experience survey.

Each questionnaire will be in Italian. For all subject-completed forms, clinic staff should ensure the subject reads the instructions and completes the questionnaires in full prior to filing in the source documentation.

Where possible, the questionnaires should be completed by the subject prior to other assessments for that visit to prevent influencing subject perceptions.

11.3. HAE Attack and Dosing Diary and Attack Confirmation

11.3.1.1. HAE Attack and Dosing Diary

The Sponsor will supply diaries to sites. Study-specific manuals will be prepared for both site staff and subjects for use of the diary for this study.

While a subject has a diary in their possession, the subject will fill out the angioedema attack diary daily, recalling whether or not symptoms of an angioedema attack were experienced in the previous 24 hours. Subjects will fill out the diary daily regardless of the presence of AAE symptoms. If the subject does report an attack, additional details about the attack will be entered into the diary including start and stop time of the attack, attack symptoms, anatomical location of swelling (if applicable), severity, treatment(s) administered and times of administration, and whether additional medical care was sought for the attack. During study drug administration, subjects will also record the times of day study drug was taken daily in the e-diary.

All subjects will fill out a diary during the run-in period of a minimum of 14 days to a maximum of 56 consecutive days from the Screening visit to enable the qualifying attack rate to be established. Subjects will continue to fill out their diary after the run-in period in advance of the baseline visit, as applicable. Subjects will continue to fill out the diary daily during the treatment and follow-up periods.

Site staff will set up the diary when a subject is initially provided a diary at the Screening visit and then as needed during the study (eg, to turn off entry into the dosing diary when not receiving study drug). Subjects should be instructed to bring their diary with them on each study visit. Once a subject completes or discontinues the study, the site should ensure that diaries are returned.

Study staff are not permitted to make any entries into the diary.

Subjects who discontinue study drug should continue to record the occurrence of attacks in their diary until the follow-up visit.

11.3.1.2. Investigator Confirmation of Attacks

For all attacks that occur from screening through the end the study the patient has to inform investigator of the occurrence, the Investigator (or appropriately trained designee) will review the diary record of the attack details. Subjects will then be contacted within approximately 2 business days of the end of the attack to discuss the clinical characteristics of the attack, any questions the Investigator has on the entered data or to gain additional attack details not included

in the diary that the Investigator deems important to clinically evaluate the event, as applicable. This information, in conjunction with the diary record, will be used by the Investigator to verify or reject the event recorded in the diary as an attack. The diary data review, subject contact summary, and Investigator verification or rejection of the attack will be documented in the source records; the Investigator attack verification (confirmed or rejected) will also be recorded.

During the study, Investigators will use their judgment to confirm or reject a reported event as an attack; however, all attacks must include symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may also include symptoms in the oropharyngeal or abdominal regions which are indicative of internal swelling.

11.3.1.3. Scheduled Telephone Contact

The Investigator (or designee) must call and talk to the subject at least weekly in between the Screening and Baseline visits and on-treatment through Week 24. Alternative forms of interactive communication such as returned email and cellular text correspondence are acceptable. During all calls, the Investigator (or designee) will assess the subject's overall wellbeing, discuss compliance (if applicable), proper recording of attack details (if applicable), or any usability issues with the e-diary. The phone call may be omitted if the subject records an attack as the Investigator must call and confirm or reject the attack.

12. ADVERSE EVENT MANAGEMENT AND REPORTING

12.1. Adverse Events

AEs will be assessed and recorded from the time of signing of the informed consent through the appropriate follow-up period.

12.1.1. Definitions

12.1.1.1. Adverse Event

An AE is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (eg, requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs. If the diagnostic procedure prompts no additional treatment, visits, or monitoring, it may not meet the definition of an AE.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period , including medical triggers resulting in an HAE attack. Emotional stress will not be considered an AE unless it results in a medical diagnosis or requires medical treatment.
- Findings from protocol-mandated interventions. This can include laboratory assessments performed in the course of the clinical trial. AEs should only be reported if the abnormalities are changed from baseline and are clinically significant as described above.

- Pre-existing medical conditions (other than the condition being studied) judged by the Investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period. When recording such events on an AE/SAE eCRF page, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (eg, "more frequent headaches").

An adverse reaction is defined in Article 2(n) of Directive 2001/20/EC as follows: all untoward and unintended responses to a study drug/IMP related to any dose administered. The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. The definition implies a reasonable possibility of a causal relationship between the event and the study drug/IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

For the purposes of this protocol, HAE attacks and their associated symptoms will not be defined as AEs, even if the subject requires hospitalization. This information, as well as HAE attacks and associated symptoms are reported in the subject's diary and are a reflection of the disease under study. The events that may trigger an HAE attack, such as an infection or trauma, are considered AEs and should be reported as such.

Hospitalization scenarios do not require reporting as an SAE where there is no occurrence of an AE. These scenarios include a planned hospitalization or prolonged hospitalization to:

- Perform a routine control screening for a preexisting illness or to diagnose a suspected illness. In the case of the latter, the symptomatology should be reported as an AE and amended if a diagnosis is confirmed.
- Undergo a diagnostic or elective surgical procedure for a preexisting medical condition that has not changed (eg, a joint replacement for which the subject was on a waiting list).
- Undergo medical observation due to AAE (eg, admission after routine dental procedure in a subject with AAE).
- Undergo medical observation without the occurrence of an AE due to standard of care in the region or hospital.

Surgical procedures should not be reported as AEs. The condition for which the surgery is required should be reported as the AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

AEs are designated as "nonserious" or "serious."

12.1.1.2. Serious Adverse Event

A SAE is an adverse event/reaction that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)

- Requires subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization (refer to Section 12.1.1.1 for details on hospitalization SAE criteria).
- Results in persistent or significant disability/incapacity (ie, there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject's health or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. For this study, examples of such events may include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in subject hospitalization.

In addition, the sponsor considers events of abortion (spontaneous or induced), fetal demise, and still birth as SAEs for reporting purposes.

Overdose will be considered an SAE only if any of the seriousness criteria are met. Any clinical complication in association with the overdose should be reported as an AE or SAE (as applicable) along with the overdose. Details of signs or symptoms, clinical management and outcome should be reported, if available. Overdose without associated signs or symptoms should not be recorded as AEs but should be recorded as protocol deviations.

12.1.1.3. Adverse Events of Special Interest

For this protocol, nonserious treatment-emergent maculopapular rashes that are deemed related to Berotralstat will be considered EOSIs. This does not include other types of rashes such as urticaria or eczema. All treatment-emergent skin conditions should be reported as AEs but only maculopapular rashes deemed related to Berotralstat should be considered EOSIs.

An EOSI event in and of itself will not be considered serious unless it meets the seriousness criteria above.

12.1.2. Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

Reports of all AEs and SAEs, regardless of Investigator attribution, are to be collected from the time of signing of the informed consent through to the last study visit (ie, through the posttreatment follow-up visit). All AEs and SAEs are to be reported on the AE CRF.

AEs should be documented on CRFs as Investigators become aware of them. AEs are to be followed until the event resolves. If an event is ongoing at the last follow-up visit, Grade 1 and 2 events do not need to be followed if the event is deemed unlikely to be related or not related to study drug (see grading section). For all Grade 3 and 4 events or events deemed possibly related to use of study drug, the event should be followed until the AE is resolved or the subject is in a clinically stable condition with regards to the AE.

The Investigator shall report all SAEs immediately to the Sponsor by communicating with the Medical Monitor (phone or email) and by submission of an SAE report form via email, and entering the event onto the AE CRF within 24 hours of their knowledge of the event (see specific Section). The SAE report form is a detailed, written report on the SAE provided by the Sponsor or designee. The Investigator should follow all unresolved SAEs observed during

the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is made during evaluation or treatment, the Investigator will update the AE record with this diagnosis. The immediate and follow-up reports shall identify subjects by unique code numbers assigned to the latter to ensure that the Sponsor shall have the necessary information to continuously assess the benefit-risk profile of the study drug in a clinical trial.

12.1.3. Definition of Severity

All AEs will be assessed (graded) for severity and classified using the DMID criteria for grading AEs. Any AEs not covered by the DMID criteria will be assessed and classified into 1 of 4 clearly defined categories as follows:

Mild:	(Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level. It may be an annoyance.
Moderate:	(Grade 2): Symptom results in mild to moderate limitations in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
Severe:	(Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
Life-threatening:	(Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required to prevent death, hospitalization; or hospice care probable.

Severity refers to the medical perspective of an event while seriousness reflects the outcome of the event (ie, hospitalization). Events of mild severity can lead to hospitalization and therefore be serious while severe events such as a headache may not meet seriousness criteria.

12.1.4. Definition of Relationship to Study Drug

The Investigator or medically qualified designee must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related:	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.
Unlikely:	The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related:	There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.
Probably Related:	The event follows a reasonable temporal sequence from drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.
Definitely Related:	The event follows a reasonable temporal sequence from study drug administration, follows a known or suspected response pattern to the study drug, is confirmed by improvement upon stopping the study drug (dechallenge), and reappears upon repeated exposure (rechallenge, if rechallenge is medically appropriate).

12.1.5. Reporting Serious Adverse Events and Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any SAE must be reported by phone or email to the Sponsor Medical Monitor and in writing via email using the SAE report form within 24 hours of the Investigator's awareness of the SAE. In addition, all SAEs must be recorded on the AE CRF in real time. All additional follow-up evaluations of the SAE must be reported to sponsor or its designee as soon as they are available. The SAE report forms should be sent to the following email addresses:

Phone (24 hours):+39 02 52774516
Email:Farmacia.PSD@grupposandonato.it

Immediate reporting should allow Sponsor to take the appropriate measures to address potential new risks in a clinical trial. Therefore, the initial report should be submitted by the Investigator within a very short period of time and under no circumstances should this period exceed 24 hours following awareness of the SAE.

The follow-up report should allow Sponsor to determine whether the SAE requires a reassessment of the benefit-risk profile of the study drug in a clinical trial, if the relevant information was not already available and provided in the initial report.

SUSARs related to the IMP(s) will be reported by the PI/Investigator to the PI Clinical Trial Pharmacy (farmacia.psd@grupposandonato.it), within 24 hours of becoming aware of the event, using the CIOMS (Council for International Organizations of Medical Sciences) form. Subsequently, the Clinical Trial Pharmacy will report the SUSARs to the relevant Competent Authority (via Eudravigilance) and the EC. For fatal and life-threatening SUSARs, the Pharmacy must report the SUSAR within 7 calendar days. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days. Treatment codes will be un-blinded for specific participants. Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current study.

12.1.5.1. Reporting Events of Special Interest

All events of diffuse maculopapular rash assessed as related to study drug/IMP, regardless of severity must be reported by phone or email to the Sponsor Medical Monitor and in writing via email using the SAE/EOSI report form within 24 hours of the Investigator's assessment of the event. High resolution photographs must also be submitted as described in specific section. In addition, the event must be recorded on the AE CRF in real time. All additional follow-up evaluations of the event must be reported to Sponsor or its designee as soon as they are available. The SAE/EOSI report form should be sent to the following email addresses:

Phone (24 hours):+39 02 52774516
Email:Farmacia.PSD@grupposandonato.it

This method of reporting will allow Sponsor to obtain more information than can be captured in the eCRF for this event. The report form will allow a full clinical description and information regarding the evaluation that cannot be documented in the electronic data capture due to free text limitations to be shared with Sponsor. Therefore, the initial report and photographs should be submitted by the Investigator within a very short period of time and under no circumstances should this period exceed 24 hours following assessment of the event.

The follow-up report should contain information about the clinical course, medical evaluation, additional photographs (if relevant), biopsy (if done), and laboratory results.

12.1.6. Pregnancy

Any female subject who becomes pregnant during the study should have study drug discontinued immediately and must be followed through the end of the pregnancy. While pregnancy is not considered an AE, all cases of fetal drug exposure via the parent as a study participant (including partners of study participants) are to be reported immediately to Sponsor or its designee. Consent from a pregnant partner of a study participant will be obtained prior to reporting any details of the pregnancy. Information related to the pregnancy must be given on a "Pregnancy Confirmation and Outcome" form that will be provided by the Sponsor or its designee so that the pregnancy may be followed and an outcome determined. Any AEs or SAEs experienced by a pregnant subject are to be reported as directed above. Any complications reported in a subject's pregnant partner should be reported on the Pregnancy Confirmation and Outcome form. All pregnancies must be followed to the outcome which occurs when an infant is delivered (live or still born), there is fetal demise, or there is an abortion (spontaneous or induced). Abortion (spontaneous or induced), fetal demise, and still birth along with congenital abnormalities in the newborn, should be reported as separate SAEs.

12.1.7. Serious Breaches of Good Clinical Practice

It is the responsibility of the Sponsor to notify the licensing authority of any serious breach of Good Clinical Practice which is likely to effect, to a significant degree, the safety or mental integrity of the subjects of the study or the scientific value of the study. All serious breaches will be notified to the relevant competent authority within 7 days. The reporting to the Sponsor will be performed by the party who suspects of the serious breach.

12.1.8. Treatment Interruptions

Treatment interruptions as a result of Investigator management of AEs potentially related to study drug are permissible. Resumption of study drug administration is also permissible upon

resolution of the event, as assessed by the Investigator, with a plan for stringent monitoring of the subject for recurrence of the AE as appropriate. In addition, other extenuating circumstances may lead to treatment interruptions such as vomiting during an abdominal attack or required fasting for medical procedures; in these cases, study drug should be resumed once the extenuating circumstance is resolved. Treatment interruptions following suspected drug-related rashes are discussed in specific Section. The exception to treatment interruption and resumption is when study drug is stopped for a rash considered study drug related (possibly, probably, definitely related). If study drug is interrupted for > 10 days due to a related drug rash, study drug may not be restarted. However, interruption for ≤ 10 days will be allowed because the study drug's long half-life would allow sufficient plasma concentrations to remain for safe reintroduction of study drug.

12.2. Toxicity Management

The Investigator (or qualified designee) will grade clinically significant events and laboratory abnormalities according to that detailed in Section above. Grade 3 and 4 clinically significant laboratory abnormalities should be confirmed by repeat testing and before any contemplated study drug discontinuation, unless such a delay is not consistent with good medical practice.

In the event that a new clinically significant safety signal emerges, investigators will evaluate risk to subjects and recommend appropriate actions. Based on the data reported, a decision will be made as to whether to halt the study or to continue dosing.

12.2.1. Aminotransferase (ALT or AST) Elevation

All baseline or treatment-emergent ALT or AST elevations > 3 × ULN (ie, Grade 3 and above) should be confirmed within 72 hours with repeat assessment of ALT and AST as well as total bilirubin, ALP, prothrombin time/INR, and complete blood count (CBC) for eosinophil levels. If subjects are asymptomatic with no other pertinent laboratory abnormality, study drug may be continued under close observation with weekly assessment of transaminase levels, total bilirubin, and ALP. These may be done at a local laboratory as long as results are reported to the Investigator as soon as they are available and the investigative site contacts the subject to ascertain any symptoms. If ALT or AST continue to increase and the subject remains asymptomatic, study drug dosing must be held if:

- Either ALT or AST is > 5 × ULN for > 2 weeks
- The ALT or AST reaches > 8 × ULN

The subject should continue weekly assessment of ALT, AST, total bilirubin, ALP, prothrombin time/INR, and CBC for eosinophil levels until ALT and/or AST are < 3 × ULN.

Provided specific criteria are met, the Investigator and subject may elect to resume Berotralstat dosing. All of the following criteria must be met for dosing to resume.

- The subject is considered to have been deriving benefit from Berotralstat prior to holding study drug.
- Transaminases return to ≤ 2 × ULN for subjects whose baseline transaminase levels were above the ULN, and ≤ ULN for those whose baseline transaminase levels were ≤ ULN.
- Subjects have not initiated or restarted androgens during the period Berotralstat was held.

- The subject agrees to continue weekly monitoring of ALT, AST, total bilirubin, ALP, CBC (eosinophil levels) and prothrombin/INR until levels appear stable and transaminase levels remain < 3 × ULN for at least 1 month after restarting BEROTRALSTAT dosing.

12.2.2. Overdose

To date there is no experience with an overdose of oral Berotralstat. Single doses of up to 1000 mg, 7 days of dosing up to 500 mg/day, and 14 days of dosing with 350 mg/day revealed no clinically significant safety concerns in healthy subjects. Safety data generated in clinical study with berotralstat with 28-day dosing of up to 350 mg/day revealed no clinically significant safety concerns in subjects with HAE. Subsequently, subjects enrolled in phase 1 study were exposed to Berotralstat 450 mg QD for 14 days without any unanticipated AEs or increased AE severity.

In the event that study personnel become aware of an overdose of study drug/IMP (> 1 dose per calendar day) that is associated with an AE, both the overdose and the resultant event should be reported as AEs. Overdose without any symptoms (ie, AEs) does not need to be reported as an AE. If overdose occurs with or without associated AEs, subjects should undergo clinical and laboratory monitoring as appropriate for their clinical condition and, if indicated, should receive clinically-indicated supportive therapy. Overdose without associated signs or symptoms should not be recorded as an AE but should be recorded as a protocol deviation.

Additional information about overdose as an AE or SAE is discussed in Section above.

13. STATISTICS

13.1. Hypotheses

Null hypothesis (H0): the attack-rate ratio between the treatment period (Day 1-168) and the run-in period equals 1.

Alternative hypothesis (H1): the attack-rate ratio between the treatment period and the run-in period is less than 1.

13.2. Sample Size Considerations

The sample size calculation is based on the methodology proposed by Sumi and Tango (Stat. Med. 2010) for inference on recurrent-event rate ratios in a matched-pairs design. In the BESAFE study, each subject serves as his/her own matched pair, with recurrent angioedema attacks observed during the run-in period being compared with those observed during the treatment period. The primary efficacy analysis evaluates the rate ratio of investigator-confirmed angioedema attacks during Berotralstat treatment relative to the run-in period. The sample size was calculated assuming:

- Baseline attack rate during the run-in period: 24 attacks per patient-year.
- Treatment-period attack rate: 17 attacks per patient-year.
- Target rate ratio (treatment/run-in): aproximately 0.65.
- Two-sided significance level (α) of 0.05.
- Statistical power of 80%.
- Run-in period duration of 28 days.
- Treatment period duration of 168 days, corresponding to an observation-period ratio (k) of 6.

The expected number of attacks during the run-in period was derived from the assumed baseline attack rate and run-in duration. The sample size was then calculated using the Sumi and Tango matched-pair recurrent-event rate-ratio formula. Under these assumptions, approximately 16 evaluable subjects are required.
The assumptions used for sample size determination are subject to substantial uncertainty because acquired angioedema due to C1-inhibitor deficiency (AAE) is an ultra-rare disorder and no prospective controlled studies of long-term prophylaxis are available in this population. Consequently, reliable estimates of attack frequency, treatment effect, and variability cannot be directly obtained from published interventional studies.
The assumed baseline attack rate of 24 attacks per patient-year was selected on the basis of the study eligibility criteria requiring at least two investigator-confirmed attacks during a run-in period of 28 to 56 days, together with clinical experience from participating AAE referral centres. This assumption is considered representative of a population with active disease requiring prophylactic treatment.
The assumed treatment-period rate ratio of approximately 0.65 was selected as a clinically meaningful reduction in attack frequency. This assumption is supported by the established role of plasma kallikrein inhibition in bradykinin-mediated angioedema and by the efficacy observed with berotralstat in hereditary angioedema, while recognizing that treatment effects in AAE may differ because of the limited available evidence in this indication.
Sensitivity evaluations performed during study planning demonstrated that required sample size varies substantially according to assumptions regarding baseline attack frequency and treatment effect. For example considering a rate ratio equal to 0.7, 23 patients are needed. Considering 20 attacks per patient-year 19 patients are needed. Accordingly, the planned enrolment of approximately 16 evaluable subjects represents a balance between statistical considerations, clinical relevance, and recruitment feasibility.
Therefore, BESAFE should be regarded as an exploratory Phase II study intended to generate preliminary estimates of efficacy, safety and variability rather than definitive confirmatory evidence.

13.3. Statistical Methods

13.3.1. Analysis Populations

The analysis populations are defined below.

13.3.1.1. Screen Failures

Subjects who give written informed consent but are not enrolled to study treatment are noted as screen failures in the eCRF are considered screen failures.

13.3.1.2. Intent-to-Treat Population

The ITT population will include all subjects who are recruited. The ITT population will be used as the primary population for efficacy analyses.

13.3.1.3. Safety Population

The safety population will include all subjects who received at least 1 capsule of study drug. This population will be used for all analyses of accountability, demographics, berotralstat drug concentrations, and safety.

13.3.1.4. Per Protocol Population

The Per Protocol (PP) population will include subjects in the safety population that complete the study. A decision will be made prior to database lock on which (if any) subjects are to be excluded from the PP population based upon major protocol deviations. In the per-protocol analysis, subjects will be assessed based on the actual treatment received on study Day 1. The PP population may be used as a secondary population for efficacy analyses.

13.3.1.5. Completers' Population

Subjects in the ITT population who complete the study will comprise the completers population.

13.3.2. General Considerations for Data Analysis

In general, descriptive summaries will include n, mean, SD, median, minimum, and maximum for continuous variables and n and percent for categorical variables. Summaries will be presented by study visit.

Given the exploratory nature of this Phase II study, no adjustment for multiplicity will be performed. The primary endpoint will be analyzed as specified in the protocol. Analyses of secondary efficacy endpoints and health-outcome endpoints will be considered exploratory and descriptive; therefore, any p-values and confidence intervals, if provided, will be interpreted descriptively and not as confirmatory evidence of treatment effect.

All individual subject data will be listed as measured. All statistical summaries and analyses will be performed using SAS® software (SAS Institute, Cary, North Carolina, USA).

13.3.3. Subject Demographic and Disposition Data

Demographic data and baseline characteristics including age, gender, race and ethnicity, height, weight, BMI, and HAE history including medication history will be summarized.

Subject disposition will be presented for all subjects. The number of subjects who complete the study and those who discontinue from the study will be provided. The reasons for early discontinuation also will be presented. A tabulation of the number of subjects exposed to study drug and duration of exposure will also be presented for each treatment group. Treatment adherence, dose interruptions, and reason for dose interruptions will be provided as summaries or listed as appropriate.

13.3.4. Analysis of Efficacy Variables

The efficacy analyses will be based on the ITT population. The analyses of the PP population and completers population will be used to support the primary efficacy analyses.

Efficacy data will be summarized by the total subjects who received Berotralstat.

The effective dosing period (Day 8 through Day 168, inclusive) is the date of first dose + 7 days to the last dose in Part 1 on Day 168 + 24 hours, or 24 hours after the last dose of the study drug, whichever is earlier.

13.3.4.1. Primary Efficacy Analysis

Categorical variables will be shown as count and percentage, normal variables as mean±SD, non-normal variables as median and interquartile range. Normality assumption will be tested in continuous variable by visual inspection of qq-plot and Shapiro test. Correlations between continuous variables will be evaluated according to Pearson R or Spearman Rho, depending on the distribution. Paired T tests will be used to compare continuous variables. T test or Wilcoxon rank sum test will be used to compare unpaired means in normally or non-normally distributed variable, respectively. Fisher's exact test or Chi- square test will be used to compare categorical data.
The primary estimand evaluates the effect of treatment with Berotralstat on the investigator-confirmed angioedema attack rate during the 24-week treatment period (Day 1-168) compared with the attack rate observed during the run-in period. All investigator-confirmed attacks occurring during Day 1–168 will be included in the primary analysis. The target population consists of all enrolled subjects meeting study eligibility criteria.
The variable of interest is the investigator-confirmed angioedema attack rate expressed as the number of attacks per unit of observation time.
Because the run-in period may vary from 28 to 56 days, the baseline attack rate will be defined for each subject as the number of investigator-confirmed attacks divided by the observed duration of the run-in period. Consequently, variability in run-in duration will be accommodated through person-time rate calculations rather than absolute event counts.
The primary treatment effect measure is the rate ratio between the treatment-period attack rate and the run-in attack rate.
The paired comparison between the attack rate post-treatment with the attack rate pre-treatment will be estimated using the Mantel-Haenszel estimator with its 95% confidence interval as described in stat.med 2010. The analysis will compare attack rates during the treatment period with attack rates during the run-in period within the same subject. Attack rates will be calculated as the number of investigator-confirmed attacks divided by the corresponding observation time. Differences in observation time between subjects and study periods will therefore be accounted for through the use of person-time rates. The treatment effect will be expressed as the rate ratio (treatment period/run-in period) together with two-sided 95% confidence intervals. The null hypothesis will be rejected if the 95% confidence interval excludes 1.

As a supportive sensitivity analysis, accounting for possible differences in follow-up and for baseline subject characteristics, the primary endpoint will additionally be analyzed using a mixed-effects negative binomial regression model. The dependent variable will be the number of investigator-confirmed angioedema attacks during each study period (run-in and treatment). Study period (run-in versus treatment) will be included as a fixed effect. Subject will be included as a random effect to account for the paired within-subject design and resulting within-subject correlation of recurrent events. The logarithm of observation time for each study period will be included as an offset term to account for possible differences in follow-up duration and exposure time. The negative binomial distribution will be used because recurrent angioedema attack counts may exhibit overdispersion, whereby the variance exceeds the mean attack rate. Treatment effect estimates will be presented as rate ratios with corresponding two-sided 95% confidence intervals and p-values. Results from this analysis will be regarded as supportive of the primary analysis and will be interpreted descriptively.

13.3.4.2. Secondary and Exploratory Efficacy Analyses

For the secondary efficacy endpoints the following estimands will be calculated: the monthly HAE attacks rate over 6 months of treatment, the mean number and the rate of attacks requiring rescue

treatment, the mean and median attack free period (defined as the time between successive attacks), the proportion of days without angioedema symptoms, the attack rate pre and post treatment for mild, moderate and severe severity will be calculated.

A supportive exploratory analysis will compare the monthly investigator-confirmed angioedema attack rate during the stable-treatment period (Weeks 20-24) with the monthly attack rate observed during the run-in period. This analysis is intended to evaluate treatment effect after achievement of steady-state exposure and sustained treatment and will be interpreted descriptively.

13.3.5. Planned Analyses

The primary analysis is planned after the last subject completes Week 24 of the study. and the study database has been finalized and locked. No formal interim analyses are planned.

13.3.6. Analysis of Safety Variables

The safety analyses will be analyzed separately will be combined for a long-term safety assessment.

Safety endpoints that will be summarized include, at a minimum, the number and proportion of subjects 1) with a TEAE; 2) who discontinue Berotralstat due to a TEAE 3) who experience a TESAE; 4) who experience a treatment-emergent Grade 3 or 4 AE; and 5) who experience a treatment-emergent Grade 3 or 4 laboratory abnormality. In addition, the proportion of subjects with a treatment-emergent, treatment-related AE consistent with a drug rash will also be summarized.

Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ class (SOC). The occurrence of TEAEs will be summarized using MedDRA preferred terms, SOC, and severity. Separate summaries of TEAEs, TESAEs, AEs considered to be related to study drug, and AEs leading to study drug interruption or to discontinuation will be generated. All AEs will be listed for individual subjects showing both verbatim and preferred terms.

Any graded abnormality that occurs following the initiation of study drug and represents at least 1-grade increase from the baseline assessment is defined as treatment-emergent. The number and percentage of subjects who experience treatment-emergent graded toxicities will be summarized. Laboratory toxicity shifts from baseline to worst postbaseline assessments will be summarized.

Clinically significant abnormal morphological ECG findings will be summarized.

The change from baseline in QTcF will be determined by routine ECGs. At each time point ECGs are analyzed, an individual subject's change from baseline will be calculated as:

$$\Delta_{ik} = (\text{QTcF for subject at time point k} - \text{Baseline QTcF})$$

Where QTcF measurements will be the average of triplicate ECGs at baseline and single values at each time point.

For routine ECGs, the number and proportion of subjects with QTcF ≤ 450, > 450 to ≤ 480,

> 480 to ≤ 500, and > 500 msec; or changes of ≤ 30, > 30 to ≤ 60, or > 60 msec will be summarized.

Physical examination findings will be listed.

Concomitant medications will be coded using the World Health Organization drug dictionary and summarized.

As applicable, safety data will be summarized by treatment group and total Berotralstat. No tests of hypothesis are planned for safety data.

13.3.7. Health Outcome Analyses

Health Outcome endpoints are as follows:

- EQ-5D-5L scores
- AE-QoL scores
- AECT scores

The between-treatment comparisons for, EQ-5D-5L, AE-QoL, and AECT will be performed using a mixed-effect model, including terms of treatment, visit, treatment and visit interaction, and baseline score. Details will be provided in the SAP.

13.3.8. Handling of Intercurrent Events

The primary efficacy estimand evaluates the effect of Berotralstat on the monthly investigator-confirmed angioedema attack rate during the 20-week treatment period compared with the run-in period.

The following strategies will be applied for intercurrent events:

Discontinuation of study drug

Subjects who permanently discontinue study drug before Week 20 will be encouraged to remain in the study and continue efficacy and safety assessments. All available efficacy data collected through Week 20 will be included in the primary analysis (treatment-policy strategy).

Temporary treatment interruptions

Investigator-confirmed attacks occurring during treatment interruptions will be included in efficacy analyses and attributed to the treatment period.

Rescue treatment

Use of rescue medications for acute angioedema attacks (e.g., icatibant or plasma-derived C1-INH) is permitted according to standard clinical practice. Attacks requiring rescue treatment will remain included in efficacy analyses. The rate of attacks requiring rescue treatment will also be summarized as a secondary endpoint.

Non-adherence

Subjects with incomplete adherence to study drug administration will remain in the ITT population and their data will contribute to the primary analysis. Treatment compliance will be summarized descriptively. Sensitivity analyses may be performed in the Per-Protocol population.

Use of prohibited medications

Initiation of prohibited medications during the study will be documented as a protocol deviation. Data collected after initiation of prohibited medication will remain included in the primary ITT analysis. The impact of such deviations may be explored through supportive Per-Protocol analyses.

Transition to compassionate use

Subjects may transition to compassionate use of Berotralstat after completion of the Week 20 visit. Since compassionate use begins after completion of the primary study period, it will not affect the primary efficacy analysis or estimand

13.3.9. Handling of Missing, Unused and Spurious Data

The primary analysis will be performed using all available efficacy data collected during the run-in and treatment periods. No imputation of missing attack counts is planned for the primary analysis. Analyses will be based on observed data and adjusted for actual observation time. Subjects will contribute person-time until the end of their available follow-up period.

Missing or Incomplete Diary Entries

Diary data constitute the primary source for attack identification and attack characteristics.

- If a diary entry is partially incomplete but sufficient information exists for the Investigator to confirm the occurrence and timing of an angioedema attack, the attack will be considered valid and included in efficacy analyses.
- If an attack is reported but key information is missing, the Investigator will attempt to clarify the information through scheduled telephone contacts or source documentation review.
- If an attack cannot be verified because of insufficient information, it will not be counted as an investigator-confirmed attack for efficacy analyses.
- Missing diary days without evidence of an attack will not be automatically interpreted as attack-free days.

Spurious Data and Data Validation

Potentially inconsistent, implausible or duplicate diary entries will be reviewed by the Investigator.

Examples include:

- duplicate attack records,
- overlapping attacks that do not satisfy protocol definitions,
- implausible dates or durations,
- contradictory diary information.

Following review, data may be corrected, confirmed or excluded from specific analyses with appropriate documentation in the study records.

Early Discontinuation of Study Drug

Subjects who permanently discontinue study treatment before Week 24 will be encouraged to continue all protocol assessments and diary recording. Consistent with the treatment-policy strategy, all investigator-confirmed attacks occurring during the planned study period will contribute to the primary analysis regardless of treatment discontinuation.

Treatment Interruptions

Temporary treatment interruptions will not result in exclusion of data. Attacks occurring during treatment interruptions will remain part of the treatment-period analysis and will contribute to efficacy endpoints.

Varying Run-in Duration

The protocol allows a run-in duration between 28 and 56 days. To account for differences in observation duration, attack rates will be calculated using the number of investigator-confirmed attacks divided by the corresponding observation time. The primary and supportive regression analyses will incorporate observation time through person-time rate calculations or offset terms, as appropriate.

Missing Follow-up Data

For subjects who discontinue study participation or are lost to follow-up:

- all available data collected up to the date of discontinuation will be included;
- no imputation of future attacks will be performed in the primary analysis;
- observation time will be censored at the last date for which reliable information is available.

Sensitivity Analyses

Sensitivity analyses may include:

- Per-Protocol analyses excluding major protocol deviations;
- analyses excluding periods following prohibited medication use;
- analyses restricted to subjects with adequate diary compliance;
- supportive negative-binomial analyses using actual observed exposure times.

The purpose of these analyses will be to evaluate the robustness of the primary findings under alternative assumptions regarding missing data and protocol deviations.

14. STUDY ADMINISTRATION

14.1. Regulatory and Ethical Considerations

14.1.1. Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; Good Clinical Practices (GCPs), including ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines (ICH E6); FDA/European Medicines Agency regulatory requirements and other national laws as applicable; and in accordance with the ethical principles of the Declaration of Helsinki. In addition, the study will be conducted in compliance with all applicable local laws and regulatory requirements relevant to the use of new therapeutic agents. The clinical trial will be conducted in compliance with Regulation [EU] No 536/2014 (CTR Annex I, section D, no. 17, letter a).

14.1.2. Institutional Review Board and Ethics Committee Approvals

Before initiation of the study at an investigational site, the protocol, the ICF, the subject information sheet (if applicable), and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study must be obtained before the study center can be initiated or the IMP can be released to the Investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained, in particular, for changes to the study, such as modification of the protocol, the ICF, the written information provided to subjects, and/or other procedures. The IRB/IEC will be promptly provided with any new information that may adversely affect the safety of the subjects or the conduct of the study. On completion of the study, the IRB/IEC will be provided with a report of the outcome of the study.

Written reports of clinical study status will be submitted to the IRB/IEC annually or more frequently if requested by the IRB/IEC. A final study notification will also be forwarded to the IRB/IEC after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. The study will be considered completed once the last subject completes the last study visit. Copies of all contact with the IRB/IEC should be maintained in the study file. Copies of clinical study status reports (including termination) should be provided to Sponsor.

14.1.3. Subject Informed Consent

A signed ICF must be obtained from each subject prior to performing any study-related procedures. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved ICF written in a language in which the subject is fluent.

The ICF that is used must be approved both by Sponsor and by the reviewing IRB/IEC. The ICF should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and Sponsor policies.

The Investigator must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons, but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and

dated copy of the ICF. The original signed ICF should be retained in the study files. The Investigator shall maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for nonenrolment.

14.1.4. Investigator Reporting Requirements

The Investigator will provide timely reports regarding safety to his/her IRB/IEC as required.

14.2. Study Monitoring

During trial conduct, Sponsor or its designee will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors will review source documents to confirm that the data recorded on CRFs are accurate. The Investigator and institution will allow Sponsor representatives, monitors, or its designees direct access to source documents to perform this verification.

It is important that the Principal Investigator(s) and their relevant personnel are available during the monitoring visits and that sufficient time is devoted to the process.

14.3. Quality Assurance

The Principal Investigator may be subject to visits by the IRB/IEC, and/or by a quality assurance group for audits performed by Sponsor, or its designee, and/or to inspection by appropriate regulatory authorities. It is important that the Investigator(s) and their relevant personnel are available during the possible audits or inspections and that sufficient time is devoted to the process.

14.4. Study Termination and Site Closure

Overall, the Sponsor may suspend enrollment into the study, suspend treatment of ongoing subjects, or terminate the study to ensure that subjects' safety and welfare are protected. The entire study, or individual sites, may be terminated for any of the following reasons:

- Changes in scientific knowledge that lead to a negative impact on the risk/benefit profile for subjects
- Request of Sponsor or competent public authorities/IRB/IEC
- If recruitment cannot be completed in a specified time frame
- If the permit to manufacture or import IMP is revoked
- If the study drug becomes commercially available or another mechanism is available to provide drug to the subject

Following study completion and where justified by the overall study results and the benefit–risk profile of berotralstat, the Sponsor will seek the appropriate regulatory approvals and/or authorizations required to enable continued access to treatment for eligible patients who may benefit from it, in accordance with applicable local regulations. The specific post-trial access mechanism will be determined following evaluation of the study outcome and the relevant regulatory framework.

Sponsor reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by Sponsor, all study materials must be collected and all CRFs completed to the greatest extent possible.

An individual trial center that is determined to be unsuitable by Sponsor, competent public authorities or IRB/IEC may be terminated, without affecting the other trial sites.

Except for those situations outlined in specific section , no other formal stopping rules for individual subjects, parts of the trial or the entire trial, will be defined. Individual subjects will be discontinued from the study following the emergence of any laboratory abnormality or AE that in the judgment of the Investigator compromises the ability to continue study-specific procedures or is considered not to be in the subject's best interest.

14.5. Records Retention

To enable evaluations and/or audits from regulatory authorities or Sponsor, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, CRFs, and medical/hospital records), all original signed ICFs, all original signed assents (where applicable), all CRFs, and detailed records of study drug accountability and treatment disposition. The records should be retained by the Investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the Investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to Sponsor. The Investigator must obtain Sponsor's written permission before disposing of any records and must notify Sponsor before transferring any records to another facility.

14.6. Confidentiality of Information and Data

Sponsor affirms the subject's right to protection against invasion of privacy and secure maintenance of the confidential nature of their personal data. Only a subject identification number and subject identifiers permitted by local regulation will identify subject data retrieved by Sponsor. However, in compliance with federal regulations, Sponsor requires the Investigator to permit Sponsor's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study, maintaining pseudo-anonymity.

All parties will abide by all applicable laws and regulations regarding subject privacy and confidentiality, including, the Health Insurance Portability and Accountability Act (HIPAA), where this rule is applicable and the requirements of the Data Protection Act in the EU, where applicable. A valid authorization and consent must meet the specifications of the applicable laws and regulations relating to such personal data and health information. It is the responsibility of the Investigator and institution to obtain such waiver/authorization in writing from the appropriate individual. HIPAA authorizations are required for US sites only.

14.7. Study Publication

All data generated from this study are the property of Sponsor and shall be held in strict confidence along with all information furnished by Sponsor. Except as provided through written agreement with Sponsor, independent analysis and/or publication of this data by the Investigator or any member of his/her staff is not permitted. Such consent will not be withheld unreasonably. Sponsor is in agreement with the principle of full disclosure of clinical trial results.

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