CLINICAL STUDY PROTOCOL

Study CRO-PK-20-344 - Sponsor code Z7251J01

Comparative bioavailability study of a new riluzole orodispersible film vs. a marketed oral reference (Rilutek® tablets) in healthy male and female volunteers

Single centre, single dose, open-label, randomised, 2-sequence, 4-period replicate cross-over study

Test formulation:	Riluzole 50 mg orodispersible film, Aquestive Therapeutics, USA
Reference formulation:	Rilutek®, 50 mg riluzole tablets, Sanofi Mature IP, France
Sponsor:	Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy Phone: +39.02.66.52.42.99 Fax: +39.02.66.52.48.87 Email: carlo.cattaneo@zambongroup.com
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Development phase:	I
Version and date:	Final version 1.0, 28APR20

This study will be conducted in accordance with the current version of Good Clinical Practice (GCP), ICH topic E6 (R2)

This document comprises 67 pages

PROTOCOL APPROVAL

SPONSOR
Zambon S.p.A., Italy

Sponsor Representatives

Carlo Cattaneo, Global Head Medical Affairs CNS

05/05/2020 _____ Carlo Cattaneo _____
Date Signature

Paola Castellani, MD, Global Chief Medical Officer and Patient's Access Head

05/05/2020 _____ Paola Castellani _____
Date Signature

INVESTIGATOR

Principal investigator

I have read this protocol and agree to conduct this study in accordance with all the stipulations of the protocol and in accordance with the Declaration of Helsinki, the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), and the applicable local law requirements, including supervising any individual or party to whom I will delegate trial-related duties and functions at the trial site.

Milko Radicioni, MD
CROSS Research S.A., Phase I Unit, Arzo, Switzerland

29 APR 2020
Date

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STUDY SYNOPSIS

Title: Comparative bioavailability study of a new riluzole orodispersible film vs. a marketed oral reference (Rilutek® tablets) in healthy male and female volunteers
Protocol number: CRO-PK-20-344 - Sponsor code Z7251J01
Clinical phase: Phase I
Study design: Single centre, single dose, open-label, randomised, 2-sequence, 4-period replicate cross-over study
Planned nr. of centres / countries: 1/Switzerland
Investigator and centre: *Principal investigator:* Milko Radicioni, MD; CROSS Research Phase I Unit, Via F.A. Giorgioli 14, CH-6864 Arzo, Switzerland
Investigational products: TEST (T): Riluzole 50 mg orodispersible film, Aquestive Therapeutics, USA REFERENCE (R): Rilutek®, 50 mg riluzole tablets, Sanofi Mautre IP, France
Dose regimen: The subjects will receive single oral doses of 50 mg of riluzole, as test orodispersible film and reference film-coated tablets under fasting conditions, in each of 4 subsequent periods separated by wash-out intervals of at least 7 days between consecutive administrations, according to a 2-treatment, 4-period, replicate cross-over design.
Objective: The objective of the study is to compare the pharmacokinetic profile of riluzole after replicate single dose of the novel orodispersible film test formulation and of the marketed reference Rilutek® tablets and to evaluate their bioequivalence.
End-points: **Primary end-point:** ➢ To evaluate the bioequivalent rate (C_{max}) and extent (AUC_{0-t}) of absorption of riluzole after replicate single dose administration of test and reference. **Secondary end-points:** ➢ To describe the pharmacokinetic profile of riluzole after replicate single dose administration of test and reference; ➢ to evaluate the test product palatability; ➢ to collect safety and tolerability data of test and reference after replicate single dose administration.
Study variables: **Primary variables:** ➢ C_{max} and AUC_{0-t} of plasma riluzole after replicate single dose administration of test and reference formulation. **Secondary variables:** ➢ $AUC_{0-\infty}$, $t_{1/2}$, t_{max}, AUC_{extra}, $\lambda_{z,}$ of riluzole after replicate single dose administration of test and reference; ➢ Palatability scores; ➢ Treatment-emergent adverse events, vital signs (blood pressure, heart rate), laboratory parameters, physical examination including body weight
Analytics: Riluzole will be determined in plasma samples at Accelera S.r.l., Italy, using a validated LC-MS/MS method with a lower quantification limit of 5 ng/mL
Safety and tolerability assessments: Treatment-emergent adverse events; vital signs (blood pressure, heart rate), physical examinations including body weight; laboratory tests
Sample size: When the sample size in each sequence group is 26 (and the total sample size is 52), a replicate crossover design will have 80% power to reject both the null hypothesis that the ratio of the test mean to the reference mean of C_{max} is below 0.800 and the null hypothesis that the ratio of test mean to the reference mean of C_{max} is above 1.250 (i.e. that the test and standard are not equivalent, in favour of the alternative hypothesis that

STUDY SYNOPSIS (cont.)

Sample size (continued):
the means of the two treatments are equivalent) assuming that data will be analysed in the natural log scale using t-tests for differences in means and that each t-test will be made at the 5.0% significance level.
In conclusion, 54 subjects will be enrolled in order to have 52 completed subjects.

Main selection criteria: Inclusion criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Sex and Age*: men/women, 18-55 years old inclusive
3. *Tobacco:* non-smokers for at least 6 months prior to study screening
4. *Body Mass Index*: 18.5-29 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting position
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
   a. A non-hormonal intrauterine device or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 2 months before the screening visit
   b. A male sexual partner who agrees to use a male condom with spermicide
   c. A sterile sexual partner

   Female participants of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.

   For all women, pregnancy test result must be negative at screening.

Exclusion criteria:

1. *Electrocardiogram (ECG) 12-leads:* (supine position) clinically significant abnormalities; QTc interval > 450 msec
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness
4. *Cotinine test*: positive cotinine test at screening
5. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
6. *Diseases*: clinically significant history or presence of renal, hepatic, gastrointestinal, cardiovascular, cerebrovascular, immunological, musculoskeletal, respiratory, skin, haematological, endocrine, psychiatric or neurological diseases or surgeries that may interfere with the aim of the study. Gastrointestinal pathologies include any clinically significant disorder of the mouth, e.g. impairment of swallowing, lesions, ulcerations, deformities, untreated dental caries
7. *Dentures:* presence of mouth jewellery, dentures, braces, piercings that may interfere with successful completion of the dosing
8. *Medications*: medications, including over the counter medications and herbal remedies for 2 weeks before study screening; central nervous system depressants, including opioids, benzodiazepines, general anaesthetics and anticonvulsants, or CYP inhibitors, including cimetidine, fluoxetine, quinidine, erythromycin, ciprofloxacin, fluconazole, ketoconazole, diltiazem and antiretroviral agents, or strong CYP inducers, including barbiturates, carbamazepine, glucocorticoids, phenytoin, St John's wort and rifampin, or hormonal oral or transdermal contraceptives for 30 days before study screening; implanted, injected, intravaginal or intrauterine hormonal contraceptives for 6 months before study screening
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 3 months before this study. The 3-month interval is calculated as the time between the first calendar day of the month that follows the last visit of the previous study and the first day of the present study
10. *Blood donation*: blood donations for 3 months before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015], caffeine (>5 cups coffee/tea/day) or tobacco use including any tobacco product like e-cigarettes and vaping products

STUDY SYNOPSIS (cont.)

Main selection criteria (continued):

12. *Drug test*: positive result at the drug test at screening or Day -1
13. *Alcohol test*: positive alcohol breath test at Day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians and vegans
15. *Pregnancy (women only)*: positive or missing pregnancy test at screening or Day -1, pregnant or lactating women

Schedule:		**Day**	**Procedures/Assessments**	**Notes**
Screening phase	**Screening – visit 1**	*From Day -14 to Day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (as S001, S002, etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Laboratory analyses: haematology, blood chemistry, cotinine, urinalysis, virology and serum pregnancy test (women only) ➢ Urine multi-drug kit test ➢ Adverse event monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	*Note:* The first two letters of the surname followed by the first two letters of the first name will be used in the Phase I Unit source document only and will not be transferred to the sponsor
Period 1	**Visit 2**	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women only) ➢ Adverse events and concomitant medications ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment and randomisation	Arrival at the Phase I Unit in the evening Confinement until the evening of Day 2 Standardised low-fat dinner and fasting for at least 10 h (overnight) up to dosing
	Visit 3	*Day 1*	➢ Investigational medicinal product administration at 08:00 ± 1 h ➢ Palatability of the test product immediately after administration ➢ Mouth visual inspections after administration of the test investigational medicinal product, at pre-dose and 0.5 and 1 h post-dose ➢ Vital signs measurement at pre-dose and 2 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at pre-dose and 15, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 6, 8 and 12 h post-dose. ➢ Adverse event monitoring and concomitant medications	Fasting until about 5 h post-dose Standardised lunch at about 13:00 Standardised dinner at about 20:00 (12 h post-dose)
		Day 2	➢ Vital signs measurement at 36 h post-dose ➢ Blood sample collection for PK analysis at 24 and 36 h post-dose. ➢ Adverse event monitoring and concomitant medications	Standardised breakfast and lunch at about 8:00 and 13:00 Discharge from the Phase I Unit in the evening after the 36-h post-dose blood sampling and vital signs check

STUDY SYNOPSIS (cont.)

Schedule (continued):				
		Day	**Procedures/Assessments**	**Notes**
Wash-out		*At least 7 days*	A wash-out interval of at least 7 days between 2 consecutive administrations	
Period 2	**Visit 4**	*Day -1*	As visit 2, excluding inclusion/exclusion criteria evaluation, enrolment and randomisation	As visit 2
	Visit 5	*Day 1*	As visit 3. Test or reference investigational medicinal product administered according to the randomisation list and cross-over design	As visit 3
		Day 2	As visit 3	As visit 3
Wash-out		*At least 7 days*	A wash-out interval of at least 7 days between 2 consecutive administrations	
Period 3	**Visit 6**	*Day -1*	As visit 2, excluding inclusion/exclusion criteria evaluation, enrolment and randomisation	As visit 2
	Visit 7	*Day 1*	As visit 3. Test or reference investigational medicinal product administered according to the randomisation list and cross-over design	As visit 3
		Day 2	As visit 3	As visit 3
Wash-out		*At least 7 days*	A wash-out interval of at least 7 days between 2 consecutive administrations	
Period 4	**Visit 8**	*Day -1*	As visit 2, excluding inclusion/exclusion criteria evaluation, enrolment and randomisation	As visit 2
	Visit 9	*Day 1*	As visit 3. Test or reference investigational medicinal product administered according to the randomisation list and cross-over design	As visit 3
		Day 2	As visit 3	As visit 3
Final phase	**Visit 10 - Final Visit or ETV**	*Day 2 of period 4 / at ETV in case of discontinuation*	➢ Full physical examination (body weight and physical abnormalities; also vital signs in case of ETV) ➢ Laboratory analyses as at screening, with the exception of cotinine, virology, urine drug test and pregnancy test ➢ Adverse events and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any adverse reactions

STUDY SYNOPSIS (cont.)

Schedule:
During each study period, the subjects will be confined from the evening preceding the IMP administration (study Day -1) until the evening of Day 2. During confinement, the subjects will not take any food or drinks (except water) for about 10 h (i.e. overnight) before each administration and for about 5 h afterwards. On Day -1 of each study period, a standardised low-fat dinner will be served. Water will be allowed as desired, except for one h before and one h after each administration. In order to maintain an adequate hydration, the subjects will be encouraged to drink at least 150 mL of still mineral water every 2 h for 5 h post-dose, starting at 1 h post-dose. On Day 1 of each study period, the subjects will remain fasted until about 5 h post-dose. Standardised lunch and dinner will be served at approximately 5 h and 12 h post-dose. On Day 2 of each study period, standardised breakfast and lunch will be served at about 8:00 and 13:00, respectively. Coffee, tea or food containing xanthines (i.e. coke, chocolate, etc.), smoking, alcohol and grapefruit will be forbidden during confinement. In particular, alcohol and grapefruit will be forbidden for 24 h before the first administration until the end of the study.
During confinement, routine ambulant daily activities will be strongly recommended.

Data analysis:
The data documented in this trial and the measured clinical parameters will be described using classic descriptive statistics for quantitative variables and frequencies for qualitative variables. The statistical analysis will be performed using SAS® version 9.3 (TS1M1) or higher (the actual versions will be stated in the final report).
The statistical analysis of pharmacokinetic parameters will be performed using Phoenix WinNonlin® version 6.3 or higher and SAS® version 9.3 (TS1M1) or higher. C_{max} and AUC_{0-t} will be compared using analysis of variance (ANOVA) for a 2-sequence, 4-period replicate cross-over design on log-transformed data. Acceptance criterion for bioequivalence is that the 90% confidence interval for the test/reference ratio of the AUC_{0-t} geometric means is within the 80.00-125.00 range, according to the current EMA guideline for bioequivalence investigations. Due to the replicate design of the study, if the within-subject variability of C_{max} of plasma riluzole after administration of the reference treatment is >30%, bioequivalence range for C_{max} will be widened. The extent of widening will be determined on the basis of the actual within-subject variability observed in study C_{max}.

STUDY SCHEDULE

ACTIVITIES	Screening	PERIOD 1, 2, 3, 4 (wash-out≥7 days)			Final visit/ETV[12]
Visit	V1	V2, V4, V6, V8	V3, V5, V7, V9		V10[11]/ETV
	Days -14/-2	Day -1	Day 1	Day 2	Day 2[11]/ETV
Informed consent	x				
Demography	x				
Lifestyle	x				
Medical/surgical history	x				
Physical abnormalities	x				x
Previous and concomitant treatments	x	x	x	x	x
Height	x				
Body weight	x				x
Laboratory analysis[1]	x				x
Cotinine	x				
Virology	x				
Drug screening	x	x			
Vital signs	x		x [2]	x [2]	x[13]
Pregnancy test	x[3]	x[4]			
ECG	x				
Inclusion/exclusion criteria	x	x[5]			
Eligibility evaluation	x	x[5]			
Alcohol breath test		x			
Enrolment and randomisation		x[5]			
Confinement		x	x		
Discharge				x	
IMP administration			x[6]		
Palatability			x[7]		
Mouth visual inspection			x[8]		
Blood samplings [9]			x	x	
Standardised meals		x	x	x	
AEs monitoring[10]	x	x	x	x	x

1. *Haematology, blood chemistry with the exception of cotinine, urinalysis*
2. *At pre-dose and 2 and 36 h post-dose*
3. *Women only - serum β-HCG test*
4. *Women only - urine test*
5. *Period 1, visit 2*
6. *At 08:00 ± 1 h under fasting conditions*
7. *Immediately after each administration of the test product*
8. *At pre-dose and 0.5 and 1 h post-dose at each administration of the test product*
9. *At pre-dose (0) and 15, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h post-dose*
10. *Adverse events monitored starting at the screening visit, immediately after informed consent, up to the final visit/ETV*
11. *Final visit on Day 2 of period 4 after the 36-h post-dose blood sampling*
12. *Early termination visit (ETV) in case of subject's premature discontinuation*
13. *At the ETV only*

TABLE OF CONTENTS

TABLES

Page

FIGURES

Page

LIST OF ABBREVIATIONS

β-HCG	human chorionic gonadotropin β
γ-GT	γ-Glutamyl transpeptidase
λ_z	Terminal elimination rate constant
ADR	Adverse Drug Reaction
AE	Adverse Event
ALCOAC	Attributable-Legible-Contemporaneous-Original-Accurate-Complete
ALS	Amyotrophic lateral sclerosis
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
AUC_{0-t}	Area under the concentration-time curve from time zero to time t
$AUC_{0-\infty}$	Area under the concentration vs. time curve up to infinity
BLQL	Below lower quantification limit
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
BW	Body weight
CA	Competent authority
CDISC	Clinical data interchange standards consortium
CI	Confidence interval
C_{max}	Peak drug concentration
CMS	Clinical Medical Service
CPL	Clinical Project Leader
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CV	Coefficient of Variation
CV_{WR}	Coefficient of Variation within-subject
CYP	Cytochrome P450
DBP	Diastolic Blood Pressure
DSU	Drug Safety Unit
EC	Ethics Committee
ECG	Electrocardiogram
EMA	European Medicine Agency
EPAR	European Public Assessment Report
ETV	Early Termination Visit
FDA	Food and Drug Administration
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMO	Glycerol monooleate
GMP	Good Manufacturing Practice
HBs Ag	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
IV	Intravenous
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LQL	Lower Quantification Limit

LSLV	Last Subject Last Visit
LSM	Least square mean
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
min	Minute
N	Normal
NA	Not Applicable
NC	Not calculated
OECD	Organisation for Economic Co-operation and Development
OTC	Over The Counter
PE	Point Estimate
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
QTc	Corrected QT interval
ROF	Riluzole oral formulation
RSI	Reference safety information
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
THC	delta-9-tetrahydrocannabinol
$t_{1/2}$	Half-life
t_{max}	Time to achieve C_{max}
USDA	United States Department of Agriculture
WHODDE	World Health Organisation Drug Dictionary Enhanced

1 INTRODUCTION

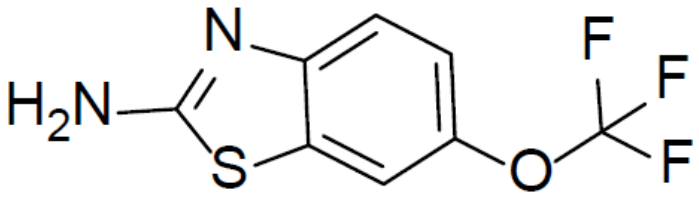
1.1 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder characterised by progressive loss of the motor neurons of the ventral horn of the spinal cord and the cortical neurons that provide their afferent input. The disorder is characterised by rapidly progressive weakness, muscle atrophy and fasciculation, spasticity, dysarthria, dysphagia and respiratory impairment. Sensory function generally is spared, as is cognitive, autonomic and oculomotor activity. ALS usually is progressive and fatal with most affected patients dying of respiratory failure and pneumonia after 2 to 3 years from the onset of symptoms, although occasional individuals have a more indolent course and survive for many years.
Although the pathogenesis of ALS is not completely elucidated, it is suggested that glutamate (the primary excitatory neurotransmitter in the central nervous system) plays a role in cell death in the disease.

1.2 Riluzole

Riluzole (2-amino-6-trifluoromethoxybenzothiazole, $C_8H_5F_3N_2OS$) is an anti-glutamatergic agent with neuroprotective properties that has been developed for the treatment of ALS (1, 2). Its structure is shown below:

Figure 1.2.1 Chemical structure of riluzole



Riluzole is an FDA- and EMA-approved active ingredient. The benzothiazole derivative has been shown to exert neuroprotective effects and prolong survival in patients with ALS (3), but has no effect on the degradation of muscular function (4, 5).

1.3 Riluzole orodispersible film and rationale for the study

Aquestive Therapeutics developed a novel riluzole formulation incorporating the substance into a proprietary polymer-based film matrix utilizing PharmFilm® technology (6).

Riluzole orodispersible film is expected to fill an important medical need since oral tablet administration in ALS patients can be a challenge. Medication that can be easily administered without water may improve the quality of life for ALS patients and improve patient care. Riluzole orodispersible film is easily administered, because the patient or caregiver needs only to place the film on the tongue, where it can immediately dissolve into the saliva and be ingested with intentional swallowing or during the normal reflex of swallowing, thus eliminating the need for swallowing a tablet with liquid or crushing it into soft food.

Zambon S.p.A., Italy, is sponsoring the present bioequivalence study in order to investigate the bioequivalence of the novel unit-dose product versus Rilutek® tablets, commercially available in Europe as 50 mg tablets. The dose strength for the test formulation is 50 mg, matching the 50 mg strength of the reference formulation.

1.4 Previous pharmacokinetic studies of riluzole orodispersible film

1.4.1 Pilot bioavailability Phase I study

In a previous Phase I, single-centre, open-label, randomised, single-dose, 3-period, 4-arm, cross-over pilot study conducted in healthy male and female non-smokers, the organoleptic effect of the test formulation administered with or without water in comparison with that of crushed Rilutek® 50 mg tablets available in USA administered with 15 mL of apple sauce and the bioavailability of plasma riluzole after administration of the test formulation with or without water in comparison with that of intact Rilutek® 50 mg tablets administered with 240 mL of water were investigated in a total of 16 subjects (6). Treatment A was riluzole orodispersible film 50 mg administered without water and Treatment B with water. Treatment C was intact Rilutek® 50 mg tablets administered with water and Treatment D was crushed Rilutek® 50 mg tablets administered with apple sauce.
Blood samples were collected at pre-dose and 0.25, 0.5, 0.75, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 20, 24, 36, and 48 h post-dose in each study period.

Table 1.4.1.1 Mean main pharmacokinetic parameters of plasma riluzole after Treatments A and C and the outcome of their statistical comparisons

Parameter (N/N)	Geometric Mean Arithmetic Mean (CV%)		Ratio of Geometric Means	90% CI	CV_{WR}%
	Treatment A	Treatment C			
AUC_{0-t} (ngxh/mL)	918.200	807.545	113.70	98.89-130.73	18.43
(13/12)	935.591 (25.10)	908.745 (38.95)			
$AUC_{0-\infty}$ (ngxh/mL)	955.116	837.189	114.09	99.08-131.36	18.62
(13/12)	971.138 (25.97)	948.391 (39.90)			
C_{max} (ng/mL)	221.157	234.384	94.36	74.27-119.88	32.13
(13/12)	236.338 (38.44)	254.237 (48.32)			
t_{max} (h)[a]	0.75	0.75	-	-	-
(13/12)	(0.50-2.50)	(0.50-2.00)			

$AUC_{0-\infty}$=area under the concentration-time curve from time 0 (dosing) extrapolated to infinity; AUC_{0-t}= area under the concentration-time curve from time 0 (dosing) to time of last measurable riluzole; CI=confidence interval; C_{max}=maximum observed plasma concentration; CV_{WR}%= within-subject variability expressed as percentage; t_{max}=time to maximum concentration.
[a]*: Presented as median and range.*
Source: Clinical Study Report 1897

Table 1.4.1.2 Mean main pharmacokinetic parameters of plasma riluzole after Treatments B and C and the outcome of their statistical comparisons

Parameter (N/N)	Geometric Mean Arithmetic Mean (CV%)		Ratio of Geometric Means	90% CI	CV_{WR}%
	Treatment B	Treatment C			
AUC_{0-t} (ngxh/mL)	876.937	807.545	108.59	93.44-126.20	18.43
(13/12)	847.053 (30.16)	908.745 (38.95)			
$AUC_{0-\infty}$ (ngxh/mL)	918.048	837.189	109.66	94.21-127.64	18.62
(13/12)	893.901 (31.00)	948.391 (39.90)			
C_{max} (ng/mL)	206.394	234.384	88.06	68.05-113.95	32.13
(13/12)	180.950 (45.93)	254.237 (48.32)			
t_{max} (h)[a]	0.75	0.75	-	-	-
(10/12)	(0.50-2.00)	(0.50-2.00)			

$AUC_{0-\infty}$=area under the concentration-time curve from time 0 (dosing) extrapolated to infinity; AUC_{0-t}= area under the concentration-time curve from time 0 (dosing) to time of last measurable riluzole; CI=confidence interval; C_{max}=maximum observed plasma concentration; CV_{WR}%= within-subject variability expressed as percentage; t_{max}=time to maximum concentration.
[a]*: Presented as median and range.*
Source: Clinical Study Report 1897

Pharmacokinetic (PK) analyses showed that the extent and the rate of absorption of riluzole were approximately 14% higher and approximately 6% lower after Treatment A than after Treatment C. After Treatment B (film with water), the extent and the rate of the absorption of riluzole were approximately 9% higher and approximately 12% lower than after Treatment C. Indeed, while the ratio of geometric means of C_{max} was 94.36% between Treatment A and C, it was 88.06% between Treatment B and C, thus indicating that the ratio between Treatment A and C was nearer the equality than the ration between Treatment B and C, in terms of rate of absorption. The ratios of geometric means of AUC_{0-t} and $AUC_{0-\infty}$ (extent of absorption) did not vary greatly between the 2 comparisons A vs. C and B vs. C.
The intra-subject variability (CV_{WR}%) observed in this pilot study namely 18.43%, 18.62% and 32.13% for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, allowed the sample size calculation of the following pivotal bioequivalence study (§ 1.4.2) and the choice of a single-dose replicate design due to the relatively large intra-subject variability observed for C_{max}.

1.4.2 Pivotal bioequivalence Phase I study

In a previous Phase I, single-centre, open-label, 3-treatment, 2-sequence, 5-period, replicate, randomised cross-over, comparative bioavailability study with evaluation of food effect in healthy male and female non-smokers, primarily the bioavailability of plasma riluzole after single dose of the test formulation compared with that of Rilutek® 50 mg tablets available in USA and secondarily the safety and tolerability of the test formulation and the effect of food on the pharmacokinetics of riluzole were investigated (6).
A total of 32 subjects were enrolled and 30 subjects completed the study. Three (3) treatment conditions were studied: the test formulation administered without water under fasting conditions (Treatment A); Rilutek® 50 mg tablets available in USA administered with water (Treatment B) and the test formulation without water under fed conditions (Treatment C).

Each subject received a total of 3 treatments in 5 periods: Treatment A and Treatment B under fasting conditions in Periods 1-4 according to the randomised replicate cross-over design. All subjects received Treatment C in Period 5.
Blood samples were collected at pre-dose and 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, and 120 h post-dose in each study period.

Table 1.4.2.1 Mean main PK parameters of plasma riluzole after Treatments A and B and the outcome of their statistical comparisons

Parameter	CV_{WR}	Geometric LSM		Ratio	90% Geometric CI		95% upper confidence bound
		Riluzole orodispersible film without water (A)	Rilutek with 240 mL water (B)		Lower	Upper	
AUC_{0-t} (ngxh/mL)	12.65	780.01	714.48	109.17%	105.67%	112.79%	-
$AUC_{0-\infty}$ (ngxh/mL)	12.49	795.98	730.02	109.04%	105.58%	112.61%	-
C_{max} (ng/mL)	32.66	-	-	115.82%	-	-	-0.0123

$AUC_{0-\infty}$=area under the concentration-time curve from time 0 (dosing) extrapolated to infinity; AUC_{0-t}= area under the concentration-time curve from time 0 (dosing) to time of last measurable riluzole; CI=confidence interval; C_{max}=maximum observed plasma concentration; LSM=least-squares mean; CV_{WR} = within-subject variability for the reference product; ratio = point estimate for geometric mean ratio; CI = confidence interval according to the reference-scaled bioequivalence approach; source: Clinical Study Report 162020

The ANOVA performed on the primary riluzole PK parameters clearly demonstrated the bioequivalence of the novel riluzole orodispersible film to Rilutek® 50 mg tablets available in US. CV_{WR} of plasma riluzole AUC_{0-t} and $AUC_{0-\infty}$ was less than 30% and since their 90% geometric confidence interval (CI) were respectively 105.67% to 112.79% and 105.58% to 112.61% both falling within the acceptance range of 80.00% to 125.00%, test and reference formulations were bioequivalent in terms of extent of exposure to riluzole.
Since CV_{WR} of C_{max} was higher than 30%, a scaled average bioequivalence approach was used according to the FDA guidance on bioequivalence studies. Therefore, also in terms of rate of absorption of plasma riluzole, the hypothesis of bioequivalence of test and reference formulation was accepted, because the 95% upper confidence bound was ≤0 and the geometric mean ratio was within the interval 80.00% to 125.00%.

1.5 Risks and benefits

Riluzole is a known drug which has been used for decades. It is usually well tolerated. Adverse events (AEs) reported in the 3 previous single-dose clinical studies in healthy volunteers of the same test formulation are: somnolence, nausea, oral hypoaesthesia, oral mucosa erythema, dizziness and transiently altered laboratory parameters. Undesired effects reported after stable therapy with the reference formulation include malaise, nausea, high alanine aminotransferase (ALT) values, headache, abdominal pain, pain, vomiting, dizziness, tachycardia, somnolence and circumoral paraesthesia. For details refer to the IB (6).

Blood sampling with cannula insertion may cause minor discomfort. The risks associated with blood draws include pain, bleeding and bruising.

No specific benefits for the participants in the current study are foreseen. Their remuneration will be paid after study completion. The remuneration covers loss of time and any inconvenience caused by the participation in the study.

2 STUDY OBJECTIVES

The objective of the study is to compare the pharmacokinetic profile of riluzole after replicate single dose of the novel orodispersible film test formulation and of the marketed reference Rilutek® tablets and to evaluate their bioequivalence.

2.1 Primary end-point

- To evaluate the bioequivalent rate (C_{max}) and extent (AUC_{0-t}) of absorption of riluzole after replicate single dose administration of test and reference.

2.2 Secondary end-points

- To describe the PK profile of riluzole after replicate single dose administration of test and reference;
- to evaluate the test product palatability;
- to collect safety and tolerability data of test and reference after replicate single dose administration.

3 CLINICAL SUPPLIES

3.1 Treatment

The subjects will receive single oral doses of 50 mg of riluzole, as test orodispersible film and reference film-coated tablets under fasting conditions, in each of 4 subsequent periods separated by wash-out intervals of at least 7 days between consecutive administrations, according to a 2-treatment, 4-period, replicate cross-over design.

3.1.1 Description of products

The analytical certificates will be supplied with the investigational medicinal products (IMPs) and enclosed in the final study report.

3.1.1.1 Test product

TEST (T)

IMP	Riluzole 50 mg orodispersible film, Aquestive Therapeutics, USA
Manufacturer (active substance)	Glenmark Life Sciences, Plot 3109 C GIDC Industrial Estate, Ankleshwar, District Bharuch, Gujarat, 393002 India (GMP compliant)
Manufacturer (finished product)	Aquestive Therapeutics, Inc, 6465 Ameriplex Drive, Portage, Indiana, 46368, USA (GMP compliant)
QP release:	Aquestive Therapeutics, Inc, 6560 Melton Road, Portage, Indiana, 46368, USA (GMP compliant)
Pharmaceutical form:	Orodispersible film
Dose	50 mg
Administration route:	Supralingual

The qualiquantitative composition of the test is shown in the table below.

Table 3.1.1.1 Quantitative composition of the test IMP

Component	% Solids (w/w)
Riluzole	27.62
Polacrilex Resin	35.18
Polyethylene oxide	1.05
Pullulan	19.24
Hypromellose, substitution type 2910	4.08
Xanthan Gum	0.31
Sucralose	1.57
Xylitol	5.49
Fructose	1.05
Glycerin	2.09
Glycerol Monooleate, Type 40 (GMO)	1.67
Flavor Honey 151a39	0.46
Flavor Juicy Lemon 160a04	0.16
FD&C Yellow #5	0.03
Purified Water	NA

Abbreviations: FD&C=Food, Drug, and Cosmetic (Act); NA=not applicable; w/w=weight per weight.

3.1.1.2 Reference product

REFERENCE (R)	
IMP	Rilutek®, 50 mg riluzole tablets
Marketing Authorisation Holder	Sanofi Mautre IP, France
Pharmaceutical form	Film-coated tablets
Dose	50 mg
Administration route	Oral

3.1.2 Dose regimen

Four single doses of 50 mg of riluzole (2 as test and 2 as reference formulation) will be administered to each volunteer in 4 subsequent study periods, separated by wash-out intervals of at least 7 days.

3.1.3 Route and method of administration

Each study dosing was performed in the morning at 08:00 ± 1 h under fasting conditions. The test formulation will be administered supralingually and the reference orally.

3.1.3.1 Test formulation

The study staff will check subjects' mouth to ensure a clean mouth before study drug administration. This will be documented in specific forms.

Before each administration, the subjects will drink 20 mL of still mineral water in order to wet their mouth.

Afterwards, using gloves, the investigator or deputy will take the orodispersible film (32.0x22.0 mm) out of the provided pouch and place the product directly on the top surface (dorsal aspect) of the subjects' tongue. The subjects will close their mouth in a natural way and gently rub the film with the tongue against the roof of the mouth to promote melting and disappearance of the film. The subjects will be allowed to swallow saliva but not to chew, bite, or swallow the film. After the subjects indicate a complete dissolution of the film (confirmed by visual inspection) by raising their hand, they will be allowed to swallow any remaining saliva. The study staff will visually inspect the films 1, 2 and 5 min post-dose until either complete dissolution is confirmed or until the subject alerts the study staff to dissolution of the film. This will be documented in specific forms. If the film is not completely dissolved within 5 min, the subjects will be allowed to swallow without water.
Dosing time is defined as the time the film is placed on the subject's tongue. The study staff will record the actual time of film placement and the actual time of dissolution. If, upon inspection, the film is dissolved, the time of mouth check will be recorded as time of dissolution.
In case a subject chews or moves the film before its complete dissolution, this occurrence will be noted. During the 5-min observation period, the subjects will inform the study staff in case

the film is accidentally swallowed before its complete dissolution and the staff will record the time the film is swallowed.

Dosing, dissolution and accidental swallowing times will be recorded in specific forms and in the subject's CRF.

3.1.3.2 Reference formulation

One film-coated tablet will be swallowed (without chewing) with 150 mL of still mineral water.

The investigator or deputy will check and confirm that all subjects take the medication appropriately.

Dosing time will be recorded in the specific source documents and in the subject's CRF.

3.1.4 Investigational product distribution

The test and reference will be administered by the investigator or by his/her deputy. The IMP will be exclusively used for the present clinical study and will only be administered to the subjects enrolled in the study.

3.2 Packaging and labelling

The test primary packaging will be a polyester/foil laminate pouch which is heat sealed.

The formulation labelling will report all the information requested according to the Annex 13 to the Good Manufacturing Practice (published by the Commission in The rules governing medicinal products in the European Community, Volume 4; 8) as follows:

a. Name, address and telephone number of the sponsor, contract research organisation or investigator (the main contact for information on the product, clinical study and emergency unblinding)
b. Pharmaceutical dosage form, route of administration, quantity of dosage units, the name/identifier and strength/potency
c. The batch and/or code number to identify the contents and packaging operation
d. A study reference code allowing identification of the study, site, investigator and sponsor if not given elsewhere
e. The study subject identification number/treatment number and the visit/period number
f. The name of the investigator (if not included in (a) or (d))
g. Directions for use (reference may be made to a leaflet or other explanatory document intended for the study subject or person administering the product)
h. "For clinical study use only" or similar wording
i. The storage conditions

j. Period of use (use-by date, expiry date or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity

Labels will be in local language (Italian).

3.3 Storage conditions

The test and the reference will be accurately kept in their pouch until ready to use and stored in the Phase I Unit pharmacy between20° and 25° C in a dry locked place, sheltered from light.

3.4 Drug accountability

Test and reference packed in individual subjects' kits will be provided directly to the investigator by STM Pharma PRO Srl., Italy (§ 16.5), in excess of the amount necessary for the study (at least 25% excess).

The reference will be purchased from the European market.

After receipt of the IMPs, the pharmacist will confirm in writing by signing and dating drug accountability forms.

At the end of the study, used, unused and partially used IMPs will either be destroyed on site (upon written authorisation) or returned to the sponsor, after final reconciliation of all IMP accountability.

4 INVESTIGATIONAL PLAN

4.1 Overall study design

Single centre, single dose, open-label, randomised, 2-sequence, 4-period replicate cross-over study.

4.2 Discussion of design

The study was designed in agreement with the EMA guideline "Guidance on the investigation of bioequivalence", CPMP/EWP/QWP/1401/98 Rev. 1 January 2010 (9). The well-known high variability of riluzole led to the choice of the replicate cross-over design as suggested by the current EMA guideline. According to the guideline, an enlargement of the acceptance interval for bioequivalence in terms of C_{max} is allowed up to 69.84-143.19% in relation to the actual intra-subject variability of riluzole C_{max} found in this study with the reference treatment, by keeping in consideration the favourable safety profile of the test formulation previously observed in 3 clinical studies. In detail, a C_{max} within-subject variability >30% was found in the previous pilot study and =32.66% in the previous bioequivalence study performed in USA (§ 1.4.2). According to the 2-arm cross-over study design, each randomised subject will be allocated to one of 2 sequences of IMP administrations in the 4 study periods according to a computer generated randomisation list (see § 8.1). Balance between the sequences will be made so that subjects have the same chances to be assigned to either sequence (either RTRT or TRTR in the 4 consecutive periods).

An open-label design will be used since the primary end-point of the study is based on objective measurements of riluzole in plasma and the outcome variables could not be influenced by the subjects or investigator being aware of the administered products. The bioanalysis will be performed under blinded conditions.

The washout period of 7 days was estimated to be adequate. Based upon a half-life of 12 h (Rilutek SmPC reference: 7), it is anticipated that minimal plasma levels would be achieved in 5 days thus allowing dosing every 7 days. Blood sampling time-points were selected on the basis of the known PK profile of riluzole and on the basis of previous Phase I studies of the same test formulation administered to healthy volunteers.

The dose for the present study was selected based on EMA Rilutek® European Public Assessment Report (EPAR) (10), which recommends a dose regimen of 50 mg (one film-coated tablet) twice a day in the common clinical practice. The dose of 50 mg is the minimal unit dose.

5 STUDY POPULATION

5.1 Target population

Healthy men and women will be enrolled in this study.

5.2 Inclusion criteria

To be enrolled in this study, subjects must fulfil all these criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Sex and Age*: men/women, 18-55 years old inclusive
3. *Tobacco:* non-smokers for at least 6 months prior to study screening
4. *Body Mass Index (BMI)*: 18.5-29 kg/m^2 inclusive
5. *Vital signs*: systolic blood pressure (SBP) 100-139 mmHg, diastolic blood pressure (DBP) 50-89 mmHg, heart rate 50-90 bpm, measured after 5 min at rest in the sitting position
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Contraception and fertility (women only)*: women of child-bearing potential must be using at least one of the following reliable methods of contraception:
    a. A non-hormonal intrauterine device [IUD] or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 2 months before the screening visit
    b. A male sexual partner who agrees to use a male condom with spermicide
    c. A sterile sexual partner

    Female participants of non-child-bearing potential or in post-menopausal status for at least 1 year will be admitted.
    For all women, pregnancy test result must be negative at screening.

5.3 Exclusion criteria

Subjects meeting any of these criteria will not be enrolled in the study:

1. *Electrocardiogram (ECG) 12-leads:* (supine position) clinically significant abnormalities; QTc interval > 450 msec
2. *Physical findings*: clinically significant abnormal physical findings which could interfere with the objectives of the study
3. *Laboratory analyses*: clinically significant abnormal laboratory values indicative of physical illness
4. *Cotinine*: positive cotinine test at screening

5. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
6. *Diseases*: clinically significant history or presence of renal, hepatic, gastrointestinal, cardiovascular, cerebrovascular, immunological, musculoskeletal, respiratory, skin, haematological, endocrine, psychiatric or neurological diseases or surgeries that may interfere with the aim of the study. Gastrointestinal pathologies include any clinically significant disorder of the mouth, e.g. impairment of swallowing, lesions, ulcerations, deformities, untreated dental caries
7. *Dentures:* presence of mouth jewellery, dentures, braces, piercings that may interfere with successful completion of the dosing
8. *Medications*: medications, including over the counter (OTC) medications and herbal remedies for 2 weeks before study screening; central nervous system depressants, including opioids, benzodiazepines, general anaesthetics and anticonvulsants, or CYP inhibitors, including cimetidine, fluoxetine, quinidine, erythromycin, ciprofloxacin, fluconazole, ketoconazole, diltiazem and antiretroviral agents, or strong CYP inducers, including barbiturates, carbamazepine, glucocorticoids, phenytoin, St John's wort and rifampin, or hormonal oral or transdermal contraceptives for 30 days before study screening; implanted, injected, intravaginal or intrauterine hormonal contraceptives for 6 months before study screening
9. *Investigative drug studies*: participation in the evaluation of any investigational product for 3 months before this study. The 3-month interval is calculated as the time between the first calendar day of the month that follows the last visit of the previous study and the first day of the present study
10. *Blood donation*: blood donations for 3 months before this study
11. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015 (11)], caffeine (>5 cups coffee/tea/day) or tobacco use including any tobacco product like e-cigarettes and vaping products
12. *Drug test*: positive result at the drug test at screening or Day -1
13. *Alcohol test*: positive alcohol breath test at Day -1
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians and vegans
15. *Pregnancy (women only)*: positive or missing pregnancy test at screening or Day -1, pregnant or lactating women

5.3.1 Not allowed treatments

No medication, including OTC and herbal remedies, will be allowed for 2 weeks before the start of the study and during the whole study duration.

Central nervous system depressants, including opioids, benzodiazepines, general anaesthetics and anticonvulsants, or CYP inhibitors, including cimetidine, fluoxetine, quinidine, erythromycin, ciprofloxacin, fluconazole, ketoconazole, diltiazem and antiretroviral agents, or

strong CYP inducers, including barbiturates, carbamazepine, glucocorticoids, phenytoin, St John's wort and rifampin, or hormonal oral or transdermal contraceptives will be forbidden for 30 days before study screening and during the whole study duration.

Implanted, injected, intravaginal or intrauterine hormonal contraceptives will be forbidden for 6 months before study screening and during the whole study duration.

Paracetamol will be allowed as therapeutic counter-measure for adverse events (AEs) according to the investigator's opinion.

The intake of any other medication will be reported as a protocol deviation. However, it will lead to subject's discontinuation from the study only if the investigator, together with the sponsor, considers it could affect the study assessments or outcome

6 STUDY SCHEDULE

The schedule of the study is summarised at page 10.

6.1 Study visits and procedures

Each study subject will undergo 10 visits.

The study protocol foresees 4 periods separated by wash-out intervals of at least 7 days. Minimum study duration will be 25 days, screening visit included. A written informed consent will be obtained before any study assessment or procedure.

The first subject first visit (FSFV) is defined as the 1st visit performed at the Phase I Unit by the 1st screened subject. The last subject last visit (LSLV) is defined as the last visit performed at the Phase I Unit by the last subject, i.e. the last visit foreseen by the study protocol, independently of the fact that the subject is a completer or a withdrawn subject, and it corresponds to the study completion.

The following phases, visits and procedures will be performed:

- **Screening phase**
  - Screening – visit 1: between Day -14 and Day -2
  - Period 1 – visit 2: Day -1

- **Interventional phase**
  - Period 1 – visit 3: Days 1-2
  - Wash-out interval of at least 7 days
  - Period 2 – visit 4: Day -1
  - Period 2 – visit 5: Days 1-2
  - Wash-out interval of at least 7 days
  - Period 3 – visit 6: Day -1
  - Period 3 – visit 7: Days 1-2
  - Wash-out interval of at least 7 days
  - Period 4 – visit 8: Day -1
  - Period 4 – visit 9: Days 1-2

- **Final phase**
  - Period 4 – visit 10: Day 2 - Final visit or early termination visit (ETV) in case of early discontinuation

		Day	Procedures/Assessments	Notes
Screening phase	**Screening – visit 1**	*From Day -14 to Day -2*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number (as S001, S002, etc.) ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Laboratory analyses: haematology, blood chemistry, cotinine, urinalysis, virology and serum pregnancy test (women only) ➢ Urine multi-drug kit test ➢ AE monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation	*Note:* The first two letters of the surname followed by the first two letters of the first name will be used in the Phase I Unit source document only and will not be transferred to the sponsor
Period 1	**Visit 2**	*Day -1*	➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Urine pregnancy test (women only) ➢ AE and concomitant medications ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment and randomisation	Arrival at the Phase I Unit in the evening Confinement until the evening of Day 2 Standardised low-fat dinner Fasting for at least 10 h (overnight) up to dosing
	Visit 3	*Day 1*	➢ IMP administration at 08:00 ± 1 h ➢ Palatability of the test product immediately after administration ➢ Mouth visual inspections after administration of the test investigational medicinal product, at pre-dose and 0.5 and 1 h post-dose ➢ Vital signs measurement (§ 7.1.2) ➢ Blood sample collection for PK analysis (§ 7.3.1) ➢ AE and concomitant medications	Fasting until about 5 h post-dose Standardised lunch at about 13:00 Standardised dinner at about 20:00 (12 h post-dose)
		Day 2	➢ Vital signs measurement (§ 7.1.2) ➢ Blood sample collection for PK analysis (§ 7.3.1) ➢ AE and concomitant medications	Standardised breakfast at about 8:00 and lunch at about 13:00 Discharge from the Phase I Unit in the evening after the 36-h post-dose blood sample collection and vital signs check

		Day	Procedures/Assessments	Notes
Wash-out		*At least 7 days*	A wash-out interval of at least 7 days between 2 consecutive administrations	
Period 2	**Visit 4**	*Day -1*	As visit 2, excluding inclusion/exclusion criteria evaluation, enrolment and randomisation	As visit 2
	Visit 5	*Day 1*	As visit 3. Test or reference IMP administered according to the randomisation list and cross-over design	As visit 3
		Day 2	As visit 3	As visit 3
Wash-out		*At least 7 days*	A wash-out interval of at least 7 days between 2 consecutive administrations	
Period 3	**Visit 6**	*Day -1*	As visit 2, excluding inclusion/exclusion criteria evaluation, enrolment and randomisation	As visit 2
	Visit 7	*Day 1*	As visit 3. Test or reference IMP administered according to the randomisation list and cross-over design	As visit 3
		Day 2	As visit 3	As visit 3
Wash-out		*At least 7 days*	A wash-out interval of at least 7 days between 2 consecutive administrations	
Period 4	**Visit 8**	*Day -1*	As visit 2, excluding inclusion/exclusion criteria evaluation, enrolment and randomisation	As visit 2
	Visit 9	*Day 1*	As visit 3. Test or reference IMP administered according to the randomisation list and cross-over design	As visit 3

		Day	Procedures/Assessments	Notes
Period 4	**Visit 9**	*Day 2*	As visit 3	As visit 3
Final phase	**Visit 10 - Final Visit or ETV**	*Day 2 of period 4 / at ETV in case of discontinuation*	➢ Full physical examination (body weight and physical abnormalities; also vital signs in case of ETV) ➢ Laboratory analyses as at screening, with the exception of cotinine, virology, urine drug test and pregnancy test ➢ AE and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any adverse reactions

6.2 Diet and lifestyle

During confinement, the subjects will not take any food or drinks (except water) for about 10 h (i.e. overnight) before IMP administration and for about 5 h afterwards. On Day -1 of each study period, a standardised low-fat dinner will be served. Water will be allowed as desired, except for one h before and one h after IMP administration. In order to maintain an adequate hydration, the subjects will be encouraged to drink at least 150 mL of still mineral water every 2 h for 5 h post-dose, starting at 1 h post-dose.

On Day 1 of each study period, the subjects will remain fasted until about 5 h post-dose. Standardised lunch and dinner will be served at approximately 5 h and 12 h post-dose.
On Day 2 of each study period, standardised breakfast and lunch will be served at about 8:00 and 13:00, respectively. The meals' composition will be standardised on Days -1 and 1 in each study period, as summarised in the table below.

Table 6.2.1 Meals' composition on Day -1 and Day 1 in the four study periods

Study day	Lunch	Dinner
Day -1	NA	Bread Tomato pasta Fruit
Day 1	Bread Pasta Bolognaise Roasted turkey Green beans	Bread Tomato pasta Bresaola and salad Fruit

NA: Not applicable
Meals will be served as standardised portions prepared by a selected catering provider

Coffee, tea or food containing xanthines (i.e. coke, chocolate, etc.), smoking, alcohol and grapefruit will be forbidden during confinement. In particular, alcohol and grapefruit will be forbidden for 24 h before the first IMP administration until the end of the study.

Only non-smokers will be included in the study.

During confinement, routine ambulant daily activities will be strongly recommended.

6.2.1 Restrictions

During each study period, the subjects will be confined from the evening preceding the IMP administration (study Day -1) until the evening of Day 2.

For the 4 h following each administration, when not involved in study activities, the subjects will remain seated. They will not be allowed to lie down.

During confinement, hazardous, strenuous or athletic activities will not be permitted.

7 DESCRIPTION OF SPECIFIC PROCEDURES

7.1 Physical examination

Full physical examinations will be performed at the screening and final visit/ ETV. Information about the physical examination will be recorded by the investigator. Any abnormalities will be recorded.

Significant findings/illnesses, reported after the start of the study and that meet the definition of an AE (see § 11), will be recorded in the subject source documents.

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be reported in the individual CRFs.

7.1.1 Body weight

Body weight will be recorded at the screening and final visit/ ETV.

Subjects will be weighed (kg) lightly clothed without shoes. Height will be measured at screening only and BMI will be recorded. BMI will be calculated as weight [kg]/(height [m] x height [m]).

7.1.2 Vital signs

Subjects blood pressure (BP) and heart rate will be measured at rest (5 min in sitting position) by the investigator or his deputy at:

- Screening
- In each period, at pre-dose and 2 and 36 h post-dose
- ETV (if applicable)

7.1.3 ECGs

One 12-Leads ECG will be performed (in supine position) at screening visit.

7.1.4 Mouth visual inspections

The investigator or deputy will inspect the subjects' mouth to check for mucosal irritation at the application site at the following times:

- After each administration of the test IMP, at pre-dose and 0.5 and 1 h post-dose

7.1.5 Palatability

The subjects will be asked about the palatability of the test product immediately after administration.
Palatability will be scored according to the following scale:

Table 7.1.5.1 Palatability evaluation scores

JUDGEMENT	SCORE
very unpleasant	0
unpleasant	1
acceptable	2
good	3
very good	4

7.2 Clinical laboratory assays

Samples of blood (12.5 mL) and urine will be collected. The following laboratory analyses will be performed at the screening visit at the centralised clinical laboratory (§ 16.4):

Haematology
Leukocytes and leukocyte differential count (percentage values and absolute values), erythrocytes, haemoglobin (conv. units), haemoglobin (IS units), haematocrit, MCV, MCH, MCHC, thrombocytes.

Blood chemistry
Electrolytes: sodium, potassium, calcium, chloride, inorganic phosphorus
Enzymes: alkaline phosphatase, γ-GT, AST, ALT
Substrates/metabolites: total bilirubin, creatinine, glucose, urea, uric acid, total cholesterol, triglycerides, cotinine
Proteins: total proteins

Serum pregnancy test (women).

Urine analysis
Urine chemical analysis (stick)**:** pH, specific weight, appearance, colour, nitrites, proteins, glucose, urobilinogen, bilirubin, ketones, haematic pigments, leukocytes
Urine sediment (analysis performed only if positive): leukocytes, erythrocytes, flat cells, round cells, crystals, cylinders, mucus, bacteria

Serum virology
Hepatitis B (HBs antigen), **Hepatitis C** (HCV antibodies), **HIV 1/2** (HIV Ag/Ab combo).

A urine drug test will be performed at the Phase I Unit at screening, using a urine multi-drug kit. The following drugs will be assayed: cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol - THC), opiates and ecstasy.

A serum pregnancy test will be performed at the clinical laboratory (§ 16.4) at screening. Urine pregnancy tests will be performed on Day -1 of each study period at the Phase I Unit.

The same analyses, with the exception of cotinine, urine drug test, virology and pregnancy test, will be performed at the final visit/ETV at the centralised clinical laboratory (§ 16.4).

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant

findings (if any) will be reported in the individual CRFs. All clinically significant abnormalities after the screening visit will be recorded as AEs. Hard copies of the laboratory print-outs will be attached to the CRFs.

7.3 Sampling for pharmacokinetic analysis

7.3.1 Venous blood sampling

In each period, venous blood samples (7 mL) will be collected from a forearm vein at the following times:

- 0 h (pre-dose), 15, 30, 45 min, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h post-dose.

Actual sampling times for each subject will be recorded in the individual case report forms (CRFs). The actual sampling times should not exceed the recommended tolerance ranges presented in the following table. Any deviation outside the recommended ranges will be verified through Data Clarification Forms and, if confirmed, will be reported as protocol deviation, although it will not automatically lead to the exclusion of the concerned subjects from the PK Sets.

Table 7.3.1.1 Tolerance ranges for the scheduled sampling times

Sampling time	Tolerance range
Pre-dose (0)	Within 30 minutes before IMP administration
0.25 h (15 min)	0 min
0.5 h (30 min)	± 1 min
0.75 h (45 min)	± 2 min
1, 1.25, 1.5 h	± 3 min
2, 3, 4 h	± 5 min
6, 8, 12, 24, 36 h	± 10 min

Blood samples for PK analysis will be collected using an indwelling catheter with switch valve. The cannula will be rinsed, after each sampling, with about 1 mL of sterile saline solution containing 20 I.U./mL Na-heparin. The first 1.5 mL of blood will be discarded at each collection time to wash the cannula out of saline solution and to avoid contamination of the sample with heparin.

The remaining 5.5 mL will be collected from the catheter and transferred with a syringe into pre-labelled Li-heparinised polypropylene tubes.

The samples will be stored on ice for a maximum of 60 min. Then the samples will be centrifuged at 4° C for 10 min at 2500 g to obtain plasma. Each plasma sample will be immediately divided into 3 aliquots, P1 (1 mL), P2 and P3 (remaining plasma), in pre-labelled polypropylene tubes, and stored frozen until analyses.

If any clinical assessment, such as vital signs measurement is foreseen at the same time-point as blood sampling for PK analysis, blood collection will be performed at the scheduled time. However, vital signs can be influenced by blood sampling. Therefore, these measurements can be performed within 30 min before the pre-dose PK time point (0 h) and within 10 min before the other scheduled PK time-points. Any deviations outside the recommended time

will be verified through Data Clarification Forms. However, since vital signs measurements will be performed for safety reasons only, deviations from the planned time schedule will be considered not relevant.

7.3.2 Analytics

The concentration of riluzole in plasma will be determined at Accelera S.r.l., Italy, (§ 16.3), using a fully validated LC-MS/MS method with a lower quantification limit (LQL) of 5 ng/mL.

Analyses will be performed according to the general Principles of "OECD Good Laboratory Practices for testing of chemicals" C(81) 30 (final) and GCP.

The method validation report and the analytical report will be attached to the final report.

7.3.3 Labelling, storage and transport of samples

7.3.3.1 Samples labelling

Each sample tube will be clearly and unequivocally identified with a label resistant to the storage temperature and reporting:

Study code	Study CRO-PK-20-344 - Sponsor code Z7251J01
Subject number	P001-P054
Tube identification	P1/P2/P3
Period	1/2/3/4
Study day	1/2
Scheduled sampling time	as h; see § 7.3.1

7.3.3.2 Samples storage and transport

At the Phase I Unit, the study samples will be stored at ≤-20° C. At the end of each collection day, aliquots 1 and 3 will be stored in freezers separate from aliquots 2.

All aliquots 1, packed in sufficient solid CO_2, will be shipped by an authorised courier from the Phase I Unit, Switzerland, to Accelera S.r.l., Italy. Aliquots 1 will remain stored at Accelera S.r.l. for a maximum time of 6 months after finalisation of the bioanalytical report. If not otherwise indicated, the samples will be destroyed and a certificate of destruction will be provided to the sponsor.

The counter-samples (aliquot 2 and 3) will remain stored at CROSS Research S.A., Switzerland. These samples could either be:

- sent to the laboratory for reanalysis should this become necessary for analytical reasons or if any problems occur during the delivery of aliquots 1, or
- destroyed at an authorised site, or
- transferred to the sponsor upon written request, or
- stored at CROSS Research S.A., for a maximum time of 5 years

No analyses different from those stated in this protocol and agreed by the subjects when signing the informed consent form will be performed unless a new informed consent and a new approval from the Ethical Committee is obtained. The subjects may ask to destroy their own samples at any time.

7.4 Total number of samples and blood withdrawn

During the study the following volume of blood will be collected:

For routine laboratories analysis:
Screening visit: 12.5 mL
Final visit/ETV: 12.5 mL

For PK analysis:
Treatment T: 15x2x7=210 mL
Treatment R: 15x2x7=210 mL

In total, 445 mL of blood (not exceeding a normal blood donation) will be withdrawn from each subject.

8 ASSIGNMENT OF STUDY TREATMENT

8.1 Randomisation

The randomisation list will be computer-generated by the Biometry Unit of the Clinical Contract Research Organization (CRO), using the PLAN procedure of the SAS® version 9.3 (TS1M1) (13) or higher (the actual version will be stated in the final report). The randomisation list will be supplied to the study site and to STM Pharma PRO Srl., Italy (§ 16.5) for the preparation of the IMP individual kits before study start and will be attached to the final clinical study report.

8.2 Treatment allocation

The study subjects will be assigned to one of 2 sequences of treatments (either RTRT or TRTR) according to their randomisation number. Randomisation number will be given to the subjects on study Day -1, period 1, as soon as they are definitely enrolled in the study.

8.3 Blinding

This is an open study. No masking procedure will be applied.

9 EVALUATION PARAMETERS

9.1 Study variables

9.1.1 Primary variables

- C_{max} and AUC_{0-t} of plasma riluzole after replicate single dose administration of test and reference formulation.

9.1.2 Secondary variables

- $AUC_{0-\infty}$, $t_{1/2}$, t_{max}, AUC_{extra}, λ_z, of riluzole after replicate single dose administration of test and reference;
- Palatability score
- TEAEs, vital signs (BP, HR), laboratory parameters, physical examination including BW

9.2 Pharmacokinetic assessments

9.2.1 Pharmacokinetic parameters

The following PK parameters will be measured and/or calculated for plasma riluzole, using the validated software Phoenix WinNonlin® version 6.3 (12) or higher (actual version will be stated in the final report):

C_{max}:	Maximum plasma concentration
t_{max}:	Time to achieve C_{max}
λ_z:	Terminal elimination rate constant, calculated, if feasible, by log-linear regression using at least 3 points
$t_{1/2}$:	Half-life, calculated, if feasible, as $\ln 2/\lambda_z$
AUC_{0-t}:	Area under the concentration-time curve from administration to the last observed concentration time t, calculated with the linear trapezoidal method
$AUC_{0-\infty}$:	Area under the concentration-time curve extrapolated to infinity, calculated, if feasible, as $AUC_{0-t} + C_t/\lambda_z$, where C_t is the last measurable drug concentration
$\%AUC_{extra}$:	Percentage of the residual area (C_t/λ_z) extrapolated to infinity in relation to the total $AUC_{0-\infty}$, calculated, if feasible, as $100\times[(C_t/\lambda_z)/AUC_{0-\infty}]$

The sampling schedule is considered adequate if the ratio $AUC_{0-t}/AUC_{0-\infty}$ equals or exceeds a factor of 0.8 (i.e. if $\%AUC_{extra}$ is <20%) for more than 80% of the individual PK profiles. This assures that the primary variable AUC_{0-t} covers a sufficient percentage of the theoretical total extent of exposure.

The quality of log-linear regression (and, consequently, the reliability of the extrapolated PK parameters) should be demonstrated by a determination coefficient $R^2 \geq 0.8$. Individual extrapolated parameters, when considered unreliable, will be reported as NC (not calculated).

9.3 Safety assessments

Safety and general tolerability of the IMP will be based on TEAEs, physical examinations including body weight, vital signs and routine haematology, blood chemistry and urinalysis laboratory tests.

10 STATISTICAL METHODS

The data documented in this study and the parameters measured will be evaluated and compared using classic descriptive statistics, i.e. geometric mean (PK data only), arithmetic mean, SD, CV (%), minimum, median and maximum values for quantitative variables, and frequencies for qualitative variables.

Not available data will be evaluated as "missing values". The statistical analysis of demographic and safety data will be performed using SAS® version 9.3 (TS1M1) (13) or higher (the actual versions will be stated in the final report).
The statistical analysis of PK parameters will be performed using Phoenix WinNonlin® version 6.3 (12) or higher and SAS® version 9.3 (TS1M1) or higher.

10.1 Analysis Sets

10.1.1 Definitions

A subject will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A subject will be defined as eligible if he/she meets all the inclusion/exclusion criteria. Otherwise he/she will be defined as a screen failure.

A subject will be defined as enrolled in the study if he/she is included into the interventional phase of the study. The enrolment will be performed through randomised allocation to one treatments sequence.

An eligible but not enrolled subject will be defined as a reserve.

A subject will be defined as randomised in the study when he/she is assigned to one randomised treatments sequence.

The following analysis sets are defined:

- Enrolled set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
- Safety set: all subjects who receive at least one dose of the investigational medicinal products. This analysis set will be used for the safety analyses
- PK set: all randomised subjects who fulfil the study protocol requirements in terms of investigational medicinal product intake and have evaluable PK data readouts for the planned treatment comparisons, with no major deviations that may affect the PK results. This analysis set will be used for the statistical analysis of the PK results

Each subject will be coded by the CRO Biometry Unit as valid or not valid for the Safety set and the PK set. Subjects will be evaluated according to the treatment they actually receive.

10.1.2 Reasons for exclusion from the PK set before bioanalysis

Reasons for the exclusion of subjects from the PK set are the following:

- vomiting and diarrhoea after drug intake which could render the plasma concentration-time profile unreliable
- intake of concomitant medications which could render the plasma concentration-time profile unreliable
- AEs which could render the plasma concentration-time profile unreliable
- administration errors which could render the plasma concentration-time profile unreliable
- other events which could render the plasma concentration-time profile unreliable

If one of these events occurs, it will be noted in the CRF as the study is being conducted.

10.1.3 Reasons for exclusion from the PK set after bioanalysis

Exclusion of subjects on the basis of pharmacokinetic reasons is possible only for:

- subjects with lack of any measurable concentrations or only very low plasma concentrations for the reference medicinal product. A subject is considered to have very low plasma concentrations if his/her AUC is less than 5% of the reference medicinal product geometric mean AUC (which should be calculated without inclusion of data from the outlying subject)
- subjects with implausible concentrations (i.e. different from the known, expected concentration profiles) for the reference medicinal product. The exclusion of these subjects must be justified on the basis of sound scientific reasons and mutually agreed between the CRO and the Sponsor
- subjects with non-zero baseline concentrations > 5% of C_{max}

The samples from the subjects excluded from the PK set should still be assayed and the results listed. Subjects should not be excluded from the PK set if the AUC_{0-t} covers less than 80% of the $AUC_{0-\infty}$.

10.2 Sample size and power considerations

Data from previous PK studies of the test product, 1897 and 162020 (§ 1.4.1and 1.4.2) were used to calculate the sample size of the present study, as shown in the table below.

Table 10.2.1 Sample size calculation

Parameter	δ	CV_{WR}	α	β	n	N=2n
C_{max}	1.11	0.3266	0.1	0.2	26	52
AUC_{0-t}	1.0917	0.1265	0.1	0.2	4	9

δ: ratio of geometric means of the considered parameter; CV_{WR}%: within-subject variability; α: type 1 error; β: type 2 error; n: number of subjects per sequence; N: total sample size

Variability of AUC_{0-t} and C_{max} (CV_{WR}) actually observed in study 162020 (§ 1.4.2) was used in the calculation. The actual ratio of geometric means of AUC_{0-t} of study 162020 was 1.0917,

whilst that of C_{max} observed in study 162020 was 1.1582. This high ratio did not prevent the demonstration of bioequivalence of the formulations in study 162020 also for C_{max}. However, the calculation of the sample size for the present study using a δ of 1.1582 would be 126 subjects. Considering that the enrolment of at least 126 subjects in a bioequivalence study would be hardly justifiable, that bioequivalence between the test IMP and Rilutek® 50 mg tablets available in US was demonstrated in study 162020 in 30 subjects, who completed the study, and that the actual δ of C_{max} obtained in the previous pilot study 1897 was 0.9436, which is notably nearer the equality, i.e. ratio = 1.00, than the ratio 1.1582 observed later in study 162020, a ratio of 1.11 was postulated in the present sample size calculation. Indeed, maintaining CV_{WR}, α and β unchanged and assuming as C_{max} ratios, alternately, 1.05 and 1.10, sample sizes of 26 and 46 subjects, respectively, would be necessary to demonstrate bioequivalence in terms of C_{max}.
Taking into account this premise, when the sample size in each sequence group is 26 (and the total sample size is 52), a replicate crossover design will have 80% power to reject both the null hypothesis that the ratio of the test mean to the reference mean of C_{max} is below 0.800 and the null hypothesis that the ratio of test mean to the reference mean of C_{max} is above 1.250 (i.e. that the test and standard are not equivalent, in favour of the alternative hypothesis that the means of the two treatments are equivalent) assuming that data will be analysed in the natural log scale using t-tests for differences in means and that each t-test will be made at the 5.0% significance level.

In conclusion, 54 subjects will be enrolled in order to have 52 completed subjects.

10.3 Demographic, baseline and background characteristics

Critical demographic characteristics will be examined according to qualitative or quantitative data. Qualitative data will be summarised in contingency tables. Quantitative data will be summarised using classic descriptive statistics.

10.4 Drug administration and analysis of film dissolution

The date and time of film placement, of film dissolution, of accidental film swallowing and reference tablet administrations will be listed. Dosing time is defined as the time the film is placed on the subject's tongue.
The actual time of film dissolution will be summarised by descriptive statistics.

The dose per body weight of riluzole will be listed and summarised by descriptive statistics. The body weight collected at the screening visit will be used for the calculation.

The minimum and maximum number of days of wash-out between periods will be presented.

10.5 Test IMP palatability

Test IMP palatability scores will be listed and summarised in tables of frequency.

10.6 Analysis of pharmacokinetic parameters

10.6.1 Descriptive pharmacokinetics

A descriptive PK will be presented. The results will be displayed and summarised in tables and figures. Individual and mean curves (+SD at sampling times), indicating inter-subject variability, will be plotted. Data below the lower quantification limit (BLQL) will be considered as 0 in the calculations and presented as BLQL in listings and tables. As a consequence of BLQL (i.e. 0) values, calculated geometric means (if requested) could be null. For this reason, in the presence of any null value, the geometric mean will be reported as not calculated (NC).

10.6.2 Statistical comparison of pharmacokinetic parameters

According to the current European Guideline (9), plasma riluzole AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} will be analysed using analysis of variance (ANOVA). A mixed linear model will be used to make statistical inferences about the values of C_{max} and AUC_{0-t}. In order to correctly assume normality and homoscedasticity, the data will be transformed prior to analysis using a neperian logarithmic transformation in compliance with the EMA guideline.

The 90% CI of the ratio of the population means (test/reference), for the parameters under consideration will be calculated. This method is equivalent to the corresponding two one-sided test procedure with the null hypothesis of bioequivalence at the 5% significance level.

- The statistical analysis will take into account treatment, period, sequence and subject within sequence as fixed effects.

- Acceptance criterion for bioequivalence in terms of extent of absorption (AUC_{0-t} as primary variable and $AUC_{0-\infty}$ as secondary variable) will be that the 90% CI of this ratio has to lie within the range 80.00-125.00%.

- Due to the replicate design of the study, if the within-subject variability (CV_{WR}%) of C_{max} of plasma riluzole after administration of the reference formulation is >30%, the reference-scaled BE approach will be used. Bioequivalence acceptance range for C_{max} will be widened, in compliance with the guideline, as exemplified in Table 10.6.2.1.

- CV_{WR}% will be calculated by applying the formula $CV_{WR}(\%) = 100\sqrt{e^{S^2_{WR}} - 1}$, where S^2_{WR} is the within-subject variation plasma riluzole after administration of the reference formulation only.

- The extent of the widening will be defined on the basis of the within-subject variability actually observed in the study according to [U, L] = exp [±k·sWR], where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 0.760 and S_{WR} is the within-subject standard deviation of the log-transformed values of C_{max} of plasma riluzole after administration of the reference formulation.

- Moreover, the widening of the acceptance range for C_{max} is justified considering the favourable safety and tolerability profile of riluzole as demonstrated for the formulations authorised on the market.

Table 10.6.2.1 Examples of widened bioequivalence acceptance limits of C_{max} according to its actual CV_{WR}

CV_{WR} %*	Lower Limit	Upper Limit
30	80.00	125.00
35	77.23	129.48
40	74.62	134.02
45	72.15	138.59
≥50	69.84	143.19

*: $CVWR(\%) = 100\sqrt{e^{S^2_{WR}} - 1}$; source: reference (9)

t_{max} and $t_{1/2}$will be compared between treatments by the non-parametric Friedman test.

10.7 Safety and tolerability evaluation

- **AEs**

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs will be classified as pre-treatment AEs (PTAEs) and TEAEs, according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of IMP and not worsening after the first dose of IMP
- TEAEs: all AEs occurring or worsening after the first dose of IMP

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs. TEAEs will be summarised by treatment and overall. The number and percentage of subjects with any TEAE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

- **Physical examination**

Date of the physical examination, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant abnormalities (if any) will be listed.

- **Laboratory data**

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be listed. All laboratory results will be listed and a table of all the abnormal values will be presented. The overall investigator's interpretation will be summarised using tables of frequency.

- **Vital signs**

Vital signs values will be listed and summarised by descriptive statistics.

- **Body weight**

Body weight values will be listed and summarised by descriptive statistics.

- **Tolerability at the application site**

Date/time of each mouth visual inspection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and clinically significant findings (if any) will be listed.

11 DEFINITION AND HANDLING OF AEs AND SAEs

11.1 Applicable SOPs

AEs definition, classification and management will follow the Sponsor's SOPs. The AE form present in the CRF and the SAE form will also follow the sponsor SOPs.

The full SOP or an operative summary will be made available to the Phase I Unit.

A brief summary of AE definition, classification and management is reported below.

11.2 Definition of Adverse Event (AE)

An AE is "*any untoward medical occurrence in a patient or clinical study subject administered a medicinal product and which does not necessarily have a causal relationship with treatment*".

AEs include:

- worsening (change in nature, severity or frequency) of conditions present at the onset of the study
- patient/subject deterioration due to the primary illness
- intercurrent illnesses
- drug interactions
- events related or possibly related to concomitant medications
- abnormal laboratory values, as well as significant shifts from baseline within the range of normal, which the investigator considers to be clinically significant.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

11.3 Definition of Adverse Drug Reaction (ADR)

An ADR is "*any untoward and unintended response to a medicinal product related to any dose administered".*
All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or matter to suggest a causal relationship.

- **Unexpected ADR**:

an unexpected ADR is: "*An adverse reaction, the nature or severity of which is not consistent with the applicable product information (Reference Safety Information - RSI)"*

- **Reference Safety Information (RSI)**: in order to assess whether an ADR is expected, the IB will be used.

11.4 Definition of Serious Adverse Events or Serious Adverse Drug Reaction

A Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (SADR) is: *"any untoward medical occurrence or effect that at any dose"*:

- results in death
- is life-threatening (i.e. the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity (where disability is defined as a permanent or substantial disruption of ability to carry out normal life functions, either reported or defined as per clinical judgement)
- is a congenital anomaly/birth defect
- is an important medical event that may not result in death, be life-threatening, or require hospitalization but, according to appropriate medical judgment, it may jeopardize the subject's health status and may require intervention to prevent any of the outcomes listed in the definition above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home or blood dyscrasias or convulsions that do not result in hospitalisation or development of drug dependency or drug abuse

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event.

A **Suspected Unexpected Serious Adverse Reaction (SUSAR)** is an ADR that is both unexpected (not consistent with the RSI) and also meets the definition of a SAE.

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event.

11.5 Definition of Severity of Adverse Events

The term "severe" is used to describe the intensity (severity) of a specific event:

- Mild: causing no limitation of usual activities; the subject may experience slight discomfort.
- Moderate: causing some limitation of usual activities; the subject may experience annoying discomfort.
- Severe: causing inability to carry out usual activities; the subject may experience intolerable discomfort or pain.

11.6 Definition of Adverse Event causality

Causality shall be determined according to the definition of ADR given in 11.3.

All AE judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions. The causality assessment given by the investigator should not be downgraded by the sponsor.

The following binary decision for causality will be used:

- Reasonable possibility that the IMP caused the event
- No reasonable possibility that the IMP caused the event

Features supportive of an association include:

- temporal plausibility
- pharmacological properties of the drug or of the substance class
- course of the adverse event after dechallenge and, if applicable, after rechallenge
- specific tests indicating involvement of the drug in the occurrence/worsening of the adverse event
- alternative explanations

11.7 Adverse Events recording

Each AE occurring to a subject, either spontaneously revealed by the subject or observed by the investigator, whether believed by the investigator to be related or unrelated to the IMP, must be recorded on the AE information page of the CRF (for SAEs information must be recorded also on the "Serious Adverse Event Form").
The investigator will also perform an evaluation with respect to seriousness and causality (relationship of any AE to IMP) of the AEs and record it on the appropriate section of the CRF and on the "Serious Adverse Event Form" (if appropriate).

11.8 AEs monitoring window

- Start of monitoring: from immediately after the signature of the informed consent
- End of monitoring: final visit/ETV

An AE occurring after the final visit/ETV and coming to knowledge of the investigator (e.g. by spontaneous reporting by study subjects) must be recorded only if it is an ADR, according to the investigator's judgment.

11.9 Adverse Events reporting

The official language for reporting is English. The investigator and clinical staff of the present study are familiar with English language.

The investigator must report all AEs which occur during the study, regardless of their relationship to the IMP.

11.10 SAEs reporting

The investigator must report the SAEs immediately and not later than 24 h from when he becomes aware of the SAE, by faxing the "Serious Adverse Event Form" (back up plan) or e-mailing as scanned attachment (backup plan) or by Electronic Data Capture to the Drug Safety Unit (DSU) personnel of Zambon (preferred method).

The community standards of confidentiality must always be maintained and any relevant national legislation on data protection must be followed.

Another copy of the "Serious Adverse Event Form" will be retained by the investigator for the investigator's file.

If the investigator becomes aware of any SAE occurred to a subject within the window established in the protocol, he will report the SAE as above. The SAE will be also reported in the CRF.

If outside the window established in the protocol the investigator becomes aware of a SAE, it is the investigator's responsibility to report the SAE. The investigator might use the "Serious Adverse Event Form" via email or fax, but the SAE must not to be reported in the CRF, as it is not an event occurred within the study period.

11.11 Follow-up for Adverse Events

A follow-up "Serious Adverse Event Form" will be filled in if important follow-up information (i.e. diagnosis, outcome, causality assessment, results of specific investigations) is made available after submission of the initial SAE Form for immediate reporting. Follow-up "Serious Adverse Event Form" will be reported to the Sponsor as above-described.

In any case of an AE that, in the opinion of the investigator, requires the subject's discontinuation, follow-up information relating to the subjects subsequent course must be collected until the event has subsided or the condition stabilised or when the subject is lost to follow up and uncontactable.

When follow-up data on non-serious AE are collected, information should be reported under "Comments" in the Final report of the CRF.

11.12 SUSARs management

The clock for initial expedited reporting starts as soon as the information containing the minimum reporting criteria has been received by the Sponsor (day 0).
For fatal and life-threatening SUSARs the EC and Competent Authority (Swissmedic) should be informed as soon as possible and in any case within 7 days.

If the initial report is incomplete, e.g. not all the information/assessments were available, a complete report should be sent within an additional 8 days. Complete follow-up report should be sent within 15 calendar days.

SUSARs which are not fatal and not life-threatening are to be reported within 15 days.

The minimum information to be reported includes:

- Sponsor study code
- One identifiable coded subject
- One identifiable reporter
- One SUSAR
- One suspect IMP (including active substance name, code)
- A causality assessment.

In addition, in order to properly process the report electronically, the following administrative information should be provided:

- the sender's (case) safety report unique identifier,
- the receipt date of the initial information from the primary source,
- the receipt date of the most recent information,
- the worldwide unique case identification number,
- the sender identifier.

11.13 Other events qualified for expedited reporting

Other safety issues also qualify for expedited reporting when they might materially alter the current benefit-risk assessment of a medicinal product or would be sufficient to consider changes in the medicinal product administration or in the overall conduct of the study, for instance:

- single case reports of an expected serious adverse reaction with an unexpected outcome (e.g.: a fatal outcome)
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
- post-study SUSARs that occur after the subject has completed a clinical study and are reported to the investigator by the subject.
- new events relating to the conduct of the study or the development of the medicinal product likely to affect the safety of the subjects, such as :
  - a SAE which could be associated with the study procedures and which could modify the conduct of the study
  - a significant hazard to the subject population such as lack of efficacy of a medicinal product used for the treatment of a life-threatening disease
  - a major safety finding from a newly completed animal study (such as carcinogenicity) or from other clinical study.

11.14 SAEs: contacts

The Phase I Unit can be contacted using the phone and fax numbers stated in this protocol or calling the mobile phone number +41.79.822.35.07 (operative 24-h/day, 365 days/year). This mobile phone can be called by the study participants to communicate to the clinical staff any SAE occurring outside the clinical facility.

The Sponsor contact for SAEs is the following:

Dr. Milko Radicioni
Phone: +41.91.64.04.450
Fax: +41.91.64.04.451
Email: milko.radicioni@croalliance.com

The investigator will report any SAE to the sponsor. The sponsor's details for SAEs are the following:
Phone: +39.02.66.524.444
Fax: +39.02.66.524.038
Email: drugsafety@zambongroup.com

11.15 Pregnancy

Subjects must be instructed that known or suspected pregnancy occurring during the study should be confirmed and reported to the investigator, who must then withdraw the subject from the study without delay. The investigator should also be notified of pregnancy occurring during the study but confirmed after its completion. In the event that a subject is subsequently found to be pregnant after inclusion in the study, then any pregnancy will be followed to term and the status of mother and child will be reported to the Sponsor after delivery through the Pregnancy Report Form provided by the Sponsor. The investigator will send pregnancy reports to DSU within the timeframes of SAEs, with follow-up information to be actively sought for the outcome of pregnancy.

- Part I of the Form is completed at the time of awareness of foetal exposure during pregnancy.
- Part II of the Form is filled in when information on pregnancy outcome becomes available.

If pregnancy results in abnormal outcome that the investigator considers to be due to the IMP, this will be treated as an expedited ADR report.

12 DATA MANAGEMENT PROCEDURES

12.1 Data collection – CRFs

The investigator must ensure that the clinical data required by the study protocol are carefully reported in the CRFs. He must also check that the data reported in the CRFs correspond to those in the subject's source documents.
To ensure legibility, the CRFs should be filled out in English, in block capitals with a ball-point pen (not pencil, felt tip or fountain pen). Any correction to the CRFs' entries must be carried out by the investigator or a designated member of staff. Incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way. A single stroke must be drawn through the original entry. Corrections have to be dated and initialled. In the interest of completeness of data acquisition, the questions which are repeated in each section of the CRFs should be answered in full, even if there are no changes from a previous examination. The investigator must provide a reasonable explanation for all missing data.
The CRFs will be completed, signed by the investigator, sent to the CRO Biometry Unit for data management procedures and finally sent to the sponsor.

12.2 Unique subject identifier

All the subjects who sign the informed consent form for the present study will be coded with "unique subject identifiers" when data are extracted from the study database into the domains of the CDISC SDTM model. The unique subject identifier consists of the sponsor study code (i.e. Z7251J01), the 3-digit site number (i.e. 001), the 4-digit screening number (e.g. S001, S002, etc.) and, if applicable, the 3-digit subject randomisation number (e.g. 001, 002, etc.). Study code, site number, screening number and subject randomisation number are separated by slashes ("/"). The last 8 digits of the unique subject identifier (enrolled subjects), corresponding to the subject screening and subject randomisation numbers separated by a slash, or the last 4 digits of the unique subject identifier (not enrolled subjects), corresponding to the subject screening number, will appear as subject identifier in the individual listings and figures of the clinical study report and will be used to identify the subjects in in-text tables or wording (if applicable).

12.3 Database management

The CRO will provide a double data entry with total re-entry of data and discrepancy resolution by a second data entrist and will update and verify the database and create the final SAS data sets. The final data file will be transferred to the sponsor in the agreed format with all the other study documentation.

12.3.1 Coding dictionaries

Medical/surgical history and underlying diseases, clinically significant physical examination abnormalities and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).
Previous and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). The version of the coding dictionaries will be stated in the study report.

13 STUDY MONITORING, QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Monitoring

The monitoring visits will be conducted by appropriate staff of Clinical Medical Services of Maria Pia Savorelli, Switzerland.

Monitoring activities, including monitoring purpose, selection and qualifications of monitors, extent and nature of monitoring, monitoring procedures, monitoring reports will comply with ICH-GCP chapter 5.18 requirements.

Adequate time and availability for monitoring activities should be ensured by the investigator and key study personnel.

Data verification is required and will be done by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality. In this respect the investigator will assure support to the monitor at all times.

The investigator agrees, by written consent to this protocol, to fully co-operate with compliance checks by allowing authorised individuals to have access to all the study documentation. In addition to the monitoring activities performed by the study monitor, the sponsor could perform some quality control activities to verify the compliance with the study procedures and the ICH-GCP guidelines.

13.2 Quality Control and Quality Assurance

The CRO has implemented and maintains a Quality System that includes quality controls and audits at different study steps with written SOPs to ensure that the study is conducted in compliance with the protocol and all effective amendments, ICH-GCP, and the applicable regulatory requirement(s) and that data have been reliably and correctly generated, recorded, processed and reported, in agreement with the ALCOAC principles (Attributable-Legible-Contemporaneous-Original-Accurate-Complete).

The clinical site(s) is responsible for implementing and maintaining quality assurance and a quality control system to ensure that the study is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH-GCP, and the applicable regulatory requirement(s).

The CROs and the sponsor will be responsible for their respective activities.

The sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor.

13.3 Applicable SOPs

The Sponsor and the CRO will follow their respective SOPs in the conduct of the respective activities as specified in the signed assignment of responsibilities. For definition and handling of AEs the Sponsor's SOP will be used. SOPs will be made available for review, if required.

13.4 Data access

The investigator and the CRO will ensure that all raw data records, medical records, CRFs and all other documentation that is relevant to this study will be made accessible for monitoring activities, audits, IEC review, and regulatory inspections.

13.5 Audits and inspections

The sponsors, independent bodies acting on behalf of the sponsor and the CRO have the right to perform audits according to ICH-GCP responsibilities.

The study may also be inspected by regulatory authorities.

- The investigator and the CRO agree, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorised individuals to have access to all the study documentation.

14 ETHICAL CONSIDERATIONS

14.1 Ethics and Good Clinical Practice (GCP)

The study will be performed in accordance with the relevant guidelines of the Declaration of Helsinki.
The approval of the study protocol by the local (Canton Ticino) IEC and by the Federal Health Authorities (Swissmedic) will be obtained before the start of the study.

The present clinical study will be carried out according to the current revision of Good Clinical Practice (GCP), ICH topic E6 (R2), and the applicable local law requirements.

14.2 Informed consent

Before being enrolled into the clinical study, the subjects must have expressed their consent to participate, after the investigator has explained to them, clearly and in details, the scope, the procedures and the possible consequences of the clinical study. Information will be given in both oral and written form. The information sheet and informed consent form will be prepared in the local language by the CRO and must be approved by the EC and regulatory authorities. It will include all the elements required by law according to the ICH-GCP recommendations.
In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organised
- the type of treatment (information on the IMP(s) and treatment procedures, as applicable)
- any potential negative effects attributable to the study product or treatment
- the freedom to ask for further information at any time
- the subjects' right to withdraw from the clinical study at any time without giving reasons and without jeopardising their further course of medical treatment
- the existence of a subject insurance cover and obligations following from this cover

Adequate time and opportunity to satisfy questions will be given to the subjects and the time will be recorded.

The investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the investigator and the subject.
A copy of the signed form will be given to the subject.

To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the investigator's study file according to the regulatory requirements (see § 15.3). The investigator will allow inspection of the forms by authorised representatives of the sponsor, EC members and regulatory authorities. He will confirm, by signing and dating the forms, that informed consent has been obtained.
A blank copy of the information sheet and the informed consent form is appended to this protocol.

14.3 Insurance policy

An insurance cover has been issued in favour of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

14.4 Withdrawal of subjects

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the primary reason for discontinuation will be recorded.

14.4.1 Primary reason for discontinuation

- **Adverse event**: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation. For the definition of AE, please refer to § 11.2.
- **death**: the absence of life or state of being dead
- **lost to follow-up**: the loss or lack of continuation of a subject to follow-up
- **non-compliance with study drug**: an indication that a subject has not agreed with or followed the instructions related to the study medication
- **physician decision**: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- **pregnancy**
- **protocol deviation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **study terminated by sponsor**: an indication that a clinical study was stopped by its sponsor
- **study terminated by Sponsor or by Swissmedic or EC**:
- **technical problems**: a problem with some technical aspect of a clinical study, usually related to an instrument
- **withdrawal by subject**: study discontinuation requested by a subject for whatever reason
- **other**: different than the ones previously specified

14.4.2 Discontinuation procedures

For any subject discontinuing the study, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening)
- arrange for alternative medical care of the withdrawn subject, if necessary

- record the subject decision about the use of collected biological samples
- report in the CRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the CRF any follow-up, if the subject is withdrawn for an AE

Subjects withdrawn from the study will retain their randomisation/study number and will not be replaced.

14.5 Study termination

The study will be considered concluded at the date of the last visit of the last subject (LSLV) or upon completion of any follow-up procedure described in protocol.

The study will be considered terminated at the date of the last visit of the last subject or upon completion of any follow-up procedure described in protocol. The investigator and the sponsor have the right to discontinue the study at any time for reasonable medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. Reasons for discontinuation have to be documented appropriately and immediately reported to Swissmedic and EC (i.e. within 15 days).

15 ADMINISTRATIVE PROCEDURES

15.1 Material supplied to the clinical centre

Beside IMPs, the following study material will be supplied to the clinical centre:

- final version of the study protocol
- CRF for each subject plus some spare copies
- copy of the investigator's brochure (IB) relative to the IMP
- informed consent forms

Moreover, before the start of the study, the investigator(s) will be provided with the following documents: ICH guidelines, confidentiality agreement (if applicable), protocol amendments (if any), declaration of Helsinki, insurance statement, SAE forms, financial agreement (if applicable), confidential subject identification code list form, drug accountability forms, investigator and study staff list form.

15.2 Protocol amendments

In order to obtain interpretable results, neither the investigator nor the sponsor will alter the study conditions agreed upon and set out in this protocol. Amendments should be made by mutual agreement between the investigator and the sponsor. Any amendment must be set out in writing, giving the reasons, and being signed by all concerned parties. The amendment becomes then part of the protocol.

All substantial amendments will be sent to EC and Swissmedic, as appropriate. The amendment will be applicable only when it is approved, unless the changes consist of urgent safety measures to protect study subjects.

Non substantial amendments will be notified according to the current regulations.

15.3 Study documentation and record keeping

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

The investigator must keep source documents for each subject in the study. All information on the CRFs must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, including laboratory data, ECGs, etc., and the original signed informed consent forms.

Data reported on the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator and the sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by the applicable regulatory requirement(s).

These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of EC, raw data of subjects including lab tests and ECG tracing, insurance contracts, certificate of analysis of the IMP(s), drug accountability records, signed informed consent forms, confidential subjects identification code, CRFs, curricula vitae of the investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.

The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the investigator and the sponsor as long as needed to comply with ICH-GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

15.4 Study subjects' recruitment

Study participants will be recruited from the volunteers' database maintained by the CRO. This database contains a pool of volunteers that are contacted whenever necessary to enrol subjects in a new study. Before the start of the new study, the principal investigator and other relevant staff discuss with the volunteers' recruiter the study recruitment needs and specific requirements. On the basis of this information, the volunteers' recruiter queries the database, contacts potential participants to propose the study and evaluate their interest and availability. In addition to the volunteers' database, new subjects often call or email the CRO asking to become a research volunteer, after hearing of the clinical site activities from other volunteers or friends or after checking the company web site.

The CRO and its clinical site have detailed SOPs on the recruitment process.

15.5 Confidentiality and data protection

By signing this protocol, the CRO and the investigators agree to keep all the information provided by the sponsor in strict confidentiality and to request he same confidentiality from his/her staff. Study documents provided by the sponsor (protocols, IB, CRFs and other materials) will be stored appropriately to ensure confidentiality. The information provided by the sponsor to the investigator and to the CRO cannot be disclosed to others without direct written authorisation from the sponsor, except for the extent necessary to obtain the informed consent from the subjects wishing to participate in the study.

In this Phase I study the CRO plays the role usually performed by the Trial Center, performing services like drug assay, clinical monitoring and related drafting of monitoring reports; clinical laboratory analysis; reporting and project management; test data management, statistics; drug/samples storage and trial document archiving directly or availing itself of authorized third parties.

Data on subjects collected in the CRFs during the study will be documented in a coded way (see § 12.2). If, as an exception, for safety or regulatory reasons identification of a subject becomes necessary, the monitor, the sponsor and the investigator will be bound to keep this information confidential.

With reference to EU Regulation no.679/2016 of European Parliament and of the Council of 27 April 2016, the General Data Protection Regulation (GDPR), such as Swiss Federal Data Protection Act of June, 19 1992, and other local law provisions the data protection roles within the Phase I study are the following:

- the Sponsor and the CRO (that plays the role of the Investigational Center in this Phase I study) are Joint Controllers. The data processing activities performed within the Phase I clinical trial are jointly designed, addressed and managed by the Parties, pursuant to Article 26 of General Data Protection Regulation (EU) 2016/679 on personal data protection (hereinafter the "GDPR"). The Joint Controllers will process the personal and study data of the participants exclusively for study related purposes and for pharmacovigilance purposes or for other legitimate purposes.
- The Principal Investigator will process the data as a Data Processor, on behalf of the CRO.

As concerns the data protection information/notice, participants must be informed properly about all the data protection elements provided by art. 13 and 14 of GDPR and similar provisions of the Swiss Federal Data Protection Act of June, 19 1992 and subsequent amendments. Investigator or his/her representative will give to the participant a proper data protection information notice compliant with GDPR, and will consequently ask to the participant a data protection consent, together with the study informed consent. According to the provisions of the GDPR and Swiss Data Protection Law, the level of disclosure in the informed consent must also be explained to the participant. The participant must be informed that his/her medical records may be examined by Auditors or other authorized personnel appointed by the sponsor, by appropriate EC members, and by inspectors from regulatory authorities.

As regards the organizational and security measures adopted, the operations of collection, storage, circulation of biological samples as well as all the data processing operations regarding the study data are performed in compliance with GDPR and Swiss Data Protection Law. The investigator or his/her representative will assign to the participants a unique identifier. Investigator will be the only one who can match the participant's identity with the data referred to the study. Any participant records or datasets that are transferred to the sponsor will contain the identifier code only; participant names or any other information which would make the participant identifiable will not be transferred to the Sponsor.

15.6 Publication policy

The sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the investigator publishing in peer reviewed journals, presenting results at scientific congresses and posting information and results on internet-based public registers and databases.
Study results will be communicated in full to the competent Health Authorities (Swissmedic) by the submission of a complete clinical study report.

As the sponsor agrees that the study results can be published by the investigator(s), the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.
This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).

The investigator will also be provided by the sponsor with the clinical study report and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).
On an exceptional basis, the sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures, etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

According to The Federal Act on Research involving Human Beings and the Ordinance on Clinical Trials in Human Research, the study will be registered and published in a WHO primary register or clinicaltrials.gov as well as in the supplementary federal database.

16 STUDY RESPONSIBLE PERSONS

16.1 Sponsor

Zambon S.p.A., via Lillo del Duca 10, I-20091 Bresso, Italy
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Email: carlo.cattaneo@zambongroup.com

Sponsor representative

Carlo Cattaneo, Global Head Medical Affairs CNS

Medical Expert
Paola Castellani, MD
Global Chief Medical Officer and Patient's Access Head

16.2 Institutes performing the study

16.2.1 Clinical centre

CROSS Research S.A. – Phase I Unit, Via F. A. Giorgioli 14, CH-6864 Arzo, Switzerland
Phone: +41.91.64.04.450
Fax: +41.91.64.04.451
Email: clinic@croalliance.com

Principal investigator
Milko Radicioni, MD

16.3 Drug assay

Accelera S.r.l., V.le Pasteur, 10, I-20014 Nerviano, Italy
Phone: +39.0331.1984.444
Fax: +39.0331.1984.200
Email: info@accelera.org

Analytics representative
Daniele Pezzetta
Phone: +39.0331.1984.355
Fax: +39.0331.1984.325
Email: daniele.pezzetta@accelera.org

Analytical facilities and procedures are in compliance with the general principles of GLP regulations.

16.4 Centralised clinical laboratory

Unilabs Ticino, via Rovere 8, CH-6932 Breganzona, Switzerland
Phone: +41.91.960.73.73
Fax: +41.91.960.73.74
Email: bmathis@unilabs.ch

16.5 Packaging and labelling

STM Pharma PRO Srl., Italy, Via Abruzzi, IT–20056, Grezzago (MI), Italy
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16.6 Co-ordination, data analysis & reporting

CROSS Research S.A.
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Quality Assurance Unit Representative
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16.7 Monitoring

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