16. APPENDICES

16.1 Study information

16.1.1 Protocol and protocol amendments

The following documents are enclosed:

- Protocol final version 1.0, 28MAY20
- Note to file, 13OCT20
- Note to file, 19JAN21

CLINICAL STUDY PROTOCOL

CRO-PK-20-345 - Sponsor code LDX0219

Influence of Food on the Oral Bioavailability of Ladarixin 200 mg Capsule in Healthy Volunteers of Both Sexes. A Single dose (400 mg), Randomized, Open Label, Two-Way Crossover Study

Single center, single dose, open label, randomized, two-way, crossover, food effect study

Test treatment (fed conditions):	Ladarixin 200 mg hard gelatin capsules, Dompé farmaceutici S.p.A., Italy
Reference treatment (fasting conditions):	Ladarixin 200 mg hard gelatin capsules, Dompé farmaceutici S.p.A., Italy
Sponsor:	Dompé farmaceutici S.p.A., via Santa Lucia 6; 20122 Milan, Italy Phone: +39 02.58383.1 Fax: +39 02.58383.324 Email: pieradelchi.ruffini@dompe.com Sponsor Medical Expert: Pier Adelchi Ruffini, MD -Global Clinical Development Director
Investigator:	Milko Radicioni, MD - Principal investigator CROSS Research S.A., 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
Development phase:	Phase I
Version and date:	Final version 1.0, 28MAY20

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

This document comprises 68 pages

PROTOCOL APPROVAL

SPONSOR
Dompé farmaceutici S.p.A., Italy

Sponsor Trial Manager
Giuseppe Terpolilli, PharmD – Clinical Operations Specialist

28/05/2020 _____
Date

Giuseppe Terpolilli _____
Signature

Sponsor Medical Expert
Pier Adelchi Ruffini, MD – Global Head Clinical Development

29/05/2020 _____
Date

Pier Adelchi Ruffini _____
Signature

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 SA, Switzerland

28 MAY 20 20
Date

Signature

CRO
CROSS Research S.A., Switzerland

Coordination
Chiara Castiglioni, Clinical Project Leader and Clinical Research Associate

29MAY2020 _____ Date

_____ Signature

On behalf of
Medical Writing
Andrea Di Stefano, Senior Medical Writer

29MAY 2020 _____ Date

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Biometry Unit Representative
Alessandra Gentili, Biometry Manager, Unit Head

29 MAY 2020 _____ Date

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Quality Assurance Unit Representative
Mario Corrado, Quality Assurance Manager, Unit Head

29 MAY 2020 _____ Date

_____ Signature

STUDY SYNOPSIS

Title: Influence of food on the oral bioavailability of ladarixin 200 mg capsule in healthy volunteers of both sexes. A single dose (400 mg), randomized, open label, two-way crossover study
Protocol number: CRO-PK-20-345 - Sponsor code LDX0219
Clinical phase: Phase I
Study design: Single center, single dose, open label, randomized, two-way, crossover, food effect on bioavailability study
Planned nr. of centers / countries: 1/Switzerland
Investigator and center: *Principal Investigator:* Milko Radicioni, MD; CROSS Research Phase I Unit, Via F.A. Giorgioli 14, CH-6864 Arzo, Switzerland
Investigational medicinal product: Ladarixin 200 mg hard gelatin capsules, Dompé farmaceutici S.p.A., Italy
Dose regimen: A single oral dose of 400 mg of ladarixin (two 200 mg capsules) will be administered to healthy male and female volunteers under fed (Test treatment) and fasting (Reference treatment) conditions in two consecutive study periods, according to a two-way crossover design, with a wash-out interval of at least 14 days between the two administrations.
Objectives: **Primary objective:** ➢ to investigate the effect of food on the bioavailability of DF 2156Y after single dose administration of 400 mg of ladarixin to healthy male and female volunteers under fed and fasting conditions. **Secondary objectives:** ➢ to investigate the effect of gender on the bioavailability of DF 2156Y and its metabolites (DF 2108Y and DF 2227Y) after single dose administration of 400 mg of ladarixin to healthy male and female volunteers ➢ to evaluate safety and tolerability of a single dose administration of ladarixin 400 mg to healthy male and female volunteers.
End-points: **Primary end-point:** ➢ to evaluate and compare the rate (C_{max}) and extent (AUC_{0-t}, $AUC_{0-\infty}$) of absorption of DF 2156Y after single dose administration of 400 mg of ladarixin under fed and fasting conditions. **Secondary end-points:** ➢ to evaluate and compare between genders the rate (C_{max}) and extent ($AUC_{0-t;}$ $AUC_{0-\infty}$) of absorption of DF 2156Y and its metabolites (DF 2108Y and DF 2227Y) after single dose administration of 400 mg of ladarixin to healthy male and female volunteers; ➢ to describe the pharmacokinetic profile of DF 2156Y (total and unbound) and its metabolites (DF 2108Y and DF 2227Y) after single dose administration of 400 mg of ladarixin under fed and fasting conditions; ➢ to collect safety and tolerability data of a single oral dose administration of 400 mg of ladarixin.
Study variables: **Primary variables:** ➢ C_{max} and AUC_{0-t} of plasma DF 2156Y, after single dose administration of 400 mg of ladarixin under fed and fasting conditions **Secondary variables:** ➢ $AUC_{0-\infty}$, t_{max}, $t_{1/2}$, λ_z and F_{rel} of plasma DF 2156Y after single dose administration of 400 mg of ladarixin under fed and fasting conditions; ➢ C_{max}, AUC_{0-t}, $AUC_{0-\infty,}$ t_{max}, $t_{1/2}$, λ_z and F_{rel} of plasma DF 2156Y and of its metabolites (DF 2108Y and DF 2227Y) after single dose of 400 mg of ladarixin under fed and fasting conditions measured and calculated in healthy men and in healthy women;

STUDY SYNOPSIS (cont.)

Secondary variables:

- Treatment-emergent adverse events, vital signs (blood pressure, pulse rate, body temperature), body weight, electrocardiograms, physical examinations, laboratory parameters (hematology, blood chemistry and urine analysis).

Analytics: The concentration of DF 2156Y (total and unbound) and its metabolites (DF 2108Y and DF 2227Y) in plasma will be determined at Dompé farmaceutici S.p.A. Bioanalytical Laboratories, L'Aquila, Italy using a fully validated HPLC-UV method. 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 clinical study report (CSR).
A total of 1512 plasma samples (756 samples for each study period) will be analyzed.

Safety and tolerability assessments: Treatment-emergent adverse events; vital signs (blood pressure, pulse rate, body temperature), physical examinations including body weight; laboratory tests (hematology, blood chemistry and urine analysis), electrocardiograms

Sample size: The sample size was not calculated through any statistical calculation. In order to have at least 32 subjects completed (16 men and 16 women), a sample size of 36 healthy volunteers (18 men and 18 women) was estimate as sufficient for the descriptive purposes of the present study. Drop-out subjects will be replaced starting from the 3rd man and/or woman discontinuing prematurely the study.

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. *Body Mass Index:* 18.5-30 kg/m2 inclusive
4. *Vital signs:* systolic blood pressure 100-139 mmHg, diastolic blood pressure 50-89 mmHg, pulse rate 50-90 bpm and body temperature 35.5-37.5° C, measured after 5 min at rest in the sitting position
5. *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
6. *Contraception and fertility (women only):* women of child-bearing potential must not wish to get pregnant within 30 days after the end of the study and must be using at least one of the following reliable methods of contraception:
   a) Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 2 months before the screening visit until 30 days after final visit
   b) 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 until 30 days after final visit
   c) A male sexual partner who agrees to use a male condom with spermicide until 30 days after final visit
   d) A sterile sexual partner

   Women participants of non-childbearing potential or in post-menopausal status for at least one year will be admitted. For all women, pregnancy test result must be negative at screening and day -1.

Exclusion criteria:

1. *Electrocardiogram (ECG) 12-leads (supine position)*: clinically significant abnormalities
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. *Allergy*: ascertained or presumptive hypersensitivity to the active principles (ladarixin or derivatives) and/or formulations' ingredients; known hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs); history of hypersensitivity to drugs (in particular methanesulfonyl propanamide) or allergic reactions in general, which the Investigator considers may affect the outcome of the study
5. *Diseases*: hypoalbuminemia or significant history of renal, hepatic, gastrointestinal, respiratory, skin, hematological, endocrine, neurological or cardiovascular diseases that may interfere with the aim of the study

STUDY SYNOPSIS (cont.)

Main selection criteria (continued):
Exclusion criteria:

6. *Medications*: medications, including over the counter drugs (in particular nonsteroidal anti-inflammatory drugs), herbal remedies and food supplements taken 14 days before the start of the study (in any case at least 5 times the half-life of the drug or a minimum of 14 days, whichever is longer), with the exception of paracetamol. Hormonal contraceptives and hormonal replacement therapy for women will be allowed.
7. *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
8. *Blood donation*: blood donations for 3 months before this study
9. *Drug, alcohol, caffeine, tobacco*: history of drug, alcohol (>1 drink/day for women and >2 drinks/day for men, defined according to the USDA Dietary Guidelines 2015-2020), caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
10. *SARS-COV2 test:* positive SARS-COV2 test on day -3 or -2 of each study period
11. *Virology:* positive Hepatitis B (HBs antigen), Hepatitis C (HCV antibodies), HIV 1/2 (HIV Ag/Ab combo) at screening.
12. *Drug test*: positive result at the drug test at screening or day -1 of each study period
13. *Alcohol test*: positive alcohol breath test at screening or day -1 of each study period
14. *Diet*: abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; vegans
15. *Pregnancy (women only)*: positive or missing pregnancy test at screening or day -1 of each study period, pregnant or lactating women

Schedule:

Screening – Visit 1	*From day -21 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, physical abnormalities) ➢ Vital signs (blood pressure, pulse rate, body temperature) measurement ➢ ECG recording ➢ Laboratory analyses: hematology, coagulation, blood chemistry, microbiology, urinalysis, serum virology and serum pregnancy test (women only) ➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Adverse events 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.
Screening Visit 1.1	*Day -3 or -2*	➢ Nasal and pharyngeal swab for SARS-COV2 test ➢ Adverse events monitoring ➢ Previous/concomitant medications	

STUDY SYNOPSIS (cont.)

Schedule (continued):			
Period 1 - Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine pregnancy test (women only) ➢ Urine multi-drug kit test ➢ ECG recording ➢ Vital signs measurement ➢ Adverse event and concomitant medications ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment and randomization	Arrival at the Phase I Unit in the evening. Confinement until the morning of day 4. Standardized low-fat dinner. Fasting for at least 10 h (overnight).
Period 1 - Visit 3	*Day 1*	➢ Investigational medicinal product administration at 8:00 ± 1 h (day 1) ➢ Vital signs measurement at pre-dose ➢ ECG recording at pre-dose ➢ Blood sample collection for pharmacokinetic analysis at pre-dose (0) and 0.25 (15 min), 0.5 (30 min), 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 18 h post-dose ➢ Adverse event and concomitant medications	Standardized high-fat and high caloric breakfast starting at 30 min pre-dose for subjects receiving the investigational medicinal product under fed conditions in this study period. Breakfast must be completed within 30 min. Subjects receiving the investigational medicinal product under fasting conditions will not have breakfast. Standardized lunch and dinner at about 13:00 (5 h post-dose) and 20:00 (12 h post-dose), respectively
	Days 2 and 3	➢ Blood sample collection for pharmacokinetic analysis at 24, 30, 36, 48, 54 and 60 h post-dose ➢ Adverse event and concomitant medications	Standardized breakfast, lunch and dinner at about 9:00, 13:00 and 20:00, respectively
	Day 4	➢ Vital signs measurement upon discharge 72 h post-dose ➢ ECG recording upon discharge 72 h post-dose ➢ Blood sample collection for pharmacokinetic analysis at 72 h post-dose ➢ Adverse event and concomitant medications ➢ Full physical examination (body weight and physical abnormalities) upon discharge	Discharge from the Phase I Unit in the morning, after the 72-h post-dose blood sample collection, ECG recording, vital signs check and full physical examination. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any adverse reactions.
Wash-out	*At least 14 days*	A wash-out interval of at least 14 days between the two administrations of the two study periods	

STUDY SYNOPSIS (cont.)

Schedule (continued):			
Period 2 - Visit 4	*Day -3 or -2*	*As visit 1.1, day -3 or -2*	
Period 2 - Visit 5	*Day -1*	As visit 2, excluding enrolment and randomization. In addition a full physical examination (body weight and physical abnormalities) will be performed.	As visit 2
Period 2 - Visit 6	*Days 1-4*	As visit 3. Investigational medicinal product administered according to the randomization list and crossover design	As visit 3
Final Visit/ETV	*Day 4 of period 2 /at Early Termination Visit in case of discontinuation*	➢ Full physical examination (body weight and physical abnormalities; also vital signs and ECG in case of Early Termination Visit) ➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Laboratory analyses as at screening, with the exception of virology, coagulation, microbiology, albumin and globulin ➢ AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalization 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.
During each study period, the subjects will be confined from the evening preceding the investigational product administration (study day -1) until the morning of day 4. On day -1 of each period, a standardized low-fat dinner will be served after confinement. On day 1 of each study period, all the subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight). The subjects allocated to the fed conditions, after the overnight fasting period, will receive a high-fat and high-caloric breakfast starting 30 min pre-doses and will complete their breakfast within 30 min, while the subjects allocated to the fasting conditions will fast overnight and then receive their treatment. Water will be allowed as desired except for 1 h before and 1 h after investigational product 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, all subjects will remain fasted until 5 h post-dose. A standardized lunch and dinner will be served at approximately 5 h and 12 h post-dose (at approximately 13:00 and 20:00). On days 2 and 3, standardized breakfast, lunch and dinner will be served to all subjects at about 9:00, 13:00 and 20:00, respectively. One cup of coffee or tea will be allowed after each meal only; any other coffee, tea or food containing xanthines (i.e. coke, chocolate, etc.), alcohol and grapefruit and alcohol will be forbidden during confinement. In particular, grapefruit will be forbidden for 24 h before the first investigational product administration until the end of the study. The subjects will be allowed to smoke 9 cigarettes during confinement, one after each meal, with the exclusion of the high-fat and high-caloric breakfast. *During confinement, routine ambulant daily activities will be strongly recommended.* *For the 4 h following the administration, when not involved in study activities, the subjects will remain seated. They will not be allowed to lie down. Hazardous, strenuous or athletic activities will not be permitted.*		

STUDY SYNOPSIS (cont.)

Data analysis :
The pharmacokinetic analysis and the statistical analysis of pharmacokinetic parameters will be performed using Phoenix WinNonlin® version 6.3 or higher, Pharsight Corporation, and SAS® version 9.3 (TS1M1) or higher. The statistical analysis of safety and tolerability data will be performed using SAS® version 9.3 (TS1M1) or higher. The data documented in this trial and the clinical parameters measured will be analyzed using classic descriptive statistics for quantitative variables and frequencies for qualitative variables.

Analysis of primary end-point
For the evaluation of food effect, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of total plasma DF 2156Y will be compared between treatments using analysis of variance (ANOVA) for a crossover design on log-transformed data. Period, treatment, sequence and subject within sequence will be taken into account as sources of variation. The 90% confidence intervals (CI) will be calculated for the point estimates (PE, i.e. the T/R ratio of least square geometric means) of the PK parameters. Established criteria for the absence of a food effect are that the 90% CI for the T/R ratio of the geometric means of the PK parameters under consideration are within the 80.00-125.00% range

Gender effect analysis
For the purpose of exploring the gender effect on bioavailability of ladarixin and its metabolites, following logarithmic transformation, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of total plasma DF 2156Y and of DF 2108Y and DF 2227Y will be analyzed using ANOVA including sequence, period, treatment, subject within sequence, sex and sex*treatment interaction as fixed effect.

Timing:
EC meeting: 16JUN20; planned clinical phase: NOV20

STUDY SCHEDULE

ACTIVITIES	Screening		PERIOD 1			(wash-out≥14 days)	PERIOD 2				Final visit/ETV[1]
Visit	V1	V1.1	V2	V3			V4	V5	V6		
	Day -21/-2	Day -3 or -2	Day -1	Day 1	Days 2, 3, 4		Day -3 or -2	Day -1	Day 1	Days 2, 3, 4	Day 4 of period 2[2] or early termination
Informed consent	x										
Demography	x										
Lifestyle	x										
Medical history and underlying disease	x										
Physical abnormalities	x				x[3]			x			x
Previous and concomitant medications	x	x	x	x	x	x	x	x	x	x	x
Height and Body Mass Index	x										
Body Weight	x				x[3]			x			x
Laboratory analysis[4]	x										x
Virology	x										
SARS-COV2 test		x					x				
Pregnancy test	x[5]		x[6]					x[6]			x[5]
Urine drug screening[7]	x		x					x			x
Alcohol breath test	x		x					x			x
Coagulation	x										
Vital signs[10]	x		x	x[8]	x[9]			x	x[8]	x[9]	x[11]
ECG	x		x	x[8]	x[9]			x	x[8]	x[9]	x[11]
Inclusion/exclusion criteria	x		x					x			
Subject eligibility	x		x					x			
Enrolment / randomization			x								
Confinement			x	x	x			x	x	x	
Discharge					x[12]						x
Study dosing				x[13]					x[13]		
Blood samplings				x[14]	x[14]				x[14]	x[14]	
Standardized meals			x[15]	x[16]	x[17]			x[15]	x[16]	x[17]	
Adverse events monitoring[18]	x	x	x	x	x	x	x	x	x	x	x

1. *Early termination visit (ETV) in case of premature discontinuation*
2. *Final visit on day 4 of period 2 after the 72-h post-dose blood sampling*
3. *On day 4 of period 1, upon discharge*
4. *Hematology, blood chemistry and urinalysis*
5. *Serum β-HCG test (women only)*
6. *Urine pregnancy test (women only)*
7. *Multi-drug kit (cocaine, amphetamine, methamphetamine, cannabinoids [Δ-9-tetrahydrocannabinol-THC], opiates and ecstasy)*
8. *At pre-dose*
9. *At 72 h post-dose (corresponding to the final visit assessment in period 2)*
10. *Blood pressure, pulse rate, body temperature*
11. *ETV only*
12. *In the morning of day 4 of period 1*
13. *At 08:00±1 h*
14. *At pre-dose (0), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 30, 36, 48, 54, 60 and 72 h post-dose*
15. *Standardized low-fat dinner served in the evening*
16. *Subjects allocated to the fed condition (T treatment) only: a high-fat and high-caloric breakfast to be started 30 min pre-dose and completed within 30 min. Standardized lunch and dinner at approximately 13:00 (5 h post-dose on day 1) and 20:00 (12 h post-dose on day 1) in both periods*
17. *Standardized breakfast, lunch and dinner served on days 2 and 3 at approximately 09:00, 13:00 and 20:00, respectively*
18. *Adverse events monitored starting at the screening visit, immediately after informed consent, up to the final visit/ETV*

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LIST OF ABBREVIATIONS

β-HCG	Human chorionic gonadotropin β
γ-GT	γ-Glutamyl transpeptidase
ApTT	Activated partial thromboplastin time
ADR	Adverse Drug Reaction
AE	Adverse Event
ALCOAC	Attributable-Legible-Contemporaneous-Original-Accurate-Complete
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
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
BT	Body temperature
C5a	Fifth Component of Complement
CCL2	Chemokine (c-c motif) ligand 2
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
eCRF	electronic Case Report Form
CRO	Contract Research Organization
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CS	Clinically Significant
CV	Coefficient of Variation
CXCL1	C-x-c motif chemokine ligand 1
CXCL8	CXC ligand 8 [formerly interleukin (IL)-8]
CXCR1/2	CXCL8 receptors
DBP	Diastolic Blood Pressure
DF 2108Y	(2R)-2-{4-[(trifluoromethanesulfonyl)oxy]phenyl} propanoic acid
DF 2156A	R-(-)-2-[(4'-trifluoromethane sulfonyloxy)phenyl]-N-methanesulfonyl propanamide, sodium salt, also named Meraxin and Ladarixin
DF 2156Y	R-(-)-2-[(4'-trifluoromethane sulfonyloxy)phenyl]-N-methanesulfonyl propanamide
DF 2227Y	(2S)-2-{4-[(trifluoromethanesulfonyl)oxy]phenyl} propanoic acid
EC	Ethics Committee
ECG	Electrocardiogram
EMA	European Medicine Agency
ETV	Early Termination Visit
FDA	Food and Drug Administration
fMLP	N-formylmethionylleucylphenylalanine
F_{rel}	Relative Bioavailability
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GLP	Good Laboratory 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 Harmonization
IL8	Interleukin-8
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
i.v.	Intravenous
MLD	Multiple Low Dose-Streptozotocin
LSLV	Last Subject Last Visit
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
N	Normal
NC	Not calculated
NCS	Not clinically significant
NOAEL	No observed adverse effect level
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PE	Point Estimate
PDCO	Pediatric Committee
PMN	Polymorphonuclear neutrophil
p.o.	Per os
PK	Pharmacokinetics
PR	Pulse rate
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
QTcV	QT interval corrected by the heart rate
R	Reference
RBC	Red Blood Cells
SAE	Serious Adverse Event
SARS-COV2	Severe acute respiratory syndrome coronavirus
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
T	Test
T1D	Type 1 Diabetes
TEAE	Treatment-Emergent Adverse Event
THC	delta-9-tetrahydrocannabinol
TNF	Tumor Necrosis Factor
$t_{1/2}$	Half-life
t_{max}	Time to achieve C_{max}
USDA	United States Department of Agriculture
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cells
WHODDE	World Health Organization Drug Dictionary Enhanced

1 INTRODUCTION

1.1 Background

DF 2156A or ladarixin is a proprietary novel small molecule that inhibits the biological activity of interleukin-8 (IL-8), more recently renamed CXC (c-x-c motif chemokine) ligand 8 (CXCL8), through inhibition of its receptors: CXCR1 and CXCR2.
Relevant pre-clinical, toxicological and clinical data are summarized below. Please also refer to the Investigator's Brochure (1) for detailed information.

1.2 Relevant non-clinical pharmacology

1.2.1 Mechanism of action and in vitro activity

Ladarixin is a potent and specific inhibitor of the biological activity of the chemokine CXCL8. Mechanism of action studies showed that ladarixin is a non-competitive allosteric inhibitor of the CXCL8 receptors: CXCR1 and CXCR2 (2).

In vitro ladarixin, in the low nanomolar range, inhibits human polymorphonuclear neutrophil (PMN) migration induced by human CXCL8 receptors activation. Chemotaxis of rodent PMN induced by mouse and rat counterparts of human CXCL8 is also inhibited, indicating that mice and rats are appropriate animal species for preclinical studies.
Interaction of DF 2156A with CXCL8 receptors inhibits the intracellular signal transduction events activated by the binding of CXCL8 to CXCR1/2. The selectivity of DF 2156A on CXCR1 and CXCR2 is proven by its lack of efficacy against PMN migration induced by N-formylmethionylleucylphenylalanine (fMLP) or C5a or PMA and NET formation and against human monocyte chemotaxis induced by CCL2.

1.2.2 In vivo studies of ladarixin mechanism of action

In vivo, ladarixin prevents by 38 up to 80% PMN infiltration and by 35 up to 90% tissue damage in experimental models of ischemia/reperfusion injury of liver and brain in rats (3). In addition, ladarixin reduces by about 60% PMN infiltration in a murine acute model of smoke exposure, whereas in a chronic model the compound completely prevents the development of pulmonary lesions.
The efficacy of ladarixin in preventing PMN infiltration and tissue damage was investigated in a passive transfer mouse model of bullous pemphigoid. In this murine model, ladarixin showed a protective effect both on clinical disease score (90% of reduction at the higher dose 16.7 mg/kg) and on PMN recruitment (60% of reduction at the higher dose 16.7 mg/kg). The inhibition was dose-dependent. When ladarixin was administered in a therapeutic schedule of treatment (co-injected with immunogrlobulin G), an inhibitory effect comparable to that obtained when the compound was administered in a preventive regimen was observed.

The antiphlogistic activity of orally administered ladarixin was proven in the acute mouse model of cantharidin-induced ear inflammation where it reduced ear edema (16% of inhibition), cell infiltration (34% of inhibition on lymphocytes T and 32% of inhibition on PMNs) and ear tissue levels of keratinocyte chemokines (4).

In addition to the above mentioned studies, more specific studies were performed to investigate the ability of the compound to modulate the onset of diabetes mellitus type 1 (T1D) after oral administration in various animal models.

1.3 A summary of toxicology data

Ladarixin was tested for toxicity in rodent and non-rodent animal species after single or repeated dose administrations. Acute and up to 1 week administration studies were conducted either by i.v. bolus or oral route. Longer (3 month) repeated dose studies were conducted only by the oral route in rats and dogs, according to the intended human administration route.
After an orally administration of ladarixin to Wistar rats (35, 70 and 150 mg/kg/day) for a 3-month period, no mortality and no relevant changes in body weight, food consumption, ophthalmology, hematology and urinalysis were observed. Changes in biochemistry parameters were only minimal and occurred mostly in males. They included increased activity of some liver enzymes (males at 150 mg/kg), dose-dependent decrease in protein levels in males, and dose-dependent decrease in cholesterol and phospholipid concentrations in males from 70 mg/kg. After a 4-week recovery period, all values were similar to those of controls and a full recovery or a clear trend to recovery was noted for all microscopic findings.
In the 3-month toxicity study followed by a 4-week treatment-free recovery period, ladarixin was administered via daily oral capsules to Beagle dogs at initial dose levels of 0, 30, 60 and 120 mg/kg/day. However, due to test item-related morbidity of one high dose male and changes in body weight and food intake of a few other animals in this group, the highest dose level was lowered from 120 to 80 mg/kg/day from Day 24 onwards.
No test item-related changes were noted in any of the remaining parameters investigated in this study (i.e. ophthalmoscopy, coagulation parameters, urinalysis, macroscopic examination and organ weights). No observed adverse effect level (NOAEL) was considered to be 60 mg/kg/day after 3 month of dosing.

Testing for mutagenic potential (Ames test, *in vitro* chromosomal aberration test, *in vivo* micronucleus test in rats) gave negative results.

No relevant changes related to the administration of the compound at doses (20, 50 and 150 mg/kg) far above those foreseen in humans were reported on renal function and on central nervous system in male rats.

As for the *in vivo* safety pharmacology, cardiovascular safety studies were conducted in dogs in 2 studies and in one study in guinea-pigs.
Treatment with ladarixin resulted in an increase in heart rate, P amplitude and QT corrected for heart rate (QTcV) intervals and a slight decrease in magnesium blood values. Vomiting of the capsules was observed in one animal one hour after dosing. No changes in blood pressure (BP) were recorded.
The results obtained indicated that ladarixin has a safe cardiovascular profile on guinea-pig. No effect was observed on BP, heart rate and QT corrected for heart rate using the Bazzet's formula up to the dose of 10 mg/kg. Only at 30 mg/kg a transient significant reduction of diastolic blood pressure (DBP), disappearing after the first minute of injection, was observed.

Ladarixin did not affect fertility either in male and female rats or rabbits.

In rats, no maternal toxicity and no developmental toxicity were observed up to the highest dose level tested (150 mg/kg/day). The NOAEL in rats was established at 150 mg/kg/day for both the maternal and developmental toxicity.
In rabbits, no maternal toxicity was observed up to the highest dose level tested (100 mg/kg/day). The NOAEL was established at 100 mg/kg/day for both the maternal and developmental toxicity.

1.4 Previous clinical experience with ladarixin

Ladarixin was previously investigated in 3 Phase I and 2 Phase II studies. Altogether, these studies involved 195 subjects. In detail, 143 subjects were exposed to ladarixin at various doses, of whom 89 healthy during the Phase I studies, 4 suffering from bullous pemphigoid and 50 suffering from T1D in the Phase II studies 26 of whom are female patients. Details of all previous clinical studies can be found in the Investigator's Brochure (1).

1.4.1 Pharmacokinetics and metabolism

The PK evaluation of ladarixin and its metabolites was performed in 89 subjects during the 3 Phase I studies and in 4 subjects during one Phase II study.

The 1st Phase I study investigated for the 1st time the PK and the safety of ladarixin administered as single ascending doses (25, 50, 100, 200 and 400 mg) to healthy men subdivided into 8 cohorts. The parent drug DF 2156Y as well as the metabolites DF 2108Y and DF 2227Y were determined in plasma, urine and feces. Blood and urine were collected up to 168 h post-dose, while feces up to 96 h post-dose.

Afterwards, in a 2nd Phase I study, the PK of ladarixin and metabolites was investigated after multiple ascending doses (50, 100 and 200 mg twice daily) for 5 days of treatment in 3 cohorts of 12 subjects each, of whom 9 received ladarixin and 3 the matching placebo. A 4th cohort of 12 subjects was recruited to investigate the drug-drug interaction of ladarixin with CYP2C9 using tolbutamide as probe drug.

The 3rd Phase I study investigated the PK of ladarixin and metabolites after single dose and at steady state, administering multiple doses (300 and 400 mg once daily) for 7 days to 2 cohorts.

1.4.2 Phase I safety, tolerability and bioavailability single ascending dose study in healthy men

Plasma, urine and faces samples were collected during a double-blind, randomized, placebo-controlled, ascending dose study to evaluate the tolerability and preliminary PK of ladarixin and its metabolites (DF 2108Y and 2227Y) after oral administration to male healthy volunteers. Five (5) dose levels were tested (25, 50, 100, 200 and 400 mg) and administered to cohorts of 8 subjects of whom 6 received ladarixin and 2 the matching placebo. Analytical determinations of the parent compound and metabolites in plasma, urine, and faces were performed by a liquid chromatography mass spectrometry (LC-MS/MS) validated method. The lower limit of quantification (LQL) in plasma was 0.1 µg/mL for DF 2156Y and DF

2108Y, respectively, and 0.05 µg/mL for DF 2227Y. In urine and faces, the LQL was 0.1 µg/mL and 0.05 µg/mL, respectively, for the 3 analytes.
Plasma samples were collected at pre-dose and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24, 36, 48, 60, 72, 84, 96, 120, 144 and 168 h post-dose. Urine samples were collected in the following time periods: 0-12, 12-24, 24-36, 36-48, and during subsequent intervals of 24 up to 168 h post-dose. Faces samples were collected in the intervals 0-24, 24-48, 48-72 and 72-96 h post-dose.

Main PK parameters of ladarixin and its metabolites measured and calculated after single dose of 400 mg of ladarixin are presented in the following table.

Table 1.4.2.1 PK parameters of ladarixin and its metabolites measured and calculated after single dose of 400 mg of ladarixin to healthy men (N=6)

Variable (Unit)	DF 2156Y (N=6)	DF 2108Y (N=6)	DF 2227Y (N=6)
C_{max} (µg/mL)	54.86±7.41	0.938±0.104	0.692±0.116
t_{max} (h)	1.75±1.12	14.92±772	45.98±15.91
AUC_{0-t} (µg/mLxh)	912.40±155.62	49.58±10.07	51.93±9.40
$AUC_{0-\infty}$ (µg/mLxh)	921.54±159.99	50.36±10.61	NC
$t_{1/2}$ (h)	13.62±2.66	18.32±2.65	NC
V_z/F (L)	8.19±1.84	ND	ND
Cl/F (L/h)	0.42±0.07	ND	ND
Ae_{0-t} (% of dose)	79.67±9.99	2.00±0.45	1.08±0.33
Cl_R (L/h)	0.34±0.05	0.131±0.03	0.068±0.023
$Ae_{Feces0-t}$ (µg)	120.76±52.31	0.0	0.0

mean±SD are reported; NC: not calculated; ND: not determined

1.4.3 Phase I safety, tolerability and bioavailability single and multiple dose study in healthy men

Twenty-four (24) men were randomized into 2 cohorts including 12 subjects (9 subjects received ladarixin and 3 subjects received placebo). Ladarixin was administered at the dose of 300 and 400 mg for 7 days. The subjects received a single dose on days 1 and 8 and twice daily every 12 h on days 3 to 7. Plasma samples were collected as follows:
Day 1: pre-dose and 0.5, 1, 1. 5, 2, 3, 4, 5, 6, 8, 10, 12, 24 h post dosing
Day 3: Day 4, Day 5, Day 6: before the first and the second daily dosing
Day 7: before and 1h after the first and the second daily dose
Day 8: pre-dose and 0.5, 1, 1. 5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60 and 72 h post dosing

Main PK parameters of ladarixin and its metabolites measured and calculated after single dose of 400 mg of ladarixin are presented in the following table.

Table 1.4.3.1 PK parameters of ladarixin and its metabolites measured and calculated after single dose of 400 mg of ladarixin to healthy men (N=9)

Variable (Unit)	DF 2156Y (N=9)	DF 2108Y (N=9)	DF 2227Y (N=9)
C_{max} (µg/mL)	81.10±13.99	0.82±0.19	0.40±0.08
t_{max} (h)	0.52 (0.50 – 1.52)	10 (8 – 12)	8.02 (5.02 – 12)
AUC_{0-48} (µg/mLxh)	977.5±152.3	32.46±5.18	19.60±3.61
$AUC_{0-\infty}$ (µg/mLxh)	1063.2±188.0	NC	NC
$t_{1/2}$ (h)	13.55±2.21	NC	NC
V_z/F (L)	7.03±0.926	ND	ND
Cl/F (L/h)	0.37±0.0734	ND	ND

mean±SD are reported except for t_{max} for which mean, minimum and maximum are reported; NC: not calculated; ND: not determined

1.5 Rationale

Considering the positive tolerability results obtained at all doses studied in the previous 3 Phase I studies and the results obtained in 2 completed randomized Phase II studies, the main aim of this study is to assess the food effect on the bioavailability of ladarixin after single dose of 400 mg of the test capsule formulation when administered under fed and fasting conditions to healthy subjects.
Since the PK profile of ladarixin in previous Phase I and II studies were evaluated only in men, the study will include healthy women in order to evaluate the PK of ladarixin and metabolites in females for the 1st time.

The study is designed according to the EMA guidelines on the investigation of bioavailability and bioequivalence (8) and on the investigation of drug interactions (9).

1.6 Risks and benefits

Ladarixin was generally well tolerated at all doses studied as derived from Phase I clinical studies. Similarly, in the Phase II clinical studies, ladarixin was generally well tolerated with only mild adverse events (AEs). Nevertheless, the limited sample size prevents any overall conclusions on the safety of the investigational medicinal product (IMP).
Overall, 95 adverse drug reactions (ADRs) were reported in a total of 48 subjects out of 143 exposed to ladarixin in all the previous clinical studies. Most frequent ADRs concerned the following System Organ Class (SOC) terms:
Gastrointestinal disorders: occurred at a frequency of about 52% and including, among most frequent ADRs: dyspepsia, dysphagia, abdominal pain, constipation, diarrhea and nausea.
Nervous System Disorders: occurred at a frequency of about 26% and including, among most frequent ADRs: headache and dizziness.

Dyspepsia and dysphagia were both considered as definitely related to ladarixin, because they occurred shortly after administration. Also dyspepsia consistently recurred after multiple drug administrations.

On the basis of these safety results, no particular safety risks are expected for healthy subjects taking part in the present study at the planned dose of 400 mg of ladarixin, which will be administered twice to each volunteer, once in each study period.

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

2.1 Objectives

2.1.1 Primary objective

Primary objective of the study is to investigate the effect of food on the bioavailability of DF 2156Y after single dose administration of 400 mg of ladarixin to healthy male and female volunteers under fed and fasting conditions.

2.1.2 Secondary objectives

- to investigate the effect of gender on the bioavailability of DF 2156Y and its metabolites (DF 2108Y and DF 2227Y) after single dose administration of 400 mg of ladarixin to healthy male and female volunteers;
- To evaluate safety and tolerability of a single dose administration of 400 mg ladarixin to healthy male and female volunteers.

2.2 Endpoints

2.2.1 Primary end-point

- to evaluate and compare the rate (C_{max}) and extent ($AUC_{0-t;}$ $AUC_{0-\infty}$) of absorption of DF 2156Y after single dose administration of 400 mg of ladarixin under fed and fasting conditions.

2.2.2 Secondary end-points

- to evaluate and compare between genders the rate (C_{max}) and extent (AUC_{0-t}; $AUC_{0-\infty}$) of absorption of DF 2156Y and its metabolites (DF 2108Y and DF 2227Y) after single dose administration of 400 mg of ladarixin to healthy male and female volunteers;
- to describe the pharmacokinetic profile of DF 2156Y (total and unbound) and its metabolites (DF 2108Y and DF 2227Y) after single dose administration of 400 mg of ladarixin under fed and fasting conditions;
- to collect safety and tolerability data of a single oral dose administration of 400 mg of ladarixin.

3 CLINICAL SUPPLIES

3.1 Treatment

3.1.1 Description of product

The analytical certificate will be supplied with the investigational medicinal product (IMP).

3.1.1.1 Test product

IMP	Ladarixin 200 mg hard gelatin capsules
Distributor	Dompé farmaceutici S.p.A., Italy
Manufacturer (active substance)	AMSA S.p.A, Como, Italy
Manufacturer (finished product)	Depo Pack s.n.c., Saronno (Va), Italy
Pharmaceutical form	Hard gelatin capsules
Dose	400 mg (two 200 mg capsules)
Administration route	Oral

3.1.2 Dose regimen

A single oral dose of 400 mg of ladarixin (two 200-mg capsules) will be administered to healthy male and female volunteers under fed (T treatment) and fasting (R treatment) conditions in 2 study periods, according to a two-way crossover design, with a wash-out interval of at least 14 days between the two administrations.

3.1.3 Route and method of administration

The IMP will be orally administered on day 1 of each period, at 8:00 ± 1 h.
In detail, at each dosing, 2 capsules will be swallowed with 240 mL of still mineral water, without chewing.

The subjects allocated to the fed conditions will receive the IMP 30 min after having started to eat a high-fat and high-caloric breakfast (§ 6.2.1), while the subjects allocated to the fasting conditions will receive the IMP under prolonged fasting conditions (at least 10 h pre-dose).

The investigator will check that all subjects take the IMP appropriately.

3.1.4 Investigational product distribution

The IMP will be administered by the investigator or by his/her deputy at the Phase I Unit. 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 labeling

The Phase I Unit will be provided with 36 subject's individual boxes (subject kits). Each subject's kit will contain 2 boxes (one for each study period), each containing 2 blisters of 1 200-mg capsule of ladarixin.

The sponsor will provide a reserve kit for each subject to be used if needed. Subject kits will be numbered from 001 up to 036. Reserve subject kits will be numbered as 001R-036R.

The formulation labeling 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; 10) as follows:

a. Name, address and telephone number of the Sponsor, contract research organization or investigator (the main contact for information on the product and clinical study)
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 and where relevant, the 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
k. "Keep out of reach of children"

Labels will be in English language.

3.3 Storage conditions

The IMP will be stored at < 30°C in a dry locked place, sheltered from light.

3.4 Drug accountability

The IMP will be provided as individual subject's kits directly to the investigator by the sponsor, in excess of the amount necessary for the study (100% excess).

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

At the end of the study, used, unused and partially used supplies of the IMP provided by the Sponsor will either be destroyed on site (upon written authorization) or returned to the Sponsor after assessment of drug accountability.
In the event that the IMP will be destroyed on site, a destruction certificate will be provided to the sponsor.

4 INVESTIGATIONAL PLAN

4.1 Overall study design

Single center, single dose, open label, randomized, two-way, crossover, food effect study.

4.2 Discussion of design

The study has been designed in agreement with the EMA Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr., 20 January 2010) (8) and FDA guidance on the Assessing the Effects of Food on Drugs in INDs and NDAs (14).

The sample size was not calculated through any statistical calculation. The planned sample size is estimated as sufficient for the descriptive purposes of the study in compliance with the relevant EMA guideline for PK studies (8).

Each randomized subject will be allocated to a sequence of treatments (fasting (R) or fed (T)) in the two study periods (TR or RT) according to a computer generated randomization list (see § 8.1).

The dose of 400 mg planned for the present study was selected because proved to be well tolerated in the previous Phase I and II clinical studies (1).

The EMA Guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1. Corr.2, 21 June 2012) (9) has been taken into account for the study administration of the IMP in fed conditions.

The wash-out interval of 14 days is considered appropriate according to the known half-life of ladarixin (§ 1.4).

The open-label design was chosen since the study endpoints are based on the objective measurement of ladarixin and its active metabolites in plasma. The outcome variables are not influenced by the subjects or investigator being aware of the administered treatment.

5 STUDY POPULATION

5.1 Target population

The study population will include healthy volunteers (men and women), aged 18-55 years inclusive.

5.2 Inclusion criteria

To be enrolled in this study, subjects must fulfill 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. *Body Mass Index (BMI)*: 18.5-30 kg/m^2 inclusive
4. *Vital signs*: systolic blood pressure (SBP) 100-139 mmHg, diastolic blood pressure (DBP) 50-89 mmHg, pulse rate (PR) 50-90 bpm and body temperature (BT) 35.5-37.5° C, measured after 5 min at rest in the sitting position
5. *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
6. *Contraception and fertility (women only)*: women of child-bearing potential must not wish to get pregnant within 30 days after the end of the study and must be using at least one of the following reliable methods of contraception:
   a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 2 months before the screening visit until 30 days after final visit
   b. 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 until 30 days after final visit
   c. A male sexual partner who agrees to use a male condom with spermicide until 30 days after final visit
   d. A sterile sexual partner

   Women participants of non-childbearing potential or in post-menopausal status for at least one year will be admitted.

   For all women, pregnancy test result must be negative at screening and day -1.

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
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. *Allergy:* ascertained or presumptive hypersensitivity to the active principles (ladarixin or derivatives) and/or formulations' ingredients; known hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs); history of hypersensitivity to drugs (in particular methanesulfonyl propanamide) or allergic reactions in general, which the Investigator considers may affect the outcome of the study
5. *Diseases*: hypoalbuminemia or significant history of renal, hepatic, gastrointestinal, respiratory, skin, hematological, endocrine, neurological or cardiovascular diseases that may interfere with the aim of the study
6. *Medications*: medications, including over the counter drugs (in particular NSAIDs), herbal remedies and food supplements taken 14 days before the start of the study (in any case at least 5 times the half-life of the drug or a minimum of 14 days, whichever is longer), with the exception of paracetamol. Hormonal contraceptives and hormonal replacement therapy for women will be allowed
7. *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
8. *Blood donation:* blood donations for 3 months before this study
9. *Drug, alcohol, caffeine, tobacco:* history of drug, alcohol (>1 drink/day for women and >2 drinks/day for men, defined according to the USDA Dietary Guidelines 2015-2020 (11)], caffeine (>5 cups coffee/tea/day) or tobacco abuse (≥10 cigarettes/day)
10. *SARS-COV2 test:* positive SARS-COV2 test on day -3 or -2 of each study period
11. Positive to one of these test: Hepatitis B (HBs antigen), Hepatitis C (HCV antibodies), HIV 1/2 (HIV Ag/Ab combo) at screening.
12. *Drug test:* positive result at the drug test at screening or day -1 of each study period
13. *Alcohol test:* positive alcohol breath test at screening or day -1 of each study period
14. *Diet:* abnormal diets (<1600 or >3500 kcal/day) or substantial changes in eating habits in the 4 weeks before this study; vegetarians; vegans
15. *Pregnancy (women only)*: positive or missing pregnancy test at screening or day -1 of each period, pregnant or lactating women

5.3.1 Not allowed treatments

No medication, including over the counter drugs (in particular NSAIDs), herbal remedies and food supplements, will be allowed for 14 days before the start of the study (in any case at least 5 times the half-life of the drug or a minimum of 14 days, whichever is longer), and during the whole study duration. Paracetamol will be allowed as therapeutic counter-measure (maximum 2 g per day) for AEs according to the investigator's opinion. Hormonal contraceptives and hormonal replacement therapy for women will be allowed.

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 summarized at page 11.

6.1 Study visits and procedures

Each study subject will undergo 8 visits.

The study protocol foresees 2 periods separated by a wash-out interval of at least 14 days. Minimum study duration will be 20 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.

The following phases, visits and procedures will be performed:

- **Screening phase**
  - Screening – visit 1: between day -21 and day -2
  - Screening – visit 1.1: day -3 or day -2
  - Period 1 – visit 2: day -1

- **Interventional phase**
  - Period 1 – visit 3: days 1-4
  - Wash-out interval of at least 14 days
  - Period 2 – visit 4: day -3 or day -2
  - Period 2 – visit 5: day -1
  - Period 2 – visit 6: days 1-4

- **Final phase**
  - Final visit/early termination visit (ETV). In case of early discontinuation, discontinued subjects will undergo an ETV.

	Day	Procedures/Assessments	Notes
Screening – Visit 1	*From day -21 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, physical abnormalities) ➢ Vital signs measurement including blood pressure (BP), pulse rate (PR) and body temperature (BT) ➢ ECG recording ➢ Laboratory analyses: hematology, blood chemistry, urinalysis, serum virology and serum pregnancy test (women only) ➢ Alcohol breath test ➢ 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.
Screening – Visit 1.1	*Day -3 or -2*	➢ Nasal and pharyngeal swab for SARS-COV2 test ➢ Adverse events monitoring ➢ Previous/concomitant medications	
Period 1 - Visit 2	*Day -1*	➢ Alcohol breath test ➢ Urine pregnancy test (women only) ➢ Urine multi-drug kit test ➢ ECG recording ➢ Vital signs measurement ➢ AE and concomitant medications ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Enrolment and randomization	Arrival at the Phase I Unit in the evening. Confinement until the morning of day 4. Standardized low-fat dinner. Fasting for at least 10 h (overnight).

	Day	Procedures/Assessments	Notes
Period 1 - Visit 3	*Day 1*	➢ Investigational medicinal product administration at 8:00 ± 1 h ➢ Vital signs measurement at pre-dose ➢ ECG recording at pre-dose ➢ Blood sample collection for PK analysis at pre-dose (0) and 0.25 (15 min), 0.5 (30 min), 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 18 h post-dose ➢ AE and concomitant medications	Standardized high-fat and high caloric breakfast starting at 30 min pre-dose for subjects receiving the investigational medicinal product under fed conditions in this study period. Breakfast must be completed within 30 min. Subjects receiving the investigational medicinal product under fasting conditions will not have breakfast. Standardized lunch and dinner at about 13:00 (5 h post-dose) and 20:00 (12 h post-dose), respectively
	Days 2 and 3	➢ Blood sample collection for PK analysis at 24, 30, 36, 48, 54 and 60 h post-dose ➢ AE and concomitant medications	Standardized breakfast, lunch and dinner at about 9:00, 13:00 and 20:00, respectively
	Day 4	➢ Vital signs measurement upon discharge 72 h post-dose ➢ ECG recording upon discharge 72 h post-dose ➢ Blood sample collection for PK analysis at 72 h post-dose ➢ AE and concomitant medications ➢ Full physical examination (body weight and physical abnormalities) upon discharge	Discharge from the Phase I Unit in the morning, after the 72-h post-dose blood sample collection, ECG recording, vital signs check and full physical examination. Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any adverse reactions.
Wash-out	*At least 14 days*	A wash-out interval of at least 14 days between the two administrations of the two study periods	
Period 2 - Visit 4	*Day -3 or -2*	*As visit 1.1, day -3 or -2*	

	Day	Procedures/Assessments	Notes
Period 2 - Visit 5	*Day -1*	As visit 2, excluding enrolment and randomization. In addition full physical examination (body weight and physical abnormalities) will be performed.	As visit 2
Period 2 - Visit 6	*Days 1-4*	As visit 3. IMP administered according to the randomization list and crossover design	As visit 3
Final Visit/ETV	*Day 4 of period 2 /at ETV in case of discontinuation*	➢ Full physical examination (body weight and physical abnormalities; also vital signs and ECG in case of ETV) ➢ Alcohol breath test ➢ Urine multi-drug kit test ➢ Laboratory analyses as at screening, with the exception of virology, coagulation, microbiology, albumin and globulin ➢ AEs and concomitant medications In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalization 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

On day -1 of each period, a standardized low-fat dinner will be served after confinement. On day 1 of each study period, all the subjects will not take any food or drinks (except water) for at least 10 h (i.e. overnight). The subjects allocated to the fed conditions, after the overnight fasting period, will receive a high-fat and high-caloric breakfast (see § 6.2.1) starting 30 min pre-dose and will complete their breakfast within 30 min, while the subjects allocated to the fasting conditions will fast overnight and then receive their treatment. Water will be allowed as desired, except for 1 h before and 1 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, all subjects will then remain fasted until 5 h post-dose. A standardized lunch and dinner will be served at approximately 5 h and 12 h post-dose (at approximately 13:00 and 20:00).
On days 2 and 3, standardized breakfast, lunch and dinner will be served to all subjects at about 9:00, 13:00 and 20:00, respectively.

One cup of coffee or tea will be allowed after each meal only; any other coffee, tea or food containing xanthines (i.e. coke, chocolate, etc.), alcohol and grapefruit will be forbidden

during confinement. In particular, grapefruit and alcohol will be forbidden for 24 h before the first IMP administration until the end of the study.

The subjects will be allowed to smoke 9 cigarettes during confinement, one after each meal, with the exclusion of the high-fat and high-caloric breakfast.

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

6.2.1 Standardized high-fat and high-caloric breakfast

As mentioned above, on day 1 of each study period after blood sample collection at pre-dose (0), subjects randomized to the Test (T) treatment will start to eat a high-fat and high-caloric breakfast 30 min pre-dose and will be instructed to complete their meal within 30 min.

Breakfast caloric content will be approximately 1000 kilocalories (kcal) and fat content will be approximately 60% of the total caloric content. The standardized breakfast will provide approximately 15%, 25% and 60% of the calories from proteins, carbohydrates and fats, respectively, and is detailed in the table below:

Table 6.2.1.1 High-caloric and high-fat breakfast composition

Food	Amount (g)	Fats (g)	Carbohydrates (g)	Proteins (g)	kcal
Whole milk	250	8	12	8	160
Two fried eggs	140	14	1	13	180
Butter	30	25	0	0	227
Two strips of bacon	50	12	0	8	138
Two slices of toast	50	0	35	4	150
Olive oil white bread	40	2	20	4	100

6.2.2 Restrictions

During each study period, the subjects will be confined from the evening preceding the IMP administration (study day -1) until the morning of day 4.

For the 4 h following the 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:

- screening visit
- on day 4 of period 1 (before discharge)
- on day -1 of period 2
- final visit/ETV.

Body weight will be recorded each time. 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]).
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 electronic case report forms (eCRF).

7.1.1 Vital signs

Subjects' blood pressure (BP), pulse rate (PR) and tympanic body temperature (BT) will be measured by the investigator or his/her deputy after 5 min at rest in sitting position at:

- screening visit
- day -1
- day 1 at pre-dose (0)
- day 4 at 72 h post-dose (in period 2, it will be considered as the final visit measurement)
- ETV, if applicable

7.1.2 ECGs

12-Lead ECGs will be performed in supine position a:

- screening visit
- day -1
- day 1 at pre-dose (0)
- day 4 at 72 h post-dose (in period 2, it will be considered as the final visit recording)
- ETV, if applicable

Date/time of the ECG recording, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and all abnormalities found (if any) will be reported in the individual eCRF.

7.1.3 Clinical laboratory assays

Samples of blood (17 mL) and urine will be collected. The following laboratory analyses will be performed at the screening visit at the clinical laboratory (§ 16.5):

Hematology
Leukocytes and leukocyte differential count (percentage values and absolute values), erythrocytes, hemoglobin (conv. units), hemoglobin (IS units), hematocrit, MCV, MCH, MCHC, thrombocytes,

Coagulation
Prothrombin time, aPTT

Blood chemistry
Electrolytes: sodium, potassium, calcium, chloride, inorganic phosphorus
Enzymes: alkaline phosphatase, γ-GT, AST, ALT
Substrates/metabolites: total bilirubin, creatinine, fasting glucose, urea, uric acid, total cholesterol, triglycerides
Proteins: total proteins, albumin, globulin (calculated by the laboratory by detracting albumin from total proteins)

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

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

Microbiological analysis
Coronavirus SARS-COV-2 (nasopharyngeal swab) will be performed at the laboratory (§ 16.5) on day -3 or -2 of the visit 1.1 (screening visit) and on day -3 or -2 of period 2

A urine drug test will be performed at the Phase I Unit at screening, day -1 of each study period and final visit/ETV using a urine multi-drug kit. The following drugs will be assessed: cocaine, amphetamine, methamphetamine, cannabinoids (delta-9-tetrahydrocannabinol - THC), opiates and ecstasy.

A serum pregnancy test will be performed at the laboratory (§ 16.5) at screening and final visit/ETV. 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 coagulation, microbiology, albumin, globulin and virology will be performed at the final visit/ETV.

Date/time of samples collection, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and all abnormalities found (if any) will be reported in the individual eCRF. All clinically significant abnormalities after the screening visit will be recorded as AEs.

7.2 Sampling for pharmacokinetic analysis

7.2.1 Venous blood sampling

Venous blood samples (8 mL) will be collected from a forearm vein at the following times:

- pre-dose (0), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 30, 36, 48, 54, 60 and 72 h post-dose

Actual sampling times for each subject will be recorded in the individual electronic case report forms (eCRF). The actual sampling times should not exceed the recommended tolerance ranges presented in the table below. 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 Set.

Table 7.2.1.1 Tolerance ranges for the scheduled sampling times

Sampling time	Tolerance range
Pre-dose (0)	Within 40 min before IMP administration
0.25 h (15 min)	0 min
0.5 h (30 min)	± 1 min
1, 1.5 h	± 3 min
2, 3, 4, 5 h	± 5 min
6, 8, 10, 12, 18, 24, 30 h	± 10 min
36, 48, 54, 60, 72 h	± 30 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 2 mL of blood will be discarded at each collection time to avoid contamination of the sample with heparin.

The remaining 6 mL will be collected from the catheter and transferred with a syringe into heparinized tubes (Li-heparin).

The samples will be stored on ice for a maximum of 15 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 (500 µL), P2 (500 µL) and P3 (the remaining amount), in pre-labeled polypropylene tubes, and stored frozen at ≤-20° C until shipment to the analytical laboratory.

If any clinical assessment, such as vital signs measurement or ECG recording, 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 and ECG parameters can be influenced by the blood sampling. Therefore, these assessments 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 and ECG recordings will be performed for safety reasons only, deviations from the planned time schedule will be considered not relevant.

7.2.2 Analytics

The concentration of DF 2156Y (total and unbound) and its metabolites (DF 2108Y and DF 2227Y) in plasma will be determined at Dompé farmaceutici S.p.A. Bioanalytical Laboratories, Italy, using a fully validated HPLC-UV method.

The concentrations of unbound DF2156Y will be determined initially only at the following time points: 1, 3, 6, 12, 24 h post dose: additional analysis could be performed at different time points only if necessary.

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 clinical study report (CSR).

A total of 1512 plasma samples (756 samples for each study period) will be analyzed.

7.2.3 Labeling, storage and transport of samples

7.2.3.1 Samples labeling

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

Study code	Study CRO-PK-20-345 - Sponsor code LDX0219
Subject number	001-036
Tube identification	P1/P2/P3
Period	1/2
Scheduled sampling time	as h; see § 7.2.1.

7.2.3.2 Samples storage and transport

At the Phase I Unit, the samples will be stored at ≤-20° C. At the end of each collection day, aliquots P1 will be stored in a freezer, separated from the other aliquots.

All aliquots P1, packed in sufficient solid CO_2, will be shipped by an authorized courier from CROSS Research S.A., Switzerland, to Dompé farmaceutici S.p.A. Bioanalytical Laboratories, Italy.

After receipt by the analytical laboratory of the first aliquots (P1) in good conditions, aliquots P2 will be shipped to the laboratory, applying the same procedures described for the shipment of aliquots P1.

All aliquots 1 and 2 will remain stored at the analytical laboratory (Dompé farmaceutici S.p.A. Bioanalytical Laboratories, Italy) at least until finalization of the CSR. Afterwards, the samples will be destroyed after authorization by the Sponsor's Project Manager and a confirmation of the disposal will be provided by email.

All aliquots P3 will remain stored (≤-20° C) at CROSS Research S.A., Switzerland. These samples could either be:

- sent to the laboratory for reanalysis if necessary for analytical reasons or if any problems occur during the delivery of aliquots P1 and/or P2, or
- destroyed at an authorised site, 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 Ethics Committee is obtained. The subjects may ask to destroy their own samples at any time.

7.3 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 coagulation:

Screening visit: 4.5 mL

for PK analysis:

Treatment T: 21 samples x 8 mL = 168 mL
Treatment R: 21 samples x 8 mL = 168 mL

In total 365.5 mL of blood (not exceeding a normal blood donation) will be withdrawn from each subject.

8 ASSIGNMENT OF STUDY TREATMENT

8.1 Randomization

The randomization list will be computer-generated by the Biometry Unit of the contract research organization (CRO), using the PLAN procedure of SAS® version 9.3 (TS1M1) (12) or higher (the actual version will be stated in the final CSR). The randomization list will be supplied to the study site before study start and will be attached to the final CSR.
Randomization will be stratified by sex in order to have the same number of men and women for every sequence of treatments.

8.2 Treatment allocation

Subjects will be assigned to one sequence of treatments (e.g. TR or RT) i.e. to receive the IMP in fed conditions (T treatment) during period 1 and in fasting conditions (R treatment) in period 2 or vice versa according to their randomization number.
Randomization number will be given to the subjects on study Day -1 of period 1 and will be used to assign the treatment sequence.

8.3 Blinding

This is an open-label trial. No masking procedure will be applied since an open-label design was considered adequate for evaluating objective measures such as pharmacokinetic parameters. All the personnel involved in the analytical determinations of ladarixin and its metabolites in plasma samples collected from the volunteers will be maintained in blind conditions.

9 EVALUATION PARAMETERS

9.1 Study variables

9.1.1 Primary variables

C_{max} and AUC_{0-t} of plasma DF 2156Y after single dose administration of 400 mg of ladarixin under fed and fasting conditions

9.1.2 Secondary variables

- $AUC_{0-\infty}$, t_{max}, $t_{1/2}$, λ_z and F_{rel} of plasma DF 2156Y after single dose administration of 400 mg of ladarixin under fed and fasting conditions
- C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, t_{max}, $t_{1/2}$, λ_z and F_{rel} of plasma DF 2156Y and its metabolites (DF 2108Y and DF 2227Y) after single dose of 400 mg of ladarixin under fed and fasting conditions measured and calculated in healthy men and healthy women
- Treatment-emergent adverse events (TEAEs), vital signs (BP, PR, BT), body weight, ECGs, physical examinations, laboratory parameters (hematology, blood chemistry and urine analysis)

9.2 Pharmacokinetic assessments

9.2.1 Pharmacokinetic parameters

The following PK parameters will be measured and/or calculated for DF 2156Y and its metabolites, DF 2108Y and DF 2227Y, using the validated software Phoenix WinNonlin® version 6.3 (13) 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 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 $100\times[(C_t/\lambda_z)/AUC_{0-\infty}]$

F_{rel}: Relative bioavailability, calculated as ratio AUC_{0-t} (T)/ AUC_{0-t} (R)

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 $R^2 > 0.8$. 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, routine hematology, blood chemistry, urinalysis laboratory tests and ECGs.

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, standard deviation (SD), coefficient of variation (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) (12) or higher (the actual version will be stated in the final report).
The statistical analysis of PK parameters will be performed using Phoenix WinNonlin® version 6.3 (13) 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 randomized allocation to one treatment sequence.

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

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

In the present study, randomized subjects will be the same as enrolled subjects.

- 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 study treatments. This analysis set will be used for the safety and tolerability analyses
- PK set 1: all subjects randomized who fulfill the study protocol requirements in terms of investigational treatment intake and have evaluable PK data readouts for DF 2156Y with no major deviations that may affect the PK results. This analysis set will be used for the statistical analysis of the PK results for DF 2156Y.
- PK set 2: all subjects randomized who fulfill the study protocol requirements in terms of investigational treatment intake and have evaluable PK data readouts for DF 2108Y and DF 2227Y, with no major deviations that may affect the PK results. This analysis set will be used for the statistical analysis of the PK results for DF 2108Y and DF 2227Y.

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

10.1.2 Reasons for exclusion from the PK sets

Reasons for the exclusion of subjects from the PK sets before the bioanalysis are the following:

- vomiting and diarrhea before or 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 eCRF as the study is being conducted.

10.2 Sample size and power considerations

The sample size was not calculated through any statistical calculation. A sample size of 36 subjects (18 men and 18 women) was estimated as sufficient for the descriptive purposes of the present study in compliance with the relevant EMA guideline for PK studies (8).

Drop-out subjects will not be replaced.

10.3 Demographic, baseline and background characteristics

10.3.1 Demography

Demographic characteristics will be listed and examined according to qualitative or quantitative data. Qualitative data will be summarized in contingency tables. Quantitative data will be summarized using classic descriptive statistics.

10.4 Analysis of pharmacokinetic parameters

10.4.1 Descriptive pharmacokinetics

A descriptive PK will be presented for plasma total and unbound DF 2156Y and its metabolites DF 2108Y and DF 2227Y. The results will be displayed and summarized in tables and figures. Individual and mean curves (+SD at sampling times), indicating inter-subject variability, will be plotted. Mean curves will be presented by treatment and by treatment and sex.

PK parameters of total plasma DF 2156Y and its metabolites DF 2108Y and DF 2227Y will be summarized by treatment and by treatment and sex.

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 NC. If for an individual PK curve, a log-linear regression with a correlation coefficient $R^2 > 0.8$ cannot be obtained, the extrapolated PK parameters will be reported as NC and considered missing in the calculations of descriptive statistics.

10.4.2 Statistical comparison of pharmacokinetic parameters

For the primary end-point evaluation of food effect, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of total plasma DF 2156Y will be compared between fed and fasting conditions (T vs. R) using analysis of variance (ANOVA) for a cross-over design on log-transformed data for evaluation of the food effect. Period, treatment, sequence and subject within sequence will be taken in account as sources of variation and they will be treated as fixed effect.

The 90% confidence intervals (CI) will be calculated for the point estimates (PE, i.e. the T/R ratio of least square geometric means) of the PK parameters.

Established criteria for the absence of a food effect are that the 90% CI for the T/R ratio of the geometric means of the PK parameters under consideration are within the 80.00-125.00% range.

For the purpose of exploring the gender effect on bioavailability of ladarixin and its metabolites, following logarithmic transformation, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of total plasma DF 2156Y and of DF 2108Y and DF 2227Y will be analyzed using ANOVA including sequence, period, treatment, subject within sequence, sex and sex*treatment interaction as fixed effect.

10.5 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 after informed consent signature by the enrolled subject but before the first dose of IMP and not negatively affected by the first dose of IMP;
- TEAEs: all AEs occurring or worsening after the administration of 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 summarized 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 all abnormalities found (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 summarized using tables of frequency.

- **Vital signs**

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

- **Body weight**

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

- **ECG**

Date/time of ECG recording, overall investigator's interpretation (as normal or abnormal and, if abnormal, clinically significant or not clinically significant) and all abnormalities found (if any) will be listed. The overall investigator's interpretation will be summarized using tables of frequency

11 DEFINITION AND HANDLING OF AEs AND SAEs

11.1 Applicable SOPs

AEs definition, classification and management will follow the Sponsor SOPs, based upon applicable local and international regulations. CRO shall submit SUSARs according to local law.

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

11.2 Definitions

11.2.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

11.2.2 Adverse Drug Reaction

An Adverse Drug Reaction (ADR) is defined as an adverse experience which is a reasonably likely to have been caused by the drug. Adverse events are to be considered unsuspected if the relationship to the study drug as described in the table in section 11.5.2 is none or unlikely; whereas any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR. The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

11.2.3 Serious Adverse Event (SAE) Definition

A serious adverse event (SAE) is defined as any untoward medical occurrence 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. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization

  (NOTE: In general, hospitalization means that the individual remained at the hospital or emergency ward for observation and/or treatment (usually involving an overnight stay) that would not have been appropriate in the physician's office or an out-subject setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious.

When in doubt as to whether "hospitalization" occurred, the event should be considered serious)

- results in persistent or significant disability/incapacity

  (NOTE: This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption)

- is a congenital anomaly/birth defect
- is medically significant or important medical condition, i.e. an important medical event that based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

  (NOTE: An important medical condition is an event that may not result in death, be life-threatening, or require hospitalization but may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the subject's wellbeing and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse

11.3 Unexpected Adverse Reaction

An ADR is considered unexpected if it is not listed in the Investigator Brochure (Reference Safety Information section). An event is unexpected also when it is not listed at the specificity or severity that has been observed and listed in the Investigator Brochure. Events that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation are considered unexpected.

The determination of expectedness shall be made on the basis of the IB Reference Safety Information (RSI) section.

11.3.1 Reference Safety Information (RSI)

As reported in the Reference Safety Information section of the Investigator's Brochure, considering the development phase of the compound and the low number of total subjects exposed to ladarixin, adverse events/reactions (notwithstanding seriousness) should be considered unexpected at this stage.

11.3.2 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is defined as an ADR that is both unexpected (not consistent with the RSI) and also meets the definition of a SAE.

11.4 Monitoring for Adverse Events

At each visit following study informed consent form signature, after the subject has had the opportunity to spontaneously mention any problems, the Investigator or appropriate designee should inquire about AEs by asking the standard questions:

- "Have you had any health problems since your last study visit?"
- "Have there been any changes in the medicines you take since your last study visit?"

AEs should be reported for any clinically relevant change in concomitant condition(s) that is the result of an untoward (unfavorable and unintended) change in a subject's medical health. Changes in any protocol-specific systemic parameter evaluated during the study are to be reviewed by the Investigator. Any untoward (unfavorable and unintended) change in a protocol-specific parameter that is clinically relevant is to be reported as an AE. These clinically relevant changes will be reported regardless of causality.

11.5 AEs recording

AEs will be collected and recorded for any untoward event that occurs in a subject from the time he or she signs the ICF for the trial until last follow-up visit/ETV. Thus, any untoward medical occurrences or unfavorable and unintended signs, symptoms, or diseases that occur in the pre-treatment [pre-treatment AE (PTAE)], in treatment or post-treatment [treatment-emergent AE (TEAE)] period are to be considered AEs and/or SAEs, and consequently recorded and reported as such. Should a non-serious AE become serious, the Investigator will then follow the same reporting procedures as for SAEs.
An AE occurring after the last follow-up 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
Each AE will be described by:

- Its duration (start and stop dates).
- Severity grade.
- Its relationship to the study drug; (suspected/unsuspected).
- Action(s) taken.
- Outcome.

11.5.1 Severity of AEs

The Investigator will grade the severity of any AE using the definitions in the table below. For each episode, the highest severity grade attained should be reported.

Mild	Grade 1 - Does not interfere with subject's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).
Moderate	Grade 2 - Interferes to some extent with subject's usual function (enough discomfort to interfere with usual activity [disturbing]).
Severe	Grade 3 - Interferes significantly with subject's usual function (incapacity to work or to do usual activities [unacceptable])

11.5.2 Relationship of AEs to the IMP

The Investigator will assess the causal relationship between the AE and the IMP, according to the criteria in table below:

None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g. subject is a passenger in a road traffic accident.
Unlikely (remote)	Relationship is not likely e.g. a clinical event including laboratory test abnormality with temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide more plausible explanations
Possible	Relationship may exist, but could have been produced by the subject's condition or treatment or other cause
Probable	Relationship is likely, the AE abates upon discontinuation of Investigational Product and cannot be due to the subject's condition
Highly Probable	Strong relationship, the event abates upon discontinuation of Investigational Product and, if applicable, re-appears upon repeat exposure

11.6 SAEs reporting

The Investigator must report all SAEs, regardless of presumed causal relationship, to Dompé Pharmacovigilance and to the CRO, by email (preferred) or fax within 24 hours of learning of the event. Contact details for SAE reporting are the following

Sponsor
Dompé Pharmacovigilance
Email: farmacovigilanza@dompe.com
Fax: +39.02.36026913

CRO:
Email: projectmanagement@croalliance.com
Fax: +41.91.630.05.11

The investigator should also report information on SAEs that continue after subject has completed his/her participation in the study (whether study completion or withdrawal), unless subject has withdrawn his/her consent.

Information on SAEs will be recorded on the SAE form. Follow-up reports (as many as required) should be completed and faxed/e-mailed following the same procedure above, marking the SAE form as "follow up Number XX".

Whenever more than one SAE is observed, the Investigator should identify which is the primary adverse event, i.e. the most relevant one. If other events are listed in the same report, the Investigator, along with their relatedness to the IMP, should identify which adverse events are serious and which are non-serious. In any case, the Investigator is requested to record his/her opinion about the relatedness of the observed event(s) with the investigational medication.

In line with CT3 Detailed Guidance and ICH E2A provisions, although the Investigator does not usually need to actively monitor subjects for AEs once the trial has ended, if the Investigator becomes aware of a SAE occurring to a subject after that subject has ended his/her participation in the study (whether study completion or withdrawal), the SAE should be reported by the Investigator to the Dompé Pharmacovigilance. Such "post-study cases" should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment and determination of expectedness are needed for a decision on whether or not expedited reporting is required.

11.6.1 Adverse events exemption

Not applicable; no adverse event reporting exemption.

11.7 Reporting Procedure to IRB/IEC and to Regulatory Authorities

During the course of the clinical trial, Dompé farmaceutici S.p.A shall report any serious unexpected ADR (SUSAR) to the Competent Authority (according to specific law requirements) while the CRO shall submit SUSARs (as per final report released by Dompé) to the concerned IEC which approved the protocol as soon as possible and in no event later than:

(a) seven (7) calendar days after becoming aware of the information if the event is fatal or life threatening;
(b) fifteen (15) calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

Dompé farmaceutici S.p.A. shall, within eight days after having informed the IEC/Competent Authority under paragraph (a), submit a complete report in respect of that information that includes an assessment of the importance and implication of any findings, made on the basis of follow up information provided by the Investigator.

The Sponsor shall be responsible to prepare and submit periodical update reports as appropriate.

Copies of all correspondence relating to reporting of any SAEs to the IEC/IRB should be maintained in the Investigator's Files.

11.8 Follow-up of Subjects with Adverse events

AE will be followed by the Investigator (by phone or visit) until resolved or considered stable.

The Investigator is responsible for adequate and safe medical care of subjects during the trial and for ensuring that appropriate medical care and relevant follow-up procedures are maintained after the trial. It is the Investigator's responsibility to assure that the subjects experiencing AEs receive definite treatment for any AE, if required.
For pharmacovigilance purposes, all SAEs should be followed-up in order to clarify as completely as possible their nature and/or causality and until all queries have been resolved. All SAEs will be followed up until the events resolve or the events or sequelae stabilize, or it is unlikely that any additional information can be obtained after demonstration of due

diligence with follow-up efforts (i.e. subject or Investigator is unable to provide additional information, or the subject is lost to follow up), unless subject has withdrawn his/her consent.

If subject was hospitalized due to a SAE, a copy of the discharge summary is to be forwarded to the Sponsor as soon as it becomes available. In addition, a letter from the Investigator that summarizes the events referred to the case, as well as results of any relevant laboratory tests and redacted section of medical records may be provided to the Sponsor, if relevant for the SAE. In case of death, a copy of the autopsy report, if performed, should also be provided.

The Investigator shall inform the Sponsor with an appropriate written communication, whenever he becomes aware of new available information regarding the SAE, once the condition is resolved or stabilized and when no more information about the event is expected. Follow-up SAE information should be processed as initial SAE notification.

11.9 Pregnancy in the Clinical Trial

Women of childbearing potential are not excluded from the study as long as adequate birth control methods are being utilized. Women of childbearing potential are defined as all women physiologically capable of becoming pregnant. Adequate birth control methods are summarized in the protocol's exclusion criteria.

Prior to enrolment in the clinical trial, female subjects of childbearing potential and their partners must be advised of the importance of avoiding pregnancy during the entire course of the study treatment and for the 30 days after the study treatment period ends and of the potential risks associated with an unintentional pregnancy. During the trial, female subjects are to be instructed to contact the Investigator immediately if they suspect they might be pregnant.

Pregnant female subject will discontinued the study. Pregnancy is not reportable as an adverse event; however, complications may be reportable and will be assessed for reportability on a case by case basis. A form prepared by the Sponsor will be utilized to capture all pregnancy-related information until the birth of the child during the study treatment period and follow-up period.

11.10 OVERDOSE

Accidental or intentional overdose, which may or may not result in serious adverse reactions, is to be reported to Dompé Drug Safety/CRO and Dompé Medical Expert, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake through different routes (e.g. ingestion) or with suicidal intentions and consequent drug overdose.
An overdose of ladarixin is defined as the administration of 6 or more capsules on any given treatment day.
The Investigator shall provide in the SAE form information about symptoms, corrective treatment and outcome of overdose. Dompé Medical Expert should be contacted to discuss corrective treatment, if necessary.

11.11 SAEs: clinical site contacts

The clinical site can be contacted by the study participant 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 contacts for SAEs for the subjects in study are the following:
Dr. Milko Radicioni
Phone: +41.91.64.04.450
Fax: +41.91.63.00.511
Email: milko.radicioni@croalliance.com and clinic@croalliance.com

12 DATA MANAGEMENT PROCEDURES

12.1 Data collection – eCRF

The study data will be recorded at the clinical site by trained study personnel in a study-specific electronic Case Report Form (eCRF) validated as per 21 CFR Part 11.
The investigator must ensure that the clinical data required by the study protocol are carefully reported in the eCRF. He must also check that the data reported in the eCRF correspond to those in the subject's source documents. The eCRF should be filled out in English. In the interest of completeness of data acquisition, the questions which are repeated in each section of the eCRF 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 eCRF will be completed during the study and will be signed by the investigator with electronic signature (ID and password) after all queries have been solved.
Data management activities will be performed by the CRO Biometry Unit. After the completion of data management procedures, data of the eCRF will be archived by the Sponsor in the format agreed with the eCRF provider (§ 16.7) and the site will remain in control and file a copy of the eCRF data, as per paragraph 8.1 of ICH-GCP.

Training of the use of eCRF will be performed before the start of the study and every user will be provided with private User ID and Password to access the eCRF, according to the assigned role (i.e. Investigator, Study Nurse, Study Monitor, Data Manager, etc.).

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. LDX0219), 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 randomization number (e.g. 001, 002, etc.). Study code, site number, screening number and subject randomization number are separated by slashes ("/") [example: LDX0219/001/S001/001]. The last 8 digits of the unique subject identifier (enrolled subjects), corresponding to the subject screening and subject randomization 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 CSR and will be used to identify the subjects in in-text tables or wording (if applicable).

12.3 Database management

Database and eCRF design will be performed by the eCRF provider (§ 16.7) before the start of the study. Verification and cross-verification of the data entered by the study personnel will be automatically performed by the system, according to predefined nature/type of the data, value ranges and rules. All rules applied by the system to check specific items will be detailed in the Data Validation Plan (DVP). Queries will be automatically generated by the system for all data found to be inconsistent, incorrect or missing. All changes performed by the Investigator/authorized user(s) will be tracked by the system.

Once all data are entered in the eCRF and all outstanding queries are solved and the Monitor complete the Source Data Verification (SDV), the Data Manager will verify the correctness of the answers and the Investigator will approve (sign) the data of the subjects' eCRF. Afterwards, it may be still necessary to issue additional queries following general quality control checks performed by the trained Data Manager of the study on the approved eCRF data that will be managed within the system. The Data Manager or the Clinical Programmer will 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 (see § 16.8).

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 authorized 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 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 requirements.

The CRO 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, the clinical center, the CRO and the eCRF provider will follow their respective SOPs in the conduct of the respective activities, unless otherwise stated in written agreements. SOPs will be made available for review, if required. AEs, SAEs, pregnancy management and SAEs reconciliation will follow Dompé SOPs.

13.4 Data access

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

13.5 Audits and inspections

The Sponsor, 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, the CRO and the eCRF provider agree, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorized 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. 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 organized
- 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 jeopardizing 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.

Subjects will be provided with an additional informed consent prepared in the local language by the CRO and already approved by the EC, regarding the information to the processing of personal data according to the Swiss Federal Law on Data Protection (Law 235.1 of 19 June 1992 and subsequent updates) and the European General Data Protection Regulation (GDPR, EU Regulation n 2016/679).

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 authorized representatives of the Sponsor, EC members and regulatory authorities. He will confirm, by signing and dating the forms, that informed consent has been obtained.

14.3 Insurance policy

An insurance cover has been issued in favor 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 complete the clinical study. If, for a subject, study treatment or observations are discontinued and 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**: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- **protocol deviation**: an event or decision that stands in contrast to the protocol
- **study terminated by Sponsor**: an indication that the clinical study was stopped by its Sponsor
- **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 eCRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the eCRF any follow-up, if the subject is withdrawn for an AE

Discontinued subjects will be replaced starting from the 3rd discontinuing man or the 3rd discontinuing woman.

14.5 Study termination

The study will be considered terminated at the date of LSLV 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.

15 ADMINISTRATIVE PROCEDURES

15.1 Material supplied to the clinical center

Beside IMP, the following study material will be supplied to the clinical center:

- final version of the study protocol
- access to the eCRF
- eCRF guideline
- 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 eCRFs and in all required reports.

The investigator must keep source documents for each subject in the study. All information on the eCRFs 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 eCRF 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 requirements.

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, eCRFs, 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 enroll 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 investigator and the CRO agree to keep all the information provided by the Sponsor in strict confidentiality and to request the same confidentiality from his/her staff. Study documents provided by the Sponsor (protocols, IB, eCRFs 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.

Data on subjects collected in the eCRFs 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.

The processing of personal data by the Sponsor, the CRO and the Clinical Site shall always be in line with the local regulations, the EU General Data Protection Regulation (GDPR; Regulation EU 679/2016), and the applicable Swiss data protection regulations (Swiss Law 235.1, Federal Act on Data Protection of June 19th, 1992). Suitable written information will be provided to the study subjects at the time of consenting.

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 by the submission of a complete clinical study report.

As the Sponsor agrees that the study results can be published by the investigator, 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

Dompé farmaceutici S.p.A. - Via Campo di Pile; I-67100 L'Aquila, Italy
Milan Offices: Via S. Santa Lucia 6, I-20122 Milan, Italy
Phone: +39.02.583831
Fax: +39.02.58383324

Trial Manager
Giuseppe Terpolilli, Pharm D. – Clinical Operations Specialist
Email: giuseppe.terpolilli@dompe.com

Medical Expert
Pier Adelchi Ruffini, MD – Global Clinical Development Director
Email: Pieradelchi.ruffini@dompe.com

Drug Safety Manager
Laura Boga, Global Head of Pharmacovigilance & EU Qualified Person for Pharmacovigilance
Email: laura.boga@dompe.com
Fax: +39.02.3602691

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 SAE reporting

Dompé farmaceutici S.p.A. Drug Safety
Email: farmacovigilanza@dompe.com
Fax: +39.02.36026913

CRO:
Email: projectmanagement@croalliance.com
Fax: +41.91.630.05.11

16.4 Drug assay

Bioanalytical Laboratories Dompé farmaceutici S.p.A. - Via Campo di Pile; I-67100 L'Aquila, Italy
Phone: +39.0862.338323
Fax: +39.0862.338367
Mobile: +39.342.7634949
Email: franca.cattani@dompe.com

Analytics representative
Franca Cattani

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

16.5 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

16.6 Co-ordination, data analysis & reporting

CROSS Research S.A., Switzerland

Via F.A. Giorgioli 14, CH-6864 Arzo, Switzerland

Coordination
Chiara Castiglioni, Clinical Project Leader and Clinical Research Associate
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Biometry Unit Representative
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Quality Assurance Unit Representative
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Email: qau@croalliance.com

16.7 Provider of eCRF system

CROS NT SRL, via Germania 2, 37136 Verona, Italy
Phone: +39. 045 820 26 66

Responsible person
Monica Pimazzoni, Director of Clinical Data Management
Email: monica.pimazzoni@crosnt.com

16.8 Monitoring

Clinical Medical Services di Maria Pia Savorelli,
Via Industria 5, CH-6850 Mendrisio, Switzerland
Mobile: +41.79.82.72.767
Email: cmed@cmed.ch

17 REFERENCES

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Note to File Nr.	CROss ALLIANCE Contract Research Organisation for Scientific Services
CRO-PK-20-345 **Sponsor code LDX0219**	**Issued: 13OCT20** **Page 1 of 1**

Study Title: Influence of Food on the Oral Bioavailability of Ladarixin 200 mg Capsule in Healthy Volunteers of Both Sexes. A Single dose (400 mg), Randomized, Open Label, Two-Way Crossover Study.

Single center, single dose, open label, randomized, two-way, crossover, food effect study

This note is related to	Affected documents/actions:
☒ TMF General section: 5 ☐ TMF/CIMF Centre section, Center nr : -- ☒ ISF Center ☐ Source Documents ☐ Other: --	Clinical Study Protocol, Final version 1.0, 28MAY20

Reason of the note

This note to file has been issued to clarify that the concentration of DF 2156Y (total and unbound) and its metabolites (DF 2108Y and DF 2227Y) in plasma will be determined using a fully validated HPLC-MS method and not HPLC-UV method as reported by a typo in the clinical Study Protocol 1.0 issued on 28MAY20.

Approvals:

SPONSOR
Dompé farmaceutici S.p.A., Italy

Sponsor Trial Manager
Giuseppe Terpolilli, PharmD – Clinical Operations Specialist

13/10/2020 _____
Date

_____
Signature

CRO
CROSS Research S.A., Switzerland

Coordination
Chiara Castiglioni, Clinical Project Leader and Clinical Research Associate

13OCT2020 _____
Date

_____
Signature

Note to File Nr. NA	CROSS ALLIANCE Contract Research Organisation for Scientific Services
CRO-PK-20-345 LDX0219	Issued: 19-JAN-2021 Page 1 of 1

Study Title: Influence of Food on the Oral Bioavailability of Ladarixin 200 mg Capsule in Healthy Volunteers of Both Sexes. A Single dose (400 mg), Randomized, Open Label, Two-Way Crossover Study

This note is related to	Affected documents/actions: drop out management according to Study Protocol Final version 1.0 dated 28MAY20
☒ TMF/CIMF General section ☐ TMF/CIMF Center section, Center nr : ☐ ISF Center ☐ Source Documents ☐ Other: NA	

Reason of the note

In the synopsis section "Sample size" of the protocol Final version 1.0 dated 28MAY20 is reported "[…] Drop-out subjects will be replaced starting from the 3rd man and/or woman discontinuing prematurely the study".

In the section 10.2 "Sample size and power considerations" of the same document, it is erroneously stated that "Drop-out subjects will not be replaced".

The correct procedure is reported in synopsis. The protocol will not be amended since the clinical phase is already concluded and this discrepancy will be described in the Clinical Study Report.

Approvals:

Angelo Vaccani, Senior Clinical Project Leader
CROSS Research SA, Switzerland

29 JAN 2021 — Date
Angelo Vaccani — Signature

Sponsor Trial Manager
Giuseppe Terpolilli, PharmD – Clinical Operations Specialist

29/01/2021 — Date
Giuseppe Terpolilli — Signature