CLINICAL STUDY PROTOCOL

Study Number: LDX0319

Study Name: GLADIATOR

EudraCT Number: 2020-001926-71

IND number: [redacted]

Investigational Product: Ladarixin

Study Phase: 2

Title: A phase 2, multicenter, randomized, double-blind, placebo-controlled study to assess the effect and safety of 400 mg twice a day oral ladarixin in patients with recent onset type 1 diabetes and a low residual β-cell function at baseline.

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Full list of coordinating investigators and investigational sites for each country involved will be kept in the Trial Master File. Updated versions will be filed chronologically. Copies will be provided to the sites.

CENTRALIZED LABORATORIES

A list of centralized laboratories will be kept in the Trial Master File. Updated versions will be filed chronologically.

PROTOCOL APPROVAL SIGNATURES

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CLINICAL CENTER: PRINCIPAL INVESTIGATOR

I have read the study protocol LDX0319 "A phase 2 , multicenter, randomized, double-blind, placebo-controlled study to assess the effect and safety of 400 mg twice a day oral ladarixin in patients with recent onset type 1 diabetes and a low residual β-cell function at baseline" and agree to conduct the study as outlined in the protocol, and in accordance with the Declaration of Helsinki, ICH-GCP and any local regulations, being responsible for personally supervise the study conduct and ensure study staff complies with protocol requirement.

Name of Principal Investigator (block letters): _____

Signature: Date:

TABLE OF CONTENTS

TABLE OF CONTENTS 4
List of Abbreviations and Definitions of Terms 8
1. STUDY SYNOPSIS AND OVERALL DESIGN 10
2. BACKGROUND INFORMATION 17
2.1 RELEVANT NON-CLINICAL PHARMACOLOGY 17
2.1.1 Mechanism of action and *in vitro* activity 17
2.1.2 In vivo general studies 17
2.1.3 Effects in models of type 1 diabetes 18
2.2 A SUMMARY OF TOXICOLOGY DATA 18
2.3 PHARMACOKINETICS AND PRODUCT METABOLISM 21
2.4 A SUMMARY OF CLINICAL DATA 21
2.4.1 Pharmacokinetics and product metabolism in humans 22
2.4.2 Efficacy 22
2.4.3 Safety 22
2.5 DISEASE REVIEW AND STUDY RATIONALE 25
2.5.1 Selection of dose and treatment schedule in the study 26
2.5.2 Alternative treatments 26
2.5.3 Risk - benefit evaluation 27
2.5.3.1 Risk related to the ladarixin 27
2.5.3.2 Blood sampling 27
2.5.3.3 Mixed Meal Tolerance Test 28
2.5.3.4 Continuous Glucose Monitoring (CGM) 28
2.5.3.5 Potential benefit 28
3. OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN 29
3.1 STUDY OBJECTIVES 29
3.2 STUDY ADMINISTRATIVE STRUCTURES, STAFF AND RESPONSIBILITY 29
3.3 OVERALL STUDY DESIGN 29
3.4 STUDY TIME TABLE 30
3.5 END OF STUDY 31
4. STUDY ENDPOINTS 32
4.1 EFFICACY ENDPOINTS 32
4.2 EXPLORATORY ENDPOINTS 32
4.3 PHARMACOKINETIC ENDPOINTS 32
4.4 SAFETY ENDPOINTS 33
5. STUDY POPULATION 34
5.1 INCLUSION CRITERIA 34
5.2 EXCLUSION CRITERIA 34
5.3 ASSIGNMENT OF PATIENT NUMBER 35

6. STUDY MEDICATION 36
6.1 PRESENTATION, STORAGE, PACKAGING AND LABELING OF THE INVESTIGATIONAL MEDICINAL PRODUCT 36
6.1.1 Presentation of Investigational Medicinal Product 36
6.1.2 Manufacturing, Packaging and Labelling of IMP 36
6.1.3 Supply, Storage and Handling of IMP 36
6.1.4 Blinding 37
6.2 IMP DISPENSATION 37
6.3 DOSE, ROUTE AND SCHEDULE OF IMP ADMINISTRATION 37
6.4 CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION OR DISCONTINUATION OF THE IMP 38
6.4.1 Criteria for schedule adjustment or dose modification 38
6.4.2 Criteria for discontinuation of the IMP 38
6.5 ACCOUNTABILITY OF THE IMP 38
6.5.1 Assessment of compliance 39
6.6 CONCOMITANT MEDICATION 39
6.6.1 Reporting of prior and concomitant medications 39
6.6.2 Restriction on allowed prior and concomitant medications 39
6.6.3 COVID-19 and other vaccination 40
6.7 INSULIN TITRATION 40
7. STUDY PROCEDURE AND ASSESSMENTS 41
7.1 ENROLMENT, SCREENING AND RANDOMIZATION 41
7.1.1 Enrolment and Intensive Diabetes Management 41
7.1.2 Run-in period 41
7.2 RANDOMIZATION & START OF DRUG ADMINISTRATION 42
7.3 TREATMENT PERIOD AND CHECK OF TREATMENT DISCONTINUATION CRITERIA 43
7.4 ADDITIONAL VISITS DURING TREATMENT AND FOLLOW-UP STUDY ASSESSMENTS 44
7.4.1 PK visits 44
7.4.2 Additional visits during treatment 44
7.4.3 Follow-up assessments 44
7.5 EARLY PATIENT WITHDRAWAL 45
7.5.1 Withdrawal criteria 45
7.5.2 Replacement policy 45
7.6 PATIENT MANAGEMENT AFTER STUDY COMPLETION 45
8. ADVERSE EVENTS 46
8.1 DEFINITIONS 46
8.1.1 Adverse Event 46
8.1.2 Adverse Drug Reaction 46
8.1.3 Serious Adverse Event/Reaction 46
8.1.4 Unexpected Adverse Events 47
8.1.5 Suspected serious unexpected adverse reaction 47
8.2 MONITORING FOR ADVERSE EVENTS 47

8.3 RECORDING 47
8.3.1 Relationship of AEs to the Investigational Product 47
8.3.2 Severity of AEs 48
8.4 SERIOUS ADVERSE EVENT REPORTING 48
8.4.1 Reporting Procedure for Investigators to Dompé 48
8.4.2 Conditions that should not be reported as serious adverse events 49
8.4.3 Follow-Up of Serious Adverse Events 49
8.4.4 Reporting Procedure to IEC and to Regulatory Authorities in the European Union 49
8.4.5 Reporting Procedure to IRB and to Regulatory Authorities in the United States 50
8.5 EXPOSURE TO INVESTIGATIONAL PRODUCT DURING PREGNANCY 51
8.6 ADVERSE EVENTS CAUSING TREATMENT DISCONTINUATION 51
8.7 OVERDOSE 52
8.8 EMERGENCY PROCEDURES 52
9. STATISTICAL ISSUES 53
9.1 SAMPLE SIZE 53
9.2 RANDOMIZATION 53
9.3 OVERVIEW OF PLANNED STATISTICAL ANALYSES 53
9.4 INTERIM ANALYSIS 54
9.5 ANALYSIS POPULATION 54
9.6 STATISTICAL METHODOLOGY 54
9.6.1 Analysis of pharmacokinetics 55
9.6.2 Demographic and baseline characteristics 55
9.6.3 Analysis of efficacy variables 55
9.6.3.1 Primary analysis 55
9.6.3.2 Sensitivity analyses 56
9.6.3.3 Secondary analyses 56
9.6.4 Analysis of exploratory variables 57
9.6.5 Analysis of safety variables 57
9.6.6 Intermediate analyses for DMC 57
9.6.7 Specification of subgroups for analysis 57
9.6.8 Missing data 58
10. ETHICAL CONSIDERATIONS 59
10.1 INDEPENDENT ETHICS COMMITTEE (IEC) / INSTITUTIONAL REVIEW BOARD (IRB) 59
10.2 INFORMED CONSENT 59
10.3 CONFIDENTIALITY 60
10.4 COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION 60
11. DATA HANDLING AND RECORD KEEPING 61
11.1 CASE REPORT FORMS 61
11.2 DIARY 61
11.3 DATA MANAGEMENT 61
11.4 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE STUDY 62

11.5 ESSENTIAL DOCUMENT RETENTION 62
12. STUDY MANAGEMENT 63
12.1 REGULATORY BODY APPROVAL 63
12.2 STAFF INFORMATION & RESPONSIBILITIES 63
12.3 MONITORING 63
12.3.1 Access to records 63
12.4 AUDIT AND INSPECTION 63
12.5 DATA MONITORING COMMITTEE 64
12.6 PROTOCOL DEVIATIONS/AMENDMENTS 64
12.7 DISCONTINUATION OF THE STUDY 65
12.8 PUBLICATIONS 65
13. REFERENCES 66
14. APPENDICES 68
14.1 APPENDIX 14.1 - PACKAGING AND LABELING DETAILS 68
14.2 APPENDIX 14.2 – STUDY FLOW CHART 69
14.3 APPENDIX 14.3 - METHODOLOGICAL DETAILS 71
14.3.1 Handling of samples for centralized assay 71
14.3.2 Details of Pharmacokinetics blood sampling and Pharmacokinetics analysis 71
14.3.3 Calculation of eGFR 73
14.3.4 Calculation of eGDR 74
14.3.5 Handling of CGM device 74
14.3.6 Mixed Meal Tolerance Test 74
14.4 APPENDIX 14.4 - CLINICAL TRIALS SUBJECT TO TRANSITION FROM EUROPEAN DIRECTIVE 2001/20/EC TO CLINICAL TRIAL REGULATION EU NO 536/2014 75

List of Abbreviations and Definitions of Terms

AE	Adverse Event
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
AUC0-12	Area Under the plasma concentration-time Curve from time zero to 12 hours post-dosing
AUCτ	Area Under the plasma concentration-time Curve within a 12-hour dosing interval at steady state)
b.i.d.	Bis in die
BMI	Body Mass Index
BP	Bullous Pemphigoid
°C	Degrees Celsius
CGM	Continuous Glucose Monitoring
CSII	Continuous Subcutaneous Insulin Infusion
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CYP2C9	Cytochrome P450 2C9
CLss/F	Apparent Clearance at steady state
Cmax	Maximum Plasma Concentration
CMED	Concomitant Medication
CONGA-n	Continuous Overall Net Glycemic Action
CRA	Clinical Research Associate
CRO	Contract Research Organization
Css,av	Average Plasma Concentration at steady state
CXCL8	CXC ligand 8 [formerly interleukin (IL)-8]
CXCR1/2	CXCL8 receptors
DCCT	Diabetes Control and Complications Trial
DMC	Data Monitoring Committee
DPP-IV inhibitor	Dipeptidyl peptidase-IV inhibitor
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
eGDR	Estimated Glucose Disposal Rate
FAS	Full Analysis Set
fMLP	formyl-met-leu-phe
GAD	Glutamic Acid Decarboxylase
HbA1c	Glycated hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen Complex
hs-CRP	High-sensitivity C-reactive protein
IA-2	Islet Antigen-2
IAA	Insulin Auto Antibody
ICF	Informed Consent Form
ICH-GCP	International Conference on Harmonization on Good Clinical Practice
IEC	Independent Ethics Committee
IL-6	Interleukin -6
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IRS	Interactive Response System
ITT	Intention To Treat
IU	International Unit
i.v.	Intravenous
LD50	Lethal Dose50
MAGE	Mean Amplitude Glycemic Excursions
MLD-STZ	Multiple Low Dose-Streptozotocin
MMTT	Mixed Meal Tolerance Test
MODD	Mean Of the Daily Differences

NET	Neutrophil Extracellular Traps
NOD	Non-Obese Diabetic
NOAEL	No Observable Adverse Effect Level
NOEL	No Observable Effect Level
PI	Principal Investigator
PK	Pharmacokinetics
PMN	Polymorphonuclear leukocyte
p.o.	per os (taken by mouth)
PP	Per Protocol
PPG	2-hour postprandial glucose
QTcF	Fridericia's corrected QT interval
Rac	Accumulation Ratio
SAE	Serious Adverse Event
SAF	Safety Population
SAP	Statistical Analysis Plan
s.c.	Subcutaneous
SD	Standard Deviation
SGLT2	Sodium-Glucose co-transporter-2
SMBG	Self-Monitoring of Blood Glucose
SUSAR	Serious Unexpected Suspected Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TESAE	Treatment Emergent Serious Adverse Event
TIR	Time in Range
t½	Elimination half life
tmax	Time to Maximum Plasma Concentration
T1D	Type 1 Diabetes
ULN	Upper Limit of Normal
$Vz_{,ss}/F$	Apparent Volume of distribution at steady state
ZnT8	Zinc Transporter Isoform 8
λz	Terminal phase rate constant

1. STUDY SYNOPSIS AND OVERALL DESIGN

Study title
A phase 2, multicenter, randomized, double-blind, placebo-controlled study to assess the effect and safety of 400 mg twice a day oral ladarixin in patients with recent onset type 1 diabetes and a low residual β-cell function at baseline.

Study Number LDX0319 **[IND: [redacted]**
[EudraCT Number: 2020-001926-71]

Study period Actual starting date (first-patient-in): 12 Jan 2021
Projected completion of patient accrual (last-patient-in): [redacted]
Projected study end date (last-patient-last-visit): [redacted]

Study design
The study will be a phase 2, multicenter, double-blind, placebo-controlled study. It will randomize approximately 130-140 patients (with up to an estimated 15-20% adolescents), with recent onset (within 180 days from 1st insulin administration) type 1 diabetes (T1D), assigned (2:1) to receive either oral ladarixin treatment (400 mg b.i.d. for 13 cycles of 14 days on/14 days off - treatment group) or placebo (control group). Recruitment will be competitive among the study sites, until the planned number of patients is randomized.

Ladarixin and placebo will be both administered for 1 year. All patients will be followed-up for 24 months from the 1st administration of the study medication. After the initial 12-m treatment period, all patients will enter into a 12-month follow-up (total period 24-month after first IMP administration). The study database (DB) will be locked when the last randomized patient has completed the month 12 visit (or being lost in follow-up), and relative data have been fully reconciled and cleaned; at that point, the DB will be unblinded and all endpoints, including the 6-month primary endpoint, will be analyzed, and the follow-up will continue under open-label conditions up to month 24.

Objectives/endpoints
The objective of this clinical trial is to assess whether ladarixin treatment has an effect to preserve β-cell function and delay the progression of T1D in adolescent and adult patients. The safety of ladarixin in the specific clinical setting will also be evaluated.

Details of study endpoints are reported below, with relevant time frame where:
Month 6 = Week 26 ±2; Month 12 = Week 52 ±2; Month 18 = Week 78 ±2; Month 24 = Week 104 ±2.

Efficacy endpoint

Primary endpoint:
Change from baseline in 2-hour area under the curve (AUC) of C-peptide response to a Mixed Meal Tolerance Test (MMTT) [Time frame: Month 6]

Secondary endpoints:
- Change from baseline in 2-hour AUC of C-peptide response to the MMTT [Time frame: Month 12, 18 and 24].
- Change in HbA1c from baseline [Time frame: Month 6, 12, 18 and 24].
- Time in range (TIR) by Continuous Glucose Monitoring (CGM) [Time frame: Month 6, 12, 18, 24]
- Proportion of patients with HbA1c of less than 7% who did not experience severe hypoglycemic events during treatment [Time frame: Month 6, 12, 18 and 24].
- Average (previous 3 days) daily insulin requirement (IU/kg/day) [Time frame: Month 6, 12, 18 and 24].

- Proportion of patients with HbA1c <7% and daily insulin requirement <0.5 (IU/kg/day) [Time frame: Month 6, 12, 18 and 24].
- Additional Glucose Variability Indices derived from CGM (glucose AUC outside the target range of 70 – 180 mg/dL, 2-hour postprandial glucose (PPG), Mean Amplitude Glycemic Excursions (MAGE), continuous overall net glycemic action (CONGA)-n, Mean Of the Daily Differences (MODD), and mean daily blood glucose, SD (Standard Deviation). [Time frame: Month 6, 12, 18 and 24].
- Number of self-reported episodes of severe hypoglycemia [Time frame: Month 6, 12, 18 and 24].
- Percentage of patients not requiring insulin therapy [Time frame: Month 6, 12, 18 and 24]
- Estimated Glucose Disposal Rate (eGDR) [Time frame: Month 6, 12, 18 and 24].

Exploratory endpoints will be:

- microRNA-375 (a marker of β-cell destruction) [Time frame: Month 6, 12, 18 and 24]
- miRNA signature [Time frame: baseline]
- IL-8 and IL-6 [Time frame: Month 6, 12, 18 and 24]
- High-Sensitivity C-Reactive Protein (hs-CRP) [Time frame: Month 6, 12, 18 and 24]
- Neutrophil Extracellular Traps (NET) [Time frame: Month 6, 12, 18 and 24]
- Proinsulin/ C-peptide ratio [Time frame: Month 6, 12, 18 and 24]

Pharmacokinetic endpoints will be:

- Plasma levels of 2156Y (acidic form of ladarixin) and relevant metabolites (DF2108Y, S-isomer; DF2227Y, R-isomer) in a subset of adolescents (14-17 years, inclusive) selected for full PK analysis [Time frame: day 1 and 14, then 24, 48 and 72 hours after the last IMP dose (morning dose) of the [redacted] treatment cycle].
- Plasma levels of 2156Y (acidic form of ladarixin) and relevant metabolites (DF2108Y, S-isomer; DF2227Y, R-isomer) in the whole population [Time frame: within 96 hours after the last IMP dose in at least 2 treatment cycles].

Safety endpoints will be:

- Vital signs (blood pressure and heart rate) [time frame: end of the 3rd (Week 11), 6th (Week 23), 9th (Week 35) treatment cycle, Month 12 and Month 24].
- Safety Laboratory Tests (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, serum albumin, total bilirubin, ALT, AST) [time frame: end of the 3rd (Week 11), 6th (Week 23), 9th (Week 35), Month 12 and Month 24 (or withdrawal)].
- Incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) [time frame: throughout the study].

Number of patients: Approximately 130-140 patients with recent onset T1D, with up to an estimated 15-20% adolescents (14-17 years inclusive).

Inclusion/exclusion criteria

Consented male and female patients aged 14-45 years, inclusive, with recent onset T1D (randomization scheduled to allow the administration of the study medication to start within 180 days from 1st insulin administration). Patients must be positive for at least one diabetes-related auto-antibody (anti-GAD; IAA, if obtained within 10 days of the onset of insulin therapy; IA-2 antibody; ZnT8); must require, or have required insulin delivered via one or more separate subcutaneous injections or Continuous Subcutaneous Insulin Infusion (CSII); must have a fasting C-peptide below 0.205 nmol/L, but must retain a β-cell function as per peak stimulated (MMTT) C-peptide level >0.2 nmol/L.

Patients will be excluded if they have a type 2 diabetes or any other unstable chronic disease for which dose adjustment of specific medication is anticipated during the trial; moderate to severe renal impairment calculated by estimated Glomerular Filtration Rate (eGFR) <60 mL/min/1.73 m^2 as determined using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation;

hepatic dysfunction (increased ALT/AST >3 x upper limit of normal and increased total bilirubin >3 mg/dL [>51.3 µmol/L]); hypoalbuminemia (serum albumin <3 g/dL); a QTcF > 470 msec.; a history of significant cardiovascular disease/abnormality; occurrence of an episode of ketoacidosis or hypoglycemic coma in the past 2 weeks; a known hypersensitivity to non-steroidal anti-inflammatory drugs. Patients on treatment with drugs metabolized by CYP2C9 with a narrow therapeutic index [i.e. phenytoin, warfarin, sulphonylurea hypoglycemics and high dose of amitriptyline (>50 mg/day)]; patients with past (within 2 weeks prior to randomization) or current use of antidiabetic agents as metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors, SGLT-2 inhibitors or amylin, or any medications known to influence glucose tolerance (e.g. β-blockers, angiotensin-converting enzyme inhibitors, interferons, quinidine antimalarial drugs, lithium, niacin, etc.) will also be excluded. Patients will be excluded as well in case of past (within 1 month prior to randomization) or current administration of any immunosuppressive medications (including oral or systemic corticosteroids and use of any investigational agents, including any agents that impact the immune response or the cytokine system. Additional exclusion criteria will be: significant systemic infection during the 4 weeks before the 1st dose of study drug (e.g., infection requiring hospitalization, major surgery, or i.v. antibiotics to resolve; other infections, e.g. bronchitis, sinusitis, localized cellulitis, candidiasis, or urinary tract infections, must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant exclusion); History of positive status for hepatitis A (IgM), hepatitis B (not due to immunization), hepatitis C and HIV. Also, pregnant or breastfeeding women or patients unwilling to use effective contraceptive measures (females and males) will be excluded.

Investigational Medicinal Product

The investigational medicinal product (IMP) will be either ladarixin OR placebo capsules for oral administration. Ladarixin will be administered orally at the dose of 400 mg twice a day at about a 12-hour interval (morning and evening) for 13 cycles of 14 days on treatment with an interval of 14 days off treatment between cycles. Placebo will be administered with the same schedule. IMP will be dispensed as Patient Kits.

Discontinuation criteria

Administration of the investigational medicinal product will be discontinued in case the QTcF becomes either >500 msec. or increases by >60 msec. from baseline measurement on two consecutive measurements 1 hour apart, or if the patient develops any significant cardiovascular disease/abnormality. Similarly, administration of the investigational medicinal product will be discontinued in the case the patient develops renal (eGFR <60mL/min/1.73 m^2) or hepatic (ALT/AST >3 x ULN and total bilirubin >3mg/dL) dysfunction as well as hypoalbuminemia (serum albumin <3 g/dL). Lastly, administration of the investigational product will be immediately discontinued if the patient becomes pregnant, develops any significant systemic infection that must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant discontinuation, ketoacidosis or hypoglycemic coma. Occurrence of any condition that qualifies the patient for treatment discontinuation will be specifically monitored through ECG readings and laboratory tests obtained at visits scheduled at the end of the 3rd (Week 11), 6th (Week 23), 9th (Week 35) treatment cycles. In addition, the investigational medicinal product will be immediately discontinued in the event of any other possibly drug related occurrences that the Investigator believes might compromise patient's safety.

Procedures

Each patient will be involved in the study for a run-in period, the randomization visit, a treatment period of 12 months and a follow-up period of 12 months after treatment completion. Patients participation in the study will include: PK visits (2 visits at Day 1 and Day 14 and then 3 visits at 24, 48, 72 hours after the last dose (morning dose) in the [redacted] treatment cycle in the subset of adolescents; at least 2 visits in the whole population within 96 hours after the last IMP dose in at least 2 treatment cycles); 5 visits during treatment scheduled at the end of the [redacted] (Week [redacted]) and [redacted] (Week [redacted]) treatment cycles, at Month [redacted], at the end of the [redacted] (Week [redacted]) treatment cycle, and at Month [redacted]; 2 follow-up visits (Month 18, Month 24) up to month 24 after the 1st IMP dose. Study period/visits are summarized in the study flow chart (see Appendix 14.2).

Screening to Randomization

Potential study patients with a recent clinical diagnosis of T1D will be identified from those referring to the site for diagnosis confirmation (e.g. auto-antibody testing) and/or disease management. Screening, including confirmation of T1D diagnosis, will be performed in consented patients.

From consent, patients will be admitted to an intensive diabetes management to ensure standardized glycemic control in the treatment groups. The Investigator will provide guidance for insulin regimen adjustment, with insulin titrated up or down to target HbA1c levels of less than 7% and self-monitored blood glucose (SMBG, fingerstick/glucose meter or CGM) of:

- pre-prandial blood glucose of 70-130 mg/dL,
- 2 hours post-prandial blood glucose <180 mg/dL,
- bed-time blood glucose of 110-150 mg/dL.

Glucose test strips and a glucose monitor will be provided to each participant for the duration of the study.

In order to optimize insulin titration, periodic controls (e.g. telephone calls and/or emails sent by the PI or sub-investigators to the patient) will be put in place to ensure timely evaluation of metabolic control and adjustment of insulin regimen. Patients will report their SMBG in the Patient Diary, at predefined intervals according to the use of the fingerstick.

Screening includes evaluation of past medical history and disease-specific clinical information, including date of the 1st insulin administration, blood sampling for measurement (centralized laboratory) of auto-antibody (anti-GAD; IAA, if obtained within 10 days of the onset of insulin therapy; IA-2 antibody; ZnT8) to confirm T1D diagnosis and measurement of fasting C-peptide that can be re-assessed once prior to Baseline in case the value at first sampling is >0.205 nmol/L.

Once the diagnosis has been confirmed and the fasting C-peptide is <0.205 nmol/L, patients will progress to the following Baseline assessments (**time frame**: within 3 weeks before treatment start):

- Vital signs.
- Body weight, height, waist and waist/hip circumference.
- Evaluation of eGDR and Body Mass Index (BMI).
- Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests".
- Evaluation of renal and hepatic function (to be derived from the safety laboratory test results).
- 12 lead ECG performed using local equipment and obtaining automatic calculation of the QTcF.
- Pregnancy test (urine dipstick or blood sample), if appropriate.
- Baseline insulin requirement (IU/kg/day averaged over the previous 3 days).
- Blood sampling for measurement (centralized laboratory) of baseline HbA1c.
- Blood sampling for measurement (centralized laboratory) of HLA DR-DQ haplotypes and Proinsulin.
- Baseline MMTT. Blood samples for measurement (centralized laboratory) of C-peptide and glucose will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120 min after the meal.
- Evaluation of Proinsulin: C-peptide ratio.
- CGM download for evaluation of baseline Glucose Variability Indices from CGM. *
- Blood sampling for measurement (centralized laboratory) of microRNA-375, miRNA signature, IL-8, IL-6, hs-CRP, NET.

* The CGM device will be positioned at least 10 days before the baseline assessment. It is recommended to place the device during the Screening Visit.

Patients fulfilling **all** the inclusion criteria and **none** of the exclusion criteria will refer to the site for a randomization visit to occur within maximum 3 days before the IMP administration is started; during

this visit a randomization number will be allocated to the patient; the Patient Diary will be delivered to the patient and the Patient Kits for the [redacted] to [redacted] treatment cycles will be dispensed.

Treatment period and check of treatment discontinuation criteria
The 1st IMP administration is recommended to happen during the randomization visit, under medical control. If this is not possible the drug can be started within 3 days after randomization. The start of IMP administration is defined as **Day 1** of the [redacted] treatment cycle. Day 1 **MUST be scheduled no later than 180 days from the 1st insulin injection** and is to be scheduled within 3 weeks from the baseline assessments.

Treatment will proceed for additional 12 cycles of 14 days on/14 days off.
Assessments to be done before the 1st dose in the [redacted] treatment cycle are part of the run-in period. Thereafter, occurrence of any condition that might prevent continuing IMP administration (as per discontinuation criteria above) will be verified at specific visits (see below) scheduled at the end of the [redacted] (Week [redacted]), [redacted] (Week [redacted]) and [redacted] (Week [redacted]) treatment cycles.

During these visits the following will be measured/assessed:
- Vital signs.
- Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests".
- Evaluation of renal and hepatic function (to be derived from the safety laboratory test results).
- 12 lead ECG, performed using local equipment that allows automatic calculation of the QTcF.
- Pregnancy test (urine dipstick or blood sample).
- Patient Diary check (e.g.: information on IMP administration, insulin requirement, severe hypoglycemia episode).

Patient Kits for the [redacted] to [redacted] cycles will be dispensed to the patient in appropriate batches, only after compliance with criteria for drug administration has been confirmed.

Results of ALT/AST, bilirubin, creatinine, serum albumin, ECG reading and pregnancy results should be made available by the local laboratory in time to allow Patient Kits dispensation as soon as possible during the visit. If this expedite timing cannot be achieved, Patient Kits might be delivered to the patient's named address by a selected courier (CRO to support shipment).

PK visits
The subset of adolescents selected for full PK analysis will attend the center on day 1 and 14 of the [redacted] treatment cycle; then 24, 48 and 72 hours after the last IMP dose (morning dose) in this cycle for PK sampling.
All patients in the study will refer to the site for at least 2 PK visits to be scheduled within 96 hours after the last IMP dose in at least 2 treatment cycles.

Other visits scheduled during treatment
During treatment, patients will attend the center for study assessments on additional 2 visits at Month 6 (Week 26) and Month 12 (Week 52). At least 10 days before each scheduled visit, the CGM sensor will be positioned.

During these visits the following will be assessed:
- Waist and waist/hip circumference and calculation of eGDR.
- Patient Diary check (e.g.: information on IMP administration, insulin requirement, severe hypoglycemia episodes)
- Insulin requirement (IU/kg/day averaged over the previous 3 days).
- Number of self-reported episodes of severe hypoglycemia in the interval.
- Blood sampling for measurement (centralized laboratory) of HbA1c.

- MMTT. Blood samples for measurement (centralized laboratory) of C-peptide and glucose will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120 min after the meal.
- Blood sampling for measurement (centralized laboratory) of microRNA-375, IL-8, IL-6, hs-CRP, NET, Proinsulin.
- CGM download for evaluation of Glucose Variability Indices from CGM.
- Blood sampling for measurement (local laboratories) of "Safety Laboratory Tests" (Month 12 only).
- Vital signs (Month 12 only).

Follow-up visits

Patients will attend the center for study assessments on 2 follow-up visits scheduled at month 18 (Week 78) and month 24 (Week 104). At least 10 days before each scheduled visit, the CGM sensor will be positioned.

At each visit, the following will be evaluated/measured:

- Waist and waist/hip circumference and evaluation of eGDR.
- Insulin requirement (IU/kg/day averaged over the previous 3 days).
- Number of self-reported episodes of severe hypoglycemia in the interval.
- Blood sampling for measurement (centralized laboratory) of HbA1c.
- MMTT. Blood samples for measurement (centralized laboratory) of C-peptide and glucose will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120 min after the meal.
- CGM download for evaluation of Glucose Variability Indices from CGM.
- Blood sampling for measurement (centralized laboratory) of microRNA-375, IL-8, IL-6, hs-CRP, NET, Proinsulin.
- Patient Diary check (information other than IMP administration e.g: insulin requirement, severe hypoglycemia episodes)
- Vital signs (Month 24 only)
- Blood sampling for measurement (local laboratories) of "Safety Laboratory Tests" (Month 24 only).

Statistics

[redacted]

The aim of this prospective study is to provide further evidence of the beneficial effect of ladarixin on C-peptide preservation in the same setting. The study will be based on approximately 130-140 randomized patients, number defined on feasibility considerations. Hypothesis testing will be based on $\log(AUC_{0\text{-}2h}+1)$ C-peptide and will be performed with an exploratory purpose. The null hypothesis is no difference between placebo and ladarixin, and the rejection of the null hypothesis will support the postulated superiority of ladarixin over placebo.

From a simulation based on the results provided in the [redacted] that showed a 15% difference in $\log(AUC_{0\text{-}2h}+1)$ C-peptide in favor of ladarixin vs. placebo, it is estimated that the sample size of 130-140 would provide >90% power to detect the same difference between treatment groups.

Summary statistics have been defined for quantitative variables (number of observations, mean, standard deviation, median, minimum and maximum) and qualitative variables (number and percentage per category). If appropriate, confidence intervals around the mean or the proportions will be presented.

The change from baseline in AUC of C-peptide at Month 6 (primary endpoint), computed using the [redacted], will be evaluated by means of Analysis of Covariance (ANCOVA) adjusting for the

baseline values, center, and baseline characteristics (age group, gender, and BMI) and their interactions with treatment. The adjusted estimated treatment differences between ladarixin and placebo at Month 6 will be presented together with the corresponding 95% confidence interval.

Primary endpoint will be tested to show superiority of ladarixin versus placebo. Independently from the results on the primary endpoint, analyses of descriptive nature will be performed on all secondary endpoints at the identified timepoints by means of descriptive statistics and by appropriate parametric tests. Change from baseline value and shift tables versus baseline will be summarized for all post-baseline visits.

AEs will be presented in terms of the number of AEs and incidence. Other safety parameters will be summarized by treatment at each available visit by means of descriptive statistics.

The Safety (SAF) and the Full Analysis Set (FAS) population will consist of all patients who will be randomized to receive at least one dose of the investigational product. Safety population will be analyzed according to the actual treatment received; FAS population will be analyzed according to the Intention to Treat (ITT) principle, i.e. by treatment allocation. The Per Protocol (PP) population will consist of all patients in the FAS population who do not have Major Protocol Deviations. Primary and secondary efficacy analyses will be conducted on the FAS population while SAF and PP populations will be used for safety and sensitivity analyses, respectively.

The Study Statistical Analysis Plan (SAP) with more technical and detailed elaboration of the principal features of statistical analyses will be finalized before the start of enrollment. Any deviation from the original statistical plan will be described in the Clinical Study Report.

2. BACKGROUND INFORMATION

Ladarixin is a novel small molecule that inhibits the biological activity of the CXC ligand 8 [CXCL8; formerly interleukin (IL)-8] through inhibition of the activation of CXCL8 receptors: CXCR1 and CXCR2. This specific inhibitor stems from a program of drug design of molecules intended to modulate chemokine action.

Original development plan of ladarixin was targeted to the dermatological area with a phase 2 study in bullous pemphigoid (BP), a rare dermatological disease. Following more recent promising results in mouse models of type 1 diabetes (T1D), development in recent onset T1D is being implemented.

Relevant pre-clinical, toxicological and clinical data are summarized below. Please also refer to the Investigator's Brochure for detailed information.

2.1 RELEVANT NON-CLINICAL PHARMACOLOGY

2.1.1 Mechanism of action and *in vitro* activity

Ladarixin is a novel, potent and specific inhibitor of the biological activity of the chemokine CXCL8. *In vitro* chemotaxis experiments showed that ladarixin, in the low nanomolar range, inhibits human polymorphonuclear leukocyte (PMN) migration following CXCL8 receptor activation. Chemotaxis of rodent PMN induced by mouse and rat counterparts of human CXCL8 was also inhibited, indicating that mice and rats are appropriate animal species for preclinical studies. Studies on the mechanism of action have shown that ladarixin is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2 [*Moriconi, 2007*]. The selectivity of ladarixin for CXCR1 and CXCR2 inhibition was proven by its lack of efficacy in PMN migration induced by fMLP or C5a and in human monocyte chemotaxis induced by CCL2.

Ladarixin (0.1 nM- 1 µM) is a potent inhibitor of IL-8-induced neutrophils extracellular trap (NET) release from isolated hPMN, with IC50 -value of ≈1 nM and ICmax -value of 1 µM. In line with the selectivity data observed in the chemotaxis assay, ladarixin (1 µM) did not affect C5a, fMLP and PMA-induced NET release. Ladarixin (1- 60 µg/mL) was also able to block IL-8-induced NET formation in human whole blood at a concentration-dependent manner with a complete inhibition at 60 µg/mL. The efficacy of ladarixin (1- 60 µg/mL) was evidenced in mCXCL1-mediated NET release in murine whole blood at a concentration-dependent manner. 60 µg/mL of ladarixin completely abolished NET release by murine PMN.

2.1.2 In vivo general studies

In vivo, ladarixin (4-16 mg/kg i.v.) prevented PMN infiltration (inhibition ranged from 38 to 80%) and tissue damage (inhibition ranged from 35 to 90%) in experimental models of ischemia/reperfusion injury of liver and brain in rats [*Garau, 2006*]. In addition, the effects of ladarixin were investigated in mouse models of acute and chronic smoke exposure. In the acute model of smoke exposure, ladarixin (3.75-15 mg/kg p.o.) reduced PMN infiltration by approximately 60%, whereas in the chronic disease model, the compound (7.5-15 mg/kg p.o.) prevented completely the development of pulmonary lesions.

The antiflogistic activity of orally administered ladarixin was demonstrated in the acute mouse model of cantharidin-induced ear inflammation where it reduced ear edema formation (16% of inhibition) and cell infiltration (34% of inhibition on lymphocytes T and 32% of inhibition on PMNs), and significantly reduced ear tissue levels of keratinocyte chemokine (37%), VEGF-A (24%) and TNF- ⟨ (44%) [*A0811/E*].

The efficacy of ladarixin in preventing PMN infiltration and tissue damage was also investigated in a passive transfer mouse model of BP. In this model, ladarixin was either co-injected with anti-mouse BP180 IgG (therapeutic treatment) or prior to disease induction (preventive treatment). Ladarixin, administered both intradermally and intraperitoneally, reduced both the clinical disease score and PMN recruitment in a dose-dependent manner and correlated with reduced levels of MPO. The clinical disease score and PMN migration were decreased by 90% and 60%, respectively, at the highest dose tested (16.7 mg/kg) [*A0811/E*].

2.1.3 Effects in models of type 1 diabetes

Ladarixin was tested in two independent models of Type 1 diabetes (T1D). In the ***Multiple Low Dose-Streptozotocin*** (MLD-STZ) ***model,*** oral administration of ladarixin (15mg/kg/day, daily for 14 days) significantly delayed the onset of diabetes in C57BL/6 mice particularly when drug administration initiated one day prior to the fist STZ injection. When ladarixin treatment started on day +5 from STZ, it still prolonged the diabetes-free period but to a lesser extent. When ladarixin treatment initiated after diabetes onset, no disease reversion was observed, however, the glycemic levels remained constantly lower in the ladarixin treated group as compared with the vehicle group for the whole 2 month observation period [*Citro, 2012a*].

Ladarixin was also tested after oral administration (15 mg/kg/day for 14 days) in ***Non-Obese Diabetic*** (NOD***) mice***. Treatment initiated at an early stage of the disease, i.e. in 4, 8 and 12 week-old animals ("prevention" setting) or in mice with early-onset hyperglycemia ("onset of diabetes" setting). In the "prevention" setting, ladarixin administration significantly reduced the incidence of T1D at all disease stages (regardless of the initial age at the time of treatment) compared to controls. In particular, when ladarixin was administered in animals at 8 weeks of age, T1D incidence reached 47% and 11% in the vehicle and ladarixin treated group, respectively (*p=0.029*). Animals presenting two consecutive blood glucose readings above 250 mg/dL in 24 hrs apart were randomized to receive either ladarixin or vehicle following the same dosing schedule as in the prevention setting. Strikingly, diabetes was reversed in 78% of ladarixin-treated NOD mice. Remission was rapid, within 72 h of treatment, and 23% reverted NOD mice remained diabetes free for >10 weeks after onset. The majority of NOD mice receiving vehicle, however, failed to undergo disease remission [*Citro, 2012b*]. Ladarixin was also able to reverse diabetes in 43%highly hyperglycemic NOD mice (glucose concentrations >350 mg/dL), but diabetes reversion did not demonstrate a long-term benefit. Mechanistically, ladarixin reduced PMN and macrophage infiltration in the pancreas and reduced insulitis as well as affected the leukocyte subpopulations that express CXCR2, among which one subpopulation of B-lymphocytes [*Citro, 2014*].

2.2 A SUMMARY OF TOXICOLOGY DATA

Ladarixin was tested for toxicity in rodent and non-rodent animal species after single and/or repeated dose administrations.

In several four-week studies performed in rats, ladarixin administration by oral gavage up to 200 mg/kg for 28 days followed by a 2-weeks recovery period did not cause any relevant toxicity and no mortality was observed. The immune system was not affected by the treatment with ladarixin. While liver and kidney weight increased in males in all dose groups, this occurred in females at 200 mg/kg. Hepatocellular hypertrophy was seen in the liver of all male treated groups and in females at 200 mg/kg that spontaneously resolved after 2 weeks. The no-observed-adverse-effect-level (NOAEL) was settled at 200 mg/kg/day.

Ladarixin oral treatment of rats for a 3 months' period with [redacted] and [redacted] mg/kg did not induce mortality or toxicologically relevant changes in body weight and food consumption, ophthalmoscopic, hematology, and urinalysis parameters. Changes in biochemistry were only minimal and occurred mostly in males. They included increased activity of some [redacted] (males at [redacted] mg/kg), dose-dependently decreased protein levels (males at all dose levels and females at [redacted] mg/kg in Week 8 only), and dose-dependent decreased cholesterol and phospholipid concentrations (males from [redacted] mg/kg). Microscopic examination revealed treatment-related findings in the [redacted]. In the liver, hepatocellular hypertrophy was noted in Week 8 in males at all treated groups and females at [redacted] mg/kg. [redacted] of the thyroid gland was seen at [redacted] mg/kg and regarded secondary to the liver hypertrophy. In the kidneys [redacted] was present at increased incidence in males at [redacted] mg/kg after 8 weeks of treatment only (interim sacrifice). This change in rates was sex (male)-specific not relevant for humans. After the 4-week recovery period, full recovery or a clear trend to recovery was noted across all findings.

Toxicology studies in dogs were conducted with up to 1 week administration by oral route, using a reduced dose from [redacted] to [redacted] mg/kg, due to poor tolerability and severe clinical signs, namely tremor, vomiting, uncoordinated movements. No macro or microscopic findings were present; organ weights

were not affected. In the 4-week oral toxicity study in dogs, ladarixin was administered up to the dose of [redacted] mg/kg. No mortality occurred. Vomiting was seen at [redacted] mg/kg. An increase in the absolute and corrected-for-heart rate QT interval was observed at the doses of [redacted] and [redacted] mg/kg. Inflammatory changes were seen in the duodenum of two males treated with [redacted] mg/kg. After 2 weeks all values returned to normal. It was concluded that the no observed-effect-level (NOEL) in dogs is [redacted] mg/kg/day.

In the 3-month study, the initial dose levels were [redacted] and [redacted] mg/kg/day. However, due to test item-related moribundity of one high dose male and changes in body weight and food intake of a few other animals in the group, the highest dose level was lowered from [redacted] to [redacted] mg/kg/day from Day 24 onward. At [redacted] mg/kg/day, one male and one female showed weight loss (up to -7%) and lower food intake during the first weeks of treatment and the clinical condition was reduced in Week 4. Food supplements were provided, which resulted in an improved clinical condition and normal body weight (gain) of these animals during the remainder of the treatment period after reduction of the dose level from [redacted] to [redacted] mg/kg/day. [redacted] was observed in males and females treated at [redacted] mg/kg/day in Weeks 4, 8 and/or 13, generally more pronounced in females. In addition, [redacted] were noted for males and (individual) females treated at [redacted] mg/kg/day in Weeks 4, 8 and/or 13. At the end of the Recovery period, only one male and one female at [redacted] mg/kg/day were still on study, and all [redacted] were returned to the normal limits. For one female at [redacted] mg/kg/day, [redacted], with [redacted] was recorded at moderate degree. There were no correlating clinical signs or necropsy alterations noted for this animal. Furthermore, no inflammation was recorded in the brain for any of the other dogs of the study; therefore, this change in a single dog at [redacted] mg/kg/day (mid dose) was regarded to be an incidental change, although rare and the origin of the lesions could not be determined. 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). Based on the results of this study, the no-observed-adverse effect level (NOAEL) was considered to be [redacted] mg/kg/day after 13 weeks of dosing.

Ladarixin testing for mutagenic potential gave negative results in three tests, the in vitro chromosomal aberration test, the Ames test, and the in vivo micronucleus assay test in rats bone marrow.

The phototoxicity potential of ladarixin was evaluated in Balb/c 3T3 cells. At dose levels of [redacted] µg/mL, in the absence or presence of UVA light, neither reductions of the cell layer nor changes in cell morphology were observed after treatment with ladarixin at any concentration tested. No reduction in neutral red uptake was seen in any treatment condition. The IC50 values were not calculable, thus the Photo Irritation Factor could not be determined. The Mean Photo Effect value was 0.009. The score values obtained with ladarixin (i.e. Photo Irritation Factor not calculable and Mean Photo Effect lower than 0.1) are predictive of no phototoxicity

A study of fertility and early embryonic development to implantation was performed in male and female Han Wistar rats at the daily oral dose of [redacted] and [redacted] mg/kg. There were no treatment-related effects in females in any dose levels and in males at [redacted] and [redacted] mg/kg/day. At the highest dose, effects were observed on spermatid counts, motility and morphology of spermatozoa. Changes were seen in the testes of some animals and consisted of degeneration/atrophy of the seminiferous tubules. According to the results obtained in this study, the fertility effects are likely associated with a stress effect, even if a secondary effect of the test item in the affected animals cannot be excluded with certainty. The NOEL was [redacted] mg/kg/day for males and [redacted] mg/kg/day for females.

In order to clarify the changes seen in the testes at [redacted] mg/kg/day, a specific study was designed in male Wistar and Sprague Dawley rats. Ladarixin was orally administered at the dose level of [redacted] mg/kg bw/day for a period of 6 weeks followed by a 4 weeks of treatment-free recovery period. In both strains, the treatment with ladarixin caused a [redacted] and a [redacted]. A [redacted], of normal complete sperms and of the mean sperm count in the epididymis was observed. After the recovery period, the decrease of the motility was not seen in Wistar rats whereas it was observed in Sprague Dawley rats. The [redacted] and the sperm analyses correlated with microscopic changes in the testes ([redacted]) and epididymides

([redacted]) of some treated animals of both strains. After 4 weeks of recovery period, none of the Wistar rats allocated to the recovery group presented any change in the testes or epididymides, while some of the Sprague-Dawley rats allocated to the recovery group had [redacted] of the [redacted] in the epididymis. The incidence of the testicular changes was very likely associated with the stress induced by the treatment with the test item. In both strains, a decrease in body weight and body weight gain (associated to a decrease in food consumption) was observed particularly in the first 2 weeks of the treatment period. Ruffled fur and weakness were also present in some treated rats and a reduction of thymus weight was observed in treated Wistar rats. All these changes indicate a condition of stress and/or distress that caused the testicular changes. At the end of the recovery period, in the Wistar rat there were no changes in any of the parameters examined as well as no changes in the testes and epididymides; in Sprague Dawleys rats, testes and epididymides changes were present in some animals at the end of the recovery period.

The prenatal developmental toxicity studies were conducted in Wistar Han rats and New Zealand White rabbits. In rats no maternal toxicity and no developmental toxicity were observed up to the highest dose level tested ([redacted] mg/kg/day). The NOAEL in rats was established as being at least [redacted] mg/kg/day for both the maternal and developmental toxicity.

In rabbits, no maternal toxicity was observed up to the highest dose level tested ([redacted] mg/kg/day). The NOAEL was established as being at least [redacted] mg/kg/day for both the maternal and developmental toxicity.

Carcinogenicity studies have not been performed.

According to the toxicology development program, a 6 and 9-month study in rats and dogs, respectively, have been recently completed to further support chronic administration in humans. After oral treatment with ladarixin for 6 months in rats at dose levels of [redacted] and [redacted] mg/kg/day, no mortality or changes were observed in food consumption, ophthalmoscopy, hematology and coagulation parameters in both sexes, and in clinical biochemistry and urinalysis in females. Only slight changes were observed in body weight in male and female and in clinical biochemistry parameters in males at ≥ [redacted] mg/kg/day and in urine parameters at [redacted] mg/kg/day. Morphologic non-adverse alterations in liver, kidneys and thyroid gland were recorded for males at ≥ [redacted] mg/kg/day, and in females at the high dose of [redacted] mg/kg/day only. The higher exposure levels to DF2156Y (acid form of ladarixin) in males may explain that males were more affected than females. Following the 4-week treatment-free period, all changes observed in the animals recovered to normal values with the exception of brown pigment (likely lipofuscin) in the cytoplasm of tubular epithelium of kidneys of males and females, and hepatocellular pigments (likely lipofuscin) in liver of males at the dose level of [redacted] mg/kg/day.

In the 9-month study, dogs were treated orally with ladarixin at doses of [redacted] and [redacted] mg/kg. No mortality occurred during the study period. The test item was clinically well tolerated up to the high dose of [redacted] mg/kg/day, as all animals were in a good general condition until the end of study. ECG recordings in Weeks 13, 26 and 38 revealed a dose-related increase in (corrected) QT intervals in both males and females at [redacted] and [redacted] mg/kg/day at 2 to 4 hours after dosing, most prominently in Week 38. In addition, heart rates were increased in [redacted] mg/kg/day treated males and females at 2 and/or 4 hours post-dose in Weeks 13, 26 and/or 38 (females were more affected than males). The QT prolongation and increased heart rates in females at [redacted] mg/kg/day were considered to be adverse, based on the maximum increase in QTcF interval of 25.5 msec in Week 38 on a group mean basis, and based on the magnitude of change for heart rate. The NOAEL for females was considered to be [redacted] mg/kg/day after 9 months of dosing. In males, the NOAEL was concluded to be at least [redacted] mg/kg/day.

In conclusion, based on the toxicology testing performed through in vivo studies, the dose of [redacted] mg/kg appears to represent the NOAEL in rats for general toxicity while in dogs in the 9-month oral toxicity study, the NOAEL was established at [redacted] mg/kg. In rats, [redacted] mg/kg is the NOAEL for developmental toxicity while in rabbits it was [redacted] mg/kg.

The toxicity studies conducted up to date support the dose schedule proposed for this study.

2.3 PHARMACOKINETICS AND PRODUCT METABOLISM

The pharmacokinetics of ladarixin (DF2156Y) was studied in rats and mice after single i.v. and oral administration and after multiple oral administrations in rats and dogs within the toxicity studies.

DF2156Y is almost completely absorbed after oral administration in rats with an absolute bioavailability higher than 90%. DF2156Y is slowly eliminated from plasma in all the three species tested (t½ ranging from 25 hours to 30 hours). Gender differences in pharmacokinetic profile after oral administration (slightly lower exposure in females than in males) were observed in rats but not in dogs.

A metabolite of DF2156Y named DF2108Y (R enantiomer) was identified, using a non-chiral analytical method, in both rats and dogs. On the basis of the chiral analytical determination, the S enantiomer (DF2227Y) was noted in the plasma of both rats and dogs. The *in vivo* interconversion of DF2108Y into DF2227Y was about 90% in rats.

In male and female rats, DF2227Y was the major and long-lasting circulating metabolite. After repeated daily oral administration of ladarixin, this metabolite showed an accumulation ratio ranging between 1.4 and 2.4. After daily oral administration of ladarixin at 200 mg/kg for 6 weeks, the parent compound and the metabolites showed comparable exposure in Wistar and Sprague Dawley rats either at day 1 or after repeated administrations.

In humans, a single oral administration of ladarixin (25 to 400 mg) provided quantifiable plasma concentration of DF2156Y within 1 hour, with peak concentration being reached between 1 and 3 hours. After a single dose, ladarixin was excreted mainly as unmodified in human urine. The presence of the two metabolites found in animal species (DF2108Y and DF2227Y) was confirmed in humans. Dose proportionality over the entire dose range investigated was observed as well as $t_{½}$ (11-19 h) remained constant across the range of doses evaluated. Renal clearance accounted for approximately 60-80% of the total clearance of DF2156Y. The dose proportionality for the main PK parameters was also seen for the two metabolites (DF2108Y and DF2227Y).

After multiple doses, DF2156Y reached the steady state around Day 5 and 6 following 50, 100, 200, 300 and 400 mg b.i.d. Systemic exposure to DF2156Y, DF2108Y and DF2227Y appeared to increase in a dose-proportional manner. The clearance and the volume of distribution of DF2156Y appeared to be dose-independent on both Days 1 and 8 for all doses. Similarly, $t_{½}$ (ranging from 11 to 18 h) remained constant on Day 1 and on Day 8 across the range of doses evaluated.

Co-administration of DF2156Y increased the exposure and urinary excretion of tolbutamide while decreasing the exposure and the urinary excretion of tolbutamide metabolites. Plasma tolbutamide AUC values increased by about 2-fold and the AUCs of the metabolites decreased by about 2-fold. Thus, ladarixin can be classified as a moderate inhibitor of CYP2C9.

In vitro ladarixin appeared to be a strong inhibitor of the enzyme CYP2C9 in humans (83%) and a moderate inhibitor in rats (51%). No inhibition was observed in dogs.

No inhibition was detected in any species with regard to CYP2D6.

Also, no inhibition was shown for CYP 1A2, 2C19, and 2E1 with respect to humans and dogs, but a moderate inhibition was shown in rats (about 50%). As to CYP3A4, a slight inhibition was seen in all species which was higher than 20% only in rats (about 28%). In humans, co-administration of ladarixin (200 mg twice a day for 5 days) approximately doubled the exposure to tolbutamide (probe for CYP2C9 isoenzyme).

Preliminary *in-vitro* protein binding studies showed that ladarixin is highly bound to plasma proteins; binding varies according to ladarixin concentrations and appears to be saturable: mice 97.7%, rats 91.8-99.4%, dogs 80.4-99.5% and humans 93.0-99.9%. Repeated at 400 mg b.i.d. administrations in humans confirmed the very high binding to plasma proteins (>99.99%).

2.4 A SUMMARY OF CLINICAL DATA

Clinical development includes three phase 1 PK and tolerability studies with single (25 to 400 mg – MEX0108) and multiple (50 to 400 mg twice a day up to 6 days, MEX0109, MEX0110) ascending dose oral administration. The first multiple ascending dose study (MEX0109) also included the evaluation of

potential interaction between ladarixin 200 mg and tolbutamide. A phase 2 efficacy and safety study was conducted with 150 mg twice a day (for 14 consecutive days) oral ladarixin in patients with moderately active BP. A phase 2 efficacy and safety study) was also conducted with 400 mg twice a day (3 cycles of 14 days on-14 days off treatment) in new onset T1D patients and recently published [MEX0114 CSR, and Piemonti L, 2022].

To date, a total of 195 subjects were involved in clinical trials, of whom 143 (89 healthy volunteers 50 T1D patients and 4 patients with BP) were exposed to ladarixin.

2.4.1 Pharmacokinetics and product metabolism in humans

Three phase 1 PK/safety/tolerability studies in healthy male volunteers were performed which included single (25 to 400 mg – MEX0108) and multiple (50 to 400 mg twice a day up to 6 days, MEX0109, MEX0110) ascending dose oral administration. An interaction evaluation for the 200 mg dose was also included in the first multiple ascending dose study. Assay of ladarixin and its metabolite DF2108Y and DF2227Y was performed in samples collected at steady state conditions, on day 5 and 8 of treatment in 3 BP patients. PK results are discussed in detail in IB version 7 Final 09 April 2020.

2.4.2 Efficacy

A phase 2, multicenter, single arm, pilot study was initiated to assess the safety and efficacy of ladarixin in patients with moderately active BP [MEX0111]. The compound was given by the oral route at 150 mg twice a day (maximum of 14 days), a relatively low dose that could be selected in line to the phase 1 trial that had been completed at that time of protocol implementation (treatment up to 200 mg twice a day). Four male patients aged 65-75 years, with either newly diagnosed or relapsing BP of mild to moderate degree, were enrolled at one Italian and three German sites. Of the 4 patients enrolled, only one completed the 14-day treatment period. The remaining 3 patients were withdrawn from the study (one patient due to treatment failure and the other 2 patients because of admission to rescue therapy). No disease remission was observed in the only patient who completed the treatment and the study was prematurely discontinued due to lack of activity.

A phase 2, multicenter, randomized, double-blind, placebo-controlled study was completed in patients with new onset T1D (first insulin within 100 days from randomization) [*MEX0114 CSR, and Piemonti L, 2022*]. The trial involved 8 sites in Italy, Germany and Belgium. Seventy-six patients (31 females, 45 males) with mean age of about 27 years have been randomized (2:1) to receive either ladarixin (400 mg twice a day for 3 cycles of 14 days on/14 days off) or placebo. The primary endpoint was the area under the curve (AUC) of C-peptide measured during a Mixed Meal Tolerance Test (MMTT) performed on week 13 from randomization. Follow-up was extended up to one year. Out of 85 patients enrolled (consent signed), 76 were randomized, 50 received ladarixin and 26 were allocated to placebo; 48 patients in the ladarixin group and 25 in the placebo completed the trial (week 52 observation).

The primary and secondary analyses were performed on the Intention to Treat (ITT) population. The primary efficacy analysis of log (AUC [0-2 hrs]+1) of C-peptide response to MMTT showed no statistically significant difference (p=0.3303) between the ladarixin and placebo group at Week 13. Overall, there were no significant differences between the treatment groups for other secondary endpoints, such as C-peptide at other time-points, overall insulin requirement, glucose levels and glucagon levels during the study. On the other hand, the proportion of patients with HbA1c <7% and absence of episodes of severe hypoglycemia was significantly higher at Week 26 (p=0.0248) in patients receiving ladarixin as compared with placebo (whole ITT population).

When considering a pre-specified subgroup analysis of patients having fasting C-peptide at screening <median value of the whole ITT population, a statistically significant difference in favor of ladarixin for both the C-peptide log($AUC_{[0\text{-}2\ hrs]}$+1) (p=0.0411) and the $AUC_{(15\text{-}120\ min)}$ (p=0.031) at Week 26 was evidenced. Moreover, the statistically significant comparison between treatment groups at Week 26 in this subgroup of patients was reinforced by data relevant to the proportion of patients with HbA1c<7% and absence of severe hypoglycemic events (p=0.0074) [*MEX0114 CSR, and Piemonti L, 2022*].

2.4.3 Safety

A total of 89 male healthy subjects aged 18 to 52 years, 4 male BP patients aged 65-75 years, and 50

T1D patients aged 18-46 years were exposed to ladarixin in the clinical trials conducted to date. Exposure included single (25 to 400 mg) as well as repeated (50 to 400 mg twice a day up to 6 days) oral administrations in healthy volunteers, 150 mg twice a day up to a maximum of 14 days in BP patients, and 400 mg twice a day for 3 cycles of 14 days on-14 days off in T1D patients. In a subset of subjects, ladarixin (200 mg twice a day) was co-administered with tolbutamide.

Overall, ladarixin was safe and well tolerated. No deaths or Serious Adverse Events (SAEs) were reported from phase 1/2 trials, as well as no safety concerns were raised during co-administration of ladarixin with tolbutamide. All the Adverse Events (AEs) were mild or moderate in intensity.

Toxicology and safety pharmacology in animals pointed out the cardiovascular system (QT prolongation) as potential safety concern in humans. Apparent isolated prolongations of the QTcF were observed at some time-points in phase 1 studies; core laboratory analysis of the ECG readings, including the review of changes in the QTcF intervals and of the pharmacokinetic-pharmacodynamic relationship, revealed no clinically significant effect of ladarixin on cardiac repolarization.

Safety was confirmed in elderly BP patients treated with several concomitant medications. Three out of 4 patients reported mild AEs which were all considered related to ladarixin with the exception of one AE in one patient (hypereosinophilia). Two patients had a series of abnormal ECGs at baseline that continued throughout the study neither of which were considered clinically significant by the investigator, supporting the cardiosafety in elderly patients. There were no other safety findings considered clinically significant by the investigator. There were no deaths, SAEs, or discontinuations from the study due to AEs.

The phase 2 trial in new onset T1D further extended the experience with ladarixin. The frequency and profile of AEs/Adverse Drug Reactions (ADRs) was similar in patients receiving ladarixin or placebo A total of 37 patients (74.0%) in the ladarixin group and 22 patients (84.6%) in the placebo group experienced TEAEs during the study. The most common Treatment Emergent AEs (TEAEs) presented by primary SOCs were infections and infestations (about 46% in both groups), followed by gastrointestinal disorders (about 35%) and nervous system disorders (34.0% ladarixin vs 26.9% placebo). The majority of the TEAEs reported in the study were considered mild in severity. A total of 3 patients in the ladarixin group and 1 patient in the placebo group reported Treatment Emergent SAEs (TESAEs). One patient in the ladarixin group reported 2 TESAEs (hyperglycemia and mental disorder); and 1 patient each in the ladarixin group reported TESAEs of gastrointestinal disorder and clavicle fracture, respectively. One patient in the placebo group reported TESAE of laceration. None of the SAEs were related to the study treatment. One patient in the ladarixin group was discontinued from the study treatment due to AEs of alanine aminotransferase increased and aspartate aminotransferase increased, and 1 patient in the placebo group was discontinued from the study treatment due to an AE of rash. Twenty patients (40.0%) in the ladarixin group had 52 ADRs and 8 patients (30.8%) in the placebo group had 17 ADRs during the study.

Cumulative **ADRs**, i.e. TEAEs judged at least possibly related to ladarixin, are presented in the Table below.

Cumulative summary of Adverse Drug Reactions (number of events and frequency) in completed phase 1 and phase 2 studies with ladarixin.

Cumulative Adverse Drug Reactions - Number of events by Terms			
Study code ref./ System Organ Class (SOC) MedDRA Preferred Term (PT)		Number of events	Frequency
Gastrointestinal disorders		48	49.48%
MEX0114	Abdominal discomfort	1	1.03%
MEX0109 - Part A MEX0110 MEX0114	Abdominal pain	3	3.09%
MEX0114	Abdominal pain upper	2	2.06%
MEX0114	Constipation	3	3.09%
MEX0109 - Part A MEX0114	Diarrhea	3	3.09%
MEX0109 - Part A	Dry mouth	2	2.06%

Cumulative Adverse Drug Reactions - Number of events by Terms			
Study code ref./ System Organ Class (SOC) MedDRA Preferred Term (PT)		**Number of events**	**Frequency**
MEX0111			
MEX0109 - Part A MEX0109 - Part C MEX0111 MEX0114	Dyspepsia	15	15,46%
MEX0109 - Part A MEX0114	Dysphagia	3	3.09%
MEX0114	Faeces hard	1	1.03%
MEX0110	Flatulence	1	1.03%
MEX0114	Hyperchlorhydria	2	2.06%
MEX0114	Gastrointestinal reflux disease	1	1.03%
MEX0108	Mouth ulceration	2	2.06%
MEX0108 MEX0110 MEX0114 LDX0219	Nausea	6	6.18%
MEX0109 - Part A	Oral Pain	1	1.03%
MEX0109 - Part A LDX0219	Vomiting	2	2.06%
General disorders and administration site conditions		**3**	3.09%
MEX0114	Asthenia	1	1.03%
MEX0109 - Part A	Feeling hot	1	1.03%
MEX0114	Sensation of foreign body	1	1.03%
Infections and infestations		**6**	**6.18%**
MEX0108 MEX0114	Oral herpes	3	3.09%
MEX0114	Viral upper respiratory tract infection	3	3.09%
Injury, poisoning and procedural complications		**1**	1.03%
MEX0114	Fall	1	1.03%
Investigations		**4**	**4.12%**
MEX0114	Alanine aminotransferase increased	1	1.03%
MEX0114	Aspartate aminotransferase increased	2	2.06%
MEX0109 - Part A	Liver function test abnormal	1	1.03%
Musculoskeletal and connective tissue disorders		**2**	2.06%
MEX0114	Muscle spasms	1	1.03%
MEX0109 - Part A	Tendonitis	1	1.03%
Nervous system disorders		**28**	**28.86%**
MEX0108 MEX0109 - Part A MEX0114	Dizziness	4	4.12%
MEX0108 MEX0109 - Part A MEX0109 - Part C MEX0110 MEX0111 MEX0114 LDX0219	Headache	23	23.71%
MEX0109 - Part A	Tremor	1	1.03%
Psychiatric disorders		**2**	**2.06%**
MEX0114	Emotional distress	1	1.03%
MEX0114	Insomnia	1	1.03%
Respiratory, thoracic and mediastinal disorders		**3**	**3.09%**
MEX0110	Oropharyngeal discomfort	1	1.03%
MEX0114	Oropharyngeal pain	2	2.06%
TOTAL REPORTS		**97**	
Subjects exposed to ladarixin* with at least an ADR		**53**	
***: figures include subjects exposed to ladarixin plus tolbutamide (MEX0109 - Part C) and excluding placebo.**			

The system organ classes most frequently (>10%) affected by ADRs were:

Gastrointestinal Disorders: (about 50%) including dyspepsia, dysphagia, abdominal pain, mouth ulceration, nausea.

Nervous System Disorders: (about 30%) including headache, dizziness.

Dyspepsia and dysphagia were both considered as definitely related to ladarixin because they occurred shortly after administration; also, dyspepsia consistently recurred on subsequent drug administration. All the ADRs resolved.

For more information refer to the latest version of the Investigator's Brochure.

2.5 DISEASE REVIEW AND STUDY RATIONALE

T1D is an organ-specific autoimmune disease of the insulin-producing β-cells. The onset of the disease typically occurs before adulthood and seriously affects a person's quality of life. Incidence of T1D is constantly increasing globally and particularly in the Western world [*Atkinson, 2014; Waldron-Lynch, 2011, Gregory 2022*].

T1D is treated with life-long daily exogenous insulin injections and requires constant monitoring of blood glucose levels. However, even optimization of glucose control through the most recent technologies cannot adequately substitute for the finely tuned normal balance of the glucose levels provided by the pancreatic beta cells. Pancreatic islets or whole pancreas transplantation is a curative option for patients with T1D but has limited success due to graft loss and immunosuppression derived side-effects. Despite marked improvements in diabetes care in recent years, insulin-dependent diabetes results in secondary long-term complications and is one of the leading causes of end-stage renal disease, blindness and amputation. Additionally, hypoglycemia unawareness is a serious consequence of recurrent hypoglycemia often requiring emergency care [*Atkinson, 2014; Waldron-Lynch, 2011*].

Maintenance of residual β-cell function (as measured by C-peptide response) was demonstrated to be associated with reduced rate of microvascular complications and hypoglycemia, improved quality of life, and overall reduction in morbidity and associated management costs. Therefore, pharmacological approaches aimed at controlling the autoimmune response and restoring self-tolerance to pancreatic β-cells had attracted clinical/scientific interest. [*Steffes, 2003; Barnard, 2010; Waldron-Lynch, 2011*].

Among these, rituximab, anti-CD3-specific monoclonal antibodies, GAD65, DiaPep277 have progressed to phase 3 clinical trials. Other agents, including cytokines modulators such as anti-TNF or anti-IL1, are under clinical evaluation [Ludvigson, 2008; MacroGenics, 2010; Mastrandrea, 2009; Peskovitz, 2009; Waldron-Lynch, 2011; Raz, 2014].

In November 2022, FDA approved the clinical use of an anti-CD3 monoclonal antibody to delay the onset of T1D in adults and pediatric patients 8 years and older, who currently have stage 2 type 1 diabetes (multiple AAb positive with glucose intolerance) [Herold KC, et al 2019].

However, this therapy only delays disease onset. Currently, new strategies are being evaluated that combine agents targeting sequential arms of the immune and inflammatory response involved in β-cell destruction. In this regard, IL-8 appears to be an important mediator in the progression of T1D. Production and secretion of pro-inflammatory IL-8 has been demonstrated from human pancreatic islets upon enterovirus infections, and LPS-induced production of IL-8 by neutrophils is increased in pre-diabetic and diabetic patients. In addition, circulating levels of IL-8 were elevated in children with T1D compared to non-diabetic controls. Levels of IL-8 correlates with glycemic control, higher level being associated to poorer or unfavorable glucose control [*Aboelasrar, 2012; Erbağci, 2001; Glowacka, 2002; Diana, 2014; Van Sickle, 2009*]

On the basis of these observations, the modulation or inhibition of CXCL8 activity is considered a valid approach to control the progression of T1D. Results obtained with ladarixin in mouse models of T1D, and particularly reversal of "diabetes" in the NOD mice, clearly shows the ability of this CXC1/2 inhibitor to protect β-cells and either prevent or delay the progression of hyperglycemia. Results obtained in the phase 2 trial in new onset T1D patients did not show a statistically significant difference (p=0.337) between ladarixin-treated and placebo groups in the primary endpoint (2-hour AUC of C-peptide response to MMTT at week 13). Notwithstanding, in a pre-specified analysis of the subpopulation with "severe T1D onset" [expressed as fasting C-peptide <0.205 nmol/L at baseline (median value of trial population)] ladarixin treated patients showed better C-peptide preservation compared to placebo treated patients (p=0.111), reaching a statistical significance at week 26 (p=0.041). Moreover, the proportion of patients with HbA1c <7% and absence of episodes of severe hypoglycemia

was significantly higher at Week 26 (p=0.0248) in patients receiving ladarixin as compared with placebo (whole ITT population). The effect of ladarixin was reinforced when considering the subgroup analysis of patients with "severe" T1D onset where the proportion of patients with HbA1c <7% and absence of severe hypoglycemic events was significantly higher in the ladarixin group as compared to placebo (p=0.0074).

[redacted]

2.5.1 Selection of dose and treatment schedule in the study

The proposed dose in this clinical study is 400 mg b.i.d. oral ladarixin twice a day for 13 cycles of 14 days on - 14 days off.

The oral route has been selected based on the very good oral bioavailability of ladarixin, the long half-life and its good tolerability when given by this route.

The 400 mg b.i.d. dosage is supported by both preclinical studies and efficacy/safety results from phase 2 clinical study MEX0114 in T1D adult patients and recently published *[Piemonti L, 2022]*.

Actually, this dose was already established to be safe in phase 1 clinical studies in adult male healthy subjects.

The resulting average steady state plasma concentration of the ladarixin unbound fraction should ensure full inhibition of PMN migration, considering that the in vitro IC_{50} is in the range of 1 ng/mL.

A regimen of 14-days on and 14-days off improved beta cell preservation in a fraction of patients in the phase 2 study *[MEX0114 CSR, and Piemonti L, 2022]*, in line with the initial observations in NOD mice *[Citro, 2015]*. In ladarixin-treated NOD mice with recent onset-diabetes, the efficacy of the compound was more pronounced during the 14 days of treatment (in these animals, the average of non-fasting glycaemia was below 200 mg/dl). Blood glucose levels progressively increased in 77% of NOD mice that showed remission after treatment discontinuation, suggesting that neutrophil recruitment islet inflammation rebounded once drug levels dropped. This was further supported by the fact that a second cycle of ladarixin treatment in diabetic NOD mice was able to re-reverse diabetes in 67% of mice, thus supporting the concept that repeated cycles of treatment are necessary for the appropriate management of this chronic condition [*Citro 2015*].

Furthermore, ladarixin prevented diabetes in a model of β-cell inflammatory injury induced by multiple low-dose streptozotocin, and the treatment for 14 days starting 1 day before the induction of diabetes appeared the most efficient in prolonging the median diabetes-free time compared to regimens in which the treatment was only administered for 7 days and/or was started few days after the induction of diabetes by streptozotocin [*Citro 2015*].

The toxicity studies conducted to date, which include the 6 and 9-month study in rats and dogs, respectively, support the dose and treatment duration proposed for this study.

The dose regimen to be used in adults (> 18 years of age) will be used also in adolescent (14-17 year-old) T1D patients. These patients are usually managed as young adults and thus no therapeutic dosage adjustment is expected to be needed [*Momper e al., 2013*]. A physiologically based pharmacokinetic modelling study has been performed using existing preclinical and clinical data on healthy adult volunteers, which has confirmed the 400 mg adult dose twice per day.

2.5.2 Alternative treatments

To date, there are no drugs that can cure T1D after the onset of symptomatic disease. However, any immunomodulation that preserves β-cell function as measured by the preservation of the remaining C-peptide levels, has shown to result in improved metabolic control and reduced microvascular complications *[Palmer, 2004]*. Despite recent advances in the number of immunomodulatory agents available and clinical trials in T1D, including drugs targeted to the cytokine system, only a few

approaches tested to date, such as an anti-CD3, DiaPep277 injections or other immunomodulatory drugs, have provided some evidence of beta cell preservation . Recently, FDA approved an anti-CD3 antibody (teplizumab) to delay the onset of T1D in adults and pediatric patients 8 years and older who currently have stage 2 type 1 diabetes (multiple AAb positive with glucose intolerance) *[Herold KC, et al N Engl J Med. 2019 Aug 15;381(7):603-613].*

Because of this, patients not willing to participate in the study may either be offered to participate in another trial of experimental treatments or will not be offered any specific alternative treatment other than insulin management.

All patients, regardless of study participation, will receive the standard of optimal care for a recent onset T1D individual.

2.5.3 Risk - benefit evaluation

2.5.3.1 Risk related to the ladarixin

Results from preclinical studies, including toxicity evaluation, support the level of drug exposure planned in this study.

Phase 1 and 2 clinical experiences with doses as high as that planned in this study provides evidence of the safety of ladarixin. The safety was confirmed in the phase 2 trials; indeed, no safety concerns were raised either in elderly patients who were on several concomitant medications due to chronic disease, or in patients with new onset T1D. No Serious Unexpected Suspected Adverse Reaction (SUSAR) or death occurred, and all the AEs encountered were mild to moderate in intensity.

Any possible risk derived from the administration of ladarixin in the specific population involved in this study will be minimized by integrated monitoring which includes clinical observations, laboratory tests and ECG readings scheduled at pre-planned interval (see Section 6.4.2 - discontinuation criteria for details).

Ladarixin inhibits enzyme CYP2C9 and may affect plasma levels of those drugs that are metabolized by this system. Restrictions of use and monitoring procedures (see Section 6.6.2 for details) will limit any possible risks derived from potential metabolic interactions.

2.5.3.2 Blood sampling

Participation in the study will require additional blood samplings other than the routine ones. In particular:

- a blood sample (about 10 mL) will be obtained at baseline, end of 3rd (Week 11), 6th (Week 23) and 9th (Week 35) treatment cycles, Month 12 and Month 24 for Safety Laboratory Test;
- blood samples for diagnostic auto-ab test and fasting C-Peptide (maximum 15 mL) and for baseline HbA1c, HLA DR-DQ haplotypes, Proinsulin, IL-8, IL-6, hs-CRP, miRNA-375, miRNA signature, NET, MMTT C-peptide and glucose (maximum 60 mL) will be taken from screening to baseline;
- blood samples for HbA1c, IL-8, IL-6, hs-CRP, miRNA-375, NET, MMTT C-peptide and glucose (maximum 60 mL) will be also taken at Month 6, 12, 18 and 24;
- blood samples for full PK analysis in the subset of adolescents will be taken on day 1 (n=12; 36 mL) and 14 of the [redacted] treatment cycle and then at 24, 48, 72 hours (total samples =15; 45 mL) after the last dose (morning dose) of this cycle. Additional blood samples (3 mL each) will be taken in the whole population within 96 hours post-last IMP dose in at least 2 treatment cycles.

Blood sampling will be done through a peripheral vein and can cause weakness and/or swelling, pain, redness, bruising or infections (infections rarely occur) at the site where the needle is inserted.

2.5.3.3 Mixed Meal Tolerance Test

Participation in the study requires testing for β-cell function through an MMTT to be performed at baseline (pre-dose) and then at the visits at month 6, 12, 18 and 24. This is not a routine test in patients with diabetes but has become a standard for the purpose of research studies and is performed frequently by investigational sites.

Apart from the risks due to blood samplings detailed above, the MMTT requires an overnight fast that may potentially increase the risk of a hypoglycemic event. However, detailed instruction for patient management provided by the Investigator will minimize the risk of metabolic unbalance.

2.5.3.4 Continuous Glucose Monitoring (CGM)

The [redacted] Continuous Glucose Monitoring System ([redacted]) will be used to perform a CGM. The sensor will be positioned subcutaneously at the site at least 10 days before the concerned visits (baseline, Month 6, 12, 18 and 24) and removed during the visit, as per Study Flowchart (see Appendix 14.2). It is recommended to place the device for the 1st time during the Screening Visit. Then CGM sensor implantation and set-up can occur at Week 23 Visit if the visit is scheduled 10 days before Week 26.

CGM via sensor positioning is commonly used in diabetic patients to monitor their glucose level.

Patients can't wear CGM (sensor, transmitter, receiver, or smart device) for magnetic resonance imaging, computed tomography scan, or high-frequency electrical heat (diathermy) treatment. Some skin care products, such as sunscreens and insect repellents, can make the plastic used in [redacted] crack. The CGM sensor placed under the skin could cause some skin irritation or skin infection if it is not inserted correctly. However, at the beginning of the study and throughout its duration the study investigator will take all necessary steps for using the device correctly and calibrating it regularly and to minimize any risk associated with the sensor.

If the patient needs assistance with sensor placement/replacement, an ad-hoc visit should be performed.

2.5.3.5 Potential benefit

To the patients: One third of the patients will be assigned to the placebo arm and will therefore obtain no disease benefit. The patients assigned to receive ladarixin may possibly benefit with control of disease progression, but this is to be ascertained.

All the patients will benefit from increased scrutiny and monitoring that comes with being part of a clinical trial.

To society: This study may identify a useful medication that may help control the progression of T1D in patients presenting a more "severe" onset of the disease (expressed as fasting C-peptide <0.205 nmol/L at baseline).

3. OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN

This study was initially designed as a phase III trial with co-primary endpoints assessing change from baseline to 12 months in (a) AUC for C-peptide and (b) HbA1c, and as such has been approved by competent authorities and local IRB, and currently ongoing. As a result of recruitment difficulties, it has been redesigned and now aims [redacted], showing that ladarixin treatment may improve β-cell function as assessed by AUC for C-peptide after 6 months of treatment in patients characterized by a low residual beta-cell function at baseline. The revised trial requires a smaller sample size than the original design, aiming to improve its feasibility.

3.1 STUDY OBJECTIVES

The objective of this clinical trial is to assess whether ladarixin treatment has an effect to preserve β-cell function and delaying the progression of recent onset T1D in adolescent and adult patients. The safety of ladarixin in the specific clinical setting will be also evaluated.

3.2 STUDY ADMINISTRATIVE STRUCTURES, STAFF AND RESPONSIBILITY

This study will be performed at up to an estimated 50 - 60 study centers in EU, US and in other countries, if appropriate. At each study center, the Principal Investigator (PI) will be responsible for ensuring that the investigation is conducted according to the signed Investigator agreement, the protocol, GCP guidelines, and local regulations.

The PI at each study center will be responsible for the management of the study, which will consist of maintaining the study file and the patient records, corresponding with the IRB (specifically for US), reporting SAEs within required timelines, completing the electronic case report forms (eCRFs) and any other study document.

The PI is responsible for supervising any individual or party to whom he/she delegates trial related duties and functions conducted at the trial site. The PI/institution should ensure that any individual or party that performs trial related duties and functions is qualified to perform those trial related duties and functions and should implement procedures to ensure the integrity of the trial related duties and functions performed and any data generated. Similarly, it is the responsibility of the PI to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

The PI will maintain a list of delegated responsibility detailing the various study tasks to be performed by each member of his/her study staff. Each staff member should sign the list, in agreement to their performing each of the tasks delegated to them on the list. Where reference is made in this protocol to the PI, either the PI and/or one or more delegated members of his/her staff are meant, according to the list of delegated responsibility.

3.3 OVERALL STUDY DESIGN

The study will be a phase 2, multicenter, double-blind, placebo-controlled study.

It has been designed to further evaluate whether ladarixin has an effect to preserve β-cell function and slowing-down the progression of T1D in patients with a more severe disease presentation.

It will involve approximately 130-140 patients with recent onset T1D, to include up to an estimated 15-20% adolescents (14-17 years). Patients will be randomly (2:1) assigned to receive either ladarixin treatment (400 mg b.i.d. for 13 cycles of 14 days on/14 days off - treatment group) or matched placebo (control group). The two groups will be balanced within centers.

Recruitment will be competitive among the study sites, until the planned number of patients is randomized. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference among study sites in the rate and timing of patient referral. Each site will recruit patients as rapidly as possible up to a maximum of 33 patients.

Each patient will be involved in the study for a run-in period (Screening and Baseline assessments) followed by a randomization visit and a post-randomization period up to 24 months from the 1st Investigational Medicinal Product (IMP) dose, as per details below:

- A run-in period including 2 or more visits scheduled to allow the IMP to start within 180 days from 1st insulin injection"; this includes the time required to confirm the diagnosis, assess fasting C-peptide and re-assess it once in case the first detection is >0.205 nmol/L, and perform baseline assessment;
- A randomization visit;
- A treatment period of 12 months, to start within 3 days from randomization and to include PK visits (2 visits at Day 1 and Day 14 and then 3 visits at 24, 48, 72 hours after the last dose (morning dose) in the [redacted] treatment cycle in the subset of adolescents; at least 2 visits in the whole population within 96 hours after the last IMP dose in at least 2 treatment cycle;
- 5 visits during treatment scheduled at the end of the [redacted] (Week [redacted]) and [redacted] (Week [redacted]) treatment cycles, at Month [redacted] (Week [redacted]) from the 1st IMP dose, at the end of the [redacted] (Week [redacted]) treatment cycle and at Month [redacted] (Week [redacted]) from the 1st dose of study treatment;
- 2 follow-up visits scheduled at Month 18 (week 78) and Month 24 (week 104) from the 1st dose of study treatment.

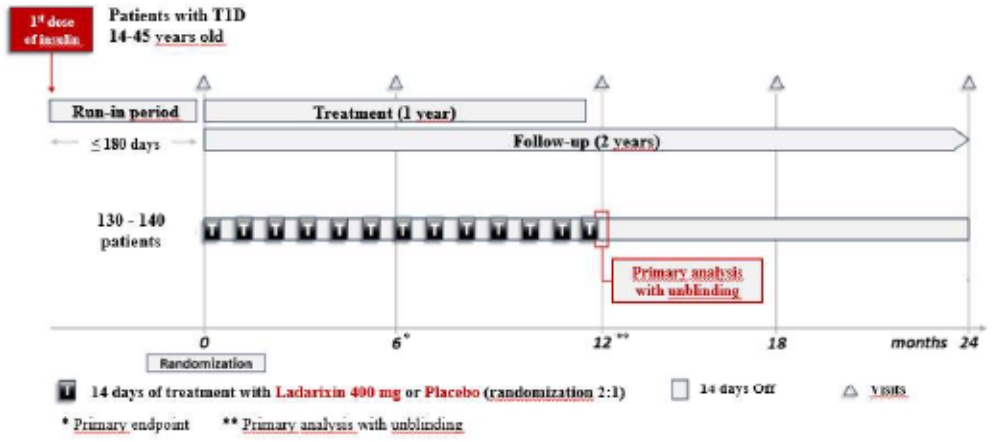
Figure 1 shows the major milestones of the study design (run-in period, treatment, follow-up visits).

Treatment administration should start within maximum 180 days from the 1st insulin dose and will continue up to one year. The proposed treatment duration is aimed to cover the time period recommended by the FDA for assessing the primary efficacy endpoint (FDA, 2008), and it is in line with the purpose of the trial in showing a preservation of β-cells at 6 months, to be confirmed at month 12.

The time frame for the primary endpoint (change from baseline in 2-hour AUC of C-peptide response to an MMTT) has been set at Month 6 (Week 26), with an additional secondary endpoint at M12 in order to blindly evaluate the potential of ladarixin on a long-term effects. Follow-up is extended up to 24 months according to the FDA recommendations (FDA, 2008) to evaluate a longer term outcome and ensure monitoring for at least 2 years of potential persistency of glycemic benefit (e.g. reduction of HbA1c, daily insulin requirement, hypoglycemic events and the control of the overall glycemic variability).

The study will proceed under double-blind conditions up to month 12 visit of the last patient randomized (or up to lost in follow-up). After this timepoint, the blind will be broken and the DB will unblinded and all endpoints, including the 6-m primary endpoint, will be analyzed. Ongoing patients will enter into an open-label follow-up up to additional 12 months, total period 24-m after first IMP administration. Thereafter, This approach will allow us to anticipate access to efficacy data without significantly affecting data integrity, since the treatment period will be blinded.

FIG.1: Design of Study LDX0319



3.4 STUDY TIME TABLE

Overall study timelines are reported below.

Actual starting date (first-patient-in): 12 Jan 2021
Projected completion of patient accrual (last-patient-in): [redacted]
Projected study end date (last-patient-last-visit): [redacted]

3.5 END OF STUDY

For the purpose of this trial, the End of Study is defined as the date of the last visit of the last patient.

4. STUDY ENDPOINTS

Study endpoints are listed below with relevant time frame, where:

Month 6 = 26±2 weeks; Month 12 = 52±2 weeks; Month 18 = 78±2 weeks; Month 24 = 104±2 weeks

4.1 EFFICACY ENDPOINTS

- Change from baseline in 2-hour AUC of C-peptide response to the MMTT [**Primary endpoint.** Time frame: Month 6].

Secondary endpoints:

- Change from baseline in 2-hour AUC of C-peptide response to the MMTT [Time frame: month 12, 18 and 24].
- Change in HbA1c from baseline [Time frame: Month 6, 12, 18 and 24]
- Time in range (TIR) by Continuous Glucose Monitoring (CGM) [Time frame: Month 6, 12, 18 and 24]
- Proportion of patients with HbA1c <7% who did not experience severe hypoglycemic events during treatment [Time frame: Month 6, 12, 18 and 24].
  For the purpose of this protocol, a severe hypoglycemic event is defined as an event with one of the following symptoms: "memory loss, confusion, uncontrollable behavior, irrational behavior, unusual difficulty in awakening, suspected seizure, seizure, loss of consciousness, or visual symptoms", in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54mg/dL or prompt recovery after oral carbohydrate, i.e. glucose, or glucagon administration.
- Average (previous 3 days) daily insulin requirement (IU/kg/day) [Time frame: Month 6, 12, 18 and 24].
- Proportion of patients with HbA1c <7% and daily insulin requirement <0.5 (IU/kg/day) [Time frame: Month 6, 12, 18 and 24].
- Additional Glucose Variability Indices derived from CGM (glucose AUC outside the target range of 70 – 180 mg/dL, 2-hour postprandial glucose (PPG), Mean Amplitude Glycemic Excursions (MAGE), continuous overall net glycemic action (CONGA)-n, Mean Of the Daily Differences (MODD), and mean daily blood glucose, SD (Standard Deviation). [Time frame: Month 6, 12, 18 and 24].
- Number of self-reported episodes of severe hypoglycemia [Time frame: Month 6, 12, 18 and 24].
- Percentage of patients not requiring insulin therapy [Time frame: Month 6, 12, 18 and 24]
- Estimated Glucose Disposal Rate (eGDR) [Time frame: Month 6, 12, 18 and 24].

4.2 EXPLORATORY ENDPOINTS

- microRNA-375 [Time frame: Month 6, 12, 18 and 24].
- miRNA signature [Time frame: baseline]
- IL-8 and d IL6 [Time frame: Month 6, 12, 18 and 24].
- High Sensitive - C Reactive -protein (hs-CRP) [Time frame: Month 6, 12, 18 and 24].
- Neutrophil Extracellular Traps (NET) [Time frame: Month 6, 12, 18 and 24]
- Proinsulin/ C-peptide ratio [Time frame: Month 6, 12, 18 and 24]

4.3 PHARMACOKINETIC ENDPOINTS

- Plasma levels of 2156Y (acidic form of ladarixin; bound and unbound) and relevant metabolites (DF2108Y, S-isomer; DF2227Y, R-isomer) in a subset of adolescents (14-17 years, inclusive) selected for full PK analysis [Time frame: day 1 and 14 of [redacted] treatment cycle and then at 24, 48, 72 hours after the last IMP dose (morning dose) in this cycle.

- Plasma levels of 2156Y (acidic form of ladarixin) and relevant metabolites (DF2108Y, S-isomer; DF2227Y, R-isomer) in the whole population [Time frame: within 96 hours after the last IMP dose in at least 2 treatment cycles].

4.4 SAFETY ENDPOINTS

- Vital signs (blood pressure and heart rate) [time frame: end of 3rd (Week 11) and 6th (Week 23) treatment cycle, Month 6, end of 9th (Week 35) treatment cycle, Month 12 and Month 24].
- Routine laboratory tests (hematology: hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count; clinical chemistry: sodium, potassium, serum creatinine, serum albumin, total bilirubin, ALT, AST) [time frame: end of 3rd (Week 11) and 6th (Week 23) treatment cycle, Month 6, end of 9th (Week 35) treatment cycle, Month 12 and Month 24 (or withdrawal)].
- Incidence of TEAEs and TESAEs [Time frame: throughout the study].

5. STUDY POPULATION

Approximately one hundred thirty / one hundred forty (130-140) patients with recent onset (within 180 days) T1D, including up to an estimated 15- 20% adolescents (14-17 years, inclusive), will be included (randomized) in the study, selected from those referring to the clinical site for diabetes diagnosis and/or management.

Each patient will be randomized provided that (s)he fully meets all of the study Inclusion Criteria and none of the Exclusion Criteria described in Sections 5.1. and 5.2. below.

5.1 INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfil the following inclusion criteria.

1. Male and female patients aged 14-45 years, inclusive;
2. Recent onset T1D (1st IMP dose within 180 days from 1st insulin administration);
3. Positive for at least one diabetes-related auto-antibody (anti-GAD; IAA, if obtained within 10 days of the onset of insulin therapy; IA-2 antibody; ZnT8);
4. Require, or has required at some time, insulin therapy through one or more separate subcutaneous injections or Continuous Subcutaneous Insulin Infusion (CSII).
5. Fasting C peptide < 0.205nmol/L;
6. Residual β-cell function as per peak stimulated (MMTT) C-peptide level >0.2nmol/L; MMTT should not be performed within one week of resolution of a diabetic ketoacidosis event;
7. Patient able to comply with all protocol procedures for the duration of the study, including scheduled follow-up visits and examinations;
8. Patients who have given written informed consent prior of any study-related procedure not part of standard medical care (participants under the age of 18, shall provide an assent for the study as per country requirements). Specific consent must be given by adolescents to be selected for the full PK analysis.

5.2 EXCLUSION CRITERIA

Patients who meet any of the following criteria are NOT eligible for inclusion in the study.

1. A type 2 diabetes diagnosis or any other unstable chronic disease for which dose adjustment of specific medication is anticipated during the trial;
2. Moderate to severe renal impairment as per estimated Glomerular Filtration Rate (eGFR) 60 mL/min/1.73m^2, as determined using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (see **Appendix 14.4.3**);
3. Hepatic dysfunction defined by increased ALT/AST > 3 x upper limit of normal (ULN) and increased total bilirubin > 3 mg/dL [>51.3 µmol/L];
4. Hypoalbuminemia defined as serum albumin < 3 g/dL;
5. QTcF > 470 msec;
6. Occurrence of an episode of ketoacidosis or hypoglycemic coma in the past 2 weeks;
7. A history of significant cardiovascular disease/abnormality;
8. Known hypersensitivity to non-steroidal anti-inflammatory drugs;
9. Concomitant treatment with drugs metabolized by CYP2C9 with a narrow therapeutic index [i.e.

phenytoin, warfarin, sulphonylurea hypoglycemics (e.g. tolbutamide, glipizide, glibenclamide/glyburide, glimepiride, nateglinide) and high dose amitriptyline (> 50 mg/day)];

10. Previous (past 2 weeks) and concomitant treatment with antidiabetic agents as metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors, SGLT2-inhibitors or amylin, or any medications known to influence glucose tolerance (e.g. β-blockers, angiotensin-converting enzyme inhibitors, interferons, quinidine antimalarial drugs, lithium, niacin, etc.);
11. Past (past month) or current administration of any immunosuppressive medications (including oral or systemic corticosteroids) and use of any investigational agents, including any agents that impact the immune response or the cytokine system;
12. Significant systemic infection during the 4 weeks before the 1st dose of the study drug (e.g., infection requiring hospitalization, major surgery, or IV antibiotics to resolve; other infections, e.g., bronchitis, sinusitis, localized cellulitis, candidiasis, or urinary tract infections, must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant exclusion);
13. History of positive status for hepatitis A (IgM), hepatitis B (not due to immunization), hepatitis C and HIV.
14. Pregnant or breast-feeding women. Unwillingness to use effective contraceptive measures up to 2 months after the end of study drug administration (females and males). Effective contraceptive measures include a hormonal birth control (e.g. oral pills, long term injections, vaginal ring, patch); the intrauterine device (IUD); a double barrier method (e.g. condom or diaphragm plus spermicide foam); abstinence.

5.3 ASSIGNMENT OF PATIENT NUMBER

At the screening visit each patient will be assigned a unique sequential patient number by an Interactive Response System (IRS). This number will be used for identification throughout the study and will not be used for any other participant.
The patient number consists of six digits and it will correspond to the concatenation of three digits for site number and three digits for incremental subject number.

After successful randomization, the patient number will be followed by the randomization number separated by a dash. The Randomization Number is constructed as nine digits: 319, then 3 digits for site number and finally 3 digits for subject number within site.

If a patient is dropped from the study for any reason, the patient's randomization number will not be reassigned.

6. STUDY MEDICATION

6.1 PRESENTATION, STORAGE, PACKAGING AND LABELING OF THE INVESTIGATIONAL MEDICINAL PRODUCT

6.1.1 Presentation of Investigational Medicinal Product

In this study the Investigational Medicinal Product (IMP) will be either ladarixin OR matched placebo.

It will be provided as hard gelatin capsules for oral administration, with the following composition:

Composition for each ladarixin unit (capsule)

NAME OF INGREDIENT	FUNCTION OF INGREDIENT	AMOUNT FOR CAPSULE	REFERENCE TO QUALITY STANDARDS
[redacted]			

Batch release certificate will be provided together with the IMP.

6.1.2 Manufacturing, Packaging and Labelling of IMP

Capsules will be manufactured by [redacted].

[redacted] or [redacted] will manage the labeling and packaging of capsules in blisters to obtain the Patient Kit.

The study medication will be provided as a Patient Kit, containing 2 Treatment wallets, covering the treatment period of 1 cycle. Each Treatment wallet will contain 28 capsules.

All labels will be prepared to meet local regulatory requirements. Details of packaging and labeling are reported in **Appendix 14.1**.

6.1.3 Supply, Storage and Handling of IMP

An appropriate number of packages will be initially sent to the site as soon as all essential documents and regulatory/ethics approvals have been obtained. IMP re-supply will be planned on demand, according to enrolment rate.

The IMP must be kept at a temperature not exceeding 30°C and must not be frozen.

A temperature probe will accompany the drug on shipment. Temperature range reached during shipment will be verified on receipt at site, so that potential stability concerns during shipment can be investigated and appropriate action taken.

Once received at the site, the Pharmacist (or designee) will check the package for accurate delivery and acknowledge receipt; any deviations from expected package content (inconsistency, damages) should be immediately reported to Dompé (or appointed CRO) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or appointed CRO).

The IMP must be stored at site in a secure location, in a temperature-controlled room. Temperature records must be available for the CRA to review at monitoring visits; any deviations from the recommended storage conditions should be immediately reported to Dompé (or appointed CRO) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or appointed CRO).

6.1.4 Blinding

Appearance, including packaging and labeling, of the IMP (capsules, packaging) will not allow recognition of actual treatment (either ladarixin or placebo).

During the trial, blinding will be broken by the Investigator for emergency purposes only, where knowledge of the blinded treatment could influence further patient care. In addition, safety reports will be unblinded, as per regulatory requirements.

Study blind will be broken after database lock when the last patient randomized has completed the Month 12 visit (or lost in follow-up) and all relative data to months 12 have been fully reconciled and cleaned.

6.2 IMP DISPENSATION

The IMP will be dispensed quarterly only by the Pharmacist (or authorized designee at site).

The IMP will be dispensed via IRS as Patient Kits during the Randomization and then during the Visits scheduled at the end of the [redacted] (Week [redacted]), [redacted] (Week [redacted]) and [redacted] (Week [redacted]) treatment cycles, after check of inclusion/exclusion criteria (at Randomization) and discontinuation criteria at the scheduled visits * (see Section 6.4.2).

A total number of 13 Patient IMP Kits will be dispensed to each randomized subject as follows:

- 3 Patient Kits of IMP for the Cycles [redacted] and [redacted] dispensed at randomization
- 3 Patient Kits for the Cycles [redacted] and [redacted] dispensed at Week [redacted] Visit
- 3 Patient Kits for the Cycles [redacted] and [redacted] dispensed at Week [redacted] Visit
- 4 Patient Kits for the Cycles [redacted] and [redacted] dispensed at Week [redacted] Visit

* Results of ALT/AST, bilirubin, creatinine, albumin, ECG readings and pregnancy results should be made available by local laboratory in time to allow Patient Kits dispensation as soon as possible during the visit. If this expedited timing cannot be achieved, Patient Kits can be delivered to patient named address by a selected courier (CRO to support shipment). Study drug dispensation will be performed as soon as possible after check of discontinuation criteria.

6.3 DOSE, ROUTE AND SCHEDULE OF IMP ADMINISTRATION

Ladarixin will be administered orally at the dose of 400 mg twice a day for 13 cycles of 14 days on / 14 days off. Placebo will be administered with the same treatment schedule.

The two daily doses will be administered at about 12 hour intervals (morning and evening; ideally between 6:30/11:30 and 18:30/23:30). At each administration, 2 capsules will be swallowed with a glass of water, at least 2 hours apart from breakfast or dinner.

6.4 CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION OR DISCONTINUATION OF THE IMP

6.4.1 Criteria for schedule adjustment or dose modification

No schedule adjustment and/or dose modification is foreseen, except for discontinuation of IMP as detailed below.

6.4.2 Criteria for discontinuation of the IMP

The IMP will be discontinued in the case:

- QTcF is either > 500 msec. or increases by > 60 msec. from baseline measurement on two consecutive ECG readings taken 1 hour apart;
- The patient develops any significant cardiovascular disease/abnormality;
- The patient develops renal (calculated eGFR < 60 mL/min) or hepatic (increased ALT/AST > 3 x ULN and increased total bilirubin > 3mg/dL [>51.3 µmol/L]) dysfunction as well as hypoalbuminemia (serum albumin <3 g/dL);
- Pregnancy occurs (female patient);
- The patient develops ketoacidosis or hypoglycemic coma;
- The patient develops any significant systemic infection that must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant discontinuation.

Occurrence of any condition that qualifies the patient to treatment discontinuation will be specifically monitored through ECG readings and laboratory tests obtained at the visits scheduled at the end of the 3rd (Week 11), 6th (Week 23) and 9th (Week 35) treatment cycles.

In addition, the IMP will be immediately discontinued in the event of any other possibly drug related occurrences that the Investigator believes might compromise patient's safety.

If the IMP administration is prematurely discontinued, the primary reason for discontinuation must be recorded in the eCRF. Patients who discontinue the treatment with the IMP will NOT be withdrawn from the study by default but will be asked to complete safety and efficacy observations as per the protocol, unless otherwise they withdraw their consent.

6.5 ACCOUNTABILITY OF THE IMP

All supplies will be maintained under adequate security by the designated member of site staff, until they are dispensed to the patients. The Investigator will ensure that study treatment is only dispensed by designated staff within the center.

When the IMP is received at the site, designated members of site staff will check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by or on behalf of Dompé and returning it to Dompé or to the appointed CRO. A copy will be retained for the Investigator/Pharmacy file.

The dispensing of the IMP will be carefully recorded on the eCRF and appropriate drug accountability forms and an accurate accounting will be available for verification by the CRA at each monitoring visit. Immediately before dispensing each Patient Kit, the kit label will be completed with the required information (i.e. Patient ID number and Investigator Name).

Drug accountability records will include:

1. the confirmation of receipt of the IMP at the trial site,
2. the dispensing of the IMP to the patient,
3. the receipt of IMP returned from the patient,

4. the disposition of unused product(s),
5. accounts of any IMP accidentally or deliberately destroyed,

They should include dates, quantities, batch numbers, expiration dates (if applicable), and any unique code numbers assigned to the IMP and/or patients. Investigators should maintain records which document adequately that:

1. the patients were provided the doses specified by the protocol/amendment(s),
2. the IMP provided was fully reconciled at the site.

The administration of the IMP (date/time for each administration) will be recorded by the patient in the Patient Diary whose data will be checked by the Investigator during the next study visit.

The CRA or site delegated personnel, if site specific procedures for drug disposal are in place, will review the drug accountability forms/eCRF and check all IMP (both unused and used) prior to making arrangements for their disposal.

IMP which has been dispensed to a patient and returned unused will not be re-dispensed to a different patient. Unused IMP (capsules) must remain in the blisters within the Patient Kit and must not be discarded or used for any purpose. Any remaining test material at the end of the trial will be returned to Dompé or disposed of, as determined by Dompé.

6.5.1 Assessment of compliance

Compliance with the study product dosing schedule will be verified by a CRA during on-site monitoring visits, as per records in the eCRF versus accountability records and actual capsules in the Patient Kits returned by the patient.

6.6 CONCOMITANT MEDICATION

6.6.1 Reporting of prior and concomitant medications

Administration of all prior (within 1 month before enrolment) and concomitant medications (CMEDs), **apart from insulin** and the agents listed below, will be reported in the appropriate section of the eCRF.

All the details as per the eCRF fields (sequential number, drug name, indication, starting dose, start/stop date, route of administration) will be recorded. Change in dose will be tracked.

It is prescribed that also the following agents will be recorded in the eCRF: homeopathic medications; elective vitamins (**including vitamin D**) and minerals; osmotic laxatives and locally acting antacids; topical medication.

6.6.2 Restriction on allowed prior and concomitant medications

The following medications **should not be used** prior to enrolment and up to the end of study participation:

Drugs that affect glucose homeostasis or its readout

- Metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors, SGLT-2 inhibitors or amylin, or any medications known to influence glucose tolerance (e.g. β-blockers, angiotensin-converting enzyme inhibitors, interferons, quinidine antimalarial drugs, lithium, niacin, etc.) [off period before randomization = 2 weeks];
- Any immunosuppressive medications, including oral or systemic corticosteroid, and any other investigational agents, including any agents that impact the immune response or the cytokine system [off period before randomization = 1 month].

Drugs metabolized by CYP2C9 with a narrow therapeutic index (drugs that may have their plasma

concentration and effect altered by inhibition of CYP2C9 by ladarixin).

- Phenytoin, warfarin, sulphonylurea hypoglycemics (e.g. tolbutamide, glipizide, glibenclamide/glyburide, glimepiride, nateglinide) and high doses of amitriptyline (> 50 mg/day).

The following medications **can be used with the restrictions detailed below**:

- Non-steroidal anti-inflammatory drugs (e.g. ibuprofen, flurbiprofen, indomethacin, piroxicam, naproxen, meloxicam, lornoxicam, celecoxib) can be used during treatment with ladarixin up to a maximum of 3 consecutive days, with a 3 days' washout before re-treatment, if any (excluded patients with known hypersensitivity to non-steroidal anti-inflammatory drugs).
- Administration of low doses amitriptyline (< 50 mg/day) is allowed under clinical monitoring for possible side effects (e.g. sedation and anticholinergic symptoms). In case a concern is raised, treatment with either amitriptyline or ladarixin should be immediately discontinued.
- Any drug that is known to be a substrate for cytochrome CY2C9 should be considered for possible drug-drug interaction as per warnings/precautions reported in the Summary of Product Characteristics. Any possible concern should be discussed with the sponsor Medical Expert prior to patient enrolment or concomitant administration with ladarixin.

6.6.3 COVID-19 and other vaccination

It is recommended that the patient, if he/she is required to undergo any vaccination during the course of the trial, promptly inform the Investigator who will evaluate any potential impact on his/her clinical condition and on the continuation/interruption of the study participation.

Considering ladarixin mechanism of action and results from previous clinical and pre-clinical studies, we expect no effects on the immune response to the COVID-19 vaccine as well as on the efficacy and safety study endpoints or interactions between ladarixin and COVID-19 vaccines.

However, where clinically possible, to avoid any potential unexpected interaction and incorrect attribution of potential adverse events, we suggest to give the COVID-19 vaccine course in the days off of ladarixin treatment and if one of the doses of vaccine would be during the days on ladarixin treatment, taking into account t1/2 of ladarixin, we suggest to stop the administration of the IMP from 48 h before to 48 h after vaccine administration.

6.7 INSULIN TITRATION

Patients will self-monitor glucose levels at least 4 times a day and will take insulin as prescribed by the Investigator throughout the study participation (see Section 7.1.1).

To ensure standardized glycemic control in the treatment groups, the Investigator will provide guidance for insulin regimen adjustment, with insulin titrated up or down to target HbA1c levels of less than 7% and self-monitored blood glucose (SMBG - fingerstick or CGM):

- pre-prandial blood glucose of 70-130 mg/dL
- 2 hours post-prandial blood glucose < 180 mg/dL
- bed-time blood glucose of 110-150 mg/Dl

Glucose test strips and a glucose monitor will be provided to study participants for the duration of the study. In order to optimize insulin titration, periodic controls (e.g. telephone calls and/or emails sent by the pi or sub-investigators to the patient) will be scheduled on a regular basis to ensure timely evaluation of metabolic control and adjustment of insulin regimen. Patients will report their SMBG in the patient diary at predefined intervals according to the use of the fingerstick.

7. STUDY PROCEDURE AND ASSESSMENTS

A schedule for the tests and evaluations to be conducted in this study is found in the flow chart in **Appendix 14.2**.

For all measurements, the actual date and time of assessment, including date of sampling, will be recorded in the eCRF. Time frame for each assessment is reported in the following sections. Month /Week match is detailed below, together with acceptable windows:

Month 6 = Week 26±2; Month 12 = Week 52±2; Month 18 = Week 78±2; Month 24 = Week 104±2.

7.1 ENROLMENT, SCREENING AND RANDOMIZATION

7.1.1 Enrolment and Intensive Diabetes Management

Potential study patients with a recent (within 180 days) diagnosis of T1D will be identified from those referring to the site for diagnosis confirmation (auto-antibody testing) and/or disease management.

Enrolment is defined as a signature of the Informed Consent Form (ICF) for study participation.

From consent, patients will be admitted to intensive diabetes management, according to current ADA recommendation [*2014*]. Patients will be instructed to self-monitor their glucose values at least 4 times a day and to report (glucose meter/log) outcome to the diabetes management team. Insulin intake will be adjusted to target HbA1c levels of less than 7% and SMBG (fingers stick or CGM):

- pre-prandial blood glucose of 70-130 mg/dL
- 2 hours post-prandial blood glucose < 180 mg/dL
- bed-time blood glucose of 110-150 mg/dL

Glucose test strips and a glucose monitor will be provided to study participants for the duration of the study. Telephone calls (outside scheduled visits) and/or emails sent by PI/sub-investigators to the patient will be scheduled on a regular basis to ensure optimization of metabolic control. It is recommended that patients are contacted at least every 2 to 3 weeks during study participation. A telephone call or email is anyway requested to be scheduled in the 2 weeks before a planned visit (as detailed in the sections below) to enable the Investigator to review glucose data and confirm/adjust insulin intake to be applied for at least 3 days before the visit.

7.1.2 Run-in period

Screening, including confirmation of T1D diagnosis, will be performed in enrolled (consented) patients.

Screening includes the following assessments, and may be performed in one or more visits, as per details below:

✔ **time frame**: from consent to baseline (clinical information)

- Past medical history and disease-specific clinical information, including date of 1st insulin administration.
- Blood sampling for measurement (centralized laboratory) of auto-antibody (anti-GAD; IAA, if obtained within 10 days of the onset of insulin therapy; IA-2 antibody; ZnT8) to confirm T1D diagnosis and of fasting C-peptide that can be measured twice in case the value at first sampling is >0.205 nmol/L;

  Samples will be obtained and stored as detailed in **Appendix 14.3.1**.

✔ **time frame**: within 3 weeks before study treatment start (baseline measurements - *consider time for centralized assay of MMTT C-peptide – results available*)

- Vital signs.

- Body weight, height, waist and waist/hip circumference.
- Evaluation of eGDR (see equation in **Appendix 14.3.4**) and Body Mass Index (BMI).
- Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests".
- Evaluation of renal and hepatic function (to be derived from the safety laboratory test results).
  - Renal function will be evaluated by eGFR, determined using CKD-EPI creatinine equation *(Levey AS. 2009* – **Appendix 14.3.3***)*
  - Hepatic function will be evaluated by bilirubin and transaminases (ALT and AST).
- 12 lead ECG, performed using local equipment that allows automatic calculation of the QTcF, otherwise manual calculation by the PI/subPI is acceptable.
- Pregnancy test (urine dipstick or blood sample), if appropriate.
- Baseline insulin requirement (IU/kg/day averaged over the previous 3 days).
- Blood sampling for measurement (centralized laboratory) of baseline HbA1c.
- Blood sampling for measurement (centralized laboratory) of HLA DR-DQ haplotypes and Proinsulin.
- Baseline MMTT. Blood samples for measurement (centralized laboratory) of C-peptide and glucose will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120 min after the meal. Procedures for MMTT and sample handling are detailed in **Appendix 14.3.6** and **Appendix 14.3.1**, respectively. MMTT should not be performed within one week of resolution of a diabetic ketoacidosis event.
- Evaluation of Proinsulin: C-peptide ratio.
- CGM download for evaluation of Glucose Variability Indices from CGM. *
- Blood sampling for measurement (centralized laboratory) of microRNA-375, miRNA signature, IL-8, IL-6, hs-CRP, NET.

* The CGM device will be positioned at least 10 days before the baseline assessment. It is recommended to place the device during the Screening Visit.

Patients who do not meet the Inclusion Criteria or meet Exclusion Criteria will be considered screen failures (screening number assigned) but could be considered for re-screening, if deemed appropriate by the PI.

7.2 RANDOMIZATION & START OF DRUG ADMINISTRATION

Compliance with inclusion/exclusion criteria will be finally verified vs demographic, laboratory test results and clinical information.

Patients fulfilling **all** the inclusion criteria and **none** of the exclusion criteria will refer to the site for a **Randomization Visit** to occur within maximum 3 days before the IMP administration is started.

During the visit the patient will be randomized (randomization number assigned by an IRS system); the Patient Diary will be delivered and the Patient Kits for the [redacted] to [redacted] treatment cycles will be dispensed.

It is the responsibility of the Investigator (or designee) to explain, and make sure the patient fully understands any appropriate treatment related information. This includes, but is not limited to treatment schedule, time of administration, time interval from meals, recording of treatment administration and other data in the Patient Diary, etc.

The 1st IMP administration is recommended to happen during the randomization visit, under medical control. If this is not possible the drug can be started within 3 days after randomization. The start of the IMP administration is defined as **Day 1.**

Day 1 MUST be scheduled no later than 180 days from the 1st insulin injection and is to be scheduled within 3 weeks from the baseline assessments and 3 days from randomization.

The administration of the IMP (date/time for each administration, number of capsules) will be recorded by the patient in the Diary which data will be checked by the Investigator during the next study visit.

7.3 TREATMENT PERIOD AND CHECK OF TREATMENT DISCONTINUATION CRITERIA

After the treatment has started, IMP administration will proceed for additional 12 cycles of 14 days on/14 days off, unless there is any reason for drug discontinuation (see Section 6.4.2). On each treatment cycle, the IMP will be administered at a 12 hour interval (morning and evening; ideally in between 6.30/11.30 and 18.30/23.30). At each administration, 2 capsules will be swallowed with a glass of water, at least 2 hours apart from breakfast or dinner.

During treatment, occurrence of any condition that might prevent continuing IMP administration will be verified at the visits scheduled during treatment (see below and discontinuation criteria in Section 6.4.2) at the end of the [redacted] (Week [redacted]), [redacted] (Week [redacted]) and [redacted] (Week [redacted]) treatment cycles.

During these visits the following will be measured/assessed (as per center practice); actual date and time of assessment, or the date of sampling, will be recorded in the eCRF:

- Vital signs.
- Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests".
- Evaluation of renal and hepatic function (to be derived from the safety laboratory test results).
- 12 lead ECG, performed using local equipment that allows automatic calculation of the QTcF, otherwise manual calculation by the PI/subPI is acceptable.
- Pregnancy test (urine dipstick or blood sample).
- Patient Diary check (e.g. information on IMP administration, insulin requirement, severe hypoglycemia episodes).

As per discontinuation criteria (see Section 6.4.2), **no IMP will be dispensed** in the case:

- QTcF is either > 500 msec or increases by > 60 msec from baseline measurement on two consecutive ECG readings taken 1 hour apart;
- The patient develops any significant cardiovascular disease/abnormality;
- The patient develops renal (calculated eGFR < 60 mL/min) or hepatic (increased ALT/AST > 3 x ULN and increased total bilirubin > 3mg/dL [>51.3 µmol/L]) dysfunction as well as hypoalbuminemia (serum albumin <3 g/dL);
- Pregnancy occurs (female patient);
- The patient develops ketoacidosis or hypoglycemic coma;
- The patient develops any significant systemic infection that must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant discontinuation.

After having confirmed the patient has not developed a condition that prevents his/her continuing participation into the study*, he/she will be provided with the Patient Kits matching his/her randomization number, together with the Patient Diary, according to the schedule reported below: (see also section 6.2).

- 3 Patient Kits for the Cycles [redacted] and [redacted] dispensed at the Week [redacted] Visit
- 3 Patient Kits for the Cycles [redacted] and [redacted] dispensed at the Week [redacted] Visit
- 4 Patient Kits of IMP for the Cycles [redacted] and [redacted] dispensed at the Week [redacted] Visit

* Results of ALT/AST, bilirubin, creatinine, albumin, ECG readings and pregnancy results should be made available by local laboratory in time to allow dispensation of Patient Kits as soon as possible during the visit. If this expedited timing cannot be achieved, Patient Kits might be delivered to a patient named address by a selected courier (CRO to support shipment).

The administration of the IMP (date/time for each administration, number of capsules) will be recorded by the patient in the Patient Diary which data will be checked by the Investigator during the next study visit.

It is the responsibility of the Investigator (or designee) to check the information the patient will insert in the Patient Diary to ensure correct intake of the IMP. The Investigator will remind, and make sure the patient still fully understands any appropriate treatment related information.

The Patient Kits containing all the blisters, either unused or empty, will be returned to and checked by the Investigator during the next study visit.

7.4 ADDITIONAL VISITS DURING TREATMENT AND FOLLOW-UP STUDY ASSESSMENTS

At each visit described below, the assessment/measurement will be done as per center practice, unless otherwise specified. Measurements, including the actual date and time of assessment, or the date of sampling, will be recorded in the eCRF.

7.4.1 PK visits

The subset of adolescents selected for full PK analysis will attend the center on day 1 and 14 of the[redacted] treatment cycle; then 24, 48 and 72 hours after the last IMP dose (morning dose) in this cycle for PK sampling.

All patients in the study will refer to the site for at least 2 PK visits to be scheduled within 96 hours after the last IMP dose in at least 2 treatment cycles.

Details of sampling during PK visits are reported in **Appendix 14.3.2**

7.4.2 Additional visits during treatment

During treatment, the patient will attend the center for study assessments on 2 additional visits at Month [redacted] (Week [redacted]) and Month [redacted] (Week [redacted]). At least 10 days before each scheduled visit, the CGM sensor will be placed. CGM sensor implantation and set-up can occur at Week [redacted] Visit if the visit is scheduled 10 days before Week [redacted].

During these visits the following will be assessed:

- Waist and waist/hip circumference and calculation of eGDR.
- Patient Diary check (e.g. information on IMP administration, insulin requirement, severe hypoglycemia episodes)
- Insulin requirement (IU/kg/day averaged over the previous 3 days).
- Number of self-reported episodes of severe hypoglycemia in the interval.
- Blood sampling for measurement (centralized laboratory) of HbA1c.
- MMTT. Blood samples for measurement (centralized laboratory) of C-peptide and glucose will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120 min after the meal.
- Blood sampling for measurement (centralized laboratory) of microRNA-375, IL-8, IL-6, hs-CRP, NET, Proinsulin.
- CGM sensor download for evaluation of Glucose Variability Indices from CGM.
- Blood sampling for measurement (local laboratories) of "Safety Laboratory Tests" (Month 12 only).
- Vital signs (Month 12 only).

7.4.3 Follow-up assessments

After having completed the treatment, patients will attend the center for study assessments on 2 follow-up visits scheduled at month 18 (Week 78) and month 24 (Week 104). At least 10 days before each scheduled visit, the CGM sensor will be placed.

At each visit, the following will be evaluated/measured:

- Waist and waist/hip circumference and evaluation of eGDR.
- Insulin requirement (IU/kg/day averaged over the previous 3 days).
- Number of self-reported episodes of severe hypoglycemia in the interval.
- Blood sampling for measurement (centralized laboratory) of HbA1c.
- MMTT. Blood samples for measurement (centralized laboratory) of C-peptide and glucose will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120 min after the meal.
- CGM download for calculation of CGM derived endpoints.
- Blood sampling for measurement (centralized laboratory) of microRNA-375, IL-8, IL-6, hs-CRP, NET, Proinsulin.
- Patient Diary check (information other than IMP administration)
- Vital signs (Month 24 only)
- Blood sampling for measurement (local laboratories) of "Safety Laboratory Tests" (Month 24 only).

7.5 EARLY PATIENT WITHDRAWAL

7.5.1 Withdrawal criteria

Patients will be informed that they have the right to withdraw from the study at any time (withdrawal of consent), without prejudice to their medical care, and are not obliged to state their reasons.

If a patient fails to return to the center for a scheduled visit, attempts should be made to contact the patient to ensure that the reason for not returning is not a SAE. Likewise, if a patient declares his/her wish to discontinue from the study e.g. for personal reasons, an attempt should be made to establish that the true reason is not a SAE (bearing in mind the patient is not obliged to state his/her reasons).

Safety laboratory tests should be performed whenever possible at patient withdrawal.

Patients who discontinue the treatment with the IMP will not be withdrawn from the study but will be asked to complete observations as per the protocol, unless otherwise they withdraw their consent. It is important that any randomized patient remains in the study and is followed for both efficacy and safety outcomes, regardless he/she has completed or discontinued the study treatment.

Investigators will be trained about the importance of patient retention through the duration of the trial.

In case of pregnancy, the patient will be withdrawn from the study, but she will be followed for safety and pregnancy outcomes monitoring, unless she withdraws her consent.

Any withdrawals must be fully documented in the eCRF.

7.5.2 Replacement policy

No patient who has been randomized and withdraws from the study for any reason will be replaced.

7.6 PATIENT MANAGEMENT AFTER STUDY COMPLETION

After completion of the week 104 (month 24) follow-up visit, patients will receive post-study care as prescribed by their health care provider. No post-study treatment will be provided by Dompé.

8. ADVERSE EVENTS

8.1 DEFINITIONS

8.1.1 Adverse Event

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.

8.1.2 Adverse Drug Reaction

An Adverse Drug Reaction (ADR) is defined as an adverse event, which is reasonably likely to have been caused by the IMP. The definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. For the purposes of IND safety reporting, "reasonable possibility" means there are facts (evidence) or arguments to suggest a causal relationship between the drug and the adverse event.

8.1.3 Serious Adverse Event/Reaction

A Serious Adverse Event (SAE)/Reaction is defined as any untoward medical occurrence that at any dose:

- results in death. Death shall always be reported as SAE and cause of death shall always be specified when known.
- is life-threatening (i.e. the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires 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-patient 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 an important medical event that, based upon appropriate medical judgment, may jeopardize the patient 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 patient 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 patient hospitalization, or the development of drug dependency or drug abuse.

8.1.4 Unexpected Adverse Events

An AE or 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 Investigator Brochure - Reference Safety Information section.

8.1.5 Suspected serious unexpected adverse reaction

A **suspected serious unexpected adverse reaction** (**SUSAR**) is defined as an adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Reaction.

8.2 MONITORING FOR ADVERSE EVENTS

At each visit following study informed consent form signature, after the subject has had the opportunity to spontaneously mention any problem, 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 or questionnaire response that is clinically relevant is to be reported as an AE. These clinically relevant changes will be reported regardless of causality.

8.3 RECORDING

AE data should be obtained through observation of the patient, from any information volunteered by the patient, or through patient questioning.

All AEs (serious and non-serious) which occur from signature of the informed consent through patient participation in the study (last planned visit or early withdrawal date) will be reported and recorded in the eCRF. It is important that the AE dedicated pages of the eCRF includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, the outcome, the action(s) taken and relevant concomitant treatments dispensed.

All AEs should be followed-up to determine the outcome of the event. The Investigator should follow up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the subjects experiencing an AE receive definite treatment for any AE, if required.

Medical conditions/diseases present before starting study treatment shall be documented in the medical history section of the eCRF; these conditions are considered AEs only if they increase either in frequency or severity once informed consent has been signed.

8.3.1 Relationship of AEs to the Investigational Product

The Investigator will assess the causal relationship between the AE and the IMP (either ladarixin or placebo), according to the criteria in **Table** below:

Relationship of the Adverse Event to the IMP

None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g. a surgical intervention for nevus removal performed during the study, but planned before patient enrolment into the study
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 patient'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 patient's condition
Highly Probable	Strong relationship, the event abates upon discontinuation of Investigational Product and, if applicable, re-appears upon repeat exposure

Any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

8.3.2 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.

Severity of the Adverse Event

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

8.4 SERIOUS ADVERSE EVENT REPORTING

8.4.1 Reporting Procedure for Investigators to Dompé

The Investigator must report all SAEs occurring during patient participation in the study, regardless of presumed causal relationship, to the CRO Pharmacovigilance contact and to Dompé Drug Safety Department, by e-mail (preferred) or fax **within 24 hours** of learning of the event. Contact details for SAE reporting by the Investigator are provided in the section "Contact Information".

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

In line with CT3 Detailed Guidance and ICH E2A provisions, although the Investigator does not usually need to actively monitor patients for AEs once the trial has ended, if the Investigator becomes aware of a SAE occurring to a patient after that patient has ended his/her participation in the study (whether study completion or withdrawal), the SAE should be reported by the Investigator to the CRO Pharmacovigilance or directly to the Dompé Global Pharmacovigilance, Safety and Surveillance department, should the whole study have been ended. 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.

Information on SAEs will be recorded on a specific SAE form. Both electronic and blank paper copies will be included in the Investigator's Site File. Follow-up reports (as many as required) should be completed and e-mailed/faxed following the same procedure above.

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 Investigational Product, 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.

8.4.2 Conditions that should not be reported as serious adverse events

The conditions listed below, that may require hospitalization of a patient, are not considered to be SAE and shall not be reported as such, but only need to be recorded in the CRF:

- Hospitalizations planned before entry into the clinical study which is part of the normal treatment or monitoring of the studied indication and not associated with any deterioration in condition.
- Hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- Hospitalization for treatments, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of SAEs given above and not resulting in hospital admission.

In addition, the following situation shall not be considered SAE:

- Abnormal lab values or test results that do not induce clinical signs and/or symptoms and require intervention/therapy, i.e. are clinically significant.

8.4.3 Follow-Up of Serious Adverse Events

If the 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 related 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 redacted 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 (see Par. 8.4.1).

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.

8.4.4 Reporting Procedure to IEC and to Regulatory Authorities in the European Union

Reporting of Suspected Unexpected Serious Adverse Reaction

Investigators must report all serious adverse events to the sponsor immediately, within 24 hours.
Dompé Global Pharmacovigilance, Safety and Surveillance Department, with the support of the CRO as appropriate, shall report any SUSAR to the concerned IEC which approved the protocol and the Regulatory Authority (via the EudraVigilance Clinical Trial module) as soon as possible, and in no event later than:

- seven calendar days after becoming aware of the information if the event is fatal or life threatening; to be followed by any relevant information within eight days.
- fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

Treatment will be unblinded by Dompé Global Pharmacovigilance, Safety and Surveillance department prior to regulatory submission of a SUSAR to Regulatory Authorities and IEC in the European Union, and only cases referred to active treatment will be considered expeditable for regulatory reporting, in line with law requirements.

If the results of an investigation show that an AE not initially determined to be reportable is reclassified as reportable, Dompé shall notify such SUSAR in a written safety report as soon as possible, but in no event later than 7/15 calendar days after the determination is made.

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

Periodical Reporting to EU Regulatory Authorities

The Dompé Global Pharmacovigilance, Safety and Surveillance Department will prepare and submit (via the CRO as applicable) to Investigators appropriate periodic safety updates as per applicable EU and local requirements and regulations. Dompé Global Pharmacovigilance, Safety and Surveillance Department shall also be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities and to IECs.

8.4.5 Reporting Procedure to IRB and to Regulatory Authorities in the United States

Reporting of Suspected Unexpected Serious Adverse Reaction

Investigators must report all serious adverse events to the sponsor immediately, within 24 hours.
Dompé Global Pharmacovigilance, Safety and Surveillance Department, with the support of the CRO as appropriate, shall report any SUSAR and potential serious risks, from clinical trials or any other source, to the FDA and all participating investigators, as soon as possible, and in no event later than:

- seven calendar days after becoming aware of the information if the event is fatal or life threatening; to be followed by any relevant information within eight days.
- fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

The Investigators in turn shall notify their IRB: Investigators are required to promptly report "to the IRB all unanticipated problems involving risk to human subjects or others," including adverse events that should be considered unanticipated problems (21 CFR 312.66).

Dompé Global Pharmacovigilance, Safety and Surveillance Department will unblind treatment prior to regulatory submission of a SUSAR to FDA and only cases referred to active treatment will be considered expeditable for regulatory reporting, in line with law requirements.

The blind should ordinarily be broken for IND safety reports submitted to FDA and all participating investigators.

If the results of an investigation show that an AE not initially determined to be reportable is reclassified as reportable, Dompé Global Pharmacovigilance, Safety and Surveillance Department shall notify such SUSAR in a written safety report as soon as possible, but in no event later than 7/15 calendar days after the determination is made.

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

To note, Dompé Global Pharmacovigilance, Safety and Surveillance Department shall also notify FDA in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible after Dompé determines that the information qualifies for reporting, in particular shall notify of:

- any suspected adverse reaction that is both serious and unexpected. Dompé must report an adverse event as a suspected adverse reaction only if there is evidence to suggest to the Sponsor a causal relationship between the drug and the adverse event.

- findings from other studies that suggest a significant risk in humans exposed to the drug. Such a finding would result in a safety-related change in the overall conduct of the clinical investigation.

- findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug

- increased rate of occurrence of serious suspected adverse reactions.

Periodical Reporting to US Regulatory Authorities

Dompé Global Pharmacovigilance, Safety and Surveillance Department will prepare and submit (via the CRO as applicable) to IRBs and Investigators periodical safety updates as per applicable local requirements and regulations.

Dompé Global Pharmacovigilance, Safety and Surveillance Department shall also be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to FDA and IRBs as applicable.

8.5 EXPOSURE TO INVESTIGATIONAL PRODUCT DURING PREGNANCY

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 exclusion criteria. Prior to enrolment in the clinical trial, female patients 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 2 months after the study treatment period ends and of the potential risks associated with an unintentional pregnancy. During the trial (treatment period or follow up period), female patients are to be instructed to contact the Investigator immediately if they suspect they might be pregnant. In the same way, male patients who become aware that the partner might be pregnant, are to be instructed to contact the Investigator immediately.

The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of learning of the pregnancy to the Drug Safety Contacts specified in the section "Contact Information", even if no AE has occurred, and follow it to term. If, however, the pregnancy is associated with an SAE (e g, if the mother is hospitalized for dehydration), in addition to the pregnancy report form, a separate SAE report form must be filed as described in Section 8.4, with the appropriate serious criterion indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE.

Any pregnancy leads to the immediate exclusion from the trial.

8.6 ADVERSE EVENTS CAUSING TREATMENT DISCONTINUATION

If a patient is withdrawn from the study as a consequence of an AE, this must be recorded and reasoned in the CRF, and the patient must be followed up until the resolution of the AE or as instructed by the medical monitor.

8.7 OVERDOSE

Accidental or intentional overdose, which may or may not result in serious adverse reactions, is to be reported to Dompé Global Pharmacovigilance, Safety and Surveillance Department/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. The Medical Expert should be contacted to discuss corrective treatment, if necessary.

8.8 EMERGENCY PROCEDURES

The treatment allocation for each patient will be provided to:

- the Investigator for emergency procedures;
- the Pharmacovigilance for safety procedures.

The treatment assignment information will be kept confidential and will not be disclosed to any other person than those ones involved in these emergency and safety procedures.

Access to individual patient treatment code will be allowed only in the event of a medical emergency where the knowledge of patient treatment is required to provide the patient with appropriate care. The investigator will be provided with a protected access to IRS so only in case of a medical emergency can open the treatment allocation for a specific patient.

Any code break and the reason behind it will be recorded (when it was opened, by whom and why) in the relevant page of the eCRF. Code break will be immediately communicated to the CRO or Dompé, as appropriate.

Dompé Global Pharmacovigilance, Safety and Surveillance department contact person will be provided with procedures to allow access to treatment codes in case of unblinding for safety procedures.

Unblinding events will be recorded and reported in the final study report.

9. STATISTICAL ISSUES

9.1 SAMPLE SIZE

[redacted]

The aim of this prospective study is to provide further evidence of the beneficial effect of ladarixin on C-peptide preservation in the same setting. The study will be based on approximately 130-140 randomized patients, number defined on feasibility considerations. Hypothesis testing will be based on $\log(AUC_{0\text{-}2h}+1)$ C-peptide and will be performed with an exploratory purpose.
The null hypothesis is no difference between placebo and ladarixin, and the rejection of the null hypothesis will support the postulated superiority of ladarixin over placebo.

From a simulation based on the results provided in the [redacted] that showed a 15% difference in $\log(AUC_{0\text{-}2h}+1)$ C-peptide in favor of ladarixin vs. placebo, it is estimated that the sample size of 130-140 would provide >90% power to detect the same difference between treatment groups.

9.2 RANDOMIZATION

Eligible Patient will be randomized in a 2:1 fashion to either ladarixin or placebo.

Randomization will be performed through IRS. Each Patient Kit number will be randomly associated with a treatment group. The randomization list will be provided to the facility responsible for IMP packaging/labelling for the purpose of IMP preparation. Each randomized patient will be allocated with randomization number according to the stratified randomization list. Dropouts after randomization will not be replaced

Randomization will be stratified by site and age group to ensure balanced assignment across treatment groups. The stratified permuted block randomization list will be generated with a computer procedure by an independent statistician not involved in the conduct of the study. A master randomization list will be generated, randomizing an excess of patients (a maximum of 33 for each site) to allow competitive recruitment within each center.

Access to individual patient treatment code will be allowed only in the event of a medical emergency where the knowledge of patient treatment is required to provide the patient with appropriate care. The investigator will be provided with a protected access to the randomization system to allow opening of the treatment allocation for a specific patient in case of a medical emergency.

Dompé Pharmacovigilance contact person will be provided with a protected access to the randomization system in case of unblinding for safety procedures.

Unblinding events will be recorded and reported in the final study report.

The randomization codes will be accessible to the independent statistician(s) who will generate the reports for the Data Monitoring Committee (DMC) evaluation. The treatment assignment information will be kept confidential and will not be disclosed to any other person than those ones involved in the handling of the listings and DMC members.

The randomization code will be broken according to study procedures after the all enrolled patients have reached their 12-month visit (or will be deemed lost in follow-up), and when all data have been cleaned and the database locked for data collected up to M12.

9.3 OVERVIEW OF PLANNED STATISTICAL ANALYSES

The study plans for the following statistical analyses:

- Main statistical analysis: this analysis will be conducted when all enrolled subjects have completed the Month 12 (or lost in follow up) visits, and the study database has been locked and unblinded.
- Addendum to Main analysis: this analysis will be conducted when all enrolled subjects have completed the open phase study post unblinding (up to 24 months).
- Analyses for the DMC: these analyses will be produced periodically according to the DMC charter.
- PK analysis: this analysis will be conducted by the CRO appointed by Dompé when the collection of PK measurements on a selected subset of subjects is completed.

9.4 INTERIM ANALYSIS

[redacted]

9.5 ANALYSIS POPULATION

The following population will be defined:

- The Full Analysis Set (FAS) population will consist of all randomized patients who received at least one dose of the investigational product (either ladarixin or placebo). FAS population will be analyzed according to the ITT principle, i.e. by treatment allocation regardless happening of intercurrent events (treatment policy strategy). The FAS population will be used for the primary analyses of the study and to present results on efficacy data.
- The Per Protocol (PP) population will consist of all randomized patients who received at least one dose of the investigational product and do not have Major Protocol Deviations. The PP population will be used for sensitivity analyses.
- The Safety (SAF) population will consist of all randomized patients who received at least one dose of the investigational product. Safety population will be analyzed according to the actual treatment received. The SAF population will be used to present results on safety data.
- The PK set population will consist of the subset of randomized adolescent patients who received at least one dose of the investigational product and who have been selected to perform PK measurements. Any deviation outside the recommended tolerance ranges (reported in a specific PK plan) will be verified and, if confirmed, will be reported as protocol deviation. The exclusion of the concerned subjects from the PK set will be evaluated case by case. The PK set population will be used to present results on PK data. A Physiologically Based Pharmacokinetic (PBPK) and a Population pharmacokinetics (POPPK) may also be developed. In that case a separated data analysis plan and report would be provided.

9.6 STATISTICAL METHODOLOGY

All patient data collected on the eCRF will be listed by patient and center.

Appropriate descriptive statistics will be produced, according to the variable. For continuous data n, mean, standard deviation, median and range (minimum and maximum) will be presented. For categorical data, frequency distributions and percentages will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

All AUC analyses will be based on actual rather than scheduled timings and will be calculated using the [redacted]. In case of partial information collected during an assessment:

- if actual time is not recorded, the scheduled time will be used instead;
- missing values will be imputed via [redacted];

- when C-peptide values are below the limit of detection, the limit value will be assumed in the calculation of area under the curve.

The last available basal sample (time and value) will be used as the first sample in the computation of the AUC. If required, inferential statistics will be conducted on log(x+1) transformed data, according to Lachin [2011].

Unless otherwise specified, the significance level used for statistical testing will be 0.05 and two-sided tests will be used.

The Statistical Analysis Plan will be issued before the start of enrolment with more technical and detailed elaboration of the principal features of statistical analyses. Additional post-hoc analysis may be produced to further allow comparison between ladarixin and placebo, according to the results obtained.

Any deviations from the original statistical plan (including unplanned analyses) will be clearly documented in the Clinical Study Report.

9.6.1 Analysis of pharmacokinetics

Plasma concentrations for DF2156Y, its metabolites DF2108Y and DF2227Y and unbound DF2156Y will be analyzed descriptively by analyte, day and treatment in a tabular and graphical display.

Cmax, Css,max, Css,min, tmax and tss,max will be obtained directly from the experimental observations.

AUC0-12, and AUCτ will be calculated using the [redacted]. For the purpose of calculating AUC0-12 and AUCτ, when 2 consecutive values less than the limit of quantification (< LOQ) are encountered after tmax or tss,max, these and all subsequent values will be excluded from the analysis. When embedded missing values occur, they will be excluded from the analysis. All values on Day 1 that are <LOQ prior to tmax will be set to zero If any, quantifiable concentrations at pre-dose on Day 1 will be set to zero.

A descriptive PK will be presented. The results will be displayed and summarised in tables and figures. Individual and mean curves (+SD at sampling times), indicating inter-subject variability, will be plotted.

The following descriptive statistics will be provided: n, arithmetic mean, SD, standard error, CV(%), geometric mean, geometric CV, 95% confidence interval (CI) for the arithmetic mean, median, minimum and maximum

9.6.2 Demographic and baseline characteristics

Demographic and baseline characteristics will be summarized for all patients in the FAS population, by treatment group.

9.6.3 Analysis of efficacy variables

9.6.3.1 Primary analysis

The change from baseline in the 2-hour C-peptide AUC post MMTT at 6 months (primary efficacy endpoint) will be analyzed by means of ANCOVA models adjusting for the baseline values, center, baseline characteristics (age group, gender, and BMI) and their interactions with treatment; additional factors may be added in the Statistical Analysis Plan (SAP). The adjusted estimated treatment differences between ladarixin and placebo at Month 6 will be presented together with the corresponding 95% confidence interval. Assumption of normal distribution will be assessed by means on Kolmogorov-Smirnov test. If required, a log transformation might be applied.

The null hypothesis H_0 is that the change from baseline in the 2-hour C-peptide AUC post MMTT at Month 6 in ladarixin ($\mu_{LADARIXIN}$) is equal to placebo ($\mu_{PLACEBO}$):

H_0: $\mu_{LADARIXIN} = \mu_{PLACEBO}$

H_1: $\mu_{LADARIXIN} \neq \mu_{PLACEBO}$

The null hypothesis will be rejected if p-value will be lower than 0.05 (two-sided test). The rejection of the null hypothesis H_0 will support the postulated explorative suggestion of superiority of ladarixin over placebo.

Since patients who discontinue the treatment with the IMP will not be withdrawn from the study but will be asked to complete safety and efficacy assessments as per the protocol, missing data under treatment policy strategy will be addressed by modeling patients with missing data after retrieved drop-outs by assumption of missing data would have been like retrieved drop-outs if they were assessed. If there are not enough retrieved dropouts, a "wash-out" analysis instead of retrieved drop-out analysis will be performed: the missing C-peptide data at month 6 will be imputed by wash-out the effect of treatment using placebo C-peptide data at month 6; this approach does not assume benefits for ladarixin in case of discontinuation and limits a post-discontinuation clinical effect to that of placebo.

9.6.3.2 Sensitivity analyses

The following sensitivity analyses are defined to assess robustness of results on primary endpoint versus adherence to protocol and presence of missing data:

- In order to assess the robustness of results to protocol deviations, comparison between ladarixin and placebo will be performed in the PP population (see section 9.5 for definition) instead of FAS;
- In order to assess the robustness of results to the method of handling missing, comparison between ladarixin and placebo will be performed in the FAS population:
    - on complete cases only, i.e. without considering patients with missing primary endpoint at Week 26 ;
    - under assumption that data are missing at random (MAR) using multiple imputation (MI) methodology for time points at which no value is observed. The imputation models will include all covariates used for primary analysis and primary endpoint assessment at each time point. Details will be provided in the SAP.

9.6.3.3 Secondary analyses

The following secondary endpoints will be evaluated:

1. Change form baseline in 2-hour AUC of C-peptide response to the MMTT [Time frame: Month 12, 18 and 24].
2. Change in HbA1c from baseline [Time frame: Month 6, 12, 18 and 24].
3. Time in range (TIR) by Continuous Glucose Monitoring (CGM) [Time frame: Month 6, 12, 18, 24]
4. Proportion of patients with HbA1c of less than 7% who did not experience severe hypoglycemic events during treatment [Time frame: Month 6, 12, 18 and 24].
5. Average (previous 3 days) daily insulin requirement (IU/kg/day) [Time frame: Month 6, 12, 18 and 24].
6. Proportion of patients with HbA1c <7% and daily insulin requirement <0.5 (IU/kg/day) [Time frame: Month 6, 12, 18 and 24].
7. Additional Glucose Variability Indices derived from CGM (glucose AUC outside the target range of 70 – 180 mg/dL, 2-hour postprandial glucose (PPG), Mean Amplitude Glycemic Excursions (MAGE), continuous overall net glycemic action (CONGA)-n, Mean Of the Daily Differences (MODD), and mean daily blood glucose, SD (Standard Deviation). [Time frame: Month 6, 12, 18 and 24].
8. Number of self-reported episodes of severe hypoglycemia [Time frame: Month 6, 12, 18 and 24].

9. Percentage of patients not requiring insulin therapy [Time frame: Month 6, 12, 18 and 24]
10. Estimated Glucose Disposal Rate (eGDR) [Time frame: Month 6, 12, 18 and 24].

The Secondary endpoints #1, #2 and #3 will be analyzed as detailed in Section 9.6.3.1

Descriptive in nature analyses will be performed on all other secondary endpoints at each available timepoints by means of descriptive statistics and by appropriate parametric tests depending on the nature of the variable and its distribution. Data transformation might be used in order to satisfy the assumption of normality requested by parametric statistical tests. In case such assumptions are not met, non-parametric counterpart tests will be used. Details will be provided in the SAP. Change from baseline value (for continuous variables) and shift tables versus baseline (for categorical variables) will also be summarized for all post-baseline visits.

Additional analyses will include the analysis of the AUCs, daily insulin requirements and HbA1c values by regression models. The adjusted least square means will be estimated for each combination of time point and treatment. The tests of the fixed effects will be presented, together with the estimated least squares means. The estimated treatment difference between ladarixin and placebo at each time point will be presented together with the corresponding 95% confidence interval.

The effect of treatment on the cumulative number of severe hypoglycemic events will be evaluated using an Andersen-Gill analysis with robust sandwich-type variance estimate.

9.6.4 Analysis of exploratory variables

In addition to descriptive statistics at each available timepoint, explorative variables will be analyzed by means of inferential tests depending on their nature and distribution (all confidence intervals and statistical tests on explorative endpoints are of descriptive nature). Change from baseline value (for continuous variables) and shift tables versus baseline (for categorical variables) might be reported for all post-baseline visits.

9.6.5 Analysis of safety variables

Safety analysis will follow the approach described in Section 9.3 with an analysis up to Month 12 visit and analysis up to Month 24.

TEAEs, ADRs and TESAE will be presented by treatment arms in terms of number of AEs and incidence by System Organ Class and Preferred Terms using MedDRA. Analyses will be provided also by severity and relationship to the study drug. In addition, time-to-event methods (Kaplan-Meier estimates) will be used to summarize the time to onset of severe hypoglycemic events.

Vital signs and laboratory tests will be presented using descriptive statistics at each available visit. Additionally, the frequency of subjects reporting an abnormal or abnormal clinically significant laboratory value will be presented for each laboratory parameter. Shift tables versus baseline will compare abnormal laboratory findings at each post-baseline visit.

9.6.6 Intermediate analyses for DMC

Safety data will be reviewed on an ongoing basis by a DMC. Full details of the activities and responsibilities of the DMC are provided in the study DMC Charter.

The DMC will give careful consideration to the appropriateness of trial continuation if there is emerging evidence that ladarixin is harmful. Since DMC does not monitor primary endpoints for early effect termination, no Type I error adjustment is necessary. Anyhow, access to unblinded information on the primary analyses is allowed in order to balance patient safety risk against a possible gain in efficacy.

9.6.7 Specification of subgroups for analysis

Statistical tests for interaction (between subgroup and treatment arm) will be performed to decide about the need to further investigate subgroups of the trial population based on the following categorical and numerical variables: age, gender, race, BMI, HLA, autoantibodies, proinsulin/c- peptide ratio, islet AAb, and microRNA-375. miRNA signature at baseline will be performed to identify patients responding to ladarixin treatment (predictive analytics).

Subgroup analysis will be performed if interaction tests are statistically significant at 15% nominal level. Statistical details and potential new subgroups definitions will be reported in the SAP.

9.6.8 Missing data

All reasonable efforts will be made to reduce the rate of missing data. Investigators will be trained about the importance of patient retention and full data capture. Also, any reasonable attempts should be made by the Investigators to emphasize continued patient's participation for the full duration of the trial. However, in order to minimize missing data, if a patient cannot refer to the site for a planned follow-up visit, the Investigator will try to obtain any relevant information from the patients, including documents/laboratory results available from local medical care.

10.ETHICAL CONSIDERATIONS

10.1 INDEPENDENT ETHICS COMMITTEE (IEC) / INSTITUTIONAL REVIEW BOARD (IRB)

It is the responsibility of the CRO appointed by Dompé or of the Investigator (US sites) to obtain approval of the trial protocol/amendments from the appropriate IEC/IRB.

Prior to the initiation of the study, the followings will be submitted to the IEC/IRB for approval:

- the study protocol,
- the ICFs,
- the current version of the Investigator's Brochure,
- Investigator's current curriculum vitae,
- Insurance certificate
- any other requested document(s).

A copy of the IEC/IRB approval will be sent to Dompé along with relevant correspondence with the IEC/IRB, a roster of IEC/IRB members or the US Department of Health and Human Services (DHHS) general assurance number.

The study will not be started until full written approval has been obtained from the appropriate IEC/IRB. The letter of approval should be dated, and should specify the type (e.g. protocol number) and the date of the documents which were reviewed and approved.

The CRO appointed by Dompé or the Investigator will submit any future amendment to the protocol to the IEC/IRB which granted the original approval. Any amendment will be implemented only when full approval has been obtained from the appropriate IEC/IRB, except for those amendments which involve only logistical or administrative aspects of the study.

The CRO appointed by Dompé or the Investigator will send to the IEC/IRB any updated Investigator's Brochure.

The CRO appointed by Dompé or the Investigator will also submit to the IEC/IRB which approved the protocol at least annually any required progress reports and study update and will inform the IEC/IRB of the termination of the study.

The CRO appointed by Dompé or the Investigator will report any serious ADRs, life-threatening problems or deaths occurred at other sites participating to this clinical trial and/or in other clinical studies conducted with ladarixin.

10.2 INFORMED CONSENT

No study-related procedures (including non-invasive and diagnostic procedures) will be undertaken prior to completion of the consenting process.

Each potentially eligible patient will be informed of the study's objectives and overall requirements. The Investigator will explain the study fully to him/her using the ICF. Although patients will be informed that they can withdraw consent at any time, the Investigator will also emphasize that missing data diminish the scientific value of all patients' contributions. Similarly, patients will be informed that safety data might have to be collected after their participation in the study has been completed. If the patient is willing to participate in the study, (s)he will be requested to give written informed consent after being given sufficient time to consider his/her participation and the opportunity to ask for further details.

The ICF will be signed and personally dated by **both** the patient and the Investigator. A copy of the signed form will be provided to the patient, and the original signed ICF will be retained and filed in the Investigator Site File. Patient consent will be documented in the hospital records.

Participants under the age of 18 (14-17 years inclusive) shall provide a consent/assent for the study using a simplified ICF template as per country requirements; parents/guardians consent/signature will also be obtained as per country requirements.

Adolescents (14-17 years inclusive - and parents/guardian, as appropriate) to whom the full PK analysis will be proposed will follow a specific consenting process, to clearly explain PK procedures. A specific PK-ICF will have to be signed by adolescents to be selected for full PK analysis.

Individual (i.e. site specific; local language) ICFs will be provided to the site once approved by the IEC/IRB. Any changes requested by the IEC/IRB must be approved by Dompé prior to the documents being used.

10.3 CONFIDENTIALITY

All information obtained during the conduct of the study will be regarded as confidential. An agreement for disclosure will be obtained in writing by the patient and will be included in the ICF. Patient's data collected during (or after completion) of the study will be handled in accordance with applicable data protection laws and European data protection Regulation (EU) No. 679/2016 of the European Parliament and of the European Council regarding the protection of natural person's personal data and the free circulation of said data (hereinafter GDPR EU No. 679/2016) and according the standards of Good Clinical Practice (Law Decree 211/2003).

On the eCRFs patients will be identified ONLY by the assigned patient number. If patient names are included on copies of documents submitted to Dompé or the CRO appointed by Dompé, the names will be obliterated or masked, and the assigned patient number added to the document.

The Investigator should keep a separate log (Patient Master List) of patient's codes, names and addresses.

10.4 COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

Before the trial formally starts, Dompé will take out a study-specific insurance covering the amount requested by the respective national laws for patients/Investigators/Institutions participating in the clinical trial.

In case of questions about medical care, cost for medical care or insurance, patients can talk to their Investigator. Contact details will be given in the ICF.

Insurance and any updates will be provided to the Investigator before trial commencement for filing into the Investigator Site File.

11. DATA HANDLING AND RECORD KEEPING

11.1 CASE REPORT FORMS

An eCRF will be used for this study and will be made available to investigational sites. eCRFs are the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé' designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

An eCRF is required and should be completed for each patient enrolled (consented). The Investigator will be responsible for the accuracy of the data entered in the eCRFs.

Source documents should be available to support all the data recorded in the eCRF; location of source documents, including those for which the eCRF might be accepted as being the sole source document, will be specified and listed at the center Initiation Visit.

The eCRF must be available for review to designated Dompé's representatives at each scheduled monitoring/audit visit.

11.2 DIARY

A Diary (local language version) will be provided to each patient randomized into the trial.

The patient will report in the Diary the details (date, time, number of capsules) of each administration of the IMP, values of daily SMBG via fingerstick, daily insulin doses, time of meals and carbohydrates intake at each meal if patient is able to apply carbs counting, hypoglycemia episodes. It is responsibility of the Investigator to explain to each patient how to enter the data in the Diary and to check the data inserted in the Diary to ensure correct completion as well as correct intake of the IMP.

The information collected in the Diary will be part of the patient's eCRF.

Diary is the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

11.3 DATA MANAGEMENT

Data management will be performed by the CRO appointed by Dompé.

The eCRF for all patients will constitute the study data-base, and the data will be verified for missing data, inconsistencies, and for any necessary medical clarifications. Queries arising from these checks will be sent by the appointed CRO to the Investigator for response and resolution.

Once all data queries have been resolved, the eCRF will be signed by the Investigators. This approval method will include applying an electronic signature, a uniquely assigned username and password that together will represent a traditional handwritten signature. After each Investigator will have signed the eCRFs related to the patients enrolled the study data-base will be declared to be "clean" and the study data will be locked ready for analysis.

After the database lock has been achieved, the Investigator may archive the pdf copies of the eCRFs to be retained at the center. All data collected in the context of this study will be stored and evaluated per regulatory requirements and any applicable guidance for electronic records. Also, data will be stored and evaluated in such a way as to guarantee patient confidentiality in accordance with the legal stipulations applying to confidentiality of data. Study records (e.g., copies of eCRFs, regulatory documents) will be retained at the study center, along with adequate source documentation, according to FDA and ICH requirements.

11.4 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE STUDY

In addition to the documents mentioned in Sections 10.1 and 12.1, the following documents will be required from the Investigator prior to the initiation visit (and during the course of the study in case of any update):

- Current, signed and dated Curriculum Vitae of Principal Investigator and any Sub-Investigators/co-workers. Updates should be provided at least every two years.
- Confidential disclosure agreement Form in accordance also with European data protection Regulation (EU) No. 679/2016 (GDPR)
- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation). Updates should be provided as soon as any reference value has changed.
- A signed page of the final protocol and any amendments.
- A signed copy of the study Financial Agreement/Clinical Study Agreement with Dompé (or CRO appointed by Dompé), including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- Form 1572 and financial disclosure form 3455 from all the persons listed on the 1572. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.

11.5 ESSENTIAL DOCUMENT RETENTION

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product. These documents should be retained for a longer period however if required by the applicable regulatory requirements. The Investigator should take measures to prevent accidental or premature destruction of these documents.

The essential documents include, but are not limited to: the signed protocol, copies of the completed eCRFs, and Diary, signed Patient Informed Consent Forms from all patients who consented, hospital records and other source documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

The Investigator will inform Dompé (or designee) of the storage location of these essential documents and must contact Dompé before disposing of any. If the Investigator wishes to assign the files to someone else or to remove them to another location, he/she should consult with Dompé about this change.

Dompé will inform the Investigator in writing when these documents no longer need to be retained.

12. STUDY MANAGEMENT

The study will be performed in accordance with the protocol, the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, October 2013) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (*ICH-GCP*) and any local regulations.

12.1 REGULATORY BODY APPROVAL

Dompé or the CRO or other consultant appointed by Dompé will obtain the necessary approval from the Competent Authorities, as needed, prior to initiation of the study.

The study will not be started until written approval from the relevant Competent Authorities (or no objection within the timeframe set by the local regulation, as applicable) has been received by Dompé.

12.2 STAFF INFORMATION & RESPONSIBILITIES

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

The Investigator will maintain a list of delegated responsibility detailing the various study tasks to be performed by each member of his/her study staff. Each staff member should sign in agreement to their performing each of the tasks delegated to them on the list.

12.3 MONITORING

Monitoring will be carried out by CRAs of CRO appointed by Dompé.

The purpose of the monitoring visit is to verify that the rights and the wellbeing of the patient are protected, that the reported data are accurate, complete and verifiable from source documents and that the conduct of the trial complies with the currently approved protocol and any amendments, with ICH GCP, and with regulatory requirements.

Prior to study start, the Investigator will be informed of the anticipated frequency of the monitoring visits. (S)He will also receive a notification prior to each monitoring visit during the course of the study. It is expected that the Investigator and/or his/her sub-Investigator(s) and other appropriate staff will be available on the day of the visit to discuss study conduct and to cooperate with the monitor to ensure that any problems detected during the course of these monitoring visits are resolved.

12.3.1 Access to records

The Investigator will allow designated Dompé representatives, including staff from the appointed CRO, and regulatory/ethics bodies to have direct access to the source documents to verify the data reported in the eCRFs. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained, if necessary, via an audit trail.

All study records must be available for audit by Dompé farmaceutici s.p.a.; its authorized representatives; and Regulatory Inspection by Regulatory Authority.

12.4 AUDIT AND INSPECTION

Audit activities will be performed by the Dompé Quality Assurance Unit or any other third party delegated by Dompé or by the CRO, as appropriate.

12.5 DATA MONITORING COMMITTEE

An independent DMC will be established and will be responsible for safeguarding the interests of trial participants, and for enhancing the integrity and credibility of the trial. The DMC will assess the safety and efficacy of the interventions during the trial and will monitor the overall conduct of the clinical trial. The DMC will provide recommendations to Dompé about stopping or continuing the trial on safety basis. To contribute to enhancing the integrity of the trial, the DMC may also formulate recommendations to Dompé relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DMC will operate independently of Dompé, and its members will not have connections to Dompé with the exception of the compensation to DMC members related to their activities.

The DMC will comprise three members. They will be a multidisciplinary group that will include:

- Two diabetologists/endocrinologists with extensive experience in the diagnosis and management of patients with T1D, mainly at disease onset;
- A Biostatistician with substantial experience in the DMC process.

The DMC:

- Will review unblinded data. To this purpose, an Independent Statistician will liaise with the CRO statistician and will have access to those components of the database necessary to generate the reports to the DMC.
- Will be responsible for the ongoing (at least every 4 months) review of safety data throughout the trial. Primary among the safety data that will be reviewed are Serious AEs.
- Will review efficacy data in an ongoing manner to enable the assessment of the acceptability of safety in the context of emerging evidence about efficacy.
- Will be advisory to Dompé and make recommendations to Dompé regarding the continuation of the trial and potential modifications to the design and conduct of the trial. These recommendations will be made in a manner to maintain confidentiality of emerging information about efficacy and safety, unless access to certain data is needed to enable Dompé to make decisions about the DMC recommendations. Dompé will be responsible for promptly reviewing the DMC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in the study conduct are required

All details of the conduct and responsibilities of the DMC will comply with Guidance for Clinical Trial Sponsors: Establishment and Operation of Clinical Trial Data Monitoring Committees and will be described in the 'DMC Charter' to be finalized during the set-up phase of the study and prior to the initiation of enrollment.

12.6 PROTOCOL DEVIATIONS/AMENDMENTS

Changes to the Protocol will be implemented only when written amendments have been signed by all individuals who signed the protocol.

Any amendment will be sent to the IEC/IRB and Competent Authority/FDA as appropriate. No deviations from or changes to the protocol will be implemented without documented approval of an amendment from the IEC/IRB which granted the original approval, except where necessary to eliminate an immediate hazard(s) to trial patient, or when the change(s) involves only logistical or administrative aspects of the trial. The deviations from or changes to the protocol implemented to eliminate an immediate hazard to the trial patient and the proposed amendment, if appropriate, should be submitted to the IEC/IRB for review and approval as soon as possible.

Any other deviation from the protocol that has not been approved by Dompé and the IEC/IR could result in a discontinuation from the study at the center involved.

Any written amendment will be sent to all recipients of the protocol.

12.7 DISCONTINUATION OF THE STUDY

Dompé reserves the right to stop the study at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative reasons.

After such a decision is made, the Investigator must inform all relevant persons e.g. study staff, potential patients etc. within 2 weeks. All delivered study materials must be collected and all eCRFs completed to the extent possible.

Study discontinuation will be notified to Competent Authorities within 15 days from decision.

12.8 PUBLICATIONS

As this study is part of a multicenter trial, publications derived from this study will be planned and agreed with the participating Investigators. Publications will include input from the Investigators, his/her colleagues, other investigators in this trial and Dompé personnel. Such input will be reflected in publication authorship. Criteria for selection of authors will be agreed. Subsequent to the multicenter publication or one year after completion of the study, whichever occurs first, an Investigator and/or his/her colleagues may publish the results of Investigator's part of the study independently.

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study must be prepared in conjunction with Dompé and must be submitted to the Dompé for review and comment at least 45 days prior to submission for publication or presentation. The Sponsor reviews proposed manuscripts prior to submission within a reasonable period of time (30-90 business days in relation with the complexity of the work). If such draft contains confidential patentable information, the Investigator will refrain from publishing any such information for a period not exceeding 180 days, to enable Dompé to file for the protection of any intellectual or proprietary property interest.

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.

In any case, study results will be communicated in full to the Competent Authority by the submission of a complete Clinical Study Report.

The Investigator(s) 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.

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14. APPENDICES

14.1 APPENDIX 14.1 - PACKAGING AND LABELING DETAILS

A Patient Kit will be prepared for each patient, containing 2 Treatment wallets, one for each treatment week. Each Treatment wallet will contain a total of 56 capsules (amount needed for one treatment cycle).

All items will have local language labels. The template of the English label is provided below. Label content will be adjusted to meet local regulatory requirements.

NOTE:

Patient Kit No. XXYY	according to the randomization list (and IRS system drug dispensation number)
Patient No. _____	to be filled by Investigator according to Patient Randomization Number
Investigator: _____	to be filled by Investigator according to PI name

Specimen Label for each Patient Kit

STUDY LDX0319 Sponsor Dompé farmaceutici s.p.a.; Via San Martino 12, Milan – Italy
INVESTIGATOR: _____

PATIENT KIT No. XXYY **PATIENT No. ____**
(contains one treatment cycle)

INVESTIGATIONAL PRODUCT: ladarixin (200 mg) or placebo oral capsules

CONTAINS: 2 TREATMENT WALLETS FOR ONE TREATMENT CYCLE; TOTAL 56 CAPSULES

coded BATCH No. coded EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C**
DO NOT FREEZE

DIRECTIONS: Dispense the Treatment Kit, corresponding to a cycle of 14 consecutive days. For any questions, please contact (*to be identified in the final label*)

For clinical trial use only. Keep out of reach of children.
Caution: New Drug-Limited by Federal law to investigational use.

Specimen Label for each Treatment Wallet

STUDY LDX0319 Sponsor Dompé farmaceutici s.p.a.; Via San Martino 12, Milan – Italy

PATIENT KIT No. XXYY **TREATMENT WALLET**

INVESTIGATIONAL PRODUCT: ladarixin (200 mg) or placebo oral capsules

CONTAINS: TREATMENT WALLET WITH A TOTAL 28 CAPSULES

coded BATCH No. coded EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C**
DO NOT FREEZE

DIRECTIONS: Take the drug twice a day: 2 capsules in the morning and 2 in the evening. See additional instructions on administration provided by the Investigator.
Contact the Investigator should you have any questions.

For clinical trial use only. Keep out of reach of children.
Caution: New Drug-Limited by Federal law to investigational use.

14.2 APPENDIX 14.2 – STUDY FLOW CHART

180 days
1st insulin [redacted]

Procedures Assay to be performed at a centralized laboratory Assay to be performed at the local laboratory	**Screening**	**Baseline (within 3 weeks to C1 start)**	**Randomization (within 3 days of C1**	**TREATMENT PERIOD (cycle = C) - 13 Cycles (C1 - C13)**									**FOLLOW-UP PERIOD**	
				C1 - C3	**Week 11** (end of 3rd cycle)	**C4 - C6**	**Week 23** (end of 6th cycle)	**C7 – C9**	**Month 6** Week 26±2 **Visit while on C7**	**Week 35** (end of 9th cycle)	**C10 - C13**	**Month 12** Week 52±2	**Month 18** Week 78 ±2	**Month 24** Week 104 ±2
Informed Consent Form	X													
Eligibility criteria Randomization	X	X	X											
Auto-antibodies	X													
Fasting C-Peptide [2]	X[2]	X[2]												
Medical history	X													
BP/HR		X			X		X			X		X		X
weight, height, waist & waist/hip circumference		X							X			X	X	X
eGDR		X							X			X	X	X
Insulin requirements previous 3 days		X							X			X	X	X
Self Reported Severe Hypoglycemia		X							X			X	X	X
HLA DR-DQ haplotypes, Proinsulin.		X												
HbA1c, microRNA-375, IL-8, IL-6, hs-CRP, NET, Proinsulin.		X							X			X	X	X
miRNA signature		X												
Safety Laboratory Test (hematology & biochemistry)[3]		X			X		X			X		X		X
MMTT [3]		X							X			X	X	X
Pregnancy Test		X			X		X			X				
Sparse PK assessment [4]				X	X	X	X	X		X	X			
12 lead ECG		X			X[6]		X[6]			X[6]				
CGM download[5]		X[5]							X[5]			X[5]	X[5]	X[5]
[redacted][6]			X		X		X			X				
Patient Diary delivery/check			X		X		X			X		X	X	X

Check discontinuation criteria [6]					X		X			X				
Patient Phone call [7]				X	X	X	X	X	X	X	X	X	X	X
AE/SAE recording	X----------X													
Prior and Concomitant Medication	X----------X													

1 anti-GAD; IAA (if obtained within 10 days of the onset of insulin therapy); IA-2; ZnT8.

2 one re-assessment of fasting C-peptide allowed in case the value at first sampling is >0.205 nmol/L.

3 tests performed at Baseline should be planned to ensure the samples are sent to the laboratory and results are available in time for randomization.

4 at least 2 assessments to be done within 96 hours after the last IMP dose in at least 2 treatment cycles.

5 **sensor to be positioned at least 10 days before the scheduled visits.**

6 [redacted] must occur ONLY after check of inclusion/exclusion criteria (at the Randomization Visit) and of discontinuation criteria (at the Visits performed at the end of the [redacted] treatment cycles). Results of ALT/AST, bilirubin, creatinine and albumin, ECG readings and pregnancy results **should be made available by the local laboratory in time to allow [redacted] as soon as possible during the visit**. If this expedite timing cannot be achieved, [redacted] might be [redacted] to patient named address by a selected courier (CRO to support shipment). As per treatment discontinuation criteria, no [redacted] will be [redacted] in the case: QTcF is either > 500 msec or has increased by > 60 msec from baseline measurement on two consecutive ECG readings taken 1 hour apart; the patient develops any significant cardiovascular disease/abnormality, renal (eGFR <60mL/min/1.73 m2) or hepatic (increased ALT/AST > 3 x ULN and increased total bilirubin > 3mg/dL [>51.3 µmol/L]) dysfunction, hypoalbuminemia (serum albumin <3 g/dL), significant systemic infection that must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant discontinuation, ketoacidosis or hypoglycemic coma; a female patient has become pregnant.

7 Telephone calls and/or emails sent by PI or Sub-Investigators to the patients will be scheduled on a regular basis to ensure optimization of metabolic control. It is expected that patients are contacted at least every 2 to 3 weeks during study participation. A telephone call will be anyway scheduled in the 2 weeks before a planned visit.

14.3 APPENDIX 14.3 - METHODOLOGICAL DETAILS

14.3.1 Handling of samples for centralized assay

One or more centralized laboratories will be involved for assay of HbA1c, HLA DR-DQ haplotypes and Proinsulin, C-peptide and glucose, miRNA signature, microRNA-375, IL-8, IL-6, hs-CRP.

The centralized laboratory performing the assay of C-Peptide and HbA1c should be certified by CLIA (or similar certification) and will perform the analyses under strict quality control. This laboratory should also participate in the National Glycohemoglobin Standardization Program with the method yearly certified at the level of Laboratory 1 Certification to ensure that the HbA1c values are traceable to the DCCT values.

A study-specific Laboratory Manual will be provided to all sites, reporting detailed instructions for centralized blood sampling, and preparation, storage and shipment of final samples. All the laboratories involved in the centralized assays will be listed in the Laboratory Manual, along with relevant contact details. The centralized laboratories will provide tubes and labels for blood withdrawal and sample storage.

Some samples will be shipped from the site to the centralized laboratories in appropriate packaging in appropriate packaging in dry ice (solid CO2) to maintain frozen conditions, if required. All samples will be shipped on an ongoing basis during the trial, according to logistics. However, screening/baseline samples and samples from the last patient visits will be shipped as soon as possible to ensure timely availability of results for randomization and database closure, respectively.

Handling of samples on receipt at the centralized laboratories and assay methods will be described in detail in the Laboratory Manual. Similarly, arrangements will be made for back-communication of the results to the sending site. Once received from the centralized laboratories, each site will enter values pertaining to its patients in the corresponding eCRF.

All steps will be tracked to ensure correct data reporting.

All samples will be destroyed after the final study report has been issued or after the patient has withdrawn his/her consent.

The CRO appointed by Dompé will be responsible for reconciling laboratory materials, for preparing/distributing storage and shipment tracking forms, and for coordinating shipment from the site to the centralized laboratories.

The protocol requires that either cell-free serum (blood allowed to clot and centrifuged) or EDTA whole blood samples are stored between -70°C and -80°C until shipment to the centralized laboratories. An appropriate refrigerated storage must therefore be available at each center. A refrigerated centrifuge will also be required.

14.3.2 Details of Pharmacokinetics blood sampling and Pharmacokinetics analysis

Venous blood sampling

Blood samples for PK analysis will be collected using an indwelling catheter with switch valve or direct venipuncture. The cannula will be rinsed, after each sampling, with about 1 mL of sterile saline solution containing 20 I.U./mL Na-heparin or similar solution as per site practice. 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.
At least 1.0 mL of plasma will be obtained and divided equally into 2 aliquots of 0.5 mL each. Each plasma sample will be immediately divided into two aliquots, P1 and P2, in pre-labelled polypropylene tubes, and stored frozen at ≤-20°C until analyses.

Blood sampling time schedule

Blood samples for full PK analysis will be collected (see Flow-chart) on:
Day 1: pre-dose and 0.25 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h (n=12; volume 36 mL);

Day 14: pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 h, then 24h, 48h, 72h (n=15; volume 45 mL). Patients will be dosed only in the morning. The evening dose will be not administrated.

Samples for "sparse" determination of DF2156Y (the acid form of ladarixin) and the metabolites DF2108Y (S-isomer) and DF2227Y (R-isomer) in all study population (adolescent and adults) should be obtained in at least 2 occasions within 96 hours after the last dose in at least 2 treatment cycles (3 mL for each sample).

Time of IMP administration and actual sampling times for each patient will be recorded in the individual eCRF.

The actual sampling times should not exceed the recommended tolerance ranges presented in a specific PK plan. Any deviation outside the recommended tolerance ranges will be verified 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.

Samples labelling (full PK and sparse PK)

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

Sponsor Study code	LDX0319
Subject number	
Tube identification	P1 (aliquot 1) /P2(aliquot 2)
Date of Sampling	(dd/mm/yyyy)
Scheduled sampling time	as h:minutes post dose (e.g. 0.25 h , 0.5 h etc.)

Samples storage, transport and analysis

During the study the samples will be stored at ≤- 20° C. Aliquots 1 and aliquots 2 will be stored in separate freezers, if possible.
All aliquots 1 (P1), packed in sufficient solid CO2, will be shipped by an authorized courier from the Clinical site to: Dompé farmaceutici S.p.A. Bioanalytical Laboratories, Via campo di Pile s.n.c., - 67100 - L'Aquila, Italy. The temperature during the shipment should be monitored.
The remaining aliquots 2 will be shipped in different shipment from the aliquots 1 in the same condition as per aliquot 1.
Plasma samples will be analyzed according to validated analytical method/s by the Dompé Bioanalytical laboratory. Aliquots 1 and 2 will remain stored at Dompé farmaceutici S.p.A. Bioanalytical Laboratories until the finalization of the clinical report would be provided. Afterwards, the samples will be destroyed and a certificate of destruction will be provided.

No analyses different from those stated in this protocol and agreed by the patients when signing the ICF will be performed unless a new ICF and a new approval from the IEC/IRB is obtained. The patients may ask to destroy their own biological samples at any time.

PK analysis (PK parameters)

Plasma concentrations for DF2156Y and the metabolites DF2108Y and DF2227Y unbound fraction (determined only in the samples collected on: Day 1: around Cmax (1h) at 12 h (pre-dose of the evening dose) and on Day 14: around Cmax (1h) at 12 h.) will be analyzed descriptively by analyte, day in a tabular and graphical display.

The following parameters for DF2156Y, DF2108Y and DF2227Y will be calculated by model independent approach (non-compartmental analysis using Phoenix WinNonlin®) for Day 1 and Day 14:
C_{max} (maximum plasma concentration)
t_{max} (time to maximum plasma concentration)
AUC0-12 (area under the plasma concentration-time curve from time zero to 12 hours post-dosing)

Data permitting, the following PK parameters for DF2156Y, DF2108Y and DF2227Y, will also be calculated by model independent methods:

Day 14
AUC_τ (area under the plasma concentration-time curve within a 12-hour dosing interval at steady state)
$C_{ss,av}$ (average plasma concentration at steady state)
λz
$t_{½}$
CLss/F (CL/F at steady state) for DF2156Y only
Vz,ss/F (apparent volume of distribution at steady state) for DF2156Y only
PTF% (peak trough fluctuation)
Rac (accumulation ratio)

A PBPK and a POPPK may also be developed.

Flow chart of time blood sampling time to assess the PK profile

VISIT DAY	Blood sample collection times (hours)															
	Pre-dose	Morning Dose	0.25	0.5	1.0*	1.5	2.0	3.0	4.0	6.0	8.0	10.0	12.0	Evening Dose		
DAY 1	X		X	X	X	X	X	X	X	X	X	X	X predose evening			
		Morning Dose	0.25	0.5	1.0*	1.5	2.0	3.0	4.0	6.0	8.0	10.0	12.0	24.0	48.0	72.0
DAY 14	X morning		X	X	X	X	X	X	X	X	X	X	X	X	X	X

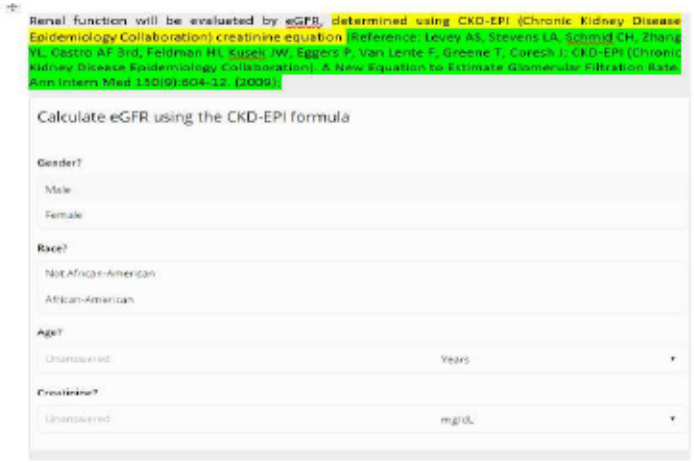
*around Cmax

Analysis will be carried out within one year from sampling. i.e. before the study blind is broken. Procedures to maintain the blind will be implemented.

The PK report(s) will be integrated in the CSR.

14.3.3 Calculation of eGFR

Renal function will be evaluated by eGFR, calculated by the following formula (*Levey AS. (2009)*:



14.3.4 Calculation of eGDR

24.31 - [12.22 × waist-to-hip ratio (WHR)] - [3.29 × hypertension status (defined as 0 = no, 1 = yes)] - [0.57 × glycated hemoglobin (HbA1c)]
The calculated eGDR units are milligrams per kilogram per minute. *(Simoniene D. et al 2020)*

14.3.5 Handling of CGM device

The [redacted] Continuous Glucose Monitoring System ([redacted]) will be used to perform a CGM. CGM measures the subject's interstitial glucose level using electrodes that measure an electric signal produced by glucose oxidase reaction. The system records data approximately every 5 minutes. CGM will be used periodically to measure the subject's interstitial glucose level according to the schedule presented in study flow chart/time and event schedule. A CGM sensor will be inserted subcutaneously and calibration performed during set-up at the site at least 10 days before the concerned visits (Baseline, Month 6, 12, 18 and 24) and removed during the day of the concerned visit, as per Study Flowchart (see Appendix 14.2).

CGM sensor implantation and set-up can occur at Screening Visit and Week 23 Visit if the visit is scheduled 10 days before Baseline and before Week 26 respectively. In addition, sites should upload the sensor data to check the subject's compliance with wearing the device. If recalibration will be needed during use at a minimum, a SMBG value obtained in the morning (e.g. fasting or pre-breakfast measurement) should be entered, as well as a value in the evening (e.g. pre- or post-dinner measurement). Subjects may be instructed to use results from the 4 daily SMBG fingerstick measures to fulfill these criteria. If the subject needs assistance with the management of the device, an ad-hoc visit should be performed, if this occurs a data upload is also recommended at this time.

According to the clinician's management of the subject, the data will remain blinded or unblinded to the subject, the investigator, and to the Sponsor during the recording and will be downloaded into a data file. The downloaded file should be printed and reviewed by the site for review of monitoring compliance. The subjects should make every attempt to be at 100% compliance with use of the device. If a subject is not compliant the reason should be discussed with the subject, documented in source notes, and the subject should be re-educated about the importance of CGM compliance. Detailed procedures (including calibration) will be described in an operations manual and site staff will be fully trained on the use of CGM. Subjects will be instructed on use of the device and calibration according to manufacturer's instructions. Subjects will wear the sensor and perform recalibration, if needed, according to manufacturer's instructions.

If a subject uses a CGM device prior to entry into the study, he/she may continue to use the device during the study in accordance with their usual diabetes management care. Such a subject will be required to also use the blinded CGM device according to protocol procedures.

14.3.6 Mixed Meal Tolerance Test

The MMTT will be performed after an overnight fast, according to Greenbaum (*2008*) at baseline (within 3 weeks prior to randomization) and at each subsequent visit at month 6, 12, 18 and 24. MMTT should not be performed within one week of resolution of a diabetic ketoacidosis event, defined as the presence of:

- hyperglycemia (blood glucose >200 mg/dL);

- pH <7.3 or HCO3 <15;

- ketones positive in the serum or urine.

Prior to the test, patients will withhold long-acting insulin on the morning of the test. Rapid-acting and short-acting insulin will be allowed up to 2 hours and 6 hours, respectively, before the test. Test will be re-scheduled if the patient has a capillary glucose value of >200mg/dL or <70mg/dL.

The test will be initiated before 10 a.m. and, for Month [redacted] visit, at least 2 hours apart from the morning dose of the IMP.

After 2 pre-meal basal samples have been drawn between -20 to 0 min (basal 1 and basal 2), patients will be given 6mL/kg of [redacted] and the equivalent [redacted]

[redacted] by [redacted] up to a maximum of 360 mL, to be drunk within 5 min. Post-meal samples will be drawn at 15±5, 30±, 60±10, 90±10, 120±15 min after the meal.

14.4 APPENDIX 14.4 - CLINICAL TRIALS SUBJECT TO TRANSITION FROM EUROPEAN DIRECTIVE 2001/20/EC TO CLINICAL TRIAL REGULATION EU NO 536/2014

LDX0319 (EudraCT 2020-001926-71) constitutes a Consolidated Protocol and the minor differences between the nationally authorized trials is summarized in the following table:

EU Member State	Minor Difference of trial conduct
Germany	The eligible patient age range for the trial is 18 - 45 years.

DocuSign

Certificato di completamento

ID busta: [redacted]

Stato: Completato

Oggetto: Complete with DocuSign: LDX0319 Study Protocol v5.1_Final_12.Oct.2023 (Clean).pdf

Busta d'origine:

Pagine documento: 75
Pagine certificato: 5
Firma guidata: Abilitato
Timbro ID busta: Disabilitato
Fuso orario: (UTC+01:00) Amsterdam, Berlino, Berna, Roma, Stoccolma, Vienna

Firme: 3
Iniziali: 0

Creatore busta:
[redacted]
Via Santa Lucia, 6
Milan, Milan 20122
[redacted]
Indirizzo IP: [redacted]

Verifica record

Stato: Originale
16/10/2023 17:47:49

Proprietario: [redacted]

Posizione: DocuSign

Firmatario - Eventi	Firma	Timestamp
[redacted] Livello di protezione: E-mail, Autenticazione account (necessaria), Accedi con SSO	[redacted] Scelta della firma: Stile preselezionato ID firma: [redacted] Mediante l'indirizzo IP: [redacted] Con autenticazione della firma mediante password DocuSign Con motivi della firma (in ogni scheda): I approve this document	Inviato: 16/10/2023 17:56:11 Rinviato: 17/10/2023 11:05:54 Visualizzato: 17/10/2023 16:42:52 Firmato: 17/10/2023 16:45:34
Record elettronico e divulgazione della firma: Accettato: 28/07/2023 01:28:54 ID: [redacted]		
[redacted] Livello di protezione: E-mail, Autenticazione account (necessaria)	[redacted] Scelta della firma: Stile preselezionato ID firma: [redacted] Mediante l'indirizzo IP: [redacted] Con autenticazione della firma mediante password DocuSign Con motivi della firma (in ogni scheda): I approve this document	Inviato: 16/10/2023 17:56:10 Rinviato: 17/10/2023 11:05:55 Visualizzato: 17/10/2023 18:42:48 Firmato: 18/10/2023 12:31:57
Record elettronico e divulgazione della firma: Accettato: 28/07/2023 10:55:35 ID: [redacted]		

Firmatario - Eventi	Firma	Timestamp
[redacted] [redacted] Livello di protezione: E-mail, Autenticazione account (necessaria)	[redacted] Scelta della firma: Stile preselezionato ID firma: [redacted] Mediante l'indirizzo IP: [redacted] Con autenticazione della firma mediante password DocuSign Con motivi della firma (in ogni scheda): I approve this document	Inviato: 16/10/2023 17:56:10 Visualizzato: 16/10/2023 18:05:44 Firmato: 16/10/2023 18:06:06

Record elettronico e divulgazione della firma:
Non disponibile tramite DocuSign

Firmatario di persona - Eventi	Firma	Timestamp

Editor - Eventi di recapito	Stato	Timestamp

Agente - Eventi recapito	Stato	Timestamp

Recapito intermedio - Eventi	Stato	Timestamp

Recapito consegna certificata - Eventi	Stato	Timestamp

Copia nascosta - Eventi	Stato	Timestamp

Firma come testimone gli eventi	Firma	Timestamp

Pubblico ufficiale - Eventi	Firma	Timestamp

Riepilogo busta - Eventi	Stato	Data e ora
Busta inviata	Con hash/Crittografato	16/10/2023 17:56:11
Consegna certificata	Controllo protezione eseguito	16/10/2023 18:05:44
Apposizione firma completata	Controllo protezione eseguito	16/10/2023 18:06:06
Completata	Controllo protezione eseguito	18/10/2023 12:31:57

Eventi di pagamento	Stato	Data e ora

Record elettronico e divulgazione della firma

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