**CLINICAL STUDY PROTOCOL**

**APPLICABLE TO THE US INVESTIGATIONAL SITES ONLY**

| | |
|---|---|
| **Study Number:** | LDX0419 |
| **EudraCT Number:** | 2020-002966-15 |
| **IND number:** | CCI [redacted] |
| **Investigational Product:** | Ladarixin |
| **Study Phase:** | 2 |

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

**Protocol Version - Date:** **Version No. 2 final– 30 July 2020**

*This protocol version results from revision of protocol version No. 1 (Final, CCI [redacted]) according to Amendment No. 1 (Final, 30 July 2020). Since Amendment No. 1 is applicable to the US sites only, also this protocol version will not be implemented in any of the other participating sites outside the US.*

**NOTE**: Even if the protocol structure remains mostly unaltered and anticipates 1 year of treatment (13 cycles), patients **will be not be treated longer than 3 months** until additional 6/9 month toxicology data in rats/dogs have been reviewed by the FDA. All procedures affected by treatment length are either modified or clearly labelled; specifically, any "grey paragraph" throughout the entire protocol text/appendices **must be read in conjunction with this NOTE**.

***STATEMENT OF CONFIDENTIALITY***

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# CONTACT INFORMATION

| | |
|---|---|
| **SPONSOR** | Dompé farmaceutici s.p.a. [Dompé]<br>Via Santa Lucia 6; 20122 Milan, Italy |
| **Medical Monitoring** | PPD |
| **Clinical Operation Manager** | PPD |
| **Project Director** | PPD |
| **CONTRACT RESEARCH ORGANIZATIONS (CROs)** | PPD<br>PPD |
| **Project Director** | PPD |
| | PPD |
| **Project Director** | PPD |
| **Pharmacovigilance:** | PPD |

**COORDINATING INVESTIGATORS AND INVESTIGATIONAL SITES**

Full list of coordinating investigators and investigational sites for each country involved will be kept in the Trial Master File. Any 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

**SPONSOR**:

Medical Monitoring

Signature: _____ Date: _____

PPD – Associate Director Clinical Development- Diabetes

Project Director

Signature: _____ Date: _____

PPD – Chief Medical Officer

**CLINICAL CENTER**: PRINCIPAL INVESTIGATOR

I have read the study protocol LDX0419 "A phase 2, multicenter, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of 400 mg twice a day oral ladarixin in patients with new-onset type 1 diabetes and preserved 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: _____

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**List of Abbreviations and Definitions of Terms**

| | |
|---|---|
| AE | Adverse Event |
| ADR | Adverse Drug Reaction |
| ALT | Alanine Aminotransferase |
| ANCOVA | Analysis of Covariance |
| AST | Aspartate Aminotransferase |
| AUC | Area Under the Curve |
| 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 |
| $C_{max}$ | Maximum Plasma Concentration |
| CMED | Concomitant Medication |
| CONGA-n | Continuous Overall Net Glycemic Action |
| CRA | Clinical Research Associate |
| CRF | Case Report Form |
| CRO | Contract Research Organization |
| CXCL8 | CXC ligand 8 [formerly interleukin (IL)-8] |
| CXCR1/2 | CXCL8 receptors |
| DPP-IV inhibitor | Dipeptidyl peptidase-IV inhibitor |
| ECG | Electrocardiogram |
| 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 |
| CCI | [redacted] |
| 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 |
| CCI | [redacted] |
| IMP | Investigational Medicinal Product |
| IRB | Institutional Review Board |

| | |
|---|---|
| ITT | Intention To Treat |
| IU | International Unit |
| i.v. | Intravenous |
| $LD_{50}$ | Lethal $Dose_{50}$ |
| MAGE | Mean Amplitude Glycemic Excursions |
| MedDRA | Medical Dictionary for Regulatory Activities |
| CCI [redacted] | [redacted] |
| MLD-STZ | Multiple Low Dose-Streptozotocin |
| MDI | Multiple Daily Injections |
| MMRM | Mixed Models for Repeated Measures |
| MMTT | Mixed Meal Tolerance Test |
| MODD | Mean Of the Daily Differences |
| CCI [redacted] | [redacted] |
| NGSP | National Glycohemoglobin Standardization Program |
| NOD | Non-Obese Diabetic |
| NOEL | No Observable Effect Level |
| PK | Pharmacokinetics |
| PMN | Polymorphonuclear leukocyte |
| p.o. | per os (taken by mouth) |
| PPG | 2-hour postprandial glucose |
| PT | Preferred Term |
| QTcF | Fridericia's corrected QT interval |
| 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 |
| SOC | System Organ Class |
| TEAE | Treatment Emergent Adverse Event |
| TESAE | Treatment Emergent Serious Adverse Event |
| TIR | Time in Range |
| T1D | Type 1 Diabetes |
| ULN | Upper Limit of Normal |
| ZnT8 | Zinc Transporter Isoform 8 |

# 1. STUDY SYNOPSIS AND OVERALL DESIGN

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

**Study Number** LDX0419 CCI
**[EudraCT Number:** 2020-002966-15]

**Study period** Projected starting date (first-patient-in): Q4-2020
Projected completion of patient accrual (last-patient-in): Q2-2022
Projected study end date (last-patient-last-visit): Q4-2023

**Study design**
The study will be a phase 2, multicenter, double-blind, placebo-controlled study. It will randomize approximately 75 adult patients with new-onset type 1 diabetes (T1D) and preserved β-cell function (fasting C-peptide ≥0.205 nmol/l) at baseline. Patients will be 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 18 months from the 1st administration of the study medication. The study database will be locked and data analyzed when the last patient randomized has completed month 12 follow-up visit. After this time point, the follow-up will continue under open-label conditions up to month 18,

**Objectives/endpoints**
The objective of this clinical trial is to evaluate whether a 12 month treatment with ladarixin is effective to improve glycemic control in newly diagnosed T1D adult patients with preserved β-cell function. The safety of ladarixin in the specific clinical setting will be also 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;

**Efficacy endpoints will be:**
- Proportion of patients with HbA1c <7% and daily insulin requirement <0.50 IU/Kg/day [**Primary endpoint.** Time frame: Month 12]

Secondary endpoints:
- Proportion of patients with HbA1c < 7% and daily insulin requirement <0.50 IU/Kg/day [Time frame: Month 6 and 18]
- Proportion of patients with a reduction in HbA1c% > 0.5% from baseline and daily insulin requirement <0.50 IU/Kg [Time frame: Month 6, 12, 18]
- 2-hour AUC of C-peptide response to the MMTT [Time frame: Month 6, 12, 18]
- Time in range (TIR) by Continuous Glucose Monitoring (CGM) [Time frame: Month 6, 12, 18]
- HbA1c levels [Time frame: Month 6, 12, 18]
- Proportion of patients with HbA1c < 7% who did not experience severe hypoglycemic events during treatment [Time frame: Month 6, 12, 18]
- 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].
- Number of self-reported episodes of severe hypoglycemia [Time frame: Month 6, 12, 18]

- Average (previous 3 days) daily insulin requirements (IU/kg/day) [Time frame: Month 6, 12, 18]
- Estimated Glucose Disposal Rate (eGDR) [Time frame: Month 6, 12, 18]

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**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 18]
- 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) treatment cycle, Month 12 and 18 (or withdrawal)]
- Incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) [time frame: throughout the study]

**Number of patients:** 75 adult (18-45 years inclusive) patients with new onset T1D and preserved residual β-cell function at baseline.

**Inclusion/exclusion criteria**

Consented male and female patients aged 18-45 years, inclusive, with new-onset T1D (randomization scheduled to allow the administration of the study medication to start within 100 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 multiple daily injections (MDI) or Continuous Subcutaneous Insulin Infusion (CSII); must have a fasting C-peptide ≥0.205 nmol/L on two occasions.

Patients will be excluded if they have any other chronic disease (including type 2 diabetes), apart from patients with autoimmune hypothyroidism requiring thyroid hormone replacement only; moderate to severe renal impairment calculated by estimated Glomerular Filtration Rate (eGFR) <60 mL/min/1.73 m2 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, sulphanylurea 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, inhaled or systemically injected steroids) 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 first 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); hepatitis A (IgM), hepatitis B (not due to immunization), hepatitis C and HIV positive serologic status. 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 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 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 m2) 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 qualify the patient to 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**
Potential study patients with a recent clinical diagnosis of T1D with a fasting C-peptide ≥0.205 nmol/L on two occasions; these patients will be considered if they fall within the definition of new-onset T1D (randomization scheduled to allow the administration of the study medication to start within 100 days from 1st insulin administration) and will have diagnosis (positive for at least one auto-antibody).

Each patient will be involved in the study for a run-in period (one or more visits) and the randomization visit, followed by a treatment period of 12 months. During treatment, 5 visits will be scheduled at the end of the 3rd (Week 11) and 6th (Week 23) treatment cycles, at Month 6 (Week 26), at the end of the 9th (Week 35) treatment cycles, and at Month 12 (Week 52) after the 1st dose of study treatment. An additional post-treatment visit will be scheduled at Month 18. Study period/visits are summarized in the study flow chart (see Appendix 14.2)

*Screening to Randomization*
Screening will be performed in patients who has given consent for this LDX0419 trial.
Patients will be admitted to an intensive diabetes management to ensure standardized glycemic control in the treatment groups. The Investigator will refer to a guidance for insulin regimen adjustment, with insulin titrated up or down to target HbA1c levels of less than 7% and self-monitoring of blood glucose (SMBG - fingerstick 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 glucose monitor will be provided to participants for the duration of the study.
In order to optimize insulin titration, periodic controls (e.g. telephone calls) will be put in place to ensure timely evaluation of metabolic control and adjustment of insulin regimen. Patients will report their SMBG (logs, glucose meter, etc.) at predefined intervals.

*Screening* includes evaluation of past medical history and disease-specific clinical information, including date of first insulin administration, and 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.

Once the criteria above have been fulfilled, 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 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).
- Screening 12 lead ECG, performed using local equipment that allows automatic calculation of the QTcF.
- Pregnancy test (urine dipstick or blood sample), if appropriate.
- Baseline daily 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 CCI [redacted].

** The CGM device will be positioned at least 10 days before the baseline assessment.

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 1st to 3rd treatment cycles will be dispensed.

*Treatment period and check of treatment discontinuation criteria*

The administration of the investigational medicinal product will start on **Day 1** of the 1st treatment cycle. **Day 1 MUST be scheduled no later than 100 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 1st treatment cycle are part of the baseline assessment. Thereafter, occurrence of any condition that might prevent continuing IMP administration (as per discontinuation criteria above) will be verified at specific visits scheduled at the end of the 3rd (Week 11), 6th (Week 23) and 9th (Week 35) 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 (IMP administration information).

Patient Kits for the 4th to 13th 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 test 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 patient named address by a selected courier (CRO to support shipment).

*Other visits scheduled during treatment*

During treatment, patient 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 (information other that IMP administration at Month 6, any information at Month 12)
- 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 CCI [redacted].
- 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).

*Post-treatment follow-up visit*

At this visit, scheduled on Month 18 (Week 78) 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 CCI [redacted].
- Patient Diary check (information other than IMP administration)
- Blood sampling for measurement (local laboratories) of "Safety Laboratory Tests".
- Vital signs.

**Statistics**

The sample size of the study is calculated on the "proportion of patients with a HbA1c < 7% and daily insulin requirement <0.50 IU/Kg/day", a post-hoc composite endpoint derived from data of the phase 2 trial CCI [redacted], considering a larger effect size expected from the longer treatment length (one year versus 3 months). Based on these assumptions, 75 patients randomized in the trial will allow to achieve a power of approximatively 80% to show superiority of ladarixin vs placebo in terms of primary endpoint, considering a one-sided alpha of 0.05 and a drop-out rate of 10%.

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 proportion of patients with HbA1c < 7% and daily insulin requirement <0.50 IU/Kg/day at Month 12 (primary endpoint) will be analyzed by means of logistic regression.

All secondary endpoints will be analysed at each available timepoint 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 and the Full Analysis Set population will consist of all patients who will be randomized and received at least one dose of the investigational product. Safety population will be analyzed according to the actual treatment received; Full Analysis Set population will be analyzed according to ITT principle, i.e. by treatment allocation. Primary and secondary efficacy analyses will be conducted on the FAS population while the SAF population will be used for safety analyses.

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

# 2. BACKGROUND INFORMATION

Ladarixin (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 CCI [redacted]. Following more recent promising results in mouse models of type 1 diabetes (T1D) and findings obtained from the phase 2 clinical trial in new-onset T1D, development in new onset T1D has been pursued.

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 have shown that ladarixin, in the low nanomolar range, inhibits human polymorphonuclear leukocyte (PMN) migration induced by human CXCL8 receptors activation. Chemotaxis of rodent PMN induced by mouse and rat counterparts of human CXCL8 is also inhibited, indicating that mice and rats are appropriate animal species for preclinical studies. 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 on CXCR1 and CXCR2 is proven by its lack of efficacy against PMN migration induced by fMLP or C5a and against human monocyte chemotaxis induced by CCL2.

DF 2156A (0.1 nM- 1 µM) results a potent inhibitor of IL-8-induced NETs release from isolated hPMN, with IC50 -value of ≈ 1nM and ICmax -value of 1 µM). Coherently with the selectivity data observed in the chemotaxis assay, DF 2156A (1 µM) did not affect C5a, fMLP and PMA-induced NETs release. DF 2156A (1- 60 µg/mL) is also able to block IL-8-induced NETs formation in human whole blood in a concentration dependent manner with a complete inhibition at 60 µg/mL. Finally, the efficacy of DF 2156A (1- 60 µg/mL) is evident on mCXCL1-mediated NETs release in murine whole blood in a concentration dependent manner being the inhibition complete at 60 µg/mL.

### 2.1.2. *In vivo* general studies

*In vivo*, ladarixin (4-16 mg/kg i.v.) prevents 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.) reduces PMN infiltration by about 60%, whereas in the chronic model the compound (7.5-15 mg/kg p.o.) completely prevents the development of pulmonary lesions.

The antiflogistic activity of orally administered ladarixin was proven in the acute mouse model of cantharidin-induced ear inflammation where it reduced the ear edema formation (16% of inhibition), the cell infiltration (34% of inhibition on lymphocytes T and 32% of inhibition on PMNs) and the ear tissue levels of keratinocyte chemokine (37%), VEGF-A (24%) and TNF-α (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 injected before disease induction (preventive treatment). Ladarixin, administered both intradermally and intraperitoneally, dose-dependently reduces both the clinical disease score and PMN recruitment as shown by reduction in MPO levels. At the highest dose tested (16.7 mg/kg), the clinical disease score and PMN migration were decreased by 90% and 60%, respectively [*A0811/E*].

### 2.1.3. Effects in models of type 1 diabetes

Ladarixin was tested after oral administration (15mg/kg/day) in the ***Multiple Low Dose-Streptozotocin*** (MLD-STZ) ***model*** of diabetes using different treatment schedules. Results showed that ladarixin significantly affected the time to diabetes development with the 14-day administration starting from day -1 of fist STZ injection being the more efficient treatment schedule in prolonging the median diabetes free time. When a 14-day treatment was started from day +5, ladarixin still maintained a good performance. Even after diabetes development, glycemic levels during the first 2 months remained constantly lower in the ladarixin treated group as compared with the vehicle group [*Citro, 2012a*].

Ladarixin was also tested after oral administration (15 mg/kg/day for 14 days) in ***Non-Obese Diabetic*** (NOD***) mice***, starting treatment at different ages to explore its effects in a "prevention" setting (animals treated at 4, 8 and 12 weeks of age) or at "onset of diabetes" (animals at about 16 weeks of age, after fasting hyperglycemia was developed). In the "prevention" setting, the incidence of diabetes was reduced by ladarixin administration, regardless of the age when the treatment was started. In particular, when ladarixin was administered in animals at 8 weeks of age, the incidence of diabetes was 47% and 11% in the vehicle and ladarixin treated group, respectively (p=0.029). The ability of ladarixin to protect damage of β-cells was confirmed in the "onset" setting. Animals presenting two consecutive blood glucose readings above 250 mg/dL over 24 h were randomized to receive either ladarixin or vehicle with the same dosing schedule as used in the prevention setting. Treatment with ladarixin at diabetes onset blocked the progression of hyperglycemia while hyperglycemia worsened in animals receiving vehicle. Three animals treated with ladarixin at diabetes onset that subsequently developed diabetes (about 30 days from end of treatment) were re-treated with the test compound starting from day 35. In 2 animals with "mild" diabetes (animals with glycaemia between 300 mg/dl and 450 mg/dl) out of the 3 animals re-treated, ladarixin apparently reverted increased glycaemia [*Citro, 2012b*]. In parallel, ladarixin modifies leukocyte infiltration in the pancreas and the inflammatory process (insulitis) as well as affects the leukocyte subpopulations that express CXCR2, among which one subpopulation of B-lymphocytes [*Citro, 2014*].

## 2.2. A SUMMARY OF TOXICOLOGY DATA

DF 2156A was tested for toxicity in rodent and non-rodent animal species after single and/or repeated dose administrations.

In the four-week studies performed in rats, the administration by gavage for 28 days up to the dose of 200 mg/kg followed by a 2-weeks recovery period, did not cause relevant toxicity. There was no mortality. The immune system was not affected by the treatment with DF 2156A. The liver and kidney weights increased in males in all dose groups and 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 resolved after 2 weeks. The no-observed-adverse-effect-level (NOAEL) was settled at 200 mg/kg/day.

Treatment of rats with DF 2156A orally for a 3 months' period at dose levels of 35, 70 and 150 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 liver enzymes (males at 150 mg/kg), dose-dependently decreased protein levels (males at all dose levels and females at 150 mg/kg in Week 8 only), and dose-dependently decreased cholesterol and phospholipid concentrations (males from 70 mg/kg). Microscopic examination revealed treatment-related findings in the liver, thyroid gland and kidneys. In the liver, hepatocellular hypertrophy was noted in Week 8 in males at all treated groups and females at 150 mg/kg. Follicular cells hypertrophy of the thyroid gland was seen at 150 mg/kg and regarded secondary to the liver hypertrophy. In the kidneys hyaline droplet accumulation was present at increased incidence in males at 150 mg/kg after 8 weeks of treatment only (interim sacrifice). This change is male rat sex-specific not relevant for humans. After the 4-week recovery period, full recovery or a clear trend to recovery was noted for all findings.

In studies with up to 1week administration in dogs by oral route, the dose of DF 2156A had to be reduced from 500 to 150 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, DF 2156A was administered up to the dose of 120 mg/kg. No mortality occurred. Vomiting was seen at 120 mg/kg. An increase in the absolute and corrected-for-heart rate QT interval was observed at the doses of 60 and 120 mg/kg. Inflammatory changes were seen in the duodenum of two males treated with 120 mg/kg. After 2 weeks all values returned to normal. It was concluded that the no observed-effect-level (NOEL) is 30 mg/kg/day.

In the 3-month study, the initial dose levels were 30, 60 and 120 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 120 to 80 mg/kg/day from Day 24 onward. At 120/80 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 120 to 80 mg/kg/day. Prolongation of the QT and/or corrected QT intervals was observed in males and females treated at 120/80 mg/kg/day in Weeks 4, 8 and/or 13, generally more pronounced in females. In addition, higher heart rates were noted for males and (individual) females treated at 120/80 mg/kg/day in Weeks 4, 8 and/or 13. At the end of the Recovery period, only one male and one female at 120/80 mg/kg/day were still on study, and all electrocardiography parameters were returned to the normal limits. For one female at 60 mg/kg/day, mixed cell inflammation of the brain parenchyma and meninges, with neuronal necrosis and gliosis 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 60 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 60 mg/kg/day after 13 weeks of dosing.

Testing of DF 2156A for mutagenic potential gave negative results for the in vitro chromosomal aberration test, for the Ames test and for the micronucleus assay test in vivo in bone marrow of rats.

Phototoxicity potential of DF2156A was evaluated on Balb/c 3T3 cells. At dose levels of 100, 50.0, 25.0, 12.5, 6.25, 3.13, 1.56 and 0.781 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 DF2156A 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 (PIF) could not be determined. The Mean Photo Effect value (MPE) was 0.009. The score values obtained with DF2156A (i.e. PIF not calculable and MPE 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 50, 100 and 200 mg/kg. There were no treatment-related effects in females in any dose levels and in males at 50 and 100 mg/kg/day. At the highest dose, effects were observed on spermatid counts, motility and morphology of spermatozoas. Changes were seen in the testes of some animals and consisted degeneration/atrophy of the seminipherous 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 100 mg/kg/day for males and of 200 mg/kg/day for females.

In order to clarify the changes seen in the testes at 200 mg/kg/day, a specific study was designed in male Wistar and Sprague Dawley rats. DF 2156A was orally administered at the dose level of 200 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 DF 2156A caused a minimal increase in incidence of reduced size of testes and epididymides and a mean decrease of testes and epididymides weights. A mean decrease of sperm motility, of normal complete sperms and of the mean sperm count in the epididymides 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 weight reduction of testes and epididymides and the sperm analyses correlated with microscopic changes in the testes (degeneration/atrophy) and epididymides (aspermia/oligospermia) 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 degeneration/atrophy of the testes and atrophy, oligospermia or cell debris in the epididymides. The incidence of the testicular changes was very likely associated to 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 (150 mg/kg/day). The NOAEL in rats was established as being at least 150 mg/kg/day for both the maternal and developmental toxicity.

In rabbits, no maternal toxicity was observed up to the highest dose level tested (100 mg/kg/day). The NOAELwas established as being at least 100 mg/kg/day for both the maternal and developmental toxicity.

Carcinogenicity studies have not been performed.

In conclusion, based on the toxicology testing performed through in vivo studies, the dose of 200 mg/kg appears to represent the NOAEL in rats for general toxicity while in dog in the 3-month oral toxicity study, the NOAEL was established at 60 mg/kg. In rats, 150 mg/kg is the NOAEL for developmental toxicity while in rabbits it was 100 mg/kg.

The toxicity studies conducted up to date support the dose schedule proposed for this study up to 3 months of continuous treatment. According to the toxicology development program, a 6 and 9 month study in rats and dogs, respectively, are ongoing to further support the chronic administration in humans.

## 2.3. PHARMACOKINETICS AND PRODUCT METABOLISM

The pharmacokinetics of 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 DF 2156A, this metabolite showed an accumulation ratio ranging between 1.4 and 2.4. After the daily oral administration of DF 2156A 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, single oral doses 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 single doses, DF 2156A was excreted mainly as unmodified in human urine. The presence of the two

metabolites found in animal species (DF 2108Y and DF 2227Y) 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 DF 2156Y. The dose proportionality for the main PK parameters was also seen for the two metabolites (DF 2108Y and DF 2227Y).

After multiple dose, DF 2156Y reached the steady state around Day 5 and 6 following 50, 100, 200, 300 and 400 mg b.i.d. Systemic exposure to DF 2156Y, DF 2108Y and DF 2227Y appeared to increase in a dose-proportional manner. The clearance and the volume of distribution of DF 2156Y 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 DF 2156Y 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 appears 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 3 phase 1 PK and tolerability studies with single CCI and multiple CCI ascending dose oral administration. The first multiple ascending dose study CCI also included the evaluation of potential interaction between ladarixin 200 mg and tolbutamide. A phase 2 efficacy and safety study was also conducted with 150 mg twice a day (for 14 consecutive days) oral ladarixin in patients with moderately active BP.

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 CCI and multiple CCI 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 DF 2108Y and DF 2227Y 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 [redacted] Final CCI.

### 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 CCI. The compound was given by the oral route at 150 mg twice a day (maximum of 14 days), a low dose that could be selected according to the phase 1 trial completed at the 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 admission to rescue therapy). No disease remissions were observed with the low dose tested in this trial and the study was prematurely discontinued due to lack of activity.

A phase 2, multicenter, randomized, double-blind, placebo-controlled study has recently been completed in patients with new onset T1D (first insulin within 100 days from randomization) CCI . 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 AUC of C-peptide measured during an 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 to received ladarixin and 26 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 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 clinically relevant treatment effect 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). Similarly, the proportion of patients with HbA1c <7% and daily insulin requirement <0.50 IU/kg/day, a post-hoc composite endpoint, was higher (76.6%) at week 26 in the ladarixin group vs. placebo (45.8%) in the whole ITT population (p=0.01).

When considering the 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 log ($AUC_{(15\text{-}120\ min)}$ +1) (p=0.0310) at Week 26 was evidenced. 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) and the post-hoc endpoint of the proportion of patients with HbA1c <7% and daily insulin requirement <0.50 IU/kg/day (p=0.004).

In the pre-specified subgroup analysis of patients having fasting C-peptide at screening ≥ median value of the whole ITT population, no statistically significant difference could be observed for any of the efficacy endpoints. However, in this subgroup of patients with fasting C-peptide ≥0.205 nmol/L at screening, the percentage of patients with HbA1c <7% and daily insulin requirement <0.50 IU/kg/day was 68.2% in ladarixin group vs. 53.3% in placebo group (p=0.361).

### 2.4.3. Safety

A total of 89 male healthy subjects aged 18 to 52 years, 4 male BP patients aged 65-75 yeas, and 50 T1D patients aged 18-46years 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.

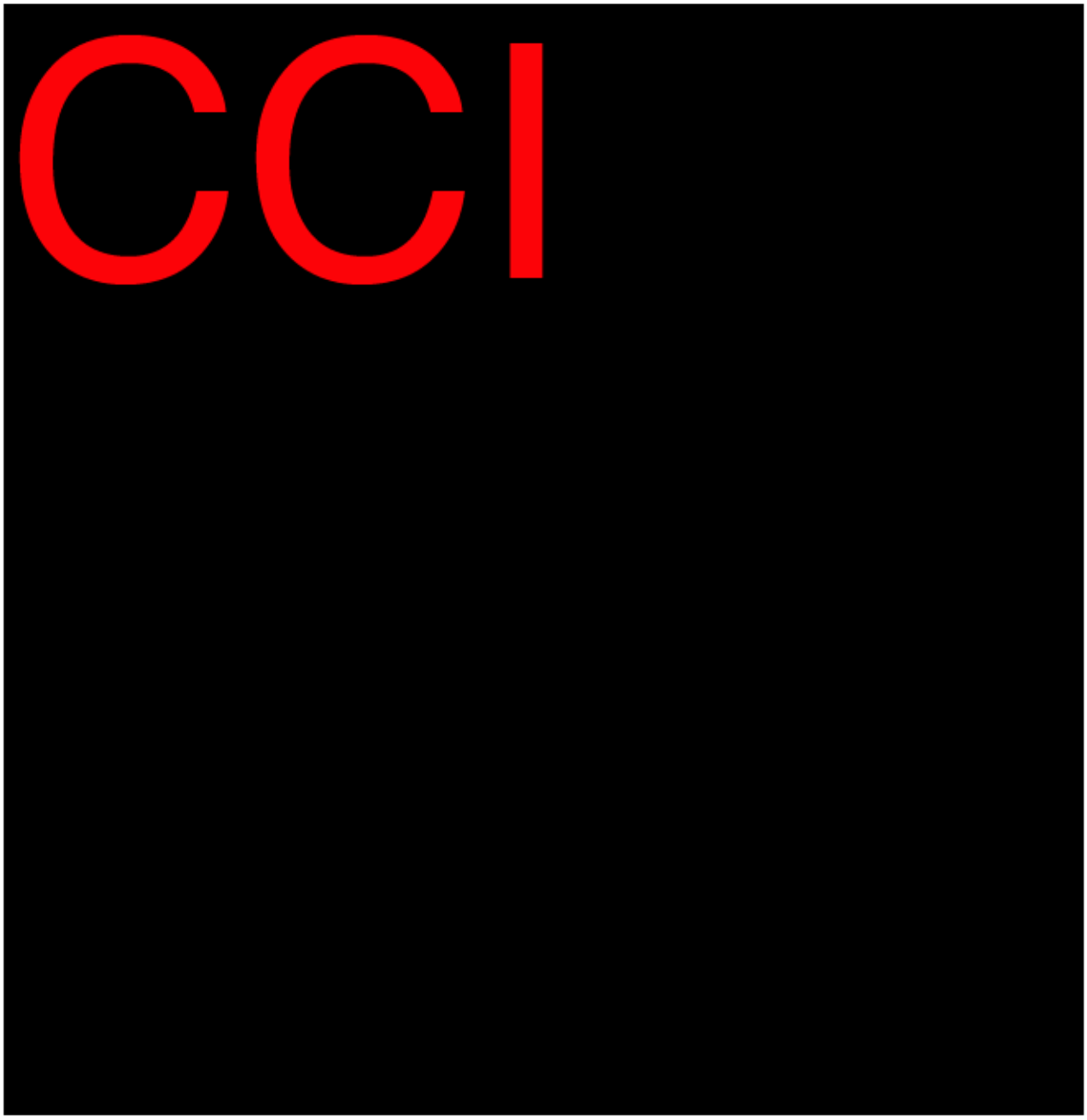
Toxicology and safety pharmacology in animals pointed out the cardiovascular system (QT prolongation) as potential safety concern in humans. Apparent isolated prolongations of the QTcF were observed at some time-points in phase 1 studies; core laboratory analysis of the ECG readings, including the review of changes in the QTcF intervals and of the pharmacokinetic-pharmacodynamic relationship, revealed no clinically significant effect of DF 2156A on cardiac repolarization.

Overall, DF2156 was safe and well tolerated. No deaths or Serious Adverse Events (SAEs) were reported from phase 1 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.

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/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 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 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 **adverse drug reactions (ADRs)**, i.e. treatment-emergent AE judged at least possibly related to ladarixin, are presented in the Table below.




CCI

The system organ classes (SOCS) 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.

## 2.5. DISEASE REVIEW AND STUDY RATIONALE

T1D is an organ-specific autoimmune disease in which the immune system attacks 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 rapidly increasing, with a predicted 70% increase in incidence over the next 15 years in Europe [*Atkinson, 2014; Waldron-Lynch, 2011*].

T1D is treated with life-long daily exogenous insulin injections and 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. Pancreatic islet or whole pancreas transplantation still has limited success due to graft loss and immunosuppression derived side-effects. Therefore, 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 the clinical/scientific interest. [*Steffes, 2003; Barnard, 2010; Waldron-Lynch, 2011*].

Among these, rituximab, 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. Unfortunately, even if safe preservation of β-cell function and improvement of glycemic control have been evidenced for some of the pharmacological approaches evaluated so far, none has been definitely approved for the "treatment" of diabetes onset [*Ludvigson, 2008; MacroGenics, 2010; Mastrandrea, 2009; Peskovitz, 2009; Waldron-Lynch, 2011; Raz, 2014*].

New strategies are being evaluated which combine agents targeting sequential arms of the immune and inflammatory response involved in β-cell disruption. In this regard, IL-8 appears to be an important mediator in the progression of type 1 diabetes. 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 type 1 pre-diabetic and diabetic patients. In parallel, circulating levels of IL-8 were elevated in children with T1D compared to non-diabetic controls. Specifically, levels of IL-8 correlate with glycemic control, higher level being associated to poorer or unfavorable glucose control [*Aboelasrar, 2012; Erbağci, 2001; Glowacka, 2002; Diana, 2014; Van Sickle, 2009*]

As a result of these findings, the modulation or inhibition of CXCL8 activity is considered a valid target for the development of innovative treatments aimed 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 could not show a statistically significant difference (p=0.337) between the two groups in the primary endpoint (2-hour AUC of C-peptide response to MMTT at week 13). 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). Similarly, the percentage of patients with HbA1c <7% and daily insulin requirement <0.50 IU/kg/day, a post-hoc composite endpoint, was higher (76.6%) at week 26 in the ladarixin group vs. placebo (45.8%) in the whole ITT population (p=0.01).

When considering the pre-specified sub-group analysis based on fasting C-peptide, a statistically significant effect of ladarixin was reached for some efficacy endpoints at week 26 only in the subpopulation with "severe T1D onset" (fasting C-peptide <0.205 nmol/L at baseline (median value of trial population). Notwithstanding, in the sub-group with fasting C-peptide at baseline ≥ 0.0205 nmol/L, the proportion of patients with HbA1c <7% and daily insulin requirement <0.50 IU/kg/day was higher in the ladarixin group at week 26, as compared to placebo. Considering that this effect is evident after a short treatment course (3 months), data obtained in the phase 2 trial support the hypothesis that a longer exposure (12 months) to ladarixin can delay disease progression also in patients with fasting C-peptide at screening ≥0.205 nmol/l).

The results obtained from the phase 2 trial coupled with the safety shown in phase 1 and 2 clinical trials, "mild" (fasting C-peptide >0.205 nmol/L provide a rationale that supports the conduct also of this Phase 2 clinical study in ) patients with less severe disease onset (fasting C-peptide >0.205 nmol/L)

### 2.5.1. Selection of dose and treatment schedule in the study

The dose and treatment schedule proposed in this phase 2 study is 400 mg oral ladarixin twice a day for 13 cycles of 14 days on - 14 days off. **However, ladarixin will NOT be administered to trial patients for longer than 3 months (3 cycles) before chronic toxicity data in rats and dogs have been deemed sufficient by the FDA to support ladarixin exposure up to one year**.

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 CCI in T1D adult patients. 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 treatment of 14 days in a regimen of 14-days on and 14-days off resulted beneficial in the phase 2 study CCI, in line with the initial observations in NOD mice. In fact, in ladarixin-treated NOD mice with recent onset-diabetes the efficacy of the compound was clearly more pronounced within the 14 days after treatment interruption (in these animals, the average of non-fasting glycemia was below 200 mg/dl) whereas blood glucose concentrations tended to progressively increase in 77% of remission NOD mice at later time after treatment discontinuation, suggesting that neutrophil recruitment as well the cross-talk with the other immune cells in the pancreas could restart after 14 days of ladarixin treatment discontinuation. This was further supported by the evidence that a second cycle of ladarixin treatment in NOD-mice with diabetes recurrence was able to reverse again diabetes in 67% of mice, thus supporting the concept that repeated cycles of treatment are necessary and appropriate for the management of the chronic condition.

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.

In the 14 days wash out period (which is the interval between two treatment cycles), the patients completely clear the drug from the body considering that ladarixin is not accumulating over repeat administration, has a low volume of distribution, it is not lipophilic and has a half-time ranging from 11 to 18 h which means that in a period of about 98 hours (i.e. about 4 days) it is completely cleared from the body.

Dompé has carried out a comprehensive nonclinical safety package, which already support the continuous administration of ladarixin for up to 3 months in human patients.

The package already available consists of acute, subacute and sub-chronic studies in rats and dogs by oral route, safety pharmacology in vitro and in vivo, pharmacokinetics and metabolism, phototoxicity in vitro, genetic toxicology in vitro and in vivo, repro-toxicity of segment I and II in two different species. These toxicity data are confirmed by safety data in human volunteers and in T1D patients where ladarixin resulted to be safe at the clinical doses to be used in this phase 2 clinical study.

Additional chronic data in rats and dogs will be collected according to ICH guideline, in order to support treatment up to 12 months.

### 2.5.2. Alternative treatments

There are no standard pharmacologic treatments, addressed to the prevention or treatment of T1D other than insulin replacement. 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 DiaPep277 injections or other immunomodulatory drugs, have provided some evidence of successful control of disease progression. No one drug has therefore reached either definite therapeutic placement or marketing authorization for this indication [*Waldron-Lynch, 2011*].

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 support the level of drug exposure planned in this study.

Phase 1 and 2 clinical experience 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 SUSAR or death occurred and all the AEs encountered were mild to moderate in intensity.

The available toxicity data supports 3 months of continuous treatment in humans. Six-month and 9-month chronic studies in rats and dogs (respectively) will be conducted, according to ICH guideline M3(R2), in order to support ladarixin administration up to 12 months. Even if the protocol anticipates, and remains structured for, 1 year of treatment (13 cycles), patients **will not be treated longer than 3 months** until such additional chronic toxicology data in rats/dogs have been reviewed by the FDA.

[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 6.5.2 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 18 for Safety Laboratory Test;
- blood samples for diagnostic auto-ab test (maximum 5 mL) and for baseline HbA1c, HLA DR-DQ haplotypes, CCI [redacted], MMTT C-peptide and glucose (maximum 100 mL) will be taken during screening to baseline;
- blood samples for HbA1c, CCI [redacted], MMTT C-peptide and glucose (maximum 100 mL) will be taken at Month 6, 12, and 18.

#### 2.5.3.3. Mixed Meal Tolerance Test

Participation in the study requires testing for β-cell function through a Mixed Meal Tolerance Test (MMTT) to be performed in basal conditions at baseline (pre-dose) and then at the visits at month 6, 12, and 18. This is not a routine test in patients with new onset 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 Dexcom G6 Continuous Glucose Monitoring System (Dexcom G6 System) will be use to perform a CGM. Sensor will be positioned subcutaneously at the site at least 10 days before the concerned visits (baseline, Month 6, 12 and 18) and removed during the visit, as per Study Flowchart (see Appendix 14.2).

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 (MRI), computed tomography (CT) scan, or high-frequency electrical heat (diathermy) treatment. Some skin care products, such as sunscreens and insect repellents, can make the plastic used in G6 crack.

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 of 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 controlling the progression of T1D in patients with preserved β-cell function at onset (fasting C-peptide ≥0.205 nmol/L at baseline).

# 3. OVERALL STUDY DESIGN AND INVESTIAGATIONAL PLAN

## 3.1. STUDY OBJECTIVES

The objective of this clinical trial is to assess whether ladarixin treatment is effectiveto improve glycemic control in newly diagnosed T1D adult patients with preserved β-cell function. The safety of ladarixin in the specific clinical setting will be also evaluated.

## 3.2. STUDY ADMINISTRATIVE STRUCTURES, STAFF AND RESPONSIBILITY

This study is planned to be performed at centers in EU and US. 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 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 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 evaluate whether ladarixin is effective in preserving β-cell function and slowing-down the progression of T1D) in patients maintaining a high residual β-cell function at onset.

It will involve 75 adult (18-45 years, inclusive) patients with new-onset T1D. 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 enrolled. 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 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 18 months from the 1st IMP dose, as per details below:

- A run-in period including 2 or more visits up to a maximum of 100 days; this includes the time required to confirm the diagnosis, assess fasting C-peptide, and perform baseline assessment;
- A randomization visit;
- A treatment period of 12 months, to include 5 visits scheduled at the end of the 3rd (Week 11) and 6th (Week 23) treatment cycles, at Month 6 (Week 26), at the end of the 9th (Week 35) treatment cycle and at Month 12 (Week 52) from the 1st dose of study treatment;
- One post-treatment follow-up visits scheduled at Month 18 (week 78) from the 1st dose of study treatment.

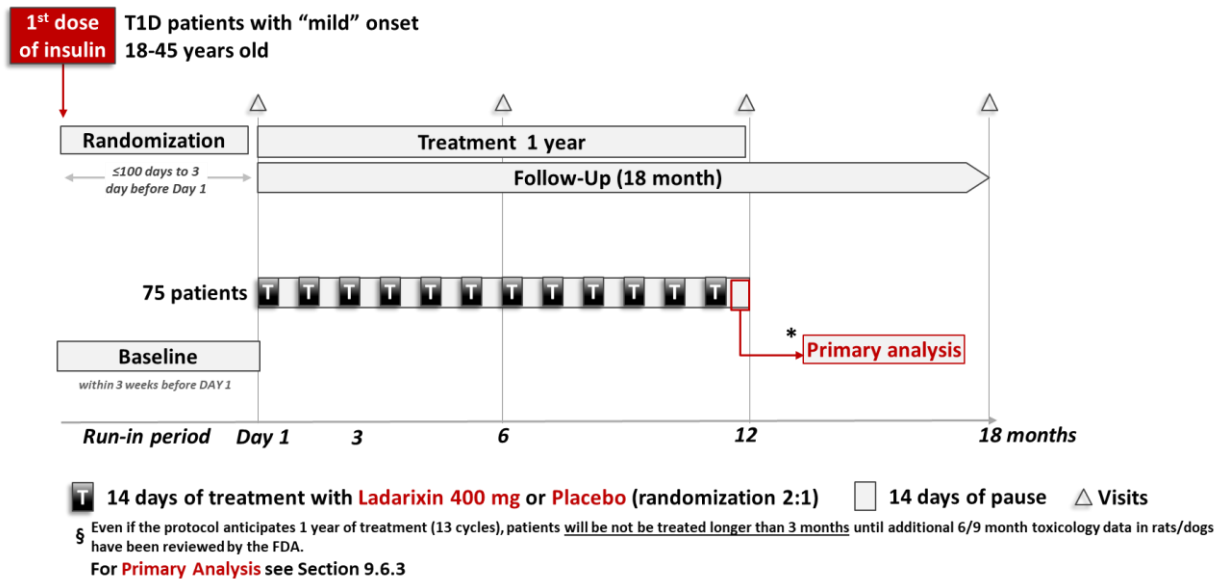
Figure 1 shows the major milestones of the study design (run-in period, treatment, follow-up visits).

**First treatment administration should start within maximum 100 days from the first insulin dose** and will continue for 3 months. Administration will be extended up to one year only after **the FDA has notified that such longer exposure to ladarixin** is acceptable after having reviewed chronic toxicology data in rats and dogs.

The time frame for the primary endpoint (HbA1c <7% and daily insulin requirement <0.5 IU/Kg/day) has been set at Month 12 (Week 52) in order to evaluate the potential of ladarixin effects on a long-term projection. Follow-up is extended up to 18 months to evaluate the potential persistency of any glycemic benefit.

The study will proceed under double-blind condition up to the month 12 visit of the last patient randomized. Thereafter, the blind will be broken and remaining follow-up will proceed in an open fashion. This approach will allow to anticipate access to safety and efficacy data without significantly affecting data integrity.

**FIG.1: Design of Study LDX0419**




## 3.4. STUDY TIME TABLE

Overall study timelines are reported below.

Projected starting date (first-patient-in): Q4-2020
Projected completion of patient accrual (last-patient-in):Q2-2022
Projected study end date (last-patient-last-visit): Q4-2023

## 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.

## 4.1. EFFICACY ENDPOINTS

- Proportion of patients with HbA1c <7% and daily insulin requirement <0.50 IU/Kg/day [**Primary endpoint.** Time frame: Month 12]

**Secondary endpoints:**

- Proportion of patients with HbA1c <7% and daily insulin requirement <0.50 IU/Kg/day [**Primary endpoint.** Time frame: Month 6 and 18]
- Proportion of patients with a reduction in HbA1c% > 0.5% from baseline and daily insulin requirement <0.50 IU/Kg/day [Time frame: Month 6, 12, 18]
- 2-hour AUC of C-peptide response to the MMTT [Time frame: Month 6, 12, 18]
- Time in range (TIR) by Continuous Glucose Monitoring (CGM) [Time frame: Month 6, 12, 18]
- HbA1c levels [Time frame: Month 6, 12, 18]
- Proportion of patients with HbA1c <7% who did not experience severe hypoglycemic events during treatment [Time frame: Month 6, 12, 18]
  *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.*
- 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]
- Number of self-reported episodes of severe hypoglycemia [Time frame: Month 6, 12, 18]
- Average (previous 3 days) daily insulin requirements (IU/kg/day) [Time frame: Month 6, 12, 18]
- Estimated Glucose Disposal Rate (eGDR) [Time frame: Month 6, 12, 18]


## 4.3. SAFETY ENDPOINTS

- Vital signs (blood pressure and heart rate) [time frame: end of 3rd (Week 11) 6th (Week 23) and 9th (Week 35) treatment cycle, Month 12 and Month 18]
- Routine laboratory tests (hematology, clinical chemistry) [time frame: end of 3rd (Week 11) 6th (Week 23) and 9th (Week 35) treatment cycle, Month 12 and Month 18 (or withdrawal)]
- Incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) [Time frame: throughout the study]

# 5. STUDY POPULATION

Seventy-five (75) adult patients with new-onset T1D will be included (randomized) in the study, selected from those having a fasting C-peptide ≥0.205 nmol/L on two occasions or those referring to the site for diagnosis confirmation (auto-antibody testing) and/or disease management. Patients can be considered for inclusion in this trial if they fall within the definition of new-onset T1D (randomization scheduled to allow the administration of the study medication to start within 100 days from 1st insulin administration) and have a confirmed diagnosis (positive for at least one auto-antibody).

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 18-45 years, inclusive;
2. New-onset T1D (1st IMP dose within 100 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 multiple daily injections (MDI) or Continuous Subcutaneous Insulin Infusion (CSII);
5. Fasting C peptide ≥0.205nmol/L on two occasions;
6. Patient able to comply with all protocol procedures for the duration of the study, including scheduled follow-up visits and examinations;
7. Patients who have given written informed consent prior of any study-related procedure not part of standard medical care.

## 5.2. EXCLUSION CRITERIA

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

1. Any other chronic disease, including type 2 diabetes, apart from autoimmune hypothyroidism requiring thyroid hormone replacement only; patients with severe (myxedema) disease potentially requiring immunosuppressive therapy will be excluded;
2. Moderate to severe renal impairment as per estimated Glomerular Filtration Rate (eGFR) 60 mL/min/1.73 m2, 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. A history of significant cardiovascular disease/abnormality
7. Occurrence of an episode of ketoacidosis or hypoglycemic coma in the past 2 weeks;
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, sulphanylurea hypoglycemics (e.g. tolbutamide, glipizide, glibenclamide/glyburide, glimepiride, nateglinide) and high dose of amitriptyline (>50 mg/day)];
10. Previous (within 2 weeks prior to randomization) 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 (within 1 month prior to randomization) or current administration of any immunosuppressive medications (including oral, inhaled or systemically injected steroids) 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 first 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. Hepatitis A (IgM), hepatitis B (not due to immunization), hepatitis C and HIV positive serologic status Serologic evidence of current infectious liver disease (hepatitis A, B, or C), including ant hepatitis A virus (immunoglobulin M), hepatitis B surface antigen, or ant hepatitis C virus 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).

## 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.

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 gelatine capsules for oral administration, with the following composition:

**Composition for each unit (capsule)**


Batch release certificate will be provided together with the IMP.

### 6.1.2. Manufacturing, Packaging and Labelling of IMP

Capsules will be manufactured by PPD will manage the labelling 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 treatment 28 capsules.

All labels will be prepared to meet local regulatory requirements. Details of packaging and labelling 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 labelling, of the IMP (capsules, packaging) will not allow to recognize 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.

## 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 Visit and then during the Visits scheduled at the end of the 3rd (Week 11), 6th (Week 23) and 9th (Week 35) 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:

- at Randomization Visit dispensed 3 Patient Kits of IMP for the Cycles 1, 2 and 3

- at Week 11 Visit dispensed 3 Patient Kits for the Cycles 4, 5 and 6

- at Week 23 Visit dispensed 3 Patient Kits for the Cycles 7, 8 and 9

- at Week 35 Visit dispensed 4 Patient Kits for the Cycles 10, 11, 12 and 13

[* 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 expedite timing cannot be achieved, Patient Kits might 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 3 cycles of 14 days on / 14 days off. Placebo will be administered with the same treatment schedule. Administration will be extended up to 12 months (13 cycles) **only after additional chronic toxicity data support such longer treatment**.

The two daily doses will be administered at about 12hour interval (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 screening 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 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.
- The patient develops ketoacidosis or hypoglycemic coma.

Occurrence of any condition that qualify 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 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 member 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 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.

The following agents do not need to be recorded: homeopathic medications; elective vitamins 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

- Twice daily pre-mixed insulin
- 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, inhaled or systemically injected steroid, 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, sulphanylurea 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 effect (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.

## 6.7. 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 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 glucose monitor will be provided to study participants for the duration of the study. In order to optimize insulin titration, telephone calls (outside scheduled visits) will be scheduled on a regular basis to ensure timely evaluation of glucose levels and adjustment of insulin regimen.

# 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.

## 7.1. ENROLMENT, SCREENING AND RANDOMIZATION

### 7.1.1. Enrolment and Intensive Diabetes Management

Potential study patients with a recent clinical diagnosis of T1D will be have a fasting C-peptide ≥0.205 nmol/L on two occasions; these patients will be considered if they fall within the definition of new-onset T1D (randomization scheduled to allow the administration of the study medication to start within 100 days from 1st insulin administration) and will have a diagnosis (positive for at least one auto-antibody).

Enrolment is defined as the signature of the Informed Consent Form (ICF) for participation in the study LDX0419.

From enrolment, patients will be admitted to an 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 glucose monitor will be provided to study participants for the duration of the study. Telephone calls (outside scheduled visits) 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 visits (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 enrolment to baseline (clinical information)

- Past medical history and disease-specific clinical information, including date of first 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;

  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 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).

- Screening 12 lead ECG, performed using local equipment that allows 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. 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 CCI [redacted].

** The CGM device will be positioned at least 10 days before the baseline assessment.

Patients who do not meet the Inclusion Criteria or meet Exclusion Criteria will be considered screen failures (screening number assigned) and will not be allowed to be re-considered for inclusion into the study.

## 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 1st to 3rd treatment cycles will be dispensed.

It is the responsibility of the Investigator (or designee) to explain, and make sure 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 day when the patient starts IMP administration is defined as **Day 1** of the 1st treatment cycle.

**Day 1 MUST be scheduled no later than 100 days from the 1st insulin injection** and is to be scheduled within 3 weeks from the baseline assessments.

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 12 hours 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 in section 7.4.1) at the end of the 3rd (Week 11), 6th (Week 23) and 9th (Week 35) 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.
- Pregnancy test (urine dipstick or blood sample).
- Patient Diary check (IMP administration information).

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 screening 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 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.
- The patient develops ketoacidosis or hypoglycemic coma.

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).

- at Week 11 Visit dispensed 3 Patient Kits for the Cycles 4, 5 and 6

- at Week 23 Visit dispensed 3 Patient Kits for the Cycles 7, 8 and 9

- at Week 35 Visit dispensed 4 Patient Kits of IMP for the Cycles 10, 11, 12 and 13

* 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 expedite timing cannot be achieved, Patient Kits might be delivered to 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 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. Additional visits during treatment

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

During these visits the following will be assessed:

- Waist and waist/hip circumference and calculation of eGDR.
- Patient Diary check (information other that IMP administration at Month 6, any information at Month 12).
- 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 CCI [redacted].
- 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).

### 7.4.2. Follow-up assessment

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

At this 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 CCI [redacted].
- Patient Diary check (information other than IMP administration).
- Blood sampling for measurement (local laboratories) of "Safety Laboratory Tests".
- Vital signs.

## 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 78 (month 18) 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 covers also 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 IB Reference Safety Information (RSI) 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 outcome of the reaction. 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<br>(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 of withdrawal), the SAE should be reported by the Investigator to the CRO Pharmacovigilance or directly to the Dompé Drug Safety 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 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 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*

Investigator must report all serious adverse events to the sponsor immediately, within 24 hours.

Dompé Drug Safety 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 Drug Safety 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 Sponsor Drug Safety Department will prepare and submit (via the CRO as applicable) to Investigators appropriate periodical safety updates as per applicable EU and local requirements and regulations. Dompé Drug Safety 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*

Investigator must report all serious adverse events to the sponsor immediately, within 24 hours.
Dompé Drug Safety 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 Drug Safety 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 Drug Safety 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 Drug Safety 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 Drug Safety 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 Drug Safety 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 Drug Safety/CRO and Dompé Medical Expert, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake through different routes (e.g. ingestion) or with suicidal intentions and consequent drug overdose.

An overdose of ladarixin is defined as the administration of 6 or more capsules on any given treatment day.

The Investigator shall provide in the SAE form information about symptoms, corrective treatment and outcome of overdose. 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 via IRS 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 Pharmacovigilance contact person will be provided with a protected access to IRS 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

The sample size of the study is calculated on the "proportion of patients with a HbA1c <7% and daily insulin requirement <0.50 IU/Kg/day", a post-hoc composite endpoint derived from data of the phase 2 trial [redacted], considering a larger effect size expected from the longer treatment length (one year versus 3 months).

Based on these assumptions, and considering a randomization ratio 2:1 (ladarixin:placebo) and a one-sided alpha of 0.05, seventy- five (75) patients randomized in the trial will allow to achieve a power of approximatively 80% to detect at least 35% of difference in terms of primary endpoint in favor of ladarixin vs placebo, assuming that the proportion of patients in the placebo group with a HbA1c <7% and daily insulin requirement <0.50 IU/Kg/day at 12 months will range between 45% and 55%.
The above sample size will take into account. a drop-out rate of approximately 10%.

## 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 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, with patients balanced between treatments/centers in accordance to randomization ratio (2:1).

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 code will be broken according to study procedures after the last enrolled patient has completed his/her follow-up visit at 12 months.

## 9.3. OVERVIEW OF PLANNED STATISTICAL ANALYSES

The study plans for the following statistical analyses:

- Final analysis: this analysis will be conducted when all enrolled subjects have completed the Month 12 follow-up visits, and the study database has been unblinded and locked.
- Addendum at Final analysis: this analysis will be conducted when all enrolled subjects have completed the open phase study post unblinding up to Month 18.

## 9.4. ANALYSIS POPULATION

The following population will be defined:

- 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 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 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.

## 9.5. 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 trapezoidal rule. 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 linear interpolation;
- 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 one-sided tests will be used.

A Statistical Analysis Plan will be issued 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.

### 9.5.1. Demographic and baseline characteristics

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

### 9.5.2. Analysis of efficacy variables

#### 9.5.2.1. Primary analyses

The proportion of patients with HbA1c < 7% and daily insulin requirement <0.50 IU/Kg/day at Month 12 (primary endpoint) will be analyzed by means of logistic regression possibly adjusting by pre-defined baseline factors that will be defined in the SAP.

The null hypothesis $H_0$ is that the proportion of patients with a HbA1c <7% and daily insulin requirement <0.50 IU/Kg/day at Month 12 in ladarixin ($\pi_{LADARIXIN}$) is lower or equal the placebo one ($\pi_{PLACEBO}$):

$$H_0: \pi_{LADARIXIN} \leq \pi_{PLACEBO}$$

$$H_1: \pi_{LADARIXIN} > \pi_{PLACEBO}$$

The null hypothesis will be rejected, and superiority of ladarixin in terms of primary endpoint concluded if p-value will be lower than 0.05 one-sided.

In case of missing assessment of the primary endpoint, primary analyses will be performed adopting a reference-based Multiple Imputation approach (Copy Reference) to consider a missing not at random mechanism for missing data: imputation of values in the ladarixin arm will be done as if the subject had been a member of the placebo arm. This approach does not assume benefits for ladarixin in case of discontinuation and limits a post-discontinuation clinical effect to that of placebo.

Sensitivity analyses will be defined in the SAP to assess the robustness of results on primary endpoints versus the assumptions on the mechanism of missingness (missing non at random vs missing at random).

#### 9.5.2.2. Secondary analyses

All secondary endpoints will be analyzed 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 repeated measurements models. The adjusted least squares 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.

### 9.5.3. 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.

### 9.5.4. Analysis of safety variables

TEAEs, ADRs and TESAE will be presented by treatment arms in terms of number of AEs and incidence by System Organ Class (SOC) and Preferred Terms (PT) 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.

### 9.5.5. Specification of subgroups for analysis

Sub-group analyses of efficacy and safety endpoints will be performed on subgroup of patients defined by age, BMI, HLA, CCI [redacted]. Statistical details and new subgroups definitions will be reported in the SAP.

### 9.5.6. 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 have 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.

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 electronic CRFs (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.

A 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, daily insulin doses, 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 database, 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 database 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. 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.

## 12.6. 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.

## 12.7. 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 CA 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 a data could, in some circumstances, prevents or negatively impacts patentability.

# 13. REFERENCES

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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 LDX0419** **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 LDX0419** **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

100 days (run-in phase)

1st insulin first IMP dose

| Procedures<br>Tests to be performed at a centralized laboratory<br>Test 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C1 - C3 | Week11 (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) | C 10 - C13 | Month 12 Week 52±2 | Month 18 Week 78 ±2 |
| Informed Consent Form | X | | | | | | | | | | | | |
| Eligibility criteria Randomization | X | X | X | | | | | | | | | | |
| Auto-antibodies[1] & Fasting C-Peptide[2] | X | | | | | | | | | | | | |
| Medical history | X | | | | | | | | | | | | |
| BP/HR | | X | | | X | | X | | | X | | X | X |
| weight, height, waist & waist/hip circumference | | X | | | | | | | X | | | X | X |
| eGDR | | X | | | | | | | X | | | X | X |
| Insulin requirement previous 3 days | | X | | | | | | | X | | | X | X |
| Self Reported Severe Hypoglycemia | | X | | | | | | | X | | | X | X |
| HLA DR-DQ haplotypes, Proinsulin, | | X | | | | | | | | | | | |
| HbA1c, CCI [redacted] | | X | | | | | | | X | | | X | X |
| Safety Laboratory Test (hematology & biochemistry)[3] | | X | | | X | | X | | | X | | X | X |
| MMTT [3] | | X | | | | | | | X | | | X | X |
| Pregnancy Test | | X | | | X | | X | | | X | | | |
| 12 lead ECG | | X | | | X[5] | | X[5] | | | X[5] | | | |
| CGM download[4] | | X[4] | | | | | | | X[4] | | | X[4] | X[4] |
| IMP dispensation [5] | | | X | | X | | X | | | X | | | |
| Patient Diary delivery/check | | | X | | X | | X | | | X | | X | X |
| Check discontinuation criteria [5] | | | | | X | | X | | | X | | | |
| Patient Phone call [6] | | | | 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 fasting C-peptide must be ≥0.205 nmol/L on two occasions.
3 tests performed at Baseline should be planned to ensure the samples are sent to the laboratory and results are be available in time for randomization.

[4] **sensor to be positioned <u>at least 10 days before the scheduled visits</u>**
(X) CGM sensor implantation can occur at Screening Visit and Week 23 Visit if the visit is scheduled 14-10 days before Baseline and before Week 26 respectively

[5] Dispensation of IMP (Patient Kits) 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 3rd, 6th, 9th treatment cycles). Results of ALT/AST, bilirubin, creatinine and albumin, ECG reading and pregnancy results **<u>should be made available by the local laboratory in time to allow Patient Kits dispensation as soon as possible during the visit</u>**. If this expedite timing cannot be achieved, Patient Kits might be delivered to patient named address by a selected courier (CRO to support shipment). As per treatment discontinuation criteria, no IMP will be dispensed 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.

[6] Telephone calls 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 CCI, C-peptide and glucose, CCI.

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 (NGSP) 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 package in dry ice (solid $CO_2$) 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.

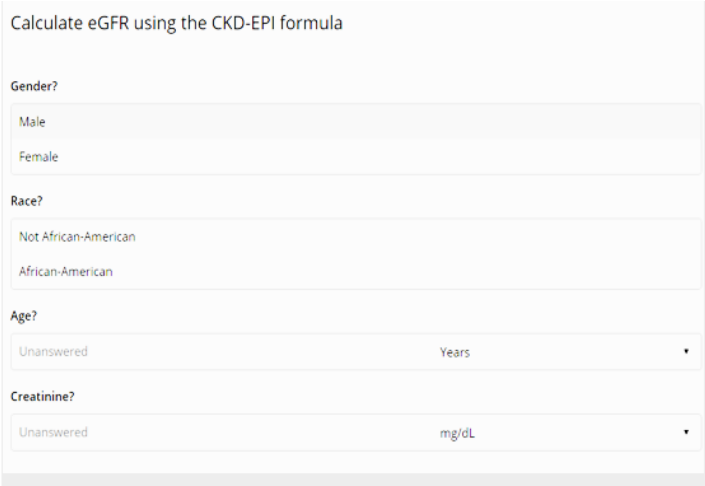
All samples will be destroyed after final study report has been issued or after the patient has withdrawn his/her consent.

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. Calculation of eGFR

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




### 14.3.3. 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)*

### 14.3.4. Handling of CGM device

The Dexcom G6 Continuous Glucose Monitoring System (Dexcom G6 System) will be use 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 at the site 14-10 days before the concerned visits (Baseline, Month 6, 12, 18) and removed during the day of the concerned visit, as per Study Flowchart (see Appendix 14.2).

CGM sensor implantation can occur at Screening Visit and Week 23 Visit if the visit is scheduled 14-10 days before Baseline and before Week 26 respectively. In addition, sites should upload the sensor data to check subject's compliance with wearing the device. The sensor requires at least one SMBG value to be entered every 12 hours for calibration 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 measures to fulfill these criteria. Every fourteen/ten (10/14) days the sensor needs to be replaced and subjects can return to the site for assistance on this if needed. If the subject needs assistance with sensor replacement, an ad-hoc visit should be performed, if this occurs a data upload is also recommended at this time. If subjects opt to perform sensor replacement at home sites should call subject 7 days after insertion as a reminder.

The data will remain blinded 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 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 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 calibration 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.

### 14.3.5. Mixed Meal Tolerance Test

The MMTT will be performed after an overnight fast, according to Greenbaum (*2008*) at baseline (within 3week prior of randomization) and at each subsequent visit at month 6, 12 and 18. 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 6 hours and 2 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. 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 Boost® High Protein Nutritional Drink (Nestlé Nutrition) up to a maximum of 360mL, to be drunk within 5 min. Post-meal samples will be drawn at 15±5, 30±, 60±10, 90±10, 120±15, 120±15 min after the meal.