CLINICAL STUDY PROTOCOL – REVISION HISTORY

Study Number: MEX0114

EudraCT Number: 2014-003968-20

Investigational Product: Ladarixin

Study Phase: 2

Title: A phase 2, multicentre, 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.

Protocol Version - Date	Change	Reason
Version No. 1 – Final 5 March 2015	---	---
Version No. 2 – Final 27 August 2015	Contact information updated	Logistics
	Study period updated in section 1 and 3.2	To match current expectation
	NOAEL in rats and toxicological details updated in section 2.2	To match the most recent (6 August 2015) "Risk Assessment of ladarixin" provided by Preclinic Expert
	Details of effective contraceptive methods added in section 5.2, bullet 14	As per request from the IEC of the Italian coordinating site and Italian Competent Authority (to match Italian insurance coverage provisions)
	Insulin added as a non-reportable concomitant medication in section 6.5.1	To make clear that insulin administration is not to be captured as a concomitant medication
	Typing errors corrected throughout the document	

CHANGE APPROVAL SIGNATURES

SPONSOR:

Medical Monitoring

Signature: _____ Date: 2 sept 2015

Andrea Vergani, MD - Medical Associate Director

Development Director – Diabetes & Transplantation

Signature: _____ Date: 2 Sept 2015

Marcello Allegretti – Chief Scientific Officer

CLINICAL STUDY PROTOCOL

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OTHER COORDINATING INVESTIGATORS AND INVESTIGATIONAL SITES

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

CENTRALIZED LABORATORIES

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

PROTOCOL APPROVAL SIGNATURES

SPONSOR:

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Andrea Vergani, MD - Medical Associate Director

Development Director – Diabetes & Transplantation

Signature: _____ Date: 2 Sept 2015

Marcello Allegretti – Chief Scientific Officer

CLINICAL CENTRE: PRINCIPAL INVESTIGATOR

I have read the study protocol MEX0114 "A phase 2, multicentre, 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 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
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
b.i.d.	Bis in die
BP	Bullous Pemphigoid
ºC	Degrees Celsius
CLcr	Calculated Creatinine Clearance (Cockcroft - Gault formula)
Cmax	Maximum Plasma Concentration
CMED	Concomitant Medication
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organization
CXCL8	CXC ligand 8 [formerly interleukin (IL)-8]
CXCR1/2	CXCL8 receptors
dL	Deciliter
DPP-IV inhibitor	Dipeptidyl peptidase-IV inhibitor
fMLP	formyl-met-leu-phe
g	Gram
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
i.v.	Intravenous
IU	International Unit
kg	Kilogram
L	Litre
LBBB	Left Bundle Branch Block
LD_{50}	Lethal $Dose_{50}$
miR-375	MicroRNA-375
mg	Milligram
mL	Millilitre
MLD-STZ	Multiple Low Dose-Streptozotocin
MMTT	Mixed Meal Tolerance Test
msec	Millisecond
ng	Nanogram
nmol	Nanomole
NOD	Non-Obese Diabetic
NOEL	No Observable Effect Level
PK	Pharmacokinetics

PMN	Polymorphonuclear leukocyte
p.o.	per os (taken by mouth)
QTc	Corrected QT interval
QTcF	Fridericia's corrected QT interval
SAE	Serious Adverse Event
s.c.	Subcutaneous
t½	Elimination half life
Tmax	Time to reach Maximum Plasma Concentration
T1D	Type 1 Diabetes
ULN	Upper Limit of Normal
µg	Microgram
µmol	Micromole

1. STUDY SYNOPSIS AND OVERALL DESIGN

Study title
A phase 2, multicentre, 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.

Study Number MEX0114 [**EudraCT Number:** 2014-003968-20]

Study period

Projected starting date (first-patient-in):	December 2015
Projected completion of patient accrual (last-patient-in):	December 2016
Projected study end date (last-patient-last-visit):	December 2017

Study design
The study will be a phase 2, multicentre, double-blind study. It will involve 72 patients with new-onset type 1 diabetes (T1D), randomly (2:1) assigned to receive either ladarixin treatment (400 mg b.i.d. for 3 cycles of 14 days on/14 days off – treatment group) or placebo (control group). The two groups will be balanced within each centre. Recruitment will be competitive among the study sites, until the planned number of patients is enrolled.

Sample size calculation has been based on the 2-hour area under the curve (AUC) of C-peptide response to a Mixed Meal Tolerance Test (MMTT) observed 12 months after randomization, as these are the only available data that best match the T1D population expected to be randomized in this trial (age, time from diagnosis, etc.) [*Lachin, 2011*]. On the other hand, the time frame for the primary endpoint (2-hour AUC of C-peptide response to the MMTT) has been set at week 13±1 in order to maximise the potential to observe an effect of ladarixin, considering the PK profile in humans as well as the reversibility of the pharmacological effect on CXCR1/2 receptors. Such an approach is appropriate for a phase 2 proof of concept trial. Follow-up is anyway extended up to 12 months.

Objectives/endpoints
The objective of this clinical trial is to investigate whether ladarixin has sufficient activity (preservation of β-cell function and slow-down of the progression of T1D) to warrant its further development (proof of concept trial). The safety of ladarixin in the specific clinical setting will be also evaluated.

Efficacy endpoints will be:
- 2-hour area under the curve (AUC) of C-peptide response to a Mixed Meal Tolerance Test (MMTT) [**Primary endpoint**. Time frame: baseline, week 13±1].
- 2-hour AUC of C-peptide response to the MMTT [Time frame: baseline, weeks 26±2 and 52±2].
- Average (previous 3 days) insulin requirements (IU/kg/day) [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- HbA1c levels [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- Basal (2 basal samples in the range between -20 to 0 min) to 180 min time course of C-peptide and glucose derived from the MMTT [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- Cumulative severe hypoglycaemic events occurring from randomization [Time frame: weeks 13±1, 26±2 and 52±2].

Exploratory endpoints will be:
- Basal (2 basal samples in the range between -20 to 0 min) to 180 min time course of glucagon derived from the MMTT [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- Auto-antibodies (GAD, IA-2, IAA, ZnT8) [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- MicroRNA-375 (miR-375) [Time frame: baseline, weeks 13±1, 26±2 and 52±2].

- T-cell response *ex vivo* to major β-cell antigens (pro-insulin, GAD65) - selected sites only [Time frame: baseline, weeks 13±1, 26±2 and 52±2].

Safety endpoints will be:
- Vital signs (blood pressure and heart rate) [time frame: screening, end of 1st treatment cycle (i.e. pre-dose visit, 2nd cycle), week 13±1].
- Routine laboratory tests (haematology, clinical chemistry) [time frame: screening, end of 1st treatment cycle (i.e. pre-dose visit, 2nd cycle), weeks 13±1 (or withdrawal)].
- Incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) [time frame: throughout the study].

Number of patients Seventy-two (72) patients with new-onset T1D

Inclusion/exclusion criteria
Consented male and female patients aged 18-45 years, inclusive, with new-onset T1D (randomization 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 (exclusion of patients taking twice daily pre-mixed insulin or on insulin pump); must have peak stimulated (MMTT) C-peptide level >0.6ng/mL (0.2nmol/L).
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 creatinine clearance < 60 mL/min according to the Cockcroft-Gault formula); 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; complete Left Bundle Branch Block (LBBB); atrio-ventricular block (mobitz II 2nd degree or 2:1 atrio-ventricular block); complete heart block; electronic pacemaker or implanted defibrillator; a history of significant cardiovascular disease; a known hypersensitivity to non-steroidal antiinflammatory drugs. Patients on treatment with phenytoin, warfarin, sulphanylurea hypoglycemics and high dose of amitriptyline (> 50 mg/day); with past (within 2 weeks prior to randomization) or current use of metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV 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. Also, pregnant or breast feeding women or patients unwilling to use effective contraceptive measures (females and males) will be excluded.

Investigational Medicinal Product
The investigational medicinal product (IMP) will be either ladarixin OR placebo hard gelatine 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 3 cycles of 14 days on / 14 days off. Placebo will be administered with the same schedule.

Discontinuation criteria
Administration of the investigational product will be discontinued in the 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 LBBB, or atrio-ventricular or heart block. Similarly, administration of the investigational product will be discontinued in the case the patient develops renal (calculated CLcr < 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. Lastly, administration of the investigational product will be immediately discontinued if the patient develops ketoacidosis or hypoglycaemic coma.

Cardiac, renal and hepatic function as well as albuminemia will be specifically monitored through ECG readings and laboratory tests obtained within 1 week before starting the 2nd and 3rd treatment cycle. 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. Results of safety laboratory tests performed after the 1st treatment cycle will be also considered.

Procedures
Study period/visits are summarized in the study flow chart (see Appendix 14.3).

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

From enrolment, patients will be admitted to an intensive diabetes management. 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. To ensure standardized glycaemic control in the treatment groups, the Investigator will provide guidance for insulin regimen adjustment, with insulin titrated up or down to target HbA1c levels of less than 7% and self-monitored (fingerstik):

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

In order to optimize insulin titration, telephone calls (outside scheduled visits) will be scheduled on a regular basis to ensure timely evaluation of metabolic control and adjustment of insulin regimen. Patients will report their self-monitored glucose levels (logs, glucose meter, etc.) as per site standard diabetes management, but at least weekly up to week 13±1 and in the 2 weeks before each follow-up visit.

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 (**time frame**: from enrolment to randomization).

The following baseline assessments (**time frame**: within 3 weeks before randomization – *consider time for centralized assay of MMTT C-peptide*) will be also performed during the screening period:

- Measurement of blood pressure, heart rate, body weight, height;
- Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests" i.e.: hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, serum albumin, blood urea nitrogen, total bilirubin, ALT, AST;
- 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 insulin requirement (IU/kg/day averaged over the previous 3 days);
- Blood sampling for measurement (centralized laboratory) of baseline auto-antibody (anti-GAD; IAA; IA-2 antibody; ZnT8 – not to be repeated if "diagnostic" test is done within this timeframe), HbA1c and miR-375 (and T-cell response *ex vivo* at selected sites only).
- Baseline MMTT. Blood samples for measurement (centralized laboratory) of C-peptide, glucose and glucagon will be drawn pre-meal (2 basal samples in the range between -20 to 0) and 15, 30, 60, 90, 120, 180 min after the meal.

Patients fulfilling **all** the inclusion criteria and **none** of the exclusion criteria will be randomized, randomization day being defined as **Study Day 0** with reference to day when IMP administration is started which is defined as **Study Day 1**.

Treatment period and in-between visits

Assessment to be done before the 1st dose in the 1st treatment cycle are part of the screening evaluation. Thereafter, patients will refer to the site within 1 week before the expected date when the 2nd or 3rd treatment cycle is due to start to confirm no condition that prevents their continuing participation into the study has arisen (as per discontinuation criteria above). The amount of IMP for each treatment cycle will be dispensed only after compliance with criteria for drug administration has been confirmed.

Follow-up visits

After completion of study treatment, patients will attend the centre for study assessments on 3 follow-up visits scheduled at weeks 13±1 (month 3), 26±2 (month 6) and 52±2 (month 12).

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

- Blood sampling for measurement (centralized laboratory) of auto-antibody (anti-GAD; IAA; IA-2 antibody; ZnT8), HbA1c and miR-375 (and T-cell response *ex vivo* at selected sites only);
- MMTT. Blood samples for measurement (centralized laboratory) of C-peptide, glucose and glucagon will be drawn pre-meal (2 basal samples in the range between -20 to 0 min) and 15, 30, 60, 90, 120, 180 min after the meal;
- Insulin requirement (IU/kg/day averaged over the previous 3 days);
- Cumulative number of severe hypoglycaemia events in the interval from: randomization to week 13±1, week 13±1 to week 26±2, week 26±2 to week 52±2;
- **On week 13 ONLY**: Measurement of blood pressure and heart rate; Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests" i.e.: hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, serum albumin, blood urea nitrogen, total bilirubin, ALT, AST.

Statistics

The Safety population will consist of all patients who received any study medication and will be based on the treatment actually received. The Intent to Treat (ITT) population will consist of all patients who are randomized and receive the IMP (either ladarixin or placebo); it will be based on the treatment randomized, regardless of the treatment actually received.

Appropriate descriptive statistics will be produced for all variables, according to the nature of the variable.

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

The primary variable [2-hour C-peptide AUC after the MMTT, log(x+1) transformed data, at week 13±1 (primary efficacy endpoint)], will be analysed with Student t test for unpaired data using PROC TTEST within SAS®, including terms for treatment and centre. The estimated treatment difference between ladarixin and placebo will be also presented together with the corresponding 95% confidence interval.

The other secondary efficacy endpoints will be analysed using appropriate parametric and non-parametric tests and appropriate 95% CI will be presented.

Post-hoc analysis may be produced to further allow comparison between ladarixin and placebo, according to the results obtained.

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 II study in bullous pemphigoid (BP), a rare dermatological disease. Following more recent promising results in mouse models of type 1 diabetes (T1D), development in new onset T1D is being implemented.

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

2.1. RELEVANT NON-CLINICAL PHARMACOLOGY

2.1.1. Mechanism of action and *in vitro* activity

Ladarixin is a novel, potent and specific inhibitor of the biological activity of the chemokine CXCL8. *In vitro* chemotaxis experiments 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.

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 oedema 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, glycaemic 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 hyperglycaemia while hyperglycaemia 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

Ladarixin was tested for toxicity in rodent and non-rodent animal species after single or repeated administrations. The repeated dose administration studies were conducted only by the oral route, according to the intended human administration route.

The acute toxicity of DF 2156A, after single administration by i.v. route to rats resulted in a LD_{50} > 500 mg/kg. In dogs, an i.v. bolus (up to 400 mg/kg) caused mortality at 400 mg/kg and clinical signs of vomiting, salivation and tremor at the lower doses.

The repeated dose administration to rats by i.v. boluses for 7 days up to the dose of 300 mg/kg did not cause mortality. Liver weight was increased, resulting in hepatocytic hypertrophy. Inflammatory and degenerative changes were seen at the injection site. In studies with up to 7 day oral administration in rats (doses up to 600 mg/kg) there was no mortality. Microscopically hepatocellular hypertrophy was present in all treated groups. In studies with up to 1 week administration in dogs, the dose had to be reduced from 500 mg/kg to 150 mg/kg due to poor tolerability and severe clinical signs.

In rats, the oral administration for 28 days up to the dose of 200 mg/kg did not cause relevant toxicity. The immune system was not affected by the treatment. Hepatocellular hypertrophy was seen in the liver of all male treated groups and in females at the highest dose that resolved after 2 weeks of free dose.

In the 4-week oral toxicity study in dogs the compound was well tolerated up to the doses of 120 mg/kg and no mortality occurred. Vomiting was seen at the highest dose. An increase in the absolute and corrected-for-heart rate QT interval was observed at the middle (60 mg/kg) and highest (120 mg/kg) doses. After 2 week washout the QT values returned to normal.

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

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

Cardiovascular safety studies were conducted in dogs by the oral route. At the highest dose tested (100 mg/kg), ladarixin prolonged the QT corrected for heart rate at 1, 2 and 12 hours post-dosing when compared to pre-dosing values. *In vitro*, ladarixin had no effect on the hERG potassium channel

when tested on hERG tail current at concentrations from 3 to 2000 μM. Two metabolites of ladarixin (DF2108Y and DF2227Y) are formed *in vivo* in rats and dogs. They were tested *in vitro* on hERG tail current at doses up to 2000 μM. A dose-related reduction of the tail current amplitudes was seen at concentrations higher than 100 μM (DF2108Y) or 500 μM (DF2227Y). The IC_{50} was: 2247.35 μM (DF2108Y) and 1957.59 μM (DF2227Y), respectively.

In conclusion based on the toxicology data, the No Observed Adverse Effect Level in rats is 200 mg/kg and the No Observed Effect Level in dogs is 30 mg/kg.

Fertility and early embryonic development to implantation was evaluated 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 at any dose levels and in males at 50 and 100 mg/kg/day. In males, at 200 mg/kg, body weight and mean body weight gain were minimally to slightly lower when compared with the controls. At the same dose level, fertility of males was minimally affected by the treatment and only few of them (4 out of 21) did not produce pregnancy in the females. The minor fertility rate was associated to degenerative changes in the testes of few treated males at 200 mg/kg/day that, however, were considered related to the stress suffered from the animals in the first period of treatment and not to a direct toxic effect of the test item. Sperm analysis in these few animals only also showed reduction in number, motility or shape. The NOEL was 100 mg/kg/day for males and of 200 mg/kg/day for females. The testicular changes in few animals were confirmed by an additional toxicity study in males only, using two rat species (Wistar and Sprague-Dawley) treated with 200 mg/kg/day over a period of 6 weeks. Also in this study the testicular changes were associated to the stress suffered in the first period of the treatment (a likely adaptation-stress to the high dose treatment). In conclusion, findings observed in male rats should not be taken as indicating a potential hazard in humans and it should be disregarded in risk assessment for clinical trials of ladarixin. Based on these results and safety assessment, a NOAEL of 200 mg/kg/day can be settled in male rats as well.

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.

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 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 (25 to 400 mg – MEX0108) and multiple (50 to 400 mg twice a day up to 6 days, MEX0109, MEX0110) ascending dose oral administration. The first multiple ascending dose study (MEX0109) also included the evaluation of potential interaction between ladarixin 200 mg and tolbutamide. A phase 2 efficacy and safety study was 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 119 male subjects were involved in clinical trials, of whom 93 (89 healthy volunteers and 4 patients with BP) were exposed to ladarixin.

2.4.1. Pharmacokinetics and product metabolism in humans

Three phase 1 PK/safety studies in healthy male volunteers were performed which included single (25 to 400 mg – MEX0108) and multiple (50 to 400 mg twice a day up to 6 days, MEX0109, MEX0110) ascending dose oral administration. An interaction evaluation for the 200 mg dose was also included in the first multiple ascending dose study. Assay of ladarixin and its metabolite 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 Section 2.3.

2.4.2. Efficacy

A phase 2, multicentre, single arm, pilot study was initiated to assess the safety and efficacy of ladarixin in patients with moderately active BP [MEX0111]. The compound was given by the oral route at 150 mg twice a day (maximum of 14 days), a 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 between 65 to 75 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 was observed with the low dose tested in this trial and the study was prematurely discontinued due to lack of activity.

2.4.3. Safety

A total of 89 male healthy subjects aged 18 to 52 years and 4 male BP patients aged 65-75 yeas 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 and 150 mg twice a day up to a maximum of 14 days in BP patients. In a subset of subjects, ladarixin (200 mg twice a day) was co-administered with tolbutamide.

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.

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.

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.

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

Cumulative Adverse Drug Reactions - Number of events by Terms			
Study code ref./ System Organ Class (SOC) **MedDRA Preferred Term** (PT)		**Number of events**	**Frequency**
Gastrointestinal disorders		**21**	**52.50%**
MEX0109 - Part A MEX0110	Abdominal pain	2	5.00%
MEX0109 - Part A	Diarrhoea	1	2.50%
MEX0109 - Part A MEX0111	Dry mouth	2	5.00%
MEX0109 - Part A MEX0109 - Part C MEX0111	Dyspepsia	7	17.50%
MEX0109 - Part A	Dysphagia	2	5.00%
MEX0110	Flatulence	1	2.50%
MEX0108	Mouth ulceration	2	5.00%
MEX0108; MEX0110	Nausea	2	5.00%
MEX0109 - Part A	Oral Pain	1	2.50%
MEX0109 - Part A	Vomiting	1	2.50%
General disorders and administration site conditions		**1**	**2.50%**
MEX0109 - Part A	Feeling hot	1	2.50%
Infections and infestations		**1**	**2.50%**
MEX0108	Oral herpes	1	2.50%
Investigations		**1**	**2.50%**
MEX0109 - Part A	Liver function test abnormal	1	2.50**%**
Musculoskeletal and connective tissue disorders		**1**	**2.50%**
MEX0109 - Part A	Tendonitis	1	2.50%
Nervous system disorders		**14**	**35.00%**
MEX0108 MEX0109 - Part A	Dizziness	2	5.00%%
MEX0108 MEX0109 - Part A MEX0109 - Part C MEX0110; MEX0111	Headache	11	27.50%
MEX0109 - Part A	Tremor	1	2.50%
Respiratory, thoracic and mediastinal disorders		**1**	**2.50%**
MEX0110	Oropharyngeal discomfort	1	2.50%

Cumulative Adverse Drug Reactions - Number of events by Terms		
Study code ref./ System Organ Class (SOC) **MedDRA Preferred Term** (PT)	**Number of events**	**Frequency**
TOTAL REPORTS	**40**	
Subjects exposed to DF 2156A* with at least an ADR	**28**	
***: figures include subjects exposed to DF 2156A plus tolbutamide (MEX0109 - Part C) and excluding placebo (8 events)**		

Overall, 40 ADRs were reported in a total of 28 subjects out of 93 exposed to ladarixin.

The most frequent (>10%) **ADRs** observed were:

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

Nervous System Disorders: (about 35%) 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, hypoglycaemia unawareness is a serious consequence of recurrent hypoglycaemia 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 hypoglycaemia, 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 III 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 glycaemic 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 glycaemic 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 hyperglycaemia. The positive effects of ladarixin, coupled with the safety shown in phase 1 studies, provide a sound rationale for a clinical study aimed at evaluating the effect of ladarixin in patients with new onset diabetes and supports the conduct of the present study.

2.5.1. Selection of dose and treatment schedule in the study

The proposed dose in this clinical study is 400 mg oral ladarixin twice a day for 3 cycles of 2 weeks on - 2 weeks off.

The oral route has been selected as the more appropriate for a 14 day continuous treatment. The PK profile support this choice.

The 400 mg dose has been selected to provide a drug exposure that would maximise the potential to observe a drug effect, while remaining within safety margins. Indeed, this dose was already established to be safe in phase 1 clinical studies in adult male healthy subjects. This dose will provide an average steady state plasma concentration of the ladarixin unbound fraction (about 100-150 ng/mL) that should ensure full inhibition of PMN migration, considering that the in vitro IC_{50} is in the range of 1 ng/mL.

The proposed treatment schedule (3 cycles of 14 days on - 14 days off) is intended to expose the patient to ladarixin for a period sufficient for such a molecule to establish a potential effect. According to International Conference on Harmonization (ICH) guidelines (guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals), the available repeated dose toxicity studies (4 weeks in rodents and dogs) support the conduct of phase 2 trials with a maximal exposure of 28 days. This proposed treatment schedule appears to exceed the duration of administration in previous clinical studies and in total will involve drug exposure over a longer period than has previously been explored in man or animals. However, each cycle of the proposed treatment can reasonably be regarded at this stage as a separate event that each is adequately supported by the available 28-day toxicity test results. Indeed, the PK in animals and man suggests clearance of the drug and its metabolites from the body of humans within 2-3 days at the most. Also, the pharmacodynamic studies in vitro and in vivo in animals and humans suggest reversibility of the effect on CXCR1/2 receptors within a short period after exposure to the drug has ceased.

The good safety profile of ladarixin in animals and humans further supports the proposed treatment schedule for this study.

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, 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 clinical experience with doses as high as that planned in this study provides evidence of the safety of ladarixin. No SAEs or death occurred and all the AEs encountered were mild to moderate in intensity. The safety was confirmed in the phase 2 trial; indeed, no safety concerns were raised even in elderly patients who were on several concomitant medications due to chronic disease.

Any possible risk derived from the administration of ladarixin in the specific population involved in this study will be minimized by integrated monitoring which include clinical observations, laboratory tests and ECG readings (see Section 7.2.2 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 screening, at the end of 1st treatment cycle (i.e. pre-dose visit, 2nd cycle) and at the 13 week follow-up visit to evaluate standard haematology and clinical chemistry;
- blood samples for diagnostic auto-ab test (maximum 5 mL) and for baseline auto-ab, HbA1c, miR-375, and MMTT c-peptide, glucose, glucagon (maximum 100 mL) will be taken during screening;
- blood samples (about 5 mL) will be obtained before the 3rd treatment cycle is started to monitor renal (creatinine) and hepatic (ALT/AST, bilirubin) function, and albumin;
- blood samples for auto-ab, HbA1c, miR-375 and MMTT c-peptide, glucose, glucagon (maximum 100 mL) will be taken during each of the 3 follow-up visits;
- T-cell response will require additional 10 mL of blood at the selected sites only.

Blood sampling will occur through a peripheral vein and may cause faintness and/or swelling, pain, redness, bruising, or infection (infection rarely happens) at the site where the needle is inserted. The volume of blood above is an amount that the body can safely replace.

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 (pre-dose) and then at follow up visits at weeks 13±1, 26±2 and 52±2. 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 hypoglycaemic event. However, detailed instruction for patient management provided by the Investigator will minimize the risk of metabolic unbalance.

2.5.3.4. 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 recent diagnosis.

3. OVERALL STUDY DESIGN AND PLAN DESCRIPTION

3.1. STUDY DESIGN

The study will be a phase 2, multicentre, double-blind study. It has been designed to investigate (proof of concept) whether ladarixin has sufficient activity (preservation of β-cell function and slow-down of the progression of T1D) to warrant its further development.

It will involve 72 patients with new-onset T1D. Patients will be randomly (2:1) assigned to receive either ladarixin treatment (400 mg b.i.d. for 3 cycles of 14 days on/14 days off - treatment group) or matched placebo (control group). The two groups will be balanced within centres.

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 site will recruit patients as rapidly as possible up to a maximum of 21 patients (as per randomization list).

Each patient will be involved in the study for a screening period of up to 100 days followed by a post-randomization period up to 52±2 weeks, as per details below:

- a screening period including 2 or more visits during a period up to 100 days; this includes the time required to confirm the diagnosis;
- a treatment period including 3 treatment cycles of 2 weeks each with 2 weeks interval between cycles and 2 corresponding pre-dose assessment visits;
- 3 follow-up visits scheduled at weeks 13±1 (month 3), 26±2 (month 6) and 52±2 (month 12).

Sample size calculation has been based on the 2-hour area under the curve (AUC) of C-peptide response to a Mixed Meal Tolerance Test (MMTT) observed 12 months after randomization, as these are the only available data that best match the T1D population expected to be randomized in this trial (age, time from diagnosis, etc.) [*Lachin, 2011*]. On the other hand, the time frame for the primary endpoint (2-hour AUC of C-peptide response to the MMTT) has been set at week 13±1 in order to maximise the potential to observe an effect of ladarixin, considering the PK profile in humans as well as the reversibility of the pharmacological effect on CXCR1/2 receptors. Such an approach is appropriate for a phase 2 proof of concept trial. Follow-up is anyway extended up to 12 months.

3.2. STUDY TIME TABLE

Overall study timelines are reported below.

Projected starting date (first-patient-in):	December 2015
Projected completion of patient accrual (last-patient-in):	December 2016
Projected study end date (last-patient-last-visit):	December 2017

3.3. 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. OBJECTIVES AND ENDPOINTS

4.1. STUDY OBJECTIVES

The objective of this clinical trial is to investigate whether ladarixin has sufficient activity (preservation of β-cell function and slow of the progression of T1D) to warrant its further development. The safety of ladarixin in the specific clinical setting will be also evaluated.

4.2. STUDY ENDPOINTS

4.2.1. Efficacy endpoints

- 2-hour AUC of C-peptide response to the MMTT [**Primary endpoint**. Time frame: baseline, week 13±1].
- 2-hour AUC of C-peptide response to the MMTT [Time frame: baseline, weeks 26±2 and 52±2].
- Average (previous 3 days) insulin requirement (IU/kg/day) [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- HbA1c levels [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- Basal (2 basal samples in the range between -20 to 0 min) to 180 min time course of C-peptide and glucose derived from the MMTT [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- Cumulative severe hypoglycaemic events occurring from randomization [Time frame: weeks 13±1, 26±2 and 52±2].

  For the purpose of this protocol, a severe hypoglycaemic event is defined as an event with one of the following symptoms: "memory loss, confusion, uncontrollable behaviour, irrational behaviour, 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.v. glucose, or glucagon administration.

4.2.2. Exploratory endpoints

- Basal (2 basal samples in the range between -20 to 0 min) to 180 min time course of glucagon derived from the MMTT [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- Auto-antibodies (GAD, IA-2, IAA, ZnT8), [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- MicroRNA-375 (miR-375) [Time frame: baseline, weeks 13±1, 26±2 and 52±2].
- T-cell response *ex vivo* to major β-cell antigens (pro-insulin, GAD65) - selected sites only [Time frame: baseline, weeks 13±1, 26±2 and 52±2].

4.2.3. Safety endpoints

- Vital signs (blood pressure and heart rate) [time frame: screening, end of 1st treatment cycle (i.e. pre-dose visit, 2nd cycle), week 13±1].
- Routine laboratory tests (haematology, clinical chemistry) [time frame: screening, end of 1st treatment cycle (i.e. pre-dose visit, 2nd cycle), weeks 13±1 (or withdrawal)].
- Incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) [time frame: throughout the study].

5. STUDY POPULATION

Seventy-two (72) patients new-onset T1D will be included (randomized) in the study, selected from those referring to the clinical site for diabetes diagnosis and/or management.

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

5.1. INCLUSION CRITERIA

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

1. Male and female patients aged 18-45 years, inclusive;
2. New-onset T1D (randomization 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, with the exclusion of patients taking twice daily pre-mixed insulin or on insulin pump;
5. Residual β-cell function as per peak stimulated (MMTT) C-peptide level >0.6ng/mL (0.2nmol/L); MMTT should not be performed within one week of resolution of a diabetic ketoacidosis event;
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. Patients taking twice daily pre-mixed insulin or on insulin pump
2. 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;
3. Moderate to severe renal impairment as per calculated creatinine clearance (CLcr) < 60 mL/min according to the Cockcroft-Gault formula (*Cockcroft-Gault, 1976*);
4. 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];
5. Hypoalbuminemia defined as serum albumin < 3 g/dL;
6. QTcF > 470 msec;
7. Complete Left Bundle Branch Block (LBBB), atrio-ventricular block (mobitz II 2nd degree or 2:1 atrio-ventricular block), complete heart block;
8. Electronic pacemaker positioned or implanted defibrillator;
9. History of significant cardiovascular disease;
10. Known hypersensitivity to non-steroidal antiinflammatory drugs;
11. Concomitant treatment with phenytoin, warfarin, sulphanylurea hypoglycemics (e.g. tolbutamide, glipizide, glibenclamide/glyburide, glimepiride, nateglinide) and high dose of amitriptyline (> 50 mg/day);
12. Previous (within 2 weeks prior to randomization) and concomitant treatment with metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV 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.);

13. 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;
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 an 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

From enrolment (consent signature) to randomization patients will be identified by a Screening number will be assigned in a sequential manner within each site, as patients are enrolled (sign the consent). It will consist of 4 digits, e.g. 0152, where first 2 digits represent site number (01 = site 1), last 2 digits, starting from 50, patient sequential enrolment number within the site (e.g. 50, 51, 52, 53, 54, etc.).

If randomized, the Randomization number will be assigned in a sequential manner within each site, as patients are found to be eligible and are randomized. It will consist of 4 digits, e.g. 0102, where first 2 digits represent site number (01 = site 1), last 2 digits, starting from 01, patient sequential randomization number within the site (e.g. 01, 02, 03, 04, etc.). 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)

NAME OF INGREDIENT	FUNCTION OF INGREDIENT	AMOUNT FOR CAPSULE (mg)		REFERENCE TO QUALITY STANDARDS
		ACTIVE	PLACEBO	
Ladarixin	Active ingredient	200mg	-	Internal monograph
Microcrystalline cellulose	Filler	up to 266.20 mg		EP current edition
Lactose monohydrate	Filler	up to 60 mg		EP current edition
Croscarmellose Sodium	Disintegrant	up to 14 mg		EP current edition
Hydroxypropyl cellulose	Binder	up to 6 mg		EP current edition
Citric acid monohydrate	Stabilising agent	up to 5.2 mg		EP current edition
Magnesium Stearate	Lubricant	up to 2 mg		EP current edition

Batch release certificate will be provided together with the IMP.

6.1.2. Manufacturing, Packaging and Labelling of IMP

Capsules will be manufactured and packaged in blisters by Patheon UK Limited, Abingdon, UK.

The study medication will be provided as a Patient Kit, containing 3 Treatment Boxes (one box for each treatment cycle). Each Treatment Box will contain 56 capsules packaged in 7 Aluminum/Aluminum blisters with 8 capsules in each blisters.

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, 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 designee) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or designee).

The IMP must be stored 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 designee) and the use of the drug suspended until authorization for its continued use has been given by Dompé (or designee).

The IMP will be dispensed only by the Pharmacist (or authorized designee). The Investigator will ensure that study treatment is only administered by designated staff within the centre.

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. 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.

The two daily doses will be administered at about 12 hour interval (morning and evening; ideally between 8.30/9.30 and 20.30/21.30). At each administration, 2 capsules will be swallowed with a glass of water, at least 2 hours apart from breakfast or dinner.

6.3. CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION/ DISCONTINUATION OF IMP

6.3.1. Criteria for schedule adjustment or dose modification

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

6.3.2. Criteria for discontinuation of 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 LBBB, or atrio-ventricular or heart block;
- The patient develops any significant cardiovascular disease;
- The patient develops renal (calculated CLcr < 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;
- Pregnancy occurs (female patient).
- The patient develops ketoacidosis or hypoglycaemic coma.

Cardiac, renal and hepatic safety will be specifically monitored through pre-planned ECG readings and laboratory tests obtained within one week before starting the 2nd and 3rd 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. Results of safety laboratory tests performed after the 1st treatment cycle will be also considered.

If the IMP administration is prematurely discontinued the primary reason for discontinuation must be recorded in the CRF. 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.

6.4. 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 centre.

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. A copy will be retained for the Investigator/Pharmacy file.

The dispensing of the IMP will be carefully recorded on the CRF 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 Treatment Box, the removable label will be detached from the Treatment Box and attached to the relevant page of the CRF.

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 on a Diary Card (see **Appendix 14.2**) which will be returned to and checked by the Investigator during the next study visit.

The CRA will review the drug accountability forms/CRF/Diary Cards 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 Treatment Box 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.

6.4.1. 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 CRF and Diary Card, versus accountability records.

6.5. CONCOMITANT MEDICATION

6.5.1. 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 CRF.

All the details as per the CRF fields (sequential number, drug name, indication, starting dose, start/stop date, route of administration) will be recorded. No 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.

6.5.2. 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 or insulin pump;
- Metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV 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 antiinflammatory 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 antiinflammatory 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.

6.6. 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 glycaemic control in the treatment groups, the Investigator will provide guidance for insulin regimen adjustment, with insulin titrated up or down to target HbA1c levels of less than 7% and self-monitored (fingerstik):

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

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 14.3**.

For all measurements, the actual date and time of assessment, including date of sampling, will be recorded in the CRF. Where a time window is acceptable, this is clearly indicated in the following sections.

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 identified from those referring to the site for diagnosis confirmation (auto-antibody testing) and/or disease management.

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

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 self-monitored (fingerstik):

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

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. Screening

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

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

✓ **time frame**: from enrolment to randomization (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; Samples will be obtained and stored as detailed in **Appendix 14.4.1**.

✓ **time frame**: within 3 weeks before randomization (baseline measurements - *consider time for centralized assay of MMTT C-peptide – results available within 5 working days*)

- Measurement of blood pressure, heart rate, body weight, height;
- Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests" i.e.: hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, serum albumin, blood urea nitrogen, total bilirubin, ALT, AST;
- Evaluation of renal and hepatic function (to be derived from the safety laboratory test results).
  Renal function will be evaluated by CLcr, calculated according to the following formula [*Cockcroft-*

Gault, 1976]:

$$\text{Male: CLcr} = \frac{[140 - \text{age (years)}] \times \text{Weight (kg)}}{\text{Serum Creatinine (µmol / L)} \times 0.815}$$

$$\text{Female: CLcr} = \frac{[140 - \text{age (years)}] \times \text{Weight (kg)}}{\text{Serum Creatinine (µmol / L)} \times 0.815} \times 0.85$$

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 3days);
- Blood sampling for measurement (centralized laboratory) of baseline auto-antibody (anti-GAD; IAA; IA-2 antibody; ZnT8 – not to be repeated if "diagnostic" test is done within this timeframe), HbA1c and miR-375 (and T-cell response *ex vivo* at selected sites only). Sample handling is detailed in **Appendix 14.4.1**;
- Baseline MMTT. Blood samples for measurement (centralized laboratory) of C-peptide, glucose and glucagon will be drawn pre-meal (2 basal samples in the range between -20 to 0 min) and 15, 30, 60, 90, 120, 180 min after the meal. Procedures for MMTT and sample handling are detailed in **Appendix 14.4.2** and **Appendix 14.4.1**, respectively. MMTT should not be performed within one week of resolution of a diabetic ketoacidosis event;

For the purpose of this protocol, a diabetic ketoacidosis event is defined as the presence of:

hyperglycemia (blood glucose >200 mg/dL);

pH <7.3 or HCO3 <15;

ketones positive in the serum or urine.

7.1.3. Randomization

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 be randomized (randomization number assigned) by an Investigator (or designee), randomization to occur within 100 days from the 1st insulin administration.

Randomization is defined as **Study Day 0** with reference to day when IMP administration is started which is defined as **Study Day 1**.

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

7.2. PRE-DOSE ASSESSMENTS & DRUG ADMINISTRATION

7.2.1. Pre-dose assessment - 1st treatment cycle

Assessment to be done before the 1st dose are part of screening evaluation and reported in Section 7.1.1 above. On the day of randomization (**Study Day 0**), patient will be provided with the Treatment Box - Cycle No. 1 matching his/her randomization number, together with the Diary Card.

Before box delivery, the removable label on the Treatment Box (see Section 6.4) will be detached and attached to the relevant CRF page.

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 administration in the Diary Card, etc.

7.2.2. Pre-dose assessment - 2nd and 3rd treatment cycle

Patient will refer to the site within 1 week before the expected date when the 2nd or 3rd treatment cycle is due to start. The following will be evaluated:

- General clinical evaluation to ensure patients has not developed concurrent clinical conditions, including pregnancy in female participants, which in the opinion of the Investigator may pose a safety risk for continuing participation in the study
- Blood sampling(s) for measurement (local laboratories), of serum creatinine, serum albumin, total bilirubin, ALT, AST. Renal and hepatic function will be evaluated as per details in Section 7.1.1 above.
- 12 lead ECG performed using local equipment that allows automatic calculation of the QTcF. If the QTcF is EITHER > 500 msec OR has increased by > 60 msec from baseline value, a second reading will be obtained 1 hour later.
- Pregnancy test (urine dipstick or blood sample), if appropriate.
- **On pre-dose 2nd cycle ONLY**: Measurement of blood pressure and heart rate; Blood sampling(s) will allow the measurement (local laboratories) of the whole set of "Safety Laboratory Tests" i.e.: hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, serum albumin, blood urea nitrogen, total bilirubin, ALT, AST.

Laboratory results and ECG reading MUST be made available before the Treatment Box for the next treatment cycles is dispensed.

As per discontinuation criteria (see Section 6.3.2), **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 LBBB, or atrio-ventricular or heart block;
- The patient develops any significant cardiovascular disease;
- The patient develops renal (calculated CLcr < 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;
- Pregnancy occurs (female patient).

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 Treatment Box - Cycle No. 2 or Treatment Box - Cycle No. 3 (as appropriate) matching his/her randomization number, together with the Diary Card.

Before box delivery, the removable label on the Treatment Box (see Section 6.4) will be detached and attached to the relevant CRF page.

It is the responsibility of the Investigator (or designee) to check the Diary Card completed for the previous administration period to ensure correct intake of the IMP. The Investigator will remind, and make sure patient still fully understands any appropriate treatment related information.

7.2.3. IMP administration

The day when the patient starts IMP administration is defined as **Study Day 1** (first day in Week 1).

On each treatment cycle, the IMP will be administered at 12 hour interval (morning and evening; ideally in between 8.30/9.30 and 20.30/21.30). At each administration, 2 capsules will be swallowed with a glass of water, at least 2 hours apart from breakfast or dinner.

Administration of the IMP will continue for 14 consecutive days, unless there is any reason for drug discontinuation (see Section 6.3.2).

The administration of the IMP (date/time for each administration, number of capsules) will be recorded by the patient on a Diary Card (see **Appendix 14.2**).

Diary Card as well as the Treatment Box containing all the blisters, either unused or empty, will be returned to and checked by the Investigator during the next study visit.

7.3. FOLLOW-UP STUDY ASSESSMENTS

Patients will attend the centre for study assessments on 3 follow-up visits scheduled at weeks 13±1 (month 3), 26±2 (month 6) and 52±2 (month 12).

At each visit, the following will be evaluated/measured as per centre practice, unless otherwise specified. Measurements, including the actual date and time of assessment, or the date of sampling, will be recorded in the CRF.

- Blood sampling for measurement (centralized laboratory) of auto-antibody (anti-GAD; IAA; IA-2 antibody; ZnT8), HbA1c and miR-375 (and T-cell response *ex vivo* at selected sites only). Sample handling is detailed in **Appendix 14.4.1**;
- MMTT. Blood samples for measurement (centralized laboratory) of C-peptide, glucose and glucagon will be drawn pre-meal (2 basal samples in the range between -20 to 0 min) and 15, 30, 60, 90, 120, 180 min after the meal. Procedures for MMTT and sample handling are detailed in **Appendix 14.4.2** and **Appendix 14.4.1**, respectively;
- Insulin requirement (IU/kg/day averaged over the previous 3 days);
- Cumulative number of severe hypoglycaemia events in the interval from: randomization to week 13±1, week 13±1 to week 26±2, week 26±2 too week 52±2;
- **On week 13 ONLY**: Measurement of blood pressure and heart rate; Blood sampling(s) for measurement (local laboratories) of "Safety Laboratory Tests" i.e.: hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, serum albumin, blood urea nitrogen, total bilirubin, ALT, AST.

7.4. EARLY PATIENT WITHDRAWAL

7.4.1. 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 centre 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.

Any withdrawals must be fully documented in the CRF.

7.4.2. Replacement policy

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

7.5. PATIENT MANAGEMENT AFTER STUDY COMPLETION

After completion of the week 52 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. Definition of an 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 unfavourable 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 [*Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3')*].

8.1.2. Definition of an 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.

8.1.3. Definition of a Serious Adverse Event/Reaction

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

- results in death,
- 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,
- results in persistent or significant disability/incapacity,
- 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.

8.2. 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 enrolment through patient participation in the study (last planned visit or early withdrawal date) will be reported and recorded in the CRF. It is important that this includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, the outcome and relevant concomitant treatments dispensed (or other action taken).

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.

8.2.1. Relationship of AEs to the Investigational Product

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

Relationship of the Adverse Event to the IMP

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

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

8.2.2. 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])

8.3. SERIOUS ADVERSE EVENT REPORTING

8.3.1. 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, 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 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 Non-Carbon Repeat 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.

CRO Pharmacovigilance will report to the Dompé Drug Safety any information received by the Investigators, by e-mail immediately and anyway within 24 hours of knowledge. Copy of the SAE form received from the Investigator will be sent by the CRO to the Dompé Drug Safety immediately, and in no case later than 6 hours from knowledge, in case of fatal or life threatening events.

8.3.2. Reporting Procedure to IEC and to Regulatory Authorities

During the course of the clinical trial, Dompé shall report any serious unexpected* ADR, life-threatening problems or deaths to the concerned IEC which approved the protocol and to the Competent Authorities as soon as possible and in no event later than:

(*a*) 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.

(*b*) fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

* For the purpose of this study, all ADRs are assumed to be unexpected.

Dompé shall follow up safety information and shall report any relevant updated findings as soon as available.

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

In addition, each IEC/Competent Authority and Investigator will receive appropriate periodic safety updates as per applicable European and local requirements and regulations.

8.4. EMERGENCY PROCEDURES

The treatment allocation for each patient will be provided in individual tamper-resistant decoding systems (sealed envelopes or scratch cards) to:

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

Individual treatment codes must be kept in a secure location accessible only to designated staff in order to prevent dissemination of the treatment to personnel involved in study conduct who must remain blind.

The Investigators will open an individual envelope in the event of an emergency only, where knowledge of the blinded treatment could influence further patient care. 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 CRF. Code break will be immediately communicated to the CRO or Dompé, as appropriate.

9. STATISTICAL ISSUES

9.1. SAMPLE SIZE

There are no data available to estimate the effect size of ladarixin in T1D patients in this study.

C-peptide decline over time have been measured in different populations of T1D patients (age, time from diagnosis, etc.) making it difficult to derive reliable data for sample size calculation in our trial.

Only the paper of Lachin [*2011*] provide figures for the population of new onset T1D patients as close as possible to that planned to be recruited in this trial. Authors also provide an algorithm for sample size calculation based on data transformed to account for homogeneous distribution.

According to the log(x+1) transformed data [initially selected by TrialNet as the appropriate transformation for MMTT C-peptide AUC] and the sample size calculated on the data stratified by an adult population (> 18 years), 72 patients will provide 85% power to detect a 50% between-group difference in the 2-hour MMTT C-peptide AUC (2:1) at 0.05 significance level (one-sided test), assuming a 24% drop-out rate.

9.2. RANDOMIZATION

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

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 21 for each site) to allow competitive recruitment within each centre.

The randomization list will be prepared by and independent statistician and provided to Dompé in a sealed envelope to prevent unblinding. Similarly, the randomization list will be provided to the facility responsible for IMP packaging/labelling for the purpose of IMP preparation.

Individual treatment codes will be provided as a tamper-resistant system (either a sealed envelope or a scratch card) to:

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

Individual treatment codes must be kept in a secure location accessible only to designated staff in order to prevent dissemination of the treatment to personnel involved in study conduct who must remain blind.

The randomization code will be broken at study completion, i.e. when the last patient has completed his/her last follow-up visit, and once the database has been locked.

9.3. ANALYSIS POPULATION

The Safety population will consist of all patients who received any study medication and will be based on the treatment actually received. The Safety population will be used to present the demographic and baseline data, and all safety data.

The Intent to Treat (ITT) population will consist of all patients who are randomized and receive the IMP (either ladarixin or placebo); it will be based on the treatment randomized, regardless of the treatment actually received. The ITT population will be used to present efficacy data.

9.4. STATISTICAL METHODOLOGY

All patient data collected on the CRF and on the Diary Card will be listed by patient and centre.

Appropriate descriptive statistics will be produced, according to the variable. For continuous data the

mean, standard deviation, standard error of the mean, median and range (minimum and maximum) and lower and upper 95% confidence limits (CI) will be presented. For categorical data, frequencies and percentages will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

All the AUC analyses will be based on actual rather than scheduled timings and will be calculated using the trapezoidal rule. If the actual time is not recorded, the scheduled time will be used instead. When C-peptide values are below the limit of detection (0.2 ng/mL), a value of 0.2 ng/mL will be assumed in the calculation of area under the curve. Missing C-peptide values will be imputed via linear interpolation, and the last available basal sample (time and value) will be used as the first sample in the computation of the AUC. 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.

Besides statistical analysis presented below, additional post-hoc analysis may be produced to further allow comparison between ladarixin and placebo, according to the results obtained. They may include change from baseline in C-peptide AUC, peak stimulated C-peptide, time to peak, etc.

A Statistical Analysis Plan will be issued describing details of all the statistical methods and analysis to be applied to trial results. Any change in the planed analysis will be documented. The data will be presented in the clinical study report.

9.4.1. Demographic and baseline characteristics

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

9.4.2. Analysis of efficacy variables

The 2-hour C-peptide AUC after the MMTT at week 13±1 (primary efficacy endpoint), will be analysed with Student t test for unpaired data using PROC TTEST within SAS®, including terms for treatment and centre. The estimated treatment difference between ladarixin and placebo will be also presented together with the corresponding 95% confidence interval.

Additional analysis will include the analysis of the AUC at the 3 time points with a single repeated measurements model using PROC MIXED within SAS®. The adjusted least squares means will be estimated for each combination of time point and treatment. The treatment effect within each time point will be compared using a two sided test at 0.05 level. The tests of the fixed effects will be presented, together with the estimated least squares means and summary statistics of the raw AUC for each of the two treatments at each time point. The estimated treatment difference between ladarixin and placebo at each time point will be presented together with the corresponding 95% confidence interval. The confidence interval will be generated at a statistical level of 0.05.

The mean in average daily insulin requirements and HbA1c value at the 3 time points will be analysed using a repeated measurements model using PROC MIXED within SAS®.

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

The other secondary efficacy endpoints will be analysed using appropriate parametric and non-parametric tests and appropriate 95% CI will be presented.

9.4.3. Analysis of exploratory variables

Exploratory variables will be presented using appropriate descriptive statistics, by treatment group.

9.4.4. Analysis of safety variables

AEs will be presented in terms of the number of AE, the incidence, severity and relationship to the study drug, overall and by body system and preferred term. SAEs will be presented in the same way.

Results for routine laboratory tests will be assessed as being below the lower limit of the normal range, within the normal range or above the upper limit of the normal range. The frequency of patients reporting an abnormal or abnormal clinically significant laboratory value at screening and follow-up will be presented for each laboratory variable.

Vital signs will be presented using descriptive statistics.

9.4.5. Missing data

All reasonable efforts will be made to reduce the rate of missing data, since any method used for imputation for missing observations would be based on untestable assumptions that likely would be invalid.

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)

It is the responsibility of the CRO appointed by Dompé to obtain approval of the trial protocol/amendments from the appropriate IEC.

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

- the study protocol,
- the ICF,
- 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 approval will be sent to Dompé along with relevant correspondence with the IEC. The study will not be started until full written approval has been obtained from the appropriate IEC. 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 will submit any future amendment to the protocol to the IEC which granted the original approval. Any amendment will be implemented only when full approval has been obtained from the appropriate IEC, except for those amendments which involve only logistical or administrative aspects of the study.

The CRO appointed by Dompé will also submit to the IEC which approved the protocol any required progress reports and study update, and will inform the IEC of the termination of the study.

Responsibility for safety reporting, including serious ADRs, life-threatening problems or deaths occurring at sites participating to this clinical trial and/or in other clinical studies conducted with ladarixin, remains within Dompé.

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) ICF will be provided to the site once approved by the IEC. Any changes requested by the IEC 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 regulations.

On the CRFs or Diary Cards, 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

CRFs will be supplied as 3-pages/ No-Carbon Required binder. CRFs 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 CRF is required and should be completed for each patient enrolled. The Investigator will be responsible for the accuracy of the data entered in the CRFs. All entries must be written in ***ENGLISH*** in black ink. Source documents should be available to support all the data recorded in the CRF; location of source documents, including those for which the CRF might be accepted as being the sole source document, will be specified and listed at the centre Initiation Visit.

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

11.2. DIARY CARD

Diary Cards will be supplied as a single-page document. They 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 Diary Card (local language version) will be provided to each patient randomized into the trial. A sample English specimen is provided in **Appendix 14.2**.

The patient will report in the Diary Card the details (date, time, number of capsules) of each administration of the IMP. It is responsibility of the Investigator to explain to each patient how to enter the data in the Diary Card and to check the Diary Card returned to ensure correct completion as well as correct intake of the IMP.

11.3. DATA MANAGEMENT

Data management of the CRFs and Diary Cards will be performed by the CRO appointed by Dompé.

The CRF and Diary Card pages for all patients will be data-entered (double data entry) into the study data-base, and the data will be verified for missing data, inconsistencies, and for any necessary medical clarifications. Queries arising from these checks will be sent to the Investigator for response and signature.

Once all data queries have been resolved, the study data-base will be declared to be "clean", and the study data will be locked ready for analysis.

After the database lock has been achieved, the Investigator may archive the copies of the CRFs and Diary Card retained at the centre. The original CRF and Diary Card will be transferred to Dompé for archiving.

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.

- 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 designee), including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).

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 CRFs, and Diary Cards, 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, October2013) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (*ICH-GCP*) and any local regulations.

12.1. REGULATORY BODY APPROVAL

The CRO appointed by Dompé will obtain the necessary approval from the Competent Authorities prior to initiation of the study.

The study will not be started until full written approval from the relevant Competent Authorities 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 CRFs. 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.

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 and Competent Authority as appropriate. No deviations from or changes to the protocol will be implemented without documented approval of an amendment from the IEC 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 for review and approval as soon as possible.

Any other deviation from the protocol that has not been approved by Dompé and the IEC could result in a discontinuation from the study at the centre 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 CRFs/Diary Cards 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 multicentre 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 multicentre 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. 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.

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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 3 Treatment Boxes, one for each treatment cycles. Each Treatment Box will contain 7 blisters of 8 pockets each, for a total of 56 capsules (amount needed for each 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. Only the label of the "Treatment Box" will be a "double - tear off" label.

NOTE: Patient No. XXYY where XX = Study site; YY = Patient sequential number at the site
Treatment cycle No. Z where "Z" can be either 1, or 2, or 3

Specimen Label for each Patient Kit

STUDY MEX0114 **Sponsor** Dompé farmaceutici s.p.a.; Via San Martino 12, Milan – Italy
INVESTIGATOR: (*name*)

PATIENT No. XXYY

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

CONTAINS: 3 TREATMENT BOXES, ONCE FOR EACH TREATMENT CYCLE; TOTAL 168 CAPSULES (IN 21 BLISTERS)

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

DIRECTIONS: Dispense the Treatment Box, corresponding to the cycle, just before the planned start of that treatment cycle. For any questions, please contact (*to be identified in the final label*)
For clinical trial use only. Box not to be dispensed to the patient.

Specimen Label for each Treatment Box [double - tear off label]

STUDY MEX0114 **Sponsor** Dompé farmaceutici s.p.a.; Via San Martino 12, Milan – Italy
INVESTIGATOR: (*name*)

PATIENT No. XXYY **TREATMENT BOX – CYCLE No. Z**

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

CONTAINS: 7 BLISTER WITH 8 POCKETS EACH; TOTAL 56 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) for 14 consecutive days. See additional instructions in the Diary Card.
Contact the Investigator (see Diary Card) should you have any questions.

For clinical trial use only. Keep out of reach of children.

Specimen Label for each blister

STUDY MEX0114 **Sponsor** Dompé farmaceutici s.p.a.
INVESTIGATOR: (*name*)

coded BATCH No. coded EXPIRY DATE mm/yyyy

PATIENT No. XXYY **Cycle No.**

For clinical trial use only. Keep out of reach of children.

14.2. APPENDIX 14.2 – SAMPLE DIARY CARD [English specimen]

Clinical Trial MEX0114 - Sponsor: Dompé farmaceutici s.p.a.

DIARY CARD

PATIENT No. ☐☐☐☐ TREATMENT CYCLE No. ☐ INVESTIGATOR NAME and CONTACT DETAILS: _____

The study doctor has provided you with a Treatment Box containing the study drug for the treatment cycle reported above.
Each day, you are requested to take **2 capsules** in the morning and **2 capsules** in the evening. Please swallow the 2 capsules with a glass of water, **2 hours apart** from breakfast or dinner, ideally in the period 8.30/9.30 and 20.30/21.30, respectively. Any time you take the study drug, please report below the number of capsules taken, and the date and time when you take the capsules. Please enter the information with indelible ink.
Please, **DO NOT DISCARD** any of the blisters provided. All the blisters, either unused or empty MUST remain in the Treatment Box. Return the Treatment Box to the study doctor during the next study visit, together with this Diary Card.

Day	**Date** day/month/year		**Time** 24 hour clock, e.g. 18.30	**No. of capsules**		**Day**	**Date** day/month/year		**Time** 24 hour clock, e.g. 18.30	**No. of capsules**
Day 1		Morning				**Day 8**		Morning		
		Evening						Evening		
Day 2		Morning				**Day 9**		Morning		
		Evening						Evening		
Day 3		Morning				**Day 10**		Morning		
		Evening						Evening		
Day 4		Morning				**Day 11**		Morning		
		Evening						Evening		
Day 5		Morning				**Day 12**		Morning		
		Evening						Evening		
Day 6		Morning				**Day 13**		Morning		
		Evening						Evening		
Day 7		Morning				**Day 14**		Morning		
		Evening						Evening		

Investigator's signature (for review): _____ date: _____

14.3. APPENDIX 14.3 – STUDY FLOW CHART

1st INSULIN ADMINISTRATION	← 100 days →				RANDOMIZATION – STUDY DAY 0 [1st cycle IMP dispensed]												
ENROLLMENT (consent signed)	VISIT	SCREENING (one or more visits)				Pre-dose visit (2nd cycle)		Pre-dose visit (3rd cycle)			FU visit 3months		FU visit 6months		FU visit 12months		
	Week	From enrolment	- 3 to 0		1-2	4	5-6	8	9-10	11 12	13±1	24 25	26±2	50 51	52±2		
	ASSESSMENTS	Past medical history and disease-specific information	BP/HR, Weight, Height Safety lab test (hematology & biochemistry) 12 lead ECG Pregnancy		Treatment cycle No.1 (start on Study Day 1)	BP/HR Safety lab test (hematology & biochemistry) 12 lead ECG (repeat 1 hour apart if QTcF is EITHER > 500 msec OR has increased by > 60 msec from screening value) Pregnancy	Treatment cycle No. 2	serum creatinine and albumin, total bilirubin, ALT/AST, 12 lead ECG (repeat 1 hour apart if QTcF is EITHER > 500 msec OR has increased by > 60 msec from screening value) Pregnancy	Treatment cycle No. 3	Pre-visit phone call	Insulin (IU/kg/day) Severe hypoglycaemia **HbA1c, Auto-Ab and miR-375**	Pre-visit phone call	Insulin (IU/kg/day) Severe hypoglycaemia **HbA1c, Auto-Ab and miR-375**	Pre-visit phone call	Insulin (IU/kg/day) Severe hypoglycaemia **HbA1c, Auto-Ab and miR-375**		
		Auto-Ab to confirm diagnosis	Baseline Insulin (IU/kg/day) Baseline$ **HbA1c, Auto-Ab** and **miR-375**								**MMTT C-peptide glucose glucagon**		**MMTT C-peptide glucose glucagon**		**MMTT C-peptide glucose glucagon**		
			Baseline **MMTT# C-peptide, glucose, glucagon**			2nd cycle IMP dispensed if no contraindication has arisen (see discontinuation criteria below*)		3rd cycle IMP dispensed if no contraindication has arisen (see discontinuation criteria below*)			BP/HR Safety lab test (hematology & biochemistry)						
			T-cell response *ex vivo* §								**T-cell response *ex vivo* §**		**T-cell response *ex vivo* §**		**T-cell response *ex vivo* §**		

Assay of green entries will be done at a centralized laboratory. Auto-Antibodies (**Auto-Ab**) include anti-GAD; IAA; IA-2 antibody; ZnT8. [$ *Baseline Auto-Ab test will not be repeated if test to confirm diagnosis is done within the -3 to 0 week timeframe*].

= Baseline MMTT should be planned to ensure the sample is sent to the centralized laboratory and C-peptide result is available (consider 5 working days) in time for randomization.

§ = T-cell to be collected at selected sites only.

* = As per Treatment discontinuation criteria, **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; Patient develops LBBB, or atrio-ventricular or heart block; or any significant cardiovascular disease; Patient develops renal (calculated CLcr < 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; Female patient has become pregnant.

Randomization = Study Day 0 with reference to **Study Day 1 = first day when the IMP administration is started**.

14.4. APPENDIX 14.4 - METHODOLOGICAL DETAILS

14.4.1. Handling of samples for centralized assay

A study-specific Laboratory Manual will be issued to all sites, reporting detailed instructions for blood sampling, and preparation, storage and shipment of samples.

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 laboratory. A refrigerated centrifuge will also be required.

Samples will be shipped from the site to the centralized laboratory in appropriate package in dry ice (solid CO_2) to maintain frozen conditions. All samples will be shipped on an ongoing basis during the trial, according to logistics. However, samples from the last patient visits will be shipped as soon as possible to ensure timely availability of results.

Handling of samples on receipt at the centralized laboratory 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 laboratory, each site will enter values pertaining to its patients in the corresponding CRF.

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

Tubes and labels for blood withdrawal and sample storage will be provided by the CRO appointed by Dompé, along with storage and shipment tracking forms. The appointed CRO will also coordinate shipment to the centralized laboratory.

All steps will be tracked to ensure correct data reporting.

Any laboratory involved in centralized assay will be listed in the Laboratory Manual, along with relevant contact details.

14.4.2. Mixed Meal Tolerance Test

The MMTT will be performed after an overnight fast, according to Greenbaum (*2008*) at baseline (within 1 week prior of randomization) and at each follow-up visit on weeks 13±1, 26±2 and 52±2. Screening 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 6hrs and 2 hrs, 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±5, 60±10, 90±10, 120±15, 180±15 min after the meal.