**CLINICAL STUDY PROTOCOL**

**Title:** A phase II randomized, placebo-controlled, double-blinded, 2-parallel arm, clinical trial evaluating ladarixin 400 mg twice a day as adjunctive therapy to improve glycemic control in overweight insulin-resistant patients with type 1 diabetes.

**Short Title**: A phase II randomized, placebo-controlled, double-blinded, 2-parallel arm, clinical trial evaluating ladarixin as adjunctive therapy to improve glycemic control in overweight insulin-resistant patients with type 1 diabetes

**Study Number:** LDX0122

**Study Name:** CONSERVA

**IND / EudraCT number:** 147145 / 2022-000743-68

**Investigational Product:** Ladarixin

**Phase of the study:** Phase II

**Protocol Version – Date:** version 3; Final 28 October 2022

*This protocol version results from revision of protocol version No. 1* CCI *according to Amendment No. 1 (Final, 28 October 2022). For Italian Investigational sites, this protocol version results from revision of protocol version No. 2 (applicable to Italian sites only)* CCI *according to Italy-specific Amendment No. 2 (Final, 28 October 2022).*

## STUDY CONTACT INFORMATION

**SPONSOR** Dompé farmaceutici s.p.a. [Dompé]
Via Santa Lucia 6; 20122 Milan, Italy

**Medical Expert** PPD

**Clinical Operation Manager** PPD
PPD

**Development Director** PPD

## CONTRACT RESEARCH ORGANIZATIONS

**Clinical** CCI

**Biometrics** CCI

**PHARMACOVIGILANCE:** CRO PhV: e-mail: PPD
PPD
Dompé PhV: e-mail: PPD
PPD

## PRINCIPAL INVESTIGATORS AND STUDY STAFF

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

## Table of Contents

## List of Abbreviations and Definitions of Terms

| | |
|---|---|
| ADR | Adverse Drug Reaction |
| AE | Adverse Event |
| ALT | Alanine Aminotransferase |
| AST | Aspartate Aminotransferase |
| AUC | Area Under the Curve |
| b.i.d. | Bis in die |
| BMI | Body Mass Index |
| BP | Bullous pemphigoid |
| ºC | Degrees Celsius |
| CGM | Continuous Glucose Monitoring |
| CKD-EPI | Chronic Kidney Disease Epidemiology Collaboration |
| CRA | Clinical Research Associate |
| CSR | Clinical Study Report |
| CXCL8 | CXC ligand 8 [formerly interleukin (IL)-8] |
| CXCR1/2 | CXCL8 receptors |
| CYP2C9 | Cytochrome P450 2C9 |
| DKA | Diabetic Ketoacidosis |
| DMC | Data Monitoring Committee |
| DPP-IV inhibitor | Dipeptidyl peptidase-IV inhibitor |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| eGFR | Estimated Glomerular Filtration Rate |
| eGDR | Estimated Glucose Disposal Rate |
| FAS | Full Analysis Set |
| fMLP | formyl-met-leu-phe |
| GAD | Glutamic Acid Decarboxylase |
| GV | Glycemic Variability |
| HbA1c | Glycated hemoglobin |
| HIV | Human Immunodeficiency Virus |
| IA-2 | Islet Antigen-2 |
| IAA | Insulin Auto Antibody |
| IB | Investigator's Brochure |
| ICF | Informed Consent Form |
| ICH-GCP | International Conference on Harmonization on Good Clinical Practice |
| IEC | Independent Ethics Committee |
| IGV | Impaired Glycemic Variability |
| IMP | Investigational Medicinal Product |
| IR | Insulin Resistance/Insulin Resistant |
| IRB | Institutional Review Board |
| ISOC | Insulin Standard of Care |
| ITT | Intention To Treat |
| i.v. | Intravenous |
| $LD_{50}$ | Lethal $Dose_{50}$ |

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|---|---|
| NET | Neutrophil Extracellular Trap |
| NGSP | National Glycohemoglobin Standardization Program |
| NOAEL | No Observed Adverse Effect Level |
| NOD | Non-Obese Diabetic |
| NOEL | No Observed Effect Level |
| PCP | Primary Care Physician |
| PK | Pharmacokinetics |
| PI | Principal Investigator |
| PMN | Polymorphonuclear leukocyte |
| p.o. | per os (taken by mouth) |
| QTcF | Fridericia's corrected QT interval |
| SAE | Serious Adverse Event |
| SAF | Safety population |
| SAP | Statistical Analysis Plan |
| s.c. | Subcutaneous |
| SD | Standard Deviation |
| SGLT2 | Sodium-Glucose co-transporter-2 |
| SUSAR | Suspected Unanticipated Serious Adverse Reaction |
| TAR | Time Above Range |
| TBR | Time Below Range |
| TEAE | Treatment Emergent Adverse Event |
| TESAE | Treatment Emergent Serious Adverse Event |
| TIR | Time In Range |
| t½ | Elimination half life |
| T1D | Type 1 Diabetes |
| T2D | Type 2 Diabetes |
| ULN | Upper Limit of Normal |
| ZnT8 | Zinc Transporter Isoform 8 |

# 1. STUDY SYNOPSIS

| **CLINICAL STUDY SYNOPSIS:** | |
|---|---|
| Study Number | LDX0122 |
| Title of Study | A phase II randomized, placebo-controlled, double-blinded, 2-parallel arm, clinical trial evaluating ladarixin 400 mg twice a day as adjunctive therapy to improve glycemic control in overweight insulin-resistant patients with type 1 diabetes. |
| Study Name | CONSERVA |
| IND – EudraCT N° | 147145 – 2022-000743-68 |
| Study Centers (Country) | USA and Italy |
| Development Phase | II |
| Objective | Primary objective: To determine whether oral ladarixin versus placebo adjunctive therapy improves glycemic control in overweight, insulin-resistant (IR) adult subjects with type 1 diabetes (T1D).<br>Secondary objectives: To ascertain the effect of ladarixin on glycemic variability as per CGM derived parameters. To determine the safety of oral ladarixin versus placebo adjunctive therapy in overweight, IR adult subjects with T1D.<br>Exploratory objectives (if site is able and deems appropriate to accommodate and conduct these objectives): CCI [redacted] |
| Study Design and Methodology | This study will be a randomized, placebo-controlled, double-blinded, 2-parallel arm, phase II trial. It will enroll up to 86 patients across all genders, 21-65 years, inclusive, with established insulin-requiring T1D and IR, assigned (1:1) to receive either oral ladarixin 400 mg b.i.d. for 7 cycles (26 weeks) of 14 days on/14 days off (treatment group) or matched placebo (control group). Patients will be involved in the trial for 5 study visits, for a total of 28 weeks.<br>Recruitment will be competitive among the study sites, until the planned number of patients is randomized. |
| Number of Patients | Up to 86 patients with established insulin-requiring T1D and with evidence of IR, aged 21-65 inclusive at the time of enrollment (patients consented and eligibility criteria confirmed) to target 76 evaluable patients. |
| Diagnosis and Main Criteria for Inclusion/Exclusion | ***Inclusion*:**<br>1. clinical diagnosis of autoimmune T1D as documented by positive T1D-related autoantibodies [the presence of at least one or more of Insulin autoantibodies (IAA), Anti-GAD (GAD65), Anti-IA2 (IA2), Zinc Transporter 8 (ZnT8)];<br>2. age 21-65 years, inclusive, at the time of consent;<br>3. T1D duration > 1 year;<br>4. detectable fasting C-peptide as per the result of screening laboratory measurement;<br>5. current insulin standard of care (ISOC), either established use of an insulin pump (closed loop system excluded) or a stable dose level and dose frequency for the last two months prior to screening, with no plans to switch the modality of insulin administration during the trial; |

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| | 6. routine use of a self-owned (if applicable) Continuous Glucose Monitoring (CGM) system that can record glucose concentrations continuously for at least 7 days;<br>7. HbA1c value ≥7.5% as per the result of screening laboratory measurement;<br>8. evidence of IR based on a total daily insulin dose >0.8 U/kg/day;<br>9. subject is overweight or obese as per body mass index (BMI) of between 24-33 kg/m$^2$, inclusive;<br>10. ability to comply with all protocol procedures for the duration of the study, including scheduled follow-up visits and examinations, and willing to be contacted by clinical trial staff;<br>11. provision of signed informed consent prior of any study-related procedure not part of standard medical care.<br><br>***Exclusion*:**<br>1. use of a "closed loop system" for integrated glucose reading/insulin infusion;<br>2. known or suspected hypersensitivity to the active pharmaceutical ingredient, non-steroidal anti-inflammatory drugs or any excipient of the investigational medicinal products (e.g. lactose and croscarmellose) as well as patients with congenital lactase deficiency, galactosaemia or glucose-galactose intolerance will have to be excluded;<br>3. use of non-insulin medications for adjunctive blood glucose control (e.g: antidiabetic agents such as metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors, SGLT-2 inhibitors or amylin) within one month of randomization as well as required in the participant's standard of care;<br>4. use of medications for weight reduction such as: Belviq (lorcaserin), Qsymia (Phentermine + topiramate), Orlistat (xenical) within one month of randomization as well as required in the participant's standard of care;<br>5. use of a medication such as stimulants, antidepressants and/or psychotropic agents that could affect weight gain or glycemic control of T1D;<br>6. treatment with drugs metabolized by CYP2C9 with a narrow therapeutic index [i.e., phenytoin, warfarin, and high dose of amitriptyline (>50 mg/day)];<br>7. use of angiotensin-converting enzyme inhibitors, interferons, quinidine antimalarial drugs, lithium, niacin;<br>8. evidence of QTcF >470 msec and a history of significant cardiovascular disease/abnormality;<br>9. any condition, including unstable diet and disordered eating behaviour, that in the judgment of the investigator will adversely affect patient's safety or the completion of the protocol or otherwise confound study outcome;<br>10. pregnancy: a) positive or missing pregnancy test (quantitative beta hCG) at screening; b) women of child-bearing potential and fertile men who do not agree to use effective contraceptive measures up to 2 months following trial discharge; lactating women;<br>11. clinical diagnosis of celiac disease that is in poor control as defined by most recent tissue transglutaminase (tTG) that is in the abnormal range; |

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| | 12. history of ≥1 Diabetic Ketoacidosis (DKA) events in the past 6 months;<br>13. hypoalbuminemia (serum albumin <3 g/dL);<br>14. 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];<br>15. moderate to severe renal impairment calculated by estimated Glomerular Filtration Rate (eGFR) <60 mL/min/1.73 m2 as determined using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation;<br>16. past (within 1 month prior to screening) or current administration of any immunosuppressive medications (including oral or systemically injected steroids) and use of any investigational agents, including any agents that impact the immune response;<br>17. a condition already known which interferes with the ability to accurately determine glycated HbA1c;<br>18. significant systemic infection during the 4 weeks before the 1st dose of study drug (e.g., infection requiring hospitalization, major surgery, or i.v. antibiotics to resolve; other infections, e.g., bronchitis, sinusitis, localized cellulitis, candidiasis, or urinary tract infections, must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant exclusion). |
| Test Product, Dosage and Mode of Administration | The Investigational Medicinal Product (IMP) will be either ladarixin OR placebo capsules for oral administration. Ladarixin will be administered orally at the dose of 400 mg b.i.d. at about 12-hour interval (morning and evening). IMP will be dispensed as Patient Kits. |
| Duration of Treatment | The IMP will be administered for 7 cycles of 14 days on treatment with an interval of 14 days off treatment between cycles, for a total duration of 26 weeks. |
| Reference product, Dosage and Mode of Administration | Placebo will be administered with the same schedule as ladarixin. |
| Study procedures | Each patient will be involved in the study for a total of 5 visits, as detailed below.<br>**Subject identification phase** (if needed as per site practice; pre-screening): The study staff will identify potential study subjects (e.g. using electronic health records, diabetes or other relevant research study registries) and will reach out to them, via their primary healthcare manager/physician, offering information about the study and how to contact the study staff. Responsive individuals will be given comprehensive details about the study and then offered an informed consent form. The potential participants will have up to 7 days to decide if they are able and willing to participate. If they are, the study staff will arrange for the potential participant to come to Visit 1 for witnessed informed consent signing and then to undergo the screening procedures.<br>**Visit 1: Screening**: The study physician or designee will present to discuss with the study subject the clinical trial and answer all questions. If the patient is able and willing to participate, after the informed consent has been obtained, study staff will assess past medical history to confirm T1D (e.g. results of past auto-antibody testing), will determine daily insulin requirement (U/kg/day - expressed as the average of the last 3 days) and its stability, will measure body weight, height, waist and waist/hip circumference, will calculate [redacted] Body Mass Index (BMI), and will record the use of concomitant medication. Screening evaluation also include establishing the presence of IR as described (inclusion criteria, bullet 8)[1-3]. Blood |

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| | samples will be obtained to assess HbA1c, fasting C-peptide, standard safety laboratory tests, pregnancy in women with reproductive potential, T1D auto-antibody (excluding IAA) should past data not be available. Vital signs (blood pressure and heart rate) will be measured and an ECG performed. Patients must ensure the CGM sensor is correctly inserted and glucose monitored for at least the 7 days prior to the next visit. Potential study subjects must meet all inclusion and exclusion criteria prior to randomization.<br>**Visit 2: Randomization and start of IMP (Day 1)**: Within 2 weeks from the screening, the study subject will come to the site in the morning for visit 2. After at least 7 days of CGM data have been downloaded for measurement of glycemic variability outcomes and all inclusion/ exclusion criteria have been finally confirmed, eligible patients (enrolled patients) will be assigned a unique sequential randomization number and randomized 1:1 into either ladarixin or placebo, according to the randomization list. Vital signs will be measured, average (previous 3 days) daily insulin requirements (IU/kg/day) will be recorded, body weight, height, waist and waist/hip circumference will be assessed [redacted] and BMI calculated.<br>CCI [redacted]<br>**IMP administration**: The patients will begin administering the IMP, while on-site under medical supervision, ideally no later than 11:30 (1st dose of cycle 1). Treatment will proceed at home for the 1st and for additional 6 cycles of 14 days on/14 days off, for a total of 26 weeks.<br>**Visit 2b: (Week 4):** An ECG will be performed and then the subject will undergo blood sampling for assessment of safety laboratory testing.<br>**Visit 3: (Week 11/12)**: Patient Kits (cycle 1 to 3) will be returned to verify treatment compliance. Medication use and Diary entries will be also ascertained. Vital signs will be measured followed by an ECG, and then the subject will undergo blood sampling for assessment of HbA1c, CCI [redacted] average (previous 3 days) daily insulin requirements (IU/kg/day) will be recorded, body weight, height, waist and waist/hip circumference will be measured [redacted] and BMI calculated. CGM data will be downloaded for measurement of glycemic variability in the previous 7 days. After IMP discontinuation criteria have been checked, Patient Kits (cycle 4 to 7) will be dispensed and the subject will be discharged from this visit.<br>**Visit 4; End of Study (Week 27/28)**: The study subject will come to the clinic for final assessment of vital signs, ECG, average (previous 3 days) daily insulin requirements (IU/kg/day), body weight, height, waist and waist/hip circumference and for calculation of [redacted] BMI. Subjects will undergo blood sampling for assessment of HbA1c, safety laboratory testing, eGFR CCI [redacted] CGM data will be downloaded for measurement of glycemic variability in the previous 7 days.<br>At the end of the visit, upon the study physician's order, the subject will be formally discharged from the study. |
| Primary Efficacy Endpoint | The proportion of responders at week 27/28 (visit 4), with responders defined as "patients with an HbA1c reduction from baseline of ≥0.50% (absolute difference) without episodes of severe hypoglycemia (level 3) |
| Secondary Efficacy Endpoints | - The proportion of responders at week 11/12 (visit 3) |

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| | - The mean difference from baseline in HbA1c assessed at week 11/12 (visit 3) and 27/28 (visit 4)<br>- Average (previous 3 days) daily insulin requirements (IU/kg/day) assessed at week 11/12 (visit 3) and 27/28 (visit 4)<br>- Glycemic Variability by CGM (previous 7 days): time in range (TIR), time above range (TAR) time below range (TBR), standard deviation and coefficient of variation assessed at week 11/12 (visit 3) and 27/28 (visit 4) |
| Exploratory Endpoints | - CCI<br>- CCI<br>- CCI |
| Safety Measures | - Vital signs (blood pressure and heart rate) & Safety laboratory tests (hematology, clinical chemistry) assessed at screening, week 11/12 (visit 3) and 27/28 (visit 4)<br>- Incidence of Treatment Emergent Adverse Events (TEAEs) recorded from the beginning of study treatment to up to the end of study participation |
| Statistical Methods | This study is designed to be a randomized, placebo-controlled, double-blinded, 2-parallel arm experiment in which subjects are randomized (1:1) to ladarixin or placebo. Sample size calculation was based on results obtained in the phase 2 trial with ladarixin in T1D onset. Considering the following assumptions:<br>• randomization ratio 1:1 (ladarixin: placebo),<br>• one-side type I error of 0.05,<br>• expected difference in favor of ladarixin in terms of responders: ~22.5%, and<br>• power of 80% to detect the expected treatment effect<br>a total of 76 evaluable patients are required; to account for a possible 10% of patients not evaluable for the primary variable after enrollment, up to 86 patients will be enrolled.<br>Summary statistics appropriate to the distribution of the primary variable will be calculated separately by treatment arm. A logistic regression model will be fit, adjusting for treatment, stratification factor, and other baseline characteristics.<br>Summary statistics are defined for quantitative variables (number of observations, mean, standard deviation, median, minimum and maximum) and qualitative variables (number and percentage per category).<br>The secondary CCI endpoints will be analyzed at each available time point by means of descriptive statistics and by appropriate inferential tests.<br>TEAEs will be presented in terms of the number of TEAEs and incidence. Other safety parameters will be summarized by treatment at each available visit by means of descriptive statistics. |

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| | The Safety and the Full Analysis Set (FAS) population will consist of all patients who will be randomized and received at least one dose of the IMP. Safety population will be analyzed according to the actual treatment received; FAS population will be analyzed according to the Intention to Treat principle, i.e. by treatment allocation. |

## 2. SCHEDULE OF EVALUATION

**The grid below** summarizes the study schedule and the patient visits of the trial. For all measurements, the actual date and time of assessment, including date of sampling, will be recorded in the Source Document and/or eCRFs. Timeframe for each assessment is also shown in the grid below.

| ACTIVITY | PRESCREENING* (Identify and reach out to potential participants and offer ICF) | Screening Visit 1 (Within week -2) | Treatment phase (week 1 to 26) | | | End of study Visit 4 (week 27/28) |
|---|---|---|---|---|---|---|
| | | | Randomization Visit 2 (Day 1) | Visit 2b (week 4) | Visit 3 (week 11/12) | |
| **Presentation of trial and ICF** | X | X | | | | |
| **Informed Consent Form (ICF) signed** | | X | | | | |
| **Medical history and pre-existing conditions** | | X | | | | |
| **Auto-antibodies evaluation[1]** | | X | | | | |
| **Fasting C-peptide** | | X | | | | |
| **Measurement of body weight, height, waist, and waist/hip circumference** | | X | X | | X | X |
| [redacted] **BMI assessment** | | X | X | | X | X |
| **Average (previous 3 days) Insulin requirement** | | X | X | | X | X |
| **HbA1c** | | X | X | | X | X |
| **CGM download for assessment of glycemic variability (previous 7 days)[2]** | | | X | | X | X |
| **Vital signs** | | X | X | | X | X |
| **Serum Pregnancy Test (blood/urine)** | | X | | | X[3] | |
| **Safety laboratory tests (hematology, biochemistry)** | | X | | X | X | X |
| CCI [redacted] | | | [redacted] | | [redacted] | X |
| **ECG (QTcF)** | | X | | X | X | X |
| **eGFR** | | X | | | X | X |
| **Randomization** | | | X | | | |
| **IMP dispensation.** | | | X | | X | |
| **Patient Diary delivery/check** | | | X | | X | X |
| **Treatment** | | | X---------- | ---------- | ----------X | |
| **Patient Phone call[5]** | | | X---------- | ---------- | ----------X | |
| **AE/SAE recording** | | | X---------- | ---------- | ----------X | |
| **Prior and Concomitant Medication** | | | X---------- | ---------- | ----------X | |

* Prescreening procedures can be performed during screening visit if applicable as per site practice

Legend:

1. To include new testing should past data not be available;

2. Secondary ;
3. Urine dipstick;
4. CCI
5. Telephone calls at least every 30-45 days.

Trial coordinators should contact participants prior to a clinic visit to review procedures as applicable.

## 2.1. 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 [5-7]. 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 CCI Following promising results in mouse models of type 1 diabetes (T1D), development in new onset T1D is ongoing[7,8].

***Relevant pre-clinical, toxicological and clinical data are summarized further below (Section 2.2-2.4). Please also refer to the Investigator's Brochure for detailed information.***

## 2.2 RELEVANT NON-CLINICAL PHARMACOLOGY

### 2.2.1. 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 receptor 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[9]. 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.

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

### 2.2.2. *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[10]. In addition, in an acute model of smoke exposure, ladarixin (3.75-15 mg/kg p.o.) reduces PMN infiltration by about 60%, whereas in the chronic exposure model the compound (7.5-15 mg/kg p.o.) completely prevents the development of pulmonary lesions.

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

of keratinocyte chemokine (37%), VEGF-A (24%) and TNF-α (44%) [See the Investigator's Brochure Reference: *A0811/E*].

In a passive transfer mouse model of BP, 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 [See the Investigator's Brochure Reference *A0811/E*].

### 2.2.3. **Effects in models of type 1 diabetes**

Ladarixin was tested after oral administration (15mg/kg/day) in the Multiple Low Dose-Streptozotocin mouse model of diabetes using different treatment schedules. Results showed that ladarixin significantly affected the time to diabetes development with the 14-day administration starting from day -1 of fist STZ injection being the more efficient treatment schedule in prolonging the median diabetes free time. When a 14 day treatment was started from day +5, ladarixin still maintained a good performance. Even after diabetes development, glycemic levels during the first 2 months remained constantly lower in the ladarixin treated group as compared with the vehicle group[7,8].

Ladarixin was also tested after oral administration (15 mg/kg/day for 14 days) in the non-obese diabetic (NOD) mouse model of autoimmune type 1 diabetes, starting treatment at 4, 8 and 12 weeks of age ("prevention" setting) 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 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 blocked the progression of hyperglycemia while hyperglycemia worsened in animals receiving vehicle. Three animals treated with ladarixin at diabetes onset that subsequently developed diabetes (about 30 days from end of treatment) were re-treated with the test compound starting from day 35. In 2 animals with "mild" diabetes (animals with glycaemia between 300 mg/dL and 450 mg/dL) out of the 3 animals re-treated, ladarixin apparently reverted increased glycaemia[7,8]. 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[7,8].

### 2.2.4. Effects of Ladarixin on an in vitro adipocyte diabetic model

The molecular mechanisms underlying the anti-diabetic effects of ladarixin were evaluated in differentiated adipocytes upon inflammatory and diabetic conditions. In high glucose (HG) conditions, the levels of expression of CXCR1 and CXCR2 are significantly increased, while ladarixin was able to counteract this effect, in particular at the highest concentrations tested, with the 10 μM concentration still significant. Moreover, to monitor glucose uptake, mature adipocytes 3T3-L1 were incubated with the fluorescent tracer 2-NBDG and in HG condition and upon inflammatory stimuli the glucose uptake is significantly decreased, contrarily the treatment with ladarixin (both 10 μM and 50 μM concentrations tested) restored to control conditions (NG). Accordingly, in high glucose (HG) conditions, the levels of GLUT4 are significantly decreased, while ladarixin at the highest concentrations tested was able to revert the GLUT4 levels to those of control, however, also the 10μM concentration was significantly efficient in counteracting HG effects. Both HG and inflammatory conditions induced elevated lipolysis (high glycerol content) while ladarixin treatment (at both concentrations tested 10 and 50 μM) was able to reduce this effect.

Furthermore, in these experimental conditions ladarixin demonstrated a significant effect on NFKB activation and cytokines release. Upon HG and inflammatory conditions, the levels of the cytokine mouse CXCL1/KC were strongly increased and ladarixin was able to significantly decrease CXCL1/KC levels and to reduce NFKB concentrations. On the other hand, the HG and inflammation-induced conditions determined lower levels of adiponectin compared to ladarixin-treated conditions, suggesting a restoration of the metabolic

activity mediated by ladarixin. In conclusion, this work reported the ability of CXCR2 inhibition through ladarixin treatment to exert a dual beneficial action in the cellular models tested (differentiated 3T3-L1 and primary human adipocytes derived from a non-obese patient), both preventing the harmful pro-inflammatory signal activated by adipose tissue in response to inflammation and high glucose (as observed in cytokine proteome array, NFKB and cytokines expressions) and restoring the adipocyte functional insulin-sensitivity (as observed in glucose transport analysis), potentially preventing insulin resistance mechanisms. On the light of the results obtained in this study, it is possible to postulate that ladarixin could be a potential treatment in insulin resistance conditions. [ CXCR1/2 Inhibitor Ladarixin Ameliorates the Insulin Resistance of 3T3-L1 Adipocytes by Inhibiting Inflammation and Improving Insulin Signaling Castelli V. et al. Cells 2021, 10, 2324. https://doi.org/10.3390/cells10092324 ].

## 2.3. A SUMMARY OF TOXICOLOGY DATA

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

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

Treatment of rats with ladarixin orally for a 3 months' period at dose levels of 35, 70 and 150 mg/kg did not induce mortality or toxicologically relevant changes in body weight and food consumption, ophthalmoscopic, hematology, and urinalysis parameters. Changes in biochemistry were only minimal and occurred mostly in males. They included increased activity of some liver enzymes (males at 150 mg/kg), dose-dependently decreased protein levels (males at all dose levels and females at 150 mg/kg in Week 8 only), and dose-dependently decreased cholesterol and phospholipid concentrations (males from 70 mg/kg). Microscopic examination revealed treatment-related findings in the liver, thyroid gland and kidneys. In the liver, hepatocellular hypertrophy was noted in Week 8 in males at all treated groups and females at 150 mg/kg. Follicular cells hypertrophy of the thyroid gland was seen at 150 mg/kg and regarded secondary to the liver hypertrophy. In the kidneys hyaline droplet accumulation was present at increased incidence in males at 150 mg/kg after 8 weeks of treatment only (interim sacrifice). This change is male rat sex-specific not relevant for humans. After the 4-week recovery period, full recovery or a clear trend to recovery was noted for all findings.

In studies with up to 1 week administration in dogs by oral route, the dose of ladarixin had to be reduced from 500 to 150 mg/kg, due to poor tolerability and severe clinical signs, namely tremor, vomiting, uncoordinated movements. No macro or microscopic findings were present; organ weights were not affected. In the 4-week oral toxicity study in dogs, ladarixin was administered up to the dose of 120 mg/kg. No mortality occurred. Vomiting was seen at 120 mg/kg. An increase in the absolute and corrected-for-heart rate QT interval was observed at the doses of 60 and 120 mg/kg. Inflammatory changes were seen in the duodenum of two males treated with 120 mg/kg. After 2 weeks all values returned to normal. It was concluded that the no observed-effect-level (NOEL) is 30 mg/kg/day.

In the 3-month study, the initial dose levels were 30, 60 and 120 mg/kg/day. However, due to test item-related moribundity of one high dose male and changes in body weight and food intake of a few other animals in the group, the highest dose level was lowered from 120 to 80 mg/kg/day from Day 24 onward. At 120/80 mg/kg/day, one male and one female showed weight loss (up to -7%) and lower food intake during the first weeks of treatment and the clinical condition was reduced in Week 4. Food supplements were provided, which resulted in an improved clinical condition and normal body weight (gain) of these animals during the remainder of the treatment period after reduction of the dose level from 120 to 80 mg/kg/day. Prolongation of the QT and/or corrected QT intervals was observed in males and females treated at 120/80 mg/kg/day in Weeks 4, 8 and/or 13, generally more pronounced in females. In addition, higher heart rates were noted for males and (individual) females treated at 120/80 mg/kg/day in Weeks 4, 8 and/or 13. At the end of the Recovery period, only one male and one female at 120/80 mg/kg/day were still on study, and all electrocardiography parameters were returned to the normal limits. For one female at 60 mg/kg/day, mixed cell inflammation of the brain parenchyma and meninges, with neuronal necrosis and gliosis was recorded at moderate degree. There were no correlating clinical signs or necropsy alterations noted for this animal.

Furthermore, no inflammation was recorded in the brain for any of the other dogs of the study; therefore, this change in a single dog at 60 mg/kg/day (mid dose) was regarded to be an incidental change, although rare and the origin of the lesions could not be determined. No test item-related changes were noted in any of the remaining parameters investigated in this study (i.e. ophthalmoscopy, coagulation parameters, urinalysis, macroscopic examination and organ weights). Based on the results of this study, the no-observed-adverse effect level (NOAEL) was considered to be 60 mg/kg/day after 13 weeks of dosing.

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

Phototoxicity potential of DF2156A was evaluated on Balb/c 3T3 cells. At dose levels of 100, 50.0, 25.0, 12.5, 6.25, 3.13, 1.56 and 0.781 µg/mL, in the absence or presence of UVA light, neither reductions of the cell layer nor changes in cell morphology were observed after treatment with DF2156A at any concentration tested. No reduction in neutral red uptake was seen in any treatment condition. The IC50 values were not calculable, thus the Photo Irritation Factor (PIF) could not be determined. The Mean Photo Effect value (MPE) was 0.009. The score values obtained with DF2156A (i.e. PIF not calculable and MPE lower than 0.1) are predictive of no phototoxicity

A study of fertility and early embryonic development to implantation was performed in male and female Han Wistar rats at the daily oral dose of 50, 100 and 200 mg/kg. There were no treatment-related effects in females in any dose levels and in males at 50 and 100 mg/kg/day. At the highest dose, effects were observed on spermatid counts, motility and morphology of spermatozoas. Changes were seen in the testes of some animals and consisted degeneration/atrophy of the seminipherous tubules. According to the results obtained in this study, the fertility effects are likely associated with a stress effect, even if a secondary effect of the test item in the affected animals cannot be excluded with certainty. The NOEL was 100 mg/kg/day for males and of 200 mg/kg/day for females.

In order to clarify the changes seen in the testes at 200 mg/kg/day, a specific study was designed in male Wistar and Sprague Dawley rats. Ladarixin was orally administered at the dose level of 200 mg/kg bw/day for a period of 6 weeks followed by a 4 weeks of treatment-free recovery period. In both strains, the treatment with ladarixin caused a minimal increase in incidence of reduced size of testes and epididymides and a mean decrease of testes and epididymides weights. A mean decrease of sperm motility, of normal complete sperms and of the mean sperm count in the epididymides was observed. After the recovery period, the decrease of the motility was not seen in Wistar rats whereas it was observed in Sprague Dawley rats. The weight reduction of testes and epididymides and the sperm analyses correlated with microscopic changes in the testes (degeneration/atrophy) and epididymides (aspermia/oligospermia) of some treated animals of both strains. After 4 weeks of recovery period, none of the Wistar rats allocated to the recovery group presented any change in the testes or epididymides, while some of the Sprague-Dawley rats allocated to the recovery group had degeneration/atrophy of the testes and atrophy, oligospermia or cell debris in the epididymides. The incidence of the testicular changes was very likely associated to the stress induced by the treatment with the test item. In both strains, a decrease in body weight and body weight gain (associated to a decrease in food consumption) was observed particularly in the first 2 weeks of the treatment period. Ruffled fur and weakness were also present in some treated rats and a reduction of thymus weight was observed in treated Wistar rats. All these changes indicate a condition of stress and/or distress that caused the testicular changes. At the end of the recovery period, in the Wistar rat there were no changes in any of the parameters examined as well as no changes in the testes and epididymides; in Sprague Dawleys rats, testes and epididymides changes were present in some animals at the end of the recovery period.

The prenatal developmental toxicity studies were conducted in Wistar Han rats and New Zealand White rabbits. In rats no maternal toxicity and no developmental toxicity were observed up to the highest dose level tested (150 mg/kg/day). The NOAEL in rats was established as being at least 150 mg/kg/day for both the maternal and developmental toxicity.

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

Carcinogenicity studies have not been performed.

According to the toxicology development program, a 6 and 9-month study in rats and dogs, respectively, have been recently completed to further support the chronic administration in humans. After oral treatment with DF 2156A for 6 months in rats at dose levels of 35, 70 and 150 mg/kg/day, no mortality or changes were observed in food consumption, ophthalmoscopy, hematology and coagulation parameters in both sexes, and

in clinical biochemistry and urinalysis in females. Only slight changes were observed in body weight in male and female and in clinical biochemistry parameters in males at ≥ 70 mg/kg/day and in urine parameters at 150 mg/kg/day. Morphologic non-adverse alterations in liver, kidneys and thyroid gland were recorded for males at ≥ 35 mg/kg/day, and in females at the high dose of 150 mg/kg/day only. The higher exposure levels to DF 2156Y (acid form of DF 2156A) in males may explain that males were more affected than females. Following the 4-week treatment-free period, all changes observed in the animals recovered to normal values with the exception of brown pigment (likely lipofuscin) in the cytoplasm of tubular epithelium of kidneys of males and females, and hepatocellular pigments (likely lipofuscin) in liver of males at the dose level of 150 mg/kg/day.

In the 9-month study, dogs were treated orally with DF2156A at doses of 30, 60 and 100 mg/kg. No mortality occurred during the study period. The test item was clinically well tolerated up to the high dose of 100 mg/kg/day, as all animals were in a good general condition until the end of study. ECG recordings in Weeks 13, 26 and 38 revealed a dose-related increase in (corrected) QT intervals in both males and females at 60 and 100 mg/kg/day at 2 to 4 hours after dosing, most prominently in Week 38. In addition, heart rates were increased in 100 mg/kg/day treated males and females at 2 and/or 4 hours post-dose in Weeks 13, 26 and/or 38 (females were more affected than males). The QT prolongation and increased heart rates in females at 100 mg/kg/day were considered to be adverse, based on the maximum increase in QTcF interval of 25.5 msec in Week 38 on a group mean basis, and based on the magnitude of change for heart rate. The No Observed Adverse Effect Level (NOAEL) for females was considered to be 60 mg/kg/day after 9 months of dosing. In males, the NOAEL was concluded to be at least 100 mg/kg/day.

In conclusion, based on the toxicology testing performed through in vivo studies, the dose of 150 mg/kg appears to represent the NOAEL in rats for general toxicity while in dog, in the 9-month oral toxicity study, the NOAEL was established at 60 mg/kg (taking into consideration the lowest in this study). In rats, 150 mg/kg is the NOAEL for developmental toxicity while in rabbits it was 100 mg/kg.

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

In humans, 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 t½ (11-19 h) remained constant across the range of doses evaluated. Renal clearance accounted for approximately 60-80% of the total clearance of ladarixin. The dose proportionality for the main PK parameters was also seen for the two metabolites (DF 2108Y and DF 2227Y).

After multiple doses, 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 ladarixin, DF 2108Y and DF 2227Y appeared to increase in a dose-proportional manner. The clearance and the volume of distribution of ladarixin appeared to be dose-independent on both Days 1 and 8 for all doses. Similarly, t½ (ranging from 11 to 18 h) remained constant on Day 1 and on Day 8 across the range of doses evaluated.

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

*In vitro* ladarixin appears to be a strong inhibitor of the enzyme CYP2C9 in humans (83%) and a moderate inhibitor in rats (51%). No inhibition was observed in dogs. No inhibition was detected in any species with regard to CYP2D6. Also, no inhibition was shown for CYP 1A2, 2C19, and 2E1 with respect to humans and dogs, but a moderate inhibition was shown in rats (about 50%). As to CYP3A4, a slight inhibition was seen in all species which was higher than 20% only in rats (about 28%). In humans, co-administration of ladarixin (200 mg twice a day for 5 days) approximately doubled the exposure to tolbutamide (probe for CYP2C9 isoenzyme).

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

## 2.5. A SUMMARY OF CLINICAL DATA

Clinical development includes 3 Phase I PK and tolerability studies with single CCI and multiple CCI ascending dose oral administration. The first multiple ascending dose study CCI also included the evaluation of potential interaction between ladarixin 200 mg and tolbutamide. A phase II efficacy and safety study was conducted with 150 mg twice a day (for 14 consecutive days) oral ladarixin in patients with moderately active Bullous Pemphigoid. A phase II efficacy and safety study CCI has been completed in patients with new onset T1D.

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

### 2.5.1. Pharmacokinetics and product metabolism in humans

Three Phase I PK/safety/tolerability studies in healthy male volunteers were performed which included single CCI and multiple CCI ascending dose oral administration. An interaction evaluation for the 200 mg dose was also included in the first multiple ascending dose study. Assay of ladarixin and its metabolite DF 2108Y and DF 2227Y was performed in samples collected at steady state conditions, on day 5 and 8 of treatment in 3 BP patients. PK results are discussed in detail in the most recent **Investigator's Brochure (**IB**)**.

### 2.5.2. Efficacy

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

A phase II, multicenter, randomized, double-blind, placebo-controlled study has recently been completed in patients with new onset T1D (first insulin within 100 days from randomization) CCI . The trial involved 8 sites in Italy, Germany and Belgium. Seventy-six patients (31 females, 45 males) with mean age of about 27 years have been randomized (2:1) to receive either ladarixin (400 mg twice a day for 3 cycles of 14 days on/14 days off) or placebo. The primary endpoint was

the AUC of C-peptide measured during an MMTT performed on week 13 from randomization. Follow-up was extended up to one year. Out of 85 patients enrolled (consent signed), 76 were randomized, 50 to received ladarixin and 26 allocated to placebo; 48 patients in the ladarixin group and 25 in the placebo completed the trial (week 52 observation). The primary and secondary analyses were performed on the ITT population. The primary efficacy analysis of log (AUC [0-2 hrs]+1) of C-peptide response to MMTT showed no statistically significant difference (p=0.3303) between the ladarixin and placebo group at Week 13. Overall, there were no clinically relevant treatment effect differences between the treatment groups for other secondary endpoints, such as C-peptide at other time-points, insulin requirement, glucose levels and glucagon levels during the study. On the other hand, the proportion of patients with HbA1c <7% and absence of episodes of severe hypoglycemia was significantly higher at Week 26 (p=0.0248) in patients receiving ladarixin as compared with placebo (whole ITT population).

A phase 3 multicenter, double-blind, placebo-controlled study with ladarixin is currently recruiting (ClinicalTrials.gov Id: NCT04628481) and aims to assess the efficacy of ladarixin in patients with new-onset T1D.

### 2.5.3. Safety

A total of 89 male healthy subjects aged 18 to 52 years, 4 male BP patients aged 65-75 yeas, and 50 T1D patients aged 18-46 years were exposed to ladarixin in the clinical trials conducted to date. Exposure included single (25 to 400 mg) as well as repeated (50 to 400 mg twice a day up to 6 days) oral administrations in healthy volunteers, 150 mg twice a day up to a maximum of 14 days in BP patients, and 400 mg twice a day for 3 cycles of 14 days on-14 days off in T1D patients. In a subset of subjects, ladarixin (200 mg twice a day) was co-administered with tolbutamide.

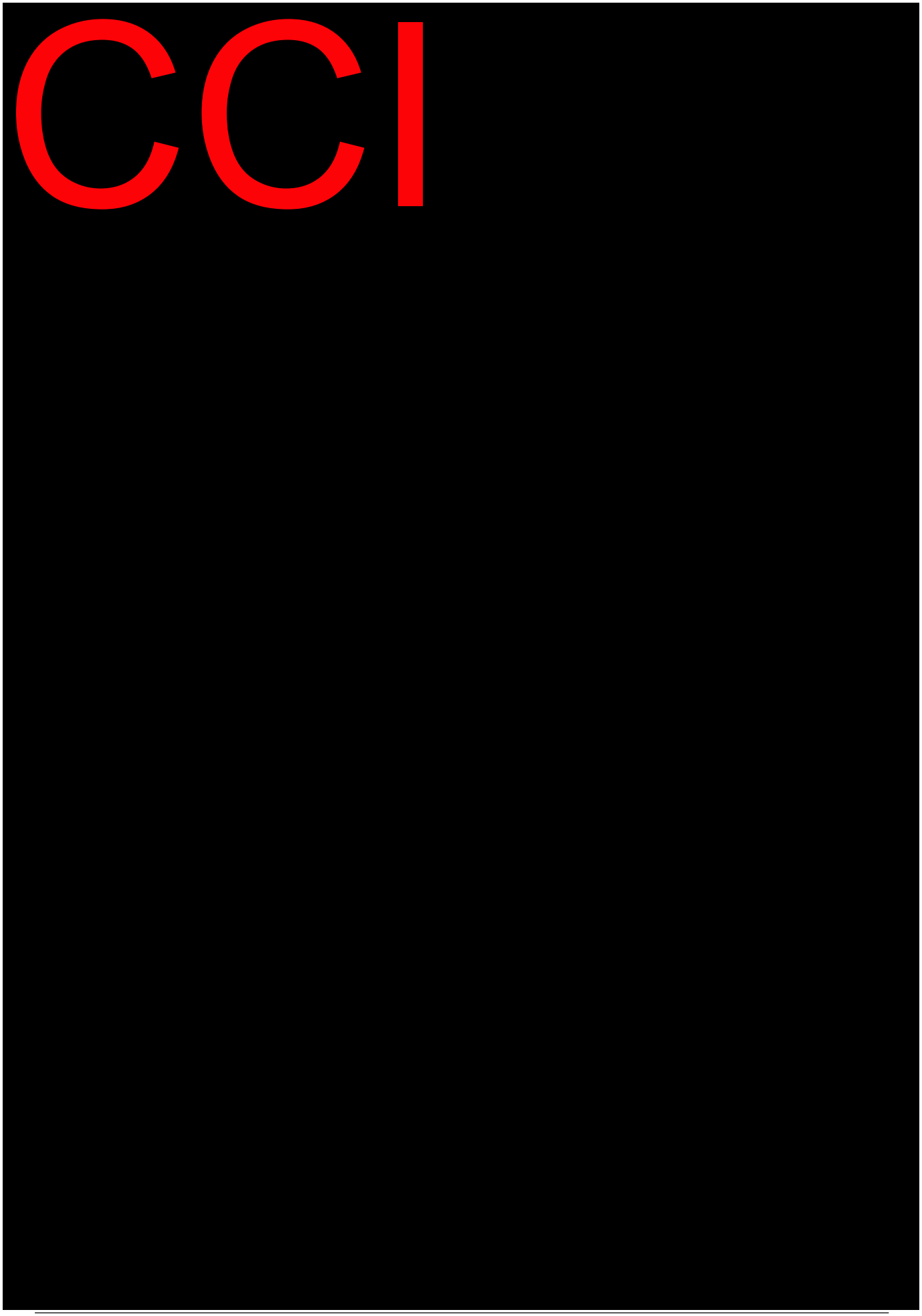
Overall, DF2156 was safe and well tolerated. No deaths or Serious Adverse Events (SAEs) were reported from Phase I trials, as well as no safety concerns were raised during co-administration of ladarixin with tolbutamide. All the Adverse Events (AEs) were mild or moderate in intensity.

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 I studies; core laboratory analysis of the ECG readings, including the review of changes in the QTcF intervals and of the pharmacokinetic-pharmacodynamic relationship, revealed no clinically significant effect of Ladarixin on cardiac repolarization.

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

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

Cumulative **adverse drug reactions** (ADRs), i.e. treatment-emergent AE judged at least possibly related to ladarixin, are presented in **Table 1**, below.


CCI

CCI

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

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

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

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

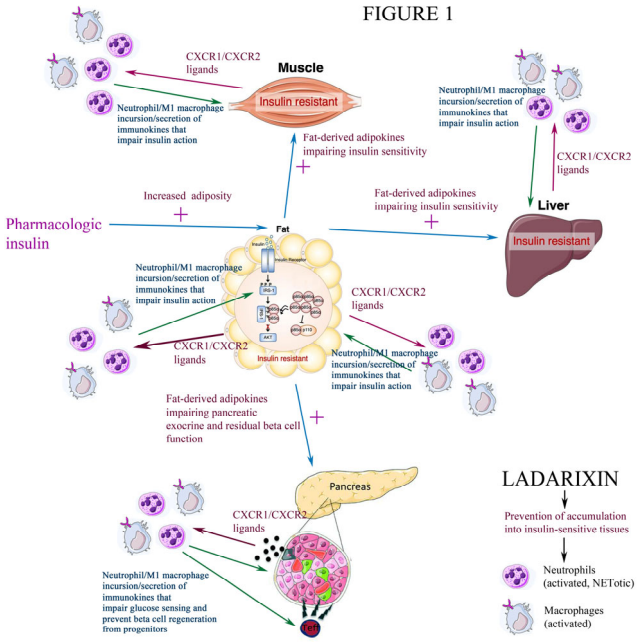
## 2.6. DISEASE REVIEW AND STUDY RATIONALE

T1D is an organ-specific autoimmune disease in which the immune system attacks the insulin-producing β-cells[11]. 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[12,13].

T1D is treated with life-long daily exogenous insulin injections and monitoring of blood glucose levels. However, even optimization of glucose control through the most recent technologies cannot adequately substitute for the finely tuned normal balance of the glucose levels. Pancreatic islet or whole pancreas transplantation still has limited success due to graft loss and immunosuppression derived side-effects. Therefore, despite marked improvements in diabetes care in recent years, insulin-dependent diabetes results in secondary long-term complications and is one of the leading causes of end-stage renal disease, blindness and amputation. Additionally, hypoglycemia unawareness is a serious consequence of recurrent hypoglycemia often requiring emergency care[11].

Many T1D patients develop insulin resistance (IR), exacerbated in part by pharmacologic insulin[14-17] and overweight/obesity. IR is a significant risk for cardiovascular and renal disease[14,16-23]. While the mechanisms leading to T1D IR are not completely-understood, a chronic low-grade systemic inflammation similar to the one in metabolic syndrome and type 2 diabetes (T2D)[24-31] has been implicated and may be responsible for IR independently of the underlying autoimmunity[24-31], as illustrated in **Figure 1**.

FIGURE 1



In spite of breakthroughs in the design and action of modern insulin and insulin pharmacotherapy[32-44], most people with T1D cannot achieve recommended HbA1c targets[45-56]. This is associated with significant risk for, and high incidence rates of long-term T1D complications (cardiovascular, neural, renal, and ophthalmic) resulting in earlier mortality, no matter how well insulin maintains their glucose control. ***This highlights the need for insulin adjunctive approaches to address the underlying metabolic imbalances beyond dysglycemia***. ***One of the under-appreciated, but emerging complication in many T1D patients is IR***[14,16-23]. It is associated with negative clinical outcomes[14,15,17-20,22,23,51-53,55-69] and there are few if any interventions targeting IR[15,57-59,61,65,66,68,69], despite an urgent clinical need[34,40,43,54,70-78]. The most common cause of death in adults with T1D is cardiovascular disease which correlates better with IR than with impaired glucose control disease[14,16-23]. Although IR is mainly observed in overweight and obese T1D patients[14,15,17,20,58,79,80], recent evidence indicates that even lean patients can exhibit IR[14,15,17,20,59-61,63,65,69,81,82].

The following observations in T1D and T2D, underlying gaps in knowledge, and limitations of previous trials inform our hypothesis and the ***scientific premise***: **i)** the IR-related exacerbation of a non-autoimmune systemic inflammation in T1D that further fuels IR in a vicious cycle[60,64,83,84]; **ii)** the role of NΦ as potential "first responders" to the accumulation of advanced glycation end products (AGEs) consequent to impaired GV [18,21,85-111] as well as in response to hypertrophying adipose under weight gain conditions[99,107,112,113]; **iii)** the role of NΦ in the impairment of insulin signaling in insulin-sensitive tissues further aggravating IR[99,114]; and **iv)** the marginal clinical outcomes of metformin as an adjunctive in IR[59,61,65,66,79-81,115,116]. In spite of its expected efficacy via improved insulin receptor signaling, metformin's outcomes were predictable considering immunokine effects at the level of the insulin receptor and the downstream transducers. On the premise that NΦ-driven inflammation exacerbates IR, we propose that ladarixin can short-circuit NΦ margination into insulin-sensitive tissues that exhibit impaired GV-mediated accumulation of "danger/damage" signals (e.g. AGEs, microcirculation fluid shear stress damage). As a consequence, ladarixin suppression of local inflammation would mitigate immunokine-driven impairment of insulin signaling. An improvement in overall insulin sensitivity and better GV/glycemic control with the potential for decreased insulin requirements is therefore anticipated.

*Inflammation, "danger", NΦ, and IR*: The chronic swings between hyper-/hypoglycemia in T1D facilitate a pro-inflammatory state associated with accumulation of microanatomical aberrations and lesions constituting "danger" signals for the immune system[117-124]. Impaired GV (IGV) outcomes can therefore trigger the activation of leukocytes of the innate arm of the immune system. One example is the oxidative stress in and around insulin-sensitive tissues, and the formation of AGEs. AGEs can potentially contribute to IR via numerous inflammatory pathways[87,90,125-135]. Reactive oxygen species (ROS) and oxidative stress are increased under hyperglycemia. Intermittent hyperglycemia exaggerates the production of ROS[136-139]. Oscillating glycemia in T2D impairs endothelial function via oxidative stress[140,141] and AGE accumulation inside skeletal muscle and adipose activates pro-inflammatory signal pathways that abrogate insulin signaling and sensitivity[142-145]. AGEs activate the innate arm of the immune system, largely by binding RAGE, expressed mainly on NΦ[105,110,111,146].

While the relevance of NΦ in the onset and progression of T2D, especially under conditions of obesity is recognized, considerably less is known about their role in the onset and progression of IR under IGV. The NΦ to lymphocyte ratio (NLR) has been strongly associated as a risk marker of metabolic disorders where the immune system has been implicated[147-150], especially in the studies by Shiny et al and Lorenzo et al in T2D[151,152]. In the former study, NLR increased with increasing severity of glucose intolerance and was found to be positively associated with IR. In the latter study, NLR was shown to be positively associated with the presence and severity of metabolic syndrome[95]. NΦ account for ~90% of granulocytes and granulocytes comprise 60-79% of human blood leukocytes thus making NΦ the largest fraction of leukocytes. Accumulating evidence implicates NΦ in T2D and T1D (reviewed in[94,96,101,109,153]). Elevated NΦ markers are present in the plasma of obese subjects[154].

Abnormal expansion of visceral adipose tissue is accompanied by influx of activated NΦ early in the process[107]. Moreover, in vitro studies have shown that NΦ physically bind adipocytes in a manner dependent on their activation state[107]. NΦ produce a number of powerful proinflammatory molecules including a variety of proteinases like NΦ elastase (NE). Genetic deletion of NE as well as its pharmacologic inhibition improved obesity-induced IR and suppressed inflammation in adipose tissue and liver in a T2D mouse model[99,107]. Intriguingly, NE can degrade IRS-1 protein and reduce insulin-induced Akt phosphorylation in adipocytes[99]. This mechanism may be involved in the NΦ- and NE-dependent effect on IR in diet-induced obesity, as the levels of IRS-1 were higher in fat and liver of NE-deficient mice, as compared to NE-sufficient mice[99].

Similar results were obtained in humans[97,155] where an imbalance between NE and α1 antitrypsin (AAT) in obesity determines, to some degree, insulin sensitivity, systemic inflammation and energy expenditure[97]. In these studies, human obese subjects exhibited a decreased serum AAT level and increased NE concentration that correlated with BMI and leptin resistance [97]. In this same study, NE accumulated in adipose throughout the development of obesity concurrent with increasing adipose inflammation[97]. These data suggest that inhibition of NE and/or the NΦ activation that precedes its production may break a vicious cycle of obesity and IR-associated inflammation. NΦ have also been implicated in the early stages of T1D in terms of their pancreatic accumulation[156,157] where they activate type I cytokine production by plasmacytoid DC and trigger the subsequent activation of the adaptive immune response[158].

*Treatment of T1D IR with adjunctive agents*: It is increasingly challenging to treat obese, T1D individuals with insulin alone. As well known, intensive insulin therapy, based on multiple daily injections, is the gold standard treatment in T1D and rationale of intensive insulin therapy is to provide tight glucose control avoiding the higher risk of hypoglycemic events and weight gain. Furthermore, with the worldwide increase of obesity in childhood and adolescence insulin resistance is now occurring more frequently also in young patients with T1D. Consequently, the rising obesity in youth seems to induce a change of T1D phenotype, defining a hybrid form of diabetes, known as "double diabetes". On this basis, to improve glycemic control and peripheral insulin sensitivity, some insulin sensitizing drugs generally used in Type 2 Diabetes (T2D), such as metformin, are more frequently used as adjuvant treatment also in patients with DD. Despite the effect on glycemic control reducing body weight and daily IR in overweight or obese T1D patients, several limitations are related to metformin.

Metformin is currently the most studied T1D adjunctive[159] however outcomes were inconsistent[59,61,65,115], as metformin faces compliance hurdles due to common AEs[160]. In fact, studies with metformin clearly showed that the drug may have many gastrointestinal side effects such as to reduce patients' treatment adherence and consequently its own effectiveness. Other T2D agents have been tested as insulin adjunctive in T1D[159,161-165]. However, marginal efficacy outcomes[15,161-163,166] and AEs[162,163,166] including ketoacidosis challenge their wider use[15,167-170].

The data supporting a non-autoimmune inflammation, very likely driven by NΦ, in the etiology of T1D IR are very compelling, justifying the adjunctive use of anti-NΦ agents to improve insulin sensitivity. Anti-inflammatory drugs that can target NΦ include corticosteroids, macrolides, cAMP-elevating agents (including cyclooxygenases), 5-lipoxygenase inhibitors, leukotriene receptor antagonists, chemokine receptor antagonists, kinase inhibitors, activators of histone deacetylase-2, and antioxidants[171-189]. Corticosteroids are not specific to NΦ and they induce glycogenolysis, raising blood glucose levels[190,191]. High dose antioxidant trials in T2D have been disappointing[192]. Other agents caused adverse events grades >2.

Although T1D and T2D pathogenesis are significantly different, with the wide increase of childhood obesity, a great number of evidence supports the hypothesis that IR plays a crucial role also in T1D pathogenesis. Consequently, the therapeutic approach in these patients has considerably changed leading to a widespread use of insulin sensitizing drugs as adjuvant treatment of insulin therapy.

In fact, in long standing T1D overweight or obese patients, the excess of white adipose tissue has been associated with low-grade chronic inflammation. Adipocytes are able to secrete adipokines such as TNF-α, IL-6, and IL-8, which due to their pro inflammatory properties, have been associated with enhanced activity of peripheral neutrophils, such as production of superoxide radicals and NET formation.

For this reason, ladarixin as potent allosteric inhibitor of IL-8, could represent a first-in-kind adjunctive therapy in long standing T1D overweight or obese patients.

Moreover, two preclinical studies were conducted with oral ladarixin (15 mg/kg/day for 28 days) in diet-induced obese (DIO) or genetically prone (db-/-) mouse models. Results obtained in the first study showed that oral ladarixin significantly improved glucose tolerance and insulin sensitivity in the DIO mice, but not in the genetically obese mice. Improved insulin sensitivity was not paralleled by change in adipose tissue inflammation. In the second study, glucose homeostasis in DIO mice was slightly affected by ladarixin, as documented by a lower glucose excursion during the intraperitoneal glucose tolerance test, a lower fasting insulin (and associated improvement in HOMA-IR) and a relative reduction in hepatic glucose output through the gluconeogenesis pathway. The effect was greater in younger and less obese mice as compared to older and more obese animals. Evidence of reduced hepatic tissue inflammation was assessed by a lower MPO

activity, a decrease in IL-6 secretion and a decreased expression of multiple inflammatory/cytokine markers CCI

On this basis, this drug can address three pressure points in T1D IR: it can suppress **i)** IGV-associated activation and progression of a NΦ -driven systemic inflammation; **ii)** NΦ margination into insulin-sensitive tissues affected by IGV-driven microanatomic anomalies; and **iii)** the ability of activated NΦ to orchestrate the progression of inflammation.

Therefore, we hypothesized that the administration of ladarixin with its anti-inflammatory activity may have a potential therapeutic role in improving glycemic control in long standing T1D IR patients undergoing intensive insulin therapy Acting through an amelioration of insulin sensitivity, it can improve insulin resistance, endothelial dysfunction, decreasing the homocysteine and inflammatory mediators' plasma levels, The purpose of this study is to evaluate the effect of ladarixin oral supplementation on glycemic control as assessed by HbA1c in overweight T1D patients undergoing intensive insulin therapy. Ladarixin is not the only NΦ antagonist to impair NΦ margination into areas of "danger". Other than ladarixin and AZD5069[7,193-205] (which have been discontinued), other anti-NΦ agents[7,193-205] do not have as extensive a developmental/clinical history as ladarixin. It is important to note that ladarixin and its infusion counterpart, reparixin, have demonstrated preclinical efficacy at the level of T1D pancreatic inflammation[7], in the prolongation of islet allograft survival[204,205].

### 2.6.1. Alternative treatments

There are no standard pharmacologic treatments, addressed to the prevention or treatment of IR in T1D other than adjustment of pharmacologic insulin dose and frequency. In fact, to achieve and maintain optimal glycemic control trough the standard insulin therapy, insulin doses are often increased causing a higher risk of hypoglycemic events, weight gain and poor glycemic control as well as exacerbating IR.

Because of this, patients not willing to participate in the study will not be offered any specific alternative treatment other than insulin management in consultation with their existing endocrinologist/diabetes specialist.

All patients, regardless of study participation, will receive the standard of optimal care for established, insulin-requiring, overweight T1D individuals in consultation with their existing endocrinologist/diabetes specialist.

### 2.6.2. Risk – benefit evaluation

2.6.2.1. Risk related to ladarixin

Results from preclinical studies support the level of drug exposure planned in this study.

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

Any possible risk derived from the administration of ladarixin in the specific population involved in this study will be minimized by integrated monitoring which includes clinical observations, laboratory tests and ECG readings, scheduled at pre-planned intervals (see Section 5.3.2 and 6).

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 4. and 5.5.2) will limit any possible risks derived from potential metabolic interactions.

2.6.2.2. Peripheral blood collection

Collection of peripheral blood for safety monitoring, HbA1c and C-peptide (up to 15 mL at visits 1, 2b, 3 and 4) as well as HbA1c at visit 2 (up to 2 mL) might be at risk of minor pain, bruising, inflammation or excessive bleeding at the venipuncture site and faintness/lightheadedness. Also, multiple punctures might be required to identify the veins. Rare risks include hematoma, infection. In some patients, additional blood

samplings (up to 40 mL) will be required at visit 2, 3 and 4 for assessment of exploratory CCI parameters, if site is able and deems appropriate to accommodate and conduct these procedures.

2.6.2.3. Continuous Glucose Monitoring (CGM)

Continuous Glucose Monitoring (CGM) via sensor positioning is commonly used in patients with diabetes to monitor their glucose level. Patients with routine use of a self-owned (if applicable) CGM system will be enrolled. In the event that the CGM system is not owned by a patient, it will be provided by the Sponsor. Therefore, participation this trial will not add risk for the patient other than those already derived from patient's standard of care. Specifically, patients can't wear CGM (sensor, transmitter, receiver, or smart device) for magnetic resonance imaging (MRI), computed tomography (CT) scan, or high-frequency electrical heat (diathermy) treatment. Some skin care products, such as sunscreens and insect repellents, can make the plastic used in G6 crack.

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

2.6.2.5. Potential benefit

To the patient: Patients during ladarixin treatment period may possibly benefit with improved glycemic control and insulin sensitivity, 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 overall metabolic control in T1D long-standing IR patients.

### 2.6.3. Description of the Investigational Products

In this study the Investigational Medicinal Product (IMP) will be either ladarixin OR matched placebo. The proposed dose in this clinical study is 400 mg oral ladarixin twice a day for 7 cycles (26 weeks) of 14 days on - 14 days off. Placebo will be administered with the same schedule.

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

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

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

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

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

# 3. OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN

## 3.1. STUDY OBJECTIVES

We propose a phase II randomized, placebo-controlled, double-blinded, 2-parallel arm, trial using the CXCR1/CXCR2 chemokine receptor antagonist ladarixin versus placebo as adjunctive therapy to insulin to first test whether it can improve glycemic control as assessed by HbA1c, in adult, insulin-requiring, overweight, IR T1D patients. Secondary study objectives include identifying the effect of ladarixin versus placebo on glycemic variability as per CGM derived parameters as well as to determine the safety in the population.

The effect of ladarixin on

## 3.2. STUDY ADMINISTRATIVE STRUCTURES, STAFF AND RESPONSIBILITY

This study will be performed at designated clinical spaces available for clinical studies, under the supervision and responsibility of the Principal Investigator (PI). The PI will be responsible for ensuring that the investigation is conducted according to the signed Investigator agreement, the protocol, GCP guidelines, institutional, federal, state and local regulations.

The PI (and the Principal Physician should the PI not be an MD), each for his own role, will be responsible for the management of the study, which will consist of maintaining the study file and the patient records, corresponding with the Independent Review Board (IRB - specifically for US), reporting SAEs within required timelines, completing the electronic case report form (eCRF) and any other study document.

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

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

## 3.3. OVERALL STUDY DESIGN

The study is a randomized, placebo-controlled, double-blinded, 2-parallel arm, phase II trial.

It will involve up to 86 adults, insulin-requiring, overweight, T1D patients with evidence of IR. After eligibility confirmation (enrollment), they will be randomly assigned to receive (1:1) either ladarixin (400 mg b.i.d. for 7 cycles of 14 days on/14 days off - treatment group) or matched placebo (control group). The two groups will be balanced within centers.

Each patient will be involved in the study for up to 30 weeks. This period consists of 5 site visits for informed consent acquisition and screening, randomization, and 3 post-randomization study visits during a maximum of 28 weeks from the first IMP dose.

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

### 3.3.1. Rationale for Selection of dose, control group and treatment schedule in the study

The dose and dose regimen have been selected according to the rationale reported in Section 2.6.3.

A double-blind, randomized study design is being adopted as the gold standard to minimize systematic bias and increase baseline comparability between treatment groups. The use of placebo control is critical to the study design for providing an accurate estimate of the IMP effect and does not prevent any standard treatment to be used.

## 3.4. STUDY TIME-TABLE.

Overall study timelines are reported below.

**Study period** Projected starting date (first-patient-in): Q2_2022

Projected completion of patient accrual (last-patient-in): Q4_2022

Projected study end date (last-patient-last-visit): Q3_2023

# 4. SELECTION OF STUDY POPULATION

**Number of patients:** 86 adult patients with established insulin-requiring T1D and evidence of IR will be enrolled, provided that they fully meet all of the Inclusion Criteria and none of the Exclusion Criteria described in Sections 4.1. and 4.2. below.

## 4.1. INCLUSION CRITERIA

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

1. clinical diagnosis of autoimmune T1D as documented by positive T1D-related autoantibodies [the presence of at least one or more of: Insulin autoantibodies (IAA), Anti-GAD (GAD65), Anti-IA2 (IA2), Zinc Transporter 8 (ZnT8) must be documented from medical records or new laboratory measurement (not including IAA)];
2. age 21-65 years at the time of consent;
3. T1D duration > 1 year;
4. detectable fasting C-peptide as per the result of screening laboratory measurement;
5. current insulin standard of care (ISOC), either established use of an insulin pump (closed loop system excluded) or a stable dose level and dose frequency for the last two months prior to screening, with no plans to switch the modality of insulin administration during the trial;
6. routine use of a self-owned (if applicable) CGM system that can record glucose concentrations continuously for at least 7 days;
7. HbA1c $\geq$7.5% as per the result of screening laboratory measurement;
8. evidence of IR based on a total daily insulin dose >0.8 U/kg/day;
9. subject is overweight or obese with a BMI of between 24-33 kg/m$^2$, inclusive;
10. ability to comply with all protocol procedures for the duration of the study, including scheduled follow-up visits and examinations, and willing to be contacted by clinical trial staff;
11. provision of written informed consent prior of any study-related procedure not part of standard medical care.

## 4.2. EXCLUSION CRITERIA

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

1. use of a “closed loop system” for integrated glucose reading/insulin infusion;
2. known or suspected hypersensitivity to non-steroidal anti-inflammatory drugs or any excipient of the investigational medicinal products (e.g. lactose and croscarmellose) as well as patients with congenital lactase deficiency, galactosaemia or glucose-galactose intolerance will have to be excluded;
3. use of non-insulin medications for adjunctive blood glucose control (e.g: antidiabetic agents such as metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors, SGLT-2 inhibitors or amylin) within one month of randomization as well as required in the participant’s standard of care;
4. use of medications for weight reduction, such as: Belviq (lorcaserin), Qsymia (Phentermine + topiramate), Orlistat (xenical) within one month of randomization as well as required in the participant’s standard of care;
5. use of a medication such as stimulants, antidepressants and/or psychotropic agents that could affect weight gain or glycemic control of T1D;
6. treatment with drugs metabolized by CYP2C9 with a narrow therapeutic index [i.e., phenytoin, warfarin, and high dose of amitriptyline (>50 mg/day)];
7. use of angiotensin-converting enzyme inhibitors, interferons, quinidine antimalarial drugs, lithium, niacin;
8. evidence of QTcF >470 msec and a history of significant cardiovascular disease/abnormality;
9. any condition, including unstable diet and disordered eating behaviour, that in the judgment of the investigator will adversely affect patient’s safety or the completion of the protocol or otherwise confound study outcome;
10. pregnancy: a) positive or missing pregnancy test (quantitative beta hCG) at screening; b) women of child-bearing potential and fertile men who do not agree to use effective contraceptive measures up

to 2 months following trial discharge [effective contraceptive measures include a hormonal birth control (e.g. oral pills, long term injections, vaginal ring, patch); the intrauterine device (IUD); a double barrier method (e.g. condom or diaphragm plus spermicide foam)]; lactating women;

11. clinical diagnosis of celiac disease that is in poor control as defined by most recent tissue transglutaminase (tTG) that is in the abnormal range;
12. history of ≥1 Diabetic Ketoacidosis (DKA) event in the past 6 months;
13. hypoalbuminemia defined as serum albumin < 3 g/dL;
14. 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];
15. moderate to severe renal impairment calculated by estimated Glomerular Filtration Rate (eGFR) <60 mL/min/1.73 m2 as determined using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation;
16. past (within 1 month prior to screening) or current administration of any immunosuppressive medications (including oral or systemically injected steroids) and use of any investigational agents, including any agents that impact the immune response;
17. a condition already known which interferes with the ability to accurately determine glycated HbA1c. Examples include: genetic variants (e.g. HbS trait, HbC trait), elevated fetal hemoglobin (HbF) and chemically modified derivatives of hemoglobin (e.g. carbamylated Hb in patients with renal failure); any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia); iron deficiency anemia, iron replacement therapy;
18. significant systemic infection during the 4 weeks before the first dose of study drug (e.g., infection requiring hospitalization, major surgery, or i.v. antibiotics to resolve; other infections, e.g., bronchitis, sinusitis, localized cellulitis, candidiasis, or urinary tract infections, must be assessed on a case-by-case basis by the investigator regarding whether they are serious enough to warrant exclusion).

### 4.3. ASSIGNMENT OF PATIENT NUMBER

At the screening visit, each patient will be assigned a unique sequential Screening number. If the patient is randomized, the Randomization number will be assigned in a sequential manner as patients are found to be eligible and are randomized. This number will be used for identification throughout the study and will not be used for any other participant.

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

# 5. STUDY MEDICATION

## 5.1. PRESENTATION, PACKAGING AND LABELING, SUPPLY, AND STORAGE OF THE INVESTIGATIONAL MEDICINAL PRODUCT

### 5.1.1. Presentation of the Investigational Medicinal Product

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

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

[redacted] CCI [redacted]

| [redacted] | [redacted] | [redacted] | [redacted] |
|---|---|---|---|
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | | [redacted] | |

Batch release certificate will be provided together with the IMP.

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

Capsules will be manufactured alternatively by PPD [redacted]

The primary packaging, labelling and kitting of capsules in blisters to obtain the Patient Kit will be done alternatively by PPD [redacted]

The study medication will be provided as 7 Patient Kits, each containing 56 capsules covering the treatment period of 14 days.

All labels will be prepared to meet local regulatory requirements. Details of packaging and labelling are reported in Section 14.1.

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

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

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

### 5.1.4. Blinding and unblinding

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. Any emergency unblinding must be notified to the CRO's medical monitor.

The sponsor's personnel from the Pharmacovigilance Department of Dompé may break the treatment code for subjects who experience a Suspected Unexpected Serious Adverse Reaction (SUSAR), in order to determine if the individual case requires expedited regulatory reporting.

With the exception of the above-mentioned episodes, the identity of the treatments will remain unknown to the subject, Investigator, site staff, CRO and Dompé's personnel until the study completion and formal unmasking

Study blind will be broken after database lock.

## 5.2. DOSE, ROUTE AND SCHEDULE OF IMP ADMINISTRATION

Ladarixin will be administered orally at the dose of 400 mg b.i.d. for 7 cycles of 14 days with an interval of 14 days off, for a total duration of 26 weeks. The two daily doses will be administered at about a 12-hour interval (morning and evening; ideally between 6:30/11:30 and 18:30/23:30). At each administration, 2 capsules will be swallowed with a glass of water, at least 2 hours apart from breakfast or dinner. Placebo will be administered with the same treatment schedule.

## 5.3. CRITERIA FOR SCHEDULE ADJUSTMENT/DOSE-MODIFICATION OR DISCONTINUATION OF THE IMP

### 5.3.1. Criteria for schedule adjustment/dose-modification

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

### 5.3.2. Criteria for discontinuation of the IMP

The IMP will be discontinued in the case:

- QTcF is either > 500 msec or increases by > 60 msec from screening measurement on two consecutive ECG readings taken 1 hour apart;
- The patient develops any significant cardiovascular disease/abnormality;
- The patient develops renal (calculated eGFR < 60 mL/min) or hepatic (increased ALT/AST > 3 x ULN and increased total bilirubin > 3mg/dL [>51.3 μmol/L]) dysfunction as well as hypoalbuminemia (serum albumin <3 g/dL);

- Pregnancy occurs (subjects of child-bearing potential);
- The patient develops Diabetic Ketoacidosis (DKA) or hypoglycemic coma.

Occurrence of any condition that qualify the patient to treatment discontinuation will be specifically monitored through ECG readings and safety laboratory tests obtained at week 4 (visit 2b) and 11/12 (visit 3) during treatment. Pregnancy (urine dipstick) will be also tested at week 11/12 (visit 3).

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

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

## 5.4. 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 center.

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

The dispensing of the IMP will be carefully recorded on the eCRF and appropriate drug accountability forms and an accurate accounting will be available for verification by the CRA at each monitoring visit.

Drug accountability records will include:

1. the confirmation of receipt of the IMP at the trial site,
2. the dispensing of the IMP to the patient,
3. the receipt of IMP returned from the patient,
4. the disposition of unused product(s),
5. accounts of any IMP accidentally or deliberately destroyed,

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

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

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

The CRA will review the drug accountability forms/eCRF and check all IMP (both unused and used) prior to making arrangements for their disposal.

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

### 5.4.1. Assessment of compliance

Compliance with the study product dosing schedule will be verified by the delegated monitoring personnel of the study during on-site monitoring visits, as per records in the specific study eCRF and actual capsules in the Patient Kits returned by the patient.

## 5.5. PRIOR AND CONCOMITANT MEDICATION

### 5.5.1. Reporting of prior and concomitant medication

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

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

The following agents do not need to be recorded: homeopathic medications; elective vitamins and minerals; osmotic laxatives and locally acting antacids; topical medication.

### 5.5.2. Prohibited medications

The following medications **should not be used** beginning from the start of the screening up to and including the end of study, unless otherwise specified:

1) Non-insulin medications for adjunctive blood glucose control (e.g. antidiabetic agents such as metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors, SGLT-2 inhibitors or amylin) within one month of randomization as well as required in the participant's standard of care;
2) Medications for weight reduction, such as: Belviq (lorcaserin), Qsymia (Phentermine + topiramate), Orlistat (xenical) within one month of randomization as well as required in the participant's standard of care;
3) Medication such as stimulants, antidepressants and/or psychotropic agents that could affect weight gain or glycemic control of T1D;
4) Drugs metabolized by CYP2C9 with a narrow therapeutic index [i.e. drugs that may have their plasma concentration and effect altered by inhibition of CYP2C9 by ladarixin such as phenytoin, warfarin, and high dose of amitriptyline (>50 mg/day)];
5) Angiotensin-converting enzyme inhibitors, interferons, quinidine antimalarial drugs, lithium, niacin;
6) Any immunosuppressive medications (including oral or systemically injected steroid), and any other investigational agents, including any agents that impact the immune response [off period before screening = 1 month]. Note that some oral, inhaled, nasal and topical corticosteroids are allowed, please speak with the study coordinator for more information.

## 5.6. INSULIN TITRATION

Patients will self-monitor blood glucose levels, as per their standard practice with self-owned (if applicable) CGM,and will take insulin as prescribed by the PI or their Primary Care Physician (PCP) and/or endocrinologist throughout the study participation.

To ensure standardized glycemic control in the treatment groups, the PI/Study Physician, together with the patient's PCP/endocrinologist will provide guidance for insulin regimen adjustment, with insulin titrated up or down to target HbA1c levels of less than 7% based on the following self- monitored glucose level:

- pre-prandial blood glucose of 70-130 mg/dL

In order to optimize insulin titration, telephone calls (outside scheduled visits) will be scheduled on a regular basis (at least every 30-45 days) to ensure timely evaluation of glucose levels and adjustment of insulin regimen.

# 6. STUDY PROCEDURE AND ASSESSMENT

A schedule for the tests and evaluations to be conducted in this study is found in the flow chart in Section 2. Details are reported below.

For all measurements, the actual date and time of assessment, including date of sampling, will be recorded in the Source Document and/or eCRFs.

Each patient will be involved in the study for a total of 5 visits, as detailed below.

## 6.1. STUDY VISITS AND STUDY EVENTS/PROCEDURES DETAILS

### 6.1.1. Identification of potential subjects

Potential subjects will be recruited after protocol approval by local Independent Ethics Committee (IEC)/IRBs.

If needed as per site practice (pre-screening), the study staff will identify potential study subjects (e.g. using electronic health records, diabetes or other relevant research study registries) and will reach out to them, via their primary healthcare manager/physician, offering information about the study and how to contact the study staff. Responsive individuals will be given comprehensive details about the study and then offered an Informed Consent Form (ICF). The potential participants will have up to 7 days to decide if they are able and willing to participate. If they are, the study staff will arrange for the potential participant to come to Visit 1 for witnessed informed consent signing and then to undergo the screening procedures.

### 6.1.2. Visit schedule and events/procedures

All subjects will follow the study procedures outlined below (see also grid in Section 2 above).

Every effort should be made to adhere to the visit schedule. Visits should be scheduled at the designated time-point. If unavoidable, an unscheduled visit may be performed, but the schedule for subsequent visits must be maintained so that the total duration of the study period per patient is as close as possible to 30 weeks. If a visit is scheduled at a time other than the protocol designated time, careful consideration must be given to the amount of study medication the subject has available.

Patients should be counseled to fast (no food or drink except water and non-study medications as prescribed) for at least 8 hours prior to all study visits where blood will be collected.

Each patient will be involved in the study for a screening visit, a randomization visit, the treatment period of 26 weeks (14 days on /14 days off) with 3 follow-up visits to the clinical research site scheduled up to week 28 after the 1st IMP dose.

Visit schedule and events/procedures are detailed in the sections below. Visits 2b and 3 are scheduled during the 14 days off treatment, while visit 4 is set in the 2 weeks after treatment completion (last IMP dose in cycle 7).

CCI [redacted]

**Visit 1: Screening**: The study physician or designee will present to/discuss with the study subject the clinical trial and answer all questions. If the patient is able and willing to participate, after the informed consent has been obtained, study staff will:

- assess past medical history to confirm T1D (e.g. results of past auto-antibody testing),
- determine daily insulin requirement (U/kg/day - expressed as the average of the last 3 days) and its stability,
- measure body weight, height, waist and waist/hip circumference,
- calculate [redacted] BMI,
- record the use of concomitant medication,
- establishing the presence of IR as described (inclusion criteria, bullet 8)[1-3].

Screening evaluation also include blood sampling for the assessment of:

- HbA1c,
- fasting C-peptide,
- standard safety laboratory tests,

- pregnancy in subjects with reproductive potential; post-menopausal women, defined as no menses for 12 months without an alternative medical cause, will not undergo pregnancy testing.
- T1D auto-antibody (excluding IAA) should past data not be available.

Vital signs (blood pressure and heart rate) will be measured and an ECG performed.

Patients must ensure the CGM sensor is correctly inserted and glucose monitored for at least the 7 days prior to the next visit. Potential study subjects must meet all inclusion and exclusion criteria prior to randomization.

The study subjects will be asked to continue regular glucose monitoring through the recording system as described in Section 5.6 to adjust their insulin intake according to T1D standard of care.

After the screening visit, the sub-investigator/trial coordinator will reach out the subject in at least one occasion to ensure compliance with daily blood glucose measurement as described in Section 5.6 and familiarize with establishing and maintaining contact with the trial staff to report/discuss any possible AEs and/or other aspects of the trial protocol.

Potential study subjects must meet all inclusion and exclusion criteria prior to randomization.

**Visit 2: Randomization and start of IMP (Day 1)**: Within 2 weeks from the screening, the study subject will come to the site in the morning for visit 2. After at least 7 days of CGM data have been downloaded for measurement of glycemic variability outcomes and all inclusion/exclusion criteria have been finally confirmed, eligible patients (enrolled patients) will be assigned a unique sequential randomization number and randomized 1:1 into either ladarixin or placebo, according to the randomization list. Dropouts after randomization will not be replaced. Vital signs will be measured, average (previous 3 days) daily insulin requirements (IU/kg/day) will be recorded, body weight, height, waist and waist/hip circumference will be assessed [redacted] and BMI calculated. Blood will be collected for the exploratory outcome measures CCI [redacted] and for HbA1c assessment (up to 2 mL).

At the end of the visit, Patient Kits for cycle 1 to 3 will be dispensed; it is the responsibility of the study physician to explain, and make sure that, patient fully understands any appropriate treatment related information. This includes, but is not limited to, treatment schedule, time of administration, time interval from meals, and recording of treatment administration in the Patient Diary.

**IMP administration**: The patients will begin administering the IMP, while on-site under medical supervision, ideally no later than 11:30 (1st dose of cycle 1). Treatment will proceed at home for the 1st and for additional 6 cycles of 14 days on/14 days off, for a total of 26 weeks.

**Visit 2b: (Week 4):** An ECG will be performed and then the subject will undergo blood sampling for assessment of safety laboratory testing.

**Visit 3: (Week 11/12)**: Patient Kits (cycle 1 to 3) and the Diary will be returned to and checked by the study staff to verify inventory and treatment compliance. Medication use will be also ascertained. Vital signs will be measured followed by an ECG, and then the subject will undergo blood sampling for assessment of HbA1c and safety laboratory testing and exploratory outcome measures CCI [redacted]; average (previous 3 days) daily insulin requirements (IU/kg/day) will be recorded, body weight, height, waist and waist/hip circumference will be measured and CGM data will be downloaded for measurement of glycemic variability in the previous 7 days. After IMP discontinuation criteria have been checked, Patient Kits (cycle 4 to 7) will be dispensed and the subject will be discharged from this visit.

**Visit 4; End of Study (Week 27/28)**: The study subject will come to the clinic for final assessment of vital signs, ECG, average (previous 3 days) daily insulin requirements (IU/kg/day), body weight, height, waist and waist/hip circumference and for . Subjects will undergo blood sampling for assessment of HbA1c, safety laboratory testing, eGFR and exploratory outcome measures CCI [redacted] CGM data will be downloaded for measurement of glycemic variability in the previous 7 days.

At the end of the visit, upon the study physician's order, the subject will be formally discharged from the study.

***REGULARLY-SCHEDULED PHONE CALLS:*** In-trial, the sub-investigator/study coordinator will proactively reach out to study subjects, at least every 30-45 days, for the entire study period from the time

of randomization visit onwards, to ensure compliance with treatment and importantly, ongoing identification of any AEs. It is the responsibility of the PI to adjust the frequency of such contacts, according to patient responsiveness and compliance with treatment.

Unscheduled visits

In addition to the scheduled visits, it is possible that subjects may need to complete an unscheduled visit if they experience an event that the PI or the Principal Physician believes to warrant follow-up. The unscheduled visit might include appropriate actions, such full physical examination and blood sample collection (less than 30 mL) that the PI or the Principal Physician feels is required, based upon the event.

## 6.2. GLUCOSE MONITORING DURING THE DURATION OF THE TRIAL

### 6.2.1. Glucose Monitoring

Glucose treatment decisions will be driven by protocol and controlled solely by the study staff.

Glycemic management for participants will follow an established titration protocol based on CGM measurements as described in Section 5.6, aiming to pre-prandial blood glucose between 70 and 130 mg/dL and a glycated HbA1c levels of levels of less than 7% without symptomatic hypoglycemia.

Blood glucose assessments will be performed for safety (to prevent hypoglycemia) according to ISOC recommendations. Pharmacologic insulin will be titrated based on results of these glucose measurements as is common in the course of usual care. Blood glucose measurements will also be performed for safety reasons by all participants in the presence of symptoms that suggest hypoglycemia, hyperglycemia, or during inter-current illness likely to affect glucose control substantially.

All participants will be followed until the end of their participation in this study.

### 6.2.1. Assessment and management of hypoglycemia

The site will review the symptoms and management of hypoglycemia with the subject. The site will counsel the subject to immediately check their glucose level if any symptoms occur that may be related to hypoglycemia (e.g., weakness, dizziness, shakiness, increased sweating, palpitations, or confusion), but also to avoid delay in treating these symptoms. Subjects should be instructed to contact the investigational site to report: any symptomatic episodes he/she believes may represent hypoglycemia, any episode of possible hypoglycemia resulting in symptoms, any episode of hypoglycemia for which assistance was required (i.e., severe hypoglycemia), any episode of self-monitored glucose of less than 70 mg/dL with or without symptoms.

Each episode should be evaluated by the PI/Principal Physician according to ADA 2022 guidelines, as per the table below.

**Table 6.4—Classification of hypoglycemia**

| | Glycemic criteria/description |
|---|---|
| Level 1 | Glucose <70 mg/dL (3.9 mmol/L) and ≥54 mg/dL (3.0 mmol/L) |
| Level 2 | Glucose <54 mg/dL (3.0 mmol/L) |
| Level 3 | A severe event characterized by altered mental and/or physical status requiring assistance for treatment of hypoglycemia |

Reprinted from Agiostratidou et al. (72).

## 6.3. EARLY PATIENT WITHDRAWAL

### 6.3.1. Withdrawal criteria

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

If a patient fails to return to the center for a scheduled visit, attempts should be made to contact the patient to ensure that the reason for not returning is not an AE or 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 an AE or SAE (bearing in mind the patient is not obliged to state his/her reasons).

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

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

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

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

Any withdrawals must be fully documented in the eCRF.

### 6.3.2. Replacement procedures

There are no plans to replace discontinued subjects.

## 6.4. 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.

## 6.5. PATIENT MANAGEMENT AFTER STUDY COMPLETION OR TERMINATION

After completion of Visit 4 (Study Discharge), or at study termination (for any other reason), patients will receive post-study care as prescribed by their PCP/endocrinologist and/or other non-study health care provider. No post-study or post study-termination treatment will be provided by the study team or Dompé.

# 7. STUDY ENDPOINTS

## 7.1. EFFICACT ENDPOINTS

**Primary endpoint:** The proportion of responders, with responders defined as "patients with an HbA1c reduction from baseline of ≥0.50% (absolute difference) without episodes of severe hypoglycemia (level 3) [timeframe: week 27/28 (visit 4)].

**Secondary endpoints:**

The following secondary endpoints will be considered:

- The proportion of responders [Timeframe: week 11/12 (visit 3)]
- The mean difference from baseline in HbA1c [Timeframe: week 11/12 (visit 3) and 27/28 (visit 4)]
- Average (previous 3 days) daily insulin requirements (IU/kg/day) [Timeframe: week 11/12 (visit 3) and 27/28 (visit 4)]
- Glycemic Variability by CGM (previous 7 days): time in range (TIR), time above range (TAR) time below range (TBR), standard deviation and coefficient of variation [Timeframe: week 11/12 (visit 3) and 27/28 (visit 4)]

**Exploratory endpoints:**

The following exploratory endpoints will be considered:

- CCI [redacted]
- CCI [redacted]

## 7.2. SAFETY ENDPOINTS

- Vital signs (blood pressure and heart rate) [Timeframe: screening, week 11/12 (visit 3), 27/28 (visit 4)]
- Laboratory tests [haematology (total WBC, neutrophils/lymphocytes/monocytes/basophils/eosinophils (% and absolute number), total RBC, MCV, hematocrit, platelet count) and clinical chemistry (sodium, potassium, serum creatinine, serum albumin, total bilirubin, ALT, AST)] [Timeframe: screening, week 11/12 (visit 3) and 27/28 (visit 4)]
- Incidence of Treatment Emergent Adverse Events (TEAEs), Adverse Drug Reaction (ADR) and Treatment Emergent Serious Adverse Events (TESAEs) [Timeframe: from the beginning of IMP administration up to the end of study participation].

# 8. ADVERSE EVENTS

## 8.1. DEFINITIONS

**Adverse event**

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

**Adverse Drug Reaction**

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

**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,

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

- results in persistent or significant disability/incapacity,

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

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

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

Pre-planned hospitalization or hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition are not considered to be serious events. These events must be recorded in the AE section (except for hospitalization for study procedures) of the eCRF where a variable will be ticked to indicate that they are not SAEs.

Death shall always be reported as SAE, and cause of death shall always be specified, when known.

**Unexpected Adverse Events**

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

According to the Investigator Brochure (Reference to Safety Information, section 6.4), for the purpose of this study all ADRs are assumed to be unexpected.

**Suspected unexpected serious adverse reaction**

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

## 8.2. MONITORING FOR ADVERSE EVENTS

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

• “Have you had any health problems since your last study visit?”

• “Have there been any changes in the medicines you take since your last study visit?”

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

## 8.3. RECORDING OF ADVERSE EVENTS

All AEs (serious and non-serious) which occur from signature of the informed consent through patient participation in the study (last planned visit or early withdrawal date) will be collected and recorded in the eCRF. It is important that the AE dedicated section of the eCRF includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, the outcome, the action(s) taken and relevant concomitant treatments dispensed. When possible, signs and symptoms indicating a common underlying pathology should be documented as one comprehensive event.

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

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

### 8.3.1. Follow-up of patients with AEs

The Investigator is responsible for adequate and safe medical care of subjects during the trial and for ensuring that appropriate medical care and relevant follow-up procedures are maintained after the trial. 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 AEs receive definite treatment for any AE, if required.

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

The Investigator shall inform the Sponsor with an appropriate written communication, whenever he becomes aware of new available information regarding the SAE, once the condition is resolved or stabilized and when no more information about the event is expected. Follow-up SAE information should be processed as initial SAE notification (see Sections 8.4, 8.5).

For pharmacovigilance purposes, all SAEs should be followed-up in order to clarify as completely as possible their nature and/or causality and until all queries have been resolved. All SAEs will be followed up until the events resolve or the events or sequelae stabilize, or it is unlikely that any additional information can be obtained after demonstration of due diligence with follow-up efforts (i.e. subject or Investigator is unable to provide additional information, or the subject is lost to follow up), unless subject has withdrawn his/her consent.

### 8.3.2. Relationship of AEs to the Investigational Medicinal Product

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

**Relationship of the AEs 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 the study drug will be considered as an ADR.

### 8.3.3. Severity of adverse events

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

**Severity of the Adverse Event**

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

## 8.4. SERIOUS ADVERSE EVENT REPORTING

### 8.4.1. Reporting Procedure for the Investigator to Dompé/CRO

The Principal Investigator must report all SAEs occurring during patient participation in the study, regardless of presumed causal relationship, to the appropriate Sponsor/CRO Pharmacovigilance contact 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" (See Page 2 of this Protocol).

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 appropriate Pharmacovigilance or directly to the Dompé Global Pharmacovigilance, Safety and Surveillance Department, should the whole study have been ended. Such "post-study cases" should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment and determination of expectedness are needed for a decision on whether or not expedited reporting is required.

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

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

### 8.4.2. Conditions that should not be reported as serious adverse events

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

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

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

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

### 8.4.3. Adverse events exemption

Not applicable.

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

*Reporting of Suspected Unexpected Serious Adverse Reaction*

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

Dompé Global Pharmacovigilance, Safety and Surveillance Department, with the support of the CRO as appropriate, shall report any SUSAR to the concerned IEC which approved the protocol and the Regulatory Authority (via the Eudravigilance Clinical Trial module) as soon as possible, and in no event later than:

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

Treatment will be unblinded by Dompé Global Pharmacovigilance, Safety and Surveillance department prior to regulatory submission of a potential SUSAR to Regulatory Authorities and IEC in the European Union. If the patient is on the active treatment, then Dompé Global Pharmacovigilance, Safety and Surveillance Department will report the case as a SUSAR. If the patient is revealed to have taken a placebo, then this would not require reporting as a SUSAR, unless there are concerns that there is a reaction to something in the placebo (such as an allergic reaction or a contaminant caused by a manufacturing issue).

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

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

*Periodical Reporting to EU Regulatory Authorities*

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

### 8.4.5. Reporting Procedure to IRB and to the FDA in the United States

The Investigator must report all SAEs to the Sponsor/CRO immediately, within 24 hours (see Section 8.4.1).

In addition to reporting the SAE to Dompé/CRO, the Investigator must also comply with the requirements related to the reporting of SAEs to the IRB which approved the study. As a minimum requirement, the Investigators must promptly report all SUSAR**s** to their IRB.

*Reporting of Suspected Unexpected Serious Adverse Reaction*

In line with provisions set forth in 21CFR312, the Sponsor shall notify the Investigators and the FDA in an IND safety report of any SUSAR and of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than:

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

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

The blind should ordinarily be broken for IND safety reports submitted to FDA and all participating investigators. Dompé Global Pharmacovigilance, Safety and Surveillance Department will unblind treatment prior to regulatory submission of a potential SUSAR to FDA and IRB and only cases referred to active treatment will be considered expeditable for regulatory reporting, in line with law requirements. In general, if the blind is broken and the subject was receiving placebo, the event should not be reported in an IND safety report because there is not a reasonable possibility that the drug caused the adverse event.

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

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

Potential serious risks arising from clinical trials or any other source, to be reported to FDA and to the Investigators, include:

- any SUSAR. Dompé must report an adverse event as a suspected adverse reaction only if there is evidence to suggest to the Sponsor a causal relationship between the drug and the adverse event.
- findings from other studies that suggest a significant risk in humans exposed to the drug. Such a finding would result in a safety-related change in the overall conduct of the clinical investigation.
- findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- increased rate of occurrence of serious suspected adverse reactions.

Based on the specific Investigator's site requirements, Dompé Global Pharmacovigilance, Safety and Surveillance Department will submit to IRBs and Investigators periodical safety updates, as per applicable local requirements and regulations.

Dompé Global Pharmacovigilance, Safety and Surveillance Department shall also be responsible to prepare and submit annual safety reports (Development Safety Update Report - DSUR) to FDA and IRBs (Director of Clinical Trial Regulatory Affairs at AHN).

## 8.5. EXPOSURE TO IMP DURING PREGNANCY

Women of childbearing potential are defined as all women physiologically capable of becoming pregnant. Prior to enrolment in the clinical trial, female patients of childbearing potential and their partners must be advised of the importance of avoiding pregnancy during the entire course of the study treatment and for the 2 months after the study discharge and of the potential risks associated with an unintentional pregnancy. During the trial (treatment period or follow up period), female patients are to be instructed to contact the Investigator immediately if they suspect they might be pregnant. In the same way, male patients who become aware that the partner might be pregnant, are to be instructed to contact the Investigator immediately.

The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of learning of the pregnancy to the Pharmacovigilance Contacts specified in the section "Contact Information", even if no AE has occurred, and follow it to term. If, however, the pregnancy is associated with an SAE (e g, if the mother is hospitalized for dehydration), in addition to the pregnancy report form, a separate SAE report form must be filed as described in Section 8.6, with the appropriate serious criterion indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE.

Any pregnancy leads to the immediate withdrawal of the study subject from the trial.

## 8.6. ADVERSE EVENTS CAUSING TREATMENT DISCONTINUATION

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

## 8.7. OVERDOSE

Accidental or intentional overdose, which may or may not result in serious adverse reactions, is to be reported to CRO Pharmacovigilance, Dompé Global Pharmacovigilance, Safety and Surveillance Department and to Dompé Medical Expert, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake through different routes (e.g. ingestion) or with suicidal intentions and consequent drug overdose.

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

The Investigator shall provide in the SAE form information about symptoms, corrective treatment and outcome of overdose. The Medical Expert should be contacted to discuss corrective treatment, if necessary.

## 8.8. EMERGENCY PROCEDURES AND UNMASKING OF STUDY TREATMENT

The treatment allocation for each patient will be provided with a protected system to:

- the Principal Investigator and study physicians for emergency procedures;
- Dompé Pharmacovigilance for safety reporting.

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

Access to individual patient treatment information will be allowed only in the event of a medical emergency where the knowledge of patient treatment is required to provide the patient with appropriate care.

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

If the treatment code needs to be broken in the interest of patient safety for a medical emergency, the Investigator is allowed to break the treatment code for the specific patient, even before informing the Sponsor. The Investigator must always notify the Sponsor, so that the reason for any premature unmasking can be documented, by means of a communication to CRO and to Dompé Medical Expert.

The Investigator will inform the Dompé representative (Dompé Medical Expert) if an emergency unmasking was performed without revealing the treatment identity, but only referring to the kit number involved in the unmasking in order to avoid a dissemination of unmasked information.

Dompé Pharmacovigilance, Safety and Surveillance Department will unmask a patient treatment only for reported SAE potentially meeting criteria of a SUSAR, in order to fulfill expedited regulatory reporting requirements. The identity of the treatment will remain unknown to the patient, Investigator, site staff, CRO and Dompé's Development personnel until the study is unmasked. Unblinding events will be recorded and reported in the clinical study report.

# 9. STATISTICAL CONSIDERATIONS

This study is designed to be a randomized, placebo-controlled, double-blinded, 2-parallel arm, phase II trial in which subjects are randomized (1:1) to ladarixin or placebo.

## 9.1. SAMPLE SIZE

Sample size calculation was based on results obtained in the phase 2 trial with ladarixin in T1D onset. Considering the following assumptions:

- randomization ratio 1:1 (ladarixin: placebo),
- one-side type I error of 0.05,
- expected difference in favor of ladarixin in terms of responders: ~22.5%, and
- power of 80% to detect the expected treatment effect

a total of 76 evaluable patients are required; to account for a possible 10% of patients not evaluable for the primary variable after enrollment, up to 86 patients will be enrolled.

## 9.2. RANDOMIZATION

The randomization list will be generated with a computer procedure by a CRO independent statistician not involved in the conduct of the study and will be provided to Dompé in a sealed envelope to prevent unblinding. The facility responsible of IMP packaging/labelling will also receive appropriate randomization codes for the purpose of IMP preparation.

Randomization will be stratified by site to ensure balanced assignment across treatment groups. A master randomization list will be generated, randomizing an excess of patients to allow competitive recruitment within each center.

Access to individual patient treatment code will be allowed only in the event of a medical emergency where the knowledge of patient treatment is required to provide the patient with appropriate care. The investigator and Dompé Pharmacovigilance will have access to the randomization code for a specific patient in case of a medical emergency or for safety reasons. Unblinding events will be recorded and reported in the Clinical Study Report (CSR). The treatment assignment information will be kept confidential and will not be disclosed to any other.

Once the study has been completed and the database has been locked, the treatment assignment information will be accessible to the study biostatistician(s) who will perform the statistical analyses and will generate reports.

## 9.3. ANALYSIS POPULATION

The following population will be defined:

- The Safety (SAF) population will consist of all randomized patients who received at least one dose of the investigational product. Safety population will be analyzed according to the actual treatment received. The SAF population will be used to present results on safety data.
- The Full Analysis Set (FAS) population will consist of all randomized patients who received at least one dose of the investigational product. FAS population will be analyzed according to the intention-to-treat (ITT) principle. The FAS population will be used for the primary analysis of the study and to present results on efficacy data.

## 9.4. STATISTICAL METHODOLOGY

Statistical analysis will be performed by the CRO appointed by Dompé.

All patient data collected during the study will be listed by patient.

Summary statistics appropriate to the distribution of the primary variable will be calculated separately by treatment arm.

For continuous data, number of observations, mean, standard deviation, median and range (minimum and maximum) will be presented. For qualitative data, frequency distributions and percentages per category will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

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

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

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

### 9.4.1. Demographic and baseline characteristics

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

### 9.4.2. Analysis of efficacy variables

9.4.2.1. Primary analysis

The following null hypothesis is defined: the proportion of patients with an HbA1c reduction from baseline of ≥ 0.50% (absolute difference) without episodes of severe hypoglycemia (level 3) at week 27/28 (visit 4) in ladarixin group is lower or equal than control:

$H_0$: $T_{LADARIXIN} \leq T_{CONTROL}$

$H_1$: $T_{LADARIXIN} > T_{CONTROL}$

where $T_{LADARIXIN}$ and $T_{CONTROL}$ are the proportions of patients with an HbA1c reduction from baseline of ≥0.50% without episodes of severe hypoglycemia for ladarixin and control groups, respectively. The null hypothesis $H_0$ will be rejected, and superiority of ladarixin declared if primary analysis p-value will be lower than 0.05.

Primary endpoint will be analyzed by means of a logistic regression model, adjusting for treatment, stratification factor and other baseline characteristics that will be defined in the SAP. A one-sided test will be used to test for differences between treatment groups.

Summary statistics appropriate to the distribution of the primary variable will be calculated separately by treatment arm.

9.4.2.2. Secondary analyses

Analyses will be performed on all secondary CCI endpoints at each available time point by means of descriptive statistics and by appropriate inferential tests, depending on the nature of the variable and its distribution. Data transformation might be used in order to satisfy the assumption of normality requested by parametric statistical tests. In case such assumptions are not met, non-parametric counterpart tests will be used. Details will be provided in the SAP.

The proportion of responders assessed at week 11/12 (visit 3) will be analyzed using the same strategy of the primary endpoint.

9.4.2.3. Analysis of safety variables

TEAEs, ADRs and TESAEs will be presented by treatment arms in terms of number of TEAEs and incidence by System Organ Class and Preferred Term using MedDRA. Analyses will be provided also by severity and relationship to the study drug.

Vital signs and safety laboratory tests will be presented using descriptive statistics at each available visit. Additionally, the frequency of subjects reporting an abnormal or abnormal clinically significant laboratory value will be presented for each laboratory parameter.

### 9.4.3. Intermediate analyses for the DMC

Safety data will be reviewed on an ongoing basis by a Data Monitoring Committee (DMC). Full details of the activities and responsibilities of the DMC are provided in the study DMC Charter (see additional details in Section 12.6).

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

### 9.4.4. Specification of subgroups for analysis

Sub-group analyses of efficacy endpoints will be performed ad hoc (e.g. subgroup of patients defined by age, HbA1c value, BMI etc.), according to the results obtained in the whole population. Statistical details on subgroup analysis will be reported in the CSR.

### 9.4.5. Missing data

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

The number of subjects with missing data will be presented under the "Missing" category. Missing values will not be included in the denominator count when computing percentages. When continuous data will be summarized, only the non-missing values will be evaluated for computing summary statistics.

For the primary analysis, since patients who discontinue the IMP will not be withdrawn from the study but will be asked to complete safety and efficacy assessments as per the protocol, missing data due to intercurrent events will be addressed by using multiple imputation based on retrieved dropouts information. Retrieved dropout patients are defined as patients who discontinue study treatment and decide to remain in the study by following the schedule of assessments and continuing to adhere to protocol requirements. If not enough data were retrieved after study treatment discontinuation for assure the convergence of the MI-RD regression model (the final decision will be done at the time of the analysis and reported in the CSR), the same multiple imputation model will be fit using data from subjects of control group, washing-out the effect of treatment. This approach does not assume benefits for ladarixin in case of discontinuation and limits a post-discontinuation clinical effect to that of placebo. Specific sensitivity analyses will be defined in the SAP to assess the robustness of results on primary endpoint versus assumptions used in the statistical model for the main estimator.

# 10. ETHICAL CONSIDERATIONS

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

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

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

- the study protocol,
- the ICF,
- the current version of the Investigator's Brochure,
- Investigator's current curriculum vitae (CV) as well as the current CVs of all key study personnel,
- any other requested document(s).

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

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

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

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

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

The CRO appointed by Dompé or the PI will report to the IEC/IRB any serious ADRs, life-threatening problems or deaths occurred in other clinical studies conducted with ladarixin.

## 10.2. ETHICAL CONDUCT OF THE STUDY

This study will be conducted in compliance with the protocol and consistent with FDA and current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312).

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the IEC/IRB. Any amendments to the protocol or consent materials must also be approved before they are implemented.

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

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

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

## 10.4. CONFIDENTIALITY

All information obtained during the conduct of the study will be regarded as confidential. Only the minimum necessary information to complete this research trial will be shared with the Sponsor and always in a de-identified manner compliant with HIPAA expectations. 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 USA data protection laws, HIPAA regulations, and European data protection Regulation (EU) No. 679/2016 of the European Parliament and of the European Council regarding the protection of natural person's personal data and the free circulation of said data (hereinafter GDPR EU No. 679/2016) and according the standards of Good Clinical Practice (Law Decree 211/2003).

On the eCRF 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.

<u>The secondary use of personal data</u>

Personal Data will be processed for the purpose of conducting any further analysis to generate new scientific hypotheses that could be verified through new research. This further analysis of personal data ("the secondary use of personal data") may deviate from this protocol and, for this reason, it may require a new clinical trial (and therefore a new legal basis for processing patient's personal data).

If the further analysis does not deviate from this protocol, there is no need to change the legal basis since the secondary use is considered to be compatible with the primary use.

Analyses of subcategories of data, for example, may be conducted consistent with the primary use.

The secondary use of data may be the subject of scientific and/or statistical publications.

## 10.5. 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 FORM (CRF)

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

An eCRF is required and should be completed for each consented patient, regardless of actual enrollment. The Investigator will be responsible for the accuracy of the data entered in the eCRF. All entries must be written in ***ENGLISH***.

Source documents should be available to support all the data recorded in the eCRF; location of source documents will be specified and listed at the center Initiation Visit.

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

## 11.2. DIARY

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

The patient will report in the Diary IMP intake, i.e. the number of capsules assumed and the date/time of assumption as well as insulin requirement in the 3 days prior to visit 3 and 4. It is responsibility of the study team to explain to each patient how to enter the data in this logbook and to check the data inserted in this Diary to ensure correct intake of the IMP.

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

## 11.3. DATA MANAGEMENT

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

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

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

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

## 11.4. DOCUMENTATION REQUIRED PRIOR TO INITIATION OF, AND DURING THE STUDY

The following documents will be required from the Investigator prior to the initiation visit (and during the course of the study in case of any update):

- Current, signed and dated Curriculum Vitae of Principal Investigator and any Sub-Investigators/co-workers. Updates should be provided at least every two years.
- Confidential disclosure agreement Form in accordance also with European data protection Regulation (EU) No. 679/2016 (GDPR)
- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or

external quality assessment or other validation). Updates should be provided as soon as any reference value has changed.

- A signed page of the most recently-IRB approved clinical protocol and any amendments.
- IRB approval documentation, IRB-approved ICFs and study materials. Documentation of continuing IRB review and annual renewals.
- A signed copy of the study Financial Agreement/Clinical Study Agreement with Dompé, including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- FDA Form 1572 and financial disclosure form 3455 from all the persons listed on the 1572. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.

## 11.5. ESSENTIAL DOCUMENT RETENTION

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

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

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

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

# 12. STUDY MANAGEMENT

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

## 12.1. REGULATORY BODY OF APPROVAL

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

As to the US, since IND 147145 is in effect, Dompé will submit to the FDA this protocol as "new protocol", according to 21 CFR Part 312.30.

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

## 12.2. MONITORING

Monitoring will be carried out by the monitor of the designated CRO.

The purpose of the monitoring 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.

It is expected that the PI 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 sponsor representative to ensure that any problems detected during the course of these monitoring visits are resolved.

## 12.3. ACCESS TO RECORD

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

All study records must be available for audit by Dompé, its authorized representatives, and Regulatory Inspection by Regulatory Authority.

## 12.4. AUDIT AND INSPECTION

In addition to the IEC/IRB, audit activities will be performed by the Dompé Quality Assurance Unit or any other third party delegated by Dompé, as appropriate.

## 12.5. DATA MONITORING COMMITTEE

An independent DMC will be established and will be responsible for safeguarding the interests of trial participants, and for enhancing the integrity and credibility of the trial. The DMC will assess the safety of the interventions during the trial and will monitor the overall conduct of the clinical trial. The DMC will provide recommendations to Dompé about stopping or continuing the trial on safety basis.

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

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

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

The DMC:

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

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

## 12.6. PROTOCOL AMENDMENTS

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

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

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

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

## 12.7. DISCONTINUATION OF THE STUDY

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

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

Study discontinuation will be notified to Competent Authority/FDA within 15 days from decision. The Investigator will inform his IRB within the same timeframe.

## 12.8. PUBLICATIONS

Publications derived from this study will be planned and agreed with the participating Study 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 initial publication or one year after completion of the study, whichever occurs first, an Investigator and/or his/her colleagues may publish the results of Investigator's part of the study independently.

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

The Sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the Investigator publishing in peer reviewed journals; presenting results at scientific congresses; and posting information and results on internet-based public registers and databases.

On an exceptional basis, the Sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures etc.) to seek necessary intellectual property protection. This is because early disclosure of such a data could, in some circumstances, prevents or negatively impacts patentability.

# 13. REFERENCES

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1. Epstein EJ, Osman JL, Cohen HW, Rajpathak SN, Lewis O, Crandall JP. Use of the estimated glucose disposal rate as a measure of insulin resistance in an urban multiethnic population with type 1 diabetes. Diabetes care 2013;36:2280-5.
2. Danielson KK, Drum ML, Estrada CL, Lipton RB. Racial and ethnic differences in an estimated measure of insulin resistance among individuals with type 1 diabetes. Diabetes care 2010;33:614-9.
3. Chillaron JJ, Goday A, Flores-Le-Roux JA, et al. Estimated glucose disposal rate in assessment of the metabolic syndrome and microvascular complications in patients with type 1 diabetes. The Journal of clinical endocrinology and metabolism 2009;94:3530-4.
4. Tam CS, Xie W, Johnson WD, Cefalu WT, Redman LM, Ravussin E. Defining insulin resistance from hyperinsulinemic-euglycemic clamps. Diabetes care 2012;35:1605-10.
5. Kemp DM, Pidich A, Larijani M, et al. Ladarixin, a dual CXCR1/2 inhibitor, attenuates experimental melanomas harboring different molecular defects by affecting malignant cells and tumor microenvironment. Oncotarget 2017;8:14428-42.
6. Carreira EU, Carregaro V, Teixeira MM, et al. Neutrophils recruited by CXCR1/2 signalling mediate post-incisional pain. European journal of pain 2013;17:654-63.
7. Citro A, Valle A, Cantarelli E, et al. CXCR1/2 inhibition blocks and reverses type 1 diabetes in mice. Diabetes 2015;64:1329-40.
8. Citro A, Cantarelli E, Piemonti L. The CXCR1/2 Pathway: Involvement in Diabetes Pathophysiology and Potential Target for T1D Interventions. Current diabetes reports 2015;15:68.
9. Moriconi A, Cesta MC, Cervellera MN, et al. Design of noncompetitive interleukin-8 inhibitors acting on CXCR1 and CXCR2. Journal of medicinal chemistry 2007;50:3984-4002.
10. Garau A, Bertini R, Mosca M, et al. Development of a systemically-active dual CXCR1/CXCR2 allosteric inhibitor and its efficacy in a model of transient cerebral ischemia in the rat. European cytokine network 2006;17:35-41.
11. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet 2018;391:2449-62.
12. Norris JM, Johnson RK, Stene LC. Type 1 diabetes-early life origins and changing epidemiology. The lancet Diabetes & endocrinology 2020;8:226-38.
13. Virtanen SM, Knip M. Type 1 diabetes-origins and epidemiology. The lancet Diabetes & endocrinology 2020;8:368-9.
14. Cree-Green M, Stuppy JJ, Thurston J, et al. Youth With Type 1 Diabetes Have Adipose, Hepatic, and Peripheral Insulin Resistance. The Journal of clinical endocrinology and metabolism 2018;103:3647-57.
15. Bacha F, Klinepeter Bartz S. Insulin resistance, role of metformin and other non-insulin therapies in pediatric type 1 diabetes. Pediatric diabetes 2016;17:545-58.
16. Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42-7.
17. DeFronzo RA, Hendler R, Simonson D. Insulin resistance is a prominent feature of insulin-dependent diabetes. Diabetes 1982;31:795-801.
18. Bulum T, Kolaric B, Duvnjak L, Vrhovac R. Association of hematological parameters with insulin resistance in type 1 diabetes. Minerva endocrinologica 2014;39:119-26.

19. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell metabolism 2011;14:575-85.
20. Nadeau KJ, Regensteiner JG, Bauer TA, et al. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. The Journal of clinical endocrinology and metabolism 2010;95:513-21.
21. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. The Journal of clinical investigation 2006;116:1793-801.
22. Orchard TJ, Olson JC, Erbey JR, et al. Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes care 2003;26:1374-9.
23. Ekstrand AV, Groop PH, Gronhagen-Riska C. Insulin resistance precedes microalbuminuria in patients with insulin-dependent diabetes mellitus. Nephrol Dial Transplant 1998;13:3079-83.
24. Battaglia M, Atkinson MA. The streetlight effect in type 1 diabetes. Diabetes 2015;64:1081-90.
25. Alman AC, Talton JW, Wadwa RP, et al. Inflammation, adiposity, and progression of arterial stiffness in adolescents with type 1 diabetes: The SEARCH CVD Study. Journal of diabetes and its complications 2018;32:995-9.
26. Babar G, Clements M, Dai H, Raghuveer G. Assessment of biomarkers of inflammation and premature atherosclerosis in adolescents with type-1 diabetes mellitus. Journal of pediatric endocrinology & metabolism : JPEM 2019;32:109-13.
27. Herder C, Hermanns N. Subclinical inflammation and depressive symptoms in patients with type 1 and type 2 diabetes. Seminars in immunopathology 2019;41:477-89.
28. Jakob P, Luscher TF. Dysfunctional HDL and inflammation: a noxious liaison in adolescents with type 1 diabetes. European heart journal 2019.
29. Ko KI, Syverson AL, Kralik RM, et al. Diabetes-Induced NF-kappaB Dysregulation in Skeletal Stem Cells Prevents Resolution of Inflammation. Diabetes 2019;68:2095-106.
30. Tsalamandris S, Antonopoulos AS, Oikonomou E, et al. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. European cardiology 2019;14:50-9.
31. Cabrera SM, Henschel AM, Hessner MJ. Innate inflammation in type 1 diabetes. Translational research : the journal of laboratory and clinical medicine 2016;167:214-27.
32. Barrio Castellanos R. Long-acting insulin analogues (insulin glargine or determir) and continuous subcutaneous insulin infusion in the treatment of type 1 diabetes mellitus in the paediatric population. Journal of pediatric endocrinology & metabolism : JPEM 2005;18 Suppl 1:1173-9.
33. Canadian Agency for D, Technologies in H. Long-Acting Insulin Analogues for the Treatment of Diabetes Mellitus: Meta-analyses of Clinical Outcomes. CADTH technology overviews 2010;1:e0113.
34. Garg SK, Giordano D. New Medications for the Treatment of Diabetes. Diabetes technology & therapeutics 2019;21:S160-S71.
35. Herring R, Russell-Jones DDL. Lessons for modern insulin development. Diabetic medicine : a journal of the British Diabetic Association 2018;35:1320-8.
36. Koehler G, Treiber G, Wutte A, et al. Pharmacodynamics of the long-acting insulin analogues detemir and glargine following single-doses and under steady-state conditions in patients with type 1 diabetes. Diabetes, obesity & metabolism 2014;16:57-62.
37. Laranjeira FO, de Andrade KRC, Figueiredo A, Silva EN, Pereira MG. Long-acting insulin analogues for type 1 diabetes: An overview of systematic reviews and meta-analysis of randomized controlled trials. PloS one 2018;13:e0194801.
38. Lee TY, Kuo S, Yang CY, Ou HT. Cost-effectiveness of long-acting insulin analogues versus intermediate/long-acting human insulin for type 1 diabetes: a population-based cohort following over 10 years. British journal of clinical pharmacology 2019.
39. Monami M, Marchionni N, Mannucci E. Long-acting insulin analogues vs. NPH human insulin in type 1 diabetes. A meta-analysis. Diabetes, obesity & metabolism 2009;11:372-8.
40. Nally LM, Sherr JL, Van Name MA, Patel AD, Tamborlane WV. Pharmacologic treatment options for type 1 diabetes: what's new? Expert review of clinical pharmacology 2019;12:471-9.
41. Nathan DM. Diabetes: long-acting insulin analogues--are benefits worth the cost? Nature reviews Endocrinology 2012;8:699-700.
42. Rendell M. Premix insulins in type 1 diabetes: the coming of degludec/aspart. Expert opinion on drug metabolism & toxicology 2019;15:341-8.
43. Sheehy DF, Quinnell SP, Vegas AJ. Targeting Type 1 Diabetes: Selective Approaches for New Therapies. Biochemistry 2019;58:214-33.

44. Tauschmann M, Hovorka R. Technology in the management of type 1 diabetes mellitus - current status and future prospects. Nature reviews Endocrinology 2018;14:464-75.
45. Wilmot EG, Choudhary P, Leelarathna L, Baxter M. Glycaemic variability: The under-recognized therapeutic target in type 1 diabetes care. Diabetes, obesity & metabolism 2019;21:2599-608.
46. Mazarello Paes V, Barrett JK, Taylor-Robinson DC, et al. Effect of early glycemic control on HbA1c tracking and development of vascular complications after 5 years of childhood onset type 1 diabetes: Systematic review and meta-analysis. Pediatric diabetes 2019;20:494-509.
47. Livingstone R, Boyle JG, Petrie JR. How tightly controlled do fluctuations in blood glucose levels need to be to reduce the risk of developing complications in people with Type 1 diabetes? Diabetic medicine : a journal of the British Diabetic Association 2019.
48. Graves LE, Donaghue KC. Management of diabetes complications in youth. Therapeutic advances in endocrinology and metabolism 2019;10:2042018819863226.
49. Dong S, Lau H, Chavarria C, et al. Effects of Periodic Intensive Insulin Therapy: An Updated Review. Current therapeutic research, clinical and experimental 2019;90:61-7.
50. Braffett BH, Dagogo-Jack S, Bebu I, et al. Association of Insulin Dose, Cardiometabolic Risk Factors, and Cardiovascular Disease in Type 1 Diabetes During 30 Years of Follow-up in the DCCT/EDIC Study. Diabetes care 2019;42:657-64.
51. Adeva-Andany MM, Martinez-Rodriguez J, Gonzalez-Lucan M, Fernandez-Fernandez C, Castro-Quintela E. Insulin resistance is a cardiovascular risk factor in humans. Diabetes & metabolic syndrome 2019;13:1449-55.
52. Adeva-Andany MM, Funcasta-Calderon R, Fernandez-Fernandez C, Ameneiros-Rodriguez E, Dominguez-Montero A. Subclinical vascular disease in patients with diabetes is associated with insulin resistance. Diabetes & metabolic syndrome 2019;13:2198-206.
53. Adeva-Andany MM, Ameneiros-Rodriguez E, Fernandez-Fernandez C, Dominguez-Montero A, Funcasta-Calderon R. Insulin resistance is associated with subclinical vascular disease in humans. World journal of diabetes 2019;10:63-77.
54. Warnes H, Helliwell R, Pearson SM, Ajjan RA. Metabolic Control in Type 1 Diabetes: Is Adjunctive Therapy the Way Forward? Diabetes therapy : research, treatment and education of diabetes and related disorders 2018;9:1831-51.
55. Gingras V, Leroux C, Fortin A, Legault L, Rabasa-Lhoret R. Predictors of cardiovascular risk among patients with type 1 diabetes: A critical analysis of the metabolic syndrome and its components. Diabetes & metabolism 2017;43:217-22.
56. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. Nature reviews Endocrinology 2016;12:144-53.
57. Abdelghaffar S, Attia AM. Metformin added to insulin therapy for type 1 diabetes mellitus in adolescents. The Cochrane database of systematic reviews 2009:CD006691.
58. Baron AD, Laakso M, Brechtel G, Edelman SV. Mechanism of insulin resistance in insulin-dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. The Journal of clinical endocrinology and metabolism 1991;73:637-43.
59. Bjornstad P, Schafer M, Truong U, et al. Metformin Improves Insulin Sensitivity and Vascular Health in Youth With Type 1 Diabetes Mellitus. Circulation 2018;138:2895-907.
60. Cleland SJ, Fisher BM, Colhoun HM, Sattar N, Petrie JR. Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? Diabetologia 2013;56:1462-70.
61. Cree-Green M, Bergman BC, Cengiz E, et al. Metformin Improves Peripheral Insulin Sensitivity in Youth With Type 1 Diabetes. The Journal of clinical endocrinology and metabolism 2019;104:3265-78.
62. DeFronzo RA, Simonson D, Ferrannini E. Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1982;23:313-9.
63. Kaul K, Apostolopoulou M, Roden M. Insulin resistance in type 1 diabetes mellitus. Metabolism: clinical and experimental 2015;64:1629-39.
64. Llaurado G, Gallart L, Tirado R, et al. Insulin resistance, low-grade inflammation and type 1 diabetes mellitus. Acta diabetologica 2012;49:33-9.
65. Nadeau KJ, Chow K, Alam S, et al. Effects of low dose metformin in adolescents with type I diabetes mellitus: a randomized, double-blinded placebo-controlled study. Pediatric diabetes 2015;16:196-203.

66. Nwosu BU, Maranda L, Cullen K, et al. A Randomized, Double-Blind, Placebo-Controlled Trial of Adjunctive Metformin Therapy in Overweight/Obese Youth with Type 1 Diabetes. PloS one 2015;10:e0137525.
67. Polsky S, Ellis SL. Obesity, insulin resistance, and type 1 diabetes mellitus. Current opinion in endocrinology, diabetes, and obesity 2015;22:277-82.
68. Priya G, Kalra S. A Review of Insulin Resistance in Type 1 Diabetes: Is There a Place for Adjunctive Metformin? Diabetes therapy : research, treatment and education of diabetes and related disorders 2018;9:349-61.
69. Szadkowska A, Pietrzak I, Mianowska B, et al. Insulin sensitivity in Type 1 diabetic children and adolescents. Diabetic medicine : a journal of the British Diabetic Association 2008;25:282-8.
70. Taylor SI, Blau JE, Rother KI, Beitelshees AL. SGLT2 inhibitors as adjunctive therapy for type 1 diabetes: balancing benefits and risks. The lancet Diabetes & endocrinology 2019;7:949-58.
71. Isaacs D, Yager S, Parker M, Wolfe L, Luxenburg J, Lekic S. Adjunct Antihyperglycemic Agents in Overweight and Obese Adults With Type 1 Diabetes. The Annals of pharmacotherapy 2019;53:371-84.
72. Tosur M, Redondo MJ, Lyons SK. Adjuvant Pharmacotherapies to Insulin for the Treatment of Type 1 Diabetes. Current diabetes reports 2018;18:79.
73. Garg SK, Rewers AH, Akturk HK. New Medications for the Treatment of Diabetes. Diabetes technology & therapeutics 2018;20:S139-S53.
74. Frandsen CS, Dejgaard TF, Madsbad S, Holst JJ. Non-insulin pharmacological therapies for treating type 1 diabetes. Expert opinion on pharmacotherapy 2018;19:947-60.
75. Doggrell SA. Do glucagon-like peptide-1 receptor (GLP-1R) agonists have potential as adjuncts in the treatment of type 1 diabetes? Expert opinion on pharmacotherapy 2018;19:1655-61.
76. Biester T, Kordonouri O, Danne T. Pharmacotherapy of type1 diabetes in children and adolescents: more than insulin? Therapeutic advances in endocrinology and metabolism 2018;9:157-66.
77. Garg SK, Giordano D, Gallo T, Shah VN. New Medications for the Treatment of Diabetes. Diabetes technology & therapeutics 2017;19:S128-S40.
78. Garg SK, Polsky S, Shah VN. New medications for the treatment of diabetes. Diabetes technology & therapeutics 2015;17 Suppl 1:S119-33.
79. Hamilton J, Cummings E, Zdravkovic V, Finegood D, Daneman D. Metformin as an adjunct therapy in adolescents with type 1 diabetes and insulin resistance: a randomized controlled trial. Diabetes care 2003;26:138-43.
80. Gin H, Messerchmitt C, Brottier E, Aubertin J. Metformin improved insulin resistance in type I, insulin-dependent, diabetic patients. Metabolism: clinical and experimental 1985;34:923-5.
81. Moon RJ, Bascombe LA, Holt RI. The addition of metformin in type 1 diabetes improves insulin sensitivity, diabetic control, body composition and patient well-being. Diabetes, obesity & metabolism 2007;9:143-5.
82. Moran A, Jacobs DR, Jr., Steinberger J, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 1999;48:2039-44.
83. Nokoff NJ, Rewers M, Cree Green M. The interplay of autoimmunity and insulin resistance in type 1 diabetes. Discovery medicine 2012;13:115-22.
84. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944-8.
85. Zand H, Morshedzadeh N, Naghashian F. Signaling pathways linking inflammation to insulin resistance. Diabetes & metabolic syndrome 2017.
86. Asghar A, Sheikh N. Role of immune cells in obesity induced low grade inflammation and insulin resistance. Cellular immunology 2017;315:18-26.
87. Ottum MS, Mistry AM. Advanced glycation end-products: modifiable environmental factors profoundly mediate insulin resistance. Journal of clinical biochemistry and nutrition 2015;57:1-12.
88. Lee CT, Harris SB, Retnakaran R, et al. White blood cell subtypes, insulin resistance and beta-cell dysfunction in high-risk individuals--the PROMISE cohort. Clinical endocrinology 2014;81:536-41.
89. Chmelar J, Chung KJ, Chavakis T. The role of innate immune cells in obese adipose tissue inflammation and development of insulin resistance. Thrombosis and haemostasis 2013;109:399-406.
90. Unoki H, Yamagishi S. Advanced glycation end products and insulin resistance. Current pharmaceutical design 2008;14:987-9.
91. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840-6.

92. Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nature medicine 2005;11:183-90.
93. Arkan MC, Hevener AL, Greten FR, et al. IKK-beta links inflammation to obesity-induced insulin resistance. Nature medicine 2005;11:191-8.
94. Rao X, Zhong J, Sun Q. The heterogenic properties of monocytes/macrophages and neutrophils in inflammatory response in diabetes. Life sciences 2014.
95. Buyukkaya E, Karakas MF, Karakas E, et al. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis 2014;20:159-63.
96. Battaglia M. Neutrophils and type 1 autoimmune diabetes. Current opinion in hematology 2014;21:8-15.
97. Mansuy-Aubert V, Zhou QL, Xie X, et al. Imbalance between neutrophil elastase and its inhibitor alpha1-antitrypsin in obesity alters insulin sensitivity, inflammation, and energy expenditure. Cell metabolism 2013;17:534-48.
98. Kaur H, Adams-Huet B, Smith G, Jialal I. Increased neutrophil count in nascent metabolic syndrome. Metabolic syndrome and related disorders 2013;11:128-31.
99. Talukdar S, Oh da Y, Bandyopadhyay G, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nature medicine 2012;18:1407-12.
100. Woo SJ, Ahn SJ, Ahn J, Park KH, Lee K. Elevated systemic neutrophil count in diabetic retinopathy and diabetes: a hospital-based cross-sectional study of 30,793 Korean subjects. Investigative ophthalmology & visual science 2011;52:7697-703.
101. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nature reviews Immunology 2011;11:519-31.
102. Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. Circulation 2010;122:1837-45.
103. Soehnlein O, Zernecke A, Weber C. Neutrophils launch monocyte extravasation by release of granule proteins. Thrombosis and haemostasis 2009;102:198-205.
104. Soehnlein O, Weber C, Lindbom L. Neutrophil granule proteins tune monocytic cell function. Trends in immunology 2009;30:538-46.
105. Toure F, Zahm JM, Garnotel R, et al. Receptor for advanced glycation end-products (RAGE) modulates neutrophil adhesion and migration on glycoxidated extracellular matrix. The Biochemical journal 2008;416:255-61.
106. Soehnlein O, Zernecke A, Eriksson EE, et al. Neutrophil secretion products pave the way for inflammatory monocytes. Blood 2008;112:1461-71.
107. Elgazar-Carmon V, Rudich A, Hadad N, Levy R. Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. Journal of lipid research 2008;49:1894-903.
108. Wang H, Meng QH, Gordon JR, Khandwala H, Wu L. Proinflammatory and proapoptotic effects of methylglyoxal on neutrophils from patients with type 2 diabetes mellitus. Clinical biochemistry 2007;40:1232-9.
109. Hatanaka E, Monteagudo PT, Marrocos MS, Campa A. Neutrophils and monocytes as potentially important sources of proinflammatory cytokines in diabetes. Clinical and experimental immunology 2006;146:443-7.
110. Wong RK, Pettit AI, Quinn PA, Jennings SC, Davies JE, Ng LL. Advanced glycation end products stimulate an enhanced neutrophil respiratory burst mediated through the activation of cytosolic phospholipase A2 and generation of arachidonic Acid. Circulation 2003;108:1858-64.
111. Wong RK, Pettit AI, Davies JE, Ng LL. Augmentation of the neutrophil respiratory burst through the action of advanced glycation end products: a potential contributor to vascular oxidant stress. Diabetes 2002;51:2846-53.
112. Vieira VJ, Ronan AM, Windt MR, Tagliaferro AR. Elevated atopy in healthy obese women. The American journal of clinical nutrition 2005;82:504-9.
113. Rensen SS, Slaats Y, Nijhuis J, et al. Increased hepatic myeloperoxidase activity in obese subjects with nonalcoholic steatohepatitis. The American journal of pathology 2009;175:1473-82.
114. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacological reviews 2010;62:726-59.

115. Al Khalifah RA, Alnhdi A, Alghar H, Alanazi M, Florez ID. The effect of adding metformin to insulin therapy for type 1 diabetes mellitus children: A systematic review and meta-analysis. Pediatric diabetes 2017;18:664-73.
116. Libman IM, Miller KM, DiMeglio LA, et al. Effect of Metformin Added to Insulin on Glycemic Control Among Overweight/Obese Adolescents With Type 1 Diabetes: A Randomized Clinical Trial. Jama 2015;314:2241-50.
117. Matzinger P. The evolution of the danger theory. Interview by Lauren Constable, Commissioning Editor. Expert review of clinical immunology 2012;8:311-7.
118. Matzinger P. The danger model: a renewed sense of self. Science 2002;296:301-5.
119. Matzinger P. An innate sense of danger. Annals of the New York Academy of Sciences 2002;961:341-2.
120. Matzinger P. Introduction to the series. Danger model of immunity. Scandinavian journal of immunology 2001;54:2-3.
121. Matzinger P. Essay 1: the Danger model in its historical context. Scandinavian journal of immunology 2001;54:4-9.
122. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Current opinion in immunology 2001;13:114-9.
123. Matzinger P. An innate sense of danger. Seminars in immunology 1998;10:399-415.
124. Matzinger P. Tolerance, danger, and the extended family. Annual review of immunology 1994;12:991-1045.
125. Biswas SK. Soluble receptor for advanced glycation end products and insulin resistance during development of type 2 diabetes mellitus. Journal of diabetes and its complications 2015;29:611.
126. Cai W, Ramdas M, Zhu L, Chen X, Striker GE, Vlassara H. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. Proceedings of the National Academy of Sciences of the United States of America 2012;109:15888-93.
127. Dozio E, Vianello E, Briganti S, et al. Expression of the Receptor for Advanced Glycation End Products in Epicardial Fat: Link with Tissue Thickness and Local Insulin Resistance in Coronary Artery Disease. Journal of diabetes research 2016;2016:2327341.
128. Kellow NJ, Savige GS. Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review. European journal of clinical nutrition 2013;67:239-48.
129. Miura K, Kitahara Y, Kajioka T, Takeuchi M, Yamagishi S. Combination Therapy with Nateglinide and Telmisartan Ameliorates Insulin Resistance in Zucker Fatty Rats by Suppressing Advanced Glycation End Product Receptor Axis. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 2011;43:e1.
130. Muellenbach EA, Diehl CJ, Teachey MK, et al. Interactions of the advanced glycation end product inhibitor pyridoxamine and the antioxidant alpha-lipoic acid on insulin resistance in the obese Zucker rat. Metabolism: clinical and experimental 2008;57:1465-72.
131. Pinto-Junior DC, Silva KS, Michalani ML, et al. Advanced glycation end products-induced insulin resistance involves repression of skeletal muscle GLUT4 expression. Scientific reports 2018;8:8109.
132. Unoki-Kubota H, Yamagishi S, Takeuchi M, Bujo H, Saito Y. Pyridoxamine, an inhibitor of advanced glycation end product (AGE) formation ameliorates insulin resistance in obese, type 2 diabetic mice. Protein and peptide letters 2010;17:1177-81.
133. Uribarri J, Cai W, Ramdas M, et al. Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. Diabetes care 2011;34:1610-6.
134. Uruska A, Gandecka A, Araszkiewicz A, Zozulinska-Ziolkiewicz D. Accumulation of advanced glycation end products in the skin is accelerated in relation to insulin resistance in people with Type 1 diabetes mellitus. Diabetic medicine : a journal of the British Diabetic Association 2019;36:620-5.
135. Yoshida T, Yamagishi S, Matsui T, et al. Telmisartan, an angiotensin II type 1 receptor blocker, inhibits advanced glycation end-product (AGE)-elicited hepatic insulin resistance via peroxisome proliferator-activated receptor-gamma activation. The Journal of international medical research 2008;36:237-43.
136. Piconi L, Quagliaro L, Assaloni R, et al. Constant and intermittent high glucose enhances endothelial cell apoptosis through mitochondrial superoxide overproduction. Diabetes/metabolism research and reviews 2006;22:198-203.

137. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes 2003;52:2795-804.
138. Risso A, Mercuri F, Quagliaro L, Damante G, Ceriello A. Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. American journal of physiology Endocrinology and metabolism 2001;281:E924-30.
139. Horvath EM, Benko R, Kiss L, et al. Rapid 'glycaemic swings' induce nitrosative stress, activate poly(ADP-ribose) polymerase and impair endothelial function in a rat model of diabetes mellitus. Diabetologia 2009;52:952-61.
140. Ceriello A, Esposito K, Piconi L, et al. Glucose "peak" and glucose "spike": Impact on endothelial function and oxidative stress. Diabetes research and clinical practice 2008;82:262-7.
141. Ceriello A, Esposito K, Piconi L, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 2008;57:1349-54.
142. Miele C, Riboulet A, Maitan MA, et al. Human glycated albumin affects glucose metabolism in L6 skeletal muscle cells by impairing insulin-induced insulin receptor substrate (IRS) signaling through a protein kinase C alpha-mediated mechanism. The Journal of biological chemistry 2003;278:47376-87.
143. Unoki H, Bujo H, Yamagishi S, Takeuchi M, Imaizumi T, Saito Y. Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes. Diabetes research and clinical practice 2007;76:236-44.
144. Kamei N, Tobe K, Suzuki R, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. The Journal of biological chemistry 2006;281:26602-14.
145. Kanda H, Tateya S, Tamori Y, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. The Journal of clinical investigation 2006;116:1494-505.
146. Collison KS, Parhar RS, Saleh SS, et al. RAGE-mediated neutrophil dysfunction is evoked by advanced glycation end products (AGEs). Journal of leukocyte biology 2002;71:433-44.
147. Stern MP. Diabetes and cardiovascular disease. The "common soil" hypothesis. Diabetes 1995;44:369-74.
148. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. International archives of medicine 2012;5:2.
149. Bhat T, Teli S, Rijal J, et al. Neutrophil to lymphocyte ratio and cardiovascular diseases: a review. Expert review of cardiovascular therapy 2013;11:55-9.
150. Lee CT, Harris SB, Retnakaran R, et al. White blood cell subtypes, insulin resistance and beta-cell dysfunction in high-risk individuals - the PROMISE cohort. Clinical endocrinology 2013.
151. Lorenzo C, Hanley AJ, Haffner SM. Differential white cell count and incident type 2 diabetes: the Insulin Resistance Atherosclerosis Study. Diabetologia 2014;57:83-92.
152. Shiny A, Bibin YS, Shanthirani CS, et al. Association of neutrophil-lymphocyte ratio with glucose intolerance: an indicator of systemic inflammation in patients with type 2 diabetes. Diabetes technology & therapeutics 2014;16:524-30.
153. Borregaard N. Neutrophils, from marrow to microbes. Immunity 2010;33:657-70.
154. Andrade VL, Petruceli E, Belo VA, et al. Evaluation of plasmatic MMP-8, MMP-9, TIMP-1 and MPO levels in obese and lean women. Clinical biochemistry 2012;45:412-5.
155. Trellakis S, Rydleuskaya A, Fischer C, et al. Low adiponectin, high levels of apoptosis and increased peripheral blood neutrophil activity in healthy obese subjects. Obesity facts 2012;5:305-18.
156. Battaglia M, Petrelli A, Vecchio F. Neutrophils and type 1 diabetes: current knowledge and suggested future directions. Current opinion in endocrinology, diabetes, and obesity 2019;26:201-6.
157. Vecchio F, Lo Buono N, Stabilini A, et al. Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes. JCI insight 2018;3.
158. Diana J, Simoni Y, Furio L, et al. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. Nature medicine 2013;19:65-73.
159. Lyons SK, Hermann JM, Miller KM, et al. Use of Adjuvant Pharmacotherapy in Type 1 Diabetes: International Comparison of 49,996 Individuals in the Prospective Diabetes Follow-up and T1D Exchange Registries. Diabetes care 2017;40:e139-e40.

160. Brufani C, Fintini D, Nobili V, Patera PI, Cappa M, Brufani M. Use of metformin in pediatric age. Pediatric diabetes 2011;12:580-8.
161. Kielgast U, Krarup T, Holst JJ, Madsbad S. Four weeks of treatment with liraglutide reduces insulin dose without loss of glycemic control in type 1 diabetic patients with and without residual beta-cell function. Diabetes care 2011;34:1463-8.
162. Ellis SL, Moser EG, Snell-Bergeon JK, Rodionova AS, Hazenfield RM, Garg SK. Effect of sitagliptin on glucose control in adult patients with Type 1 diabetes: a pilot, double-blind, randomized, crossover trial. Diabetic medicine : a journal of the British Diabetic Association 2011;28:1176-81.
163. Hari Kumar KV, Shaikh A, Prusty P. Addition of exenatide or sitagliptin to insulin in new onset type 1 diabetes: a randomized, open label study. Diabetes research and clinical practice 2013;100:e55-8.
164. Biester T, Aschemeier B, Fath M, et al. Effects of dapagliflozin on insulin-requirement, glucose excretion and ss-hydroxybutyrate levels are not related to baseline HbA1c in youth with type 1 diabetes. Diabetes, obesity & metabolism 2017;19:1635-9.
165. Kim J, Kim SM, Nguyen HC, Redondo MJ. Therapeutics in pediatric diabetes: insulin and non-insulin approaches. Part of a series on Pediatric Pharmacology, guest edited by Gianvincenzo Zuccotti, Emilio Clementi, and Massimo Molteni. Pharmacological research 2012;65:1-4.
166. Prasad-Reddy L, Isaacs D. A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. Drugs in context 2015;4:212283.
167. Bloomgarden ZT. Sodium-glucose cotransporter 2 inhibitors and diabetic ketoacidosis. Journal of diabetes 2016;8:175-6.
168. Peters AL, Buschur EO, Buse JB, Cohan P, Diner JC, Hirsch IB. Euglycemic Diabetic Ketoacidosis: A Potential Complication of Treatment With Sodium-Glucose Cotransporter 2 Inhibition. Diabetes care 2015;38:1687-93.
169. Pfutzner A, Klonoff D, Heinemann L, Ejskjaer N, Pickup J. Euglycemic ketosis in patients with type 2 diabetes on SGLT2-inhibitor therapy-an emerging problem and solutions offered by diabetes technology. Endocrine 2017;56:212-6.
170. Wang AY, Hou SK, Li SJ, Kao WF. Euglycemic diabetic ketoacidosis in type 2 diabetes with sodium glucose cotransporter 2 inhibitors. The American journal of emergency medicine 2017;35:379 e5- e6.
171. Anderson R, Theron AJ, Gravett CM, Steel HC, Tintinger GR, Feldman C. Montelukast inhibits neutrophil pro-inflammatory activity by a cyclic AMP-dependent mechanism. British journal of pharmacology 2009;156:105-15.
172. Barletta KE, Ley K, Mehrad B. Regulation of neutrophil function by adenosine. Arteriosclerosis, thrombosis, and vascular biology 2012;32:856-64.
173. Barnes PJ. New molecular targets for the treatment of neutrophilic diseases. The Journal of allergy and clinical immunology 2007;119:1055-62; quiz 63-4.
174. Barnes PJ. Glucocorticosteroids: current and future directions. British journal of pharmacology 2011;163:29-43.
175. Gaudreau R, Le Gouill C, Venne MH, Stankova J, Rola-Pleszczynski M. Threonine 308 within a putative casein kinase 2 site of the cytoplasmic tail of leukotriene B(4) receptor (BLT1) is crucial for ligand-induced, G-protein-coupled receptor-specific kinase 6-mediated desensitization. The Journal of biological chemistry 2002;277:31567-76.
176. Gravett CM, Theron AJ, Steel HC, et al. Interactive inhibitory effects of formoterol and montelukast on activated human neutrophils. The European respiratory journal 2010;36:1417-24.
177. Hatzelmann A, Schudt C. Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. The Journal of pharmacology and experimental therapeutics 2001;297:267-79.
178. Lazaar AL, Sweeney LE, MacDonald AJ, Alexis NE, Chen C, Tal-Singer R. SB-656933, a novel CXCR2 selective antagonist, inhibits ex vivo neutrophil activation and ozone-induced airway inflammation in humans. British journal of clinical pharmacology 2011;72:282-93.
179. Leonardi C, Matheson R, Zachariae C, et al. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. The New England journal of medicine 2012;366:1190-9.
180. Nair P, Gaga M, Zervas E, et al. Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 2012;42:1097-103.
181. Ohnishi H, Miyahara N, Gelfand EW. The role of leukotriene B(4) in allergic diseases. Allergology international : official journal of the Japanese Society of Allergology 2008;57:291-8.

182. Sivertson KL, Seeds MC, Long DL, Peachman KK, Bass DA. The differential effect of dexamethasone on granulocyte apoptosis involves stabilization of Mcl-1L in neutrophils but not in eosinophils. Cellular immunology 2007;246:34-45.
183. Stankova J, Turcotte S, Harris J, Rola-Pleszczynski M. Modulation of leukotriene B4 receptor-1 expression by dexamethasone: potential mechanism for enhanced neutrophil survival. Journal of immunology 2002;168:3570-6.
184. Steel HC, Theron AJ, Cockeran R, Anderson R, Feldman C. Pathogen- and host-directed anti-inflammatory activities of macrolide antibiotics. Mediators of inflammation 2012;2012:584262.
185. Steel HC, Theron AJ, Tintinger GR, Anderson R. Posaconazole attenuates leukotriene B4 release and uptake of calcium by chemoattractant-activated human neutrophils: a potential strategy to control neutrophil-mediated inflammation. The Journal of antimicrobial chemotherapy 2009;64:1008-12.
186. Tannheimer SL, Sorensen EA, Haran AC, Mansfield CN, Wright CD, Salmon M. Additive anti-inflammatory effects of beta 2 adrenoceptor agonists or glucocorticosteroid with roflumilast in human peripheral blood mononuclear cells. Pulmonary pharmacology & therapeutics 2012;25:178-84.
187. Tintinger GR, Feldman C, Theron AJ, Anderson R. Montelukast: more than a cysteinyl leukotriene receptor antagonist? TheScientificWorldJournal 2010;10:2403-13.
188. Tintinger GR, Steel HC, Theron AJ, Anderson R. Pharmacological control of neutrophil-mediated inflammation: strategies targeting calcium handling by activated polymorphonuclear leukocytes. Drug design, development and therapy 2009;2:95-104.
189. Tsuchihashi Y, Oishi K, Yoshimine H, et al. Fourteen-member macrolides suppress interleukin-8 production but do not promote apoptosis of activated neutrophils. Antimicrobial agents and chemotherapy 2002;46:1101-4.
190. den Uyl D, van Raalte DH, Nurmohamed MT, et al. Metabolic effects of high-dose prednisolone treatment in early rheumatoid arthritis: balance between diabetogenic effects and inflammation reduction. Arthritis and rheumatism 2012;64:639-46.
191. Rafacho A, Ortsater H, Nadal A, Quesada I. Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. The Journal of endocrinology 2014;223:R49-R62.
192. Huang HY, Caballero B, Chang S, et al. The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National Institutes of Health state-of-the-science conference. Annals of internal medicine 2006;145:372-85.
193. Sun L, Clavijo PE, Robbins Y, et al. Inhibiting myeloid-derived suppressor cell trafficking enhances T cell immunotherapy. JCI insight 2019;4.
194. Roberts G, Chen S, Yates P, et al. Randomized, Double-Blind, Placebo-Controlled Study of the Safety, Tolerability, and Clinical Effect of Danirixin in Adults With Acute, Uncomplicated Influenza. Open forum infectious diseases 2019;6:ofz072.
195. Madan A, Chen S, Yates P, et al. Efficacy and Safety of Danirixin (GSK1325756) Co-administered With Standard-of-Care Antiviral (Oseltamivir): A Phase 2b, Global, Randomized Study of Adults Hospitalized With Influenza. Open forum infectious diseases 2019;6:ofz163.
196. Iida T, Matsuzawa Y, Ogura H, et al. Evaluation of the Safety, Tolerability, Pharmacokinetics, and Food Effect of Danirixin Hydrobromide Tablets in Japanese Healthy Elderly Participants. Clinical pharmacology in drug development 2019.
197. Lazaar AL, Miller BE, Tabberer M, et al. Effect of the CXCR2 antagonist danirixin on symptoms and health status in COPD. The European respiratory journal 2018;52.
198. Tavares LP, Garcia CC, Machado MG, et al. CXCR1/2 Antagonism Is Protective during Influenza and Post-Influenza Pneumococcal Infection. Frontiers in immunology 2017;8:1799.
199. Busch-Petersen J, Carpenter DC, Burman M, et al. Danirixin: A Reversible and Selective Antagonist of the CXC Chemokine Receptor 2. The Journal of pharmacology and experimental therapeutics 2017;362:338-46.
200. Mozaffari S, Nikfar S, Abdollahi M. Inflammatory bowel disease therapies discontinued between 2009 and 2014. Expert opinion on investigational drugs 2015;24:949-56.
201. Russo RC, Guabiraba R, Garcia CC, et al. Role of the chemokine receptor CXCR2 in bleomycin-induced pulmonary inflammation and fibrosis. American journal of respiratory cell and molecular biology 2009;40:410-21.
202. Coelho FM, Pinho V, Amaral FA, et al. The chemokine receptors CXCR1/CXCR2 modulate antigen-induced arthritis by regulating adhesion of neutrophils to the synovial microvasculature. Arthritis and rheumatism 2008;58:2329-37.

203. Barsante MM, Cunha TM, Allegretti M, et al. Blockade of the chemokine receptor CXCR2 ameliorates adjuvant-induced arthritis in rats. British journal of pharmacology 2008;153:992-1002.
204. Pawlick RL, Wink J, Pepper AR, et al. Reparixin, a CXCR1/2 inhibitor in islet allotransplantation. Islets 2016;8:115-24.
205. Citro A, Cantarelli E, Maffi P, et al. CXCR1/2 inhibition enhances pancreatic islet survival after transplantation. The Journal of clinical investigation 2012;122:3647-51.
206. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine 2009;150:604-12.
207. Ferreira-Hermosillo A, Ibarra-Salce R, Rodriguez-Malacara J, Molina-Ayala MA. Comparison of indirect markers of insulin resistance in adult patients with Double Diabetes. BMC endocrine disorders 2020;20:87.
208. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. The American journal of physiology 1979;237:E214-23.

# 14. APPENDICES

## 14.1. APPENDIX 1 - SPONSOR APPROVAL PAGE

A phase II randomized, placebo-controlled, double-blinded, 2-parallel arm, clinical trial evaluating ladarixin 400 mg twice a day as adjunctive therapy to improve glycemic control in overweight insulin-resistant patients with type 1 diabetes.

Medical Expert: _____ Date: _____/_____/_____
PPD

Clinical Operation Manager: _____ Date: _____/_____/_____
PPD

Development Director: _____ Date: _____/_____/_____
PPD

## 14.2. APPENDIX 2 - INVESTIGATOR'S SIGNATURE PAGE

**Investigator's Statement**

I have read study protocol LDX0122 *(A phase II randomized, placebo-controlled, double-blinded, 2-parallel arm, clinical trial evaluating ladarixin 400 mg twice a day as adjunctive therapy to improve glycemic control in overweight insulin-resistant patients with 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 and/or Principal Physician: _____

(block letters)

Signature: _____ Date: _____/_____/_____

## 14.3. **APPENDIX 3 -** PACKAGING AND LABELING DETAILS

A Patient Kit will be prepared for each patient, containing the treatment for 14 days, i.e. a total of 56 capsules.

All items will have local language labels. Sample label content is summarized below and will be adjusted to meet local regulatory requirements.

**NOTE**:

Patient No. where XXX is the unique site number and ZZZ is patient randomization number

Kit No. where XXX-ZZZ-YY is the unique Kit identifier for that patient

**Content of the Label for each Patient Kit**

| |
|---|
| **STUDY CODE:** LDX0122 |
| **Sponsor** Dompé farmaceutici s.p.a.; Via Santa Lucia 6, Milan – Italy -Phone: +39 02 583831 |
| **INVESTIGATOR:** |
| **PATIENT No.** XXX-ZZZ **KIT** No. XXX-ZZZ-YY |
| **INVESTIGATIONAL PRODUCT**: ladarixin (200 mg) or placebo oral capsules |
| **CONTAINS**: 7 blisters of 8 capsules each. Total 56 CAPSULES |
| **coded BATCH No.** **coded EXPIRY DATE** mm/yyyy |
| **DO NOT STORE AT >30°C** **DO NOT FREEZE** |
| **DIRECTIONS**:<br>Take the drug twice a day: 2 capsules in the morning and 2 in the evening, for 14 consecutive days. See additional instructions on administration provided by the Investigator.<br>Contact the Investigator should you have any questions |
| **For clinical trial use only** |
| **CAUTION: New Drug-Limited by Federal (or United States) law to investigational use*** |
| **Keep out of reach of children** |

*For US labels only

**Content of the Label for the Blister**

| |
|---|
| **STUDY CODE:** LDX0122 |
| **Sponsor** Dompé farmaceutici s.p.a. |
| **KIT** No. XXX-ZZZ-YY |
| **INVESTIGATIONAL PRODUCT:** ladarixin (200 mg) or placebo - 8 oral capsules |
| **coded BATCH No.** |
| **DO NOT STORE AT >30°C** **DO NOT FREEZE** |
| **DIRECTIONS**: See directions in the label of the Patient Kit |
| **CAUTION: New Drug-Limited by Federal (or United States) law to investigational use*** |
| **Keep out of reach of children** |

* For US labels only

## 14.4. METHODOLOGICAL DETAILS

### 14.4.1. Handling of samples for assays

One or more laboratories will be involved for assay of HbA1c, safety laboratory tests, C-peptide, , and characterization by flow cytometry of

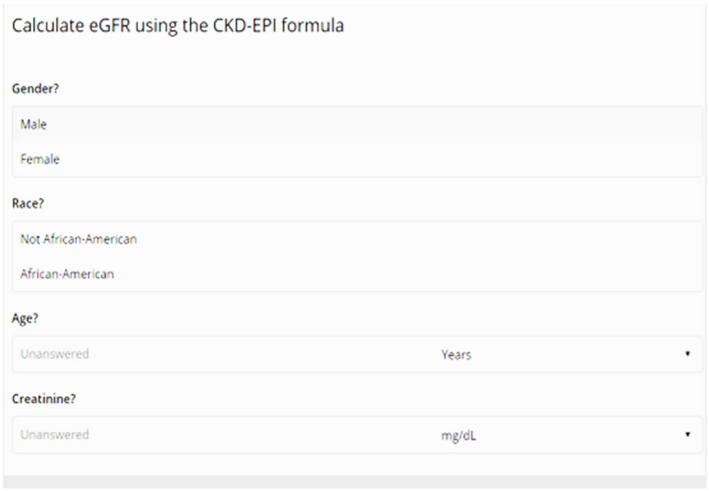
The laboratory performing the assay of HbA1c should be certified by CLIA (or similar certification) and will perform the analyses under strict quality control.

All steps will be tracked to ensure correct data reporting.

All samples submitted to these labs will be destroyed after the CSR has been issued or after the patient has withdrawn his/her consent.

### 14.4.2. Calculation of eGFR

Renal function will be evaluated by estimated Glomerular Filtration Rate (eGFR), calculated by the following formula[206]:




### 14.4.3. Calculation of [redacted]