AMENDMENT No. 1

To the CLINCIAL STUDY PROTOCOL version No. 1 – Final 11 October 2013

APPLICABLE TO SITE No. 01 ONLY

[Schulze Diabetes Institute; University of Minnesota Medical School]

Study Number: REP0112

IND Number: 117390

Investigational Product: Reparixin

Title: A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation.

Amendment No. 1 Version – Date Version No. 1 – final 18 July 2016

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NOTES:
This Amendment does not result in a change to either the CRF or the ICF.

PROTOCOL AMENDMENT APPROVAL SIGNATURES

SPONSOR:

Medical Expert

Signature: _____ Date: _____

Pier Adelchi Ruffini, MD – Development Director

CLINICAL CENTRE: PRINCIPAL INVESTIGATOR [PRIMARY INVESTIGATOR]

I have read study protocol REP0112 "A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation".

Name of Principal Investigator: Melena Bellin, MD

Signature: _____ Date: _____

3. OVERALL STUDY DESIGN AND PLAN DESCRIPTION

3.1. STUDY DESIGN

The following paragraph [protocol, page 20] has been changed from:

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites. Each centre will enroll patients as rapidly as possible, up to a maximum of 40 patients (as per the randomization list).

To:

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites. Each centre will enroll patients as rapidly as possible, up to a maximum of 40 patients (as per the randomization list). A maximum of 48 patients is allowed for the site of the Primary Investigator.

Reason: The study design has been changed considering current patient enrollment status. Specifically, since the first patient randomized to the trial back in February 2014, recruitment is extending considerably longer than originally expected. To date, 86 patients have received TP-IAT, out of 89 randomized. New sites in addition to those originally involved have joined the trial with Cincinnati and UCSF having been initiated early in 2016.
Despite this continuous effort, overall enrollment rate across the 9 participating sites is lower than predicted, mainly due to the very rare condition of the TP-IAT procedure, coupled with a non-homogeneous distribution of this procedure across the US states/sites. In particular, enrollment rate has significantly decrease since the site of the Primary Investigator (UMN) has reached the cap of 40 patients (out of 89 randomized to date) allowed at a single site as per protocol. The high enrollment rate of this site actually reflects the high volume of TP-IAT procedures specific for Minnesota.
Therefore, considering the importance of:

- maintaining study continuity;
- achieving a timely completion of the trial in order to avoid "dilution" over time of data obtained and potential bias on data reproducibility;
- distribution of TP-IAT procedures across different US sites;

the study design has been changed to allow enrollment at the site of the Primary Investigator up to a total of 48 patients.

9. STATISTICAL ISSUES

9.2. RANDOMIZATION

The following paragraph [protocol, page 42] has been changed from:

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment (in blocks of 4) will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 40 for each site) to allow competitive recruitment within each centre.

To:

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment (in blocks of 4) will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 40 for each site – additional 8 patients for the site of the Primary Investigator) to allow competitive recruitment within each centre.

Reason: To account for the change in study design (see section 3.1.)

CLINICAL STUDY PROTOCOL - CONFIDENTIAL

Study Number: REP0112

IND Number: 117390

Investigational Product: Reparixin

Title: A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation.

Protocol Version – Date Version No. 2 – Final 18 July 2016

APPLICABLE TO SITE No. 01 ONLY

[Schulze Diabetes Institute; University of Minnesota Medical School]

This protocol version results from revision of protocol version No. 1 (Final, 11 October 2013) according to Amendment No. 1 (final 18 July 2016).

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CONTACT INFORMATION

SPONSOR

Dompé s.p.a. [Dompé]
Via Campo di Pile; 67100 L'Aquila, Italy
Milan Offices: Via S. Lucia 6, 20122 Milan, Italy
Phone: +39 02 583831; Fax: +39 02 58383324

Medical Expert

Pier Adelchi Ruffini, MD - Development Director
Dompé s.p.a. - Milan Offices;
Direct line: +39 02 58383500; Mobile: +39 346 8745908
e-mail:

Clinical Trial Manager

Luisa Daffonchio, PhD - Clinical Development Manager
Dompé s.p.a. - Milan Offices;
Direct line: +39 02 58383246;
e-mail: luisa.daffonchio@dompe.it

Pharmacovigilance

Dr. Laura Boga, QPPV
Dompé s.p.a. - Milan Offices
Direct line: +39 02 58383462; Mobile: +39 335 219580
Fax: +39 02 36026913
e-mail:

CLINICAL RESEARCH ORGANIZATION

inVentive Health Clinical [inVentive]
504 Carnegie Center; Princeton, NJ 08540, USA
Phone: +1 609 951 6800; Fax: +1 609 514 0390

Project Manager

Fiona Wingrave - Manager, Clinical Research
5515 North Service Rd, Suite 200; Burlington, ON L7L 6G4, Canada
Phone: +1 289 313 3037; Fax: +1 937 637 9086
e-mail: fiona.wingrave@inventivhealth.com

INVESTIGATIONAL SITES

Primary Investigator

Melena Bellin, MD; Assistant Professor, Pediatric Endocrinology and Diabetes
Schulze Diabetes Institute; University of Minnesota Medical School
MMC 404, 420 Delaware St SE - Minneapolis, MN 55455
Phone: 612-625-4686 or 612-626-5716
Fax: 612-624-0420 (SDI), 612-626-5262 (Pediatric Endocrinology)

Full list of investigational sites will be kept in the Trial Master File. Updated versions, if any, will be filed chronologically.

CENTRALIZED LABORATORIES

List of centralized laboratories will be kept in the Trial Master File. Updated versions, if any, will be filed chronologically.

PROTOCOL APPROVAL SIGNATURES

SPONSOR:

Medical Expert

Signature: _____ Date: _____

Pier Adelchi Ruffini, MD - Development Director

Clinical Trial Manager

Signature: _____ Date: _____

Luisa Daffonchio, PhD – Clinical Development Manager

CLINICAL CENTRE: PRINCIPAL INVESTIGATOR [PRIMARY INVESTIGATOR]

I have read study protocol REP0112 "A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation".

Name of Principal Investigator: Melena Bellin, MD

Signature: _____ Date: _____

TABLE OF CONTENTS

List of Abbreviations and Definitions of Terms

AE	Adverse Event
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
ATG	Anti-Thymocyte Globulin
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
AUC_{0-t}	Area under the plasma concentration-time curve from time zero to time t (time of last quantifiable plasma concentration) of reparixin and metabolites
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from time zero to infinity of reparixin and metabolites
BP	Blood Pressure
C	Degrees Celsius
CL	Clearance of reparixin and metabolites
CLcr	Calculated Creatinine Clearance (Cockcroft - Gault formula)
$C_{initial}$	Plasma concentration of reparixin and metabolites just prior to the end of administration
Cmax	Maximum Plasma Concentration
CMED	Concomitant Medication
cmH_2O	Centimeters of Water
CPB	Cardiopulmonary Bypass
CRA	Clinical Research Associate
CRF	Case Report Form
CRP	C-reactive Protein
Css	Steady State Concentration
CXCL8	CXC ligand 8 [formerly interleukin (IL)-8]
ddPCR	Droplet Digital PCR
DMC	Data Monitoring Committee
g	Grams
GCP	Good Clinical Practice
HbA1c	Glycated hemoglobin
HR	Heart rate
kg	Kilogram
IAT	Islet Auto-Transplantation
ICH	International Conference on Harmonization
IRB	Institutional Review Board
IEQ	Islet Equivalent
IND	Investigation New Drug
INR	International Normalized Ratio
ITT	Intent to Treat
i.v.	Intravenous
LD_{50}	Lethal $Dose_{50}$
mg	Milligram
miR-375	microRNA-375
mL	Millilitre
mmHg	Millimeters of mercury
MMTT	Mixed Meal Tolerance Test
ng	Nanogram
NGSP	National Glycohemoglobin Standardization Program
NSAID	Non Steroidal Anti-Inflammatory Drug
PICD	Patient Informed Consent Document
PMN	Polymorphonuclear leukocyte
p.o.	per os (taken by mouth)
PTT	Partial Thromboplastin Time
SAE	Serious Adverse Event
s.c.	Subcutaneous
t½	Elimination half life
tmax	Time of maximum plasma concentration of reparixin and metabolites
TPN	Total Parenteral Nutrition
U-CRA	Unblinded-CRA
ULN	Upper Limit of Normal
Vz	Volume of distribution of reparixin
XDP	Fibrin Degradation Products
µg	Microgram
λ_z	Terminal phase rate constant reparixin and metabolites

1. STUDY SYNOPSIS AND OVERALL DESIGN

Study title

A phase 2/3, multicenter, randomized, double-blind, placebo-controlled, parallel assignment study to assess the efficacy and safety of reparixin in pancreatic islet auto-transplantation.

Study Number REP0112 [**US IND #** 117390]

Study period Projected starting date (first-patient-in): December 2013
Projected completion of patient accrual (last-patient-in): March 2015
Projected study end date (last-patient-last-visit): March 2016

Study design

The study will be a phase 2/3, multicenter, double-blind, parallel assignment study. It will involve 100 adult recipients of an intra-hepatic pancreatic Islet Auto-Transplantation (IAT).

Patients will be randomly (1:1) assigned to receive either reparixin treatment (continuous i.v. infusion for 7 days – treatment group) or placebo (control group). The two groups will be balanced within centres. Recruitment will be competitive among the study sites, until the planned number of patients is enrolled.

Objectives/endpoints

The objective of this clinical trial is to assess whether reparixin leads to improved transplant outcome as measured by the proportion of insulin-independent patients following IAT. The safety of reparixin in the specific clinical setting will be also evaluated.

Efficacy endpoints will be:

- The proportion of insulin-independent patients following IAT [**Primary endpoint**. time frame: day 365±14 after the transplant].
- Area Under the Curve (AUC) for the serum C-peptide level during the first 4 hours of an MMTT, normalized by the number of Islet Equivalent (IEQ)/kg [Time frame: day 75±14 and 365±14 after the transplant].
- Average daily insulin requirements [time frame: day 75±14 and 365±14 after the transplant].
- Basal (2 basal samples in the range between -20 to 0) to 240 min time course of glucose, C-peptide and insulin derived from the MMTT [time frame: day 75±14 and 365±14 after the transplant].
- β-cell function as assessed by β-score [time frame: day 75±14 and 365±14 after the transplant].
- The proportion of patients with an HbA1c ≤6.5% [time frame: day 365±14 after the transplant].
- Cumulative number of severe hypoglycemic events [time frame: from day 75±14 to day 365±14 after the transplant].
- The proportion of patients with an HbA1c ≤6.5% at day 365±14 **AND** are free of severe hypoglycemic events from day 75±14 to day 365±14 inclusive [time frame: day 365±14 and from day 75±14 to day 365±14 after the transplant].

Safety endpoints will be:

- Incidence and severity of Adverse Events and Serious Adverse Events [time frame: throughout the study up to day 365±14 after the transplant].

- Standard laboratory tests including hematology (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count), clinical chemistry (sodium, potassium, serum creatinine, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and coagulation (International Normalized Ratio (INR), partial thromboplastin time (PTT)) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- Vital signs, i.e. blood pressure (BP) and heart rate (HR) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- ALT/AST, INR/PTT, fibrin degradation products (XDPs), C-reactive protein (CRP) [time frame: all daily from day 1 up to day 7 after the transplant; ALT/AST also on day 75±14 after the transplant].
- Weight loss from pre-transplant value [time frame: day 75±14 and 365±14 after the transplant].
- Serum level of albumin and pre-albumin (absolute and change from pre-transplant value) [time frame: day 75±14 and 365±14 after the transplant].
- Severity of steatorrhea [time frame: day 75±14 and 365±14 after the transplant].
- Malnutrition risk (poor prognosis, significant risk, increased risk, normal) according to pre-albumin level [time frame: day 75±14 and 365±14 after the transplant].
- Cumulative number of episodes of documented hypoglycemia (documented symptomatic; asymptomatic) [time frame: from day 75±14 to day 365±14 after the transplant].
- Cumulative number of diabetic ketoacidosis-related events [time frame: from day 75±14 to day 365±14 after the transplant].

Exploratory endpoints will be:

- Time course of inflammatory chemokines/cytokines as assessed by serum level of CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), CXCL9 (MIG), IL-6, IL-10, INF-γ, TNF-α, and IL-1β [time frame: pre-infusion hospital admission (2 basal samples collected 6 to 24 hrs apart; both samples will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].
- Proportion of patients who are randomized but do not receive IAT [time frame: transplant day].
- Time course of serum microRNA-375 (miR-375) [time frame: pre-infusion hospital admission (sample will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].

Pharmacokinetic endpoints will be:

- Plasma levels of reparixin (total and unbound) and relevant metabolites (DF2243Y and ibuprofen) [time frame: post-operative day 1, 3, and 5 (steady state) in all patients; just prior to, and then at 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration in a subset of at least 20-24 patients].

Number of patients

The goal of this study is to reach a total of 100 patients who are randomized and receive IAT after total or completion pancreatectomy.

Inclusion/exclusion criteria

Patients aged ≥ 18 years given written informed consent who are eligible for an IAT following total (or completion) pancreatectomy will be included in this study.

Recipients of a previous IAT (if completion pancreatectomy) as well as patients undergoing total pancreatectomy due to pancreatic cancer or pancreatic benign diseases (e.g. pancreatic pseudocystis, insulinomas, etc.) will be excluded.

Patients who have inadequate renal reserve (calculated creatinine clearance < 60 mL/min according to the Cockcroft-Gault formula), hepatic dysfunction (increased ALT/AST > 3 x upper limit of normal **or** increased total bilirubin above upper limit of normal at local laboratory, except patients with Gilbert's syndrome), a preoperative International Normalized Ratio (INR) > 1.5 or any known coagulopathy, hypersensitivity to ibuprofen or to more than one non steroidal anti-inflammatory drug or to more than one medication belonging to the class of sulfonamides (e.g. sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib - hypersensitivity to sulphanilamide antibiotics alone, e.g. sulfamethoxazole, does not qualify for exclusion) will be also excluded.

Also, patients will be excluded from study participation if they have concurrent sepsis (as per positive blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome), have received a treatment with systemic steroids in the 2 weeks prior to enrolment (except for the use for physiological replacement only), or any investigational agent (including any anti-cytokine/chemokine agents) or any immune modulators in the 4 weeks prior to enrolment, if they have pre-existing diabetes or evidence of impaired β-cells function (pre-operative fasting blood glucose >115 mg/dL and/or a HbA1c > 6.5%) or require treatment with any anti-diabetic medication (e.g. insulin, metformin, etc) within the 2 weeks prior to enrollment, if they have past or current history of alcohol abuse, if they have evidence of pre-operative portal hypertension (clinical history **and** abdominal/liver imaging by ultrasound techniques).

Pregnant or breast-feeding women or patients unwilling to use effective contraceptive measures (females and males) will not participate in the study.

Investigational Product

The investigational drug will be reparixin or matching placebo. Reparixin will be administered as a continuous i.v. infusion into a (high flow) central vein at a dose of 2.772 mg/kg body weight/hour for 7 days (168hrs), starting approximately 12hrs (6-18 hrs) before pancreatic islet infusion. Placebo (0.9% w/v sodium chloride solution) will be infused at matching volume/rate.

Procedures

Each patient will be involved in the study for a 7 day hospital stay during pancreatectomy followed by islet transplantation, for all required measurements up to hospital discharge and for 2 post-transplant visits scheduled at day 75±14 and 365±14 after the transplant.

Statistics

All patient data collected on the CRF and on the Diary will be listed by patient, treatment group and centre. The data will be presented in the Clinical Study Report.

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

For the primary efficacy endpoint, the proportion of insulin-independent patients following IAT over the time frame of day 365±14 after the transplant, the differences in proportions will be assessed using the Pearson Chi-square statistic, stratified by the three groups at baseline: < 2500 IEQ/kg vs. 2500-5000 IEQ/kg vs. > 5000 IEQ/kg.

A Statistical Analysis Plan will be issued describing details of all the statistical methods and analysis to be applied to trial results. Any deviations from the original statistical plan will be described in the Clinical Study Report.

2. BACKGROUND INFORMATION

Reparixin is a specific inhibitor of CXC ligand 8 [CXCL8; formerly interleukin (IL)-8] biological activity, stemming from a program of drug design of molecules intended to modulate chemokine action.

Reparixin is the first low molecular weight blocker of CXCL8 biological activity in clinical development. Relevant pre-clinical, toxicological and phase 1 and 2 clinical data are summarized below. Please also refer to the Investigator's Brochure for more detailed information.

Reparixin (formerly repertaxin) was granted orphan drug designation for the "prevention of delayed graft function after (solid) organ transplantations" by the European Commission of Orphan Medicinal Products in September 2001 and by the Food and Drug Administration in January 2003. More recently orphan drug designation has been granted in EU (Septemebr 2011) for the "prevention of graft loss in pancreatic islet transplantation" and in the US (September 2012) for the "prevention of graft loss in islet cell transplantation".

2.1. RELEVANT NON-CLINICAL PHARMACOLOGY

Reparixin is *in vitro* a potent and specific inhibitor of CXCL8 biological activity. *In vitro* chemotaxis experiments have shown that reparixin inhibits CXCL8-induced chemotaxis of human polymorphonuclear leukocytes (PMN) in the low nanomolar range. Studies to elucidate the mechanism of action have shown that reparixin is a non-competitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2. Interaction of reparixin with CXCL8 receptors inhibits the intracellular signal transduction events activated by binding of CXCL8 to CXCR1 and CXCR2 [*Bertini, 2004; Allegretti, 2005*].

In vivo, reparixin prevented PMN infiltration into the transplanted kidney and reduced creatinin levels in a rat model of kidney transplantation. Similarly, in a rat model of lung transplantation, reparixin improved isolated graft oxygenation, decreased pulmonary oedema, and significantly reduced neutrophil infiltration into transplanted lungs. Moreover, reparixin prevented PMN infiltration and tissue damage in other animal models of ischemia/reperfusion injury of liver, brain, intestine, heart and spinal cord. In these models, *in vivo* inhibition of PMN recruitment ranged from 40 to 90%, and inhibition of tissue damage ranged from 50 to 80%. Efficacy was seen in all models at reparixin dose of 9.90 mg/kg [*Bertini, 2004; Cugini, 2005; Souza, 2004; Cavalieri, 2005; Garau, 2005; Villa, 2007; Gorio, 2007*].

More recently, reparixin lysine salt was evaluated in different models of intrahepatic pancreatic islet transplantation in mice, which include syngeneic and allogeneic settings. Reparixin was administered by s.c. continuous infusion for 7 or 14 days starting from day -1 of islet transplantation. A dose of 5.28 mg/kg/hour was administered in all experiments. Reparixin was able to significantly improve islet engraftment, as demonstrated by its ability to increase the likelihood of and to reduce the time to gain non-fasting blood glucose levels less than 200 mg/dl (normo-glycaemia) in marginal mass syngeneic islet transplantation model. In the fully mismatched allogeneic model, reparixin not only protected islets from early graft failure, but was also able to increase the time to rejection, as shown by post-transplant prolongation of normo-glycaemia. Graft function was indefinitely prolonged in 20/30% of mice treated with reparixin and rapamycin, suggesting possible tolerance induction. In parallel, reparixin treatment reduced intrahepatic infiltration of PMNs, macrophages, T helper and dendritic cells [*Citro, 2012a*].

Reparixin also delayed the onset of diabetes induced in mice by multiple low doses of streptozotocin. Also, mice treated with reparixin retained a better glycemic control (lower glycemic levels) even after diabetes development [*Citro, 2012b*].

2.2. A SUMMARY OF TOXICOLOGY DATA

Reparixin was tested for toxicity in rodent and non-rodent animal species after single and repeated i.v. doses. The repeated dose administration studies were conducted by i.v. continuous infusion, according to the intended human administration route.

The general toxicological profile of i.v. reparixin, in the studies conducted to date, is characterized by a low toxicity after single or repeated dose administrations in rats (LD_{50} = 229.68 mg/kg i.v.; 660.00 mg/kg/day as No Observed Adverse Effect Level from 4 weeks studies) and mice (401.94 mg/kg i.v.). Continuous i.v. administration to dogs for 2 weeks resulted in a safe dose of 39.60 mg/kg/day.

Continuous i.v. infusion of reparixin to the male and female rat at dose levels of up to 660.00 mg/kg/day did not have any significant adverse effects on mating performance and fertility.

Reparixin poses no genotoxic hazard for humans.

Reparixin lysine salt, at doses in excess of those intended to be used in humans, has a safe pharmacology profile in the renal, cardiovascular and respiratory systems of rats and dogs.

The local tolerability of reparixin lysine salt was assayed in the rabbit ear lateral vein. The compound was well tolerated in concentrations up to 4.95 mg/mL (1 mL/kg) infused over a minute.

In order to provide evidence of the safety of DF2243Y, the main metabolite of reparixin excreted in urine in humans, safety pharmacology and toxicity studies have been performed at doses 2 to 3 times higher than those reached in man, as may occur during the treatment of patients receiving kidney transplantation.

2.3. PHARMACOKINETICS AND PRODUCT METABOLISM

PK studies by i.v. injection revealed that reparixin is very rapidly eliminated in rats and humans ($t_{1/2}$ 0.5-3hrs and 1.0-1.5hrs, respectively) whereas elimination is slower in dogs (12-28hrs). The PK of reparixin was linear in rats and in dogs but linearity was less evident in humans.

Reparixin undergoes complete metabolism (oxidation + conjugation) in all the species tested. The *in vitro* human hepatic, phase I metabolism of reparixin is catalysed by CYP2C9 and to a lesser extent by CYP2C19. DF2243Y, DF2188Y, methanesulfonamide and ibuprofen are the metabolites detected in human plasma and urine, with DF2243Y being the major metabolite. Exposure to ibuprofen after administration of reparixin 2.77 mg/kg/h for 48hrs (the highest dose tested in humans) was similar or lower than that obtained after a standard therapeutic single dose of ibuprofen (300mg).

Due to extensive metabolism, unchanged reparixin was poorly or not excreted into the urine of rat, dogs and humans so that the PK profile of reparixin is not influenced by renal impairment.

In vitro protein binding of [^{14}C]-reparixin showed that reparixin is highly bound (approximately 99%) to plasma proteins in rats, dogs, rabbits, cynomolgus monkeys and humans. Albumin is likely to be the major binding protein in plasma in all species, accounting for 99.2% in humans.

Reparixin has some potential *in vitro* for a non-competitive inhibition of the human hepatic enzyme CYP3A4 that is involved in the metabolism of cyclosporine A, tacrolimus and rapamycin. However, since inhibition is evident at concentration far higher than the free plasma concentration of reparixin at steady state in humans, it is predicted that the clinical relevance of such inhibition is remote. Indeed, reparixin does not affect to a clinically relevant extent the activity of CYP3A4 and CYP2C9 (enzyme involved in reparixin metabolism), as revealed by an interaction study where the PK of midazolam and tolbutamide (probe substrates for these enzymes) was evaluated in healthy subjects receiving single oral doses of the probes alone or in combination with reparixin.

2.4. A SUMMARY OF CLINICAL DATA

Clinical development includes phase 1 pharmacokinetics, tolerability and pharmacodynamic studies and 2 phase 2 studies conducted in lung and kidney transplant patients. An independent study was also carried out in cardiopulmonary bypass (CPB) patients.

To date, a total of 363 subjects were involved in completed clinical studies, of whom 230 were exposed to reparixin.

A pilot phase 2 study is ongoing in islet transplantation and is addressed below.

2.4.1. Pharmacokinetics and product metabolism in humans

Three phase 1 PK/safety studies were performed which included 30min to 48h i.v. infusion of ascending doses in healthy male subjects and 6h i.v. infusion of a selected dose in male and female subjects with normal to severely impaired renal function [*REP0101; REP0102; REP0203*]. An additional interaction study was also conducted in healthy subjects [*REP0103*]. PK results are discussed in paragraph 2.3.

2.4.2. Efficacy

In the first US-Canada phase 2 study [*REP0104*], 101 patients (46 on reparixin, 55 on placebo) undergoing single or bilateral lung transplant were included in the intent to treat (ITT) and safety population out of 114 enrolled. The patients were randomized to receive 48h i.v. continuous infusion (loading: 4.488 mg/kg/h for 30min, maintenance: 2.772 mg/kg/h for 47.5hrs) of either reparixin or placebo starting a few hours before the transplant. The study failed to show any statistically significant difference between the reparixin and placebo groups in the primary efficacy variable (PaO_2/FiO_2 ratio) and in any of the secondary efficacy variables assessed up to month 12 post-transplant, except survival. Indeed, there was a statistically significant difference in patient survival at Month 12 post-transplant between the placebo (7 deaths) and reparixin (no deaths) groups (p-value = 0.0111 [Log-Rank]).

The second phase 2 study [*REP0204*] was conducted in US, Italy, Spain and France and involved patients undergoing kidney transplantation at increased risk of developing Delayed Graft Function. This was a pilot study designed to explore the efficacy and safety of two different dosing schedules selected to minimize the impact of possible post-transplant renal dysfunction on reparixin PK. Out of 80 patients randomized, 74 patients (25 reparixin continuous infusion; 23 reparixin intermittent infusion; 26 pooled placebo) and 73 patients (24 reparixin continuous infusion; 23 reparixin intermittent infusion; 26 pooled placebo) were included in the safety and ITT analysis, respectively. The patients were randomized to receive reparixin or placebo as continuous infusion of 2.772 mg/kg/h for 12hrs or 12 intermittent i.v. infusions of 2.244 mg/kg for 30min with 1.5 hour intervals over a total period of 22.5hrs or matched placebo. Overall, the study failed to show a significant difference between the treatment groups in the primary efficacy variable (early CLcr) and in any of the secondary efficacy variables assessed up to month 12 post-transplant. However, a trend for a better graft function, measured by early (1-3 and 10-12hrs after transplant) CLcr and day 1 to 7 serum Cr was observed in the reparixin intermittent infusion group, which however did not reach a statistical significance. Also a higher, even not significant, percentage of patients in this group had immediate graft function, with only one patient experiencing graft failure.

The Medical University of Vienna conducted an additional independent placebo-controlled, randomized pilot trial in 32 patients undergoing elective coronary artery bypass grafting with CPB. Reparixin was infused at a loading dose of 4.488 mg/kg/h for 30min followed by a maintenance dose of 2.772 mg/kg/h for 8h. The rise in neutrophil count after CPB was less marked in the reparixin than in the placebo group ($p < 0.05$). Significant group differences were also detected at 4 hours post-CPB.

A phase 2 multicenter, randomized, open-label, parallel-assignment, pilot study is ongoing and is assessing the efficacy and safety of reparixin following a single-infusion islet allo-transplantation [REP0110; NCT01220856]. Patients are on an immunosuppression regimen consisting of induction with ATG followed by maintenance immunosuppression. Patients are randomly (1:1) assigned to receive either no additional experimental intervention (control group) or reparixin treatment (2.772 mg/kg body weight/hour i.v. continuous infusion for 7 days) – treatment group). Inclusion criteria restrict enrolment to patients who are expected to receive an islet mass (4000 to 7000 islet equivalent (IEQ)/kg body weight) in the lower range of the currently accepted transplantable islet amount.

Due to these preliminary results obtained in 7 patients, protocol REP0110 was amended to allow randomization to the reparixin treatment group only. Also, patients already treated with reparixin with a functioning graft were allowed to receive a 2nd islet infusion. Follow-up was re-scheduled to provide measurements up to one year after the 2nd islet infusion [*Citro, 2012a*].

A total of 9 patients have been enrolled: 6 have been randomized to reparixin treatment and 3 to the control group. Preliminary data for the measurements at month 1 post-1st infusion are summarized in the table below.

REP0110 – Summary preliminary data (demographics and month 1 measurements)

	Reparixin (n=6)	**Control** (n=3)
Gender (F/M) - Age (mean±SD)	3/3 - 46.2±8.8	2/1 - 48.0±7.0
Total IEQ/kg (mean±SD)	4911±897	4528±398
Peak C-peptide ng/mL (mean±SD)	1.47±1.37	0.20±0
C-peptide AUC (MMTT) normalized by IEQ/kg (meanx10^{-4}±SD)*	1.92±1.62	0.44±0.04
Insulin requirement IU/kg/day [percent decrease] (mean±SD)	0.42±0.32 [-41.87±39.89]	0.62±0.34 [0±0]
HbA1c % [percent decrease] (mean±SD)	7.60±1.07 [16.72±4.46]	7.03±1.02 [11.17±5.40]
β-score (mean±SD)	1.83±1.17	1.33±1.53
Transplant Estimated Function (mean±SD) (*Caumo, 2008*)	111.93±64.09	13.73±49.39
Number of patients with graft loss	2	3

* AUC has been calculated on the -10 to 120 min time course of C-peptide during an MMTT. Where the C-peptide value was below the detection limit (0.2 ng/mL), a value of 0.2 ng/mL has been used for calculation.

None of the patients in the control group expressed a β-cell function at month 1 post-transplant (no decrease in insulin requirement coupled with C-peptide levels < 0.3 ng/mL) and all were withdrawn due to graft loss. On the other hand, all but two of the patients treated with reparixin experienced improved transplant outcome as measured by glycemic control, decreased insulin requirement and appearance of detectable levels of C-peptide above 0.3 ng/mL. Out of 4 patients who have received a 2nd infusion to date, 3 have achieved insulin-independence, with two patients retaining insulin-independence 1 year post last (2nd) transplant.

The development of reparixin in islet allo-transplantation is being progressed and a phase 3 clinical trial is ongoing in EU and US [REP0211; NCT01817959].

2.4.3. Safety

A total of 230 subjects have been exposed to reparixin in the clinical studies completed to date which include 103 adult healthy subjects (100M/3F), 17 patients with different grades of renal impairment (12M/5F), 16 patients undergoing cardiopulmonary by pass (10M/6F), 46 patients undergoing lung transplantation (23M/23F) and 48 patients undergoing kidney transplant (31M/17F). Exposure included short or prolonged i.v. infusion up to 10.6 mg/kg over 30min or 133 mg/kg over 48h.

Overall, reparixin was safe and well tolerated in both healthy subjects and critically ill patients.

In phase 1 studies, no deaths, Serious Adverse Events (SAEs) or Adverse Event (AE)-related withdrawals were reported. The majority of AEs reported were of mild intensity. All subjects had recovered completely or had ongoing adverse events of mild intensity when they were discharged. The safety of reparixin was confirmed also in patients with different grades of renal impairment. In the interaction study no safety concerns were raised during co-administration of midazolam/tolbutamide with reparixin.

During phase 2 studies, AE and SAE profile was similar for both placebo and reparixin groups and no particular safety concerns were raised.

In the lung transplant study there were no AE-related withdrawals or deaths for either placebo or reparixin groups. A total of 28 patients experienced SAEs. Death occurred in 7 patients, all in the placebo group. Death was due to infections in 4 patients leading to sepsis/bacteraemia (3 patients) or pneumonia (1 patient). One patient died due to a cardiovascular accident. Coronary artery disease or unknown origin was the cause of death reported for the other 2 patients for whom minimal information was available.

In the kidney transplant study there was 1 AE-related withdrawal in the reparixin continuous infusion group. AEs leading to discontinuation of study drug were reported for 3 patients in the reparixin continuous infusion group and for 1 patient in the placebo group. One patient in the placebo group had an AE (septic shock, pneumonia) leading to death. SAEs were reported in 21 patients. Death occurred in 2 patients, 1 in the placebo group (septic shock, pneumonia) and 1 in the reparixin intermittent infusion group (no information available).

No SAEs were reported from the CPB study.

As to Adverse Drug Reactions (ADRs), infusion site reactions, mainly erythema or aseptic thrombophlebitis, were one of the most common ADRs. The relatively high frequency was due to a cluster of events occurring in cohort 1 of the phase 1 48h-infusion study. Local toxicity was clearly related to drug concentration since the use of a more diluted solution at a higher infusion rate markedly reduced the incidence, type and severity of infusion site reactions.

Cumulative summary tabulations of ADRs are reported in **Appendix 14.1**.

The most frequent (>10%) ADRs observed in the phase 1 and phase 2 studies were:

Nervous system disorders (about 27%), including headache, dizziness, hypoaesthesia, somnolence.

General disorders and administration site conditions (about 23%), including cannula site reaction, injection site thrombosis, infusion site oedema and peripheral oedema, fatigue, lethargy.

Gastrointestinal disorders (about 14%), including nausea, vomiting, abdominal pain, dyspepsia, flatulence, gastroesophageal reflux disease.

Overall, there was no correlation between the incidence of ADRs and the extent of exposure. Moreover, the incidence of ADRs was variable among studies regardless of the clinical condition of the population exposed to reparixin, i.e. patients under critical care (lung/kidney transplant or CPB patients) did not experience increased frequency of adverse events attributable to reparixin.

Preliminary data obtained in the ongoing pilot trial in islet allo-transplantation further support the safety of reparixin in this clinical setting. In patients treated with a 7 day course of reparixin a few AEs were reported which were judged to be at least possibly related to reparixin treatment. Most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these were mild to moderate in nature and none required discontinuation if the Investigational Product. A total of 5 SAEs were reported to date in 4 patients. Two patients (one randomized to the control group, one to the reparixin group) experienced intraperitoneal bleeding which was judged to depend on the islet infusion procedure (not-related to the study treatment) in both cases. One patient who had received reparixin during the first islet transplant was hospitalized for diabetic ketoacidosis (due to graft failure) and diarrhea (possibly due to MMF administration). Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion; upon case evaluation, a medical error was evidenced, i.e. the patient received a dose of reparixn 3 times as high as that foreseen in the protocol; infusion of reparixin was immediately discontinued and the patient recovered. About 2 months later, this same patient required hospitalization for diarrhea and worsening of kidney function which were due to immunosupressive medications (MMF, tacrolimus).

2.5. DISEASE REVIEW AND STUDY RATIONALE

Total pancreatectomy is a surgical procedure to treat end-stage pancreatic diseases, including, but not limited to, chronic pancreatitis. Despite improvement in surgical techniques, total pancreatectomy is associated with long term complications which are mostly accounted for by endocrine insufficiency. While exocrine insufficiency is considered easier to overcome, the ensuing surgical brittle diabetes presents with a high risk of hypoglycemic unawareness that is a leading cause of patient death [*Sarr, 1993; Berney, 2000; Gruessner, 2004; Garcea, 2009*].

After total pancreatectomy, the absence of endogenous insulin is coupled with the lack of other regulatory hormones, such as glucagon, resulting in the well known poor post-operative diabetes control [*Farley, 1995; Linhean, 1988*]. Segmental pancreatic or islet auto-transplantation (IAT) has therefore been considered since the 1970s as feasible adjunctive procedures to prevent severe post-surgical diabetes in patients undergoing total pancreatectomy for chronic pancreatitis. IAT offers advantages over heterotopic pancreas auto-transplantation as intraportal islet infusion is a smaller procedure and should be favoured whenever the patient has lost pancreatic exocrine function [*Sutherland, 1978; Hogle, 1978; Gruessner, 2004; Dong, 2011*].

Despite β-cell mass, as estimated by Islet Equivalent (IEQ)/kg body weight, is less than for islet allografts, long term successful auto-graft function can be achieved in most patients, with a rate of insulin independence as high as >80% of recipients, even if lower figures have been reported, according to the total amount of IEQ infused [*Sutherland, 2012; Pollard, 2011; Dong, 2011*].

In addition to end-stage chronic pancreatitis, IAT post pancreatectomy has been utilized for an expanded series of pancreatic benign diseases including, pancreatic pseudocystis, insulinomas, etc. More recently, patients bearing pancreatic cancer were admitted to IAT with no evidence of hepatic recurrence of pancreatic disease up to almost one year follow-up, offering some neoplastic patients a chance for post-pancreatectomy glycemic control [*Dong, 2011; Balzano, 2011*].

In contrast to allo-transplantation in type 1 diabetes patients, where immunological mechanisms involving allo- and auto-antibodies affect long-term graft function, outcome in IAT is independent from immunological processes and does not require immunosuppression management. On the other hand, early inflammatory events intrinsic to the intra-portal islet infusion have been demonstrated to impact islet engraftment. Among possible mechanisms, PMNs have been found to be the predominant cell types infiltrating the liver in a syngeneic model of islet transplantation in mice. Also, the intra-hepatic mRNA for CXCL1/KC, the murine counterpart of human CXCL8, was strongly induced immediately after islet infusion. Consistent with this results, transplanted CXCR2 knock out mice had a significant improvement in islet function [*Citro, 2011*]. Thus, CXCL8 might represent a relevant therapeutic target to prevent early graft failure after IAT.

Data obtained in experimental models of islet transplantation in mice demonstrate a clear effect of reparixin in improving graft survival and function. Protection from the loss and/or deterioration of transplanted islets was evident regardless of the immunological mechanisms involved in islet damage, suggesting that the ability of reparixin to modulate early inflammatory responses readily impact graft outcome. Results obtained with reparixin in the experimental model of multiple low doses of streptozotocin further emphasize the ability of reparixin to protect β-cells from an inflammatory damage which appears to impact subsequent graft outcome.

Thus, the use of reparixin may emerge as a potential useful medication in the control of non specific inflammatory events surrounding the early phases of IAT.

Preliminary data obtained in transplanted patients recruited in the ongoing pilot trial coupled with the safety shown in human phase 1 and 2 studies provide a sound rationale for further development of reparixin also in IAT and prompt the conduct of this phase 2/3 clinical study aimed at assessing the efficacy and safety of reparixin in improving graft outcome in patients undergoing total (or completion) pancreatectomy followed by IAT.

2.5.1. Selection of dose and treatment schedule in the study

The dose of reparixin proposed for this trial (2.772 mg/kg body weight/hour) is the same being administered in the ongoing clinical trials in islet allo-tranplantation. It was originally derived from the effective reparixin concentrations found both in "in vitro" inhibition of CXCL8-induced chemotaxis of human PMN and in experiments in mouse models of syngeneic and allogeneic transplantation. Such a dose was found safe in previous phase 1 and 2 studies. Also, preliminary data obtained in the ongoing pilot trial in islet allo-transplantation confirm the efficacy and the safety of such a dose.

Consistent with experimental data in animals and preliminary data in the ongoing pilot trial in islet allo-transplantation, the proposed schedule of administration (continuous infusion for 7 days) is intended to expose the patient to reparixin throughout the time-window of relevant inflammatory post-transplant events after both allo-transplantation and IAT [*Pollard, 2011; Citro, 2011*].

2.5.2. Alternative treatments

There are no specific pharmacologic treatments addressed to the prevention of graft failure after IAT. A trial is currently ongoing to test the potential effect of sitagliptin in IAT (NCT01186562). Because of this, patients not willing to participate in the study may either be offered to participate in another trial of experimental treatment or will not be offered any specific alternative treatment.

All patients, regardless of study participation, will receive the standard of optimal care for the transplanted organ (procurement, storage, isolation, infusion) and the recipient.

2.5.3. Risk - benefit evaluation

2.5.3.1. General risks

Transplantation of islets is associated with several potential risks. These are intrinsic to the procedure and may result from the intra-hepatic islet infusion as well as testing involved in the care and evaluation of transplanted subjects. All patients receiving IAT are at risk of complication regardless of study participation.

2.5.3.2. Risk related to the Investigational Product

Results from preclinical studies support the level of drug exposure planned in this study. Also, past clinical experience with the same dose as planned in this study provides a clear cut evidence of reparixin safety. Very few SAEs were reported with this dose even from clinical trials conducted in severe clinical conditions such as patients undergoing lung or kidney transplantation.

Preliminary data obtained in the ongoing pilot trial in islet allo-transplantation further support the safety of reparixin in this clinical setting. In patients treated with a 7 day course of reparixin a few AEs were reported which were judged to be at least possibly related to reparixin treatment. Most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these were mild to moderate in nature and none required discontinuation if the Investigational Product. A total of 5 SAEs were reported to date in 4 patients. Two patients (one randomized to the control group, one to the reparixin group) experienced intraperitoneal bleeding which was judged to depend on the islet infusion procedure (not-related to the study treatment) in both cases. One patient who had received reparixin during the first islet transplant was hospitalized for diabetic ketoacidosis (due to graft failure) and diarrhea (possibly due to MMF administration). Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion; upon case evaluation, a medical error was evidenced, i.e. the patient received a dose of reparixn 3 times as high as that foreseen in the protocol; infusion of reparixin was immediately discontinued and the patient recovered. About 2 months later, this same patient required hospitalization for diarrhea and worsening of kidney function which were due to immunosupressive medications (MMF, tacrolimus).

The very short half life of reparixin ($t_{1/2}$ 1.0-1.5hrs) represents an important safety factor as plasma levels decline rapidly after drug discontinuation. This PK profile, coupled with the mechanism of reparixin action (reversible inhibition), makes readily effective appropriate stopping rules.

Administration via a high flow central vein, which was proven to be safe in lung and kidney recipients, as well as in patients undergoing islet transplant, is proposed for the study to minimize the risk of infusion-site toxicity seen in a cohort of volunteers receiving i.v. infusion via a peripheral vein.

2.5.3.3. Central line

The study drug will be administered as a continuous i.v. infusion over 7 days with a catheter inserted in a (high flow) central vein. The risks of a central line are not considered study-related risks since a central line is routinely placed in patients undergoing post-pancreatectomy IAT for their routine clinical care. This required access will be maintained for a period of time that might be slightly longer than that routinely used. Nevertheless, central line care as routinely practiced by the participating sites will minimize the risk of thrombosis and infections.

2.5.3.4. Blood sampling

In addition to the routine blood sampling, participation in the study will require an amount of blood slightly higher than routine sampling at some time points (not exceeding 5mL at each time point), to allow study specific measurements and centralized assay of C-peptide, HbA1c, inflammatory chemokines/cytokines, miR-375 and PK samples.

2.5.3.5. Other study related procedures

No other procedures specifically associated to the study are required. Type, timing and frequency of some measurements, e.g. stimulation test by a Mixed Meal Tolerance Test (MMTT), might be guided by the protocol, but such measurements are basically part of standard patient care and would be performed regardless of study participation.

2.5.3.6. Potential benefit

To the patients: According to preliminary data obtained in islet allo-transplantation, patients receiving reparixin are likely to benefit with improved auto-graft function, but this is to be confirmed. Patients assigned to the control arm are expected to obtain no additional benefit other than that established in IAT recipients.

To society: This study may identify a useful medication that will make post-pancreatectomy IAT more effective for future recipients.

3. OVERALL STUDY DESIGN AND PLAN DESCRIPTION

3.1. STUDY DESIGN

The study will be a phase 2/3, multicenter (US), randomized, double-blind, placebo-controlled, parallel assignment pilot study.

The goal of this study is to reach a total of 100 adult patients who are randomized and receive IAT after total or completion pancreatectomy. Patients will be randomly (1:1) assigned to receive either reparixin [continuous i.v. infusion for 7 days (168hrs)], or matched placebo (control group),starting approximately 12hrs before islet infusion. The two groups will be balanced within each centre. All patients who are randomized and receive the Investigational Product (either reparixin or placebo) will be inlcuded in the ITT analysis. Patients will be in the ITT analysis whether or not they receive IAT, because exclusions cannot be made for events occurring after randomization that could be influenced by the randomized assignment.

Recruitment will be competitive among the study sites, until the planned number of patients is enrolled. Competitive recruitment has been chosen to increase the speed of recruitment and to account for any difference in transplant rate among study sites. Each centre will enroll patients as rapidly as possible, up to a maximum of 40 patients (as per the randomization list). A maximum of 48 patients is allowed for the site of the Primary Investigator. Each patient will be involved in the study for 7 day hospital stay during pancreatectomy followed by islet transplantation, for all required measurements up to hospital discharge and for 2 post-transplant visits scheduled @ day 75$\pm$14 and 365$\pm$14 after the transplant.

3.2. STUDY TIME TABLE

The planned patients are expected to be recruited in a 12 month period. A follow-up of 12 months is planned for each patient. Overall study timelines are reported below.

- Projected starting date (first-patient-in): December 2013
- Projected completion of patient accrual (last-patient-in): March 2015
- Projected study end date (last-patient-last-visit): March 2016

3.3. END OF STUDY

For the purpose of this trial, the end of study is defined as the date of the last visit of the last patient.

4. OBJECTIVES AND ENDPOINTS

4.1. STUDY OBJECTIVES

The objective of this clinical trial is to assess whether reparixin leads to improved transplant outcome as measured by the proportion of insulin-independent patients following IAT. The safety of reparixin in the specific clinical setting will be also evaluated

4.2. STUDY ENDPOINTS

4.2.1. Primary efficacy endpoint

- The proportion of insulin-independent patients following IAT [time frame: day 365±14 after the transplant].

  For the purpose of this protocol, insulin-independence is defined as freedom from the need to take exogenous insulin for 14 or more consecutive days, with adequate glycemic control, as defined by:
  - a glycated hemoglobin (HbA1c) level ≤6.5%;
  - fingerstick fasting blood glucose not exceeding 126 mg/dL more than three times in the past week (based on a minimum of one daily measurement;
  - a 2 hour post-prandial blood glucose not exceeding 180 mg/dL more than four times in the past week (based on a minimum of one daily measurement);
  - a laboratory fasting glucose in the non-diabetic range (<126 mg/dL).

4.2.2. Secondary efficacy endpoints

- Area Under the Curve (AUC) for the serum C-peptide level during the first 4 hours of an MMTT, normalized by the number of Islet Equivalent (IEQ)/kg [Time frame: day 75±14 and 365±14 after the transplant].
- Average daily insulin requirements [time frame: day 75±14 and 365±14 after the transplant].

  For the purpose of this protocol, daily insulin is reported as IU/kg and intake averaged over the previous week.
- Basal (2 basal samples in the range between -20 to 0) to 240 min time course of glucose, C-peptide and insulin derived from the MMTT [time frame: day 75±14 and 365±14 after the transplant].
- β-cell function as assessed by β-score [time frame: day 75±14 and 365±14 after the transplant].
- The proportion of patients with an HbA1c ≤6.5% [time frame: day 365±14 after the transplant].
- Cumulative number of severe hypoglycemic events [time frame: from day 75±14 to day 365±14 after the transplant].

  For the purpose of this protocol, a severe hypoglycemic event is defined as an event with one of the following symptoms: "memory loss, confusion, uncontrollable behavior, irrationale behavior, unusual difficulty in awakening, suspected seizure, seizure, loss of consciousness, or visual symptoms", in which the subject was unable to treat him/herself and which was associated with either a blood glucose level <54mg/dL or prompt recovery after oral carbohydrate, i.v. glucose, or glucagon administration.
- The proportion of patients with an HbA1c ≤6.5% at day 365±14 **AND** are free of severe hypoglycemic events from day 75±14 to day 365±14 inclusive [time frame: day 365±14 and from day 75±14 to day 365±14 after the transplant].

4.2.3. Safety endpoints

- Incidence and severity of Adverse Events and Serious Adverse Events [time frame: throughout the study up to day 365±14 after the transplant].

- Standard laboratory tests including hematology (hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count), clinical chemistry (sodium, potassium, serum creatinine, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and coagulation (International Normalized Ratio (INR), partial thromboplastin time (PTT)) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- Vital signs, i.e. blood pressure (BP) and heart rate (HR) [time frame: pre-transplant hospital admission and post-transplant hospital discharge].
- ALT/AST, INR/PTT, fibrin degradation products (XDPs), C-reactive protein (CRP) [time frame: all daily from day 1 up to day 7 after the transplant; ALT/AST also on day 75±5 after the transplant].
- Weight loss from pre-transplant value [time frame: day 75±14 and 365±14 after the transplant].
- Serum level of albumin and pre-albumin (absolute and change from pre-transplant value) [time frame: day 75±14 and 365±14 after the transplant].
- Proportion of patients falling into one of the following levels of steatorrhea severity [time frame: day 75±14 and 365±14 after the transplant].

  For the purpose of this protocol, levels of steatorrhea severity (evaluated in the 4 weeks prior to day 75±14 and 365±14), are defined as:
  - No steatorrhea;
  - Steatorrhea few times per week;
  - Steatorrhea daily;
  - Stool incontinence.
- Proportion of patients falling into one of the following malnutrition risk levels (poor prognosis, significant risk, increased risk, normal) according to pre-albumin level [time frame: day 75±14 and 365±14 after the transplant].

  For the purpose of this protocol, malnutrition risk levels are defined as [adapted from *Bernstein, 1995*]:
  - Poor prognosis = pre-albumin level <5.0 mg/dL
  - Significant risk = pre-albumin level 5.0 to 10.9 mg/dL
  - Increased risk = pre-albumin level 11.0 to 15.0 mg/dL
  - Normal = pre-albumin level > 15.0 (up to 35.0) mg/dL
- Cmulative number of episodes of documented hypoglycemia (documented symptomatic; asymptomatic) [time frame: from day 75±14 to day 365±14 after the transplant].

  For the purpose of this protocol, the following definition applies [*Diabetes Care, 2005*]:
  - Documented symptomatic hypoglycemia = An event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration ≤70mg/dL.
  - Asymptomatic hypoglycemia = An event not accompanied by typical symptoms of hypoglycemia, but with a measured plasma glucose concentration ≤70mg/dL.
- Cumulative number of diabetic ketoacidosis-related events [time frame: from day 75±14 to day 365±14 after the transplant].

  For the purpose of this protocol, a diabetic ketoacidosis event is defined as the presence of [*Pediatrics, 2004*]:
  - hyperglycemia (blood glucose >200 mg/dL);
  - pH <7.3 or HCO3 <15;
  - ketones positive in the serum or urine.

4.2.4. Exploratory endpoints

- Time course of inflammatory chemokines/cytokines as assessed by serum level of CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), CXCL9 (MIG), IL-6, IL-10, INF-γ, TNF-α, and IL-1β [time frame: pre-infusion hospital admission (2 basal samples collected 6 to 24 hrs apart;

both samples will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].

- Proportion of patients who are randomized but do not receive IAT [time frame: transplant day].
- Time course of serum microRNA-375 (miR-375) [time frame: pre-infusion hospital admission (sample will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion].

4.2.5. Pharmacokinetic endpoints

- Plasma levels of reparixin (total and unbound) and relevant metabolites (DF2243Y and ibuprofen) [time frame: post-operative day 1, 3, and 5 (steady state) in all patients; just prior to, and then at 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration in a subset of at least 20-24 patients]. This number of patients should guarantee post-treatment sampling in at least 10-12 patients randomized to reparixin (base on a 1:1 randomization). These patients will be enrolled at 2 of the participating sites.

5. STUDY POPULATION

The goal of this study is to reach a total of 100 patients who are randomized and receive IAT after total (or completion) pancreatectomy, each being randomised (1:1) to receive either reparixin or placebo.

Patients will be selected from those eligible for IAT following total or completion pancreatectomy. In addition to locally accepted eligibility criteria for the IAT (see below), each prospective patient will be randomized provided that (s)he fully meets all of the study-specific Inclusion Criteria and none of the study-specific Exclusion Criteria described in Sections 5.1. and 5.2. below.

5.1. INCLUSION CRITERIA

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

1. Patients eligible for an IAT following total (or completion) pancreatectomy.

Eligibility to both total (or completion) pancreatectomy and IAT will be based on locally accepted criteria and guidelines (see also exclusion below). The most restrictive criteria will be applied.

2. Ages ≥ 18 years.

3. Patients willing and able to comply with the protocol procedures for the duration of the study, including scheduled follow-up visits and examinations.

4. Patients who have given written informed consent, prior to any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to their future medical care.

5.2. EXCLUSION CRITERIA

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

1. Recipients of a previous IAT (if completion pancreatectomy).

2. Patients undergoing total pancreatectomy due to either pancreatic cancer or pancreatic benign diseases other than chronic pancreatitis, including insulinomas, etc.

3. Patients with inadequate renal reserve as per calculated creatinine clearance (CLcr) < 60 mL/min according to the Cockcroft-Gault formula (*1976*).

4. Patients with hepatic dysfunction as defined by increased ALT/AST > 3 x upper limit of normal (ULN) **or** increased total bilirubin above the upper limit at local laboratory). Patients with Gilbert's syndrome (elevated unconjugated bilirubin levels in the absence of any evidence of hepatic or biliary tract disease) are not excluded.

5. Patients with a preoperative International Normalized Ratio (INR) > 1.5 or any known coagulopathy.

6. Hypersensitivity to:

a) ibuprofen or to more than one non steroidal anti-inflammatory drug (NSAID).

b) more than one medication belonging to the class of sulfonamides, such as sulfamethazine, sulfamethoxazole, sulfasalazine, nimesulide or celecoxib; hypersensitivity to sulphanilamide antibiotics alone (e.g. sulfamethoxazole) does not qualify for exclusion.

7. Concurrent sepsis (as per positive blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome).

8. Treatment with systemic steroids in the 2 weeks prior to enrolment (except for the use of ≤5mg prednisone daily or equivalent dose of hydrocortisone, for physiological replacement only) or with any immune modulators in the 4 weeks prior to enrolment.

9. Patients with pre-existing diabetes or evidence of impaired β-cells function, based on pre-operative fasting blood glucose >115 mg/dL and/or a HbA1c > 6.5%, or requiring treatment with any anti-diabetic medication (e.g. insulin, metformin, etc) within the 2 weeks prior to enrolment.

10. Use of any investigational agent in the 4 weeks prior to enrolment, including any anti-cytokine/chemokine agents.

11. Pregnant or breast-feeding women; unwillingness to use effective contraceptive measures (females and males). (NB: pregnancy should be avoided in patients or partners during the first month after completing the treatment with the Investigational Product; no other specific warnings are described, considering the treatment course of the Investigational Product, its PK profile, and the lack of significant adverse effects on mating performance and fertility in animal studies).

12. Patients with past or current history of alcohol abuse based on clinical history and/or past treatment for alcohol addiction.

13. Patients with evidence of pre-operative portal hypertension as per clinical history **and** abdominal/liver imaging by ultrasound techniques.

Sites will comply with any additional or more restrictive exclusion criteria locally accepted, as per centre practice.

5.3. ASSIGNMENT OF PATIENT NUMBER

Patient number will be assigned in a sequential manner as a patient is found to be eligible for entry into the study and is randomized. It will consist of the site number, and the patient number e.g. 0102, where first 2 digits represent site number, last 2 digits patient number. If a patient is dropped from the study for any reason, the patient's number will not be reassigned.

6. INVESTIGATIONAL PRODUCT

The Investigational Product will be either reparixin [R(-)-4-Isobutyl-alpha-methylphenylacetyl-methanesulfonamide] or matched placebo.

6.1. PRESENTATION, STORAGE, PACKAGING AND LABELING

6.1.1. Presentation of Investigational Products

Reparixin will be provided as concentrate for solution for i.v. infusion packaged into 250 mL clear Glass Vials with the following composition per single (250 mL) unit:

Reparixin composition - 33mg/mL

NAME OF INGREDIENT	PER-UNIT FORMULA	FUNCTION OF INGREDIENT	REFERENCE TO QUALITY STANDARDS
Reparixin (DF1681Y)	8.25 g	Drug substance	Manufacturer monograph and specification
Sodium Dihydrogen Phosphate Dihydrate	1.96 g	Buffer	European Pharmacopoeia - current edition
L-lysine monohydrate	4.78 g	Solubilizer	German Pharmacopoeia - current edition
Sodium hydroxide	qs to pH 8.0	Buffer	European Pharmacopoeia - current edition
Water for injections	qs to 250 mL	Solvent	European Pharmacopoeia - current edition

Placebo will be physiologic salt solution (0.9% w/v sodium chloride solution) provided as commercially available 250 mL clear Glass Vials.

Batch release certificate will be provided together with the Investigational Products.

6.1.2. Manufacturing, Packaging and Labelling of Investigational Product

Reparixin will be manufactured by Patheon (Italy). Both Investigational products will be packaged and labelled by an authorized facility which will be identified before trial commencement.

Reparixin and placebo vials will be supplied along with sterile empty Infusion Bags and corresponding Infusion Bag labels.

All the materials will have trial-specific labels. Details of packaging and labelling are reported in **Appendix 14.2**.

6.1.3. Supply, Storage and Handling of Investigational Product

An appropriate number of packages will be initially sent to the site as soon as all essential documents and FDA/ethics approvals have been obtained. Additional supplies (reparixin, placebo, Infusion Bags) will be sent on demand, according to enrolment rate.

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

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

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

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

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

Pharmacists will be provided with the 'Instructions to the Pharmacy', a booklet detailing instructions for Investigational Product handling and preparation of the dosing solution.

6.1.4. Preparation of the Dosing Solution

The dosing solution for infusion will be prepared at the designated Pharmacy or authorized location within each centre according to local guidelines for sterile re-consitution of i.v. injectable solutions.

For each 750 mL dosing solution volume, the content of a Vial (250 mL) will be diluted with 500 mL of 0.9% sterile saline to dispense reparixin as 11.00 mg/mL solution. The dosing solution will be placed in a 1000 mL sterile empty Infusion Bag. Dosing solutions will be prepared and used within 72 hours from preparation, unless the site has more restrictive rules.

A double-tear off label will be attached to each prepared bag, reporting patient number and relevant blinded information. Infusion Bags for the same patient/administration course will be numbered in a sequential manner, starting from No. 1. Labels for the Investigation product administration during the 2nd islet infusion will be clearly identified as such.

The Pharmacist (or designee) will make the Infusion Bags available to the hospital ward at the requested time.

6.1.5. Blinding

Reparixin solution in the vial (33mg/mL) has a slightly different appearance (pale yellow colour) as compared with placebo. Also, a transient foamy layer occasionally results from the preparation of the reparixin dosing solution. However, once the foamy layer, if any, had subsided, the dosing solution of reparixin in the Infusion Bag will be indistinguishable from that of placebo.

Individual treatment codes will be provided in tamper-resistant system (either sealed envelopes or scretch cards) to the Pharmacist (or designee). They must be kept in a secure location accessible only to designated staff in order to prevent dissemination of the treatment to personnel involved in study conduct who must remain blind.

The Pharmacist (or designee) will break the treatment code only after the patient-specific "IP Preparation Order Form" is received from the Investigator. The individual code matching the patient randomization number will be opened to prepare the dosing solution matching the patient randomization number.

The Investigators can request to an independent facility (either by telephone or web based system) an individual code in the event of an emergency only, where knowledge of the blinded treatment for that patient could influence further patient care. Any potential unauthorized code break and the reason

behind it will be recorded.

6.2. DOSE, ROUTE AND SCHEDULE OF INVESTIGATIONAL PRODUCT ADMINISTRATION

Patients will receive either reparixin at a dose of 2.772 mg/kg body weight/hour for 7 days (168 hrs) or matched (flow rate/length of infusion) placebo, according to the randomization number.

The dosing solution (if reparixin, 11mg/mL) will be administered as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump adequate to provide reliable infusion rates (see below), as per treatment schedule.

Infusion of the Investigational Product will start approximately 12hrs (allowed range 6-18hrs) before the anticipated time when islet infusion is started. The Investigator will identify the time to start study drug administration.

The pump rate will be adjusted to provide an infusion rate of approximately 0.25mL/kg/hour. Actual infusion rate (mL/hour), adjusted to body weight, is tabulated in **Appendix 14.3**. Figures in the appendix represent mathematical rounding of original infusion rates derived from the following formula:

$$\text{Infusion rate (mL/hour)} = \frac{\text{dose (mg/kg/hour) x body weight (kg)}}{\text{11.00 mg/mL}}$$

Such a rounding affects actual administered dose/kg/hour by less than 1%.

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

6.3.1. Criteria for schedule adjustment or dose modification

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

6.3.2. Criteria for discontinuation of Investigational Product

Phase 1 studies in patients with end stage renal disease have shown that renal function has a profound effect on plasma concentrations of a major, marginally active metabolite, carboxy-reparixin (DF2243Y), which was found to accumulate over time along with the increased elimination half-life. Even if toxicological results to date suggest that DF2243Y does not raise any safety concern, limited experience in humans recommends discontinuation of the drug in case of renal impairment as it would not be possible to predict the risk associated with elevated plasma levels.

Because reparixin undergoes extensive hepatic metabolism, hepatic dysfunction should also be monitored to avoid an increase in plasma levels. An asymptomatic, self-limited transaminitis is consistently seen after islet transplantation into the liver. This is characterized by elevation in AST/ALT which peaks at about 7 days post transplant and spontaneously normalizes within 4 weeks. Bilirubin and other liver function tests remained within the normal range, suggesting no major hepatic dysfunction that would impact reparixin metabolism. [*Rafael, 2003; Barshes, 2005*].

Therefore, the Investigational Product should be immediately discontinued in case the patient develops renal or hepatic dysfunction defined as:

Renal dysfunction: the occurrence of both CLcr < 60 mL/min **and** urine output < 0.5 mL/kg/hour on two consecutive samplings. Transient alterations of serum creatinine or urine output, other than defined above, do not indicate a renal dysfunction that would cause impaired excretion of DF2243Y.

Hepatic dysfunction: the occurrence of ALT/AST > 3 x ULN **and** total bilirubin > 3mg/dL [> 51.3 μmol/L].

Renal and hepatic function will be evaluated daily from post-operative day 1 during Investigational Product infusion. Samples will be processed immediately and results made available as soon as possible to the Investigator.

Additionally, the Investigational Product should be immediately discontinued in the event of any other possibly drug related occurrences that the Investigator believes might compromise patient safety.

Lastly, the Investigational Product will be immediately discontinued in the case the patient withdraw his/her consent or has not received the transplant.

Patients who discontinue the treatment with the Investigational Product will not be withdrawn from the study by default, but will complete observations as per the protocol, unless otherwise they withdraw their consent.

If the Investigational Product therapy is prematurely discontinued the primary reason for discontinuation must be recorded in the CRF.

6.4. ACCOUNTABILITY

All supplies will be maintained under adequate security by the responsible member of the Pharmacy staff. The Investigator will ensure that study treatment is only administered by those named as sub-investigators on the FDA form 1572 and designated staff.

When the Investigational Product is received by the Pharmacist (or designee), (s)he will check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by Dompé (or designee) and returning it to Dompé (or designee). A copy will be retained for the Investigator/Pharmacy file.

The dispensing of the Investigational Product will be carefully recorded on appropriate drug accountability forms provided by Dompé (or designee) and an accurate accounting will be available for verification by an U-CRA at each monitoring visit.

Drug accountability records will include:

- confirmation of receipt of the Investigational Product at the trial site,
- the inventory at the site (vials),
- details of each bag preparation,
- the use by each patient,
- disposition of unused vial(s),
- account of any Investigational Product accidentally or deliberately destroyed.

They should include dates, quantities, batch numbers, use by date information, and any unique code numbers assigned to the Investigational Product and/or patients.

Records should be maintained to adequately document that:

- the patients were provided the doses specified by the protocol/amendment(s),
- all Investigational Product provided was fully reconciled.

The U-CRA will review the drug accountability forms for consistency with drug administraiton (as per CRF) and remaining unused Investigational Product vials.

Investigational Product which has been dispensed to a patient and returned unused will not be re-dispensed to a different patient.

Unused Investigational Product must not be discarded or used for any purpose other than the present study. Any remaining test material at the end of the trial will be returned to Dompé (or designee) or disposed of, as determined by Dompé (or designee).

6.4.1. Assessment of compliance

Compliance will be assured by the person(s) within the centre in charge of Investigational Product administration.

Immediately before the use of an Infusion Bag is started, the removable label on the bag will be detached and attached to the relevant page of the CRF. Actual date and time of infusion start and end for each container will be recorded in the CRF, as well as the infusion rate(s). Temporary interruption of > 30 min duration (cumulative within each 24 hours) during drug administration should also be reported.

Compliance with the study product dosing schedule will be verified by a CRA during on-site monitoring visits, as per records in the CRF, versus accountability records.

6.5. CONCOMITANT THERAPY

Any medications required for the patient's welfare are permitted and will be given at the discretion of the Investigator, except for drugs described in paragraphs below.

Administration of all prior (4 weeks prior to enrolment) and concomitant medications (CMEDs), apart from the agents listed below, during hospital stay will be reported in the appropriate section of the CRF. Only medications used for SAE, oral hypoglycemic drugs as well as medications that should not be used (see Section 6.5.1 below) will be reported after hospital discharge.

All the details as per the CRF fields (sequential number, drug name, indication, starting dose, start/stop date, route of administration) will be recorded. Change in dose will not be tracked.

The following agents do not need to be recorded:

Saline and other hydration solutions (including additional electrolytes)
Total parenteral nutrition and enteral feeds
Homeopatic medications
Elective vitamins and minerals
Topical agents with no or negligible systemic absorption
Osmotic laxatives and locally acting antacids

6.5.1. Other treatments

Drugs required during surgery will be administered as per centre practice.

The following prophylactic anticoagulation is suggested to be used in patients enrolled in this study

- Heparin will be administered at a dose of 70 U/kg, divided equally between the islet bags, followed by 3 U/kg/hour infused i.v. for the next 4 hours. From the 5th to the 48th hour post-transplant, heparin will be titrated to achieve and maintain PTT in the range 50-60 seconds. Comparable marker other than PTT will be used as per site experience.

- Enoxaparin will be administered at a dose of 30 mg subcutabeously from day 2 to 7 post-transplant.

Prophylactic anticoagulation management (choice of medication and/or doses) might be adjusted on the basis of clinical requirements (e.g. occurrence of bleeding) and/or centre practice. Low Molecular Weight Sulfate Dextran cannot be used in this trial.

Glycemic control in the early post-transplant period will be achieved by insulin administration to target glucose levels in the range 80-180mg/dL. Patients will be tested for blood sugar by fingerstick a minimum of 4 times per day. Insulin will be titrated to maintain fasting and pre-meal blood sugars in the range 80-125 mg/dL, and 2 hour post-prandial glucoses <180 mg/dL. For patients on continuous enteral or parental feeds, insulin will be titrated to maintain glucose in the range 80-140 mg/dL.

The following medications **should not be used** in this study:

- Systemic steroids (i.e. intravenous, intramuscular, oral; intra-articular injection allowed): from 2 weeks prior to enrolment up to the end of the study; use of ≤5mg prednisone daily or equivalent dose of hydrocortisone, for physiological replacement only is allowed.
- Any investigational agents: from 4 weeks prior to enrolment up to the end of the study;
- Any anti-cytokine/chemokine medications (e.g. anti-TNFα): from 4 weeks prior to enrolment up to the end of the study;
- Any immune modulators: from 4 weeks prior to enrolment up to the end of the study;
- Low Molecular Weight Sulfate Dextran;
- Oral hypoglycemic agents and any drugs that influence insulin sensitivity.

Since exposure to ibuprofen after administration of reparixin 2.772 mg/kg/h for 48hrs is in the range of that obtained after a standard therapeutic single dose of ibuprofen (300mg), concomitant administration of NSAIDs, including cyclo-oxygenase-2 specific inhibitors, should be considered with caution during infusion with the Investigational Product, as this may increase the risk of cyclo-oxygenase-dependent adverse affects.

7. STUDY PROCEDURE AND ASSESSMENTS

A schedule for the tests and evaluations to be conducted in this study is found in the flow chart in **Appendix 14.4**. A list of acceptable assessment/procedure time windows is detailed in **Appendix 14.5**. Methodological details are reported in **Appendix 14.6**.

7.1. HOSPITAL STAY

7.1.1. Screening and randomization

Potential study patients will be identified among those referring to the investigational site and found eligible as per site standard criteria for total (or completion) pancreatectomy followed by IAT. Consented patients will undergo the following screening evaluation, within 72 hours prior to randomization.

BP, HR, body weight (kg) and height (m) will be measured.

A baseline blood sample will be taken prior to any intervention for safety laboratory tests (assay as per centre practice). These include hematocrit, hemoglobin, red blood cells, platelets, white blood cells, differential white blood cells count, sodium, potassium, serum creatinine, blood urea nitrogen, total bilirubin, ALT, AST, INR, PTT. Albumin and pre-albumin will be also measured.

Pregnancy test (urine dipstick or blood test) will be performed, if appropriate.

Renal reserve will be assessed by CLcr (*Cockcroft-Gault; 1976*; see details in **Appendix 14.6.2**). Hepatic function will be assessed by ALT/AST and bilirubin.

Concurrent sepsis will be diagnosed by blood culture(s) and/or fever associated with other signs of systemic sepsis syndrome.

Pre-operative portal hypertension will be diagnosed as per clinical history **and** abdominal/liver imaging (e.g. presence/absence of varices, splenomegaly, Doppler waveforms and flow direction) by ultrasound techniques (e.g. duplex ultrasonography, spectral Doppler imaging and Color Doppler imaging, power Doppler imaging).

Two basal serum samples collected 6 to 24 hrs apart will be stored for centralized assay of pre-transplant chemokines/cytokines; similarly, a basal sample will be stored for centralized assay of pre-transplant miR-375 (see **Appendix 14.6.3**). All basal samples will be obtained before surgery and before Investigational Product administration is started.

Compliance with inclusion/exclusion criteria will be verified vs demographic, laboratory test results and clinical history/information available as per local standard clinical practice.

Eligible patients will be randomized (randomization number assigned) by an Investigator (or designee) before starting the infusion of the Investigational Product.

A screening form will be completed for all patients who signed the Patient Informed Consent Document, regardless of subsequent entry into the study. Patients will be identified by their age and sex; patient number if enrolled or reasons for exclusion from the study will be recorded.

7.1.2. Start of infusion with the Investigational Product

Infusion of the Investigational Product will start approximately 12hrs (6-18hrs) before the anticipated time of islet infusion. The Investigator will identify the time to start study drug infusion. Infusion may be halted during pancreatic surgery if required, provided that interruption is not longer than 6 hours.

The infusion pump will be set to provide the infusion rate corresponding to the planned dose. Infusion rate (mL/hour) corresponding to each unit increment (kg) of body weight, is reported in **Appendix 14.3**.

Immediately before the start of infusion, the removable label on the Infusion Bag No. 1 will be detached and attached to the relevant page of the CRF. Starting time of drug administration and infusion rate will be recorded in the CRF.

7.1.3. Islet isolation and transplantation

Techniques for pancreas removal will be used, as per centre practice, including requirement for concomitant splenectomy.

Islet preparation: Site will process islets by enzymatic digestion using an FDA approved collagenase and neutral protease as per site specific standards. The pancreas will be distended by intraductal infusion of collagenase/neutral protease; if duct disruption prohibits intraductal infusion, enzyme will be manually injected into the pancreatic parenchyme. Pancreatic digestion will be performed using the semi-automated method of Ricordi. Purification will be performed when necessary to reduce tissue volume, dependent on site specific standards for islet manufacturing, using density centrifugation with COBE 2991. Samples will be taken from the pancreatic preservation solution and final islet product for sterility testing by gram stain and culture.

Islet mass will be assessed by manual counts as detailed in **Appendix 14.6.1** and quantified as total IEQ and IEQ/kg body weight. The final product is suspended in a 200 mL suspension of CMRL 1066 Transplant Media or equivalent solution. The final product is supplied in one to three 200 mL infusion bags, containing a minimum dose of >10,000 IEQ total.

Islet infusion: At all sites, islets will be transplanted on the same day as the total pancreatectomy procedure.

The islet mixture will be delivered slowly by gravity drainage into the portal vein or a tributary of the portal vein. Access to the portal vein may be obtained by direct cannulization during laparotomy or by percutaneous access by fluoroscopic, ultrasonographic, or real-time CT guidance.

At minimum, portal venous pressure will be monitored (direct venous measurement) before initiation, mid-way, and after completion of islet infusion. In case the portal pressure reaches >35 cmH_2O, islet infusion will be put on hold, and restarted when portal pressure has dropped. In the rare event that portal pressure does not lower (within a 30 min interval), infusion is definitely stopped and any remaining tissue either discarded or placed in another tissue (e.g. peritoneal cavity, duodenal wall, underneath the kidney capsule, etc.).

The following isolation and operative details will be recorded in the CRF, as per documentations provided by the local Islet Processing Facility and centre information:

- Date and time of isolation (procedure completed).
- Date and time of infusion (start and end of infusion);
- IEQ/kg infused. Islet will be counted as detailed in **Appendix 14.6.1**;
- Total islet infusion volume;
- Pre-, mid-way, and post- infusion portal pressure.

7.1.4. Drug administration during hospital stay

Administration of the Investigational Product will continue during hospital stay.

Infusion Bags will be used in a sequential manner (No. 1, No. 2, No. 3 etc.) and infusion of the Investigational Product will progress non-stop up to a total of 7 day (168hrs) administration.

The removable label on each new Infusion Bag will be detached and attached to the relevant page of the CRF. Start date/time and stop date/time for each Bag will be recorded in the CRF, along with accidental interruptions of study drug infusion for > 30min (cumulative within each 24 hours).

7.1.5. Post-operative assessments

The following will be assessed during hospital stay and recorded in the CRF. Measurement will be performed as per centre practice, unless otherwise specified.

- Renal function (CLcr, urine output): Renal function will be assessed daily from post-operative day 1 up to the end of study drug infusion. Details of Clcr calculation are reported in **Appendix 14.6.2**. There is no need to have a urine catheter in place, if this is not required by standard of care. Urine volume will be measured at urination (measuring cylinder); corresponding collection time will be calculated from the previous urination. Depending from feasibility and patient compliance, ideally more than one measurement should be done over a 24-hour period to get the best estimate of urine output.

- Total bilirubin: Total bilirubin will be assessed daily from post-operative day 1 up to the end of study drug infusion as part of hepatic function evaluation.

- XDP, ALT/AST, INR/PTT, C-reactive protein (CRP): These will be measured daily from day 1 up to day 7 post-transplant.

- Chemokines/cytokines [CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), CXCL9 (MIG), IL-6, IL-10, INF-γ, TNF-α, and IL-1β]: A blood sample will be obtained @ 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion. Samples will be handled and stored for the centralized assay, as detailed in **Appendix 14.6.3**.

- miR-375: A blood sample will be obtained @ 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion. Samples will be handled and stored for the centralized assay, as detailed in **Appendix 14.6.3**.

- Pharmacokinetics [plasma levels of reparixin (total and unbound) and relevant metabolites (DF2243Y and ibuprofen)]: A blood sample will be obtained in all patients randomized and treated with the Investigational product on post-operative day 1, 3 and 5, ideally in the morning. In a subset of at least 20-24 patients, treated at 2 selected sites, a blood sample will be also obtained just prior to, and then at 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration. Samples will be handled and stored for centralized assay, as detailed in **Appendix 14.6.3.**

- Safety laboratory tests: Laboratory tests will be performed before hospital discharge.

- Vital signs, i.e. blood pressure (BP) and heart rate (HR) will be measured before hospital discharge.

During hospital stay, all AEs (see details in Sections 8.2.) and CMEDs (see details in Sections 6.5.) will be recorded.

For all measurements above, the actual date and time of assessment, including date of sampling, will be recorded in the CRF.

Apart from compliance with protocol procedures up to day 7 post-transplant, hospital discharge will be primarily based on criteria related to total pancreatectomy (the major surgical procedure in total pancreatectomy followed by IAT) and will comply with each site's hospital standards. However, at all sites, at least the following criteria will be complied with: "A patient will be discharged if he/she at least is ambulating, has adequate nutrition (oral, enteral, or Total Parenteral Nutrition (TPN), is not requiring any intravenous medications which require inpatient monitoring, pain is adequately treated, and he/she has a stable plan for glycemic control (management of blood glucose testing and insulin administration at home)".

7.2. FOLLOW-UP PROCEDURES AND ASSESSMENTS

Since hospital discharge, patients will self monitor glucose levels and insulin intake according to standard clinical instructions at the site. Insulin will be administered to target fasting and pre-meal glucose levels in the range 80-125 mg/dL, and 2 hour post-prandial glucoses <180 mg/dL. For patients on continuous enteral or parental feeds, insulin will be titrated to maintain glucose in the range 80-140 mg/dL.

The Investigator will identify if- and the date when- it is appropriate for the patient to stop insulin intake, according to standard of centre practice. Regardless the patient is on or off insulin, he/she will report on a Diary Card (see **Appendix 14.7**) his/her self monitored glucose levels for 14 consecutive days prior to the follow-up visits on day 75±14 and 365±14 after the transplant: glucose levels will be measured at least two times a day: 1) after an overnight fast (or anyway before breakfast if the patient is still on a tube feed and TPN); 2) within 2 hours post-prandial. Also, patients still on insulin will report on the Diary Card their daily insulin intake.

Patients will attend the centre for study assessments on 2 follow-up visits scheduled on day 75±14 and 365±14 after the transplant.

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

- Patient weight.
- Retrospective self-measured glucose and insulin requirement data in the 2 weeks prior to the visit will be reviewed and average (previous week) insulin requirement at each time point as well as insulin-independence / insulin-dependence status will be assessed.
- Retrospective episodes of severe hypoglycaemia in the interval will be assessed as per centre practice. Events of documented hypoglycemia in the interval will be obtained from review of the patient's home glucometer download from between visits and/or patient glucose logs.
- Retrospective severity of steatorrhea in the 4 weeks prior to the visit will be evaluated.
- A blood sample will be taken prior to the meal administration to measure HbA1c, albumin and pre-albumin. ALT/AST will be also measured on day 75±5.
- A MMTT test will be performed as detailed in **Appendix 14.6.4**. Blood samples will be drawn @ 0 (2 basal samples in the range between -20 to 0 prior to the meal) and 15, 30, 60, 90, 120, 180, 240 min after the meal for glucose, C-peptide and insulin measurement.
- β-score will be calculated according to *Ryan (2005)* (see **Appendix 14.6.5** for details).

C-peptide and HbA1c will be measured by a centralized laboratory with CLIA certification and National Glycohemoglobin Standardization Program (NGSP) certified assay. Details of handling and storage of samples as well as data handling are reported in **Appendix 14.6.3**.

Any SAEs that occurred after hospital discharge will be recorded at the visits, as well as CMEDs, as appropriate.

Patients should be strongly motivated to attend the centre for the planned follow-up visits. However, if a patient cannot refer to the site for protocol assessments, the Investigator will try to obtain any relevant information from the patients, including documents/lab results available from local medical care.

7.3. EARLY PATIENT WITHDRAWAL

7.3.1. Criteria for withdrawal from the study

Patients will be informed that they have the right at any time to withdraw from further participation in the study (*withdrawal of consent*), without prejudice to their medical care, and without being obliged to state their reasons. However, during the consent process, patients will be also educated about the scientific relevance of their continued participation as well as the deleterious effect that missing data will have on trial integrity.

If a patient fails to return to the centre for a scheduled visit, all reasonable attempts should be made to contact the patient to ensure that the reason for not returning is not a SAE. Likewise if a patient declares his/her wish to discontinue from the study e.g. for personal reasons, an attempt should be made to establish that the true reason is not a SAE (bearing in mind the patient is not obliged to state his/her reasons). The term *withdrawal of consent* should be used only when the patient no longer wishes to participate in the trial and no longer authorizes the Investigators to make efforts to continue to obtain his/her outcome data. Ideally, if patients withdraw their consent, it should be done in writing.

Patients who have discontinued the study treatment should be anyway followed to ensure that primary and secondary outcome measures are assessed, unless they formally have withdrawn 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 and full data capture. Also, all reasonable attempts should be made by the Investigators to emphasize continued patient's participation for the full duration of the trial.

Any withdrawals must be fully documented in the CRF.

7.3.2. Replacement policy

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

8. ADVERSE EVENTS

8.1. DEFINITIONS

8.1.1. Definition of an Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any 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 [*Clinical safety data management: Definitions and Standards for Expedited Reporting*].

8.1.2. Definition of a Serious Adverse Event

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

- results in death,
- is life-threatening (i.e. the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event that based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

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.

8.2. EMERGENCY PROCEDURES

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

The Investigator can request treatment allocation for a patient to an independent facility (either by telephone or web based system) in case of emergency where knowledge of the double-blind treatment may influence the further care of the patient.

If a code is opened for any reason, the reason behind it will be recorded .

8.3. RECORDING

AE data should be obtained through observation of the patient, from any information volunteered by the patient, or through patient questioning. Specifically, patients will be asked about any episode of documented hypoglycemia and ketoacidosis-related events occurring from the transplant day up to day 75$\pm$14 post-transplant. Any episodes will be reported as an AE or SAE, as appropriate. In addition, the Investigator will capture and report episodes of non-documented hypoglycemia throughout study participation.

All AEs (serious and non-serious) encountered during the clinical study will be recorded in the appropriate section of the CRF as per the recording period defined in paragraph 8.3.1. It is important that this includes the duration of the AE (onset/resolution dates), the relationship to the drug, the severity, the outcome and relevant concomitant treatments dispensed (or other action taken) (see Sections 8.3.2. and 8.3.3. below).

8.3.1. AE recording period

All AEs (serious and non-serious) which occur during each hospital stay will be recorded in the CRF. In addition, SAEs and relevant untoward events that occur subsequent to each hospital discharge will be recorded in the CRF.

8.3.2. Relationship of AEs to the Investigational Product

The Investigator will assess the possible relationship between the AE and the investigational medication, according to the criteria in the **Table** below:

Relationship of the Adverse Event to the Investigational Product

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

An **Adverse Drug Reaction (ADR)** is defined as an adverse experience which is a reasonably likely to have been caused by the drug. Any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

8.3.3. Severity of AEs

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

Severity of the Adverse Event

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

8.4. SERIOUS ADVERSE EVENT REPORTING

8.4.1. Reporting Procedure for Investigators to Dompé (or designee)

The Investigator must report all SAEs, regardless of presumed causal relationship, to Dompé (or designee) by fax or e-mail within 24 hours of learning of the event. Contact details for SAE reporting are provided in the section "Contact Information".

Information on SAEs will be recorded on a specific Non-Carbon Repeat SAE form. Both electronic and blank paper copies will be included in the Investigator's Site File. Follow-up reports (as many as required) should be completed and faxed/e-mailed 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. Reporting Procedure for Investigators to IRB

In addition to reporting the SAE to Dompé, the Investigator must also comply with the requirements related to the reporting of SAEs to the IRB which approved the study.

The requirements of IRB varies from one state and indeed one IRB to another; however, as a minimum requirement, the Investigators must promptly report all serious unexpected* ADRs, life-threatening problems or deaths to their IRB.

Dompé (or designee) will inform Investigators of all serious unexpected ADRs which are reported to Dompé from other Investigators. These SAEs should also be reported promptly to the IRB in compliance with the local regulations.

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

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

8.4.3. Reporting Procedures to the FDA

During the course of the clinical trial, Dompé (or designee) shall inform the FDA of any serious unexpected ADR* as soon as possible and in no event later than:

(*a*) seven calendar days after becoming aware of the information if the event is fatal or life threatening; and

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

Dompé (or designee) shall, within 8 days after having informed the FDA under paragraph (*a*), submit a complete report in respect of that information that includes an assessment of the importance and implication of any findings made.

Furthermore, Dompé (or designee) shall follow up safety information and shall report final findings in a written safety report as soon as the relevant information is available.

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

Dompé (or designee) will inform the FDA of all serious ADRs which are reported from other Investigators.

8.5. ADVERSE EVENT EXEMPTION

The following events will neither require recording nor reporting, as they are considered routinely associated to the pancreatectomy or to the transplant procedures:

- Delayed Gastric Emptying requiring insertion of a central line for Total Parenteral Nutrition or insertion of a nasojejunal tube for Enteral Nutrition;
- Increased ALT/AST levels up to 5 x ULN, inclusive, within 2 weeks post transplant;
- Abnormal PTT and INR values during prophylactic anticoagulation.

9. STATISTICAL ISSUES

9.1. SAMPLE SIZE

The primary efficacy endpoint of the clinical trial will be the proportion of insulin-independent patients following IAT over the time frame: day 365±14 after the transplant.

In the following considerations, statistical significance for this endpoint will be achieved if the Pearson Chi-Square Statistic yields a two sided p-value less than 0.05. The corresponding false positive error rate is (one-sided) 0.025. In addition, 95% confidence interval for the difference of success (failure) rates between the reparixin and placebo groups will be provided, based on the normal distribution approximation to the standardized difference in estimated success rates.

Based on published data from a retrospective series (*Sutherland, 2012*), it appears that the likelihood of achieving insulin independence at 12 month follow-up in the placebo control arm depends upon the number of IEQ/kg, with patients categorized in three groups:

- < 2500 IEQ/kg: 13% insulin independence
- 2500-5000 IEQ/kg: 23% insulin independence
- > 5000 IEQ/kg: 55% insulin independence

In ther overall series, the yields were <2500 IEQ/kg in 36%, 2500-5000 IEQ/kg in 39% and > 5000 IEQ/kg in 24% of patients. In the more recent series of 217 patients receiving IAT from 2006 to 2011, corresponding yields were <2500 IEQ/kg in 34%, 2500-5000 IEQ/kg in 42% and > 5000 IEQ/kg in 24%:

We initially focus on the cohort that is in the range 2500-5000 IEQ/kg, since these participants will be the largest fraction of enrollees. It is anticipated that the percentage of such patients achieving insulin independence will be increased from 23% in the placebo control arm to 55% in the reparixin arm. Using standard statistical formulas based on the approximation of the binomial distribution by the normal distribution, the sample size of 44 patients per arm provides 90% power to detect a 23% vs. 55% true difference when using a Pearson Chi-Square Statistic having the traditional (one-sided) 2.5% false positive error rate. Even accounting for the discreteness, the power should be in the 87%-90% range. Furthermore, with 44 patients per arm, statistical significance (i.e., one-sided p=0.025) will be obtained if the estimated difference is 22.7% (10/44) vs. 43.2% (19/44), which is an estimated 20.5% increase in patients achieving insulin independence. Hence, with 44 patients per arm, statistical significance is achieved with an estimated effect size that is clinically meaningful.

Even though the largest percentage of patients will be in the cohort receiving IEQ/kg in the range 2500-5000 IEQ/kg, the trial will also enroll participants in two additional strata: <2500 IEQ/kg and >5000 IEQ/kg. As noted above, we are presuming the middle strata would have an increase from 23% to 55% in percentage of patients achieving insulin independence. This is an odds ratio higher than 4. If we have an odds ratio of approximately 4.3 to 4.5 in the other 2 strata and if approximately ½ of the patients would be from the 2500-5000 IEQ/kg stratum, then only a modest increase of about 12 additional patients beyond the 88 patient sample size would be needed to maintain the statistical power of the trial. An increase in success rate from 13% to 40% in the <2500 IEQ/kg stratum would be an odd ration of 4.4 and an increase in success rate from 55% to 84% in the >5000 IEQ/kg stratum would be an odd ration of 4.3.

Based on these considerations, it is planned to enroll 100 patients in this trial who receive IAT, with the expectation that there will be approximately 5 others who do not receive IAT. Under such a plan, the trial would not be overpowered even if all participants were in the 2500-5000 IEQ/kg stratum since statistical significance (i.e., one-sided p=0.025) would be obtained with 52 patients per arm if the

estimated difference is 23.1% (12/52) vs. 42.3% (22/52), which is an estimated clinically significant 19.2% increase in percentage of patients achieving insulin independence.

The sample size of 100 to 105 patients also provides high statistical power for the key secondary endpoint, C-peptide AUC (MMTT) normalized by IEQ/kg. Based on preliminary data reported from the ongoing pilot trial (see table on page 14), month 1 post transplant means and standard deviations for this endpoint are 1.92±1.62 in the reparixin group and 0.44±0.04 in the control group. With 52 patients in each treatment group, the trial will have greater than 95% power to detect a difference in means of at least 1.48 (the difference between a treated mean of 1.92 and a control mean of 0.44) assuming that the common standard deviation is 1.37 and using an 0.0025 two-sided significance level.

9.2. RANDOMIZATION

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

The randomization list will be generated with a computer procedure by the method of random permuted blocks in which treatment (in blocks of 4) will be balanced within centres. A master randomization list will be generated, randomizing an excess of patients (a maximum of 40 for each site – additional 8 patients for the site of the Primary Investigator) to allow competitive recruitment within each centre.

The randomization list will be prepared by and independent statistician and provided to Dompé in a sealed envelope to prevent unblinding.

Similarly, individual treatment codes will be provided as a tamper-resistant system (either a sealed envelope or a scratch card). Such system will definite allow to recognize if the code has been broken and guarantees detection of any code misuse. Individual treatment codes will be provided to:

- the Pharmacist (or designee) within each participating site for Investigational Product preparation;
- the Dompé Pharmacovigilance department for safety procedures.

The Investigators can request to an independent facility (either by telephone or web based system) an individual code in the event of an emergency only, where knowledge of the blinded treatment for that patient could influence further patient care. Any potential unauthorized code break and the reason behind it will be recorded.

The randomization codes will also be accessible to an Independent Statistician (liaison between the CRO database and DMC Biostatistician) who will generate the reports for the Data Monitoring Committee (DMC) evaluation (see Section 12.5).

The randomization code will be broken at study completion, i.e. when the last patient has completed his/her last follow-up visit (planned 365 days after islet infusion), and once the database has been locked.

9.3. ANALYSIS POPULATION

The Intent to Treat (ITT) population will consist of all patients who are randomized and receive the Investigational Product (either reparixin or placebo); it will be based on the treatment randomized, regardless of the treatment actually received. Patients will be in the ITT analysis whether or not they receive IAT, because exclusions cannot be made for events occurring after randomization that could be influenced by the randomized assignment. The primary and secondary efficacy analyses will be presented primarily for the ITT Population.

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

9.4. STATISTICAL METHODOLOGY

All patient data collected on the CRF and on the Diary will be listed by patient, treatment group and centre.

Appropriate descriptive statistics will be produced, according to the variable. For continuous variables, the data will be presented according to a clinically relevant discretization. For categorical data, frequencies and percentages will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented.

All the AUC analyses will be based on actual rather than scheduled timings and will be calculated using the trapezoidal rule. If the actual time is not recorded, the scheduled time will be used instead. For ease of interpretation, the AUC value obtained will be divided by the total time the scale is assessed for reporting purposes.

The data will be presented in the Clinical Study Report. A Statistical Analysis Plan will be issued describing details of all the statistical methods and analyses to be applied to trial results, including alpha spending and testing for multiple endpoints. Any deviations from the original statistical plan will be described in the Clinical Study Report.

All reasonable efforts will be made to prevent missing data. Thoughtful methods for imputation of missing data will be presented in the Statistical Analysis Plan for the clinical trial

9.4.1. Demographic and baseline characteristics

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

9.4.2. Primary and Secondary Endpoint analysis

For the primary efficacy endpoint, the proportion of insulin-independent patients over the time frame of day 365±14 after the transplant, the differences in proportions will be assessed using the Pearson Chi-square statistic, stratified by the three groups at baseline: < 2500 IEQ/kg vs. 2500-5000 IEQ/kg vs. > 5000 IEQ/kg. The significance level used for statistical testing will be 2.5% and one-sided test will be used. The 95% confidence interval for treatment proportions and for the difference in treatment proportion between reparixin and placebo will be produced. The primary analysis will be performed using both ITT population with PP analysis serving as a sensitivity analysis.

For secondary endpoints that will be tested both at 75±14 and 365±14 days after the transplant, only the day 365±14 time point will be tested for superiority in the fixed sequence testing procedure.

The C-peptide AUC after the MMTT normalized by IEQ/kg will be analyzed at the two time points by a repeated measurements model using PROC MIXED within SAS®, including terms for treatment, time point and center. The treatment effect within each time point will be compared using a two-sided test at the 5% level. The estimated treatment difference between reparixin and placebo at each time point will be presented together with the corresponding 95% confidence interval. The confidence interval will be generated using α=0.05.

The mean in average daily insulin requirements at the two time points will be analyzed using a repeated measurements model using PROC MIXED within SAS®. The model will include fixed effect

terms for center, time point and treatment. Time point will be specified as a repeated measurement. The treatment effect within each time point will be compared using a two sided test at the 5% level.

The proportion of patients with an HbA1c $\leq$6.5% will be analyzed at day 365$\pm$14. Additionally, the proportion of patients with an HbA1c $\leq$6.5% at day 365$\pm$14 will be analyzed again, but patients are included in this second analysis if they are also free of severe hypoglycemic events from day 75$\pm$14 to day 365$\pm$14 inclusive. Treatment effect will be analyzed using Pearson Chi-square.

The effect of treatment on the rate of recurrent episodes of severe hypoglycaemia will be evaluated using an Andersen-Gill analysis with robust sandwich-type variance estimate.

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

9.4.3. Safety analysis

Safety variables will be presented for the Safety population, by treatment group.

All AEs will be coded using the most up-to-date version at the time of database lock of the MedDRA and will be presented by primary system organ class and preferred term. AEs will be presented in terms of the incidence, severity and relationship to the study drug, overall and by body system and preferred term. SAEs will be presented in the same way.

Results for laboratory test at screening and hospital discharge will be assessed as being within the normal range or outside the normal range and clinically-significant outside results. The following parameters will be also presented at different time points: AST/ALT, INR/PTT, fibrin degradation products (XDPs), C-reactive protein (CRP) will be presented for all daily from day 1 up to day 7 after transplant and on day 75$\pm$5 after transplant for AST/ALT. Serum level of albumin and pre-albumin will be summarized as absolute values and change from pre-transplant value at day 75$\pm$14 and 365$\pm$14 after transplant.

Vital signs at each time point and the change in vital signs from pre-transplant value, and weight loss will be presented using descriptive statistics.

Steatorrhea and malnutrition risk will be graded as described in 4.2.3. Analysis will be performed separately at day 75$\pm$14 and 365$\pm$14 after transplant. 2x4 contingency table will be performed at each time point. 95% confidence intervals of proportion will be also done.

Analysis of documented hypoglycemia episodes and diabetic ketoacidosis-related events, recorded as cumulative number of episodes per patient, will be performed from day 75$\pm$14 to 365$\pm$14 after islet infusion. Mean, median, SD, min, max will be calculated for the interval

9.4.4. Analysis of exploratory endpoints

Chemokines/cytokines will be presented using appropriate descriptive statistics, by treatment group.

The relationship between inflammatory chemokines/cytokines response and exposure to reparixin will be explored.

Serum level of miR-375 will be presented using appropriate descriptive statistics, by treatment group.

The comparison will be made between the reparixin and the placebo control arms regarding the rates of patients who are randomized but do not receive IAT.

9.4.5. Interim analysis

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

Primary endpoint data will not be available until each patient reaches one year post randomization. Hence, it is expected that the majority of enrolment and treatment will be completed before meaningful evidence is available regarding treatment effect on the primary endpoint. Thus, traditional group sequential methods would have more limited utility in this setting.

The DMC will give careful consideration to the appropriateness of trial continuation if there is emerging evidence that Reparixin is harmful. One component of this assessment will be the rate of unexpected deaths. Based on historical experiences from the published Minnesota series for 1977-2011 that includes 356 adults, 217 underwent pancreatectomy and IAT from 2006-2011. There was 1 unexpected death within 12 months of transplant. This translates to an estimated 0.5% rate.

The DMC will consider the following guidelines regarding early termination based on the occurrence of unexpected deaths. Early termination will be considered if:

- there are at least 2 unexpected deaths in the first 33 reparixin patients (an outcome with 1.2% chance if the true event rate is 0.5%);
- there are at least 3 unexpected deaths in reparixin patients at any time in the trial (an outcome with 0.2% chance in 50 reparixin patients if the true event rate is 0.5%).

In making any recommendations about termination, the totality of data will be considered, including the number of unexpected deaths in the control group, and the available evidence about efficacy and the overall safety profile.

The DMC will also give particular attention to clinically significant coagulation abnormalities, including but not limited to intra-abdominal or gastrointestinal hemorrhage. Early termination would be considered if the rate of clinically significant post-surgical complications that require reoperation reliably exceeds the rate expected currently in standard practice settings. Insights about that rate in standard practice settings will be provided by a literature review, and will include the publication for the Minnesota series, where intra-abdominal as well as gastrointestinal hemorrhage have been reported to be the most common reasons for reoperation (*Sutherland, 2012*).

The DMC will also consider early termination if the quality of conduct of the trial is such that the trial will not be able to provide a timely and reliable answer to the questions it was designed to address.

9.4.6. Missing data

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

Investigators will be trained about the importance of patient retention and full data capture. Also, any reasonable attempts should be made by the Investigators to emphasize continued patient's participation for the full duration of the trial (see details in Section 7.3.1 and 10.2). 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.

Subjects who die before the primary endpoint assessment at 12 months will be treated as failures in the efficacy analysis. Additionally, for the primary analysis, patients who are lost to follow-up by 12

months will be considered as failures, with the recognition that the procedures described in this section have been developed to ensure the number of patients who are lost to follow-up will be very small.

9.4.7. Methods for multiplicity correction in analyses

If the primary analysis of the primary endpoint leads to rejection of the null hypothesis, the null hypotheses for the secondary endpoints will be tested in a conditional sequential manner. A null hypothesiswill be rejected if and only if the primary analysis of that endpoint and all primary analyses of preceding primary and secondary endpoints result in a rejection of the respective null hypotheses. This procedure protects the family-wise false positive error rate at the overall one-sided 0.025 level.

It is noted that for secondary endpoints that will be tested both at 75±14 and 365±14 days after the transplant, only the day 365±14 time point will be tested for superiority in the fixed sequence testing procedure.

10. ETHICAL CONSIDERATIONS

10.1. INSTITUTE REVIEW BOARD (IRB)

It is the responsibility of the Investigator to obtain approval of the trial protocol/amendments from the appropriate IRB.

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

- the study protocol,
- the Patient Informed Consent Document,
- the current version of the Investigator's Brochure,
- Investigator's current curriculum vitae,
- any other requested document(s).

A copy of the IRB approval will be sent to Dompé along with **all** other correspondence with the IRB, including the submission documents. The Investigator should file copies of all correspondence with the IRB in the Investigator Site File.

The study will not be started until full written approval has been obtained from the appropriate 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 Investigator will submit any future amendment to the protocol to the IRB which granted the original approval. Any amendment will be implemented only when full approval has been obtained from the appropriate IRB, except for those amendments which involve only logistical or administrative aspects of the study.

The Investigator will send to the IRB any updated Investigator's Brochure received from Dompé (or designee).

The Investigator will submit required progress reports to the IRB which approved the protocol at least annually, as well as report any serious ADRs, life-threatening problems or deaths.

The Investigator will also inform the IRB of reports of serious ADRs occurred at other sites participating to this clinical trial and/or in other clinical studies conducted with reparixin.

The Investigator must inform the IRB of the termination of the study.

10.2. INFORMED CONSENT

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

Each potentially eligible patient will be informed of the study's objectives and overall requirements. The Investigator will explain the study fully to him/her using the Patient Informed Consent Document (PICD). 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. 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 PICD will be signed and personally dated by **both** the patient and the Investigator. Although nursing staff may be involved in describing the trial to a patient, the Principal Investigator (or a Sub-Investigator) must participate in discussions with the patient **and sign** and personally date the PICD.

A copy of the signed form will be provided to the patient, and the original signed PICD will be retained and filed in the Investigator Site File. Patient consent will be documented in the hospital records.

Individual (i.e. site specific) PICD will be based on a master document provided by Dompé and must be approved by Dompé prior to submission to the IRB. Any changes requested by the IRB must be approved by Dompé prior to the documents being used. Translation into a language different from English will be provided, if required.

10.3. CONFIDENTIALITY

All information obtained during the conduct of the study will be regarded as confidential. An agreement for disclosure will be obtained in writing by the patient and will be included in the PICD. Patient's data collected during the study will be handled in accordance with applicable data protection laws and regulations. The patient's privacy associated with the use and disclosure of the patient's protected health information will be safeguarded under applicable country laws (e.g. HIPAA).

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

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

10.4. COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

Before the trial formally starts, Dompé will take out a study-specific insurance contract 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 PICD.

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

11. DATA HANDLING AND RECORD KEEPING

11.1. CASE REPORT FORMS COMPLETION

CRFs will be supplied by the CRO appointed by Dompé. CRFs are the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé'designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

A CRF is required and should be completed for each included patient. The Investigator will be responsible for the accuracy of the data entered in the CRFs. All entries must be written in ***ENGLISH*** in black ink. Source documents should be available to support all the data recorded in the CRF.

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

11.2. DIARY CARD

Diary Cards will be supplied by the CRO appointed by Dompé. They are the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé'designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

A sample Diary Cards is provided in **Appendix 14.7**. Translation into a language different from English, will be provided, if required.

Diary Cards are required and should be completed for each patient. It is responsibility of the Investigator to explain to each patient how to enter the data in the Diary Card.

11.3. DATA MANAGEMENT

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

The CRF and Diary Card pages for all patients will be data-entered (double data entry) into the study 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 to the Investigator for response and signature.

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

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

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

In addition to the documents mentioned in Sections 10.1 and 12.1, the following will be required from the Investigator prior to the initiation visit:

- Current, signed and dated Curriculum Vitae, GCP qualification, and Financial Disclosure Statement of Principal Investigator and any Sub-Investigators. Updates should be provided at lest every two years.

- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation). Updates should be provided as soon as any reference value has changed.
- A signed page of the final protocol and any amendments.
- A signed original of the study Financial Agreement/Clinical Study Agreement with Dompé, including Pharmacy, laboratory etc (i.e. all study specific costs).
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- Form 1572 and financial disclosure form 3455 from all the persons listed on the 1572.

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 at least: the signed protocol, copies of the completed CRFs, and Diary Cards, signed Patient Informed Consent Forms from all patients who consented, hospital records and other source documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

The Investigator will inform Dompé 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 (59th WMA General Assembly, Seoul, October 2008) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (*ICH-GCP*) and any local regulations.

12.1. REGULATORY BODY APPROVAL

The study will not be started until review from FDA has been positively completed.

12.2. STAFF INFORMATION & RESPONSIBILITIES

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

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

12.3. MONITORING

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

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

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

Monitoring at the Pharmacy will be carried out by the U-CRA in order to maintain study blind with all other CRO/site staff involved.

12.3.1. Access to records

The Investigator will allow designated Dompé representatives and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. Source documents should be available to support all the data recorded in the CRF. Location of source data, including those for which the CRF might be accepted as being the sole source document, will be specified and listed at the centre Initiation Visit.

12.4. AUDIT AND INSPECTION

Audit activities will be performed by the Quality Assurance of the CRO appointed by Dompé, except for audit to Protocol/Amendments, Patient Informed Consent Document (template) and CRF that will be done by the Dompé Quality Assurance Unit.

On one or more occasions the study site may be audited by the CRO appointed by Dompé. The Investigator will be informed in advance of such a visit.

Additionally the study site may be inspected by a regulatory agency on one or more occasions.

12.5. DATA MONITORING COMMITTEE

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

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

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

- Two surgeons/physicians with extensive experience in islet transplantation and critical care medicine (e.g. a. Medical Director of a Transplant Program);
- 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. In particular, the DMC will monitor the number of deaths for both arms of the study to assess whether mortality is consistent with historical data. The DMC also will give attention to post-surgical reoperation and clinically significant coagulation abnormalities (intra-abdominal as well as gastrointestinal hemorrhage have been reported to be the most common reasons for reoperation).

- Will review efficacy data in an ongoing manner to enable the assessment of the acceptability of safety in the context of emerging evidence about efficacy, i.e. measures of metabolic control and graft function.

- Will be advisory to Dompé and make recommendations to Dompé regarding the continuation of the trial and potential modifications to the design and conduct of the trial. These recommendations will be made in a manner to maintain confidentiality of emerging information about efficacy and safety, unless access to certain data is needed to enable Dompé to make decisions about the DMC recommendations. Dompé will be responsible for promptly reviewing the DMC recommendations, to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in the study conduct are required

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

12.6. PROTOCOL DEVIATIONS/AMENDMENTS

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

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

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

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

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 CRFs completed to the extent possible.

Study discontinuation will be notified to the FDA within 5 days from decision.

12.8. PUBLICATIONS

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

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study must be prepared in conjunction with Dompé and must be submitted to the Dompé for review and comment at least 45 days prior to submission for publication or presentation. If such draft contains confidential patentable information, the Investigator will refrain from publishing any such information for a period not exceeding 180 days, to enable Dompé to file for the protection of any intellectual or proprietary property interest.

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14. APPENDICES

14.1. APPENDIX 1 – CUMULATIVE SUMMARY OF ADVERSE DRUG REACTIONS[1]

Adverse Drug Reactions - Number of Reports by Terms			Frequency
MedDRA Body System / LLT	**Non Serious**	**Serious**	
Blood and lymphatic system disorders	**3**	**1**	**4.30%**
Anaemia	2		
Coagulopathy		1	
Lymphadenopaty	1		
Cardiac Disorders	**1**	**0**	**1.08%**
Tachycardia	1		
Gastrointestinal Disorders	**13**	**0**	**13.98%**
Abdominal pain NOS	1		
Dyspepsia	1		
Flatulence	2		
Gastroesophageal reflux disease	1		
Nausea	6		
Vomiting	2		
General disorders and administration site conditions	**21**	**0**	**22.58%**
Cannula site reaction	13		
Fatigue	1		
Injection site thombosis	3		
Infusion site oedema	2		
Lethargy	1		
Oedema peripheral	1		
Immune system disorders	**0**	**1**	**1.08%**
Lung transplant rejection		1	
Injury, poisoning and procedural complications	**1**	**0**	**1.08%**
Complications of transplanted kidney	1		
Investigations	**2**	**0**	**2.15%**
Blood amylase increased	1		
Liver function test abnormal	1		
Metabolic and nutrition disorders	**1**	**0**	**1.08%**
Hyperkalaemia	1		
Musculoskeletal and connective tissue disorders	**1**	**0**	**1.08%**
Arthralgia	1		
Nervous system disorders	**25**	**0**	**26.88%**
Dizziness	3		
Headache	8		
Hypoaesthesia	3		
Somnolence	11		
Psychiatric disorders	**4**	**0**	**4.30%**

Adverse Drug Reactions - Number of Reports by Terms			Frequency
MedDRA Body System / LLT	**Non Serious**	**Serious**	
Abnormal dream	1		
Restlessness	1		
Euphoric mood	2		
Renal and urinary disorders	**4**	**0**	**4.30%**
Renal failure	1		
Renal tubular necrosis	2		
Urinary retention	1		
Respiratory, thoracic and mediastinal disorders	**6**	**1**	**7.53%**
Bradypnoea	1		
Cough	2		
Nasopharyngitis	2		
Respiratory failure		1	
Sore throat	1		
Skin and subcutaneous system disorders	**4**	**0**	**4.30%**
Erythema	1		
Infusion site erythema	2		
Pruritis	1		
Vascular disorders	**1**	**3**	**4.30%**
Haemorrhage		1	
Hypotension	1		
Retroperitoneal heamorrhage		2	
TOTAL	**87**	**6**	

1 = table does not include ADRs reported from the ongoing pilot trial in islet allo-transplant patients

The most frequent (>10%) ADRs observed in the phase 1 and phase 2 studies were:

Nervous system disorders (about 27%), including headache, dizziness, hypoaesthesia, somnolence.

General disorders and administration site conditions (about 23%), including cannula site reaction, injection site thrombosis, infusion site oedema and peripheral oedema, fatigue, lethargy.

Gastrointestinal disorders (about 14%), including nausea, vomiting, abdominal pain, dyspepsia, flatulence, gastroesophageal reflux disease.

In patients undergoing islet allo-transplantation and treated with a 7 day course of reparixin most frequent ADRs were erythema, hypotension, nausea, vomiting; great majority of these events were mild to moderate in nature and none required discontinuation of the Investigational Product.

Nausea, vomiting and severe gastrointestinal bleeding associated with anaemia developed in a female patient early after the beginning of reparixin infusion; upon case evaluation, a medical error was evidenced, i.e. the patient received a dose of reparixn 3 times as high as that foreseen in the protocol; infusion of reparixin was immediately discontinued and the patient recovered.

14.2. APPENDIX 2 - PACKAGING AND LABELING DETAILS

The template of label specimens is provided below.

Specimen label for the REPARIXIN Vial

STUDY REP0112

Sponsor Dompé s p.a.; Via Campo di Pile, L'Aquila – Italy
Phone: + 39 346 8745908

REPARIXIN

INVESTIGATOR:

CONTAINS: 250 mL REPARIXIN (33 mg/mL) CONCENTRATE FOR SOLUTION FOR I.V. INFUSION

BATCH No. EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C (86°F)**

DIRECTIONS: Transfer into an INFUSION BAG the content of the vial + 500 mL of 0.9% NaCl, according to procedures detailed in the "Instructions to the Pharmacy".

Caution: New Drug-Limited by Federal law to investigational use.

Specimen label for the PLACEBO Vial

STUDY REP0112

Sponsor Dompé s p.a.; Via Campo di Pile, L'Aquila – Italy
Phone: + 39 346 8745908

PLACEBO

INVESTIGATOR:

CONTAINS: 250 mL Physiologic salt solution (0.9% w/v sodium chloride)

BATCH No. EXPIRY DATE mm/yyyy **DO NOT STORE AT >30°C (86°F)**

DIRECTIONS: Transfer into an INFUSION BAG the content of the vial + 500 mL of 0.9% NaCl, according to procedures detailed in the "Instructions to the Pharmacy".

Caution: New Drug-Limited by Federal law to investigational use.

Specimen label for Infusion Bag - "double tear off" label

Each of the 2 sections of the double label will have identical information as per specimem below.

STUDY REP0112

Sponsor Dompé s p.a.; Via Campo di Pile, L'Aquila – Italy
Phone: + 39 346 8745908

PATIENT No. ☐☐☐☐ **INFUSION BAG No.** ☐

INVESTIGATOR:

CONTAINS: 750 mL OF REPARIXIN (11.00 mg/mL) or PLACEBO I.V. INJECTABLE DOSING SOLUTION

PREPARED ON |__|__|/|__|__|/|__|__|__|__| |__|__| : |__|__|
dd / mm / yyyy h : min

DO NOT STORE AT >30°C (86°F)

USE BY |__|__|/|__|__|/|__|__|__|__| |__|__| : |__|__|
dd / mm / yyyy h : min

DIRECTIONS: Administer as a continuous i.v. infusion into a (high flow) central vein, by an infusion pump. Infusion will start approximately 12 hours (6 to 18) before islet infusion. Pump rate will be adjusted according to patient body weight as per Appendix 3 of Study Protocol.

Caution: New Drug-Limited by Federal law to investigational use.

NOTE: Shadowed field are those to be completed by the pharmacist at the time of preparation.

Patient No. Report the four digit number derived from the "IP Preparation Order Form" received from the Investigator.

Infusion Bag No. Report the sequential bag number within the series for that patient.

Preparation date/time Report the date and time when the preparation of the dosing solution has started.

Use by date/time Calculate the use by date and time as date/time of preparation + maximum 72 hours (expiry date/time of the dosing solution), unless the site has more restrictive rules for reconstituted solutions (e.g. 24 hours).

14.3. APPENDIX 3 - STUDY DRUG INFUSION RATE BY BODY WEIGHT

Reparixin dose = 2.772 mg/kg/hour for 7 days (168 hours)

Dosing solution = 11 mg/mL

BODY WEIGHT(kg) each figure is from 0.00 to 0.99	INFUSION RATE (mL/hour)	24 hours INFUSION VOLUME (mL)
45	11.3	271.2
46	11.6	278.4
47	11.8	283.2
48	12.1	290.4
49	12.3	295.2
50	12.6	302.4
51	12.9	309.6
52	13.1	314.4
53	13.4	321.6
54	13.6	326.4
55	13.9	333.6
56	14.1	338.4
57	14.4	345.6
58	14.6	350.4
59	14.9	357.6
60	15.1	362.4
61	15.4	369.6
62	15.6	374.4
63	15.9	381.6
64	16.1	386.4
65	16.4	393.6
66	16.6	398.4
67	16.9	405.6
68	17.1	410.4
69	17.4	417.6
70	17.6	422.4
71	17.9	429.6
72	18.1	434.4
73	18.4	441.6

BODY WEIGHT(kg) each figure is from 0.00 to 0.99	INFUSION RATE (mL/hour)	24 hours INFUSION VOLUME (mL)
74	18.6	446.4
75	18.9	453.6
76	19.2	460.8
77	19.4	465.6
78	19.7	472.8
79	19.9	477.6
80	20.2	484.8
81	20.4	489.6
82	20.7	496.8
83	20.9	501.6
84	21.2	508.8
85	21.4	513.6
86	21.7	520.8
87	21.9	525.6
88	22.2	532.8
89	22.4	537.6
90	22.7	544.8
91	22.9	549.6
92	23.2	556.8
93	23.4	561.6
94	23.7	568.8
95	23.9	573.6
96	24.2	580.8
97	24.4	585.6
98	24.7	592.8
99	24.9	597.6
100	25.2	604.8

14.4. APPENDIX 4 - STUDY FLOW CHART

Test/Examination	HOSPITAL STAY											FOLLOW-UP	
	Screening	day -1	day 0 Tx day	day 1	day 2	day 3	day 4	day 5	day 6	day 7	Hospital discharge	day 75±14 post transplant	day 365±14 post transplant
Informed consent	X												
Inclusion/exclusion criteria	X												
BP, HR, weight, height[1]	X										X[1]	X[1]	X[1]
Safety laboratory tests	X										X		
Pregnancy test	X												
Renal reserve/function[2] & Total bilirubin	X			X	X	X	X	X	X				
Albumin and pre-albumin	X											X	X
Concurrent sepsis	X												
Pre-operative portal hypertension	X												
miR-375; Inflammatory chemokines/cytokines[3]	XX[3]		XXX[3]	X[3]		X[3]		X[3]		X[3]			
Study drug administration[4]		X----------								X			
PK sampling[4]				X		X		X	X[4]				
ALT/AST				X	X	X	X	X	X	X		X	
XDP, INR/PTT, CRP				X	X	X	X	X	X	X			
Average daily insulin; HbA1c												X	X
Severe, Documented Symptomatic, Asymptomatic hypergycemia events												X----------	X
MMTT[5]: Glucose, C-peptide, insulin												X	X
β-score – TEF calculation												X	X
Steatorrhea[6] and malnutrituion risk[6]												X	X
Adverse Events		X----------									Record **all** AEs up to each hospital discharge Then, only SAEs		----------X
Concomitant Medication	X-------Record **all** CMEDs up to each hospital discharge Then, only drugs used for SAEs, oral hypoglycemic drugs and forbidden medications -------X												

1 = Measure only BP and HR at hospital discharge. Measure only weight at follow-up visits.
2 = Assess Creatinine clearance on pre-transplant. Assess creatinine clearance AND urine output from day 1 to day 6 post-transplant.
3 = Store a serum sample for centralized assay as detailed in **Appendix 14.6.3**. Take the 2 chemokines/cytokines pre-transplant samples 6 to 24 hours apart; obtain all basal samples before surgery and before the Investigational Product administration is started. Take post-transplant samples @ 6, 12, 24, 72, 120 and 168hrs after islet infusion.
4 = Start study drug infusion approximately 12 (6-18) hours before islet infusion. Obtain PK ideally in the morning (day 1, 3, 5 post-transplant). Obtain (if you are one of the 2 selected sites) a sample just prior to, and then 1, 3, 5, 6, 8, and 12 hours after the end of study drug administration.
5= Mixed Meal Tolerance Test: measure glucose, C-peptide and insulin prior to (2 basal samples in the range between -20 to 0 prior to the meal) AND @ 15, 30, 60, 90, 120, 180 and 240 min after the meal, as detailed in **Appendix 14.6.4**. Store a blood sample for centralized assay of C-peptide and HbA1c as detailed in **Appendix 14.6.3**.
6= Evaluate steatorrhea in the 4 weeks preceeding the follow-up visit. Evaluate malnutrition risk level as per pre-albumin value.

14.5. APPENDIX 5 - ACCEPTABLE TIME WINDOWS FOR ASSESSMENTS/ PROCEDURES

Assessment/Procedure	Window
Investigational Product administration	
Start of Investigational Product infusion @ 12hrs prior to islet transplant.	6-18hrs prior to start of islet infusion
End of Infusion @ 7 days (168hrs).	± 12hrs
Follow-up visits	
Visit @ day 75 and 365after the transplant	± 14 days
Mixed Meal Tolerance Test	
Sampling @ 15, 30, 60, 90, 120, 180, 240 mins after the meal	± 5 mins (15 min and 30 min) or ± 10 mins (60 to 240 min)
Chemokine/cytokines; miR-375	
Sampling @ 6, 12, 24, 72, 120 and 168hrs after islet infusion	± 10 mins (6 to 24 hrs) or ± 30 mins (72 to 168hrs)
PK sampling	
Sampling on post-operative day 1, 3 and 5, ideally in the morning	Between 5.30 a.m. and 12.30 p m.
Sampling just prior to termination of Investigational Product administration	Within 6 hrs before the end of Investigational Product administration
Sampling @ 1, 3, 5, 6, 8, and 12 hrs after termination of Investigational Product administration	± 10 mins

14.6. APPENDIX 6 - METHODOLOGICAL DETAILS

14.6.1. Islet determination

The following method will be used to quantify isolated and infused IEQ.

After staining dithizone, islets will be counted by phase contrast microscope and grouped in classes according to their size (islet diameter; μm). IEQ will be derived from the islet number in each class integrated by a conversion factor, as detailed in the table below, where "n" is the number of islet in each class.

Class [islet diameter range (μm)]	Conversion into IEQ [islets of 150 μm diameter]
50-100	n / 6
101-150	n / 1.5
151-200	n x 1.7
201-250	n x 3.5
251-300	n x 6.3
301-350	n x 10.4
>350	n x 15.8

14.6.2. Calculation of creatinine clearance

Renal function will be evaluated by creatinine clearance (CLcr), calculated by the following formula (*Cockcroft-Gault, 1976)*:

$$\text{Male: CLcr} = \frac{[140 - \text{age (years)}] \bullet \text{Weight (kg)}}{\text{Serum Creatinine (mmol / L)} \bullet 815}$$

$$\text{Female: CLcr} = \frac{[140 - \text{age (years)}] \bullet \text{Weight (kg)}}{\text{Serum Creatinine (mmol / L)} \bullet 815} \bullet 0.85$$

14.6.3. Handling of samples for centralized assays and assay methods

Detailed instructions will be provided for preparation, storage and shipment of samples. Tubes and labels for storage will be provided by the CRO appointed by Dompé, along with storage and shipment tracking forms.

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

Arrangements will be made with each centralized laboratory to provide appropriate procedures for receipt and analysis of the samples and, apart from PK analysis (see section "PK samples below"), back-communication of the results to the sending site. All steps will be tracked to ensure correct data reporting.

Once received from the centralized laboratory, each site will enter values pertaining to its patients in the corresponding CRF. All samples will be destroyed after final study report has been issued or after the patient has withdrawn his/her consent.

The centralized laboratory for the assay of C-Peptide, HbA1c and Inflammatory chemokines/cytokines will be:
Northwest Lipid Metabolism and Diabetes Research Laboratories (Santica Marcovina, Ph.D., D.Sc.); University of Washington - 401 Queen Anne Ave North; Seattle, WA 98109 - US. Phone: +001 (206) 685 3331; smm@u.washington.edu. The laboratory is certified by CLIA and all the analyses are performed under strict quality control. Also, the laboratory participates in the National Glycohemoglobin Standardization Program (NGSP) and the method is yearly certified at the level of Laboratory 1 Certification to ensure that the HbA1c values are traceable to the DCCT values.

The centralized laboratory for the assay of miR-375 will be:
Diabetes Research Institute, β-cell Biology Unit (Lorenzo Piemonti, MD); IRCCS Ospedale San Raffaele; 1st floor, Lot Q L33; Via Olgettina, 60; 20132 Milan, Italy.

The centralized laboratory for the assay of PK samples will be:
CDMO Department (Gaetano D'Anniballe BSc), Quality Control Laboratory; Dompé s.p.a. - Via Campo di Pile; 67100 L'Aquila - Italy. Phone: +39 0862 338361; e-mail: gaetano.danniballe@dompe.it

- C-peptide

Blood samples will be kept at room temperature for at least 30 min to allow clotting. They will then be centrifuged at 700 × g within one hour from sampling. Cell free serum samples for each time-point will be aliquoted (at least 2 x 0.5mL aliquots) and stored between -70°C and -80°C until shipment to the centralized laboratory. Determination of C-peptide levels will be performed by a two site immuno-enzymometeric assay using a Tosoh 2000 auto-analyzer (TOSOH, Biosciences, Inc., South San Francisco, CA).

- HbA1c

EDTA whole blood samples will be collected and at least 2 x 1 mL aliquots will be stored between -70°C and -80°C until shipment to the centralized laboratory. Measurement of the relative proportion of hemoglobin subclasses and calculation of the HbA1c levels are performed by a dedicated analyzer (TOSOH, Biosciences, Inc) using non-porous ion exchange high performance chromatography to achieve rapid and precise separation of stable HbA1c from other hemoglobin fractions.

- Inflammatory chemokines/cytokines

Blood samples, obtained before (2 basal samples collected 6 to 24 hrs apart; both samples will be obtained before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion, will be immediately kept at 4°C, protected from the light and centrifuged within 2 hrs at 700 × g at 4°C. Cell free serum will be aliquoted (4 x 100µL aliquots) and stored between -70°C and -80°C until shipment to the centralized laboratory and analysis.

Starting from 25µL of thawed serum the following chemokines/cytokines will be determined by a multiplex technology that uses magnetic bead sets (BioRad BioPlex instrument using a Human Cytokine Panel; Millipore Inc., HCYTOMAG-60K): CXCL8, CCL2 (MCP-1), CCL3, CCL4, CXCL10 (IP-10), IL-6, IL-10, INF-γ, TNF-α, and IL-1β. Due to cross-reactivity with the Human Cytokine Panel (HCYTOMAG-60K), analysis of CXCL-9 (MIG) is performed on a BioRad BioPlex system using MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel III (Millipore, Inc., HCYP3MAG-63K).

- miR-375

Blood samples, obtained before (one basal sample collected before surgery and before Investigational Product administration is started) and 6, 12, 24, 72, 120 and 168hrs after the end of islet infusion, will be immediately kept at 4°C, protected from the light and centrifuged within 2 hrs at 700 × g at 4°C. Cell free

serum samples for each time-point will be aliquoted (at least 2 x 300µL aliquots) and stored between -70°C and -80°C until shipment to the centralized laboratory and analysis.

Circulating miR-375 levels will be measured by droplet digital PCR (ddPCR); serum samples will be extracted with commercially available kits and the recovered miRNA fraction will be first reverse transcribed and then partitioned into approximately 20000 droplets using the droplet generator of the Bio-Rad QX100 droplet digital PCR system. After PCR amplification in a conventional thermal cycler, each individual droplet from each sample will be read and its fluorescent status assessed using the QX100 droplet reader. The output of the QX100 reader will be analyzed using the QuantaLife 1.3 software.

- PK samples

Blood samples will be taken into lithium heparin monovettes and centrifuged ideally within 15 minutes at 2500 x g at 4°C for 10 minutes. If that is not possible then samples can be stored at room temperature for not more than 2 hours or at 4°C for not more than 24 hours before centrifugation. Plasma will be aliquoted (2 x 1.5mL aliquots) and stored at a temperature in the range -20°C to -80°C into stopped tubes (glass or plastic), until shipment to the centralized analytical laboratory.

Analaysis will be carried out within one year from sampling. i.e. before the study blind is broken. Therefore, all samples obtained during the conduct of the trial will be sent to the centralized laboratory and analyzed in order to maintain the study blind. Appropriate procedures will be implemented by the centralized laboratory to guarantee the blinding; also, results will be released only after study blind has been broken.

Analysis will be performed according to standardized and validated methods as per Good Laboratory Practices. In particular, the following PK parameters will be determined:

$C_{initial}$	Plasma concentration of reparixin and metabolites (DF 2243Y and ibuprofen) just prior to the end of infusion;
C_{max}	Maximum plasma concentration of reparixin and metabolites;
t_{max}	Time of maximum plasma concentration reparixin and metabolites;
λ_z	Terminal phase rate constant of reparixin and metabolites;
$t_{½}$	Terminal half life of reparixin and metabolites;
AUC_{0-t}	Area under the plasma concentration-time curve from time zero ($C_{initial}$) to time t (time of last quantifiable plasma concentration) of reparixin and metabolites;
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from time zero ($C_{initial}$) to infinity of reparixin and metabolites
V_z	Volume of distribution of reparixin
CL	Clearance of reparixin

Data will be summarised in a separate report that will be integrated into the final study report.

14.6.4. Mixed Meal Tolerance Test

The MMTT will be performed on all patients possibly after an overnight fast, according to Greenbaum (*2008*). The test will be initiated before 10 a m. The Boost High Protein will be used for the MMTT.

Patients will be given 6mL/kg of Boost preparation up to a maximum of 360mL, to be drunk within 5 min. Blood samples for glucose, C-peptide and insulin measurement will be withdrawn in basal condition (two samples in the range between -20 to 0 prior to the meal) and then @ 15, 30, 60, 90, 120, 180 and 240 min after the meal.

14.6.5. β-score calculation

β-score will be calculated on a 0-8 scoring system (*Ryan, 2005*) which gives 0-2 points each for glucose, HbA1c, stimulated C-peptide and insulin requirement, as per the table below.

Components	Score 2	Score 1	Score 0
Fasting (or before breakfast) plasma glucose (mg/dL OR mmol/L)	≤99 OR ≤5.5	100 – 124 OR 5.6-6.9	≥125 OR >7.0
HbA1c (%)	≤6.1	6.2 – 6.9	≥7.0
Daily average (previous week) insulin (IU/kg/day)	---	0.01 – 0.24	≥0.25
Stimulated C-peptide (ng/mL)[1]	≥0.9*	0.3 – 0.89	<0.3#

1: C-peptide level in the blood sample taken 90 min after drinking the mixed meal. *: If fasting C-peptide is ≥0.9 ng/mL, then the stimulated C-peptide level is assumed to be ≥0.9 ng/mL. #: If stimulated C-peptide is <0. 3 ng/mL, then an overall score of 0 is awarded

14.7. APPENDIX 7 – SAMPLE DIARY CARD

Clinical Trial REP0112

PATIENT DIARY CARD

PATIENT No. ☐☐☐☐

Sponsor: Dompé s.p.a.

Please report in the table below your blood glucose (fingerstick) for 14 days before the planned follow-up visit. You are required to report at least one value taken after an overnight fast (or anyway before breakfast) and one value taken within 2 hours after one of the two meals. Also, please report the total amount of insulin you have taken each day.
Please add the date as day/month/year (example 25/07/2012 for 25 July 2012) and the time as 24 hours clock (example 9.35 in the morning, 21.35 in the afternoon). Please enter the information requested with indelible ink.

Day	**Breakfast glucose**		**Lunch glucose**		**Dinner glucose**		**Daily Insulin**
	Time	Value Before	Time	Value After	Time	Value After	
Monday - date:							
Tuesday - date:							
Wednesday - date:							
Thursday - date:							
Friday - date:							
Saturday - date:							
Sunday - date:							

Investigator's signature: _____ date: _____