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ORIGINAL ARTICLE

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Targeting CXCR1/2 in the first multicenter, double-blinded, randomized trial in autologous islet transplant recipients

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Several cytokines and chemokines are elevated after islet infusion in patients undergoing total pancreatectomy with islet autotransplantation (TPIAT), including CXCL8 (also known as interleukin-8), leading to islet loss. We investigated whether use of reparixin for blockade of the CXCL8 pathway would improve islet engraftment and insulin independence after TPIAT. Adults without diabetes scheduled for TPIAT at nine academic centers were randomized to a continuous infusion of reparixin or placebo (double-blinded) for 7 days in the peri-transplant period. Efficacy measures included insulin independence (primary), insulin dose, hemoglobin A_{1c} (HbA_{1c}), and mixed meal tolerance testing. The intent-to-treat population included 102 participants (age 39.5 ± 12.2 years, 69% female), n = 50 reparixin-treated, n = 52 placebo-treated. The proportion insulin-independent at Day 365 was similar in reparixin and placebo: 20% vs. 21% (p = .542). Twenty-seven of 42 (64.3%) in the reparixin group and 28/45 (62.2%) in the placebo group maintained HbA_{1c} ≤6.5% (p = .842, Day 365). Area under the curve C-peptide from mixed meal testing was similar between groups, as were adverse events. In conclusion, reparixin infusion did not improve diabetes outcomes. CXCL8 inhibition alone may be insufficient to prevent islet damage from innate inflammation in islet autotransplantation. This first multicenter clinical trial in TPIAT highlights the potential for future multicenter collaborations.

KEYWORDS
clinical trial, cytokines / cytokine receptors, innate immunity, insulin / C-peptide, islets of Langerhans, pancreatitis, TPIAT, total pancreatectomy

Abbreviations: BG, blood glucose; CCL3, chemokine ligand 3; CCL4, chemokine ligand 4; CXCL8, interleukin 8 / CXC ligand 8; CXCL9, CXC ligand 9; CXCL10, CXC ligand 10; HbA_{1c}, hemoglobin A_{1c}; Boost HP, Boost High Protein; IEQ, islet equivalents; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-10, interleukin 10; INF-γ, interferon-γ; IEQ/kg, islet equivalents per kilogram; MMTT, mixed meal tolerance testing; MCP-1, monocyte chemoattractant protein-1; SHE, severe hypoglycemic episodes; TNF-α, tumor-necrosis factor α; TPIAT, total pancreatectomy and islet autotransplantation.

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1 | INTRODUCTION

Total pancreatectomy with islet autotransplant (TPIAT) is performed for patients with severe chronic or recurrent acute pancreatitis. In this procedure, the pancreas is removed to treat pancreatic pain, the islets are isolated and transplanted back into the portal vein of the recipient.[1,2] Patients undergoing TPIAT generally do not have diabetes before the procedure. Although islet autotransplant (IAT) is very successful at maintaining some endogenous beta cell function in the majority, only 30%–40% of patients achieve insulin independence.[3–7] The infusion of islets into the portal vein elicits an instant blood mediated inflammatory response that compromises islet survival and engraftment.[8,9] Strategies that reduce this detrimental inflammatory response may improve diabetes outcomes after TPIAT.[10]

Several cytokines and chemokines are elevated early after islet infusion in clinical patients undergoing TPIAT, including the cytokine CXCL8 (also known as interleukin-8).[11] Blockade of CXCR1/2, the receptors for CXCL8, improved islet graft survival in mice models of both autologous and donor islet transplant, creating enthusiasm for inhibiting this pathway in clinical islet transplant recipients including those undergoing TPIAT.[11] Although the investigational drug reparixin, which inhibits CXCR1/2, failed to provide a significant benefit on insulin secretion in a phase 3 trial of 45 patients (27 treated, 18 placebo) with type 1 diabetes mellitus undergoing cadaveric donor alloislet transplant, further analyses suggested that treatment effect may correlate with the intensity of the peri- and post-inflammatory reaction.[12] However, islet transplant success in allotransplantation is complicated by immune-mediated rejection and beta cell toxicity of immunosuppressive agents, and therefore a simultaneous trial was initiated to investigate reparixin in islet autotransplant recipients.

We investigated the use of reparixin in a phase 2/phase 3 randomized placebo-blinded trial of patients undergoing TPIAT, a setting in which there is no risk of alloimmunity or autoimmunity and patients are not subject to immunosuppression treatment. In this manuscript, we present our results with this first multicenter, randomized clinical trial conducted in patients undergoing TPIAT.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

This phase 2/3 multicenter, randomized, double-blind, parallel-assignment study conducted at eight US centers and one Canadian center with a targeted enrollment of 100 subjects age ≥18 years undergoing total/completion pancreatectomy and autologous islet transplantation (TP-IAT) (ClinicalTrials.gov: NCT01967888). Study design was specifically discussed during a Specific Protocol Assessment in agreement with the US Food and Drug Administration. The protocol was approved by each institution's Institutional Review Board. All participants provided informed consent before screening. Exclusion criteria included: previous IAT (if completion pancreatectomy) or pancreatectomy due to pancreatic cancer or benign disease other than pancreatitis, significant kidney or liver disease, coagulopathy, pre-existing diabetes.

2.2 | Study treatment, randomization, and masking

Subjects were randomized to either reparixin at a dose of 2.772 mg/kg body weight/hour by continuous infusion through a high flow vein, or matched (flow rate/length of infusion) placebo, starting approximately 12 h before islet infusion. To maintain blinding, the dosing solution of reparixin in the infusion bag was indistinguishable from that of placebo. An independent statistician generated the master randomization list, balancing reparixin and placebo in a 1:1 (block size = 4) fashion within each center. Individual treatment codes were provided in sealed envelopes to the pharmacist within each participating center to be used for the preparation of the dosing solution and to the sponsor pharmacovigilance team for safety purposes.

The dose of reparixin used for this trial (2.772 mg/kg body weight/hour) was the same being administered in the ongoing clinical trials in islet allotranplantation. Dosing 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, and preliminary data from an ongoing pilot trial in islet allotransplantation supported the efficacy and the safety of such a dose.[11]

2.3 | Pancreatectomy and islet infusion

All sites used their standardized methods for operative management and islet processing. Open or robotic pancreatectomy were performed. Sites processed pancreases for islet isolation by enzymatic digestion using an FDA-approved collagenase and neutral protease, followed by mechanical dissociation using the semi-automated Ricordi technique. Purification was performed when necessary to reduce tissue volume by tissue separation using density gradient in a COBE 2991 cell processor. Islet volume was assessed by manual counts quantified as total islet equivalents (IEQ) and IEQ/kg body weight. The islets were infused on the same day as the pancreatectomy procedure into the portal vein (or a tributary vein) by gravity, with monitoring of portal pressures. In the rare event that portal pressure exceeded 35 cm H_2O and did not decrease (within 30 min), the infusion was discontinued, and any remaining islets were either discarded or placed elsewhere (i.e., peritoneal cavity, small bowel mesentery, etc.).

2.4 | Concomitant medications and perioperative anticoagulation

All subjects received perioperative medications as per center practice. Prophylactic anticoagulation was administered as heparin 70

U/kg in the islet media, followed by a low-dose heparin continuous infusion transitioned to enoxaparin for postoperative days 2–7 or per center practice. Low molecular weight dextran sulfate was prohibited.

2.5 | Perioperative glycemic control and medication restrictions

Glycemic control in the early posttransplant period was achieved by insulin administration to target blood glucose (BG) levels ranging 80–180 mg/dl. Subjects tested BG by fingerstick ≥4 times per day with targets of fasting and pre-meal BG 80 to 125 mg/dl, and 2-hour post-prandial BG <180 mg/dl (or BG 80–140 mg/dl if on enteral or parenteral feeds). Use of non-insulin medications affecting glycemic control, anti-TNFα, IL-1 RA, or corticosteroids (>5 mg prednisone/day) were not allowed.

2.6 | Postoperative follow-up and study endpoints

Study visits occurred preoperatively, on postoperative Days 75 ± 5 and 365 ± 14. The primary outcome was the proportion of insulin-independent subjects at 365 ± 14 days posttransplant, defined as no exogenous insulin for 14 or more consecutive days, with adequate glycemic control (HbA_{1c} of ≤6.5%, a fasting BG not exceeding 126 mg/dl more than three times per week, and a 2-hour post-prandial glucose not exceeding 180 mg/dl more than four times per week or 90-minute BG not exceeding 180 mg/dl on mixed meal tolerance test MMTT) based on a 14-day insulin and glucose diary. Secondary endpoints included the AUC for the serum C-peptide level during four hours of the MMTT, average daily insulin dose (from 14-day diary), β-cell function as assessed by β-score, and the proportion of subjects with an HbA_{1c} ≤6.5% and freedom from severe hypoglycemic events. Markers of exocrine pancreatic insufficiency, documented hypoglycemia, and ketoacidosis events were also monitored. Adverse events were defined by any untoward medical occurrence in a patient or clinical investigation subject who received study drug treatment. As an exploratory endpoint, inflammatory chemokines/cytokines were drawn at baseline (average of two samples drawn 6–24 h apart) and at 6, 12, 24, 72, 120, and 168 h after the end of islet infusion, including: CXCL8, monocyte chemoattractant protein-1 (MCP-1), chemokine ligand 3 (CCL3), chemokine ligand 4 (CCL4), CXC ligand 10 (CXCL10), CXC ligand 9 (CXCL9), interleukin 6 (IL-6), interleukin 10 (IL-10), interferon-γ (INF-γ), tumor-necrosis factor α (TNF-α), and interleukin 1β (IL-1β).

2.7 | Mixed Meal Tolerance Tests (MMTTs)

MMTTs were performed at Days 75 and 365 posttransplant. Glucose and C-peptide were measured at time 0 (just prior to Boost High Protein [HP]) and at 30, 60, 90, 120, 180, and 240 min following Boost HP administration (6 ml/kg, maximum 360 ml). The area under the curve (AUC) for glucose (AUC glucose) and C-peptide (AUC C-peptide) were calculated. AUC C-peptide to AUC glucose ratio was calculated to normalize C-peptide to glucose values. In addition, AUC C-peptide measures were normalized to IEQ and weight by dividing by IEQ and IEQ and weight, respectively.

2.8 | Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median with range (min–max), according to their distribution, while categorical data are presented using counts and percentages. Sample size was powered on the primary endpoint of insulin independence at Day 365. The proportion of insulin-independent subjects was compared between treatment groups using the Cochran-Mantel-Haenszel test stratified by IEQ/kg at IAT (<2500 IEQ/kg, 2500–5000 IEQ/kg, and >5000 IEQ/kg, with these thresholds for islet mass and expected outcomes defined *a priori* for sample size calculations based on the largest series of insulin independence data in the TPIAT literature at the time of protocol development[7]). The significance level used for statistical testing was 0.025, and a one-sided test was used. As a sensitivity analysis, the primary endpoint analysis was repeated including Site 01 only, which was the largest site with the greatest number of subjects receiving IAT. Secondary endpoints were analyzed for descriptive purposes. The C-peptide AUC after the MMTT normalized by IEQ/kg and the mean in average daily insulin requirements were analyzed by a repeated measurements model, including terms for treatment, time point and center. For the MTTT measures, least squares means, standard errors, and confidence intervals come from a mixed repeated measures model which includes AUC as the response; treatment group, time, and study site as fixed main effects; the treatment by time interaction; the treatment by study site interaction if significant at the 0.10 α-level; patient as a random effect; and uses a compound symmetry covariance structure. The treatment effect within each time point was compared using a two-sided test at the 5% level. The proportion of patients with an HbA_{1c} ≤6.5% was analyzed using Pearson Chi-square. The effect of treatment on the rate of recurrent episodes of severe hypoglycemia was evaluated using an Andersen–Gill analysis with robust sandwich-type variance estimate. The other secondary efficacy endpoints were analyzed using appropriate parametric and nonparametric tests and appropriate 95% confidence intervals (Cis) were presented.

AEs and SAEs were presented in terms of the incidence, severity and relationship to the study drug, overall and by body system and preferred term. Results for laboratory test at each follow-up were presented using descriptive statistics.

3 | RESULTS

3.1 | Patient disposition and baseline characteristics

Details of patient disposition and inclusion in analysis sets are shown in Figure 1. One hundred and four patients were enrolled and randomized into the trial between February 2014 and December 2016 based on TPIAT eligibility criteria. Two subjects were excluded (not treated) since did not meet the study criteria prior to the surgical procedure. The remaining 102 patients received the study treatment (50 reparixin; 52 placebo) and were included in the Intention To Treat Population (ITT, *N* = 102) analyses.

3.2 | Patient characteristics and interventions

Patient characteristics of the ITT Population did not differ between both groups (Table 1).

Overall, there were more females 71 (70%), mean age was 39.5 (SD 12.2) years, and mean BMI was 27.1 (SD 6.7). The most common indication for surgery was chronic pancreatitis in 92 (90%) patients of unknown or idiopathic etiology 35 (34%). Ninety one (89%) patients had a total pancreatectomy, whereas 11 (11%) a completion pancreatectomy. All but one patient from the placebo group received IAT. Mean IEQ was 311 010 (SD 187 251) and mean IEQ/kg was 4,158 (SD 2371).

Ninety one (89%) patients completed the treatment over 7 days: 42 (84.0%) versus 49 (94.2%) in the reparixin versus placebo group, respectively (p = .119). Ten patients (20%) in the reparixin group and six patients (11.5%) in the placebo group received <70% of the intended dose of study drug (p = .284); this includes patients for whom treatment was stopped early and those who completed treatment but had interruptions in the continuous infusion that resulted in a lower total dose of drug. The most common reasons for discontinuation of Investigational Product were "other" (7/13 patients, 53.8%) and "adverse event" (4/13 patients, 30.8%).

3.3 | Primary endpoint—Insulin independence at Day 365 for ITT Population

Overall, 10 (20.0%) versus 11 (21.2%) patients were insulin-independent at Day 365 ± 14 in the reparixin versus placebo group (p = .524, Table 2). The proportion of insulin-independent patients at Day 365 in relation to the ranges of islet mass transplanted (<2500 IEQ/kg, 2500–4999 IEQ/kg, ≥5000 IEQ/kg) also did not differ statistically in both groups. No patient was insulin-independent when transplanted islet mass was below 2500 IEQ/kg. The odds of achieving insulin independence at Day 365 after IAT were greater for patients with islet mass transplanted of 2500–5000 IEQ/kg or >5000 IEQ/kg compared with patients with <2500 IEQ/kg (OR = 13.8 and OR 16.4, respectively, both p = .002).

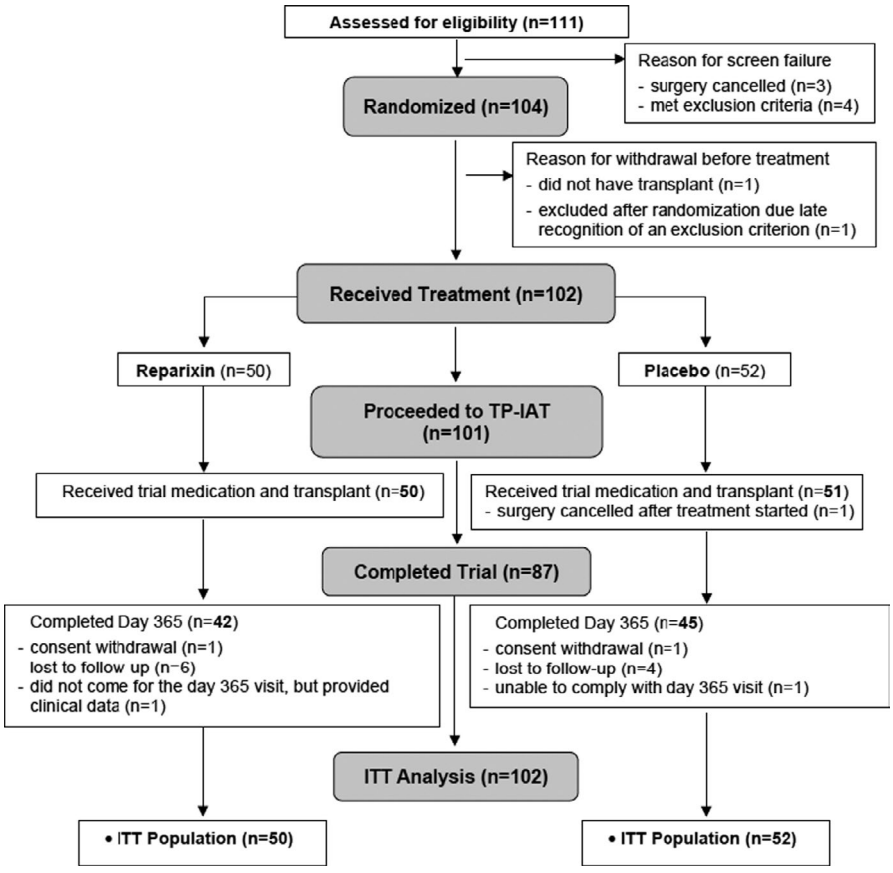

FIGURE 1 Consort diagram of study enrollment, randomization, and follow-up

TABLE 1 Patient characteristics and interventions

	Reparixin (*N* = 50)	Placebo (*N* = 52)	*p*-value
Age at screening (years), *n*			.684
Mean (SD)	40.0 (14.4)	39.0 (10.0)	
Median (min, max)	41.0 (18, 67)	39.0 (20, 61)	
Gender, *n* (%)			.520
Male	17 (34.0)	14 (26.9)	
Female	33 (66.0)	38 (73.1)	
Race, *n* (%)			.187
White/Caucasian	50 (100)	46 (88.5)	
Black or African American	0	2 (3.8)	
Asian	0	1 (1.9)	
Mixed	0	1 (1.9)	
Other	0	2 (3.8)	
Ethnic origin, *n* (%)			.618
Hispanic or Latino	1 (2.0)	3 (5.8)	
Not Hispanic or Latino	49 (98.0)	49 (94.2)	
Height at screening (cm), *n*			.735
Mean (SD)	167.72 (9.35)	167.14 (7.90)	
Median (min, max)	167.70 (139.7, 188.0)	167.55 (149.8, 182.9)	
Weight at screening (kg), *n*			.732
Mean (SD)	75.74 (22.42)	77.20 (20.43)	
Median (min, max)	69.00 (43.5, 135.4)	72.50 (43.1, 132.0)	
BMI at screening (kg/m^2), *n*			.632
Mean (SD)	26.78 (7.39)	27.42 (6.04)	
Median (min, max)	25.6 (15.96, 50.16)	25.93 (17.37, 41.66)	
Indication, *n* (%)			.521
Chronic pancreatitis	44 (88.0)	48 (92.3)	
Acute recurrent pancreatitis	6 (12.0)	4 (7.7)	
Etiology, *n* (%)			.389
Idiopathic or unknown	20 (40.0)	15 (28.8)	
Hereditary or genetic disease	17 (34.0)	17 (32.7)	
Pancreas divisum	7 (14.0)	9 (17.3)	
Sphincter of Odi dysfunction	3 (6.0)	9 (17.3)	
Other	3 (6.0)	2 (3.8)	
Type of pancreatectomy, *n* (%)			1.000
Total pancreatectomy	45 (90.0)	46 (88.5)	
Completion pancreatectomy	5 (10.0)	6 (11.5)	
IEQ, *n*			.924
Mean (SD)	312,823 (177,451)	309,268 (197,936)	
Median (min, max)	288,745 (7,695, 724,019)	271,894 (0, 895,092)	
IEQ/kg, *n*			
Mean (SD)	4,272 (2,353)	4,049 (2,406)	.637
Median (min, max)	3,736 (113, 9,959)	3,640 (0–9,520)	
Viability (%), *n*			.871
Mean (SD)	88.8 (7.9)	89.0 (7.5)	
Median (min, max)	88.5 (72.0, 99.0)	90.0 (77.0, 99.0)	

(Continues)

TABLE 1 (Continued)

	Reparixin (*N* = 50)	Placebo (*N* = 52)	*p*-value
Pellet volume of islet product (ml), *n*			.413
Mean (SD)	13.9 (13.2)	12.2 (6.4)	
Median (min, max)	10.0 (1.5, 80.0)	13.0 (0.8, 30.0)	
Study drug exposure, *n* (%)			
Completed 7 days treatment	42 (84.0)	49 (94.2)	.119
Less than 70% of intended dose	10 (20)	6 (11.5)	.284

One patient from placebo group did not received any IAT.
p-values were referred to a two-sided *t*-test for quantitative variables and to a two-sided Fisher's exact test for frequencies.
Differences between groups are nonsignificant.

To determine if variations in center practice could be masking an effect, we performed a sensitivity analysis at the largest site (Site 1). Similar to the entire cohort, insulin independence rates at Day 365 in did not differ: 6 (28.6%) versus 8 (36.4%) patients were off insulin in reparixin versus placebo group (NS).

3.4 | Average daily insulin requirements and glycemic control by HbA1c

Least square (LS) means for insulin daily dose were similar in the reparixin and placebo groups (Table 3). At Day 75, the mean daily insulin requirement was 0.22 (SE 0.03) versus 0.21 (0.03) IU/kg/day for the reparixin versus placebo group (p = .675), whereas at Day 365 it was 0.17 (0.03) versus 0.18 (0.03), respectively (p = .761). The proportion of patients with an HbA1c level ≤6.5% at Day 365 and free of severe hypoglycemic episodes (SHE) was similar, 25/39 (64%) versus 26/44 (59%) in the reparixin and in the control group, respectively (p = .640), as well as the proportion of patients with an HbA_{1c} level ≤7.0% at Day 365 and free of SHE: 28/39 (71.8%) versus 29/44 patients (65.9%), respectively (p = .564).

3.5 | Assessment of islet graft function

Islet engraftment was assessed based on islet graft function at Day 75 and 365 (Table 3). Islet function was measured by beta score and area under the curve (AUC) C-peptide normalized to IEQ/kg from the 4-hour MMTT, AUC C-peptide normalized by AUC glucose, AUC C-peptide normalized by AUC glucose and IEQ, and beta score. In addition, BETA-2 score and BETA-2 normalized for IEQ were added in the post hoc analysis.

β-score was similar in both groups: 5.7 ± 1.6 versus 5.2 ± 1.9 (p = .176) on Day 75, and 5.9 (±2.0) versus 5.4 (±2.4) (p = .403) on Day 365 for the reparixin group versus placebo group, respectively. BETA-2 and BETA-2/IEQ also did not differ between the groups. MMTT AUC C-peptide normalized to IEQ/kg was lower on Day 75 in reparixin versus placebo group: the LS mean (SE) ([ng/ml]/[IEQ/kg]×1000) was 0.455 (0.07) versus 0.62 (0.07), respectively (p = .046), but the difference did not reach statistical difference on Day 365: 0.47 (0.08) versus 0.59 (0.07), respectively (p = .172). Islet graft function by MMTT calculations were otherwise similar between the two groups (Table 3).

3.6 | Time course of glucose, C-peptide, and insulin derived from the MMTT

In the model estimates over all time points, the LS for C-peptide and insulin mean difference (reparixin–placebo) was not statistically significant for any of the parameters at Day 75 or at Day 365 (Figure 2). However, at Day 365, the LS mean values for glucose were statistically significantly higher in the placebo group compared to the reparixin group at 30 min (p = .047), 60 min (p = .020), and 90 min (p = .028).

TABLE 2 Proportion of patients who were insulin independent at Day 365 after islet autotransplantation (ITT Population)

Insulin independent at Day 365, *n* (%)	Reparixin	Placebo	*P*-value
ITT Population (*N* = 102)	N = 50	N = 52	
Overall	**10 (20%)**	**11 (21.2%)**	.542
Islet mass infused			
<2500 IEQ/kg	0	0	
2500–5000 IEQ/kg	6 (31.6%)	7 (33.3%)	
>5000 IEQ/kg	4 (21.1%)	4 (22.2%)	
Site 01 (*N* = 43)	N = 21	N = 22	
Overall	**6 (28.6%)**	**8 (36.4%)**	.409
Islet mass infused			
<2500 IEQ/kg	0	0	
2500–5000 IEQ/kg	2 (25.0%)	4 (37.4%)	
>5000 IEQ/kg	4 (50.0%)	4 (57.1%)	

Bold indicates the overall insulin independence rates.

TABLE 3 Secondary efficacy endpoints; assessment of glucose control and islet graft function in ITT Population

	Reparixin	Placebo	P
Insulin requirement [IU/kg/day] (SE)			
Day 75.	0.22 (0.030)	0.21 (0.030)	.675
Day 365	0.17 (0.030)	0.18 (0.030)	.761
HbA_{1C}, Day 365			
Mean (SE)	6.4 (0.2)	6.8 (0.3)	.772
≤6.5%	27/42 (64.3%)	28/45 (62.2%)	.842
≤6.5% and free of SHE	25/39 (64.1%)	26/44 (59.1%)	.640
<7.0% and free of SHE	28/39 (71.8%)	29/44 (65.9%)	.564
Beta score (0–8) (SE)			
Day 75	5.7 (0.248)	5.2 (0.270)	.176
Day 365	5.9 (0.353)	5.4 (0.377)	.403
BETA 2			
Day 75			
Mean (SE)	13.3 (0.908)	12.5 (0.934)	
Median (min–max)	14.7 (0.6–27)	12.10 (1.3–28.2)	.432
Day 365			
Mean (SE)	15.6 (1.352)	14 (9.2, 1.457)	
Median (min–max)	18.1 (1.7–26.5)	16.28 (0.6–34.3)	.306
BETA 2/IEQ			
Day 75			
Mean (SE)	77.6 (21.9)	55.2 (5.3)	
Median (min–max)	50.93 (3.5–947.8)	47.78 (6.7–186.9)	.609
Day 365			
Mean (SE)	65.8 (8.9)	54.8 (5.1)	
Median (min–max)	54.09 (8.8–291.1)	55.97 (5.9–131.1)	.447
AUC c-peptide [ng/ml]			
Day 75			
Mean (SE)	1.86 (0.17)	2.08 (0.19)	
Median (min–max)	1.79 (0.06–4.33)	1.81 (0.32–6.04)	.553
Day 365			
Mean (SE)	1.99 (0.18)	2.22 (0.19)	
Median (min–max)	1.89 (0.06–4.88)	2.37 (0.09–5.92)	.364
AUC c-peptide/ IEQ [ng/ml/IEQ × 10 × 8]			
Day 75			
Mean (SE)	749 (72)	876.9 (74)	
Median (min–max)	711.9 (85–2,121)	718.2 (112–2087)	.319
Day 365			
Mean (SE)	819.4 (76.5)	871.8 (81.1)	
Median (min–max)	877.5 (88–1,723)	790 (125 –2683)	.937
AUC c-peptide/IEQ/kg			

(Continues)

TABLE 3 (Continued)

	Reparixin	Placebo	P
LS mean (SE) [ng/ml]/[IEQ/kg]×1000			
Day 75	**0.46 (0.07)**	**0.62 (0.07)**	**.046**
Day 365	0.47 (0.08)	0.59 (0.07)	.172
AUC c-peptide/AUC glucose [ng/ml]/[mg/dl] × 10^4			
Day 75			
Mean (SE)	149 (14)	161 (16)	
Median (min–max)	144 (4–361)	139 (11–452)	.920
Day 365			
Mean (SE)	167 (93)	177 (19)	
Median (min–max)	167 (4–331)	197 (4–585)	.837
AUC c-peptide/AUC glucose/ IEQ [ng/ml]/[mg/dl]/IEQ × 10 × 8			
Day 75			
Mean (SE)	5.9 (0.6)	6.2 (0.5)	
Median (min–max)	5.9 (0.4–15.3)	5.7 (0.80–19.1)	.896
Day 365			
Mean (SE)	6.8 (0.7)	6.5 (0.7)	
Median (min–max)	6.3 (0.2–14.5)	6.2 (0.7–24.7)	.695

Data are expressed as mean (SE) or number (%). Bold indicates the significant difference between groups.

3.7 | Cytokine profiles

There was no overall difference in the trend of inflammatory cytokines/chemokines post-islet infusion in reparixin versus placebo-treated groups. The largest increases in inflammatory cytokines occurred for IL-6 and IL-10, with a smaller peak for IL-8 and MCP-1 (Figure 3).

3.8 | Safety endpoints

No notable differences between treatment groups were observed in any of the posttransplant safety endpoints, vital signs, body weight, steatorrhea, or hypoglycemic episodes (Table S1). Severe hypoglycemia was rare in the entire study, with only 0.1 episodes per person-years in the reparixin group and none in the placebo (NS). Steatorrhea daily or few times per week was reported in approximately 40% of patients at Day 365 in both groups. Body mass loss on Day 75 comparing to screening was 6.5 kg and persisted as 7 kg on Day 365 in both groups.

3.9 | Adverse events

Adverse events (AE) and serious adverse events (SAE) were similar in reparixin and placebo groups (Table S2). There were no deaths.

Forty-nine patients (98.0%) in the reparixin group reported 489 AEs, and 50 patients (96.2%) in the placebo group reported 533 AEs. There were 100 versus 109 SAEs reported in 29 (58.0%) patients in the reparixin and 30 (57.7%) in placebo group, respectively; none were probably or highly probably related.

4 | DISCUSSION

Total pancreatectomy with islet autotransplantation offers relief from severe pain for those individuals diagnosed with chronic pancreatitis, but at the expense of postoperative diabetes mellitus.[1] The instant blood mediated inflammatory response triggered by intraportal islet infusion is postulated to negatively impact islet engraftment and survival.[8] In this phase 2/3, multicenter, randomized, blinded and placebo-controlled trial, reparixin therapy administered for 7 days post-islet infusion to block the CXCL8 inflammatory pathway was not effective to improve insulin independence or islet graft function in adults undergoing TPIAT.

The instant blood mediated inflammatory response is a complex upregulation of multiple cytokines and chemokines, complement, and coagulation pathways.[10] Our data suggested marked elevations for IL-6, IL-10, and MCP-1 after islet infusion, while only an approximately threefold increase in CXCL8 levels was observed. Indeed, overall cytokine and chemokine profiles were similar in reparixin-treated and placebo-treated subjects. In the mouse model, serum concentration of CXCL1, the murine counterpart of CXCL8 after syngeneic islet transplantation was about sixfold higher[11] than CXCL8 in humans, whereas MCP-1 and IL-6 levels were about sixfold and threefold, respectively more elevated in the human blood. Interestingly, isolated islets are known to produce *in vitro* abundant MCP-1 on cytokine stimulation, which has been linked to poor islet graft survival,[13] and increased circulating levels of MCP-1 in response to IBMR have been reported.[8] In addition, preclinical studies have focused on a single effect of reparixin on hand-picked pure islets in the absence of other treatments, whereas standard TPIAT clinical protocols deliver impure islet tissue and incorporate heparin, which is known to have a strong inhibitory effect on IBMIR.[14,15]

The comparison between clinical and preclinical data suggest a non-crucial role of the IL-8/PMN axis in the TPIAT clinical setting that is coherent with the observed lack of efficacy of the treatment.

It is noteworthy that, in contrast to our data, elevated levels of IL-8 during the first 24 h following TPIAT were reported in control arm patients from a retrospective clinical study investigating the effect of the combination of anakinra and etanercept to reduce IBMIR.[16] Since these patients underwent TPIAT from 2006 to 2009, it is possible that progressive improvement in the islet isolation and infusion procedure have contributed to mitigate the early PMN-mediated local inflammatory reaction with a moderate impact on the stimulation of monocyte-directed mediators possibly produced by the islets themselves.[17]

Although an early open label pilot study suggested efficacy of reparixin in islet allotransplant,[11] our results are similar to those more recently reported findings from a phase 3 randomized trial in islet allotransplantation for type 1 diabetes. In that study of 39 patients with type 1 diabetes, receiving cadaveric donor islets under cover of immunosuppression, no differences in C-peptide response to a mixed meal (the primary endpoint in the type 1 diabetes study) were observed in reparixin versus placebo.[12] However, the induction immunosuppression was found to strongly influence the intensity of the inflammatory reaction, with anti-thymocyte globulin (ATG) triggering a much higher cytokine/chemokine reaction as compared to basiliximab. In line with the mechanistic hypothesis, a higher proportion

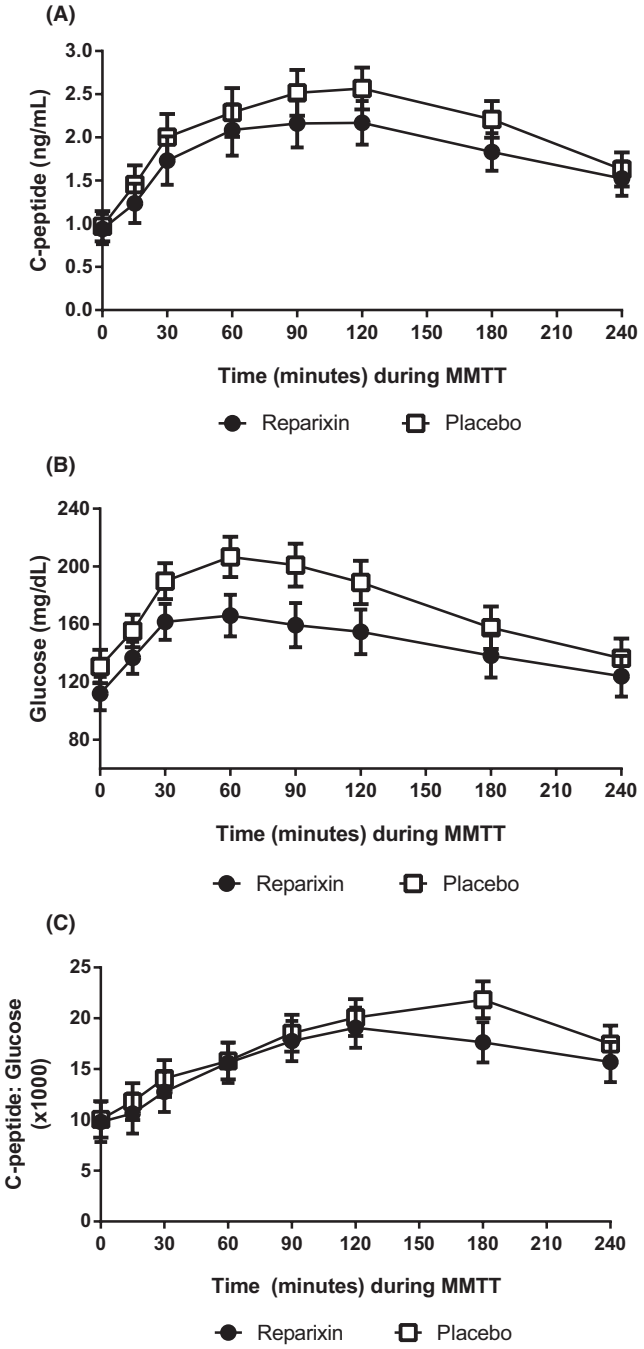

FIGURE 2 Mixed meal tolerance testing at Day 365 with (A) least squares (LS) means C-peptide, (B) LS means glucose, and (C) LS means for C-peptide to glucose ratios for thereparixin (black circles) and placebo (open squares) groups

of insulin-independent subjects was observed in the ATG subgroup treated with reparixin as compared to placebo. Similar to the basiliximab subgroup in allotransplantation, in our TPIAT recipients who do not require immunosuppression, we found no benefit of reparixin alone on posttransplant islet function in 102 subjects treated. Of note, our clinical trial in islet autotransplantation was begun while the allotransplant trial was ongoing, and thus these results and subgroup analyses from the randomized clinical trial in islet allotransplantation for type 1 diabetes were not available until near completion of the current study. It is possible that reparixin could have a role in alloislet transplantation specifically in the setting of ATG therapy, given the cytokine release in this setting may exaggerate IBMIR, but this would need to be evaluated in future studies with larger numbers of ATG-treated allogeneic islet transplant recipients.[11,18]

Another cytokine inhibitor, etanercept, a TNF-α inhibitor, appears to convey some benefit on long-term outcomes in islet allotransplantation.[17] However, preliminary data with clinical use of a "double cytokine blockade" with etanercept and an IL-1 inhibitor suggest a potential modest benefit on glycemic control and C-peptide-based markers only when used in combination, compared to etanercept alone.[16] Thus, we cannot exclude the possibility that reparixin could have a role for study in a combined drug approach. However, no such studies are underway at this time.

Of note, while we observed no differences in insulin independence or insulin secretion (C-peptide) between the reparixin and placebo groups, there was a lower meal-stimulated glucose excursion in reparixin treated as shown in Figure 2. While this does suggest somewhat better post-prandial glycemic control in the reparixin group, glycemic control is complex and is influenced by both insulin secretion and insulin sensitivity. AUC C-peptide/AUC glucose ratio was similar between groups supporting that insulin secretion in each group was appropriate for blood glucose levels. It is likely that the small differences in glucoses in the reparixin group displayed in this figure are resulting from non-treatment factors such as insulin sensitivity, which was not assessed in this study.

It is notable that despite the lack of benefit from reparixin, outcomes from our TPIAT patients managed across nine different centers supports the benefit of performing an islet autograft when pancreatectomy is necessary for treatment of pancreatitis. Over 20% of patients were insulin-independent using strict study criteria. And nearly 70% were meeting American Diabetes Association defined glycemic control goals at 1 year based on HbA_{1c} level <7%. While glycemic data are sparse in the literature in those undergoing total pancreatectomy alone, as a comparison, fewer than 30% of patients (age 18–50 years) with complete insulin deficiency due to type 1 diabetes registered in the type 1 diabetes exchange registry had a HbA_{1c}<7%.[19]

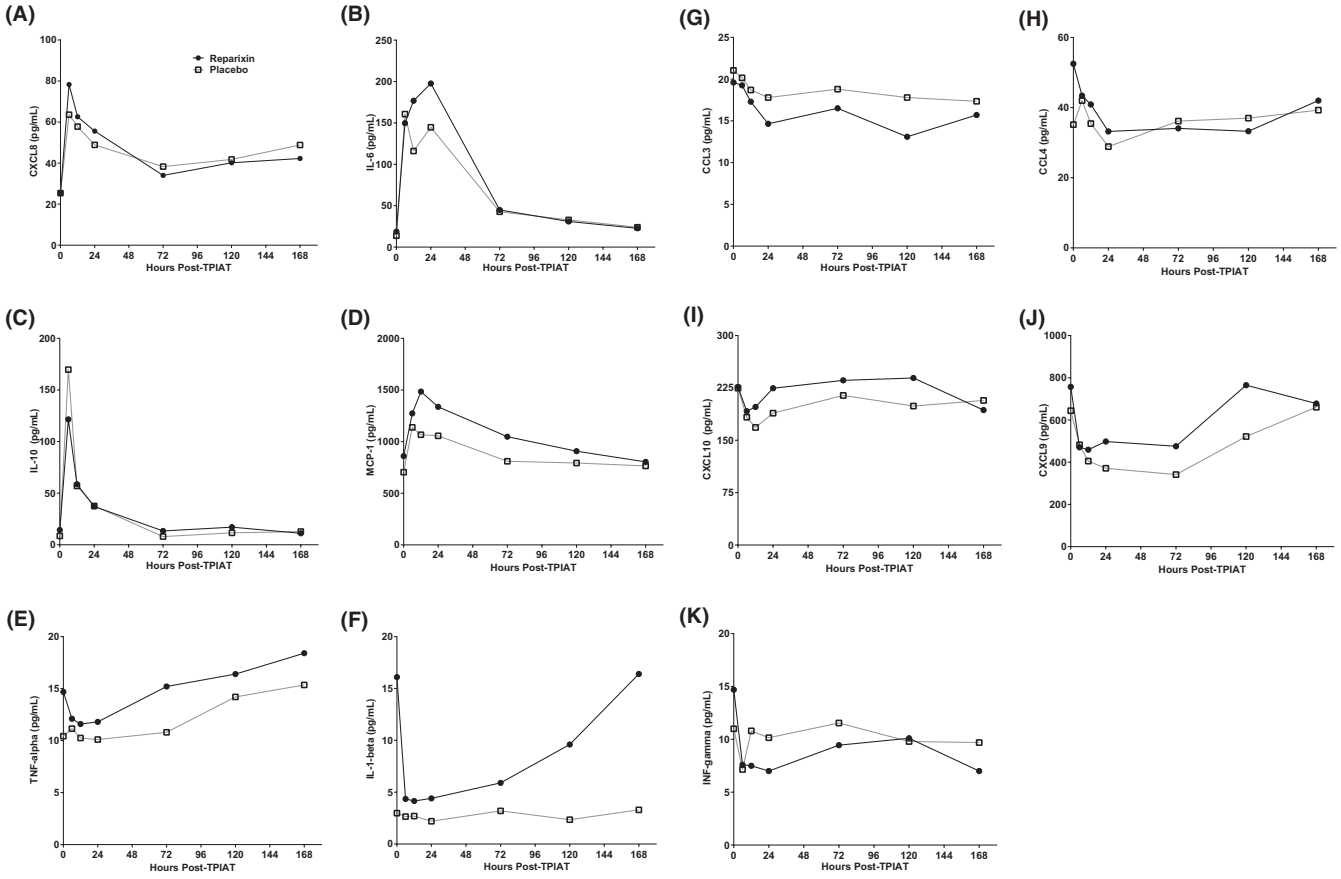

FIGURE 3 Cytokine profiles after TPIAT in Reparixin (black circles with black line) or Placebo (open squares with gray line) groups, for CXCL8 (A), IL-6 (B), IL-10 (C), MCP-1 (D), TNF-alpha (E), IL1-beta (F), CCL3 (G), CCL4 (H), CXCL10 (I), CXCL9 (J), INF-gamma (K). Plot reflects median values. Time 0 is the average of two pre-TPIAT baseline samples

While insulin independence rates did differ across sites, insulin independence is multi-factorial and may be potentially influenced by severity of disease at time of TPIAT, patient characteristics, lifestyle choices, and site insulin management protocols. It is unlikely that a treatment effect was obscured by site differences for a couple reasons. First, randomization was stratified by site to ensure equal representation of sites in the placebo and reparixin group. Second, even a sensitivity analysis of the primary endpoint at the largest site (almost half of participants) showed no evidence of benefit.

This is the first multicenter, randomized clinical trial in TPIAT performed to date, and highlights the potential for future multicenter collaborations. As TPIAT is a rare procedure, other new therapeutics designed to improve TPIAT success may require such multicenter collaboration to reach sufficient power. Although we are limited by some missing laboratory data for subjects who did not complete the Day 75 or Day 365 visits in person, 87 of 104 subjects completed all protocol visits as planned, and the majority of subjects received the drug infusion as planned. Correlative studies of this trial provide relevant information on the nature of the inflammatory reaction following TPIAT, possibly paving the way for further research in the field. While reparixin does not appear to have a role in islet autotransplantation, future studies could reassess a role in allotransplantation, where ATG-mediated cytokine release may exaggerate IBMIR.[11,18]

In conclusion, in this multicenter clinical study, treatment with reparixin did not lead to an improved transplant outcome compared to placebo, as measured by the proportion of patients who were insulin-independent Day 365 after TPIAT nor based on the secondary measurements of glycemic control and islet graft function. Reparixin was found to be safe and well-tolerated in this patient population.

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DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Melena Bellin also receives support from Dexcom, Viacyte (Research) and Insulet (DSMB membership). Piotr Witkowski was a consultant for Sernova and Dompé farmaceutici (liver transplantation trial), and currently is a consultant for Vertex Pharmaceuticals. The other authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Aggregated results are publicly available at clinicaltrials.gov (NCT01967888). All data can be found in the Clinical Study Report available at Dompe farmaceutici, S.p.A., Milan, Italy.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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