# Targeting CXCR1/2 Does Not Improve Insulin Secretion After Pancreatic Islet Transplantation: A Phase 3, Double-Blind, Randomized, Placebo-Controlled Trial in Type 1 Diabetes

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## OBJECTIVE

**Reparixin is an inhibitor of CXCR1/2 chemokine receptor shown to be an effective anti-inflammatory adjuvant in a pilot clinical trial in allotransplant recipients.**

## RESEARCH DESIGN AND METHODS

**A phase 3, multicenter, randomized, double-blind, parallel-assignment study (NCT01817959) was conducted in recipients of islet allotransplants randomized (2:1) to reparixin or placebo in addition to immunosuppression. Primary outcome was the area under the curve (AUC) for C-peptide during the mixed-meal tolerance test at day 75 ± 5 after the first and day 365 ± 14 after the last transplant. Secondary end points included insulin independence and standard measures of glycemic control.**

## RESULTS

**The intention-to-treat analysis did not show a significant difference in C-peptide AUC at both day 75 (27 on reparixin vs. 18 on placebo, $P = 0.99$) and day 365 (24 on reparixin vs. 15 on placebo, $P = 0.71$). There was no statistically significant difference between treatment groups at any time point for any secondary variable. Analysis of patient subsets showed a trend for a higher percentage of subjects retaining insulin independence for 1 year after a single islet infusion in patients receiving reparixin as compared with patients receiving placebo (26.7% vs. 0%, $P = 0.09$) when antithymocyte globulin was used as induction immunosuppression.**

## CONCLUSIONS

**In this first double-blind randomized trial, islet transplantation data obtained with reparixin do not support a role of CXCR1/2 inhibition in preventing islet inflammation-mediated damage.**


Pancreatic islet transplantation has become a feasible option in the treatment of uncontrolled type 1 diabetes (T1D) that allows long-term sustained function and improved metabolic control even when exogenous insulin is required (1). Although significant progress has been made in islet transplantation, several limitations remain that preclude its widespread application (2). Among others, the loss of up to 75% of

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**Members of the REP0211 Study Group are listed in the Appendix.*



**See accompanying article, p. 698.**

islets during engraftment in the liver is a main obstacle, requiring a very large number of islets from more than one donor (3,4). Moreover, the detrimental impact of the inflammatory response and preexisting as well as transplant-induced auto- and allo-immunoreaction are not fully addressed by current immunosuppression protocols (5). Growing evidence demonstrates the contribution of innate inflammatory events to islet injury occurring posttransplant. Some groups have suggested enhanced engraftment when combining the tumor necrosis factor-α (TNF-α) inhibitor etanercept with the T-lymphocyte–depleting agent antithymocyte globulin (ATG) for induction of immunosuppression (6,7). In the same direction, blockade of the CXCL1-CXCR1/2 axis was recently demonstrated to promote fully allogeneic islet survival after transplantation to prevent and revert T1D in a NOD mouse model and to lower inflammation-mediated cell damage (8). Reparixin is an investigational low-molecular-weight, allosteric inhibitor of CXCR1/2 chemokine receptors (9). Data obtained in experimental models of islet transplantation in mice demonstrate an effect of reparixin in improving graft survival and function, a protective effect on transplanted islets confirmed in a pilot clinical trial in patients with T1D receiving allogeneic islet transplantation (10,11). Thus, the use of reparixin may emerge as a potential key component in the sequentially integrated approach to immunomodulation and control of the inflammatory events surrounding the early phases of islet engraftment. This finding prompted the conduct of a phase 3 clinical study aimed at assessing the efficacy and safety of reparixin in preventing graft dysfunction after islet transplantation in patients with T1D.

## RESEARCH DESIGN AND METHODS

### Study Design and Patients

This phase 3 clinical trial was registered with ClinicalTrials.gov (NCT01817959) and conformed with all applicable regulatory requirements. Study design (sample size, primary end point, backbone immunosuppression, etc.) was specifically discussed during a Scientific Advice with European Medicines Agency (EMA)/Committee for Medicinal Products for Human Use. The protocol, protocol amendments, and consent documents were approved by appropriate ethics committees or institutional review boards. All participants provided written, informed consent. This was a multicenter, randomized, double-blind, parallel-assignment study conducted at eight European Union centers (two in Milan, one each in Stockholm, Uppsala, Malmö, Göteborg, Newcastle, and Prague) and one U.S. center (Chicago) in recipients of allogeneic pancreatic islet transplants from donors who were brain-dead. Considering the ultra-rare nature of the clinical condition, sample size was determined by feasibility (number of islets transplanted across countries/sites), with at least 42 patients expected to be enrolled. As a minimum, inclusion criteria included age 18–70 years, T1D with insulin dependence for at least 5 years, and stimulated C-peptide $<0.3$ ng/mL. Exclusion criteria included any previous transplant, insulin requirement $>1$ IU/kg/day, $HbA_{1c}>97$ mmol/mol (11%), creatinine clearance $<60$ mL/min, ALT/AST more than three times the upper limit of normal and total bilirubin $>3$ mg/dL, and other significant comorbid conditions or administration of concomitant medications that could have biased the efficacy outcome/readout. Additional or slightly modified criteria were listed for the U.S. site, being that allogeneic islet transplantation in the U.S. is an Investigational New Drug research procedure.

### Study Treatment, Randomization, and Masking

Patients received either reparixin at a dose of 2.772 mg/kg body weight/h or matched (flow rate/length of infusion) placebo according to their randomization number. Study drugs were administered on top of immunosuppression by continuous infusion through a high-flow vein for 7 days starting ~12 h before each islet infusion. An independent statistician generated the master randomization list, balancing reparixin and placebo in a 2:1 fashion (block of 3) within each center. Individual treatment codes were provided as a sealed envelope to the pharmacist within each participating center to be used for the preparation of the dosing solution. The investigators and sponsor pharmacovigilance service also received individual codes for emergency/safety purposes only. Reparixin was supplied as concentrate (33 mg/mL) solution, and placebo was a commercially available 0.9% w/v sodium chloride solution. The dosing solution for final infusion (if reparixin, 11 mg/mL) was prepared at the designated pharmacy or authorized location within each center according to local guidelines for sterile reconstitution of intravenous (i.v.) injectable solutions. To maintain the blind, the dosing solution of reparixin in the infusion bag was indistinguishable from that of placebo.

### Backbone Immunosuppression, Anticoagulation, and Other Medication Restriction

All subjects were to receive backbone immunosuppression, which included induction with either a T-cell–depleting agent (ATG) or an anti-interleukin (IL)-2 receptor (anti-CD25) monoclonal antibody (only basiliximab was used) before the first islet infusion and anti-CD25 before the second islet infusion. ATG was to be administered i.v. (central vein) at a total dose of 6 mg/kg divided into four or five administrations up to day 4 posttransplant, with the first dose to start between 48 and 8 h before islet infusion. Basiliximab was to be administered i.v. at a first dose of 20 mg within 2 h prior to islet transplant and a second dose of 20 mg 4 days posttransplant. Maintenance was to be achieved with one cell proliferation inhibitor, either rapamycin or mycophenolate mofetil, and one calcineurin inhibitor, preferentially tacrolimus, but cyclosporine could also be used. As accepted by the EMA/Committee for Medicinal Products for Human Use, choice of medication and doses could be adjusted on the basis of clinical requirements and/or center practice. Prophylactic anticoagulation was to be achieved with heparin 70 units/kg divided equally between the islet bags, followed by 3 units/kg/h infused i.v. for the next 4 h, then titrated to target partial thromboplastin time in the range of 50–60 s. From day 2 to 7 posttransplant, enoxaparin was to be administered at a dose of 30 mg subcutaneously. Use of noninsulin medications affecting glycemic control, low-molecular-weight sulfate dextran, anti-TNF-α, IL-1 receptor agonist (IL-1Ra), and steroids was not allowed, apart from a 500-mg bolus of methylprednisolone prior to ATG or $\leq$5 mg prednisone daily for physiological replacement only.

### Procedures and End Points

Patients enrolled in this trial could receive a maximum of two islet infusions, the second occurring between months 3 and 15 after the first. Protocols for

procuring and preserving donor pancreas and for isolation were used as per center practice, provided that islet preparation met at least 70% viability, >20% purity, <10 mL pellet volume, and a yield of ≥3,000 islet equivalents (IEQ)/kg (islets counted by phase-contrast microscope and grouped in classes according to their size). To be eligible for a second islet infusion, a patient should have received <10,000 IEQ/kg at the first transplant and should have been on insulin with a still-functioning graft, defined as fasting/stimulated C-peptide >0.3 ng/mL and/or the presence of a clinical benefit (e.g., decreased insulin requirement, reduced hypoglycemia) that justified maintenance immunosuppression to continue. Following hospital discharge, patients had to self-monitor glucose levels and insulin intake according to standard clinical instructions at the site. Follow-up assessments were scheduled during the posttransplant hospital stay and then on day 75 ± 5 after each islet infusion and on day 365 ± 14 after the last islet infusion. Prespecified primary outcome was the area under the curve (AUC) for the serum C-peptide level during 2 h of a mixed-meal tolerance test (MMTT), normalized by the number of IEQ/kg at day 75 ± 5 after the first islet infusion and day 365 ± 14 after the last islet infusion. AUC analyses were based on actual rather than scheduled timings and calculated using the trapezoidal rule. Secondary end points included the proportion of insulin-independent patients, defined as freedom from the need to take exogenous insulin for ≥14 consecutive days, with adequate glycemic control per $HbA_{1c}$ <53 mmol/mol (7%), fasting glucose not exceeding 140 mg/dL more than three times a week, and 2-h postprandial glucose not exceeding 180 mg/dL more than four times a week; time to achieve insulin independence was defined as the interval from transplant to the first day off insulin for ≥14 consecutive days. The proportion of patients who achieved and maintained an $HbA_{1c}$ <53 mmol/mol (7%) and were free of severe hypoglycemic events, change in average daily insulin requirements, and $HbA_{1c}$ levels, β-cell function, autoantibodies (GAD, IA2, optional: zinc transporter 8), and anti-HLA antibodies were other secondary end points. Serum level of chemokines/cytokines (CXCL8, CCL2/MCP-1, CCL3, CCL4, CXCL10/IP-10, CXCL9/MIG, IL-6, IL-10), coagulation/complement activation markers (C3a, sC5b-9, thrombin-antithrombin complexes, D-dimer, polymorphonuclear leukocyte activation), and miRNA-375 were measured posttransplant up to 7 days. In addition to adverse event (AE) recording and measurement of standard laboratory parameters (hematology, clinical chemistry, and coagulation), ALT/AST, prothrombin time/partial thromboplastin time, fibrin degradation products or D-dimer, and C-reactive protein were measured as specific posttransplant safety end points. Details of assessment and time points are reported in Supplementary Fig. 1.

### Statistical Analysis

Data are presented as mean ± SD or median according to their distribution. Variables with a normal distribution were compared using unpaired Student *t* test. Variables with a nonnormal distribution were compared using Mann-Whitney *U* test. Categorical variables were compared using the $\chi^2$ test or Fisher exact test, as appropriate. Survival was estimated according to Kaplan-Meier method. The primary outcome was analysis by ANOVA, including terms for treatment, center, and treatment-by-center interaction. Centers that recruited fewer than four subjects were pooled and counted as a single center for analysis purposes. Treatment effect was compared using a two-sided 0.0025-level Student *t* test estimated from the ANOVA model (statistical significance adjusted for one pivotal trial). All secondary end points and the supportive analyses were considered as descriptive evidence of efficacy and were analyzed without any procedures to account for multiple comparisons. Because of potential confounding factors that became evident after treatment unblinding, alternative approaches were explored, as described in the sections below.

### Data and Resource Availability

No data outside that published in the current article and its Supplementary Data are available for sharing.

## RESULTS

### Patient Disposition and Baseline Characteristics

Fifty-one patients with T1D were randomized from October 2012 to March 2015, selected among those eligible for intrahepatic islet transplantation according to local practice and criteria applicable at each participating center. Two patients were randomized to placebo in deviation from exclusion criteria: one patient was receiving liraglutide, a prohibited medication (exemption was granted provided that liraglutide was immediately discontinued and never readministered); the other patient had a prior pancreas transplant that, however, occurred 14 years prior to enrollment in the REP0211 trial. (The graft was lost in 5 days because of portal vein thrombosis, and immunosuppression was similarly discontinued; the patient had no abnormal hematology results and no panel-reactive antibody at the time of enrollment). Forty-six patients of the 51 randomized received the investigational product and had at least one islet infusion. Five subjects withdrew after the first transplant, including one subject on reparixin withdrawn just after the first islet infusion because of a serious AE (SAE) and one in each treatment group who had lost the graft. Thirty-three subjects had a second islet transplant, and 41 subjects completed the trial (day 365 after the last islet infusion), with the last patient visit occurring in December 2016. Details of patient disposition and inclusion in analysis sets are shown in Fig. 1. Backbone immunosuppression varied among sites (Supplementary Table 1) that specifically clustered as to the induction used for the first islet infusion (Supplementary Table 2): ATGs versus basiliximab in 25 versus 21 patients, respectively (ATGs included either Thymoglobulin or ATG-Fresenius). Also, rapamycin was used in combination with tacrolimus only in three of the nine participating sites. Mean exposure (percent of scheduled total mg/kg dose) to reparixin was 94.3 ± 16.8% and 97.1 ± 5.3% during the first and second islet infusion, respectively. This includes two patients who received 45.3% and 26.0% of the planned dose because of premature treatment discontinuation as a result of SAE occurrence that led to patient withdrawal in the latter case. Duration of drug/placebo administration did not impact any of the outcome measures (data not shown).

The intention-to-treat (ITT) population consisted of the 45 subjects who completed at least day 75 after the first islet infusion. Of these, 41 completed day 365 after the last islet infusion. Recipient, donor, and graft characteristics of the ITT patients are reported in Supplementary Tables 2 and 3. No difference was detected between treatment groups for any

demographic and baseline values apart from a significantly lower donor BMI (second transplant) in the reparixin group.

### Metabolic Outcomes in the Overall Population

The proportions of subjects with a functioning islet graft, defined as a basal or stimulated serum C-peptide level >0.3 ng/mL, were 80% and 87% at day 75 after the first and day 365 after the last infusion, respectively. Eighteen (40%) of 45 patients reached insulin independence after either one or two islet infusions, which was maintained up to 1 year after the last islet infusion in 13 subjects (29%). The median number of severe hypoglycemic events was 0 (25th–75th percentile 0–0) at day 365 after the last infusion versus 5 (0–12) at baseline ($P < 0.001$). The proportion of subjects with an $HbA_{1c}$ level <53 mmol/mol (7%) increased from 20% at baseline to 78% at day 75 after the first infusion and to 71% at the last visit (511 ± 196 days from first infusion, $P = 0.031$). Median $HbA_{1c}$ decreased from 65 mmol/mol (8.1%) at baseline to 45 mmol/mol (6.3%)

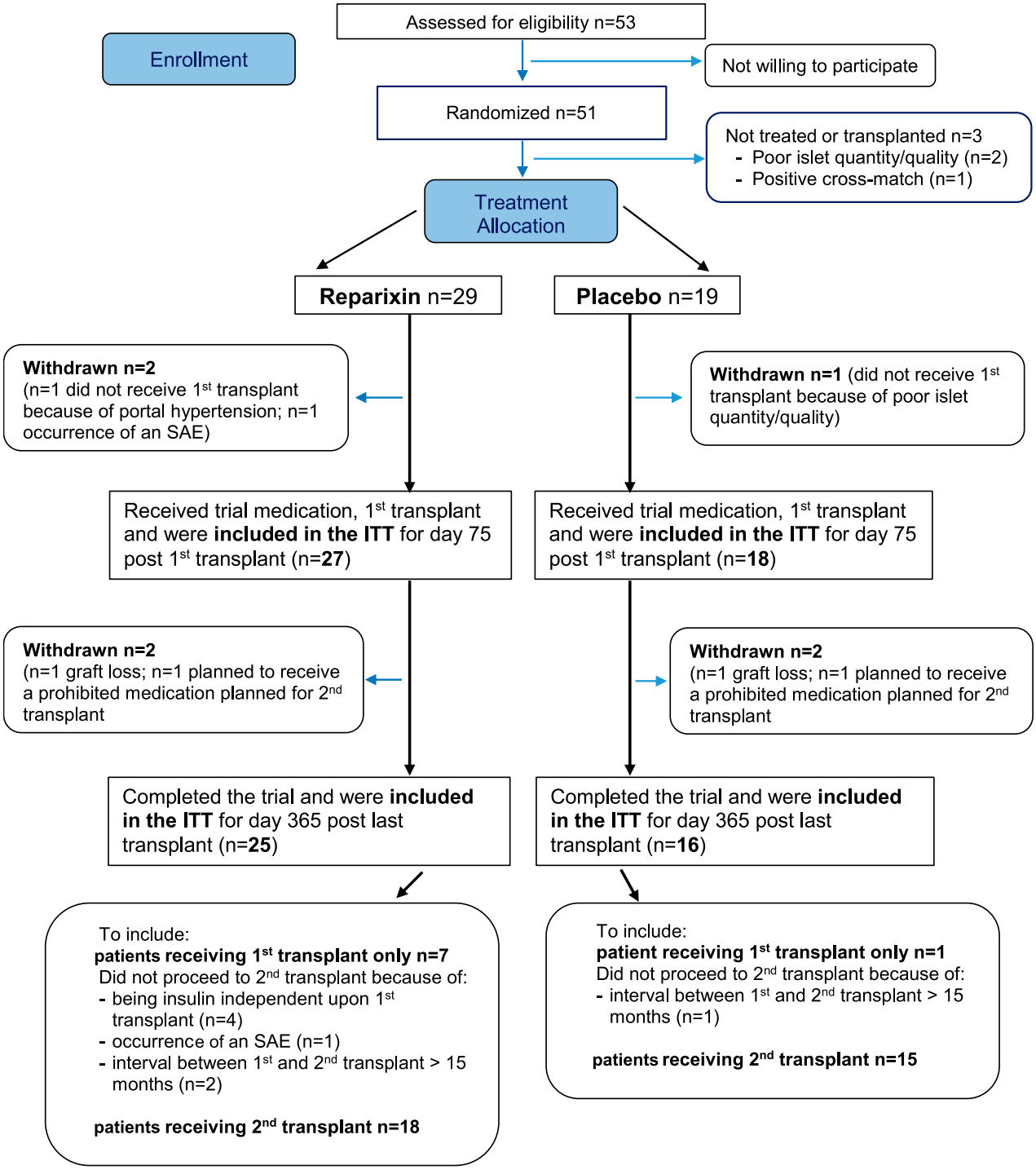


**Figure 1**—Patient disposition diagram showing patient recruitment, random assignment to treatment, discontinuation, and inclusion in analysis sets.

and 42 mmol/mol (6.0%) at day 75 after the first infusion and at the last visit, respectively ($P < 0.0001$). Median daily insulin dose dropped from 0.53 IU/kg/day at baseline to 0.30 IU/kg/day at day 75 after the first infusion and 0.20 IU/kg/day at the last visit (0.00–0.41, $P < 0.001$). A β-score of ≥6 was present in 31% and 38% of subjects at day 75 after the first infusion and at the last visit, respectively. The median β-score increased significantly from 0 at baseline to 4 (2–6) at day 75 after the first infusion and to 5 (2–7) at the last visit ($P < 0.0001$).

### Preplanned Efficacy and Exploratory Outcomes

The ITT data set analyzed for the primary efficacy outcome included 45 subjects (27 on reparixin, 18 on placebo) for day 75 after the first islet infusion and 39 subjects (24 on reparixin, 15 on placebo) for day 365 after the last islet infusion. One subject in each treatment group, out of 41 completing day 365 after the last islet infusion, did not provide data for the primary variable (MMTT refused). As shown in Fig. 2*A*, there was no statistically significant difference between treatments in C-peptide AUC at both day 75 after the first islet infusion ($P = 0.99$) and day 365 after the last transplant ($P = 0.71$). At day 75 after the first islet infusion, insulin independence was reported in five (18.5%) subjects in the reparixin group compared with one (5.6%) in the placebo group. The number of insulin-independent subjects increased after the second transplant, with eight (32.0%) and five (31.3%) subjects maintaining insulin independence up to day 365 after the last islet infusion in the reparixin and placebo groups, respectively. As shown in Fig. 2*B*, patients in the reparixin group showed a weak trend for a higher probability to gain insulin independence after the first islet infusion. Four subjects randomized at site 09, all in the reparixin group, reached and maintained insulin independence for up to 1 year after a single islet infusion ($P = 0.14$, Fisher exact test). There were no statistically significant differences between treatment groups when examining all the other secondary end points (Table 1).

Since the time when the protocol was generated, advances in the scientific and clinical approach to islet transplant evaluation have been discussed within the islet transplant community. As a result, the $\beta_2$-score (12) and the "Igls score" (13) have emerged as new tools to define the islet transplant outcome. Using available data, both indexes were calculated for the REP0211 patients as a post hoc analysis. As shown in Fig. 3*A*, no statistical difference was detected in the $\beta_2$-score calculated for both day 75 after the first islet infusion ($P = 0.94$, Mann-Whitney *U* test) and day 365 post last islet infusion ($P = 0.4$, Mann-Whitney *U* test). The Igls score is shown in Fig. 3*B*. The percentage of patients falling in the optimal category at day 75 after the first islet infusion trended greater in the reparixin than in the placebo group, but the difference did not reach statistical significance ($P = 0.13$). The proportion of patients fitting the optimal category at day 365 after the last islet infusion was superimposable in the two treatment groups. No clear differences were apparent between treatment groups in the time courses of inflammatory chemokines/cytokines, miRNA-375, coagulation/complement mediators, and quantity/distribution of autoantibodies and anti-HLA antibodies after the first or second infusion (data not shown).

### Analysis of Patient Subsets

Chemokines/cytokines increased in the peritransplant to posttransplant interval, with a time-concentration profile in the placebo group higher in patients receiving ATGs at first islet infusion, as compared with subjects receiving basiliximab (Supplementary Fig. 2). According to the impact of induction on chemokines/cytokines, efficacy end points relevant to the first islet infusion were also evaluated in the subsets of patients receiving either ATGs or basiliximab. As shown in Supplementary Table 4, there was a weak trend for a higher percentage of patients gaining and retaining insulin independence after the first transplant in those receiving ATGs as compared with patients receiving basiliximab (20.8% vs. 4.8% [$P = 0.19$] and 16.7% vs. 0% [$P = 0.11$], respectively), with the highest proportion of insulin independence in the subset of patients receiving ATGs and reparixin. Concordantly, the percentage of subjects falling into the optimal category as defined by Igls score was higher in ATG-treated than in basiliximab-treated patients (25% vs. 4.8%, respectively, $P = 0.1$). Since C-peptide $AUC_{120\ min}$ could not

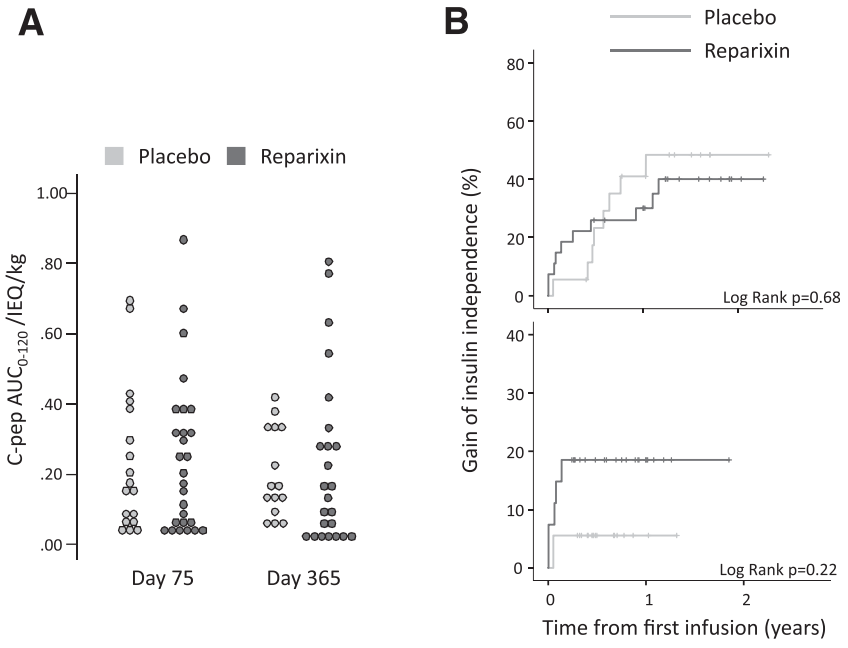


**Figure 2—**Reparixin treatment and islet transplantation outcome in patients with T1D. *A*: Box plots representing AUC of C-peptide (C-pep) after a 2-h MMTT normalized by the number of IEQ/kg. Box = 25th and 75th percentiles; bars = 5th and 95th percentiles. MMTT was performed at day 75 after the first islet infusion and at day 365 after the last islet infusion. MMTT was performed after an overnight fast, as described by Greenbaum et al. (35). Comparison was made using a Student *t* test estimated from the ANOVA model. *B*: Probability of insulin independence after islet transplantation according to Kaplan-Meier method. Top panel: gain of insulin independence after either the first or the second islet infusion. Bottom panel: gain of insulin independence after the first islet infusion. *P* value determined by log-rank test.

**Table 1—Reparixin treatment and islet transplantation outcome in patients with T1D: secondary end points**

| Variable | Time point | Reparixin | Placebo | *P* value |
|---|---|---|---|---|
| Insulin independence, *n/N* (%) | Day 75 post 1st Tx | 5/27 (18.5) | 1/18 (5.6) | 0.38 |
| | Day 365 post last Tx | 8/25 (32.0) | 5/16 (31.3) | 0.91 |
| Insulin requirement (% change from baseline), median (25th–75th percentile) | Day 75 post 1st Tx | −48 (21–80) | −33 (5–61) | 0.39 |
| | Day 365 post last Tx | −65 (29–100) | −86 (42–100) | 0.6 |
| $HbA_{1c}$ (% change from baseline), median (25th–75th percentile) | Day 75 post 1st Tx | −22 (14–25) | −21 (16–30) | 0.26 |
| | Day 365 post last Tx | −17 (6–25) | −23 (14–26) | 0.24 |
| Median severe hypoglycemic episodes | Cumulative from 1st Tx to day 365 post last Tx | 0 | 0 | — |
| MMTT peak C-peptide level (ng/mL), mean ± SD | Day 75 post 1st Tx | 2.09 ± 1.93 | 2.03 ± 1.70 | 0.84 |
| | Day 365 post last Tx* | 2.75 ± 2.74 | 3.64 ± 1.94 | 0.08 |
| β-Score, median (25th–75th percentile) | Day 75 post 1st Tx | 4.0 (2–6) | 4.5 (2–6) | 0.79 |
| | Day 365 post last Tx | 5.0 (2.5–7) | 5.0 (3–7) | 0.94 |

Tx, treatment. *One subject in each Tx group (of 41 completing day 365 after the last islet infusion) refused to undergo the MMTT.

be the best estimate of engrafted islet mass (since it accounts for neither the time-to-peak nor the glucose exposure that can result in very different islet responses interpreted as having the same value), we also examined the C-peptide $AUC_{30\ min}$ (as a marker of early-phase insulin secretion), the incremental AUC (which may be more appropriate when evaluating a response metric), and the C-peptide AUC divided by the corresponding glucose AUC over the same time intervals (Supplementary Tables 5 and 6). Reparixin treatment did not significantly modify any of these parameters. Finally, reparixin did not affect the time-concentration profile of inflammatory chemokines/cytokines in patients receiving either ATGs or basiliximab (Supplementary Fig. 3).

### Safety Results

No clear differences between treatment groups were observed for rates, severity, and distribution of AEs or SAEs. Specifically, 29 (100%) and 18 (95%) patients in the reparixin and placebo groups, respectively, reported an AE, with 17 (59%) patients on reparixin and 12 (63%) on placebo reporting an SAE. Similarly, 14 (48%) patients receiving reparixin and 8 (42%) patients receiving placebo reported an AE that was judged to be treatment related. Three (10%) patients in the reparixin group and two (11%) patients on placebo experienced an AE that led to discontinuation of study treatment; there was one subject who withdrew from the trial because of an SAE that was judged as not related to study drug administration. As detailed in Supplementary Table 7, the great majority of subjects ($n = 38$, 79%) experienced an event of gastrointestinal disorders in both treatment groups. No clear differences were seen between treatment groups in any laboratory values (hematology, biochemistry, coagulation) at screening and hospital discharge, and there were no apparent differences between treatment groups in any posttransplant-specific safety end points.

## CONCLUSIONS

To the best of our knowledge, this is the first randomized, double-blind, phase 3 study conducted to evaluate the efficacy of an investigational anti-inflammatory small molecule in preventing graft dysfunction after islet transplantation in patients with T1D. In summary, there were no statistically significant differences between reparixin and the placebo in the primary, secondary, or exploratory efficacy end points. This is in contrast with preclinical results and the previous pilot experience in humans (10). The field of islet transplantation has evolved significantly from the first report in humans in 1977, and advances in islet isolation and engraftment, together with improved immunosuppressive strategies, have been reported (14). Despite this, only two large-scale phase 3 clinical trials in islet transplantation have been published to date: Clinical Islet Transplantation Consortium Protocol 07 (CIT-07) (multicenter, single-arm) and Trial Comparing Metabolic Efficiency of Islet Graft to Intensive Insulin Therapy for Type 1 Diabetes's Treatment (TRIMECO) (multicenter, open-label, randomized) (15,16). Both these studies were designed to demonstrate that human islets transplanted in subjects with T1D with impaired awareness of hypoglycemia and severe hypoglycemic events can safely and efficaciously maintain glycemic balance and eliminate one of the most severe complications associated with insulin therapy. Few phase 1/2 controlled trials were developed to validate different islet isolation processes, islet infusion, and, more importantly, immunosuppressive strategies (17–20). Consequently, the clinical practice in this field is mainly based on single-center/network observational studies of local practice and results, or on retrospective data analysis of the Collaborative Islet Transplant Registry (CITR), rather than on prospective interventional trials (6,10,21–30). Growing evidence has demonstrated the important contribution of innate inflammatory events to islet injury posttransplantation and, according to the CITR, anti-inflammatory agents have become keystone components of peritransplant management in at least 80% of transplants performed. Adoption of an anti-inflammatory strategy is generally empirical and driven by the availability of drugs on the market rather than by an appropriate and robust supportive validation in the preclinical phase or in clinical study (31). We previously demonstrated that genetic and pharmacological blockade of the CXCL1-CXCR1/2 axis in mice improves intrahepatic islet engraftment and reduces intrahepatic recruitment of polymorphonuclear leukocytes and natural killer T cells after islet infusion (10). The comparison with other available anti-inflammatory agents (TNF-α inhibitor, anti-IL-1Ra, and dexamethasone) showed

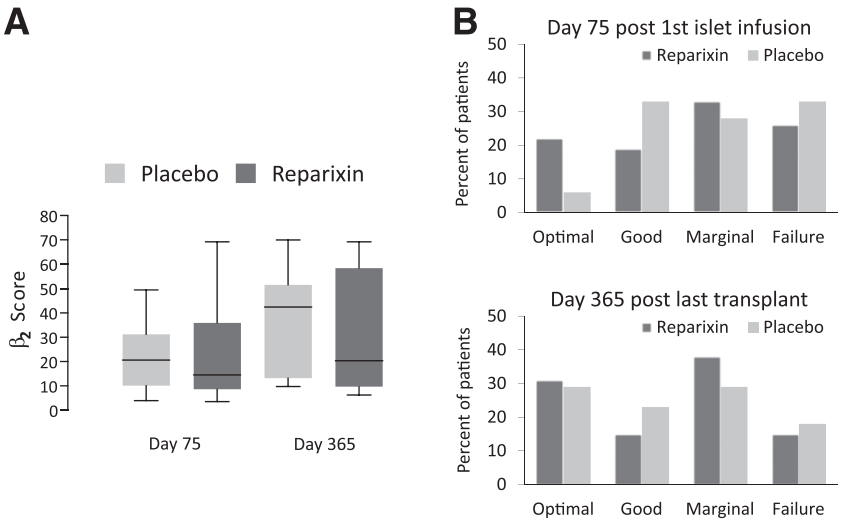


**Figure 3**—Reparixin treatment and islet transplantation outcome. *A*: $\beta_2$-Score was evaluated at day 75 after first islet infusion and at day 365 after the last islet infusion. Comparison was made using Mann-Whitney *U* test. *B*: Metabolic outcomes according to Igls criteria in transplant recipients. Optimal β-cell graft function: $HbA_{1c} \leq 48$ mmol/mol (6.5%), the absence of severe hypoglycemia, the absence of an exogenous insulin requirement or other glucose-lowering (antihyperglycemic) drugs, and documentation of an increase in C-peptide levels compared with the pretransplant condition. Good β-cell graft function: $HbA_{1c} < 53$ mmol/mol (7.0%), the absence of severe hypoglycemia, a reduction by >50% from baseline in insulin requirements or the use of noninsulin glucose-lowering drugs, and documentation of an increase in C-peptide levels compared with the pretransplant condition. Marginal β-cell graft function: failure to achieve $HbA_{1c} < 53$ mmol/mol (7.0%), occurrence of any severe hypoglycemia, or <50% reduction in insulin requirements when there is documentation of an increase over pretransplant measurement of C-peptide that reached >0.17 nmol/L. Failure of β-cell graft function: absence of any evidence for a clinical impact with C-peptide ≤0.17 nmol/L, even if quantifiably higher than before transplant.

that the inhibition of the CXCR1/2 pathway is the most efficient way to improve islet engraftment in the hepatic site and to delay the time to rejection in the mouse model (11). Moreover, blockade of the CXCL1-CXCR1/2 axis plays a key role in preserving β-cell function in a spontaneous mouse model of T1D (8). Concordantly, the CXCR1/2 allosteric inhibitor reparixin improved outcome in a phase 2 randomized, open-label pilot study with a single infusion of a marginal mass of allogeneic islets in subjects with T1D (10). This evidence prompted the testing of reparixin in this phase 3 trial that recruited subjects from a small population of patients with T1D who were insulin dependent for ≥5 years and required a pancreatic islet transplant because of unstable glycemia. This limited the number of subjects eligible for enrollment and, accordingly, the final sample size, even if demographic and baseline characteristics of the subjects randomized in the trial reflect those of the general population of patients with T1D eligible for islet transplantation. Unfortunately, the result of the phase 3 study was negative. More than one explanation could justify this result. The primary and secondary efficacy end points may not have been appropriate to detect differences between treatment groups. The samples size, which was based on feasibility, might not have been sufficient to detect treatment differences. Considering the ultra-rare condition, a sample size dictated by actual feasibility as well as a backbone immunosuppression with a lower degree of standardization were agreed with EMA to ensure participation of those sites in Europe with larger transplant volumes that could provide the best experience in islet transplant. Unbalanced patient distribution across sites and some site-specific differences in the population/procedures, including type of induction used for the first transplant, might have affected the overall results. In fact, the peri- and posttransplant inflammatory reaction was clearly different according to the induction used, with a cytokine/chemokine time-concentration profile higher in patients receiving ATGs at first islet infusion, as compared with subjects receiving basiliximab. Of note, there was a weak trend for better results in terms of insulin independence achievement after the first infusion in patients receiving ATGs as compared with basiliximab, with the higher proportion of insulin-independent subjects in the subset of patients receiving ATGs and reparixin. This experimental condition (single infusion of a marginal mass of allogeneic islets with ATG as induction) is that used in the previous phase 2 pilot study where reparixin was shown to be effective in improving islet function and survival (10). Targeting inflammation may be more important when T-lymphocyte–depleting agents are used for induction of immunosuppression, because of cytokine release from lysed T cells, than when inhibitors of T-lymphocyte activation (anti-CD25) are used. However, reparixin did not affect the release of inflammatory cytokines, leaving unanswered the question of whether this finding accounts for the lack of clinical effect because of possible underdosing, wrong time/duration of study treatment administration, or incorrect hypothesis. On the other hand, the systemic levels of cytokines could be poor biomarkers to evaluate the ability of reparixin to modulate the inflammatory environment of liver after islet infusion.

Regardless of the speculations, we cannot also exclude that the use of an anti-inflammatory therapy does not actually add a real advantage to islet survival. In this direction, anti-TNF-α was integrated into clinical islet transplantation largely on the basis of a single publication and was supported by a single-donor islet transplant series in which etanercept was used both in islet culture and during peritransplant management (32,33). Indeed, even if adopted as a standard therapy by some groups, other studies failed to demonstrate a significant benefit of anti-TNF-α (34). Similarly, adjuvant IL-1Ra anti-inflammatory therapy has been integrated within clinical islet transplantation in combination with a TNF-α inhibition strategy, despite preclinical results showing a role of this combinatory therapy in a syngeneic marginal mass model of islet transplant under the kidney capsule without supporting data in the allogeneic model or in the intrahepatic setting (34).

Although conducted in a limited number of patients (an intrinsic limitation in the islet transplantation field) and negative for the primary outcome, this trial has generated additional valuable knowledge. First, we confirmed the feasibility

and the safety of a treatment with the CXCR1/2 allosteric inhibitor in patients with T1D in the presence of immunosuppression. The safety profiles in interventional and control groups were identical, with no differences in any safety assessment or any clear differences in clinically significant laboratory findings. Second, we confirmed that transplantation of human islets is effective in treating severe hypoglycemic events in patients with T1D with an acceptable $HbA_{1c}$ target. In our trial, >70% of subjects with transplants had an $HbA_{1c}$ level of <53 mmol/mol (7%) and were free from severe hypoglycemia, which was consistent with previous findings (15,16). Insulin independence was achieved in 40% of our patients, which was a slightly lower proportion than in the cohort described in the TRIMECO and CIT-07 trials. This difference is probably explained by the fact that the median $HbA_{1c}$ and insulin requirement values at baseline were higher in our study than in CIT-07 and by the fact that the criteria for the insulin independence definition were more stringent in our study than in TRIMECO.

In summary, previous preclinical and clinical evidence has provided important supportive data on the role of CXCR1/2 inhibition as a possible target to prevent islet inflammatory-mediated damage. This finding is not confirmed by the first randomized, double-blind, phase 3 study conducted to evaluate the efficacy of a drug in preventing graft dysfunction after islet transplantation. Further randomized and controlled studies are needed in the field of islet transplantation to verify whether an anti-inflammatory treatment is indeed able to improve islet transplantoutcome. The choice of a cohort of patients treated with ATG appears to be the most appropriate for any future studies.

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**Author Contributions.** P.M., T.L., G.T., E.R., J.A.M.S., A.L., F.S., P.W., K.G., F.B., and B.G. researched data and contributed to the discussion. L.D. and P.A.R. reviewed and edited the manuscript. L.P. wrote the manuscript, researched data, and contributed to the discussion. L.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented in abstract form at the 17th World Congress of the International Pancreas & Islet Transplant Association, Lyon, France, 2–5 July 2019.

## Appendix

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