Induction of immune education in type 1 diabetes through controlled allogeneic islet rejection at onset: a monocentric open-label pilot study

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Summary

Background Current immunotherapies for type 1 diabetes (T1D) have shown limited success in durably preserving β-cell function. We tested a novel strategy that repurposes allogeneic islet transplantation not for metabolic replacement, but as a platform for antigen-specific immune education.

Methods In this monocentric, open-label pilot study (April 2015–April 2023), six patients with recent-onset T1D received a minimal islet mass (median 3452 IEQ/kg; range 2980–4050) combined with short-term immunomodulation (ATG, transient mTOR inhibition, and G-CSF). The transplanted islet mass was intentionally insufficient for metabolic replacement. The primary endpoint was the change in stimulated 2-h C-peptide AUC at 52 weeks. Exploratory endpoints included immune cell phenotyping, cytokine/chemokine profiling, miR-375 release kinetics, analysis of islet-related autoantibodies, and monitoring of donor-specific HLA antibodies. ClinicalTrials.gov Identifier: NCT02505893.

Findings The protocol was safe and well-tolerated. At 12 months, the median stimulated C-peptide AUC was preserved at 91–100% of baseline with all participants achieving a partial clinical remission (IDAA1c ≤ 9). At 5 years, median C-peptide AUC declined to 44–56% of baseline, with 2 patients maintaining stable secretion and 2 retaining ∼50% of initial function. Exploratory analyses demonstrated a structured pattern of immune resetting, with early lymphodepletion followed by memory and regulatory T-cell expansion; transient increases in IL-2 and IL-10; sustained early elevation of sCD25; biphasic miR-375 peaks indicating early β-cell stress; transient increases in autoantibodies without epitope spreading; and donor-specific class I HLA antibodies in five patients, with persistent class II DSA in two. No expansion of $CD3^+CD8^+$ T cells specific for GAD65 was detected.

Interpretation This study introduces a paradigm shift in the use of islet transplantation—transforming it from a metabolic intervention into a tolerogenic stimulus through controlled antigen exposure. Further potentiation with stem-cell–derived islets may enable improved matching, graft modification, and iterative antigen delivery.

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Keywords: Type 1 diabetes (T1D); Immunotherapy; β-cell function preservation; Allogeneic islet transplantation; Antigen-specific immune education; Minimal islet mass; Immunomodulatory regimen; T cell depletion; mTOR inhibition; G-CSF; Regulatory T cells (Treg) expansion; Immune regulation; C-peptide preservation; Partial clinical remission; Immune reset; Cytokine profiling; IL-2; IL-10; sCD25; miR-375; Autoantibodies; Donor-specific alloantibodies (DSA); HLA matching; Tolerogenic stimulus; Stem cell-derived islets; Immune tolerance;

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Antigen-specific therapy; Immune modulation; Lymphodepletion; Clinical remission; Transient inflammation; Immune rewiring; Immunosuppression alternatives; Personalized antigen delivery; Autoimmune diabetes treatment

Research in context

Evidence before this study

We searched PubMed, Embase, and CENTRAL up to April 1, 2015, using terms related to type 1 diabetes, islet transplantation, immune tolerance, and regulatory T-cell–based interventions. Standard immunotherapies (e.g., anti-CD3, low-dose IL-2, T-cell depletion) transiently preserved C-peptide but did not induce lasting immune tolerance, while antigen-specific approaches were safe but minimally effective. No clinical studies evaluated allogeneic islets specifically as controlled immune triggers rather than metabolic replacement.

Added value of this study

This is the first clinical study to test minimal allogeneic islet infusion combined with short-term immunomodulation as an antigen-delivery strategy rather than as cellular replacement. All participants achieved partial remission, with preserved C-peptide at one year and sustained secretion in a subset after 5 years without chronic immunosuppression. Immune profiling revealed structured modulation with Treg expansion and cytokine dynamics consistent with controlled engagement rather than broad immunosuppression.

Implications of all the available evidence

These findings support controlled antigen exposure combined with transient immune modulation as a potentially viable approach to reshaping autoimmunity in T1D. Integration with stem-cell–derived islets could allow better matching, graft engineering, and repeated antigen delivery. Larger controlled trials are needed to validate this strategy and define optimal patient selection and timing.

Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing beta cells in the pancreas, leading to insulin deficiency and hyperglycemia.[1] The disease is driven by an imbalance between autoreactive T effector cells (Teffs) and regulatory T cells (Tregs), resulting in immune-mediated destruction of the beta cells.[2] Current treatments for T1D rely on insulin replacement addressing blood glucose management, but disregarding the underlying autoimmune process.[3] Efforts to develop immune-based therapies for T1D have generally been met with limited success.[4] Numerous studies have focused on patients with recent-onset stage 3 T1D, typically enrolling participants in their early teenage years.[5] These studies have primarily aimed to measure improvements in stimulated C-peptide responses compared to placebo controls.[6] The therapies tested have targeted a variety of pathways,[7,8] including cytokine antagonists (such as IL-6, IL-1, IL-21, TNF, IL-12/23, and IL-8) and agonists (such as IL-2 and IL-10), depletion and exhaustion of T effector cells (using agents like anti-CD3, ATG, and anti-CD2), inhibition of T cell activation (through blockade of CTLA-4/CD28, CD40/CD154, and CD2), and activation of immune checkpoints (via antibodies targeting PD-1, CTLA-4, and beta cell-expressing PD-L1). Other approaches have included regulatory cell-based therapies (such as CAR/TCR-Tregs, Tr1 cells, MDSCs, Bregs, FOXP3$^+$ programmed cells, and tolerogenic dendritic cells), B cell depletion (like anti-CD20), strategies for beta cell regeneration (such as Verapamil and GLP-1 agonists), microbiome-targeted treatments (including BCG, probiotics, and coxsackie B vaccines), and interventions in the tissue microenvironment (like IDO, adenosine agonists, and tyrosine kinase inhibitors). Immunosuppressive agents, like anti-CD3 monoclonal antibodies, have shown some promise[9] in early stages but have not achieved durable beta cell preservation in clinical trials. Antigen-specific therapies, which aim to induce immune tolerance by targeting specific islet antigens,[10] have demonstrated excellent safety profiles in clinical studies but have not been sufficiently effective in altering disease progression.[11] Given these challenges, there is growing interest in combination therapies that offer a more comprehensive approach to modulating the immune response in T1D.[12] Our study proposes a novel combination therapy approach to enhance the effectiveness of antigen-specific immunotherapy for T1D. We aim to assess the safety and efficacy of minimal islet transplantation combined with a targeted, short-term drug regimen in patients with recent-onset T1D. This approach includes several innovative modifications: using a depleting antibody as induction therapy,[13] administering a transient drug treatment with pegylated granulocyte colony-stimulating factor (G-CSF) to promote Treg expansion,[14] utilizing whole pancreatic islets to provide a broad range of antigens, delivering antigens via the liver to leverage its immunoregulatory properties,[15] and applying a mammalian target of rapamycin (mTOR) inhibitor to further support immune tolerance.[16] By integrating these strategies, we aim to induce a more robust and lasting immune control of autoimmunity to islet antigens, potentially altering the course of beta cell decline and offering a new therapeutic avenue for patients with T1D. This

monocentric, open-label pilot study seeks to explore the potential of this combined approach to provide durable benefits without the long-term risks associated with traditional immunosuppression.

Methods

Study design

This is a prospective, single-arm, mono-center pilot study designed to investigate whether administering a combination therapy consisting of minimal islet transplantation (>1500 IEQ/kg body weight), ATG, rapamycin, and pegfilgastrim to 6 patients with T1D at onset was safe and, secondarily, whether it would preserve insulin production. Each patient was involved in the study for about 5 years, including a screening period and a post-transplant phase with a 12-week treatment cycle. Follow-up visits were scheduled at 2 and 4 weeks, 3, 6, 12 and 18 months, and annually thereafter at 2-, 3-, 4-, and 5-year post-transplant.

Participants

Potential participants were screened and, if deemed eligible, were placed on a waiting list. When human pancreatic islets became available, patients were selected based on blood group and HLA matching, with a focus on maximizing the mismatch of HLA A and B and maximizing the match of HLA DR. This specific HLA matching strategy was employed to balance two competing immunological concerns: maximizing HLA A and B mismatch to potentially reduce recurrent autoimmunity against transplanted islets, while maximizing HLA DR match to minimize allorecognition and rejection. This approach acknowledges the dual immunological challenge in T1D islet transplantation—preventing both the recurrence of autoimmune destruction and the rejection of transplanted donor tissue. To be eligible for the study, patients had to be between 18 and 45 years old, able to provide written informed consent, mentally stable, and willing to adhere to the study protocol, including attending all follow-up visits and examinations. Participants needed to have either new-onset T1D (diagnosed and on insulin treatment within the past 180 days) with residual beta cell function (fasting C-peptide >0.3 ng/ml and glucose levels between 70 and 200 mg/dL) or T1D for over 180 days but less than 2 years, with a stimulated C-peptide peak of 0.3 ng/ml or greater during a 4-h mixed-meal tolerance test (MMTT). They were required to test positive for at least one T1D specific autoantibody among anti-GAD (GADA), anti-IA2 (IA2A), anti-zinc transporter 8 (ZnT8A), and anti-insulin (IAA), and to currently require or have required insulin for at least 7 days between diagnosis and the start of the study drug. Patients were excluded if they had chronic conditions beyond T1D or autoimmune hypothyroidism, significant renal impairment (creatinine clearance below 90 ml/min), or macroalbuminuria. Hepatic dysfunction, defined by ALT/AST levels exceeding three times the upper limit of normal, and a negative Epstein–Barr Virus (EBV) IgG test also excluded participants. Those with serious cardiovascular disease, active infections, or a history of malignancy were not eligible. Additionally, a body mass index (BMI) of 32.0 kg/m^2 or higher, or a weight of 50 kg or less were considered exclusion criteria. Participants requiring more than 1.0 IU/kg of insulin daily or with an HbA1c level above 10% were also excluded. Recent or current use of immunosuppressive drugs or investigational agents, as well as pregnancy or breastfeeding, also disqualified patients. Lastly, individuals unwilling to use effective contraceptive methods were excluded from the study.

"The study was approved by the Institutional Ethics Committee of IRCCS Ospedale San Raffaele (approval number: protocollo DRI-MITO 2014. 01/04/2015) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent."

Intervention

The study involved administering 30–40 ml sterile suspension containing at least 70% viable and 30% pure allogeneic human islets, suspended in Ringer's lactate solution plus human serum albumin. Each dose provided a minimum of 1500 IEQ/kg of the recipient's body weight. The islets were delivered through a catheter inserted into the portal vein or its tributaries, with access achieved percutaneously and guided by fluoroscopy or ultrasonography. Portal pressure was monitored before and after the infusion. For immunosuppression, Rabbit Anti-Thymocyte Globulin (ATG, Thymoglobulin®) was administered intravenously at a total dose of 6 mg/kg, divided into seven doses over six days. The first dose of 1.5 mg/kg was given 24 to 8 h before the islet infusion, preceded by a 500 mg bolus of methylprednisolone. The subsequent doses of 0.75 mg/kg were administered as 18-h infusions, accompanied by oral paracetamol and diphenhydramine. Granulocyte Colony-Stimulating Factor (G-CSF, Neulasta®) was given as six doses of 6 mg each, administered subcutaneously every two weeks, starting before discharge after the Thymoglobulin® infusion. Sirolimus (Rapamune®) was initiated at 0.2 mg/kg orally on the day of the transplant, followed by 0.1 mg/kg daily. The dose was adjusted to maintain a target trough level of 8–10 ng/ml and was discontinued one month after the transplant. All participants were treated with multiple daily insulin injections (MDI).

Study endpoints

The primary efficacy endpoint was the change in stimulated 2-h plasma C-peptide area under the curve (AUC) from baseline, measured during a mixed meal tolerance test (MMTT) at week 52. This endpoint

specifically assessed the ability of the transplanted islets to produce insulin in response to a meal stimulus, serving as a direct indicator of beta cell function and insulin production. Secondary endpoints included the change in stimulated C-peptide levels at other time points, such as weeks 4, 12, 26, 78, and annually for up to five years post-transplant, as well as the maximum stimulated C-peptide response observed during these tests. The study also evaluated overall glycemic control, including HbA1c and insulin requirements. Exploratory endpoints focused on the impact of islet transplantation on immune function in T1D. This involved tracking changes in cytokine and chemokine levels, immune cell profiles, T cell responsiveness, and B cell activity. Additionally, the study sought to identify biomarkers predictive of treatment success, such as serum levels of hormones related to metabolic status and markers of beta cell preservation. These exploratory assessments were conducted from baseline and extended over different time post-transplant. The study also monitored the incidence and severity of adverse events (AEs) associated with both the islet transplant procedure and immunosuppressive therapy. For the transplant, AEs included significant bleeding, portal vein thrombosis, biliary puncture, wound complications, and elevated liver enzymes. For immunosuppression, AEs encompassed allergic reactions, kidney function decline, increased urinary protein, and systemic issues such as infections and malignancies. Safety was assessed throughout the study, with additional monitoring for immune sensitization through the detection of new anti-HLA antibodies at 4, 12, and 52 weeks.

Sample size

This pilot study, primarily designed to assess the safety of minimal islet transplantation in patients with new-onset T1D, utilized a convenient sample size of six patients, reflecting the exploratory nature of the research.

Analysis population

The Safety Population encompasses all patients who received at least one transplant with >1500 IEQ/kg and is used for demographic, baseline, and safety data. The Efficacy Population consists of patients who underwent transplantation and received short-term immunosuppression. Efficacy analyses were primarily conducted on intention-to-treat (ITT) population, with potential confirmation from the per-protocol (PP) population.

Detection of Anti-HLA antibodies

Anti-HLA antibodies were screened using LABScreen™ Mixed (LSM12, One Lambda). Positive samples were further analyzed for antibody specificity with LABScreen™ Single Antigen Class I (LS1A04) and Class II (LS2A01) kits. All assays were Luminex-based and performed according to the manufacturer's protocols. A mean fluorescence intensity (MFI) ≥1000 was considered positive. Data were analyzed using HLA Fusion™ version 4.7 software.

Exploratory end points: cytokine, chemokines and hormones

Blood samples were collected at designated time points and centrifuged within 30 min. Cell-free serum aliquots (2 × 0.5 ml) were prepared and stored at –20 °C. Protein levels in the sera were measured using magnetic bead-based multiplex assays compatible with the Luminex® platform (R&D Systems), including the following panels: LXSAHM-34 (TNFα, IL-6, Insulin, IL-3, IL-8, CCL7, IL-7, IL-10, IL-16, VEGFA, IL-1β, IFNɣ, IL-1RA, LIF, CCL3, CCL4, IL-1A, IL-4, IL-17, IL-2, TNFβ, GM-CSF, CD25, Resistin, CXCL9, IL-5, G-CSF, VCAM1, ICAM1, CCL2, IFNα, IL-12, TRAIL, CCL27); LXSAHM-16 (Visfatin, CXCL10, SCF, BNGF, CXCL1, FGF-B, M-CSF, Leptin, MIF, IL-12p70, IL-15, IL-13, HGF, SCGF, CCL11, IL-18); LXSAHM-02 (PAI-1, Adiponectin); LXSAHM-03 (PDGF-BB, RANTES, Adipsin); and FCSTM19-03 (Ghrelin, Active GLP-1, Glucagon). All assays were performed according to the manufacturer's instructions. Plates were read using a Luminex® 200 xMAP instrument (Luminex Corporation), and data were analyzed with xPONENT® 3.1 software (Luminex Corporation).

Measurement of T1D-associated autoantibodies

GADA, IA-2A, ZnT8A and IAA were measured in serum samples collected pre-transplant and at multiple post-transplant timepoints (1, 3, 6, 12, 18 months and 2, 3, 4, 5 years). Autoantibodies were measured using our optimized Luciferase Immunoprecipitation System (LIPS) assays. These assays utilize recombinant nanoluciferase-tagged autoantigens and were conducted as previously described.[17–19]

Monitoring of circulating miR-375 by digital PCR (dPCR)

Circulating microRNAs were extracted from serum samples collected pre-transplant and at sequential post-transplant intervals (1, 3, 6, 12, 24 h; 3, 5, 7, 15 days; 1, 3, 6, 12, 18 months; and 2, 3, 4, 5 years) using the Maxwell® RSC miRNA from Plasma and Serum kit with the Maxwell® RSC Instrument (Promega), according to manufacturer's instructions. All serum miRNAs underwent universal reverse transcription to cDNA using the miRCURY LNA RT Kit (Qiagen). Quantification of circulating miR-375 and miR-16 (used as a non-tissue-specific reference miRNA) was performed on the QIAcuity Digital PCR (dPCR) System (Qiagen) with corresponding miRCURY LNA miRNA PCR assays, as detailed in Appendix p 2. The QIAcuity Software Suite (Qiagen) quantified the positive partitions for each target, and results were converted to

miRNA copies per ml of serum after normalizing by sample volume inputs at each analytical step (extraction, reverse-transcription, and dPCR).

Immune profiling by flow cytometry

Whole blood samples were obtained from patients. Red blood cells were lysed using red blood cell lysis buffer (Merck) and subsequently stained using the following monoclonal antibodies (mAbs): CD3 PE-Cy7 (clone SP34-2), CD4 Pacific Blue (clone RPA-T4), CD8 BV450 (clone SK1), CD45RA allophycocyanin (APC)-H7 (clone HI100), CD62L PE (clone 150503), CD25 PE (clone M-A251), CD19 PE (clone HIB19), CD15 PerCP-Cy5.5 (clone HI98), CD14 AF488 (clone M5E2), CD56 APC-Cy7 (clone B159), CD16 pacific blue (clone 3G8), all from BD biosciences. CD127 eFluor 450 (clone eBioDDR5) from eBioscience Vα24-Jα18. APC (clone 6B11) from Biolegend. Ki-67 AF488 (clone SOLA15) from Thermofisher Scientific. For antigen-specific T-cell detection, we used APC-labeled HLA-A*0201/GAD65114-123 dextramers from Immundex (Copenhagen, Denmark). Zombie UV Fixable Viability Dye (BioLegend) was used to exclude dead cells. Cell debris and doublets were excluded based on side versus forward scatter. Samples were acquired on a Becton Dickinson LSR-Fortessa X-20 with FACS Diva software and analyzed using FlowJo software version 9.3.2 (Tree Star).

Statistical analysis

Descriptive statistics were used to summarize the data. Continuous variables are reported as mean (range or 95% CI) or median (interquartile range), as appropriate, and categorical variables as frequencies and percentages. The Wilcoxon test was used to assess efficacy outcomes at 1-year and 5-year time points. Because most longitudinal variables were not normally distributed, the Friedman test was applied for repeated-measures analysis. When significant differences were detected ($p < 0.05$), Dunn's multiple-comparison test was used for post-hoc pairwise comparisons. For exploratory endpoints with occasional missing values or approximately normal residuals, a mixed-effects model was applied to account for within-subject correlation, using the Geisser–Greenhouse correction when sphericity assumptions were violated. Given the exploratory nature of the study, pairwise comparisons were not adjusted for multiple testing. All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant. Analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY) and GraphPad Prism version 10 (GraphPad Software, San Diego, CA).

Role of the funding source

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Results

Baseline characteristics

Between April 2015 and April 2018, ten patients were enrolled in the trial. Table 1 presents the baseline characteristics of the study participants. The average age of participants was 28.2 years, with a range from 18 to 43 years. The cohort consisted of 6 females and 4 males. The mean BMI was 22.4 (range: 20.4–23.4). The duration from T1D onset to screening varied from 138 to 683 days, with a mean of 350.8 days. Baseline diabetes characteristics include the mean daily insulin requirement (DIR) of 15.1 U/day (range: 3–30 U/day) and DIR normalized to body weight of 0.24 U/kg/day (range: 0.04–0.4 U/kg/day). Fasting C-peptide levels had a mean of 1.04 ng/ml (range: 0.2–1.38 ng/ml), and HbA1c levels averaged 6.2% (range: 5.3%–8.1%). Serum creatinine (Crea) levels averaged 0.74 mg/dL (range: 0.55–1.07 mg/dL), with an estimated glomerular filtration rate (eGFR) mean of 122.6 ml/min (range: 93–154 ml/min). All patient tested positive for at least one autoantibody, including IAA in 4 out of 10 patients, GADA in 6, IA-2A in 5, and ZnT8A in 7.

Participant flow

Fig. 1 depicts the flow of participants throughout the trial. Out of the ten patients initially enrolled, four were excluded: #02 and #07 withdrew consent while awaiting islet preparation, #03 surpassed the two-year threshold from T1D onset, and #09 was negative for islet autoantibodies during screening. Consequently, six patients proceeded with islet infusions and a short-term drug regimen. Details regarding the islet preparation are outlined in Table 2. Among these six patients, three had an HLA-A match, one had an HLA class B match, five matched for DRB1, and two matched for DRQ1. All patients who received the islet infusion completed the study without any loss to follow-up or withdrawals for other reasons.

Safety outcomes

Transient transaminitis was observed in three patients (#01, #04, #05), but no severe complications such as bleeding or portal vein thrombosis were reported after islet infusion. All participants experienced transient moderate leukopenia within the first two weeks post-infusion. Patient #08 developed a mild rash on the lower limbs and abdomen, which resolved spontaneously during ATG treatment. Patient #10 experienced mild fever, myalgia, and bilateral cervical lymph node swelling two weeks post-infusion, leading to

	#01	#02	#03	#04	#05	#06	#07	#08	#09	#10
Age/sex	19/M	28/F	41/F	24/F	29/F	27/F	22/F	18/M	27/M	43/M
Weight (kg)	56	63.9	75	61	68.6	59.5	66.5	61.4	57	61
BMI (kg/m^2)	21.3	22.6	23.1	21.4	22.9	21.6	21.7	23.4	20.4	23.2
Time to screening (days)	223	408	683	191	371	252	249	185	138	393
DIR (U/day)	18	9	30	15	3	17	20	5	12	7
DIR (U/kg/day)	0.31	0.14	0.4	0.25	0.04	0.29	0.3	0.08	0.21	0.11
FBG (mg/dl)	88	89	84	102	183	93	135	70	96	108
Fasting C-pep (ng/ml)	0.83	0.72	0.2	0.67	1.18	0.81	0.96	1.38	1.22	1.27
HbA1c (%)	5.7	5.6	5.7	5.3	8.1	6	6	6	5.4	6.7
Crea (mg/dl)	0.55	0.77	0.86	0.84	1.07	0.72	0.71	0.74	0.82	0.59
eGFR (ml/min)	147	130	120	117	99	130	154	120	93	119
IAA	+	+	+	+	+	+	+	+	–	–
GADA	–	+	+	+	+	+	+	+	–	+
IA-2A	–	+	+	+	+	–	+	–	–	–
ZnT8A	–	+	+	+	+	+	+	–	–	+
HLA-A	2/3	nt	nt	2/24	2/26	1/58	nt	3/29	nt	1/2
HLA-B	7/37	nt	nt	75/60	8/35	8/51	nt	8/38	nt	8/18
DRB1	3/4	nt	nt	9/11	17/4	17/8	nt	17/4	nt	17/17
DQB1	nt	nt	nt	nt	nt	1/58	nt	8/84	nt	2/138

Table 1: **Patient characteristics at screening.**

readmission. Following the suspension of immunosuppressive therapy, the patient was treated with high-dose Amoxicillin/clavulanate, IV Metronidazole, and prophylactic low molecular weight heparin. Symptoms improved, inflammatory markers normalized, and lymph node swelling decreased, allowing discharge after 5 days. All infection tests were negative, but rapamycin and G-CSF were not resumed. No further adverse events related to the study intervention were reported during follow-up, including no cases of allergies, reductions in GFR, increases in urinary albumin excretion, changes in anti-hypertensive or anti-hyperlipidemic therapy, oral ulcers, lower extremity edema, gastrointestinal toxicity, hematological abnormalities (neutropenia, anemia, or thrombocytopenia), or infections and neoplasms. Two episodes of severe hypoglycemia were reported by patient #01, and hypoglycemic awareness was reported by patient #08 at the five-year follow-up; these were deemed unrelated to the study intervention. Biochemical parameters after islet infusion are reported in Appendix p 3.

Efficacy outcomes

At week 52, the median area under the curve (AUC) for C-peptide values was 203 (94–264) ng/ml/120 min for the intention-to-treat (ITT) analysis and 244 (75–281) ng/ml/120 min for the per-protocol (PP) analysis. These values represented 91% (45–104%) and 100% (48–105%) of pre-transplant levels, which were 268 (202–299) ng/ml/120 min for ITT ($p = 0.31$) and 242 (180–304) ng/ml/120 min for PP ($p = 0.62$) (see Table 3 and Fig. 2). Among the secondary endpoints (Table 3), significant changes from baseline to week 52 were observed in fasting C-peptide, fasting insulin, and insulin dose-adjusted HbA1c (IDAA1C) in the ITT analysis. A trend toward an increase in DIR was also noted. In the PP analysis, similar trends were observed in fasting C-peptide, fasting insulin, and IDAA1C, although these did not achieve statistical significance. However, no significant changes were observed in maximum stimulated C-peptide and insulin levels or fasting glucose, during the MMTT, nor in HbA1c. Notably, all patients achieved an IDAA1C ≤ 9, and all but one patient (Patient #4) maintained a HbA1c below 7.0% and a DIR of less than 0.5 U/kg/day.

Throughout the entire 5-year follow-up (Fig. 2 and Appendix p 4 and p 5), AUC C-peptide declined significantly in the ITT analysis ($p = 0.008$), with a similar trend noted in the PP analysis ($p = 0.063$). At the 5-year mark, the median AUC C-peptide values were 110 (12–245) ng/ml/120 min for ITT and 176 (30–249) ng/ml/120 min for PP analysis, corresponding to 44% (4–103%, $p = 0.094$) and 56% (19–107%, $p = 0.19$) of pre-transplant levels, respectively. Additionally, significant reductions in fasting C-peptide and insulin levels were observed, alongside increases in DIR, IDAA1C, and weight (Table 3). The analysis of individual patients revealed unique outcome patterns. Two of the six patients (#5 and #10) maintained stable basal C-peptide secretion over five years, while another two (#1 and #6) retained approximately 50% of their pre-treatment secretion levels at the five-year assessment. In contrast, the remaining two patients (#4 and #8) demonstrated a near-complete loss of C-peptide secretion, approaching 0%.

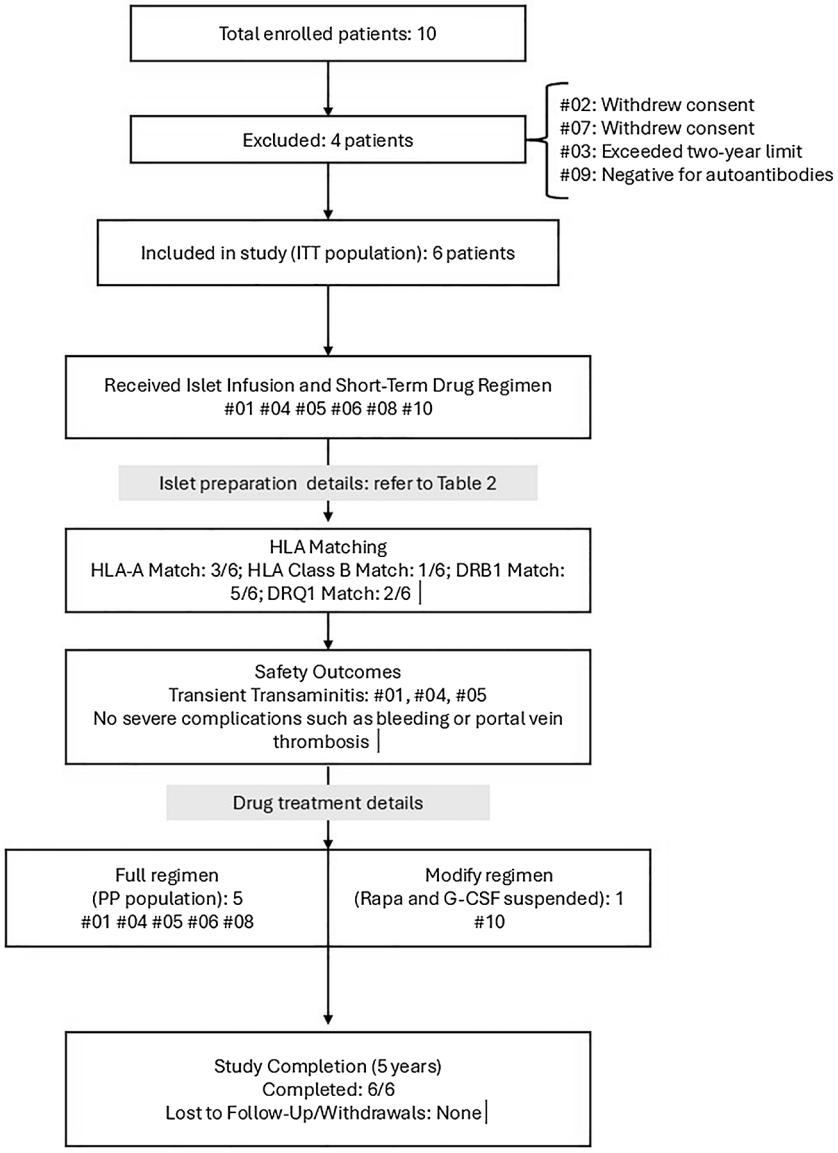

Fig. 1: **Participant flow throughout the trial.** Flow diagram detailing participant progression through the trial, including screening, enrollment, treatment initiation, and study completion. The figure reports the number of participants assessed for eligibility, excluded, enrolled, and who completed the study, along with reasons for withdrawal or discontinuation at each stage.

Circulating miRNA profiles following islet transplantation

To assess beta cell damage post-transplantation, circulating levels of miR-375 and miR-16 were longitudinally evaluated in plasma samples. miR-375, a microRNA enriched in pancreatic islet cells and associated with beta cell injury, showed a significant increase within the first hour following islet infusion, followed by a second distinct peak around day 5 (Fig. 3 A). This biphasic kinetic pattern was consistently observed across most subjects, indicating a temporally structured release profile. In contrast, miR-16, a ubiquitously expressed and non–tissue-specific miRNA, did not exhibit significant changes over time, supporting the specificity of the miR-375 signal.

Modification of anti-islet autoantibodies following islet transplantation

The presence and dynamics of islet autoantibodies were longitudinally evaluated in peripheral blood samples from patients undergoing minimal islet transplantation (Fig. 3C). Autoantibody levels against GAD65, IA-2, insulin, and ZnT8 were specifically measured. All patients initially negative for these autoantibodies

ID	#01	#04	#05	#06	#08	#10
Donor age (yrs)	51	63	47	46	49	49
Donor BMI (kg/m^2)	24.22	25.95	32.66	27.78	24.49	34.77
Cause of death	Cranial Trauma	Cerebral Hemorrhage	Post-Anoxic Encephalopathy	Cerebral Hemorrhage	Cerebral Hemorrhage	Cerebral Hemorrhage
Islet infusion	Yes	Yes	Yes	Yes	Yes	Yes
Time at islet infusion (days)	259	219	458	420	340	595
Pancreas weight (g)	103	120	132	150	84	138
Islet number (IEQ/kg)	3222	3250	2906	3768	2743	3770
Tissue volume (ml)	0.5	1.5	1.5	3.5	1	0.7
Purification (%)	70	40	50	30	40	65
Vitality (%)	90	90	95	90	90	90
Pre-Tx culture (h)	45	17	42	15	47	17
Delta Portal pressure (mmHg)	3	0	0	2	1	0
Donor HLA-A	2/31	2/66 (10)	2/69 (28)	2/24 (99)	2/30	32/33
Donor HLA-B	18/60 (40)	35/35	39 (16)/55 (22)	38 (16)/58 (17)	8/35	7/64 (14)
Donor DRB1	4/11 (5)	11 (5)/14 (6)	4/11 (5)	17 (3)/11 (5)	4/11	7/13 (6)
Donor DQB1	4/7 (3)	5 (1)/7 (3)	2/7 (3)	2/7 (3)	7 (3)/8 (3)	2/2
MHC Class I Match	1/4	1/4	1/4	0/4	1/4	0/4
MHC Class II Match	1/2	1/2	1/2	1/2	2/4	1/4
Bleeding[a]	No	No	No	No	No	No
Portal Vein Thrombosis	No	No	No	No	No	No
Increased Transaminase Levels[b]	Yes	Yes	Yes	No	No	No
Other Complications	No	No	No	No	No	No

[a]>2 g/dL decrease in hemoglobin concentration. [b]>5 times the upper limit of normal.

Table 2: **Characteristics of islet infusions.**

remained negative, indicating no antigen spreading post-transplant. Although baseline levels varied, most autoantibodies showed a rapid and significant increase after transplantation, followed by a return to baseline or, in some cases, a reduction below baseline levels. Specifically, IA-2 antibodies remained negative in four patients, while two experienced transient increases that normalized over time. Among GAD antibodies, one patient stayed negative, while the others showed transient elevations that returned to baseline. For ZnT8 antibodies, two patients remained negative, whereas four demonstrated significant increases. Anti-insulin antibodies were negative in one patient, increased in three, and fluctuated in two. Notably, no significant expansion of GAD-specific CD8+ T cells was detected in two of three HLA-A2 positive patients assessed by dextramer staining (Fig. 3B).

Development of donor specific anti-HLA antibodies (DSA) post-transplantation

Five out of six patients developed at least one class I anti-HLA DSA within the first year following transplantation. Additionally, two of these patients exhibited reactivity against class II antigens during follow-up. The timing, persistence, and scope of DSA responses varied considerably among individuals (Fig. 3D). Class I anti-HLA DSA appeared transiently, while class II DSA emerged and persisted.

Exploratory endpoints

As expected, ATG treatment led to marked alterations in the circulating leukocyte landscape (Fig. 4). Total T cells, including CD4$^+$ T helper and CD8$^+$ cytotoxic subsets, exhibited a sharp decline immediately following islet infusion and ATG administration, with levels returning to baseline within the first month. Analysis of T cell subpopulations (Appendix p 6) indicated that naïve T cells (Tn; CD45RA$^+$CD62L$^+$) were preferentially depleted within both CD4$^+$ and CD8$^+$ compartments, whereas memory subsets (Tcm and Tem) demonstrated relative resistance to depletion. A significant rise in Ki-67$^+$ T cells was observed within the first three months post-treatment, particularly among CD8$^+$ cells (Fig. 5), indicative of homeostatic proliferation aimed at restoring T cell homeostasis following lymphodepletion.[20] This proliferative response was paralleled by an increase in IL-7 and IL-15, cytokines essential for T cell survival and homeostatic expansion, further supporting the occurrence of homeostatic reconstitution.[21] Regulatory T cells (Tregs; CD3$^+$CD4$^+$CD25$^+$CD127^{low}) expanded significantly from the first month post-infusion and remained

	Pre TX	Follow-up				
		Week 52		5 years		Multiple comparison[b]
			p[a]		p[a]	p
C-pep AUC_{120}						
ITT	268 (202–299)	203 (94–264)	0.31	110 (12–245)	0.094	0.0080
PP	242 (180–304)	244 (75–281)	0.62	176 (30–249)	0.19	0.063
% C-pep AUC_{120}						
ITT	100	91 (45–104)	0.31	44 (4–103)	0.094	0.008
PP	100	100 (48–105)	0.62	56 (19–107)	0.19	0.063
C-pep AUC_{240}						
ITT	496 (424–557)	449 (244–577)	0.84	244 (21–561)	0.16	0.060
PP	479 (385–524)	509 (194–615)	0.81	365 (75–573)	0.31	0.24
C-pep max (ng/ml)						
ITT	2.9 (2.1–3.4)	2.4 (1.3–2.9)	0.81	1.3 (0.11–3)	0.16	0.15
PP	2.5 (1.9–3.5)	2.6 (1.1–3.2)	0.83	1.9 (0.4–3)	0.31	0.45
C-pep fasting (ng/ml)						
ITT	1.15 (0.95–1.56)	0.7 (0.27–0.98)	0.031	0.31 (0.03–0.66)	0.031	<0.0001
PP	1.12 (0.89–1.4)	0.87 (0.19–1)	0.06	0.54 (0.06–0.77)	0.06	0.0012
HbA1C (%)						
ITT	5.6 (5.4–6.4)	6.4 (5.9–6.7)	0.12	6.2 (6–6.3)	0.41	0.25
PP	5.4 (5.3–6.2)	6.4 (5.7–6.6)	0.25	6.2 (5.9–6.4)	0.31	0.34
DIR (U/kg/Day)						
ITT	0.19 (0.03–0.28)	0.35 (0.14–0.46)	0.062	0.50 (0.18–0.61)	0.0312	<0.0001
PP	0.25 (0.02–0.28)	0.34 (0.09–0.54)	0.12	0.48 (0.13–0.61)	0.062	0.0004
HbA1c < 7.0% and DIR <0.5 U/kg/day						
ITT	6/6 (100)	5/6 (83)	–	3/6 (50)	–	–
PP	5/5 (100)	4/5 (80)	–	3/5 (60)	–	–
IDAA1C (%)						
ITT	6.4 (5.9–7.1)	8 (6.9–8.2)	0.031	8.3 (6.5–8.7)	0.031	0.0124
PP	6.3 (5.7–7.1)	7.9 (6.6–8.1)	0.062	8.1 (6.4–8.8)	0.062	0.042
IDAA1C $\leq$ 9						
ITT	6/6 (100)	6/6 (100)	–	5/6 (83)	–	–
PP	5/5 (100)	5/5 (100)	–	4/5 (80)	–	–
FBG (mg/dl)						
ITT	109 (84–150)	105 (100–122)	0.93	87 (74–148)	0.69	0.21
PP	103 (83–137)	103 (99–113)	0.56	86 (71–167)	0.99	0.18
Gly max (mg/dl)						
ITT	229 (149–293)	243 (197–312)	0.31	266 (236–378)	0.15	0.082
PP	227 (132–293)	206 (195–331)	0.43	257 (223–359)	0.31	0.078
Gly AUC_{120} ($x10^3$)						
ITT	18 (14–28.9)	23.7 (19.7–29.9)	0.31	26.3 (19.5–32.3)	0.21	0.16
PP	19.8 (13.3–29.2)	21.6 (19.4–30.9)	0.62	24.4 (19.3–36.2)	0.43	0.20
Insulin fasting (mUI/l)						
ITT	6.5 (3.4–10.2)	3.3 (2.3–4.2)	0.031	0.7 (0.4–2.4)	0.031	0.0003
PP	5.2 (3.1–10.7)	3.1 (2.1–4.7)	0.062	0.8 (0.5–2.6)	0.062	0.0033
Insulin Max (mUI/l)						
ITT	14.4 (12.7–36.5)	16.9 (11.8–23)	0.84	3.9 (1.1–6.3)	0.03	0.0005
PP	14.4 (12.7–40.9)	17.1 (11.5–25.5)	0.99	4.8 (2.2–7.1)	0.062	0.0039
Weight (kg)						
ITT	59.5 (57.2–62.2)	60.5 (57.4–64.4)	0.12	63 (59.2–66.7)	0.031	<0.0001
PP	59 (56.5–64.5)	61 (57–66.3)	0.12	64 (58.5–67.5)	0.062	<0.0001

Data are expressed as median and 25–75 percentile. [a]Wilcoxon test. [b]Friedman test considering 1, 3, 6, 12, 18, 24, 48, 60 months.

Table 3: **Efficacy outcomes.**

elevated throughout follow-up (Fig. 6). Concurrently, transient increases in IL-10 and IL-2 were detected within hours of transplantation; however, these changes were not sustained. In contrast, soluble CD25 (sCD25) levels rose early and remained elevated during the first month before returning to pre-transplant levels

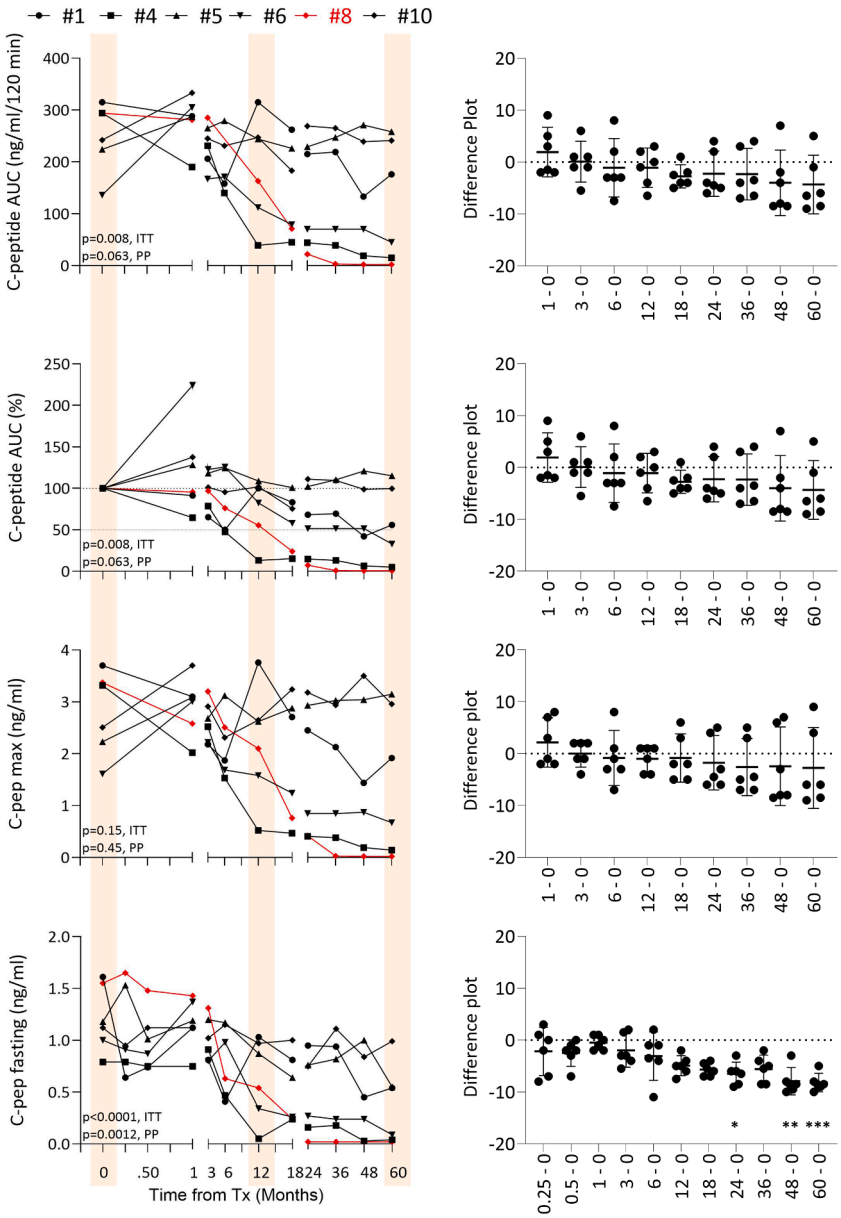

***Fig. 2*: C-peptide AUC, maximum C-peptide, and fasting C-peptide during MMTT over follow-up.** Longitudinal profiles of C-peptide Area Under the Curve (AUC), maximum C-peptide, and fasting C-peptide measured during the Mixed Meal Tolerance Test (MMTT) over the follow-up period in six patients. The left panels show individual patient values across time points; the patient highlighted in red did not complete the pharmacological treatment. The yellow shaded area indicates the time window for the primary endpoint (see Table 3). P-values were calculated using the Friedman test and are reported for the Intent-to-Treat (ITT, n = 6) and Per-Protocol (PP, n = 5) populations. Difference plots on the right display pairwise comparisons with baseline (time 0), based on rank differences. Each dot represents the rank difference for an individual patient between a given time point and baseline. The Y-axis displays unitless rank differences, with mean values and 95% confidence intervals. Pairwise comparisons were performed using Dunn's test with correction for multiple comparisons; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

(Fig. 6). Additional leukocyte analysis revealed relative expansions in B lymphocytes and monocyte/granulocyte populations, with minimal changes observed in NK and NKT cell frequencies. Circulating cytokine and chemokine profiles displayed distinct temporal dynamics (Appendix p 7). A subset of inflammatory mediators, including TNF-α, IL-8, IL-6, M-CSF, CCL2, CCL3, CCL4, CXCL10, and CCL11, exhibited rapid and

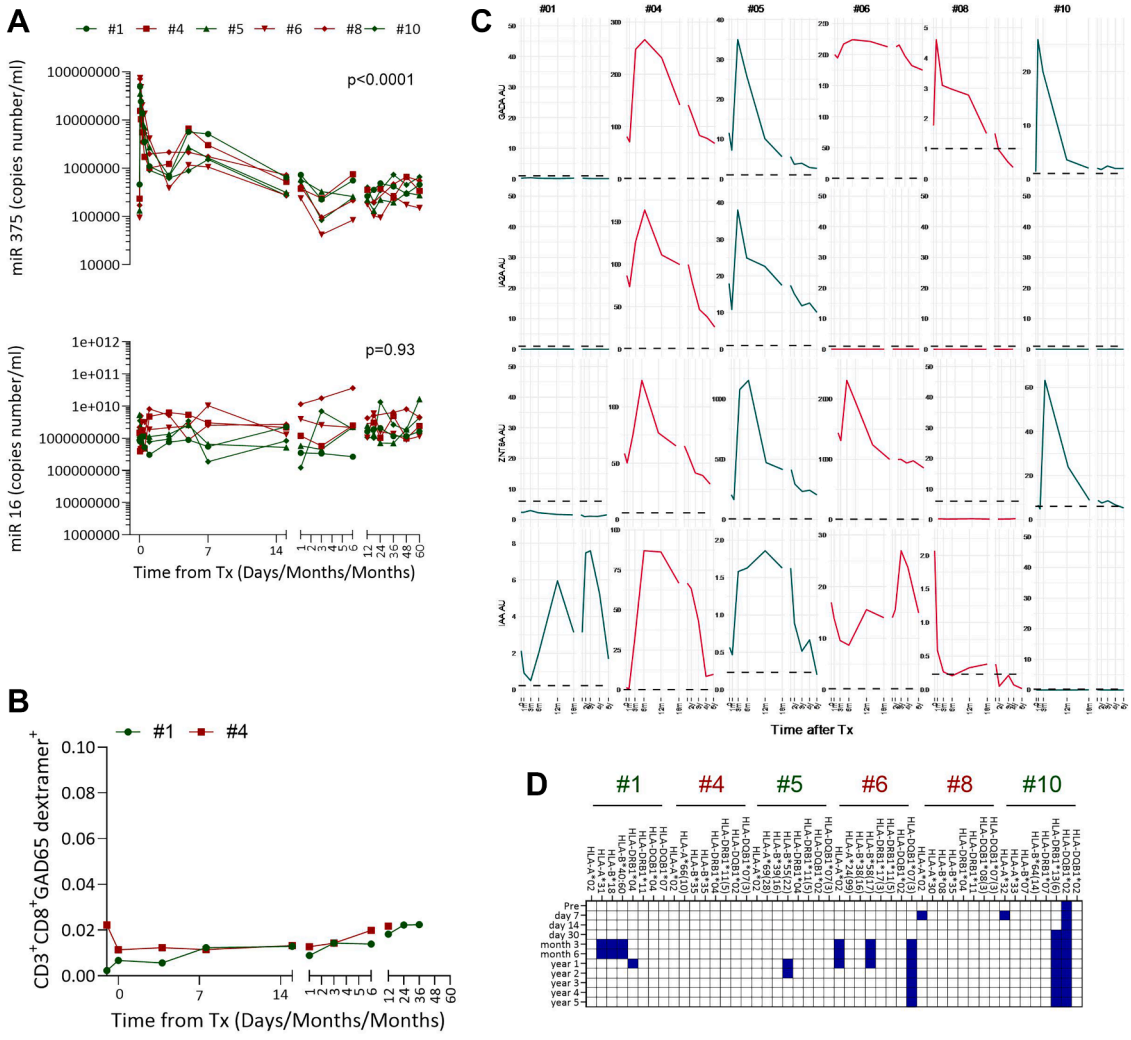

***Fig. 3:* Longitudinal dynamics of circulating miRNA and immune responses following islet transplantation.** Longitudinal profiles of circulating miR-375, miR-16, anti-islet autoantibodies, and donor-specific anti-HLA antibodies (DSA) following minimal islet transplantation. Patients highlighted in red experienced a greater-than-median decline in C-peptide secretion at 5 years; patients in green showed a lesser-than-median decline. *Panel A.* Serum levels of miR-375, a miRNA associated with beta cell injury, and miR-16, a non-tissue-specific miRNA, were measured at multiple time points following islet infusion to assess changes in response to transplantation. Each line represents an individual patient. Data was analyzed using the Friedman test. *Panel B.* GAD Dextramer $CD8^+$ T Cells: Peripheral blood samples were analyzed for the presence of GAD-specific $CD8^+$ T cells using dextramer staining in two out of three patients with known HLA-A2 positivity. *Panel C.* Levels of GADA (glutamic acid decarboxylase autoantibodies), IA-2A (insulinoma-associated protein 2 autoantibodies), ZnT8A (zinc transporter 8 autoantibodies), and IAA (insulin autoantibodies) measured at multiple timepoints over 5 years post-treatment. The first 18 months after treatment are displayed with an expanded time scale to highlight early changes. Dashed horizontal lines indicate the clinical threshold for autoantibody positivity (GADA: 1 AU; IA-2A: 1 AU; ZnT8A: 6 AU; IAA: 0.2336 AU). Autoantibody levels are shown in arbitrary units (AU). *Panel D.* Donor-specific anti-HLA antibodies (DSA) across all patients (#1, #4, #5, #6, #8, #10). Columns represent individual antibody measurements, and rows represent time points ranging from pre-transplant to 1-year post-transplant. Color blue indicated positivity (>1500 MFI).

transient elevations within hours post islet infusion, typically returning to baseline within one week. In contrast, markers such as VCAM-1, IL-1α, HGF, IL-1RA, IL-16, and ICAM-1 showed early increases that remained elevated for up to one month, indicating sustained endothelial activation and ongoing modulation of immune responses. A second wave of immune modulation was observed between days 7 and 14, characterized by peaks in cytokines such as CCL7, G-CSF, IFN-γ, TRAIL, SCGF, and SCF (Appendix p8), suggesting a delayed phase of immune activity potentially related to tissue adaptation or secondary immune

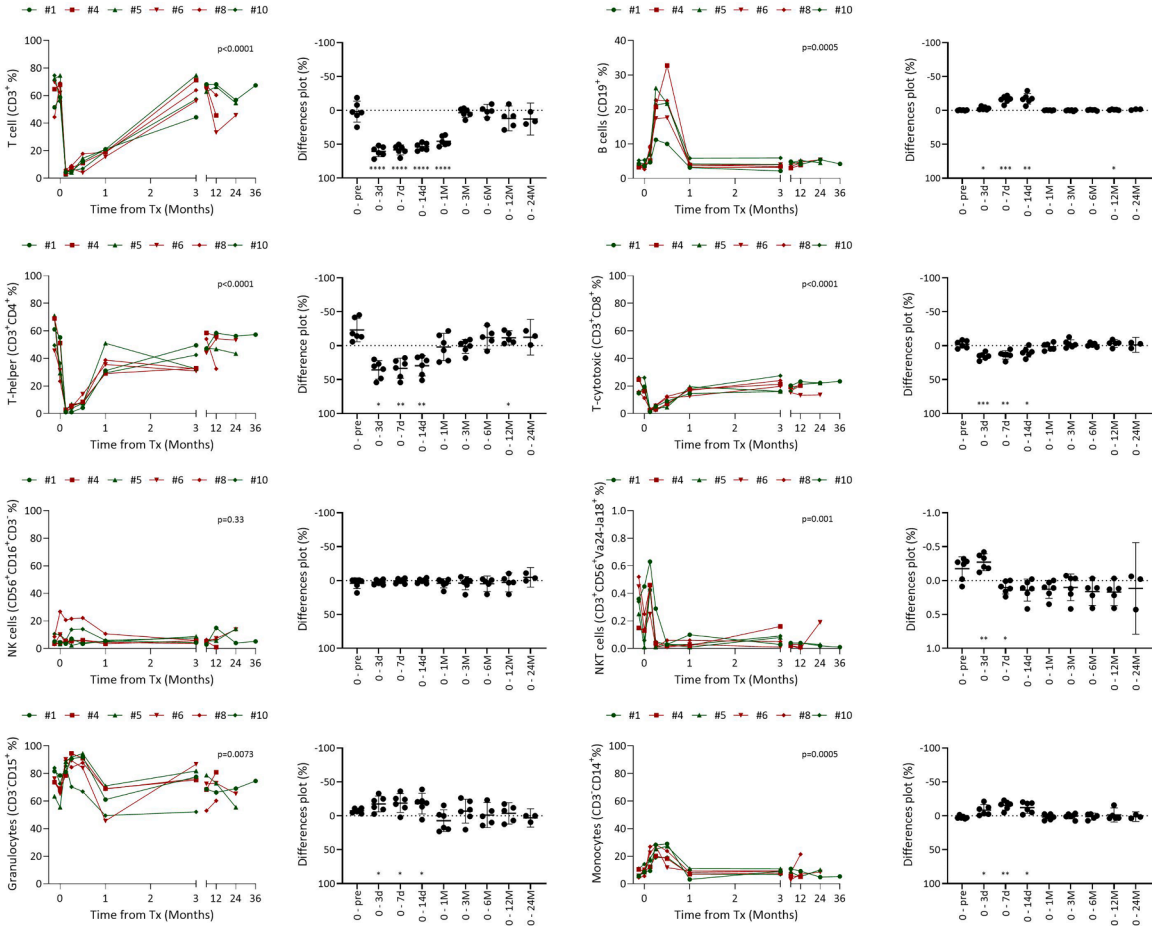

Fig. 4: **Long-term follow-up of circulating leukocyte subsets over 3 years post-islet transplantation.** Longitudinal analysis of circulating leukocyte subsets over a 3-year follow-up in six patients after minimal islet transplantation. Parameters include T cells ($CD3^+$), T-helper cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), B cells ($CD19^+$), NK cells ($CD56^+CD16^+CD3^-$), NKT cells ($CD3^+CD56^+Va24\text{-}Ja18^+$), granulocytes ($CD3^-CD15^+$), and monocytes ($CD3^-CD14^+$), measured in peripheral blood. Each line represents an individual patient. The patient highlighted in red experienced a greater-than-median decline in C-peptide secretion at 5 years; the patient in green showed a lesser-than-median decline. P-values were calculated using a mixed-effects model and are reported for the Intent-to-Treat (ITT, n = 6) population. Difference plots on the right display pairwise comparisons with baseline (time 0), based on estimated marginal means (LS Means). Each dot represents the difference in raw values for an individual patient between a given time point and baseline. The Y-axis shows actual value differences, with mean values and 95% confidence intervals. Pairwise comparisons were performed using Fisher's LSD test without correction for multiple comparisons; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

responses. Conversely, the downregulation of CCL27, PDGF-BB, and RANTES was noted during the first week post-infusion. Other cytokines—including CXCL9, VEGF, IL-18, IL-3, IL-13, and GM-CSF—displayed heterogeneous and non-reproducible kinetics. A subset of 12 cytokines (CXCL11, FGF-2, MIF, IL-12p70, IL-12p40, IL-1β, LIF, IL-4, IL-17, IL-5, TNF-β, IFN-α) did not exhibit consistent modulation following transplantation (Appendix p 9). Lastly, a panel of hormonal and adipokine markers was longitudinally assessed to explore potential metabolic changes (Appendix p 10). An early, transient suppression of adipsin, PAI-1, and adiponectin was observed within the first 15 days post-transplant, suggesting a short-term impact on adipose tissue signaling. In contrast, circulating levels of glucagon and resistin increased during the same period. Leptin levels gradually increased over time, in parallel with progressive weight gain observed in participants.

Discussion

In this first-in-human pilot study, we investigated the feasibility, safety, and immunometabolic effects of a minimal islet transplantation protocol combined with short-term immunomodulation in recent-onset T1D.

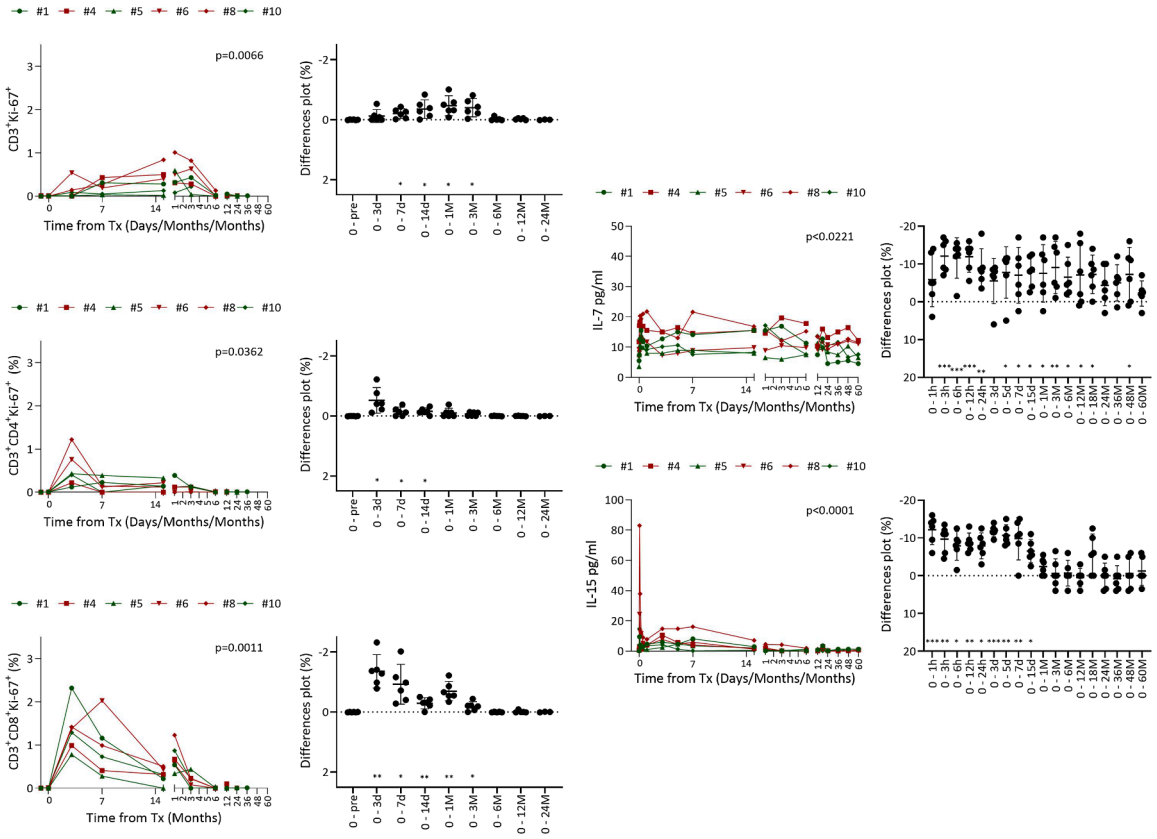

Fig. 5: **Long-term follow-up of regulatory T cells and related cytokines over 5 years post-islet transplantation.** Longitudinal analysis of circulating regulatory T cells (Tregs, $CD3^+CD4^+CD25^+CD127^{low}$), IL-10, IL-2, and soluble CD25 over a 5-year follow-up in six patients after minimal islet transplantation. Each line represents an individual patient. The patient highlighted in red experienced a greater-than-median decline in C-peptide secretion at 5 years; the patient in green showed a lesser-than-median decline. Cytokine data were analyzed using the Friedman test; Treg data were analyzed using a mixed-effects model to account for missing values. Difference plots on the right show pairwise comparisons with baseline (time 0), based on rank differences for cytokines and estimated marginal means for Tregs. Each dot represents the individual difference—unitless ranks for cytokines and raw values for leukocytes—between a specific time point and baseline. The Y-axis displays these differences with mean values and 95% confidence intervals. Pairwise comparisons were performed using uncorrected Dunn's test (cytokines) and Fisher's LSD test (Tregs), without correction for multiple comparisons; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

The results suggest that the approach is well tolerated and biologically active, with a subset of patients achieving durable preservation of beta cell function and immunologic signatures consistent with effective but non-exhaustive immune modulation. This study introduces a novel conceptual approach to antigen-specific immunomodulation in T1D, leveraging controlled exposure to allogeneic islets as a source of beta cell autoantigens. Unlike traditional islet transplantation strategies aimed at graft survival and long-term metabolic replacement,[22] the goal here was fundamentally different: to induce a regulated immune interaction with donor-derived antigens, with the intent of promoting functional immune modulation towards shared autoantigens in the recipient. This model intentionally accepts, and even relies on, the occurrence of a controlled graft rejection, redirecting the immunological focus from suppression to adaptation.[23]

From a safety perspective, the procedure was generally well tolerated, with no major infusion-related complications or long-term adverse events. The observed transient transaminitis and leukopenia are consistent with the known safety of ATG and rapamycin-based regimens and were self-limited in all cases. Notably, while two participants experienced transient systemic symptoms requiring clinical attention, no infectious etiologies were identified, and symptoms resolved following supportive therapy and immunosuppressive adjustment. These findings support the feasibility of this low-intensity protocol in carefully selected patients. Anti-HLA antibody monitoring revealed transient class I donor-specific

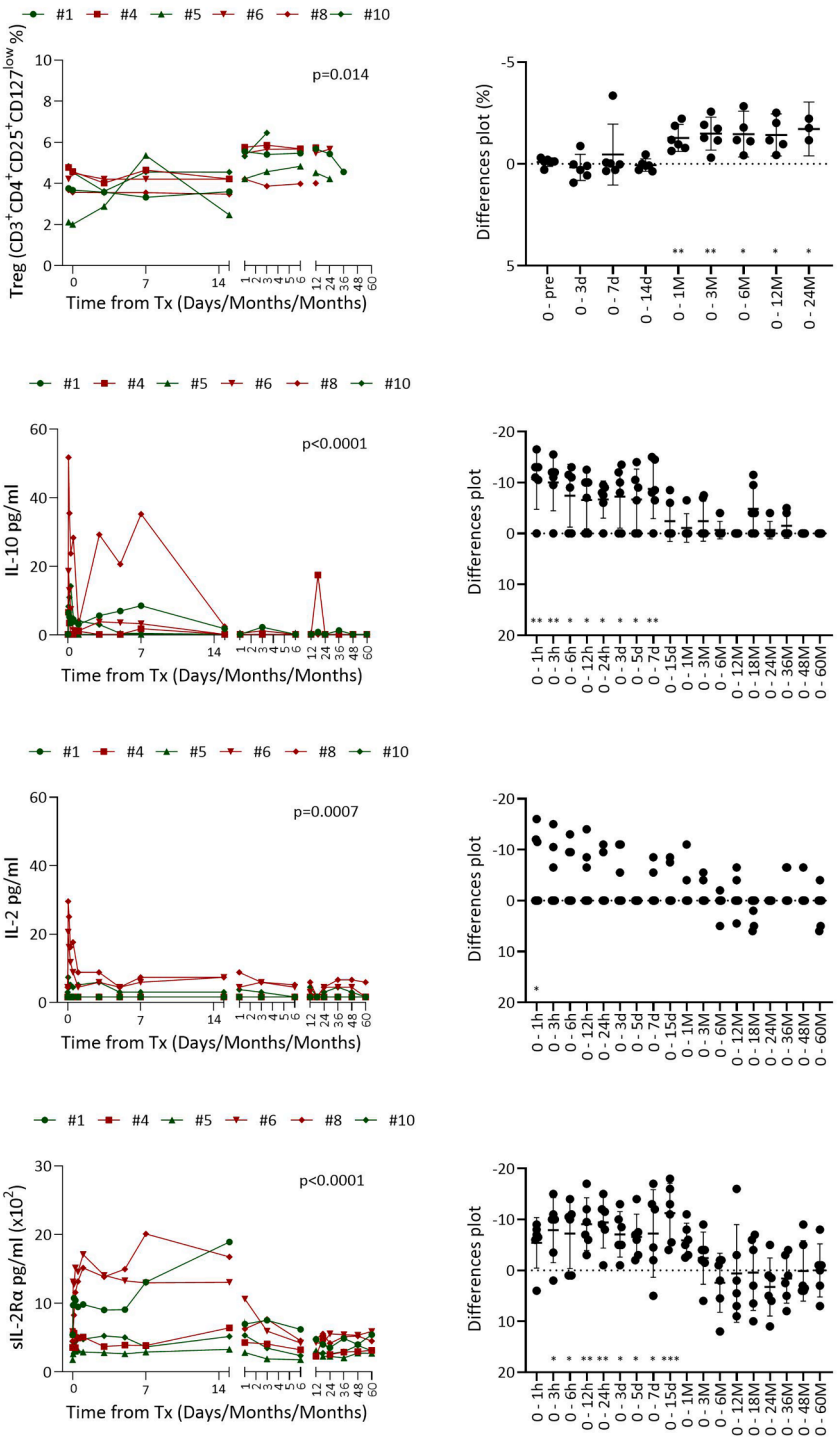

Fig. 6: **Long-term follow-up of homeostatic proliferation and related cytokines over 5 years post-islet transplantation.** Longitudinal assessment of homeostatic proliferation, evaluated by Ki-67$^+$ expression in CD3$^+$, CD4$^+$, and CD8$^+$ T cell subsets, and circulating levels of IL-7 and IL-15, measured over 5 years in six patients who underwent minimal islet transplantation. Each parameter is plotted over time, with individual trajectories representing each patient. The patient highlighted in red experienced a greater-than-median decline in C-peptide secretion at 5 years; the patient in green showed a lesser-than-median decline. Cytokine data were analyzed using the Friedman test; Ki-67$^+$ T cell data were analyzed using a mixed-effects model to account for missing values. Difference plots on the right display pairwise comparisons with baseline (time 0), based on rank differences for cytokines and estimated marginal means for T cell proliferation. Each dot represents the

responses that resolved within the first year, and low-level class II DSA persisting in two subjects without clinical or metabolic consequences. Although formal PRA testing was not performed, the longitudinal anti-HLA analysis showed no evidence of broad or progressive sensitization, indicating that alloimmune activation was limited, non-evolving, and unlikely to affect future transplant eligibility.

Functionally, islet infusion appeared to stabilise β-cell secretory capacity during the first year, as reflected by C-peptide AUC values that remained close to pre-transplant levels. This observation must be interpreted with caution, given the absence of a concurrent control arm. As a first-in-human, proof-of-concept study, the primary aim was to assess safety and biological activity rather than efficacy; a conventional randomised design would have required infusing large numbers of participants before any safety data were available, which was neither feasible nor ethically justified. To better contextualise our findings, we compared post-transplant C-peptide trajectories with two complementary reference cohorts presented in Supplementary Figure S9. The first included five participants recruited in this study who, during donor matching, underwent serial MMTTs while awaiting transplantation; in all, stimulated C-peptide declined progressively. Among the three later infused, trajectories diverged—patient #10 showed a reversal of decline, patient #6 a shallower slope, and patient #8 a similar rate before and after infusion. The second cohort comprised 26 adults with new-onset T1D who received placebo in the contemporaneous Ladarixin trial,[24] showing a consistent reduction in stimulated C-peptide over 12 months despite comparable demographics and metabolic features. Together, these internal and external data suggest that C-peptide stabilisation in our treated participants was more sustained than expected for adults at a similar stage of disease. All participants achieved IDAA1c $\leq$ 9, a recognised surrogate marker of partial remission,[25] and most maintained low insulin requirements and satisfactory glycaemic control during the first year. Endogenous insulin secretion then declined gradually, consistent with progressive autoimmunity or β-cell exhaustion, yet two of six participants retained substantial secretion at five years—indicating possible sustained immunometabolic benefit in a subset of responders. In the TrialNet cohort,[26] only about 20% of adults maintained IDAA1c $\leq$ 9 and fewer than 5% preserved baseline C-peptide after four years, while INNODIA[27] data show 70% partial remission at one year. Against this background, the relative preservation of β-cell function observed here may reflect a transient but relevant modulation of the autoimmune process. No clear association could be established between interval from diagnosis and β-cell outcomes, partly because donor availability constrained infusion timing. The feasibility of shorter intervention windows may be improved in the future by using autologous or HLA-matched iPSC-derived islets, allowing treatment to be initiated closer to disease onset.

The analysis of circulating microRNAs revealed a reproducible, biphasic increase in plasma miR-375 following islet infusion, consistent with early β-cell injury and a secondary wave of release around day 5. The specificity of this pattern—absent in the ubiquitous control miRNA miR-16—supports its utility as a dynamic biomarker of islet stress.[28] These findings underscore the potential of miRNA profiling as a non-invasive approach to monitor graft integrity.[29]

The emergence of anti-HLA antibodies in most patients—although variable in timing and persistence—indicates expected alloimmune activation following islet infusion.[30] However, within the framework of this protocol, such activation appears to have been regulated rather than suppressed, consistent with controlled immune engagement. The pattern observed in patient #1 is particularly illustrative: the transient class I donor-specific antibody response, followed by spontaneous alloantibody downregulation, aligns with a controlled rejection process. This suggests that immune activation occurred but was effectively contained, potentially through the induction of regulatory mechanisms. Similarly, the transient increases in autoantibodies against islet antigens—such as GAD65, IA-2, insulin, and ZnT8—followed by normalization or reduction to baseline, reflect a regulated immune response rather than uncontrolled autoimmunity or antigen spreading. This controlled modulation of autoantibodies further supports the concept of active but contained alloimmune and autoimmune engagement. Importantly, the presence of these allo- and autoantibodies did not correlate with metabolic deterioration, underscoring a central premise of this strategy: the transplanted islets were not intended to persist or function long-term. Instead, the observed glucometabolic trajectory likely reflects residual native islet function, while the allogeneic islets serve primarily as immune triggers. These islets provide transient antigen exposure intended to modulate, rather than suppress, the host immune response, promoting recalibration of immune recognition in the context of islet

individual difference—unitless ranks for cytokines and raw values for leukocytes—between a specific time point and baseline. The Y-axis shows these differences with mean values and 95% confidence intervals. Pairwise comparisons were performed using uncorrected Dunn's test (cytokines) and Fisher's LSD test (leukocytes), without correction for multiple comparisons; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

autoimmunity. In this setting, "controlled exposure" does not refer to any physical protection of the islets but rather to their temporally limited survival under brief immunosuppression. By discontinuing all immunosuppressive therapy after one month, the grafts were allowed to undergo predictable immune rejection, thereby creating a short and regulated window of antigen presentation. This temporal control ensured transient, rather than persistent, alloantigen stimulation—defining the "controlled" nature of the exposure. Within this framework, temporary modifications of antibody profiles should not be interpreted solely as evidence of rejection or renewed autoimmunity, but rather as signs of controlled immune engagement that might favour regulatory adaptation.[31] Supporting this interpretation, no measurable expansion of circulating autoreactive $CD8^+$ T cells (GAD65 $dextramer^+$) was detected, indicating that if activation occurred, it remained below the threshold of detection or was rapidly controlled. This pattern reinforces the notion of contained immune engagement rather than sustained autoreactive amplification.

Importantly, the immunological landscape post-transplant was marked by profound and temporally structured alterations. ATG-induced lymphodepletion preferentially affected naïve T cells, while sparing central and effector memory subsets. The subsequent expansion of Ki-67^+ T cells and sustained elevation of IL-7 and IL-15 levels suggest a coordinated homeostatic rebound aimed at reconstituting T cell compartments.[20,32] As expected after lymphodepletion, regulatory T cells expanded rapidly during this rebound phase as part of homeostatic proliferation driven by increased IL-2 availability. However, in our study this expansion was accompanied by a sustained rise in IL-10 and soluble IL-2Rα, indicating not only quantitative recovery but also qualitative regulatory activation. Concordantly, regulatory T cells expanded significantly within the first month and remained elevated, indicative of a favorable immunoregulatory milieu.[33,34]

The early elevation of IL-2 and IL-10—two cytokines pivotal to immune regulation and tolerance induction—indicates activation of regulatory pathways during the immediate post-transplant window. These transient surges are indicative of a tightly regulated, time-sensitive immune response. IL-10, known for its potent anti-inflammatory properties,[35] and IL-2, essential for the expansion and maintenance of regulatory T cells (Tregs), together suggest the early engagement of tolerogenic mechanisms.[36] In parallel, the sustained rise in soluble IL2Rα, a surrogate marker of T cell activation and particularly associated with regulatory T cell activity,[37] persists over the first month post-infusion. This prolonged elevation may reflect ongoing immunomodulation and the maintenance of a regulatory milieu beyond the initial cytokine wave. The temporal disconnect—short-lived IL-2 and IL-10 versus prolonged sIL2Rα elevation—suggests that while IL-2 and IL-10 may initiate the regulatory cascade, markers like sIL2Rα may better capture the downstream persistence of immune modulation, potentially supporting longer-term immune reprogramming.

The cytokine and chemokine response exhibited two main phases. The initial surge in TNF-α, IL-6, and CXCL10 likely reflects an acute inflammatory response to islet infusion and ATG-mediated innate activation. A second wave, peaking between days 7 and 14 (including IFN-γ, TRAIL, and G-CSF), may represent adaptive immune recognition and tissue adaptation. Endothelial and repair mediators such as VCAM-1, ICAM-1, and HGF remained elevated longer, possibly reflecting ongoing vascular activation and attempts at immune resolution. These dynamics highlight the interplay between innate and adaptive responses in the early post-transplant phase and may inform optimisation of peri-transplant immunotherapy.[38,39]

Interestingly, the early post-infusion phase was also marked by distinct hormonal and adipokine changes, including transient increases in resistin and glucagon and reductions in adipsin, adiponectin, and PAI-1. This pattern suggests an acute, time-limited metabolic and inflammatory shift, possibly reflecting adipose stress or transient insulin resistance after infusion. The elevation of resistin and glucagon may represent a compensatory response to early metabolic stress,[40–42] while the decline in adiponectin and adipsin—both linked to insulin sensitivity and β-cell support—may signal temporary impairment of adipocyte-endocrine signalling.[43,44] The reduction in PAI-1, typically elevated in pro-inflammatory states, could reflect early regulatory adaptation aimed at limiting coagulation and matrix remodelling.[45] Taken together, these findings define a coordinated signature of post-exposure immune engagement, consistent with a state of immune modulation aligned with regulatory activation. As no validated assay currently exists to directly demonstrate tolerance in human islet autoimmunity, interpretation must necessarily rely on surrogate immunological markers.

This study has several limitations. First, the inability to directly assess transplanted islet survival or function limits our ability to distinguish between effects from the graft versus native islet activity. Second, the monocentric design and small sample size reduce the generalizability of the findings. Additionally, reliance on surrogate immunological markers, rather than direct measures of antigen-specific tolerance, limits the interpretation of immune regulation. Third, the lack of histological graft and pancreas data prevent conclusions about the long-term durability of the observed immune modulation. Finally, we recognise that the immunomodulatory effects observed could also result, at least in part, from the concomitant lymphodepletion and G-CSF/sirolimus regimen. The design of this first-in-

human pilot study does not allow the relative contribution of each component to be distinguished. However, the coordinated immune and serological patterns observed—linking alloimmune engagement, transient cytokine surges, and Treg activation—suggest that islet exposure contributed specifically to antigen-directed modulation. Future controlled studies comparing ATG + G-CSF with and without islet infusion will be required to formally address this question.

In conclusion, this work advances a paradigm shift in the treatment of autoimmune diabetes: from attempts to rescue functionally doomed islets, to using them deliberately as tolerogenic agents. It opens the door to rethinking transplantation not as a means of cellular replacement, but as a tool for immune education, redefining the landscape of antigen-specific tolerance in T1D. This concept, initially constrained by the limited availability of donor tissue, could now be significantly expanded and refined through the use of pluripotent stem cell–derived islets.[46] These novel sources enable extensive HLA matching, genetic modification of the graft to enhance immunoregulatory properties, and, crucially, the feasibility of repeated antigen exposure through serial infusions—thus providing a more versatile and potent platform for inducing durable immune tolerance.

Contributors

LP: Conceptualization; Formal Analysis, Project administration, Writing—original draft. AB: Resources, Investigation, Data curation; AC: Resources, Investigation, Data curation; RM: Resources, Investigation, Data curation; AM: Resources, Data curation; VS: Resources, Investigation, Data curation; PM: Resources, Investigation, Data curation; PMa: Data curation; VL: Resources, Investigation, Data curation; IM: Resources, Investigation, Data curation; PMaf: Data curation; MR: Resources, Investigation, Data curation; NC: Data curation; EP: Data curation; DC: Data curation; MC: Resources, Investigation, Data curation; RC: Supervision; Writing—review & editing; EB: Conceptualization, review & editing, LP is responsible for final submission of the manuscript for publication and all authors approved the final version before submission. LP and RC had full access to all the data and verified the accuracy of the analyses.

Data sharing statement

De-identified participant data are not available. The study protocol, statistical analysis plan, and analytical code will be shared upon reasonable request to the corresponding author (Lorenzo Piemonti; lorenzo.piemonti@hsr.it) following publication, subject to institutional and ethical approvals.

Declaration of interests

Lorenzo Piemonti reports institutional funding from Fondazione Italiana Diabete (FID) to the IRCCS Ospedale San Raffaele for the specific research project *"Induction of immune education in type 1 diabetes through controlled allogeneic islet rejection at onset"*; the funder had no role in study design, data collection, analysis, interpretation, or writing of the report.

Outside the submitted work, LP serves on advisory boards for Vertex Pharmaceuticals, Novo Nordisk, and Sanofi. Emanuele Bosi reports consulting and/or advisory roles for Sanofi, Roche, and Medtronic. Paolo Monti reports research grants from Telethon and Breakthrough T1D outside the submitted work. All other authors (Andrea Mario Bolla, Amelia Caretto, Raffaella Melzi, Alessia Mercalli, Valeria Sordi, Paola Magistretti, Vito Lampasona, Ilaria Marzinotto, Paola Maffi, Miriam Ramondetta, Nicoletta Cagni, Erica Pedone, Davide Catarinella, Massimo Cardillo, Rossana Caldara) declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2025.103685.

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