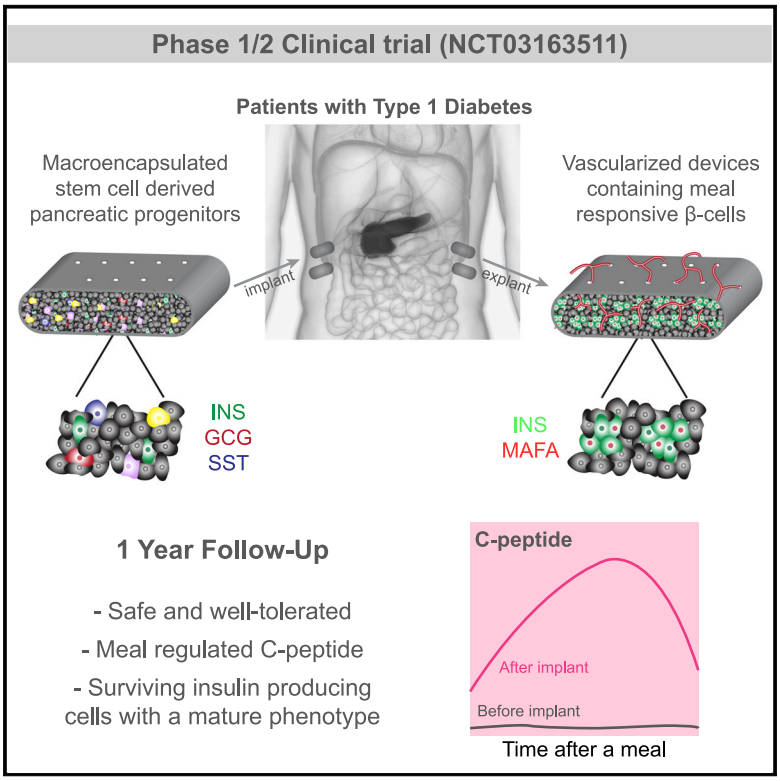
Cell Stem Cell

Clinical and Translational Report

Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes

Graphical abstract



Highlights

- Stem-cell-derived pancreatic progenitors were well tolerated by human subjects
- Patients developed meal-response C-peptide production 26 weeks post-implantation
- Explants were dominated by α cells, but a subset had a mature β cell phenotype

Authors

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In brief

Kieffer and colleagues assessed the implant of stem-cell-derived pancreatic cells for the treatment of type 1 diabetes. Patients developed meal-responsive insulin secretion post-implantation and retrieved grafts contained cells with a mature β cell phenotype. Stem-cell-derived cells can mature into glucose-responsive functional β cells in patients with diabetes.

Ramzy et al., 2021, Cell Stem Cell *28*, 2047–2061
December 2, 2021
https://doi.org/10.1016/j.stem.2021.10.003

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Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes

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https://doi.org/10.1016/j.stem.2021.10.003

SUMMARY

An open-label, first-in-human phase 1/2 study is being conducted to evaluate the safety and efficacy of pancreatic endoderm cells (PECs) implanted in non-immunoprotective macroencapsulation devices for the treatment of type 1 diabetes. We report an analysis on 1 year of data from the first cohort of 15 patients from a single trial site that received subcutaneous implantation of cell products combined with an immunosuppressive regimen. Implants were well tolerated with no teratoma formation or severe graft-related adverse events. After implantation, patients had increased fasting C-peptide levels and increased glucose-responsive C-peptide levels and developed mixed meal-stimulated C-peptide secretion. There were immunosuppression-related transient increases in circulating regulatory T cells, PD1^{high} T cells, and IL17A^{+}CD4^{+} T cells. Explanted grafts contained cells with a mature β cell phenotype that were immunoreactive for insulin, islet amyloid polypeptide, and MAFA. These data, and associated findings (Shapiro et al., 2021), are the first reported evidence of meal-regulated insulin secretion by differentiated stem cells in patients.

INTRODUCTION

Diabetes mellitus is a chronic disease that affects over 460 million people worldwide (International Diabetes Foundation, 2019) and bears a significant financial, disability, and mortality cost for health care systems and patients globally (Anderson et al., 2001). Almost 130,000 new cases of type 1 diabetes are diagnosed each year, and patients face a lifetime of exogenous insulin therapy (Anderson et al., 2001). In 2000, a scientific breakthrough occurred in Edmonton, Canada, with the development of an islet transplantation protocol that successfully yielded insulin independence for seven patients with type 1 diabetes (Shapiro et al., 2000). Since then, over 1,500 islet transplantation procedures have been performed worldwide, and between 2007 and 2010, 44% of patients registered with the Clinical Islet Transplant Registry achieved insulin independence at 3 years post-transplantation (Barton et al., 2012). Median HbA1c levels dropped more than 1.5%, and 87.5% met HbA1c goals 1 year post-transplantation (Hering et al., 2016; NCT00434811), leading to reduced risks of both acute hypoglycemic events and progression of chronic complications (Warnock et al., 2008). Despite these encouraging findings, widespread adoption of this procedure remains limited because of the paucity of islets from deceased donors.

Given the potential for an islet cell therapy to give patients insulin independence and to mitigate the complications of diabetes, there is a need for an abundant alternate supply of insulin-producing cells. Alongside interest in porcine islets, development of insulin-secreting cell lines, and *in situ* cellular reprogramming strategies, use of human pluripotent stem cells has made tremendous progress toward becoming a viable clinical option for the mass production of insulin-producing cells (Ellis et al., 2017). In 2001, Assady et al. reported spontaneous *in vitro* differentiation of human pluripotent embryonic stem cells (ESCs), which included the generation of cells with characteristics of insulin-producing β cells (Assady et al., 2001). Based upon prior knowledge of pancreas development and empirical determination, stepwise protocols were created to control and direct ESCs to definitive endoderm (D'Amour et al., 2005) and subsequently pancreatic endoderm (D'Amour et al., 2006). Pancreatic endoderm cells (PECs), marked by the transcription factors homeobox protein Nkx-6.1 (NKX6.1) and pancreatic

and duodenal homeobox protein 1 (PDX1), produce little insulin but can adequately complete differentiation into pancreatic islet cells after implantation into mice to prevent onset of hyperglycemia following destruction of endogenous mouse β cells (Kroon et al., 2008) and to reverse established diabetes in mice (Rezania et al., 2012), including when implanted subcutaneously in macroencapsulation devices (Bruin et al., 2013). Some protocols have been developed to differentiate ESCs to a more mature β cell phenotype *in vitro* (Loo et al., 2018; Rezania et al., 2014), but challenges with lower cell yield, longer culture time, higher oxygen requirements, and higher cost (Iworima et al., 2021; Memon and Abdelalim, 2020) have been motivations to explore the clinical use of less mature PECs.

In 2014, ViaCyte launched a phase 1/2 prospective, multicenter, open-label trial (clinicaltrials.gov: NCT02239354) to investigate the safety, tolerability, and efficacy of their VC-01 product candidate. VC-01 is a combination of PECs (the drug candidate PECs produced by ViaCyte have been named "PEC-01") in macroencapsulation devices (named "PEC-Encap" by the manufacturer) designed to be immunoprotective via use of a cell-impermeable layer. Although full results have not been released, the first 19 patients were reported to tolerate the product well and had few complications (Henry et al., 2018). Although insulin-immunoreactive cells were identified in some explanted grafts 2 years post-implantation, cell survival was inconsistent because of a foreign body response to the encapsulation devices, and there was no reported evidence of insulin secretion (Henry et al., 2018).

In an effort to mitigate cell loss due to device fibrosis, ViaCyte initiated a follow-up trial in 2017 (clinicaltrial.gov: NCT03163511) to investigate the safety, tolerability, and C-peptide production of PEC-01s macroencapsulated in non-immunoprotective devices that include portals designed to enable direct capillary vascular permeation into the device interior (the VC-02 combination product has been named "PEC-Direct" by the manufacturer). This approach requires use of immunosuppression to limit alloimmune, and possibly autoimmune, reactions to implanted cells. We present our initial results from a single site from this trial and provide evidence that PEC-01s survived and matured into glucose-responsive insulin-secreting cells within 26 weeks post-implantation and that patients spent more time in targeted blood glucose range. These findings support the conclusion that PEC-01s contained within vascularizing macroencapsulation devices can survive and mature into functional β-like cells when implanted subcutaneously in patients with type 1 diabetes.

RESULTS

Patients underwent screening (full inclusion and exclusion criteria and the Consolidated Standards of Reporting Trials [CONSORT] flow diagram available; Methods S1 and S2) and were enrolled between November 2017 and March 2020. Patients had been diagnosed with diabetes for 10 to 54 years, were both sexes (male = 7, female = 8), had ages ranging from 36 to 56 years, predominantly identified as white (n = 14, one patient identified as "Hawaiian or native pacific islander"), and had variable degrees of chronic complications of diabetes (Table 1). During the first year of follow-up, all patients reported adverse reactions (total 175), of which three were serious adverse reactions (Table S1). Two patients terminated the study before 1 year because they elected to withdraw consent to take immunosuppressive medications after having serious adverse reactions: patient 04 withdrew consent after 6 months because of typhlitis (grade four) and a liver abscess (grade four), and patient 02 withdrew consent after 10 months because of a parvovirus B19 infection associated with aplastic anemia requiring treatment with intravenous immunoglobulin (grade three). Complications were documented as being "possibly related to immunosuppression," given that tacrolimus and mycophenolate mofetil (MMF) are well documented to cause bone marrow suppression and increased risk for infections (Hafiz et al., 2005; Nowacka-Cieciura et al., 2020). Five other patients (03, 08, 10, 11, and 12) were recommended to withdraw after 9 months because of failed risk-benefit assessment by the clinical trial sponsor, based primarily on undetected C-peptide by the contract research organization (limit of detection 33 pM) and histological assessment, as well as consideration of patient HbA1c, exogenous insulin requirements, and Clarke hypoglycemia awareness score. All data collected during the first year of patient enrollment and prior to final patient withdrawal have been included.

Patient compliance with presenting for scheduled clinical appointments was 100% (some visits were canceled because of the coronavirus disease 2019 (COVID-19) pandemic), while five visits were missed by the academic research team, leading to missing data. At six visits for 4-h mixed meal tolerance test, patients did not complete the full test because of elevated blood glucose (n = 4), patient discomfort (n = 1), or technical difficulties with sample collection (n = 1). At designated visits, patients were weighed (Figure 1A) and reported their Clarke hypoglycemia awareness score (Figure 1B) (Clarke et al., 1995). Patients had stable body weight ($B = -0.035$, $SE = 0.028$, $t(113.1) = -1.25$, $p = 0.213$) and improved hypoglycemic awareness over the duration of follow-up ($B = -0.018$, $SE = 0.0064$, $t(51.8) = -2.79$, $p = 0.007$). Patient HbA1c was also monitored and was stable over the duration of the study ($B = -0.0055$, $SE = 0.0042$, $t(39.4) = -1.363$, $p = 0.193$) with patients reporting good glucose control based on mean HbA1c <7.0% at all time points (Figure 1C). Patients had stable creatinine from baseline to 26 weeks post-implantation (baseline: 95% CI 70.7–88.7 μM, week 26: 95% CI 78.7–98.7 μM), and one patient with a documented history of proteinuria pre-enrollment had an elevated albumin/creatinine ratio at week 26 (10:112.8 mg/g), but no albumin/creatinine ratio was collected at baseline for comparison.

Patient total daily insulin requirements were logged both pre-implantation and throughout the study (Figure 1D). Patients had reduced insulin requirements post-implantation ($B = -0.220$, $SE = 0.03$, $t(186.3) = -7.25$, $p < 0.001$), and the decrease in insulin requirements over the study duration was still significant if the post-implantation steroid-associated increase in insulin requirements (methylprednisolone 125 mg intravenously (i.v.) for 5 days) was excluded from the analysis ($B = -0.156$, $SE = 0.03$, $t(171.2) = -5.71$, $p < 0.001$). There was no association between absolute fasting serum C-peptide and insulin requirements ($B = 0.0073$, $SE = 0.028$, $t(106.8) = 0.254$, $p = 0.80$), nor any relative change to serum C-peptide and insulin requirements ($B = 0.212$, $SE = 0.044$, $t(101.4) = 0.005$, $p = 0.996$). Only one patient (11) had a >50% reduction in insulin requirements within 1 year post-implantation, and no patients achieved insulin

Table 1. Patient demographics collected at the time of enrollment

Patient ID	ID in Shapiro et al.	Age	Sex	Diabetes duration (years)	Insulin therapy	BMI (kg/m^2)	ACR (mg/g)	Creatinine (μM)	Total cholesterol (mM)	HDL cholesterol (mM)	Blood pressure (mmHg)	Ongoing chronic medical history at the time of enrollment (year of diagnosis)
01	N/A	40	M	10	MDI	28.8		116.6	5.6	1.5	130/81	lipohypertrophy (2012), hyperlipidemia (2017), weight control (2012), hearing loss (2017)
02	E-002	49	F	31	MDI	25.0	*	73.4	5.2	2.3	94/58	pityriasis (2017), hyperlipidemia (2017)
03	E-003	43	M	20	pump	29.0	2.2	86.6	6.3	1.1	130/77	asthma (1983), eczema (1990), elevated BMI (2012), migraines (1980)
04	E-005	56	F	43	pump	25.5	0	73.4	4.3	2.3	110/70	hyperlipidemia (2000), elevated BMI (2012)
05	E-006	54	M	33	pump	27.3	*	76.9	4.6	1.8	132/84	hypertension (2013), hyperlipidemia (2005), diabetic retinopathy (2014), coronary artery disease (2013)
06	E-007	45	M	12	pump	28.4	3.3	90.2	4.5	1.4	108/68	sinus bradycardia (2018), peripheral neuropathy (2015), erectile dysfunction (2013)
07	E-008	49	F	44	pump	27.3	0	64.5	4.6	1.8	103/71	hypothyroidism (2015), iron deficiency (2015)
08	N/A	58	F	53	MDI	30.5	0	71.6	5.5	1.9	146/74	diabetic retinopathy (1982)
09	N/A	55	F	54	pump	22.2	4.2	53.9	4.1	1.7	171/77	psoriatic arthritis (2012), depression (2008), hypothyroidism (1992), psoriasis (2008), obstructive sleep apnea (2017), hypertension (2017)
10	N/A	55	M	46	pump	32.4	*	103.4	3.5	1.7	152/73	sleep apnea (2018), hypertension (1999), proteinuria (1999), GERD (2010), proliferative retinopathy (1998), hypercholesterolemia (1998)
11	N/A	52	M	37	pump	30.9	3.1	74.3	3.5	1.3	138/78	depression (2005), sinus bradycardia (2019)
12	N/A	36	F	20	MDI	30.6	4.7	67.2	4.4	1.7	111/77	dermatographic urticaria (2013), IBS (1999)
13	N/A	42	M	29	pump	23.5	9.4	101.7	6.3	1.7	139/78	autonomic neuropathy (2008), diabetic neuropathy (2006), diabetic retinopathy (2012), dyslipidemia (2017)
14	N/A	54	F	43	pump	27.8	28.5	69.8	4.5	1.4	139/74	endometriosis (2006),
15	N/A	42	F	25	pump	25.5	39.4	72.5	4.3	2.0	130/80	diabetic retinopathy (1995), gastresophageal reflux disease (2018), irritable bowel syndrome (2019)

*No ACR sample was collected, but patients had negative protein on a urine dipstick test.
BMI, Body mass index; ACR, albumin-creatinine ratio; MDI, multiple daily injections.

independence (secondary endpoint). Additionally, patients had clinically relevant improved glucose regulation (Figure 1E) with no change to time in the hypoglycemic range (<3.9 mM; B = −0.004, SE = 0.04, t(170.6) = −0.096, p = 0.924) but significantly more time in the target range (3.9 to 10 mM; B = 0.666, SE = 0.18, t(171.1) = 3.66, p < 0.001) and significantly less time in the hyperglycemic range (>10 mM; B = −0.699, SE = 0.16, t(170.4) = −4.38, p < 0.001) (Danne et al., 2017). There were no significant

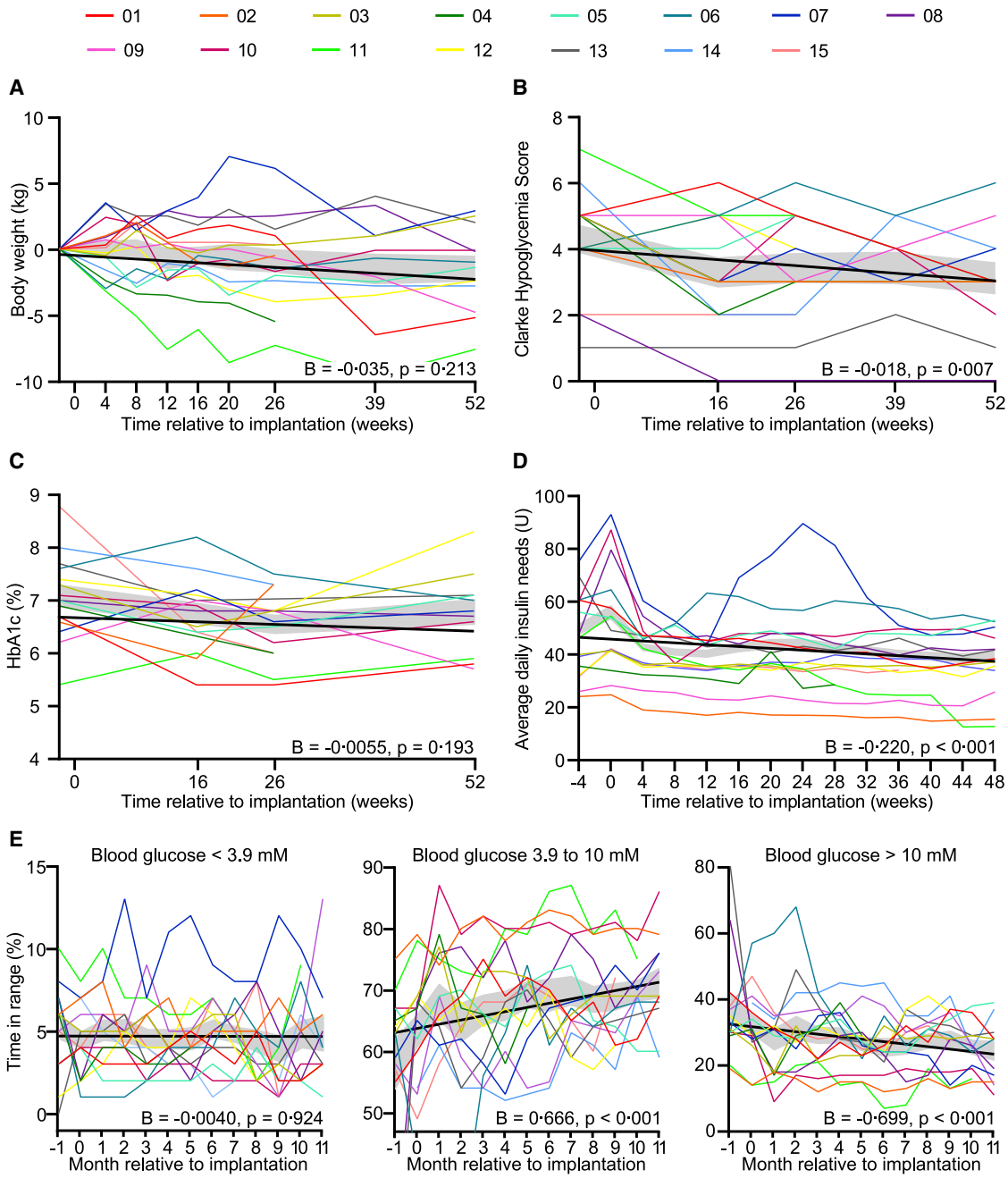

Figure 1. Assessment of changes to patient body weight, hypoglycemic awareness, glucose control, insulin requirements, and blood glucose control over 1 year post-implantation

(A) Body weight of individual patients tracked relative to baseline for 1 year after graft implantation.

(B) Patients' hypoglycemic awareness over the study duration was assessed using the Clarke hypoglycemia awareness score.

(C) At designated visits, blood samples were collected for HbA1c analysis.

(D) Average daily insulin usage over 4-week intervals during the study.

(E) Patient blood glucose was tracked using continuous glucose monitoring and time in a hypoglycemic range (<3.9 mM), euglycemic target range (3.9–10 mM), or hyperglycemic range (>10 mM) and was calculated in 4-week intervals.

In all panels, the multilevel regression is shown by a bolded black line and the mean ± SEM is shaded in gray. HbA1c, hemoglobin A1c.

associations between either absolute fasting serum C-peptide or patient change in C-peptide and time in each blood glucose range. We performed supplemental analyses and compared the change in insulin requirements and time in target glucose range pre-implant to the time point of the primary endpoint (C-peptide at 26 weeks) in those with higher and lower C-peptide

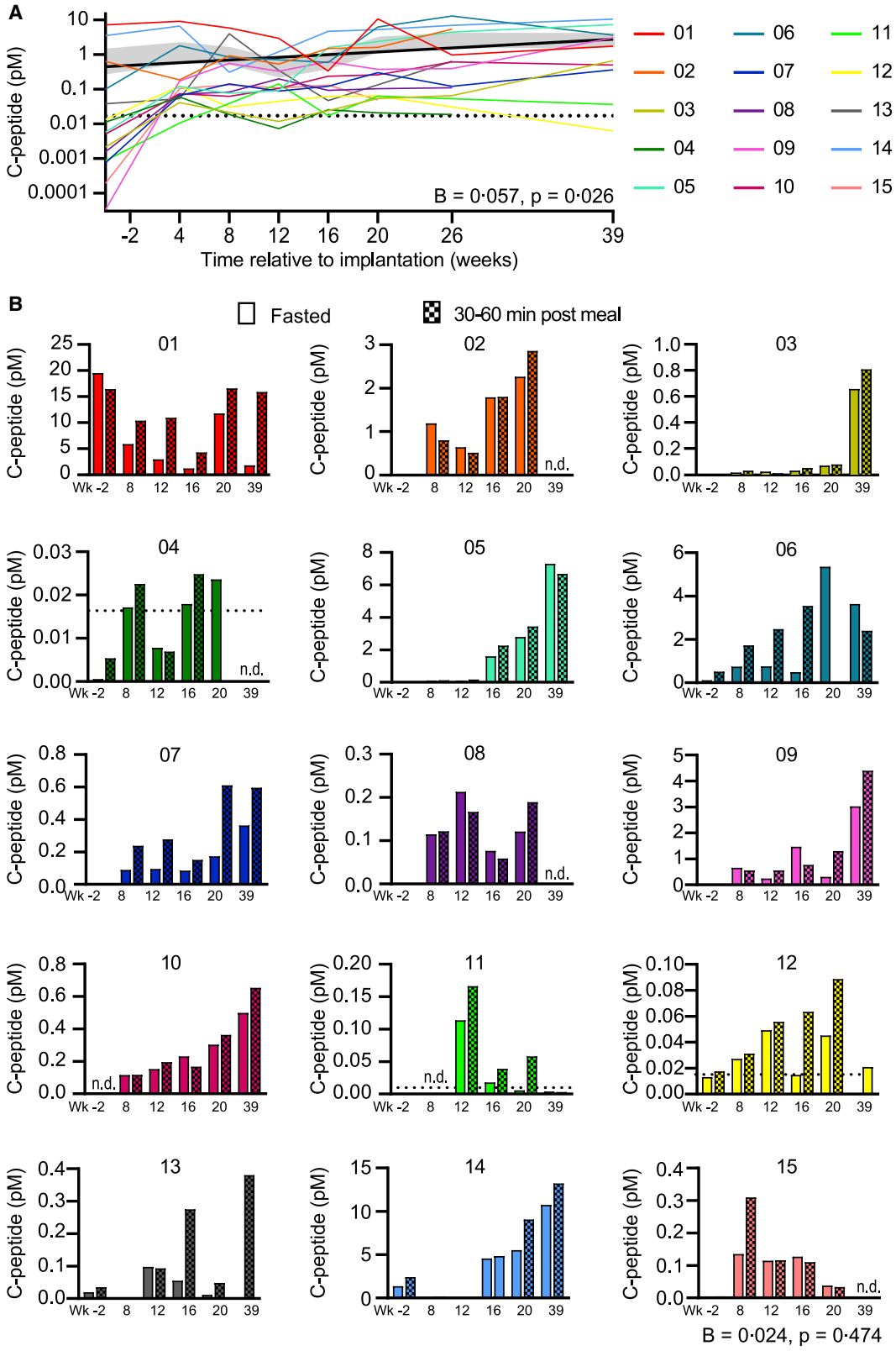

Figure 2. Assessment of fasting and rapid meal-responsive circulating C-peptide pre- and post-implantation

From pre-implantation to 39 weeks post-implantation, patient serum was collected after an 8-h fast and 30–60 min after a mixed meal and assayed for C-peptide. (A) Fasting C-peptide levels over the study duration with the multilevel regression shown by a bolded black line and the mean ± SEM shaded in gray.

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levels (Figure S1; peak C-peptide levels > or <3 pM during the week 26 mixed meal tolerance test). There were no differences between groups.

At visits from baseline to 39 weeks post-implantation, fasting serum samples were collected for C-peptide analysis (Figure 2A) and glucose assay (Figure S2). There was no effect of week of collection on fasting blood glucose over the study duration (B = −0.019, SE = 0.034, t(95.02) = −0.56, p = 0.58). We assessed whether there was an increase in C-peptide over time, and our final model accounted for patient heterogeneity by allowing for variable baseline C-peptide and, given variable blood glucose at each time point, including group-mean-centered blood glucose. There was a significant effect of time on C-peptide (B = 0.057, SE = 0.018, t(88.90) = 3.10, p = 0.0026) but no interactive effect of week and blood glucose on C-peptide, suggesting that the relationship between C-peptide and blood glucose did not change over time (B = −0.0071, SE = 0.0065, t(96.33) = −1.09, p = 0.281). In supplementary analyses, we assessed whether there was an effect of patient age, sex, volume of implanted cells, or use of an insulin pump on C-peptide levels. There was an interactive effect of age and week on C-peptide (B = 0.0057, SE = 0.0025, t(92.79) = 2.33, p = 0.022), suggesting that patients with greater age had greater increases in C-peptide over the study duration. Additionally, patients using pumps had a greater increase in C-peptide over the study duration compared to patients using multiple daily injections of insulin (B = 0.098, SE = 0.036, t(92.63) = 2.76, p = 0.0070). In the other models, there was still a significant increase in C-peptide over time, but there were no interactive effects of sex or volume of cells and week on C-peptide.

We analyzed the frequent simplified oral meal challenge tests to determine whether and when patients developed rapid meal-responsive C-peptide secretion in an attempt to dissect the timeline of development (Figure 2B). Notably, there were highly heterogenous blood glucose responses to the meal both within and between participants; thus, we included group-mean-centered blood glucose in our models (Figure S2B). First, we assessed whether there were meal-responsive elevations in serum C-peptide concentrations at any point during the study and found that patients did not have rapid meal-responsive C-peptide production (Figure S2C). Next, we ran a multilevel model assessing the interactive effect of meal and week on C-peptide to determine whether there was increased meal responsivity over time (Figure 2B). Our model revealed no effect of time on fasting C-peptide (B = 0.038, SE = 0.023, t(143.30) = 1.63, p = 0.106), but patients developed increased post-meal C-peptide over the study duration (B = 0.057, SE = 0.022, t(148.68) = 2.56, p = 0.011). Finally, given heterogenous blood glucose throughout the study, we assessed the interactive effect of week and blood glucose to determine whether patients had greater glucose-stimulated C-peptide over the study duration. When accounting for variable glucose control, although patients did not have increased rapid meal-responsive C-peptide, we found that patients had both more C-peptide over the study duration (B = 0.059, SE = 0.018, t(145.34) = 3.31, p = 0.0012) and a significant increase in the relationship between blood glucose and C-peptide over the study duration (B = 0.0081, SE = 0.0041, t(147.80) = 1.99, p = 0.049), demonstrating development of glucose-responsive C-peptide secretion.

We assessed the development of meal-responsive C-peptide during 4-h mixed meals by comparing baseline, week 26, and week 52 data (Figure 3). Blood glucose levels during mixed meals were similar at all time points (Figures S2D–S2G), and there were no differences in the area under the curve. Given that the primary endpoint was C-peptide production by 26 weeks post-implantation, we first determined whether patients gained meal-responsive C-peptide secretion at the 26-week time point. Our model revealed a significant interactive effect of week and hour on C-peptide (B = 1.40, SE = 0.57, t(177.32) = 2.47, p = 0.014), demonstrating that, at the later week time point (post-implantation), there was a stronger increase in C-peptide after the meal. Importantly, we assessed the meal responsiveness of C-peptide pre- and post-implantation and found that, at pre-implantation, there was no impact of the meal on C-peptide (B = −0.22, SE = 0.42, t(177.27) = −0.52, p = 0.63). Conversely, at week 26, there was a significant positive meal responsiveness of C-peptide over the hours post-meal (B = 1.18, SE = 0.43, t(177.26) = 2.73, p = 0.0069). In follow-up models, we included the week 52 post-implantation time point and found that unlike at baseline, patients had meal-responsive C-peptide at week 52 (B = 0.86, SE = 0.27, t(251.26) = 3.15, p = 0.0018), and there was no difference in the meal responsiveness of C-peptide between the week 26 and week 52 time points (B = −0.69, SE = 0.67, t(248.28) = −1.04, p = 0.30).

PEC-01-containing devices were implanted in non-immunoprotective macroencapsulation devices; thus, patients were given immunosuppression using a regimen based upon that used for islet transplantation (Methods S3). We interrogated the immunological changes during the study by assessing changes in absolute numbers and proportions of T cell subsets from peripheral blood over the study duration (Figures 4 and S3). Consistent with known effects of anti-thymocyte globulin (ATG) (Haller et al., 2019), patients had reduced numbers of $CD3^+$ T cells after induction of immunotherapy (Figure S3A). The numbers of $CD4^+$, $CD8^+$, conventional T cells, and T regulatory cells (Tregs) were similarly reduced (Figures S3B–S3E), and there was an overall decreased ratio of $CD4^+$ to $CD8^+$ T cells (Figure 4A). Nevertheless, patients had an increased ratio of Tregs (defined as $CD127^-CD25^+$ $FOXP3^+$ cells) to conventional T cells (Figure 4B) as well as a transient increase in $Helios^+$ Tregs (Figure S3F). They also had increased proportions of $PD1^{high}CD4^+$ and $PD1^{high}CD8^+$ T cells (Figures 4C and 4D) and effector memory $CD4^+$ and $CD8^+$ T cells (Figures 4E and 4F), with a concomitant reduction in proportions of naive $CD4^+$ and $CD8^+$ T cells (Figures S3G and S3H). There was no change to the proportion of central memory $CD4^+$ T cells (Figure S3I) but a delayed reduction in the proportion of central memory $CD8^+$ T cells (Figure S3J). Notably, there was a transient increase in the proportion of IL17A-producing $CD4^+$ T cells (Figure 4G) but

(B) Individual patient fasted and post-meal C-peptide levels. An interaction between the week of monitoring and the pre- or post-meal status of the patient was included.

Assay sensitivity is shown with a dotted line, and blood glucose was included in models presented in (A) and (B). (n.d. = not done because there was no available sample)

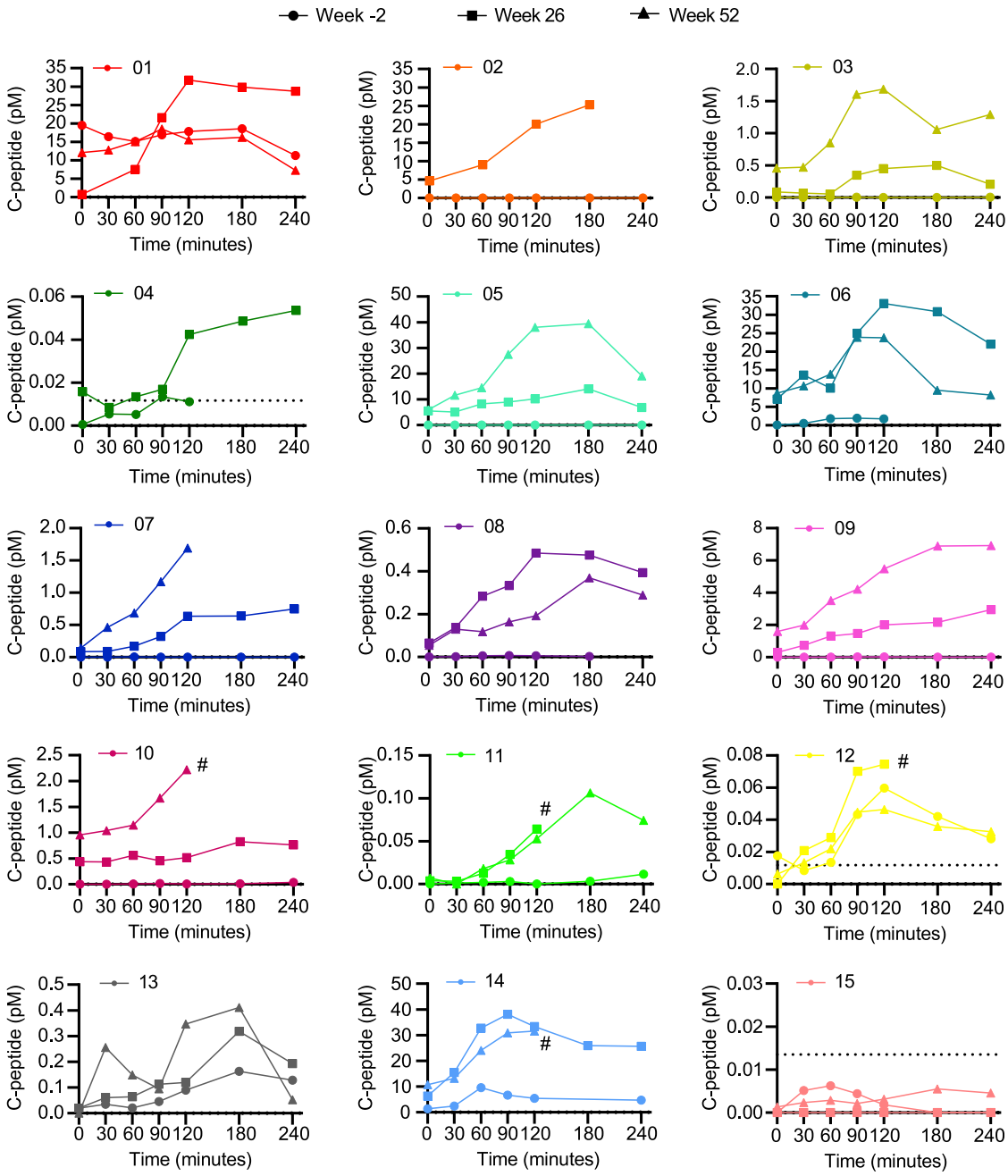

Figure 3. Assessment of meal-responsive C-peptide production pre- and post-implantation

Individual patient serum C-peptide levels over 4 h in response to a mixed meal consumed at baseline (week −2), 26 weeks post-implantation, and 52 weeks post-implantation. At baseline, patients did not have increased C-peptide post-meal ($p = 0.63$), but patients developed positive meal-responsive C-peptide at 26 weeks post-implantation ($p = 0.0069$), and meal responsiveness was similar at repeat test at 52 weeks post-implantation ($p = 0.30$). There was a significant interaction of week of study and hour relative to meal intake from baseline to week 26 ($p = 0.014$). Blood glucose was included in all models. Assay sensitivity is shown with a dotted line. #Data are from abbreviated 2-h mixed meal tolerance tests at 39 weeks (patients 11, 12, and 14) or 65 weeks (patient 10) post-implantation.

a decrease in IL2-producing $CD4^+$ and $CD8^+$ T cells (Figures 4H and S3K) and no change to IFN-γ-producing $CD4^+$ T cells (Figure 4I). We investigated whether the proportion of proinsulin reactive $CD4^+$ T cells changed using an activation-induced marker assay (Cook et al., 2021; Zaunders et al., 2009) but observed no significant change over time (Figures S3L–S3O). There were no relationships between proportions of $IL17A^+$ T cells, $PD1^{high}$ T cells, Treg cells, or proinsulin-specific $CD4^+$ T cells and fasting C-peptide over time.

Given findings that patients developed meal-responsive C-peptide secretion after implantation, we aimed to determine whether implanted stem cells were the source of C-peptide. First,

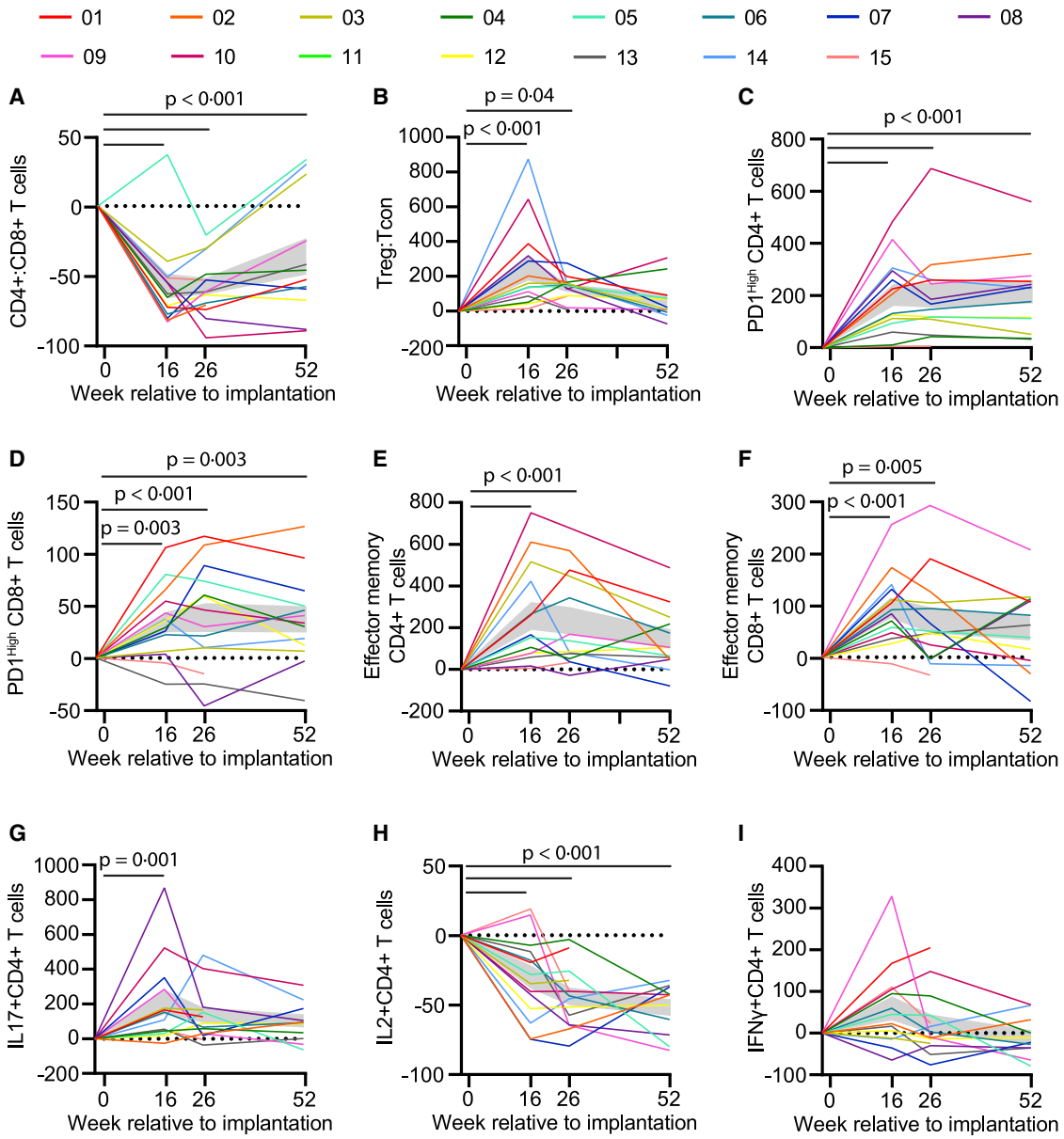

Figure 4. Increased proportions of Tregs and PD1-expressing T cells post-implantation

Whole blood was collected and stained with DURAClone tubes (using panels detailed in Methods S6) to quantify the indicated populations. Data in all panels are presented as percent change from baseline. In all panels, baseline is shown with a dotted line and the mean ± SEM is shaded in gray.

(A and B) Ratio of the absolute numbers of $CD4^+$:$CD8^+$ T cells (A) and of Treg:T conventional cells (Tcon) (B).

(C–I) Proportions of $PD1^{high}CD4^+$ T cells (C), $PD1^{high}CD8^+$ T cells (D), effector memory (defined as $CD45RA^-CCR7^-$) $CD4^+$ T cells (E), effector memory $CD8^+$ T cells (F), IL-17A^+CD4^+ T cells (G), $IL2^+CD4^+$ T cells (H), and IFNγ^+CD4^+ T cells (I). 52-week time point data for patients 02 and 04 were collected after early study termination (approximately 10 and 8 months post-implantation, respectively). Data were analyzed by mixed-effects model, and post-implant time points were compared to baseline using Dunnett's test.

we determined whether patients had preserved C-peptide production after full explantation of all devices. Two patients completed mixed meal tolerance tests after explantation (01 on the day of explantation and 06 2 months post-explantation). Post-explantation, both patients had C-peptide levels at or below their pre-implantation levels (Figure S4A). Next, we determined whether explanted grafts had surviving cells with a mature β cell phenotype. We performed immunohistochemistry on cell-loaded devices explanted 35 to 57 weeks post-implantation from patients 01 to 10 (n = 14 devices; note that we do not present data from patients 11 to 15 because their explant surgeries were delayed because of the COVID-19 pandemic). A practicing board-certified pathologist reviewed hematoxylin- and eosin-stained tissue sections (three sections from each device) and observed no evidence of teratoma formation. The devices contained cells of the pancreatic lineage, and the pathologist

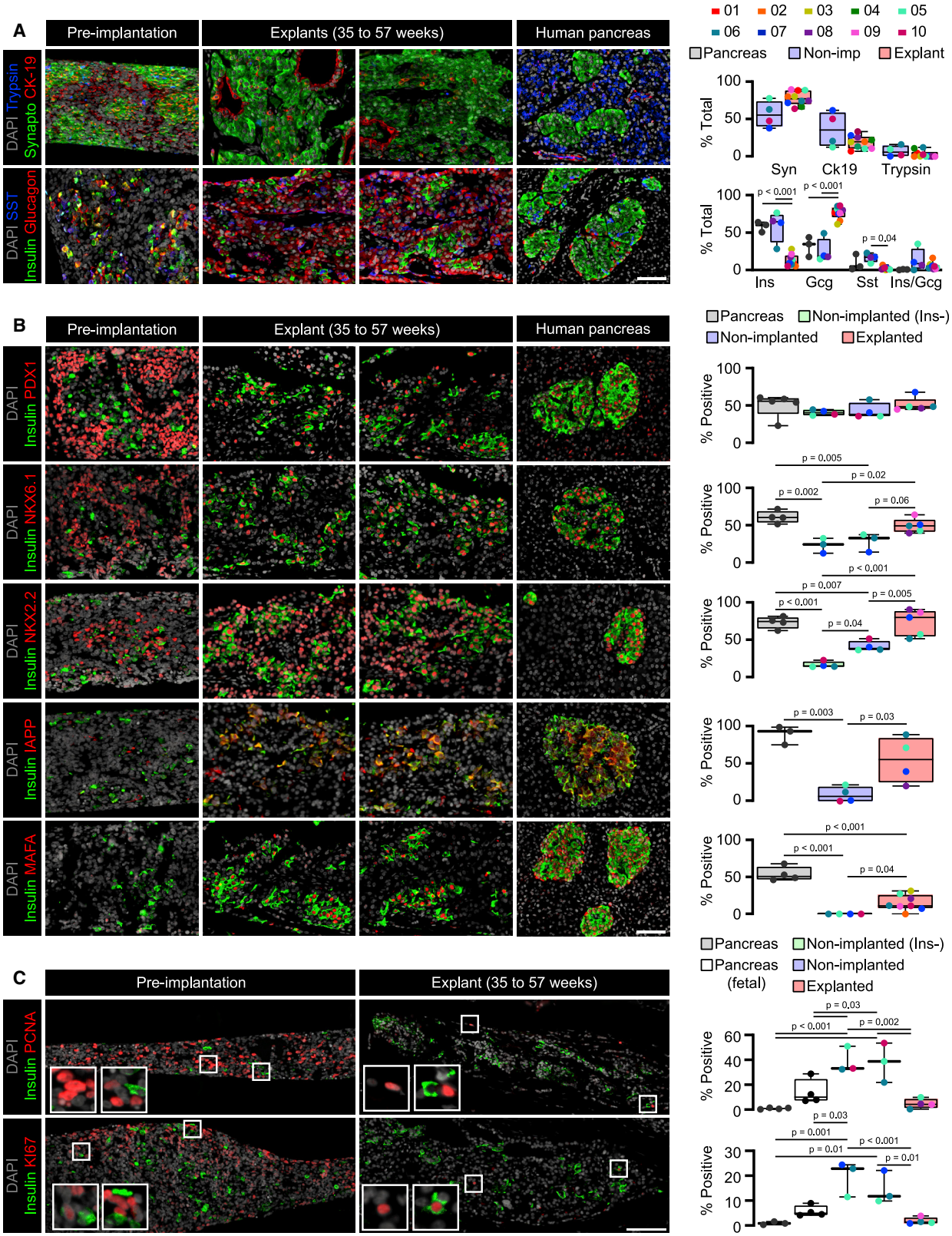

Figure 5. Histological assessment of stem cell graft composition and β cell maturation

Devices loaded with PEC-01s were collected prior to implantation (n = 4, patients 05, 06, 07, and 10) or 35 to 57 weeks post-implantation (n = 10, patients 01 to 10). Human pancreas biopsies from donors without diabetes are shown as a control (representative images of n = 3–5).

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observed no formation of non-pancreatic endoderm tissue or any non-endodermal tissue by the implanted cells (Table S2). The proportion of pancreatic cells that adopted an endocrine cell fate was associated with the area under the curve of C-peptide production at the week 26 mixed meal tolerance test for patients 02 to 10 (data from patient 01 were excluded given that their baseline C-peptide levels were an outlier at >1,000× greater than the interquartile range; $p = 0.04$, adjust $R^2 = 0.46$; Figure S4B). All devices contained pancreatic tissue, blood vessels, and a background of fibrous tissue, but the amount of pancreatic tissue visible (cellularity) was highly heterogenous. The presence of foamy histiocytes may represent evidence of local inflammation and hemorrhage due to the surgical procedure.

We performed a detailed assessment of the graft composition and maturity of graft cells by immunostaining for markers of pancreatic tissue types, endocrine cells, and mature β cells. Using human pancreas as a control, cell-loaded yet never implanted devices (n = 4) contained cells from all three pancreatic cell lineages with a predominance of endocrine tissue (synaptophysin immunoreactive) and ductal tissue (Cytokeratin-19 [CK-19] immunoreactive), but few acinar cells (trypsin immunoreactive; Figures 5A and S5). Post-explant, grafts (n = 10 patients) were heterogenous, and we observed scattered CK-19-positive duct-like structures surrounded by more abundant synaptophysin-positive cell populations. Proportions of synaptophysin immunoreactive cells were higher than the estimated proportion of endocrine cells on pathologist review, potentially attributable to the similar appearance of acinar cells versus endocrine cells on hematoxylin and eosin staining (per review of the results with the pathologist). Additionally, in low-cellularity devices (Table S2), there were as few as nine cells with immunoreactivity for the pancreatic cell lineages, thus introducing expected heterogeneity between tissue sections.

Unlike the un-implanted devices that contained mostly cells that were not immunoreactive for insulin, glucagon, or somatostatin (<10% of cells were immunoreactive for each hormone), the explanted devices had an endocrine cell population that was significantly enriched in glucagon-positive cells that were not immunoreactive for insulin, as well as a significantly lower proportion of cells that were immunoreactive for insulin but not glucagon (Figures 5A and S5). There was no association between the ratio of insulin:glucagon immunoreactive cells and patient C-peptide area under the curve at the week 26 mixed meal tolerance test (Figure S4C). Pre-implantation PEC-01s had extensive PDX1 and NKX6.1 immunoreactivity, whereas in the explanted grafts (a subset with the highest cellularity were analyzed), bright nuclear immunoreactivity was limited to insulin-immunoreactive cells, with rates of immunoreactivity comparable to human pancreas (Figures 5B and S5). Immunoreactivity for homeobox protein Nkx-2.2 (NKX2.2) was present in both insulin-immunoreactive cells and other non-insulin-producing endocrine cells in both grafts. The proportion of insulin-immunoreactive cells that were PDX1, NKX6.1, and NKX2.2 immunoreactive in explanted cells was similar to human pancreas ($p > 0.05$). Unlike pre-implantation PEC-01s wherein insulin-immunoreactive cells lacked islet amyloid polypeptide (IAPP), explanted insulin-immunoreactive cells took on a mature β cell phenotype with abundant IAPP immunoreactivity, similar to human β cells (Figure 5B). Further, non-implanted insulin-immunoreactive cells had almost no V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA) immunoreactivity, whereas explanted cells (a full set of n = 10 devices were analyzed, but insulin-immunoreactive cells were detected in only eight devices) had higher levels of MAFA ($p = 0.04$ versus non-implanted cells), but the proportion of insulin-immunoreactive cells with MAFA immunoreactivity was still lower than adult human pancreas ($p < 0.001$). There was no association between the proportion of insulin-immunoreactive cells with nuclear MAFA immunoreactivity and patient C-peptide area under the curve at the week 26 mixed meal tolerance test (Figure S4D). Finally, we assessed cell proliferation and observed that pre-implantation cells had a high proportion of cells with proliferation cell nuclear antigen (PCNA) or antigen KI-67 (KI67) immunoreactivity (Figure 5C), similar to studies on other stem-cell-derived pancreatic endoderm cells (Rezania et al., 2012). Rates of PCNA and KI67 immunoreactivity in non-implanted cells were higher than human fetal pancreas (n = 4; 14 to 21 weeks gestational age), and explanted cells had replication rates similar to mature human pancreas ($p > 0.05$). In support of the pathologist review (Table S2), abundant immunoreactivity for the endothelial marker von Willebrand factor around the device and adjacent to insulin-immunoreactive cells confirmed direct vascularization into devices, and we did not detect any extensive immune cell infiltration by CD3 immunostaining (Figure S5).

DISCUSSION

With islet transplantation from cadaveric organ donors setting a precedent as an effective therapy for patients with diabetes, identifying an alternative insulin-producing cell source is now warranted. ∼20% of patients on exogenous insulin meet glycemic control targets, whereas with current islet transplantation protocols, almost 90% meet glycemic control targets, have improved quality of life, and have freedom from severe hypoglycemic events (NCT01668485, NCT01148680, NCT00434811). Implantation of PEC-01 cells was well tolerated, and the serious

(A) Grafts contained mixed pancreatic cell lineages including acinar tissue (trypsin), ductal tissue (cytokeratin-19), and endocrine cells (synaptophysin), the latter group of which was composed of insulin-, glucagon-, and somatostatin-immunoreactive cells. Shown on the right is the proportion of cells immunoreactive for each target out of all cells positive for any pancreatic lineage (top row) or any hormone (bottom row).

(B) Grafts and human pancreas were co-immunostained for insulin and PDX1, NKX6.1, NKX2.2, islet amyloid polypeptide (IAPP; a subset of n = 3–4 with the highest cellularity were used), or MAFA (devices from all 10 patients were used but insulin-immunoreactive cells were only detected in 8 devices). Shown on the right is the proportion of all insulin-immunoreactive cells (excepting the group in green, which represents all other cells in the non-implanted device) with immunoreactivity for the target of interest in the nucleus (PDX1, NKX6.1, NKX2.2, or MAFA) or cytoplasm (IAPP).

(C) Grafts, adult human pancreas, and human fetal pancreas (gestational age 14 to 21 weeks) were co-immunostained for insulin and markers of cellular replication (PCNA or KI67) to assess for rates of replication. Quantification of the proportion of cells with nuclear immunoreactivity is shown on the right.

All panels show individual data points in coded color symbols on box and whisker plots, scale bars represent 100 μm (A–B) or 250 μm (C), and insets are enlarged 4×.

adverse events that impacted two patients have been previously documented to be associated with the immunosuppression protocol (Hafiz et al., 2005; Nowacka-Cieciura et al., 2020) and appear unrelated to distant implantation of a cell product in the subcutaneous space. Importantly, as supported by pre-clinical studies (Robert et al., 2018), ultrasound examination and pathological review confirmed that there was no evidence for teratoma formation, and PEC-01-derived cells appeared to be of a pancreatic lineage and cellular replication rates were low. In addition to demonstrating safety of the VC-02 combined product, for the first time, we describe meal-stimulated C-peptide secretion by implanted stem-cell-derived insulin-producing cells in patients with type 1 diabetes. We confirm the presence of surviving insulin-producing cells that were immunoreactive for markers of mature β cells, including the transcription factor MAFA, and demonstrate that meal-responsive C-peptide was lost following graft explantation, thus reducing the likelihood of alternate sources of C-peptide. Taken together, histological and biochemical findings suggest that implanted PEC-01s survived and matured into glucose-responsive bona fide human β cells in patients with type 1 diabetes.

Over the follow-up period, which lasted up to 1 year, patients had 20% reduced insulin requirements, spent 13% more time in target blood glucose range, had stable average HbA1c <7.0%, and had improved hypoglycemic awareness (average Clarke hypoglycemia awareness score decreased ~1 point) associated with C-peptide levels that were, on average, ~1/100th normal levels. Separating the contributions of study enrollment with regular follow-up and intensive glucose monitoring from the impact of low-level C-peptide production by PEC-01s on clinical outcomes is challenging. Observations that there were no differences in clinical outcomes between those with higher and lower C-peptide levels, as well as no clear relationships between changes in C-peptide levels and clinical outcomes, suggest that C-peptide (and inferred insulin production) secretion from PEC-01s was not the major cause for improved clinical outcomes. However, residual C-peptide as low as 5–30 pM in patients with type 1 diabetes was associated with improved hypoglycemic awareness, fewer severe hypoglycemic episodes, and decreased insulin requirements compared to patients with C-peptide <5 pM (Jeyam et al., 2021). Furthermore, improved hypoglycemic awareness has been achieved in patients post-islet transplant despite not achieving insulin independence (Hering et al., 2016). Achieving insulin independence requires an average meal-stimulated C-peptide of 1,000–1,500 pM (Uitbeijerse et al., 2021), but early studies suggest that stimulated C-peptide levels only 20% of normal (approximately 250 pM) may be sufficient for some patients to nearly achieve insulin independence (Rickels et al., 2005), suggesting that low engrafted β cell mass may still offer substantial physiological benefit. A subset of patients in the current study (01, 05, 06, and 14) achieved stimulated C-peptide levels of >30 pM, suggesting that the engrafted PEC-01 population is adequate for some clinical benefit (Jeyam et al., 2021) but may be only 1/10th to 1/30th of that required for insulin independence.

Each patient received only 250–500 million PEC-01s, which can be approximated as an average islet equivalent (IEQ) (Ramachandran et al., 2015) dose of 5,300 IEQ/kg body weight. Because islet transplant recipients have better outcomes with >5,000 IEQ/kg body weight from each donor (Shapiro, 2012; Shapiro et al., 2017) and a total islet dose of >11,000 IEQ/kg body weight (Shapiro et al., 2000), it is likely that implanting more PECs and improving cell survival could be approaches to further enhance outcomes. Given the modest size of devices (3 cm × 9 cm × 1 mm), implantation of more devices is a readily feasible step to double or triple the implanted cell number to reach comparable cell numbers to islet transplantation studies. Nonetheless, with C-peptide levels reaching less than 10% of levels associated with insulin independence (Uitbeijerse et al., 2021), improving cell survival by upward of 10-fold is needed. Optimization of cell preparation, device design, implantation approach, modifiable patient physiology, and immunosuppressive regimen are complimentary avenues worth investigating to achieve this target.

In the absence of a physical or intrinsic barrier to immune rejection, immunosuppression was critical to cell survival by preventing rejection of allogeneic cells. Since the publication of the Edmonton protocol in 2000 (Shapiro et al., 2000), immunosuppressive regimens used in islet transplantation have continued to be optimized (Barton et al., 2012). In the current study, patients had induction therapy with ATG identical to previous islet transplantation protocols (total 6 mg/kg) (Hering et al., 2005, 2016) and were also treated with etanercept as an anti-inflammatory agent. A previous study reported that treatment with low doses of ATG (defined as 2.5 mg/kg) may be of therapeutic benefit in new-onset diabetes because there was preservation of β cell function associated with an increased ratio of Tregs to conventional CD4$^+$ T cells, as well as increased proportions of PD1^{high}CD4$^+$ T cells, a marker of T cell exhaustion (Haller et al., 2019). In contrast, another trial using higher doses of ATG (6.5 mg/kg) reported no benefit for preserving islet function and toxicity to Tregs (Gitelman et al., 2016). Despite the use of 6.0 mg/kg of ATG here, we found immunological changes largely consistent with those previously reported at the lower dose, including a higher proportion of Tregs and PD1^{high}CD4$^+$ T cells, a decreased CD4 to CD8 ratio, and increased effector (but not central) memory T cells. The variable effects on T cell cytokine production, with a significant and long-lasting decrease in IL2, a transient increase in IL17A, and no change in IFN-γ-secreting CD4$^+$ T cells, require more investigation and are likely related to both the effect of the immunosuppression and the presence of an allograft. Beyond these immunological profiling studies, long-term immunosuppression carries risks and was the most likely cause of the high-grade adverse events experienced by two patients (Hafiz et al., 2005; Nowacka-Cieciura et al., 2020). Overall, adverse events and altered immune profile results indicate that optimizing the dose of ATG and other immunosuppressive drugs is an important parallel approach to devising ways to protect the allogenic PECs from alloimmune, and potentially also autoimmune, attack.

Patients' fasting C-peptide increased, on average, by 2.3 pM over the study duration, and patients developed meal-responsive C-peptide by 26 weeks post-implantation. In support of the conclusion that engrafted PEC-01 cells were the source, C-peptide was meal responsive and there were insulin-immunoreactive cells within explanted devices with a mature β cell phenotype. Additionally, after full graft explantation, two patients lost meal-responsive C-peptide and had a return to C-peptide

levels at or below pre-implantation levels. We also note that despite theoretical benefit of immunosuppressive medications on the protection of endogenous β cells, there is only limited data that immunosuppression may (Feutren et al., 1986; Pescovitz et al., 2009) or may not (Gottlieb et al., 2010) preserve β cell mass in new-onset type 1 diabetes, let alone support expansion of β cell mass in patients with diabetes for 10 to 54 years. Additionally, we are aware of no reports of increased C-peptide in patients with type 1 diabetes after solid organ transplant (e.g., kidney, lung, or heart) that required use of an immunosuppressive regimen similar to the one used in the current study. Taken together, these observations support the conclusion that implanted PEC-01s were the source of meal-responsive C-peptide and limit the likelihood that the endogenous pancreas or another endogenous tissue contributed.

C-peptide levels were highly variable between participants, and we probed the etiology of this heterogeneity by assessing whether there was an association between the implanted cell volume and C-peptide. Limited by small sample sizes, we did not detect any effects, thus suggesting that cell number did not substantially impact C-peptide levels. Given the capability to study many explanted grafts, we determined whether features of the explanted cell population had predictive value for variable C-peptide levels. Interestingly, we detected a correlation between the proportion of cells in the pancreatic lineage (by pathologist review; patients 02 to 10) and the area under the curve of C-peptide levels at the week 26 mixed meal tolerance test, suggesting that patients with a greater proportion of endocrine cells developed higher levels of meal-stimulated C-peptide. In contrast, there was no association between the ratio of insulin:glucagon cells and patient C-peptide, nor an association between the proportion of MAFA immunoreactive insulin-producing cells and patient C-peptide. These observations are limited by acknowledgment that reviewing only up to three tissue sections from a sentinel device characterizes a tiny fraction of the implanted cell population in each patient. Nonetheless, it is noted that for the subset of patients with multiple explanted devices (02, 03, and 04), there was a tendency to have similar low cellularity in all devices. We hypothesize that unknown host environment factors or cell preparation factors favor the endocrine fate in implanted cells, and further exploration of potential drivers including patient thyroid status (Bruin et al., 2016; Rezania et al., 2014) could be insightful for optimization of the study protocol.

Despite substantial unexplained heterogeneity, patients displayed meal-responsive C-peptide secretion at 26 and 52 weeks post-implantation. This contrasts with findings of only weak interactive effects of week and meal on C-peptide during simplified oral meal challenge tests. Interestingly, blood glucose rose in the 30–60 min post-meal, but peak C-peptide was not observed until 90–180 min post-meal, potentially due to progressive accumulation of secreted C-peptide by a small, engrafted cell mass and/or a weak or delayed response of C-peptide production by graft cells compared to the normal rapid first-phase insulin secretion dynamics of endogenous β cells. Though islet transplant recipients have dramatically blunted insulin secretion dynamics to intravenous glucose, their more impressive response to oral mixed meals suggests that incretin signaling on islets adjacent to the portal circulation may contribute to normal insulin secretion (Rickels et al., 2005), a factor that may be reduced when PECs are implanted subcutaneously. Furthermore, studies on encapsulated islets support the logical conclusion that diffusion of nutrients across large distances delays insulin secretion dynamics in response to glucose (Korsgren, 2017). Notably, over time, the vascularizing devices should avoid this diffusion delay as blood vessels penetrate the devices. Alternatively, it is possible that slow maturation of the PEC-01s contributed to the delayed development of rapid glucose-responsive C-peptide until 39 weeks after implantation. However, this interpretation is unlikely given preclinical results of meal-responsive C-peptide within 10–14 weeks of implantation (Kroon et al., 2008).

Critically, we provide evidence for the presence of surviving PEC-01-derived endocrine cells by immunostaining explanted grafts for markers of pancreatic lineage, endocrine cells, and mature β cells, indicating that it is possible for PEC-01 derivatives to survive for up to 55 weeks post-implantation. Pre-implantation, only a small subset of PEC-01s were immunoreactive for insulin, glucagon, and/or somatostatin, but most had critical endocrine cell precursor transcription factors NKX6.1 and PDX1 (Rezania et al., 2013), as well as NKX2.2, which is known to act downstream of the endocrine lineage specifier neurogenin-3 (Rukstalis and Habener, 2009). The explanted grafts contained a majority of endocrine cells by synaptophysin immunoreactivity, and a subset of these cells were surviving insulin-immunoreactive cells. Insulin-immunoreactive cells had a mature β cell phenotype with nuclear immunoreactivity for the prototypical mature β cell marker MAFA (Nishimura et al., 2015; Wang et al., 2007), albeit at rates lower than adult human pancreas, as well as other critical β cell proteins IAPP, NKX2.2, NKX6.1, and PDX1 (Vieira et al., 2017). Without a lineage trace in these cells, it is not possible to determine whether PEC-01 cells with insulin immunoreactivity at the time of implantation are the same cells with insulin immunoreactivity at the time of explantation. Overall, the presence of insulin-immunoreactive cells with a mature β cell appearance in retrieved grafts lends support for the conclusion that PEC-01s can differentiate into mature β cells. The survival of PEC-01-derived cells with a mature β cell phenotype in conjunction with observations of post-explant loss of meal-responsive C-peptide together strengthen the conclusion that implanted PEC-01s are the source of long-term meal-responsive C-peptide in patients.

Of importance to assessing potential optimizations of future studies, grafts were heterogeneous with an insulin:glucagon ratio ranging from 0.06 to 0.46, suggesting that PECs can favor the α cell fate and/or survival over the β cell fate and/or survival in humans compared to preclinical studies in mice (Kroon et al., 2008; Rezania et al., 2012). Early endocrine fate, as evidenced by high rates of synaptophysin immunoreactivity in pre-implanted devices, may have favored the α cell fate (Rezania et al., 2011; Veres et al., 2019). We have previously observed that PECs derived using different protocols can also be biased to an α cell fate over a β cell fate when implanted in mice compared to rats (Bruin et al., 2015; Rezania et al., 2011) and further in mice with hypothyroidism (Bruin et al., 2016). Given the known dysregulated production of glucagon in patients with diabetes, including a lack of glucose suppression of glucagon secretion (Greenbaum et al., 2002; Hare et al., 2010), excess differentiation

of PEC-01s into glucagon-producing cells may impede the ability of PEC-01s to regulate patient glycemia. Additionally, though a subset of explanted devices had rates of MAFA immunoreactivity in β cells similar to rates in normal adult human pancreas, many explanted grafts had lower rates of MAFA immunoreactivity, which suggests that insulin-producing cells were not fully mature β cells. Further studies aimed at optimizing the PEC product and/or host factors that impact endocrine cell fate of implanted PECs are needed to ensure that a greater proportion of PECs differentiate into mature MAFA-immunoreactive insulin-producing β cells.

Patients with diabetes report substantial improvements in quality of life and reduced rates of diabetic complications after islet transplantation (Foster et al., 2019). For the first time, we provide evidence that stem-cell-derived PEC-01s can mature into glucose-responsive, insulin-producing mature β cells *in vivo* in patients with type 1 diabetes. Patients developed meal-responsive C-peptide post-implantation with PEC-01s, and explanted grafts contained insulin-immunoreactive cells with a mature β cell phenotype. In this open-label phase 1/2 trial, patients tolerated the cell therapy without serious adverse events likely attributable to the cell product and had reduced insulin requirements, improved glucose control, and improved patient hypoglycemic awareness. These early findings support future investment and investigation into optimizing cell therapies for diabetes.

Limitations of the study

The present work reports on an early open-label phase 1/2 clinical trial without a control group. Given that interventions are not controlled or blinded, we are unable to decisively determine the causative role of independent study components on patient outcomes. This includes a limited ability to determine whether improved clinical outcomes are due to secretory products of the stem cell graft or attributable to enrollment in an intensive clinical trial with regular follow-up. Beyond clinical outcomes, another limitation is the extent of characterization and investigation of the stem cell grafts. As all grafts were formalin fixed per the clinical trial sponsor's registered protocol, we present data from immunostained sections within the graft that represent one to three regions of the graft. Given that there is expected substantial heterogeneity both within a single device and between the multiple devices implanted within each patient, we are unable to assess the total cell mass within the grafts. Incomplete characterization of a heterogeneous graft limits our ability to comment decisively on any potential relationships between engrafted mass and circulating C-peptide levels. Further, quoted from the study protocol, "Histological results of explanted units and any associated tissue capsule as evaluated for cell viability, vascularization, immune response, and/or cell maturation and differentiation" was registered as an exploratory endpoint. Finally, outcomes are highly heterogeneous, and despite presenting data from all patients at the largest clinical trial site of the largest trial investigating a stem cell therapy for diabetes, sample size limits our power to detect potential moderating variables.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stem.2021.10.003.

ACKNOWLEDGMENTS

The authors express their gratitude to the patient volunteers for their immeasurable contributions by volunteering to partake in the study. Our appreciation to Barbara Allan, Travis Webber, VGH nursing, and the VGH clinical research unit team for their time and assistance. A.R. gratefully acknowledges studentship support from the CIHR (Vanier Canada Graduate Scholarship) and Vancouver Coastal Health (CIHR-UBC MD/PhD Studentship). K.A.W.H. is supported by fellowships from CIHR, the Canadian Society of Gastroenterology, and JDRF. L.C. was supported by fellowships from BC Children's Hospital Research Institute and JDRF. M.K.L. receives a salary award from the BC Children's Hospital Research Institute. This research was supported by funding from the Stem Cell Network, JDRF, Canadian Institutes of Health Research, ViaCyte, Inc., and California Institute for Regenerative Medicine.

AUTHOR CONTRIBUTIONS

A.R. prepared the manuscript with contributions from D.M.T., K.A.W.H., P.T.W.K., G.L.W., M.K.L., and T.J.K. All authors contributed to study design, sample or data collection, and/or data analysis or interpretation. A.R., P.T.W.K., and G.L.W. performed surgical procedures. All authors approved the final version of the manuscript.

DECLARATION OF INTERESTS

T.J.K. has received research funding from CRISPR Therapeutics, funding and research contracts from Aspect Biosystems, consulting fees from Sigilon Therapeutics, and research support from ViaCyte, Inc. and has filed patent applications related to the subject of this manuscript. M.K.L. has received research funding from CRISPR Therapeutics. Subsequent to the completion and submission of this manuscript, T.J.K. became an employee of ViaCyte, Inc.

Received: December 3, 2020
Revised: April 29, 2021
Accepted: October 8, 2021
Published: December 2, 2021

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CCR4 PE/Dazzle594	BioLegend	RRID: 2564095
CCR6 PECy7	Beckman Coulter	RRID: 2892544
CD127 APC-700	Beckman Coulter	RRID: 2889979
CD134 APC	Beckman Coulter	RRID: 28922545
CD14 FITC	Beckman Coulter	RRID: 314186
CD19 APC	Beckman Coulter	RRID: 314242
CD25 PE	Beckman Coulter	RRID: 2892546
CD3 Krome Orange	Beckman Coulter	RRID: 2892547
CD3 PE	Beckman Coulter	RRID: 10640418
CD39 PECy5.5	Beckman Coulter	RRID: 2892548
CD4 Pacific Blue	Beckman Coulter	RRID: 2892549
CD45 Krome Orange	Beckman Coulter	RRID: 2888654
CXCR3 FITC	Beckman Coulter	RRID: 2892550
CCR4 PE/Dazzle594	BioLegend	RRID: 2564095
Cytokeratin-19	Agilent Technologies	RRID: 2234418
Glucagon	Sigma	RRID: 259852
Homeodomain transcription factor 6.1 (NKX6.1)	Betalogics (Johnson and Johnson), produced by Lifespan Biosciences	RRID: 2716793
Homeodomain transcription factor 2.2 (NKX2.2)	Developmental Studies Hybridoma Bank (DSHB)	RRID: 531794
IL-2 PE	BD Biosciences	RRID: 400426
Insulin	Thermo Fisher Scientific	RRID: 794668
Insulin	Cell Signaling	RRID: 2126503
Insulin	Cell Signaling	RRID: 10949314
Islet amyloid polypeptide	AbCam	RRID: 297677
Ki-67 (for immunohistofluorescence)	Abcam	RRID: 443209
Ki-67 PE/Dazzle594	BioLegend	RRID: 2566228
Pancreatic and duodenal homeobox 1 (PDX1)	Abcam	RRID: 777178
Proliferating cell nuclear antigen (PCNA)	Abcam	RRID: 444313
Somatostatin	Beta Cell Biology Consortium (BCBC)	RRID: 10014609
Synaptophysin	Novus Biologicals	RRID: 792140
TNF AF700	ThermoFisher Scientific	RRID: 10671335
Trypsin	R & D Systems	RRID: 884531
V-maf muscoloapo-neurotic fibrosarcoma oncogene homolog A (MAFA)	Betalogics (Johnson & Johnson), produced by Lifespan Biosciences	RRID: 2665528
Biological samples		
Human pancreas biopsies	University of Alberta IsletCore	https://www.ualberta.ca/alberta-diabetes/core-services/isletcore.html
Human pancreas biopsies	Ike Barber Human Islet Laboratory	http://surgery.med.ubc.ca/research/labs/ike-barber-human-islet-transplant-laboratory/
Chemicals, peptides, and recombinant proteins		
Recombinant human proinsulin	Dr. Jing Jin, Northwestern University, Chicago	N/A

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Staphylococcal enterotoxin B (SEB) from *Staphylococcus aureus*	Sigma-Aldrich	S4881
Critical commercial assays		
Simoa© C-peptide Advantage Kit (HD-1/HD-X)	Quanterix	100199
Glucose Oxidase assay	Sekisui Diagnostics	DCL 235-60
Fixable Viability Dye Aqua	ThermoFisher	L34957
Software and algorithms		
MetaXpress® High-Content Image Acquisition & Analysis Software	Molecular Devices Corporation	https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metaxpress
Odyssey Infrared Imaging System Application Software Version 3.0.16	LI-COR Biosciences	https://www.licor.com/
GraphPad Prism Version 8	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
R (Version 4.0.3)	R	https://www.r-project.org/
R Studio (Version 1.3.1093)	RStudio	https://rstudio.com/
Glooko	Glooko Inc	https://www.glooko.com/
Other		
Antibody Diluent	Agilent Technologies	S0809
Fetal Calf Serum (FCS)	ThermoFisher Scientific	12483020
Flow-Count Fluorospheres	Beckman Coulter	7547053
Flow-Check Pro Fluorospheres	Beckman Coulter	A63493
Flow-Set Pro Fluorospheres	Beckman Coulter	A63492
Iscove's Modified Dulbecco's Medium (IMDM)	ThermoFisher Scientific	12440053
IOTest 3 Fixative Solution	Beckman Coulter	A07800
IsoFlow Sheath Fluid	Beckman Coulter	8546859
OptiLyse C Lysing Solution	Beckman Coulter	A11895
Phosphate Buffered Saline (PBS)	ThermoFisher Scientific	14190-144
PerFix-nc kit	Beckman Coulter	B31168
Protein block, serum free	Agilent Technologies	X0909
VersaLyse Lysing Solution	Beckman Coulter	A09777
DURAClone IM T Cell Subsets Tube	Beckman Coulter	B53328
DURAClone IM Treg Tube	Beckman Coulter	B53346
DURAClone IF T Helper Cell Tube	Beckman Coulter	C04666
Clinicaltrials.gov identifier	NIH	NCT03163511

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be sent to the lead contact, Timothy J. Kieffer (tim.kieffer@ubc.ca).

Materials availability

Reagents utilized in this study that are not commercially sold will be made available by the lead contact and shared upon reasonable request (consideration will be given to limited quantity and availability of suitable alternatives).

Data and code availability

Individual deidentified participant data that underlie the results reported in this article alongside the detailed study protocol will be made available indefinitely to researchers who provide a methodologically sound proposal to the clinical trial sponsor 18 months after trial completion, currently scheduled for March 2023. Composite data will be released by the sponsor to clinicaltrials.gov within 12 months of study completion.

METHOD DETAILS

We present results from one of seven sites (Vancouver, Canada) of a non-randomized, open-label, phase 1/2, multi-center trial that began enrollment in July 2017 and with ongoing recruitment at the time of this manuscript preparation. Eligible patients were non-pregnant adults (18 to 65 years old) with a $\geq$ five-year history of type 1 diabetes, one to five severe hypoglycemic episodes in the year prior to enrollment, ongoing insulin therapy without oral antihyperglycemic medications, no severe chronic neurovascular complications of diabetes, stable diabetic treatment for at least three months, and hypoglycemia unawareness or significant glycemic lability (full eligibility criteria in online supplemental file). Pre-implantation, patients were screened for humoral alloreactivity by human leukocyte antigen panel-reactive antibody. Pre-enrollment insulin therapy was not part of the eligibility criteria, and thus some patients were using insulin pumps (n = 11) while others used multiple daily insulin injections (n = 4). Patients were directed to continue comparable therapy and were required to use continuous glucose monitoring throughout the planned two-year follow-up period. Here, we present data from the first year of post-implantation monitoring for an uninterrupted series of 15 patients (patients 01 to 15; three additional patients screened did not meet eligibility criteria; to enable cross-referencing with Shapiro et al., we note the overlapping patient IDs in Table 1). Patient demographics and baseline bloodwork are summarized in Table 1. Informed consent was obtained from all subjects. The studies were approved by the University of British Columbia Clinical Research Ethics Board.

Procedures and sample collection

All patients were enrolled by invitation and underwent baseline screening two to four weeks prior to implantation to ensure they met inclusion criteria. After confirmation of eligibility, patients began induction immunotherapy with anti-thymocyte globulin (ATG; total dose 6 mg/kg) prior to implantation and maintenance immunotherapy included tacrolimus and mycophenolate mofetil (full immunotherapy regimen summarized in Methods S3). To assess the impact of steroids on graft survival and function, two patients were trialed on high dose oral prednisone (08 and 10). Patients received a total of two to four PEC-01-containing devices (VC-02-300) and six smaller PEC-01-containing sentinel devices (VC-02-20; Methods S4). Sentinel devices are approximately 1 cm × 1.5 cm × 1 mm and contained 6 – 8 million cells and larger devices are approximately 3 cm × 9 cm × 1 mm and contained 90 – 120 million cells (diagrams shown in Shapiro et al., 2021). Macroencapsulation devices were a multilayer structure with a semipermeable polytetrafluoroethylene membrane that had vascularizing portals and an external polyester mesh to give the device structure (Faleo et al., 2016). PEC-01s were generated at ViaCyte as using protocols similar to those previously described (Schulz, 2015; Schulz et al., 2012) (Methods S7) including thawing lots of cells known to meet release criteria and loading cells suspended in enriched media into devices via access ports before overnight shipping to Vancouver the day prior to implant in temperature controlled packaging. Quality control release criteria included mycoplasma and endotoxin testing, cell composition assessment by flow cytometry, pluripotency assessment (OCT4 immunocytochemistry), a gram stain and sterility assessment by approved laboratories during formulation, device filling, and upon final production of finished products. Implanted devices had portals engineered to enable direct neovascularization into the device. On the day of implantation, patients were placed under general anesthesia, and subcutaneous pockets were dissected superficial to the deep fascia overlying the volar forearm muscles, and the external obliques on the lateral abdominal wall through up to four incisions on each site (maximum six total incisions per patient). Combined cell products were washed in phosphate buffered saline and then slotted into the dissected space using ribbon retractors or curved forceps.

Patients had regular follow-up including physical exams, blood collection, C-peptide analysis, immunological characterization, and ultrasound assessment of the grafts to assess for signs of cellular expansion (any increase in graft thickness to greater than 1.5 mm; Methods S5). For visits with serum sample collection, patients fasted for eight hours and withheld short-acting insulin for at least four hours prior to the appointment. At designated visits, patients underwent simplified oral meal challenge tests during which serum was collected fasted and 30-60 min after consumption of a mixed meal and at other visits patients underwent 4-hour mixed meal tolerance tests during which serum samples were collected fasted and at 30, 60, 90, 120, 180, and 240 min post-meal (6 mL Boost High Protein®/kg body weight, max 360 mL. Macronutrients per 100 mL: 13.5 g carbohydrates (of which 6.3 g was sugar), 6.3 g protein, and 2.5 g fat, total 101 kcal). During 4-hour mixed meal tests patients used basal insulin as usual but continued to withhold short acting insulin for the duration of the test. Samples for immune cell characterization were also collected fasted at pre-implantation baseline and weeks 4, 16, 26, and 52 after implantation. Patients had planned surgical removal of sentinel devices and occasional larger devices (Methods S5).

Primary and secondary endpoints

The registered primary endpoint of this clinical trial for the patients presented here was change in C-peptide levels from baseline to the week 26 MMTT. Secondary endpoints were implant tolerability, all reported adverse events, the incidence of immune sensitization (not reported), rate of premature explant, change to insulin requirements, percent of subjects who achieved a >50% reduction in insulin requirements, rates of insulin independence, and time in target glucose range. All other data including histological study of explanted units were not registered endpoints and should thus be considered exploratory in nature.

Serum assays

Serum samples were analyzed for C-peptide using digital ELISA technology (single molecule array - SIMOA; Quanterix; Billerica, MA). SIMOA enabled sensitive C-peptide detection (sensitivity 0.017 pM) with inter-assay variability of 14.2% and had no cross-reactivity

to human insulin, Lantus®, Novolog®, nor Humalog® (< 0.00001%) and minimal cross-reactivity to human proinsulin (0.39%). The same samples were analyzed for glucose levels by glucose oxidase assay (Sekisui Diagnostics, Charlottetown, Canada). Hemoglobin A1c (HbA1c) were analyzed by ACM laboratories (Rochester, NY).

Immunological characterization

Unfractionated blood was collected at weeks −2, 4, 16, 26, and 52, and stained using the indicated DURAClone tubes (Beckman Coulter; Brea, CA; Methods S6) according to the manufacturers protocol. Panels focused on analysis of T cell subsets (IM T Cell Subsets tube, Beckman Coulter; B53328), regulatory T cells (IM Treg tube, Beckman Coulter; B53346) with added CD127 APC-700 (Beckman Coulter; A7116) and Ki-67 PE/Dazzle594 (BioLegend, 350533; San Diego, CA)), and cytokine production (IF T Helper Cell tube, Beckman Coulter; C04666) with added IL2 PE (BD Biosciences, 340450; Mississauga, Canada), TNF AF700 (ThermoFisher, 56-7349-42; Waltham, MA), Ki67 PE/Dazzle594 (BioLegend) antibodies and Fixable Viability Dye Aqua (ThermoFisher, L34957) (Methods S6). Cell counts were performed using flow-count fluorospheres according to the manufacturers' instructions with the addition of CD45-Krome Orange, CD3-PE, CD19-APC and CD14-FITC mAbs and red cell lysis with VersaLyse (Beckman Coulter) and IOTest 3 Fixative (Beckman Coulter). The activation-induced marker assay was performed by stimulating whole blood with 100 μg/mL recombinant human proinsulin (generously provided by Dr. Jing Jin, Northwestern University, Chicago, IL) and compared to negative controls (unstimulated blood) and positive controls (1 μg/mL Staphylococcal enterotoxin B (Sigma-Aldrich)). After 44-48 h, staining was performed with the mAb panel in Methods S6, red blood cells lysed with OptiLyse C (Beckman Coulter) and data acquired on a Navios flow cytometer (Beckman Coulter). $OX40^{+}CD25^{+}$ $CD4^{+}$ T cells were defined as antigen-specific (Zaunders et al., 2009) and assay cut offs and data analysis were performed as previously described (Cook et al., 2021). All flow cytometry data were acquired on a Navios flow cytometer (Beckman Coulter).

Histology

Tissue samples were fixed in 10% formalin before immunohistofluorescence as previously described (Asadi et al., 2015). Slides stained by standard hematoxylin and eosin by ViaCyte, Inc. were sent for review by a board certified pathologist actively practicing in Vancouver, Canada. All immunohistofluorescence images were captured and analyzed with an ImageXpress® Micro XLS System (Molecular Devices Corporation; Sunnyvale, CA) including cell scoring using a multiwavelength cell scoring journal to determine the proportion of cells with immunoreactivity for targets of interest including the proportion of cells ± insulin immunoreactivity with immunoreactivity for β cell expressed transcription factors. Primary antibodies used are listed in the STAR methods table. Human pancreas tissue biopsies from patients without diabetes were provided by the Islet Core (University of Alberta; Edmonton, Canada).

QUANTIFICATION AND STATISTICAL ANALYSIS

Multilevel statistical analyses were carried out in R version 3.5.3 (R Core Team, 2021) using statistical packages Tidyverse, Psych, lme4, and lmertest. Body weight, Clarke Hypoglycemia Awareness score, HbA1c, average daily insulin needs, continuous glucose monitoring time in low, target, or high blood glucose, and fasting blood glucose were analyzed using multilevel regression models with time as the predictor. Fasted serum C-peptide data were analyzed using a multilevel regression model with C-peptide as the outcome variable, time as the predictor, and group mean centered blood glucose as a predictor. The intercept was allowed to be random and the effect of time on the outcome was modeled as fixed.

Simplified oral meal challenge test and 4-hour mixed meal tolerance test data had dependency with multiple time points for each individual; thus, we conducted multilevel regression analyses. In the simplified oral meal challenge test model, serum C-peptide was the outcome variable and the predictors we included were pre versus post meal (pre = 0, post = 1), week relative to implantation, group mean centered blood glucose, and an interactive term for pre/post-meal × week. We allowed a random intercept and given a substantial random effect of meal this was also included in our final model. We assessed meal responsiveness at each time point using Sidak's multiple comparisons test. In supplementary models, we assessed the interaction of week and blood glucose on C-peptide by including group mean centered blood glucose and an interactive term of week × blood glucose in the model. In the 4-hour mixed meal tolerance test model, C-peptide was the outcome and the predictors included were the hour relative to meal (0 to 4 hour), week relative to implantation, group mean centered blood glucose, and an interactive term for hour × week. In supplementary models, we dummy-coded week −2 [1 0 0] and week 52 [0 0 1] and included interactive terms for Week −2 × hour and Week 52 × hour. We allowed a random intercept and did not include random slopes for hour, week, or week × hour. B coefficients of multilevel models can be interpreted as representing the slope of the association.

Immunological data and C-peptide stratified group comparisons were analyzed using mixed effects models with time as the predictor, and post-implantation time points were compared to baseline using Dunnett's test (Graphpad Prism v8.2.0; San Diego, CA).

Role of the funding source

ViaCyte, Inc. prepared and delivered all cell-containing devices to sites in Vancouver, BC at which point the authors, with funding from the Stem Cell Network, JDRF, and the Canadian Institutes of Health Research, oversaw all monitoring, implantation, sample collection, assays, and data analysis. This manuscript was prepared independently by the authors and made available to the clinical

trial sponsor before submission for publication. The corresponding author had complete access to all data and analyses presented and takes responsibility for the integrity of the work presented.

ADDITIONAL RESOURCES

This study was preregistered and can be reviewed at clinicaltrials.gov, NCT03163511.