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National Institutes of Health–Sponsored Clinical Islet Transplantation Consortium Phase 3 Trial: Manufacture of a Complex Cellular Product at Eight Processing Facilities

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ISLET STUDIES

Eight manufacturing facilities participating in the National Institutes of Health–sponsored Clinical Islet Transplantation (CIT) Consortium jointly developed and implemented a harmonized process for the manufacture of allogeneic purified human pancreatic islet (PHPI) product evaluated in a phase 3 trial in subjects with type 1 diabetes. Manufacturing was controlled by a common master production batch record, standard operating procedures that included acceptance criteria for deceased donor organ pancreata and critical raw materials, PHPI product specifications, certificate of analysis, and test methods. The process was compliant with Current Good Manufacturing Practices and Current Good Tissue Practices. This report describes the manufacturing process for 75 PHPI clinical

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See accompanying article, p. 3243.

lots and summarizes the results, including lot release. The results demonstrate the feasibility of implementing a harmonized process at multiple facilities for the manufacture of a complex cellular product. The quality systems and regulatory and operational strategies developed by the CIT Consortium yielded product lots that met the prespecified characteristics of safety, purity, potency, and identity and were successfully transplanted into 48 subjects. No adverse events attributable to the product and no cases of primary nonfunction were observed.

Previous clinical trials assessing treatments for unstable type 1 diabetes have demonstrated restoration of pancreatic β-cell function by allogeneic islet transplantation (1–4). In 2000, the University of Alberta reported that transplantation of allogeneic purified islet products resulted in sustained C-peptide levels, improved glycemic control, and prevention of hypoglycemia in seven subjects with type 1 diabetes (5). The results were later confirmed by Brennan et al. (6) in a multicenter trial reporting the 12-year follow-up of seven subjects. One subject experienced graft failure 10.9 years post–islet transplantation. The remaining six continued to have sustained C-peptide levels and improved glycemic control. No episodes of severe hypoglycemia, opportunistic infections, or lymphomas were reported. Although significant progress has been made since the initial trials, development pathways for cellular therapy have required convergence toward regulatory guidelines on clinical trial design. Manufacturing differences at the various processing facilities prevented comparison of clinical results across centers, making it difficult to derive overall conclusions and limiting islet transplantation use in medical practice. Factors affecting the quality of islet products, including organ donor acceptance criteria (7,8), organ recovery techniques, cold ischemia time (9–11), organ preservation and transport solutions (12,13), and methods for islet isolation (14), purification, and culture, have been previously reported (15–17).

Protocol CIT-07 was a prospective, multicenter, single-arm, pivotal phase 3 trial conducted at eight academic institutions in North America (Clinical trial reg. no. NCT00434811, www.clinicaltrials.gov) (18). The trial evaluated the safety, tolerability, and efficacy of the purified human pancreatic islet (PHPI) product in a defined population of subjects with type 1 diabetes and supported product licensure in the U.S. The trial was conducted under a Division of Allergy, Immunology and Transplantation/National Institute of Allergy and Infectious Diseases (DAIT/NIAID)–sponsored U.S. Investigational New Drug application and a Clinical Trial Application in Canada. Manufacturing information was submitted to a DAIT/NIAID-sponsored U.S. Drug Master File. A chemistry, manufacturing, and controls monitoring committee (CMC MC), with representatives from each manufacturing facility and DAIT/NIAID, was responsible for the harmonization of manufacturing processes, including in-process controls, qualification of manufacturing facilities, definition of product specifications, and quality oversight.

This article describes the manufacturing process and the in-process and lot release test results for the 75 clinical lots transplanted into 48 subjects enrolled in the trial. Each product was manufactured and transplanted at the same institution. We report the results of the first successful standardization of manufacturing processes for the allogeneic PHPI product executed at multiple facilities for transplantation to support a pivotal trial. The Clinical Islet Transplantation (CIT) standard operating procedures (SOPs) and the master production batch record (MPBR) developed for product manufacturing are publically available (19–40). The clinical outcomes are reported elsewhere (18).

RESEARCH DESIGN AND METHODS

Participation in the National Institutes of Health (NIH)-sponsored CIT Consortium required a demonstrated record of robust clinical and islet product manufacturing success (Supplementary Data). The CMC MC developed the common MPBR (23), product specifications (32), and interim and final certificates of analysis (COAs) (30,31) and agreed on the deceased donor organ acceptance criteria (Table 1). Each manufacturing facility was responsible for its own quality control, quality assurance, and compliance with Current Good Manufacturing Practices (cGMP) and Current Good Tissue Practices. The CMC MC provided further quality oversight by qualifying the manufacturing facilities for study participation and conducting batch record reviews. Assays used to determine product viability (fluorescein diacetate/propidium iodide [FDA/PI]) (33,34,37), identity/quantity (dithizone [DTZ]) (35), and biological potency (glucose stimulated insulin release [GSIR] by ELISA) (41) were qualified at each facility before that facility's participation in the trial. Manufacturers were required to meet the predefined assay acceptance criteria for accuracy, precision, selectivity, sensitivity, and reproducibility of each assay. All operations were conducted using aseptic processing in accredited cGMP facilities (Supplementary Data).

Pancreas Processing

After arrival of the donor pancreas at the manufacturing facility, acceptance criteria were verified (Table 1), and a sample of the preservation solution was taken for sterility testing. Excess pancreas tissue was removed, and the pancreas was weighed (initial weight). The pancreas was then decontaminated by placing it in Hanks' balanced salt solution containing 1 g/L cefazolin or 10% povidone iodine solution.

Perfusion and Distension

The pancreas was divided at the neck to separate the head from the body and tail. A cannula was placed in the main pancreatic duct of each section. On the basis of the initial pancreas weight, the volume of the CIT Enzyme Solution

Table 1—Pancreas donor qualifications

Requirement	Yes	No	NA
A qualified donor must have yes responses to all the inclusion criteria (A), and no responses to all the exclusion criteria (B and C).			
Container label must specify human pancreas, and an UNOS or a DDD number must be present.			
The organ procurement organization must be identified.			
A. Inclusion criteria (The donor or pancreas must meet these criteria.)			
1. Pancreas preservation in (i) UW, (ii) PFC/UW, (iii) HTK, or (iv) PFC/HTK solutions			
2. Maximum 12-h cold ischemia time			
3. Donor age 15–65 years			
4. Cause and circumstances of death acceptable to the transplant team			
B. Exclusion criteria (Is there evidence of the following conditions?)			
1. History or biochemical evidence of type 1 or 2 diabetes (Transplant teams may consider donor HbA_{1c} >6.1% in the absence of transfusions in the week before death as an indication for exclusion, with discretion for donors who have received transfusions.)			
2. Pancreas from non—heart-beating cardiac death donors			
3. Malignancies, other than resected basal squamous cell carcinoma or intracranial tumor as the cause of death			
4. Suspected or confirmed sepsis			
5. Evidence of clinical or active viral hepatitis (A, B [HBcAg], C). HBsAb positive is acceptable if there is a history of vaccination.			
6. AIDS			
7. HIV seropositivity (HIV-I or HIV-II) or HIV status unknown			
8. HTLV-I or HTLV-II (optional)			
9. Syphilis (RPR or VDRL positive)			
10. Active viral encephalitis or encephalitis of unknown origin			
11. TSE or Creutzfeldt-Jakob disease			
12. Suspected rabies diagnosis			
13. Treated or active tuberculosis			
14. Individuals who have received pituitary human growth hormone			
15. Any medical condition that, in the opinion of the transplant team, precludes a reasonable possibility of a favorable outcome of the islet transplant procedure			
16. Clinical history and/or laboratory testing suggestive of West Nile virus, vaccinia, or SARS			
C. Exclusion criteria—behavioral profiles (Is there evidence of the following conditions?)			
17. High-risk sexual behavior within 5 years before time of death: men who have had sex with men, individuals who have engaged in prostitution, and individuals whose sexual partners have engaged in high-risk sexual behavior			
18. Nonmedical intravenous, intramuscular, or subcutaneous drug use within the past 5 years			
19. Persons with hemophilia or related clotting disorders who have received human-derived clotting factor concentrates			
20. Findings on history or physical examination consistent with an increased risk of HIV exposure			
21. Current inmates of correctional systems and individuals who have been incarcerated for >72 consecutive hours during the previous 12 months			

DDD, donor-derived disease; HBcAg, hepatitis B core antigen; HBsAb, hepatitis B surface antibody; HTK, histidine-tryptophan-ketoglutarate; HTLV, human T-cell lymphoma; NA, not applicable; PFC, perfluorocarbon; RPR, rapid plasma reagin; SARS, severe acute respiratory syndrome; TSE, transmissible spongiform encephalopathy; UNOS, United Network for Organ Sharing; UW, University of Wisconsin; VDRL, venereal disease research laboratory.

(28,29,36) was determined. The pancreas was perfused with one of three enzyme blend solutions containing a collagenase and a protease (Table 2). Each enzyme lot was qualified by three CIT manufacturers by assessing enzyme activity before its introduction into product manufacture (23). Each pancreas segment was distended intraductally by perfusion with cold CIT Enzyme Solution (28,29,36) either manually or by controlled perfusion (42–44). Perfusion occurred for 4–10 min at 60–80 mmHg followed by 4–6 min at 160–180 mmHg at 4–14°C. Final trimmed pancreas weight was calculated by subtracting the weight of tissue trimmed during perfusion from the initial pancreas weight. The perfused pancreas was cut into 5–15 pieces and placed in a Ricordi digestion chamber (Biorep Technologies, Inc., Miami Lakes, FL).

Digestion Step

Tissue digestion began with recirculation of the Enzyme Solution (phase 1) to dissociate the pancreas progressively followed by a dilution phase (phase 2) in which the enzymes were neutralized by cooling and dilution with fresh RPMI medium. For the recirculation phase, the circuit was primed with CIT Enzyme Solution, and the temperature was set at 32–38°C. The flow rate was set to 210–250 mL/min until the set temperature was reached and then reduced to 90–130 mL/min. Freed islets were eluted in

Table 2—Enzyme combinations used in the manufacture of PHPI product

Activity (units) and manufacturer	*N* = 75	Mean (SD)	Median	Minimum–maximum
Collagenase NB 1 (Wünsch) SERVA Electrophoresis, Heidelberg, Germany	46	2,008 (439)	1,829	1,600–3,842
Neutral Protease NB (DMC units) SERVA Electrophoresis		244 (65)	229	200–581
Cl*zyme* (Wünsch) VitaCyte, Indianapolis, IN	18	2,472 (542)	2,201	1,620–3,740
Neutral Protease NB (DMC units) SERVA Electrophoresis		199 (45)	183	147–270
Liberase Collagenase I/II MTF (Wünsch) Roche Diagnostics, Basel, Switzerland	11	2,678 (302)	2,632	2,426–3,500
Liberase Thermolysin (neutral protease units) Roche Diagnostics		99,552 (55,097)	71,577	51,075–187,275

DMC, dimethylcasein; MTF, mammalian tissue free.

the diluting buffer and collected. Samples were taken throughout digestion and assessed by DTZ staining (35) to determine the time to switch from phase 1 to phase 2 (45). The decision to switch to dilution was based on the number of islets, percent free islets, and percent fragmented islets (23).

During dilution (phase 2), the flow rate was increased back to 210–250 mL/min, and the temperature was reduced to ≤30°C. If a large number of embedded islets was observed in samples of the digested product, the temperature was maintained between 30 and 38°C. Dilution was complete when minimal tissue remained in the chamber and no islets were observed in the DTZ-stained remaining tissue. The eluate containing the islets was pooled, centrifuged, washed with cold CIT Wash Solution (27), and resuspended in cold CIT Purification Solution (22).

Purification Step

Twenty-five milliliters or less of digested tissue were layered over an iodixanol-based continuous density gradient (20), ranging from 1.060 to 1.100 ± 0.01 g/mL, and were centrifuged at 1,800–2,000 rpm in a COBE 2991 cell processor (Terumo BCT, Lakewood, CO) at 4–8°C. Twelve 25-mL gradient fractions were collected in 250 mL conical tubes prefilled with 225 mL CMRL 1066, Supplemented, CIT Modification Solution (Mediatech, Manassas, VA) (38). Islet purity from each fraction was estimated by DTZ staining. Purified fractions were classified as high (≥70%), middle (40–69%), and low (30–39%) purity, and those with the same purity were pooled. If >50,000 islets were present in fractions with purity <30%, supplementary purification was performed using Biocoll (Biochrome AG, Berlin, Germany) (25), polysucrose discontinuous gradients (Mediatech) (24), or OptiPrep (Nycomed/Takeda, Osaka, Japan) (26). Purity of the fractions obtained by supplementary purification was assessed by DTZ staining, and each fraction was combined with fractions of equivalent purity to those obtained by using the iodixanol-based gradient. From this point forward, the high-, medium-, and low-purity fractions were processed separately and combined only after culture and immediately before filling the infusion bags.

Culture Step

Purified islets were cultured for 36–72 h in nontreated T-175 vented flasks (SARSTEDT AG & Co., Nümbrecht, Germany) at 10,000–30,000 islet equivalents (IEQ)/30 mL CIT Culture Media (19). High-purity islet fractions were cultured at 37°C/5% CO_2 for the first 12–24 h and at 22°C/5% CO_2 for the remaining time. The middle- and low-purity fractions were cultured at 22°C/5% CO_2 at all times as previously reported (3,46,47). The media was removed after the first 24 h and fresh media added.

The cultured islet tissue was collected, washed, pooled, and resuspended in 50–250 mL CIT Transplant Media (40) according to islet fraction purity range. The settled tissue volume was estimated by aspirating each pellet from the high-, middle-, or low-purity fractions with a 10-mL glass pipette and allowing the aspirate to sediment by gravity for 5 min. If the combined total settled tissue volume was ≤7.5 mL, all purity fractions were pooled into a single infusion bag. If the total settled tissue volume was >7.5 and ≤15 mL, the tissue was divided into two or three infusion bags, with a maximum tissue volume of 7.5 mL/bag. The pooled tissue for each infusion bag was allowed to sediment, washed, and resuspended in 100 mL CIT Transplant Media (40). The final product was maintained at room temperature and transplanted within 6 h of bag fill. Samples for lot release tests were obtained before filling the infusion bags with the PHPI final product.

Lot Release

Tests assessed product safety, purity, identity, and potency. Because sterility and biological potency results were not available at the time of lot release, lots were released for transplantation by using an interim COA (ICOA) (Supplementary Data) (31). Safety assessments at the time of release included microbial contamination by Gram staining and endotoxin using the limulus amoebocyte lysate Endosafe-PTS or -KTA^2 (Charles River, Charleston, SC). The final COA (30) was issued when the sterility and postculture GSIR test results became available (Table 3). The sterility test was performed compliant with section 610.12 of the

Table 3—Final COA

Test	Method	Requirement
Identity		
Recipient identity	Visual inspection	Recipient study ID and recipient medical record number on this COA and on each infusion bag label identical to that in the production batch record, section 12.3
Islet identity	DTZ stain and microscopic examination	Islets present in each product bag
Volumes in bags		
Suspension volume	Direct measurement	200 mL per product bag ≤600 mL total in three product bags
Settled tissue volume	Direct measurement after 5-min settling	≤7.5 mL per product bag ≤15.0 mL total in three product bags
Potency		
GSIR (high-purity islets, preculture sample)	ELISA	For information only, report stimulation index
GSIR (high-purity islets, postculture sample)	ELISA	Stimulation index >1
Islet quantity	DTZ stain and microscopic examination	First infusion: ≥5.0×10^3 IEQ/kg recipient BW (total IEQ/ infusion) Subsequent infusions: ≥4.0×10^3 IEQ/kg recipient BW (total IEQ/infusion)
Viability	FDA/PI stain and microscopic examination	≥70% in each product bag
Purity		
Islet concentration	DTZ stain and microscopic examination	≥20,000 total IEQ/mL total settled tissue volume
Safety		
Appearance	Visual inspection	Light yellow to amber liquid with visible aggregates in each product bag
Endotoxins	LAL	≤5.0 EU/kg of recipient BW (total EU/infusion)
Sterility	21 CFR 610.12	No growth in each product bag

CFR, Code of Federal Regulations; EU, endotoxin unit; LAL, limulus amoebocyte lysate.

Code of Federal Regulations Title 21. A preemptive plan that included the criteria for reporting product contamination, if a contaminated lot needed to be transplanted for clinical reasons, was agreed on with the Food and Drug Administration.

Purity of the PHPI product was defined, not histologically, but as total IEQ per milliliter of total settled tissue volume. Viability was assessed by staining with FDA/PI (Sigma-Aldrich, St. Louis, MO) and expressed as the percent of viable cells (33,34,37) among 50 consecutive islets. DTZ staining was used for identification of islets, quantification of IEQ, purity determination, and categorization by size ranges (35). Biological potency was determined by the GSIR by ELISA (41), which quantitates the concentration of insulin secreted in vitro by islets in response to stimulation with low (2.8 mmol/L) and high (28 mmol/L) glucose concentrations. The results are expressed as the stimulation index.

Statistical Methods

Recovery is defined as the quantity of IEQ retrieved during the manufacturing process and expressed as IEQ per gram of trimmed pancreas. Process yield refers to the proportion of IEQ recovered relative to the starting IEQ. Continuous variables are displayed as number of observations, mean ± SD, or median (interquartile range [IQR]). Categorical variables are displayed as number and percent. Relationship of donor characteristics to IEQ recovery was analyzed by stepwise regression. Comparisons of variables among enzyme solutions and manufacturing centers were performed using F tests. Statistical significance was defined at $P < 0.05$. SAS for Windows versions 9.3–9.4 software (SAS Institute, Cary, NC) was used for all data management and statistical analyses.

RESULTS

A total of 324 pancreata were processed for use in all CIT clinical protocols. The overall success rate in producing PHPI lots that met product release criteria was 52.5% (170 of 324 total CIT lots), which is consistent with previously reported rates (48–50). Manufacture success among the centers ranged from 24.5 to 89.5%. The main factor associated with manufacturing failure was insufficient number of islets to meet the minimum required product dose. Subjects in Protocol CIT-07 received 75 of the 170 successfully manufactured PHPI lots, and the remainder of this report focuses on these 75 lots. Table 4 shows a side-by-side comparison of the data for each center and for all centers together and displays the

Table 4—Manufacturing success rate and results from 75 PHPI lots by facility and for all facilities

	Center ID								
	1 (*n* = 9)	2 (*n* = 4)	3 (*n* = 17)	4 (*n* = 15)	5 (*n* = 7)	6 (*n* = 12)	7 (*n* = 6)	8 (*n* = 5)	All facilities (*N* = 75)
All CIT isolations*									
No. lots prepared	57	27	44	39	35	58	19	37	324
PHPI lots transplanted	29 (50.9)	12 (44.4)	26 (59.1)	22 (56.4)	16 (45.7)	32 (55.2)	17 (89.5)	9 (24.3)	170 (52.5)
Protocol CIT-07									
Donor characteristic									
Age (years)	46 (9)	50 (6)	39 (19)	37 (22)	49 (27)	43 (21)	37 (7)	53 (26)	42 (19)
Height (cm)	172.0 (5.0)	167.8 (11.6)	182.9 (7.0)	180.0 (12.4)	175.0 (14.9)	175.1 (13.5)	174.4 (9.8)	172.7 (15.3)	177.8 (12.7)
Weight (kg)	85.0 (28.5)	93.0 (28.1)	113.4 (13.7)	90.7 (28.0)	113.0 (54.9)	112.5 (16.6)	113.6 (29.0)	93.0 (32.0)	107.0 (31.0)
BMI (kg/m^2)	27.1 (7.7)	31.9 (5.6)	34.7 (4.6)	28.2 (11.2)	36.0 (14.8)	36.3 (5.2)	38.4 (12.9)	28.6 (13.6)	33.4 (8.2)
Cold ischemic time (h)	6.0 (5.4)	8.5 (4.6)	7.4 (2.5)	6.7 (3.2)	7.9 (2.7)	8.9 (5.4)	7.8 (0.7)	7.8 (0.7)	7.7 (3.4)
Product manufacture									
Final trimmed pancreas weight (g)	95 (37)	93 (46)	109 (28)	103 (40)	101 (63)	102 (64)	112 (31)	124 (19)	106 (40)
Total packed tissue volume (mL)	45 (6)	45 (5)	44 (14)	45 (30)	40 (46)	39 (18)	25 (1)	40 (10)	44 (16)
Postdigestion IPN	865,000 (720,723)	718,000 (404,000)	1,183,000 (585,500)	542,000 (556,000)	421,250 (30,500)	351,000 (145,524)	548,500 (231,000)	853,125 (111,250)	537,500 (528,000)
Postdigestion IEQ	732,250 (128,250)	728,842 (314,642)	1,074,360 (277,000)	693,520 (615,069)	613,534 (447,954)	523,218 (359,927)	787,250 (397,160)	666,068 (93,080)	708,470 (382,000)
Postdigestion index†	1.10 (0.40)	1.00 (0.20)	0.95 (0.15)	1.30 (0.10)	1.30 (0.30)	1.70 (1.40)	1.55 (0.10)	0.70 (0.00)	1.20 (0.60)
Postpurification IPN	451,750 (188,000)	362,000 (177,000)	506,000 (243,500)	368,250 (216,000)	268,750 (188,750)	233,475 (150,900)	350,750 (205,000)	630,000 (80,000)	390,500 (287,750)
Postpurification IEQ	620,000 (258,506)	641,275 (273,010)	672,547 (226,290)	556,181 (280,726)	588,602 (338,136)	503,459 (245,830)	569,038 (438,968)	492,736 (96,845)	582,370 (267,931)
Postpurification index	1.40 (0.50)	1.70 (0.90)	1.30 (0.40)	1.50 (0.50)	1.90 (0.70)	1.95 (0.85)	1.75 (0.70)	0.90 (0.30)	1.50 (0.60)
Postculture IPN	396,000 (186,000)	242,688 (115,188)	265,250 (180,750)	282,000 (145,750)	197,000 (197,095)	219,250 (103,500)	210,500 (81,690)	484,000 (223,510)	276,000 (171,000)
Postculture IEQ	469,400 (120,380)	579,915 (139,065)	637,280 (243,020)	455,098 (235,652)	402,600 (168,601)	422,665 (284,797)	598,874 (168,600)	370,034 (43,550)	490,174 (226,835)
Postculture (index)	1.40 (0.50)	1.55 (0.60)	1.30 (0.90)	1.30 (0.40)	1.30 (0.40)	1.85 (0.90)	1.90 (0.40)	0.80 (0.20)	1.40 (0.70)
Process recovery (IEQ/g trimmed pancreas)									
Postdigestion	8,135 (2,300)	6,531 (3,812)	8,958 (2,876)	6,270 (4,417)	5,708 (2,919)	4,720 (2,042)	7,778 (3,064)	6,422 (1,527)	6,813 (3,672)
Postpurification	5,877 (4,544)	5,715 (3,029)	6,601 (1,883)	5,884 (3,942)	5,442 (1,594)	4,634 (1,614)	6,201 (3,501)	4,371 (1,160)	5,471 (2,709)
Postculture	4,925 (2,730)	5,235 (1,845)	5,749 (1,273)	5,305 (2,614)	4,525 (1,565)	4,064 (662)	5,662 (2,427)	3,319 (1,154)	4,730 (2,156)
Process yield (%)									
Postpurification	73 (24)	84 (6)	77 (19)	81 (25)	106 (42)	92 (31)	84 (11)	79 (13)	82 (22)
Postculture	81 (17)	82 (17)	91 (13)	88 (11)	80 (27)	89 (20)	96 (30)	75 (8)	86 (17)
Overall	61 (25)	71 (13)	68 (17)	71 (20)	73 (25)	85 (39)	79 (7)	60 (12)	71 (22)
Final product release test results									
GSIR index (high-purity islets, preculture)‡	2.1 (0.7)	1.6 (1.7)	3.5 (3.9)	2.3 (1.2)	2.9 (3.7)	2.1 (2.0)	2.6 (2.9)	2.0 (0.2)	2.1 (2.0)
GSIR index (high-purity islets, postculture)	2.6 (0.4)	3.8 (2.7)	2.2 (1.1)	1.6 (1.1)	2.9 (2.4)	1.7 (1.8)	2.2 (1.2)	2.5 (0.7)	2.3 (1.6)
Settled tissue volume (mL)	5.0 (1.5)	3.9 (1.7)	6.0 (4.2)	3.0 (4.0)	2.9 (2.6)	2.5 (1.1)	3.9 (3.4)	2.2 (0.8)	4.0 (3.8)
Total transplant (IEQ)	462,510 (119,531)	573,116 (169,216)	614,578 (190,868)	467,135 (245,378)	396,926 (166,745)	406,982 (281,925)	573,643 (178,843)	366,390 (49,896)	480,500 (231,139)
Islet quantity dose/batch (IEQ/kg recipient BW)	6,535 (1,390)	8,387 (2,943)	7,813 (2,416)	6,561 (3,338)	5,924 (2,267)	6,359 (1,903)	9,035 (1,518)	5,277 (1,892)	6,694 (2,800)
Viability bag #1	97.00 (6.20)	93.10 (7.50)	89.80 (7.70)	93.00 (1.00)	96.24 (2.32)	94.11 (3.48)	100.00 (0.00)	96.60 (4.90)	93.80 (6.34)
Endotoxins (EU/kg recipient BW)	0.07 (0.05)	0.58 (0.38)	0.66 (0.72)	0.12 (0.54)	0.02 (0.06)	1.14 (1.53)	3.41 (1.89)	0.88 (0.20)	0.44 (0.86)
Islet concentration total (IEQ/mL total settled tissue volume)	78,512 (31,467)	155,391 (141,985)	89,580 (105,652)	173,613 (101,180)	134,813 (188,556)	210,443 (178,965)	146,832 (131,407)	218,409 (110,943)	134,813 (131,407)
Gram stain	All negative	All negative	All negative	All negative	All negative	All negative	All negative	All negative	All negative
Sterility									
Negative	7 (77.8)	4 (100)	16 (94.1)	15 (100)	7 (100)	11 (91.7)	4 (66.7)	5 (100)	69 (92)
Positive	2 (22.2)		1 (5.9)			1 (8.3)	2 (33.3)		6 (8)
PHPI calculated microscopic purity (%)	70 (3)	63 (20)	66 (14)	60 (15)	57 (27)	75 (32)	70 (32)	80 (13)	69 (17)

Data are *n* (%) or median (IQR). EU, endotoxin units; IPN, islet particle number. *The total number of lots includes Center 9, which did not manufacture PHPI for Protocol CIT-07. †Postdigestion index: IPN values were available for 41 of 75 lots. ‡GSIR preculture result was for information only and not used for lot release.

rate of successful PHPI preparations. Table 4 also provides the characteristics of the pancreas donors, intermediate and final IEQ recovery, and lot release results. The intermediate product results included those for the postdigestion, postpurification, and postculture manufacturing steps. Values are expressed as median (IQR) as a measure of variability.

Donor Characteristics

Tables 4 and 5 summarize the pancreas donor physical characteristics, cause of death, preservation method, and cold ischemia times. Final recovery of IEQ correlated with donor sex and BMI (P = 0.0002 and 0.0082, respectively). No other donor or pancreas characteristics were significantly related to IEQ recovery after adjustment for donor sex and BMI. Differences in donor BMI among centers led to differences in weight of the trimmed pancreas and total IEQ recovered per pancreas, but pairwise comparisons among centers of IEQ recovery per gram trimmed pancreas revealed no significant differences after adjustment for multiple comparisons.

Pancreas Perfusion

Lots were manufactured by using a collagenase and a protease (Table 2). The mean CIT Enzyme Solution volume used during perfusion was 403 ± 52 mL (range 350–500 mL), and the mean perfusion time was 11 ± 2 min. There was no significant difference among enzyme blends in the final recovery of IEQ per gram of trimmed pancreas.

Digestion and Dilution Phases

The mean digestion time (recirculation, phase 1) was 14 ± 3 min (range 8–23 min). The decision to switch to dilution (phase 2) was based on visual examination of 1–2-mL samples of digested tissue collected from the Ricordi chamber. The following factors were considered: *1*) total amount of digested tissue in the sample, *2*) estimated total number of islets, *3*) percent free islets, and *4*) percent fragmented islets. The mean duration of phase 2 was 34 ± 9 min (range 8–57 min). The median (IQR) packed tissue volume after phase 2 was 44 (16) mL, and contained 708,470 (382,000) IEQ, representing a step recovery of 6,813 (3,672) IEQ/g trimmed pancreas (Table 4).

Table 5—Additional donor and pancreas characteristics

Category	*n* (%)
Sex	
Male	53 (70.7)
Female	22 (29.3)
Cause of death	
Anoxia	4 (5.3)
Cerebrovascular accident	33 (44)
Head trauma	34 (45.3)
Other	4 (5.3)
Cardiac arrest	
No	59 (78.7)
Yes	7 (9.3)
Unknown	9 (12)
Preservation method	
UW	47 (62.7)
PFC/UW	8 (10.7)
HTK	14 (18.7)
PFC/HTK	3 (4)
Not specified	3 (4)

HTK, histidine-tryptophan-ketoglutarate; PFC, perfluorocarbon; UW, University of Wisconsin.

Islet Purification

Islet purification was performed by continuous density gradient centrifugation with a COBE 2991 cell processor. The maximum tissue volume purified in each COBE run was 25 mL. All COBE operations were performed at 2–8°C. Of the 75 lots manufactured and transplanted, 52 (69.3%), 10 (13.3%), 9 (12%), and 4 (5.3%) required two, three, one, and four COBE runs, respectively, for islet purification from the digested tissue. Postpurification islet recovery (total IEQ) was 582,370 (267,931) IEQ, representing 5,471 (2,709) IEQ/g trimmed pancreas. Before culture, fractions were combined based on relative purity (high ≥70%, medium 40–69%, low ≤40%) as determined by DTZ staining (35). The purification step median yield was 82% (22%) of the islets present in the postdigestion intermediate product (Table 4).

Islet Culture

Purified islets were cultured for 36–72 h before transplantation. For the first 12–24 h, high-purity islet fractions were incubated at 37°C/5% CO_2, and middle- and low-purity islet fractions were incubated at 22°C/5% CO_2 as previously described (3,46,47). All fractions were cultured at 22°C/5% CO_2 for the remainder of the culture period. Median total islet count postculture was 490,174 (226,835) IEQ, representing a total islet recovery of 4,730 (2,156) IEQ/g trimmed pancreas. Median islet yield for the culture step was 86% (17%). Median total process yield, calculated from postdigestion to postculture, was 71% (22%) (Table 4).

PHPI Product Characteristics and Lot Release

PHPI final product was released on the basis of the results reported on the ICOA (Supplementary Data) (31). Each PHPI product lot was defined as the purified pancreatic islets isolated during a single purification run from a single deceased donor pancreas and administered to a single recipient. Twenty-two subjects received one dose of PHPI by intraportal infusion, and 25 and 1 received two and three doses, respectively. Additional details of doses for first, second, and third infusions are included in the Supplementary Data. The initial transplant required a dose of ≥5,000 IEQ/kg recipient body weight (BW), and the second and third required a dose of ≥4,000 IEQ/kg recipient BW (Table 6). The median total PHPI dose per subject was 11,972 IEQ/kg (range 5,227–25,553 IEQ/kg), with 42 of 48 subjects achieving clinical success (18).

Table 6—Final product formulation for PHPIs

Ingredient	Quantity
Active ingredient	
Human pancreatic islets	First infusion: $\geq 5.0 \times 10^3$ IEQ /kg recipient BW (total IEQ/infusion) Subsequent infusion: $\geq 4.0 \times 10^3$ IEQ /kg recipient BW (total IEQ/infusion)
Inactive ingredients	
CMRL 1066 transplant media (n = 12) containing HEPES without sodium bicarbonate	q.s. to 200 mL per bag
Albumin human, USP	2.5%

q.s., sufficient quantity; USP, U.S. Pharmacopeia.

Each of the 75 PHPI lots was released for transplantation in 1 (n = 54), 2 (n = 20), or 3 (n = 1) infusion bags. The median settled tissue volume was 4.0 (3.8) mL and contained 6,694 (2,800) IEQ/kg recipient BW at a concentration of 134,813 (131,407) IEQ/mL settled tissue. Median viability was 94% (6%) in the high-purity sample, and the median GSIR stimulation index was 2.3 (1.6) (Table 4). For all 75 PHPI lots, Gram stain was negative, and the endotoxin concentration was $\leq$5.0 endotoxin units/kg recipient BW.

Sterility tests were performed in samples of the preservation solution and the final product (Supplementary Data); 22 of 75 preservation solution samples were contaminated with skin saprophytes and 1 of 75 contained *Candida albicans*. For the final product, the sterility tests of 5 of 75 lots showed skin saprophytes, and 1 lot contained *C. albicans* (for the same lot that reported the presence of *C. albicans* in the preservation solution). Per CIT Consortium and site-specific procedures, in all cases, the site investigators were immediately notified. The subject who received the lot contaminated with *C. albicans* received fluconazole prophylactically. None of the recipients of these six lots exhibited signs or symptoms of infection posttransplantation. No adverse effects attributable to the investigational product were reported for any recipient. All lots met the interim lot release criteria. After transplantation, positive sterility test results were reported for six product lots, and two lots failed the GSIR specification. All other lots met the final release criteria.

DISCUSSION

The methods for large-scale isolation and purification of adult human pancreatic islets have substantially improved over the past three decades as a result of major collaborative efforts among several centers in North America and Europe (1,51–53). Advancements in islet processing technology, immunosuppression, immunomodulation, and peritransplant anti-inflammatory strategies have contributed to progressive improvement of clinical results. However, the lack of standardization of islet processing methods may have contributed to the variability of outcomes across centers and reduced the ability to compare results, even when the same clinical protocol and immunosuppressive regimen were used.

Previously reported differences in outcome among centers highlighted the challenges of the manufacturing process for pancreatic islets in addition to those related to immunosuppression management in islet allotransplantation (4,16). The Edmonton trial conducted at nine centers resulted in a variable success rate (0–100%) for the primary end point, with all cases of primary nonfunction occurring at two of the three centers at which no subject achieved the primary end point (4). Islet products were transplanted immediately after processing, which may not have allowed time to evaluate the quality of the product. Given previous experience, to minimize the risk of primary nonfunction, purified islets were cultured to permit quality control before product release for transplantation (45,46).

Quality of the islet products was affected by donor characteristics, organ recovery and preservation, islet isolation, purification, and culture methods (7,10,14–17). The CIT investigators recognized the need for a major effort in standardization of the PHPI manufacturing process to allow for meaningful comparison of clinical trial outcomes across sites. The investigators also recognized the need to define stringent donor selection criteria and to define and develop process improvements based on acquired experience that would consistently yield a high-quality islet product (53).

We report the methods and results of a common manufacturing protocol, representing the convergence of several years of teamwork by eight academic institutions to optimize and standardize processes, criteria, and test methods across islet transplantation centers. A major standardization effort was undertaken to ensure product and process consistency and to test method reproducibility across participating manufacturers. The CIT collaborative effort resulted in the implementation of a defined set of critical process parameters and in-process controls for PHPI production reflected in the common MPBR and SOPs executed at the eight CIT processing facilities.

Among the key raw materials, we identified the donor pancreas and the enzyme blends as critical components of the PHPI manufacturing process. In 2007, the most widely used enzyme for manufacture of islet products became unavailable. This unforeseen limitation forced our group to reevaluate, test, and qualify additional proteolytic replacement enzymes. The CIT efforts resulted in the successful identification and testing of new enzyme blends by the manufacturing centers. The results presented here show that the enzymes used in digestion yielded lots that met the product specification and, therefore, could be used in manufacturing the PHPI product.

During the trial, potency was assessed by viability and identity of islets as determined by FDA/PI (33,34,37) and DTZ stain (35), respectively. The CMC MC developed and standardized a GSIR by ELISA (41) for quality control and as an in vitro assay for potency assessment of PHPI products. The GSIR assay provided a quantitative measure of the biological activity by determining the amount of insulin released in response to glucose stimulation (Table 4).

During the conduct of Protocol CIT-07, all lots were released on the basis of an ICOA and met lot release specifications (Supplementary Data). As previously stated, the GSIR results of the final product were not available before transplantation. Additional studies may determine whether the GSIR should become a component of the matrix of functional assays for lot release.

The clinical trial results show that the PHPI product was well-tolerated, safe, and effective in the specific type 1 diabetes population (18). These results were obtained despite a range of donor characteristics (BMI 33.4 [8.2] kg/m^2, donor ages 42 [19] years, cold ischemia times 7.7 [3.4] h) and median PHPI dose (11,972 [range 5,227–25,553] IEQ/kg).

This report is the first of the successful standardization of the manufacture of a defined complex biological cellular product for the treatment of type 1 diabetes with hypoglycemia across multiple manufacturing facilities for a license-enabling trial. The cGMP manufacturing process defined in the common CIT documents yielded product lots that met the prespecified criteria for safety, purity, potency, and identity. These results show that the CIT manufacturers achieved consistent and reproducible results across participating centers. No case of primary nonfunction was observed after transplantation of PHPI lots (18). The manufacturing and clinical data generated in this study will be available to CIT and non-CIT sites to facilitate licensure by the Food and Drug Administration. Product licensure will be important to improve patient access (with third-party coverage) to PHPI transplantation and to facilitate the development of second-generation islet products (e.g., stem cells, xenogeneic, encapsulated).

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Duality of Interest. The following authors reported potential conflicts of interest: C.R. is the coinventor on patents related to islet isolation processing aspects that are in part used for current islet cell product manufacturing (does not receive any royalty or financial benefit from these patents or from islet cell processing activities); J.F.M., ViCapsys (consultant); J.O., Novartis (speaker, grant), Pfizer (grant), Novo Nordisk (grant), and Semma Therapeutics (consultant); B.J.H., Sanofi (consultant), Novartis (consultant), and Dompé (consultant); and P.G.S., Genzyme (personal fees), Sanofi (personal fees), Eli Lilly (personal fees), Novo Nordisk (grant, personal fees), and Conatus (nonfinancial support). No other potential conflicts of interest relevant to this article were reported.

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Appendix

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