NIH-sponsored Clinical Islet Transplantation Consortium Phase 3 Trial: Manufacture of a Complex Cellular Product at Eight Processing Facilities

Running title: NIH-CIT Phase 3 Trial - Islet Manufacturing

Camillo Ricordi[1,15], Julia S. Goldstein[2,15], A. N. Balamurugan[3,15,†],Gregory L. Szot[4,15], Tatsuya Kin[5,15], Chengyang Liu[6,15], Christine W. Czarniecki[2,15], Barbara Barbaro[7], Nancy D. Bridges[2], Jose Cano[8], William R. Clarke[9], Thomas L. Eggerman[10], Lawrence G. Hunsicker[9], Dixon B. Kaufman[11,†], Aisha Khan[1], David-Erick Lafontant[9], Elina Linetsky[1], Xunrong Luo[11], James F. Markmann[12], Ali Naji[6], Olle Korsgren[13], Jose Oberholzer[7], Nicole A. Turgeon[8], Daniel Brandhorst[14], Andrew S. Friberg[13], Ji Lei[12], Ling-jia Wang[11,†], Joshua J. Wilhelm[3], Jamie Willits[9], Xiaomin Zhang[11], Bernhard J. Hering[3,16], Andrew M. Posselt[4,16], A. M. James Shapiro[5,16]

[1.*] Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL

[2] National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

[3.*] Schulze Diabetes Institute and Department of Surgery, University of Minnesota, Minneapolis, MN

[4.*] Department of Surgery, University of California, San Francisco, CA

[5.*] Clinical Islet Transplant Program and Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta

[6.*] Institute for Diabetes, Obesity and Metabolism and Departments of Surgery and Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

[7.*] Division of Transplantation, University of Illinois Hospital and Health Sciences System, Chicago, IL

[8.*] Emory Transplant Center, Emory University, Atlanta, GA Division of Transplantation, Department of Surgery,

[9] Clinical Trials Statistical and Data Management Center, University of Iowa, Iowa City, IA

[10] National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

[11.*] Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, IL

[12] Division of Transplant Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA

[13] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden.

Diabetes Publish Ahead of Print, published online July 27, 2016

[14] Department of Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden

[15] Primary authors contributed equally to this paper. [16] Senior authors contributed equally to this research project.

* Facilities that manufactured PHPI product transplanted to Protocol CIT-07 subjects

† Present addresses: A.N. Balamurugan, Islet Cell Laboratory, Cardiovascular Innovation Institute, Department of Surgery, University of Louisville, Louisville, KY; Dixon B. Kaufman, University of Wisconsin, Madison, WI; Ling-jia Wang, Division of Transplantation, University of Illinois Hospital and Health Sciences System, Chicago, IL.

Additional members of the CIT Consortium are listed in the Acknowledgements.

Corresponding authors

Correspondence and requests for materials should be addressed:

Camillo Ricordi, M.D.
Diabetes Research Institute
1450 NW 10 Avenue
Miami, FL 33136
Phone: 305 243-6913 Fax: 305 243-4404
ricordi@miami.edu

Julia S. Goldstein, M.D.
DAIT, NIAID, NIH
5601 Fishers Lane - Rm 7B29 - MSC 9828
Bethesda, MD - 20892 (for USPS)
Rockville, MD – 20852 (UPS/Fed Ex)
Phone: 240 627-3509 Fax: 301 480-1537
goldsteinj@niaid.nih.gov

Word Count: 3,811

Number of Tables: 6

Number of Figures: 0

Abbreviations:

BMI	Body Mass Index
BW	Body Weight
cGMP	Good Manufacturing Practice
cGTP	Good Tissue Practice
CIT	Clinical Islet Transplantation
CMC MC	Chemistry, Manufacturing and Controls Monitoring Committee
CO_2	Carbon Dioxide
CoA	Certificate of Analysis
DAIT/NIAID	Division of Allergy, Immunology and Transplantation/National Institute of Allergy and Infectious Diseases
DMC	Dimethylcasein
DTZ	Dithizone
FDA	Food and Drug Administration
FDA/PI	Fluorescein Diacetate/ Propidium Iodide
ELISA	Enzyme-linked Immunosorbent Assay
GSIR	Glucose Stimulated Insulin Release
HBSS	Hanks' Balanced Salt Solution
HTK	Histidine-Tryptophan-Ketoglutarate
ICoA	Interim Certificate of Analysis
IEQ	Islet Equivalents
IND	Investigational New Drug
IPN	Islet Particle Number
IQR	Interquartile Range
LAL	Limulus Amoebocyte Lysate
MPBR	Master Production Batch Record
N	Number
PFC	Perfluorocarbon
PHPI	Purified Human Pancreatic Islets
SOPs	Standard Operating Procedures
UW	University of Wisconsin

Abstract

Eight manufacturing facilities participating in the NIH-sponsored Clinical Islet Transplantation (CIT) Consortium jointly developed and implemented a harmonized process for manufacture of the 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 (MPBR), standard operating procedures including acceptance criteria for deceased donor organ pancreata and critical raw materials, PHPI product specifications, certificate of analysis (CoA) and test methods. The process was compliant with current Good Manufacturing Practices (cGMP) and Good Tissue Practices (cGTP).

This report describes the manufacturing process for 75 PHPI clinical lots and summarizes the results of the manufacturing process including lot release. The results demonstrated the feasibility of implementing a harmonized process at multiple facilities for manufacture of a complex cellular product. The quality systems, regulatory and operational strategies developed by the CIT consortium yielded product lots that met the pre-specified characteristics of safety, purity, potency and identity, and were successfully transplanted into 48 subjects. No adverse events attributable to the product and no case of primary non-function were observed.

Introduction.

Previous clinical trials, assessing treatments for unstable type 1 diabetes, demonstrated restoration of pancreatic beta-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, 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 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 required convergence towards regulatory guidelines on clinical trial design. Manufacturing differences at the different processing facilities prevented comparison of clinical results across centers, making it difficult to derive any overall conclusions and limited 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, multi-center, single-arm, pivotal Phase 3 trial conducted at 8 academic institutions in North America (www.clinicaltrials.gov; NCT00434811) [18]. The trial was designed to evaluate the safety, tolerability and efficacy of the PHPI product in a defined population of subjects with type 1 diabetes and to support product licensure in the US. The trial was conducted under a Division of Allergy, Immunology and Transplantation/National Institute of Allergy and Infectious Diseases (DAIT/NIAID)-sponsored US Investigational New Drug (IND) application and a Clinical Trial Application (CTA) in Canada. Manufacturing information was submitted to a DAIT/NIAID-sponsored US Drug Master File. A Chemistry, Manufacturing and Controls Monitoring Committee (CMC MC), with representatives from each manufacturing

facility and DAIT/NIAID, was responsible for harmonization of manufacturing processes including in-process controls, qualification of manufacturing facilities, definition of product specifications, and quality oversight.

This manuscript describes the manufacturing process, and the in-process and lot release test results for the 75 clinical lots transplanted to the 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 Allogeneic, Purified Human Pancreatic Islet (PHPI) product executed at multiple facilities for transplantation to support a pivotal trial. The CIT Standard Operating Procedures (SOPs) and the MPBR developed for product manufacturing are publically available [19-40]. The clinical outcomes are reported elsewhere [18].

RESEARCH DESIGN AND METHODS

Participation in the CIT Consortium required a demonstrated record of robust clinical and islet product manufacturing success (Online Supplemental Materials - Section I). The CMC MC developed the common MPBR [23], Product Specifications [32], Interim and final CoA [30, 31], and agreed on the deceased donor organ acceptance criteria (Table 1). Each manufacturing facility was responsible for its own quality controls, quality assurance and compliance with cGMP and cGTP. 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 prior to that facility's participation in the trial. Manufacturers were required to meet the pre-defined assay acceptance criteria for accuracy, precision, selectivity, sensitivity, and reproducibility of each assay. All operations were conducted using aseptic processing in accredited cGMP facilities (Online Supplemental Materials - Section II).

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 (HBSS) 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. Based on the initial pancreas weight, the volume of the CIT Enzyme Solution [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 prior to its introduction in 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 minutes at 60 – 80 mmHg, followed by 4 – 6 minutes at 160-180 mm Hg at 4 – 14 °C. Final trimmed pancreas weight was calculated from 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, 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 1640. 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 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 – 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: ≤ 25 mL of digested tissue were layered over a iodixanol-based continuous density gradient [20] ranging from 1.060 – 1.100 g/mL ± 0.01g/mL, and were centrifuged at 1800 – 2000 rpm in a COBE® 2991 (Terumo BCT, Lakewood, CO) cell processor at 4 – 8 ˚C.

Twelve 25 mL gradient fractions were collected in 250 mL conical tubes prefilled with 225 mL CMRL 1066 Supplemented Solution, CIT Modifications (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 fractions with the same purity were pooled. If > 50,000 islets were present in fractions with purity < 30%, supplementary purification was performed using Biocoll [25] (Biochrome AG, Germany), Polysucrose discontinuous gradients [24] (Mediatech, Manassas, Virginia), or OptiPrep™ [26] (Nycomed/Takeda, Osaka, Japan). Purity of the fractions obtained by supplementary purification was assessed by DTZ, and each fraction was combined with fractions of equivalent purity to those obtained 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 prior to filling the infusion bag(s).

Culture Step: Purified islets were cultured for a total of 36 – 72 hr in non-treated T-175 vented flasks (SARSTEDT AG & Co, Germany) at 10,000 – 30,000 IEQ/30 mL CIT Culture Media [19]. High purity islet fractions were cultured at 37° C / 5 % CO_2 for the first 12 – 24 hr and at 22° C / 5 % CO_2 for the remainder 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 hr 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 pipet and allowing the aspirate to sediment by gravity for 5 minutes. 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 > 7.5mL and ≤ 15 mL, the tissue was divided into 2 or 3 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 hr of bag fill. Samples for lot release tests were obtained before filling the infusion bag(s) 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 using an Interim COA [31] (ICoA) (Online Supplemental Materials - Section III). Safety assessments at the time of release included microbial contamination by Gram staining and endotoxin using the Limulus Amoebocyte Lysate (LAL) Endosafe®-PTS™ or -KTA^2 (Charles River Endosafe, Charleston, SC). The final CoA [30] was issued when the sterility and post-culture GSIR test results became available (Table 3). The sterility test was performed compliant with 21 CFR 610.12. A preemptive plan that included the criteria for reporting product contamination, if a contaminated lot needed to be transplanted for clinical reasons, was agreed upon with the Food and Drug Administration (FDA).

Purity of the PHPI product was defined, not histologically, but as total IEQ/mL of total settled tissue volume. Viability was assessed by staining with FDA/PI (Sigma-Aldrich, St. Louis, Missouri), and expressed as the percent of viable cells [33, 34, 37] among fifty consecutive

islets. DTZ staining was used for identification of islets, quantification of islet equivalents (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 mM) and high (28 mM) glucose concentrations. The results are expressed as the stimulation index.

Statistical Methods: "Recovery" is defined as the quantity of IEQs retrieved during the manufacturing process and expressed as IEQ/g 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 (N), and mean ± standard deviation or median [interquartile range (IQR)]. Categorical variables are displayed as number (N) 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 as a p-value < 0.05. SAS for Windows version 9.3 – 9.4 (SAS Institute, Cary, NC) was used for all data management and statistical analyses.

RESULTS

A total of 324 pancreases 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), consistent with previously reported rates [48-50]. Manufacture success among the centers ranged from 24.5 – 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 compares side-by-side the data for each center and for all centers together and displays the 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 products results included those for the post-digestion, post-purification and post-culture 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 gender and body mass index (BMI) ($p=0.0002$ and 0.0082, respectively). No other donor or pancreas characteristics were significantly related to IEQ recovery after adjustment for donor gender 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/g trimmed pancreas revealed no significant differences after adjustment for multiple comparison.

Pancreas perfusion: Lots were manufactured 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/g 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: (i) total amount of digested tissue in the sample, (ii) estimated total number of islets, (iii) % free islets, and (iv) % 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 of trimmed pancreas (Table 4).

Islet purification: Islet purification was performed by continuous density gradient centrifugation using 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 lots (69.3 %), 10 lots (13.3 %), 9 lots (12 %), and 4 lots (5.3 %) required 2, 3, 1 and 4 COBE runs, respectively, for islet purification from the digested tissue. Post-purification islet recovery (total IEQ) was 582,370 [267,931] IEQ, representing 5,471 [2,709] IEQ/g of trimmed pancreas. Prior to 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 post-digestion intermediate product (Table 4).

Islet culture: Purified islets were cultured for 36 – 72 hr prior to transplantation. For the first 12 – 24 hr 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 post-culture was 490,174 [226,835] IEQ, representing a total islet recovery of 4,730 [2,156]

IEQ/g of trimmed pancreas. Median islet yield for the culture step was 86 [17] %. Median total process yield, calculated from post-digestion to post-culture, was 71 [22] % (Table 4).

PHPI product characteristics and lot release: PHPI final product was release based on the results reported on the ICoA [31] (Online Supplemental Material - Section III). 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. Each subject received one (22 subjects), two (25 subjects) or three (1 subject) doses of PHPI product by intraportal infusion. Additional details of doses for first, second, and third infusions are included in the Online Supplemental Material - Section IV. The initial transplant required a dose of ≥ 5,000 IEQ/kg recipient's BW, and the second and third required a dose of ≥ 4,000 IEQ/kg recipient's BW (Table 6). The median total PHPI dose per subject was 11,972 (Range: 5,227 – 25,553) IEQ/kg, with 42 of 48 subjects achieving clinical success [18].

Each of the 75 PHPI lots was released for transplantation in 1 (n=53), 2 (n=22), 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 recipients' 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 ≤ 5.0 EU/kg of recipients' BW.

Sterility tests were performed in samples of the preservation solution and the final product (Online Supplemental Materials - Section V); 22/75 preservation solution samples reported

contamination with skin saprophytes and 1/75 identified *C. albicans*. For the final product, the sterility tests of 5/75 lots reported skin saprophytes and 1 lot identified *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 that identified *C. albicans* received fluconazole prophylactically. None of the recipients of these six lots exhibited signs or symptoms of infection post-transplantation. No adverse effects attributable to the investigational product were reported for any recipient. All lots met the interim lot release criteria. Following transplantation, positive sterility test results were reported for 6 product lots, and 2 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 peri-transplant 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 utilized.

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 9 centers, resulted in variable success rate (0 – 100%) for the primary endpoint, with all cases of primary non-function occurring at 2 of the 3 centers at which no subject achieved the primary endpoint [4]. Islet products were transplanted immediately after processing, which may not have allowed time to evaluate the quality of the product. Based on previous experience, to minimize the risk of primary non-function purified islets were cultured permitting quality controls to be performed prior to 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 the 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 team work 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 methods 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 that were reflected in the common MPBR and SOPs, executed at the 8 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 enzyme that had been most widely used 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 in this manuscript show that the enzymes used in digestion yielded lots that met the product specification and, therefore, could be used in manufacturing of the PHPI product.

During the trial, potency was assessed by viability and identity of islets, as determined by FDA/PI [33, 34, 37] and by 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 based on an ICoA and met lot release specifications (Online Supplemental Materials - Section III). As previously stated, the GSIR results of the final product were not available prior to 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 showed 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] hours), and median PHPI dose of 11,972 IEQ/kg recipient ranging from 5,227 – 25,553 IEQ/kg.

This is the first report of 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 lots of product that met the pre-specified 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 non-function 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 FDA. Product licensure will be important to improve patient access (with third-party coverage) to PHPI transplantation and to facilitate development of second generation islet products (e.g., stem cells, xenogeneic, encapsulated, etc.).

Author Contributions. The following authors contributed to the design and realization, analysis of the data and writing of this paper: C.R., J.S.G., A.N.B., G.L.S., T.K., C.L., C.W.C., B.B., N.D.B, J.C., W.R.C., T.L.E., L.G.H., D.B.K., A.K., D-E.L., E.L., X.L., J.F.M., A.N., O.K., J.O., N.A.T., D.B., A.S.F., J.L., L.W., J.J.W., J.W., X.Z., B.J.H., A.M.P., A.M.J.S.

C.R., J.S.G., A.N.B drafted the primary manuscript. G.L.S, T.K, C.L, C.W.C. are co-primary authors of the study and contributed substantially to the design, analysis, and interpretation of PHPI manufacturing results, and to the preparation of the final manuscript for publication.

All members of the CMC MC contributed equally to the concept and development of the MPBRs, process SOPs, product Specifications, CoA, and participated in the study conduct, data acquisition, and interpretation of results. J.S.G. led the CMC MC, which also provided oversight for product (PHPI) manufacturing and qualified sites for participation in the protocol. C.W.C. and J.S.G. developed the regulatory strategy and led the development of the manufacturing process for PHPI. L.G.H., N.D.B., and T.L.E. made significant editorial contributions to the final manuscript. All authors reviewed and approved the final version of the manuscript.

Acknowledgements. We would like to thank Nordmark-Pharma/SERVA Electrophoresis GmbH and Biorep Technologies, Inc. for contributing their products. We would like to thank our collaborators throughout the CIT Consortium, particularly those involved with the manufacture of PHPI.

Guarantor Statement. C.R., A.N.B. and J.S.G are the guarantors of the work and, as such, had full access to all the manufacturing data and take responsibility for the integrity of the data and the accuracy of the data analysis.

Consortium Members

The NIH CIT CMC MC: J. Ansite, A.N. Balamurugan, B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, T. Granderson, E.

Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, K. McElvany, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G.L. Szot, L. Wang, J.J. Wilhelm, J. Willits, X. Zhang

The NIH CIT Consortium

Emory University: J. Avila, B. Begley, J. Cano, S. Carpentier, E. Holbrook, J. Hutchinson, C.P. Larsen, J. Moreno, M. Sears, N.A. Turgeon, D. Webster

Massachusetts General Hospital: S. Deng, J. Lei, J.F. Markmann

NIAID: N.D. Bridges, C.W. Czarniecki, J.S. Goldstein, T. Granderson, G. Putz

NIDDK: T.L. Eggerman

Northwestern University: P. Al-saden, J. Battle, X. Chen, A. Hecyk, H. Kissler, X. Luo, M. Molitch, N. Monson, E. Stuart, A. Wallia, S. Wang, X. Zhang

University of Alberta, Edmonton: D. Bigam, P. Campbell, P. Dinyari, T. Kin, N. Kneteman, J. Lyon, A. Malcolm, D. O'Gorman, C. Onderka, R. Owen, R. Pawlick, B. Richer, S. Rosichuk, D. Sarman, A. Schroeder, P.A. Senior, A.M.J. Shapiro, L. Toth, V. Toth, W. Zhai

University of California–San Francisco: K. Johnson, J. McElroy, A.M. Posselt, M. Ramos, T. Rojas, P.G. Stock, G.L. Szot

University of Illinois, Chicago: B. Barbaro, J. Martellotto, J. Oberholzer, M. Qi, L. Wang, Y. Wang

University of Iowa (Data Coordinating Center): L. Bayman, K. Chaloner, W. Clarke, J.S. Dillon, C. Diltz, G.C. Doelle, D. Ecklund, D. Feddersen, E. Foster, L. G. Hunsicker, C. Jasperson, D-E Lafontant, K. McElvany, T. Neill-Hudson, D. Nollen, J. Qidwai, H. Riss, T. Schwieger, B. Shields, J. Willits, J. Yankey

University of Miami: R. Alejandro, A. Alvarez, A.C. Corrales, R. Faradji, T. Froud, A.A. Garcia, E. Herrada, H. Ichii, L. Inverardi, N. Kenyon, A. Khan, E. Linetsky, J. Montelongo, E. Peixoto, K. Peterson, C. Ricordi, J. Szust, X. Wang

University of Minnesota: M.H. Abdulla, J. Ansite, A.N. Balamurugan, M.D. Bellin, M. Brandenburg, T. Gilmore, J. V. Harmon, B.J. Hering, R. Kandaswamy, G. Loganathan, K. Mueller, K.K. Papas, J. Pedersen, J.J. Wilhelm, J. Witson

University of Pennsylvania: C. Dalton-Bakes, M. Kamoun, J. Kearns, Y. Li, C. Liu, E. Luning-Prak, Y. Luo, E. Markmann, Z. Min, A. Naji, M. Palanjian, M. Rickels, R. Shlansky-Goldberg, K. Vivek, A.S. Ziaie

University of Wisconsin: L. Fernandez, D.B. Kaufman, L. Zitur
Uppsala University: D. Brandhorst, A. Friberg, O. Korsgren

Funding. This research was supported by grants from the National Institute of Allergy and Infectious Diseases and the National Institute for Diabetes and Digestive and Kidney Diseases to the following institutions: Emory University (Atlanta, GA), U01-AI-089317; Northwestern University (Evanston, IL), U01-AI-089316; University of Alberta (Edmonton, Alberta, Canada),U01- AI-065191; University of California, San Francisco (San Francisco, CA)U01-DK-085531;University of Illinois, Chicago (Chicago, IL), 5U01-DK-070431-10; University of Iowa (Iowa City, IA), U01-DK-070431; University of Miami (Miami, FL), U01-DK- 070460; University of Minnesota (Minneapolis, MN), U01-AI-065193; University of Pennsylvania (Philadelphia, PA), U01-DK-070430; and Uppsala University (Uppsala, Sweden), U01-AI-065192. In addition, the study was supported by the following General Clinical Research Center Awards and Clinical and Translational Science Awards: Emory University, UL1-TR-000454; Northwestern University, 5UL1-RR-025741 and 8UL1-TR-000150; University of California, San Francisco, UL1-TR-000004; University of Illinois, Chicago, UL1-TR-000050; University of Miami, 1UL1-TR-000460; University of Minnesota: 5M01-RR-000400 and UL1-TR-000114; and University of Pennsylvania, M01-RR-00040 and UL1-TR-000003.

Duality of Interest. The following authors reported potential dualities of interest: B.J.H., Sanofi (consultant), Novartis (consultant), Dompe (consultant); J.F.M., Vicapsys (consultant); J.O., Novartis (speaker, grant), Pfizer (grant), Novo Nordisk (grant), and Semma Therapeutics (consultant); C.R., 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); 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.

References

1. Ricordi, C. and T.B. Strom, *Clinical islet transplantation: advances and immunological challenges.* Nat Rev Immunol, 2004. **4**(4): p. 259-68.
2. Markmann, J.F., et al., *Insulin independence following isolated islet transplantation and single islet infusions.* Ann Surg, 2003. **237**(6): p. 741-9; discussion 749-50.
3. Hering, B.J., et al., *Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes.* JAMA, 2005. **293**(7): p. 830-5.
4. Shapiro, A.M., et al., *International trial of the Edmonton protocol for islet transplantation.* N Engl J Med, 2006. **355**(13): p. 1318-30.
5. Shapiro, A.M., et al., *Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen.* N Engl J Med, 2000. **343**(4): p. 230-8.
6. Brennan, D.C., et al., *Long-Term Follow-Up of the Edmonton Protocol of Islet Transplantation in the United States.* Am J Transplant, 2016. **16**(2): p. 509-17.
7. Lakey, J.R., et al., *Variables in organ donors that affect the recovery of human islets of Langerhans.* Transplantation, 1996. **61**(7): p. 1047-53.
8. Kin, T., *Islet isolation for clinical transplantation.* Adv Exp Med Biol, 2010. **654**: p. 683-710.
9. Brandhorst, H., et al., *Impact of cold ischemia and timing of intraductal collagenase distension on human islet yield, purity, viability, and survival in low temperature culture.* Transplant Proc, 1994. **26**(2): p. 590-1.
10. Kin, T., et al., *Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon.* Transplantation, 2006. **82**(10): p. 1286-90.
11. Yamamoto, T., et al., *Quality control for clinical islet transplantation: organ procurement and preservation, the islet processing facility, isolation, and potency tests.* J Hepatobiliary Pancreat Surg, 2009. **16**(2): p. 131-6.
12. Brandhorst, H., et al., *Comparison of histidine-tryptophane-ketoglutarate (HTK) and University of Wisconsin (UW) solution for pancreas perfusion prior to islet isolation, culture and transplantation.* Transplant Proc, 1995. **27**(6): p. 3175-6.
13. Iwanaga, Y., et al., *Pancreas preservation for pancreas and islet transplantation.* Curr Opin Organ Transplant, 2008. **13**(2): p. 135-41.
14. Lakey, J.R., P.W. Burridge, and A.M. Shapiro, *Technical aspects of islet preparation and transplantation.* Transpl Int, 2003. **16**(9): p. 613-32.
15. Noguchi, H., et al., *Islet Culture/Preservation Before Islet Transplantation.* Cell Med, 2015. **8**(1-2): p. 25-9.
16. Balamurugan, A.N., et al., *Prospective and challenges of islet transplantation for the therapy of autoimmune diabetes.* Pancreas, 2006. **32**(3): p. 231-43.
17. Shapiro, A.M. and C. Ricordi, *Unraveling the secrets of single donor success in islet transplantation.* Am J Transplant, 2004. **4**(3): p. 295-8.
18. Hering, B.J., et al., *Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia.* Diabetes Care, 2016. Published ahead of Print April 18, 2016.

19. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Culture Media – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B04 CIT Culture Media.* CellR4, 2014. **2**(3): p. e981.
20. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Purification Density Gradient – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B10 CIT Purification Density Gradients.* CellR4, 2014. **2**(3): p. e991.
21. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Digestion Solution – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B01 CIT Digestion Solution.* CellR4, 2014. **2**(3): p. e985.
22. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Purification Solution – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B02 CIT Purification Solution.* CellR4, 2014. **2**(3): p. e993.
23. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, Master Production Batch Record – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B04 CIT Culture Media.* CellR4, 2014. **2**(2): p. e891.
24. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, Supplementary Purification, Discontinuous Polysucrose Procedure & Record – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium.* CellR4, 2014. **2**(3): p. e997.
25. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T.

Wilson, X. Zhang, *Purified Human Pancreatic Islets, Supplementary Purification, Biocoll Procedure & Record – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium.* CellR4, 2014. **2**(3): p. e995.

26. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, Supplementary Purification, OptiPrep Procedure and Record – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium.* cellR4, 2014. **2**(3): p. e999.
27. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, , *Purified Human Pancreatic Islets, CIT Wash Solution – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium, SOP 3106 B12.* CellR4, 2014. **2**(3): p. e989.
28. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Enzyme Solution Roche Enzymes– Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B14 CITRoche Enzyme Solution.* CellR4, 2015. **3**(1): p. e1360.
29. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Enzyme Solution -VitaCyte Enzymes and Vitacyte/ServaEnzymes Combination– Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B13 VitaCyte Enzyme Solution.* CellR4, 2015. **3**(1): p. e1350.
30. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, Certificate of Analysis (Product Code PHPI-A-01) – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP B02.* CellR4, 2015. **3**(1): p. e1448.
31. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, Interim Certificate of Analysis (Product Code PHPI-A-01) – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium* - CellR4, 2015. **3**(1): p. e1446.

32. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, Specification (Product Codes PHPI-A-01, PHPI-L-01, PHPI-E-01) – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP3100, A01.* CellR4, 2015. **3**(1): p. e1437.
33. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islet, Viability Estimation of Islet Using Fluorescent Dyes (FDA/PI) – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP3104 A02* CellR4, 2015. **3**(1): p. e1378.
34. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islet, Viability Estimation of Islet Using Fluorescent Dyes, Attachment II, Islet Viability WOrksheete – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3104, A02, A01 FDA-PI Attach II.* CellR4, 2015. **3**(1): p. e1374.
35. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islet: Qualitative and Quantitative Assessment of Islets Using Dithizone (DTZ) – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP3104, A01.* . CellR4, 2015. **3**(1): p. e1360.
36. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islets, CIT Enzyme Solution Serva Enzymes Proportional Units Collagenase & Neutral Protease – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B11.* CellR4, 2015. **3**(1): p. e1342.
37. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Purified Human Pancreatic Islet, Viability Estimation of Islet Using Fluorescent Dyes, Attachment I, Preparation of Fluorescein Diacetate and Propidium Iodine Solutions – Standard Operating Procedure of the NIH Clinical Islet*

Transplantation Consortium SOP3104, A02, A01 FDA-PI Attach I. CellR4, 2015. **3**(1): p. e1374.

38. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Raw Material Specification, CMRL 1066, Supplemented, CIT Modifications – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium - SOP 3106, B10 CIT Purification Density Gradient.* CellR4, 2016. **4**(3): p. e1895.
39. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Raw Material Specification, Human Pancreas, Deceased Donor – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium, SOP3102, A18, Human Pancreas, Deceased Donor.* CellR4, 2016. **4**(3): p. e1932.
40. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, SOP 3106, B06, *Purified Human Pancreatic Islets, CIT Transplant Media – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium.* CellR4, 2015. **3**(1): p. 1523.
41. J. Ansite, A.N.B., B. Barbaro, J. Battle, D. Brandhorst, J. Cano, X. Chen, S. Deng, D. Feddersen, A. Friberg, T. Gilmore, J.S. Goldstein, E. Holbrook, A. Khan, T. Kin, J. Lei, E. Linetsky, C. Liu, X. Luo, K. McElvaney, Z. Min, J. Moreno, D. O'Gorman, K.K. Papas, G. Putz, C. Ricordi, G. Szot, T. Templeton, L. Wang, J.J. Wilhelm, J. Willits, T. Wilson, X. Zhang, *Functional Assessment of Purified Human Pancreatic Islets: Glucose Stimulated Insulin Release by ELISA – A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium.* CellR4, 2014. **2**(2): p. e900.
42. Lakey, J.R., et al., *Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion.* Cell Transplant, 1999. **8**(3): p. 285-92.
43. Warnock, G.L., et al., *Tissue banking of cryopreserved islets for clinical islet transplantation.* Transplant Proc, 1994. **26**(6): p. 3438.
44. Papas, K.K., T.M. Suszynski, and C.K. Colton, *Islet assessment for transplantation.* Curr Opin Organ Transplant, 2009. **14**(6): p. 674-82.
45. Kissler, H.J., et al., *Validation of methodologies for quantifying isolated human islets: an Islet Cell Resources study.* Clin Transplant, 2010. **24**(2): p. 236-42.
46. Hering, B.J., et al., *Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody.* Am J Transplant, 2004. **4**(3): p. 390-401.
47. Froud, T., et al., *Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience.* Am J Transplant, 2005. **5**(8): p. 2037-46.

48. Cross, S.E., et al., *Collagenase penetrates human pancreatic islets following standard intraductal administration.* Transplantation, 2008. **86**(7): p. 907-11.
49. Shapiro, A.M., *Strategies toward single-donor islets of Langerhans transplantation.* Curr Opin Organ Transplant, 2011. **16**(6): p. 627-31.
50. Balamurugan A.N., L.G., Bellin M.D., Wilhelm J.J., Harmon J., Anazawa T.,, R.D.M. Soltani S.M., Yuasa T., Tiwari M., Papas K.K., McCarthy R., Sutherland, and H.B.J. D.E., *A new enzyme mixture to increase the yield and transplant rate of autologous and allogeneic human islet products.* Transplantation, 2012. **93**(7): p. 693-702.
51. Piemonti LP, *25 years of the Ricordi automated Methods for islet isolation.* CellR4, 2013. **1**: p. e128.
52. Ricordi, C., *Islet transplantation: a brave new world.* Diabetes, 2003. **52**(7): p. 1595-603.
53. Barton, F.B., et al., *Improvement in outcomes of clinical islet transplantation: 1999-2010.* Diabetes Care, 2012. **35**(7): p. 1436-45.

Table 1: Pancreas Donor Qualification

REQUIREMENTS A qualified donor must have "Yes" responses to all of the Inclusion Criteria (A), and "No" responses to all of the Exclusion Criteria (B & C).	**Yes**	**No**	**NA**
Container Label must specify Human Pancreas, and a UNOS or DDD number must be present.			
The Organ Procurement Organization (OPO) must be identified.			
A. Inclusion Criteria (The donor or pancreas must meet these criteria.)			
1. Pancreas Preservation in (i) UW, (ii) PF/UW, (iii) HTK, or (iv) PF/HTK Solution(s)			
2. Maximum 12 hour 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 Diabetes mellitus Type 1 or 2 (Transplant teams may consider donor HbA1C > 6.1% in the absence of transfusions in the week prior to 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			

REQUIREMENTS A qualified donor must have "Yes" responses to all of the Inclusion Criteria (A), and "No" responses to all of the Exclusion Criteria (B & C).	**Yes**	**No**	**NA**
5. Evidence of clinical or active viral Hepatitis [A, B (HBcAg), C]. HBsAb+ is acceptable, if there is a history of vaccination.			
6. Acquired Immunodeficiency Syndrome (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-Jacob Disease			
12. Suspected Rabies Diagnosis			
13. Treated or Active Tuberculosis			
14. Individuals who have received pit-hGH (pituitary 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 prior to time of death: men who			

REQUIREMENTS A qualified donor must have "Yes" responses to all of the Inclusion Criteria (A), and "No" responses to all of the Exclusion Criteria (B & C).	Yes	No	NA
have had sex with men, individuals who have engaged in prostitution, and individuals whose sexual partners have engaged in high-risk sexual behavior			
18. Non-medical intravenous, intramuscular, or subcutaneous drug use within the past five 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 more than 72 consecutive hours during the previous 12 months			

Table 2: Enzyme Combinations Used in Manufacture of PHPI Product

Activity (Units)/Manufacturer	N=75	Mean (SD)	Median	Min-Max
Collagenase NB 1 (Wünsch) SERVA Electrophoresis GmbH, Germany	46	2,008 (439)	1,829	1,600-3,842
Neutral Protease NB (DMC Units) SERVA Electrophoresis GmbH, Germany		244 (65)	229	200-581
Cl*zyme*™ (Wünsch) VitaCyte, LLC, Indianapolis, IN	18	2,472 (542)	2,201	1,620-3,740
Neutral Protease NB (DMC Units) SERVA Electrophoresis GmbH, Germany		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 (Wünsch) Roche Diagnostics, Basel, Switzerland		99,552 (55,097)	71,577	51,075-187,275

Table 3: Final Certificate of Analysis

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 are identical to that in the Production Batch Record, Section 12.3
Islets Identity	DTZ Stain and Microscopic Examination	Islets are 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-minute settling	≤7.5 mL per product bag ≤15.0 mL total in three product bags
Potency		
Glucose Stimulated Insulin Release (High Purity Islets, Pre-culture Sample)	Enzyme-linked Immunosorbent Assay (ELISA)	For Information Only, Report Stimulation Index
Glucose Stimulated Insulin Release (High Purity Islets, Post-culture Sample)	ELISA	Stimulation Index >1

Islets Quantity	DTZ Stain & Microscopic Examination	First Infusion: ≥ 5.0 x 10^3 IEQ/kg of Recipient's Body Weight (Total IEQ/ infusion) Subsequent Infusions: ≥4.0 x 10^3 IEQ/kg of Recipient's Body Weight (Total IEQ/infusion)
Viability	FDA/PI Stain & Microscopic Examination	≥70% in each product bag
Purity		
Islets Concentration	DTZ Stain & 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's Body Weight (Total EU/infusion)
Sterility	21CFR 610.12	No growth in each product bag

DTZ, Dithizone; ELISA, Enzyme-linked Immunosorbent Assay; FDA/PI, fluorescein diacetate/propidium iodide; LAL, Limulus Amoebocyte Lysate

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									
# 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 Characteristics									
Donor Age (Yrs)	46 [9]	50 [6]	39 [19]	37 [22]	49 [27]	43 [21]	37 [7]	53 [26]	42 [19]
Donor Height	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]
Donor 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]
Donor BMI	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 (hours)	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]
Post-digestion 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]
Post-digestion 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]
Post-digestion 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]
Post-purification 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]
Post-purification 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]
Post-purification 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)
Post-culture 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]
Post-culture 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]

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)
Post-culture (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)									
Post-digestion	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]
Post-purification	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]
Post-culture	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 (%)									
Post-purification	73 [24]	84 [6]	77 [19]	81 [25]	106 [42]	92 [31]	84 [11]	79 [13]	82 [22]
Post-culture	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, pre-culture) †	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, post-culture)	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 recipients' 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 recipients' 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]

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)
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]

Values are reported as Median [IQR] or N(%); *Post-digestion Index: IPN values were available for 41 of 75 lots. † GSIR, pre-culture result was for information only and not used for lot release.

N, Number of lots; BMI, Body Mass Index; IPN, islet particle number, IEQ, islet equivalent; Islet Index, IEQ/IPN; IEQ/g, islet equivalents per gram trimmed pancreas; GSIR, glucose stimulated insulin release; IEQ/kg, islet equivalents per kg recipients' body weight; BW, body weight; EU, endotoxin units; PHPI, purified human pancreatic islets.

Table 5: Additional Donor and Pancreas Characteristics

Category	N (%)
Gender	
M	53 (70.7)
F	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	
University of Wisconsin (UW)	47 (62.7)
Perfluorocarbon (PFC)/UW	8 (10.7)
Histidine-tryptophan-ketoglutarate (HTK)	14 (18.7)
PFC/HTK	3 (4)
Not Specified	3 (4)

Table 6: Final Product Formulation for Purified Human Pancreatic Islets

Ingredient	**Quantity**
Active Ingredient	
Human Pancreatic Islets	First Infusion: ≥ 5.0 x 10^3 IEQ /kg of Recipient's Body Weight (Total IEQ/infusion)
	Second Infusion: ≥ 4.0 x 10^3 IEQ /kg of Recipient's Body Weight (Total IEQ/infusion)
Inactive Ingredients	
CMRL 1066 Transplant Media[12], contains HEPES and without Sodium Bicarbonate	q.s. to 200 mL per bag
Albumin Human, USP	2.5%

IEQ, islet equivalent

Online Supplementary Materials:

Authors' Justification:

The authors believe that the submitted manuscript is complete as it stands. However additional information was requested by the reviewers, to include: the criteria for qualification of centers to participate in CIT trials, the methods used to minimize the risk of product contamination, the criteria for release of the product for transplantation (contained in the Interim Certificate of Analysis), details of IEQs in each of the PHPI infusions, and specifics concerning the contaminating microbial species identified in the initial pancreas perfusion solution and the final PHPI products. Editorial limitations precluded including all this information in the submitted manuscript itself. To make the above information available to the readers of Diabetes, in addition to the reviewers, this information has been included in the Online Supplemental Materials.

I: Qualification and Oversight of Manufacture

This section describes our approach to ensure a consistent and reproducible product manufacture and a safe, pure, potent and efficacious product.

The initial Drug Master File submission contained lot release testing data for a minimum of 3 lots manufactured at each processing site using a production process comparable (as

determined by the CMC Monitoring Committee) to that described in the master batch record.

Each site was responsible for Quality Systems oversight to ensure cGMP compliance. Lot-to-lot consistency at each site and site-to-site comparability of product manufacture was controlled through the use of a common Master Production Batch Record (MPBR), common Product Specifications and common Certificates of Analysis (COA). These documents were controlled by DAIT/NIAID and approved by a CMC Monitoring Committee comprised of representatives from the manufacturing sites and DAIT/NIAID. Addition of new sites to the clinical study followed the same qualification plan and the data were submitted in amendments to the Drug Master File.

Any change proposed to the MPBR, the Product Specifications, or the Certificates of Analysis required the concurrence of all sites and NIAID before implementation. Any changes proposed to a site's SOPs impacted the identity, potency, purity or safety of the product also required the concurrence of all sites and NIAID before implementation of the change. Changes to the manufacturing process were described in amendments to the DMF and submitted by NIAID as appropriate.

Each Manufacturing Site:

1. Submitted to an initial facility assessment of site capabilities and evaluation of cGMP compliance to ensure regulatory requirements were satisfied.
2. Accepted the common MPBR and product specification documents, common assay methods for in-process testing and lot release testing, and common process controls and operational ranges.
3. Agreed to utilize the above-mentioned documents with no modification without the prior concurrence of DAIT and the CMC Monitoring Committee.
4. Agreed to the review of its manufacturing and testing process records by DAIT ORA Quality Assurance (QA) for each lot of product transplanted, and to address observations of inconsistencies and non-compliance with the cGMP and CIT-defined requirements recorded in the Audit Reports.
5. Provided to the CMC Monitoring Committee (for review and approval) cell processing and cell product test records demonstrating 3 batches of islets were manufactured by a process similar to that defined in the MPBR and those 3 batches met the in-process limits and final product release specifications.

II: Aseptic Processing and Measures to Minimize Contamination Risk

Prevention of contamination was implemented by design. As it is standard practice in manufacture of biological products, all manufacturing operations were conducted using aseptic processing in accredited cGMP facilities. All open operations were conducted in class 100 (ISO5) isolators equipped with positive air pressure and unidirectional air flow. Air quality was monitored and tested according to the plans of each manufacturing

facility and compliant with 21CFR 211.42. Air testing included viable, non-viable particulates under static and dynamic conditions. Adjacent areas to the class 100 were either class 1000 or 10,000 and monitored accordingly. Personnel and material flow were compliant with the facility written processes. All containers that contacted the product and its intermediaries were disposable (e.g., tubing, pipettes, infusion bags, etc.) except for the Ricordi chamber, which was sterilized and stored per the facilities' SOPs.

Sterility deviation reports were considered critical and reviewed by the CMC MC. Corrective and preventive actions (CAPA) were approved by the CMC-MC prior to implementation and required re-training of the appropriate manufacturing personnel. The manufacturers/suppliers of sterile media and of the digestion enzymes were qualified by an independent auditor contracted by DAIT/NIAID. Qualification of the PHPI manufacturers for participation in the CIT consortium included cGMP assessment of the manufacturing facility by an independent cGMP expert consultant contracted by DAIT/NIAID. Finally, per protocol, all subjects received antimicrobial prophylaxis prior to transplantation. Please refer to Section 5.3 of the clinical Protocol CIT-07 (www.islet study.org).

III: Interim Certificate of Analysis

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 are identical to that in the Production Batch Record, Section 12.3
Islets Identity	DTZ Stain and Microscopic Examination	Islets are present in each product bag
Volumes in Bags		
Suspension Volume	Direct Measurement	200 mL per product bag
Settled Tissue Volume	Direct Measurement after 5-minute settling	≤7.5 mL per product bag ≤15.0 mL total in three product bags
Potency		
Glucose Stimulated Insulin Release (High Purity Islets, Pre-culture Sample)	Enzyme-linked Immunosorbent Assay (ELISA)	For Information Only, Report Stimulation Index
Islets Quantity	DTZ Stain & Microscopic Examination	First Infusion: ≥ 5.0 x 10^3 IEQ/kg of Recipient's Body Weight (Total IEQ/ infusion) Subsequent Infusions: ≥4.0 x 10^3 IEQ/kg of Recipient's Body Weight (Total IEQ/infusion)
Viability	FDA/PI Stain & Microscopic Examination	≥70% in each product bag
Purity		

Islets Concentration	DTZ Stain & 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's Body Weight (Total EU/infusion)
Gram Stain (Islet Purity Levels Pre-combination Samples)	Gram Stain	No Organisms Seen

IV: Total IEQ Infused per PHPI Transplant

Table IV-A: Total IEQ Infused per PHPI Transplant

Category	Number of PHPI Transplants	Mean (SD)	Median	Min-Max
Subjects with 1 PHPI Transplant Only	22	588,756 (193,284)	582,895	286,565-1,005,822
Subjects with 2 or more PHPI Transplants				
1st Transplant	26	487,703 (118,491)	464,823	322,092-716,720
2nd Transplant	26	479,781 (130,341)	460,073	282,156-771,338
3rd Transplant	1	585,590	585,590	585,590-585,590

IEQ, islet equivalent; PHPI, purified human pancreatic islets

V: Sterility Test Results for Pancreas Preservation Solution and Final Product

Preservation Solution: The identity of the contaminating microorganism in the 23/75 samples reported to be positive showed saprophytes of the skin (*Bacillus, sp*. not anthracis or ceruleus, gram positive rods) in 22/23 cases. The remaining case (1/23), identified C. *albicans* (see below).

Final Product Results: The sterility test results of 6/75 final product lots were reported to be positive. In 5/6 cases speciation showed skin saprophytes (Propionibacteria (2 lots); and Bacillus species, Diphtheroids, S. maltophilia*)* and in 1/6 case *C albicans* (for the

same lot that reported the presence of *C.albicans* in the preservation solution). The investigations conducted concluded that in 5/6 cases there was an operator sampling error. The corrective and preventive action (CAPA) in each of these cases included additional personnel training on aseptic processing. None of the recipients of these six lots exhibited signs or symptoms of infection post-infusion. The subject who received the lot that identified *C. albincans* received fluconasol prophylactically but never exhibited signs or symptoms of infection.

Data analysis showed that there was no significant difference among manufacturing centers in the frequency of contamination of the preservation solution or the final product among the eight manufacturing facilities.

Table V-A: Sterility Test Results for Pancreas Preservation Solution and Final Product

Parameter	**Preservation Solution N (%)**			**PHPI Final Product N (%)**	
Center	**Negative**	**Positive**	**Not Available**	**Negative**	**Positive**
03	7 (77.8)	2 (22.2)		7 (77.8)	2 (22.2)
04	3 (75)	1 (25)		4 (100)	
05	10 (58.8)	7 (41.2)		16 (94.1)	1 (5.9)

Parameter	Preservation Solution N (%)			PHPI Final Product N (%)	
Center	Negative	Positive	Not Available	Negative	Positive
06	10 (66.7)	5 (33.3)		15 (100)	
10	2 (28.6)	4 (57.1)	1 (14.3)	7 (100)	
17	11 (91.7)	1 (8.3)		11 (91.7)	1 (8.3)
18	4 (66.7)	2 (33.3)		4 (66.7)	2 (33.3)
19	4 (80)	1 (20)		5 (100)	
All Centers	51 (68.0)	23 (30.7)	1 (1.3)	69 (92)	6 (8)
Fisher's Exact Test p-value: 0.268				**Fisher's Exact Test p-value: 0.174**	