CIT ISLET-ALONE PROTOCOLS

CIT-04: LABORATORY MANUAL
FOR CENTRAL LABORATORY ASSESSMENTS

VERSION 7.0

JANUARY 18, 2012

CONFIDENTIAL

TABLE OF CONTENTS

List of Appendices

1. ADDITIONAL STUDY CONTACT INFORMATION

PPD Development	**Tandem Labs**	**Protocol Coordinator**
Attn: Maria Edwards 2244 Dabney Rd Richmond, VA 23230 Phone: 804-359-1900 Fax: 804-253-1104	Attn: Dania Yaskanin 115 Silvia Street West Trenton, NJ 08628 Phone: 609-228-0243 Fax: 609-434-1028	Cynthia Diltz, RN, BSN, CCRC University of Iowa Department of Biostatistics Clinical Trials Statistical and Data Management Center 2400 University Capitol Centre Iowa City, IA USA 52242-5500 Phone: +1-319-353-4982 Fax: +1-319-353-3960 cynthia-diltz@uiowa.edu

2. CIT-04 CENTRAL LABORATORY SPECIMEN SCHEDULE

Central Laboratory Assessments					
Assessment	Laboratory	Visit / Time-point	Volume	Collection Container	Shipping Instructions
Albumin/Creatinine Ratio	University of Minnesota	V08,10,21 (Subs Tx Day 75[3], Day 365)[2] [V56, 63][6]	5 mL Urine	Sterile Urine Container	Ship in batches weekly, frozen on dry ice. Ship on Monday – Thursday.
Alloantibodies	University of Pennsylvania	V03,10,15,18,21 (V24,27,30)[1] (Subs Tx Day 0, 75[3], 365)[2] [V56, 63][6]	2 mL Blood	(1) 3-mL Red-top Vacutainer	Ship on dry ice in batches at least quarterly. Ship Monday – Thursday only.
Atherogenic Profile	University of Washington	V21 (Sub Tx Day 365)[2] [V63][6]	8.5 mL Blood	(1) 8.5 mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
Autoantibodies	Barbara Davis Center	V10,15,18,21 (V24,27,30)[1] (Subs Tx Day 75[3], 365)[2] [V56, 63][6]	2 mL Blood	(1) 3-mL Red-top OR (1) 3.5 mL Tiger top SST	Ship on dry ice in batches at least quarterly .Ship Monday – Wednesday only. If collected on Thursday or Friday, freeze serum at -20°C or -70°C until Monday, Tuesday, or Wednesday.
Belatacept Trough Level	PPD Development	V04,05,06,07,08,09,10 11,12,13,14,15,16,17,18,19, 20,21 (V22,23,24,25,26,27, 28,29,30)[1] (Subs Tx Day 4, 7, 75[3], 365)[2] [V51,52,53,54,55,56,57,58, 59,60,61,62,63][6]	2 mL Blood	(1) 3.5 mL Gold SST	Ship in batches bi-monthly on dry ice. Ship Monday – Thursday.
Fasting serum glucose and c-peptide / serum creatinine	University of Washington	V08,09,10[4],12,13,14, 15,16,17,18,19,20,21 (V22,23,24,25,26,27, 28,29,30)[1] (Subs Tx Day 75[3], 365)[2] [V56,63,64][6]	2 mL Blood	(1) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.

Central Laboratory Assessments					
Assessment	Laboratory	Visit / Time-point	Volume	Collection Container	Shipping Instructions
GFR	University of Minnesota	V08,10,21 (Subs Tx Day 75[3], 365)[2] [V63][6]	10 mL Blood 2 mL each at 120, 150, 180, 210 and 240 minutes	(5) 2-mL Na Heparin Tube	Ship in batches weekly on dry ice. Ship Monday – Thursday
Hemoglobin A1c (HbA1c)	University of Washington	V10,15,18,21, (V24,27,30)[1] (Subs Tx Day 75[3], 365)[2] [V53, 56, 60, 63, 64][6]	2 mL Blood	(1) 2-mL Lavender top EDTA Vacutainer	Ship on cold pack within 24 hours of collection. Ship Monday-Thursday only.
Immunogenicity Sample	Tandem Labs	V02,14,21 (V27)[1] (Subs Tx Day 365)[2] [V63, 64][6]	4 mL Blood	(1) 4-mL Gold SST	Ship in batches quarterly on dry ice. Ship Monday – Wednesday
Insulin Modified FSIGT	University of Washington	V10[4],21 (Subs Tx Day 75[3], 365)[2]	48 mL Total Blood 2 mL each at -10, -5, and -1 minutes pre-injection of glucose 2 mL each at 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes post injection of glucose	(24) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
MMTT: Stimulated serum glucose and c-peptide	University of Washington	V10[4],15,18,21 (V24,27,30)[1] (Subs Tx Day 75[3], 365)[2] [V56, 63, 64][6]	4 mL Total Blood • 2 mL at 60 minutes (*ONLY if checking for graft failure*) • 2 mL at 90 minutes (Note: the Fasting serum glucose and c-peptide/serum creatinine is the 0 hour sample for the MMTT)	(2) 3.5-mL Gold SST	Ship on dry ice in batches at least weekly. Ship Monday – Thursday only.
PBMC / Plasma to Archive	ITN Central Cell Processing Core Facility	V10,15,18,21 (V24,27,30)[1] (Subs Tx Day 75[3], 365)[2]	30 mL Blood	(3) 10-mL Na Heparin Tube	Ship ambient daily.
RNA to Archive	Expression Analysis	V10,15,18,21 (V24,27,30)[1] (Subs Tx Day 75[3], 365)[2]	9 mL Blood	(3) 3-mL Tempus RNA Tube	Ship in batches quarterly on dry ice.

Central Laboratory Assessments					
Assessment	Laboratory	Visit / Time-point	Volume	Collection Container	Shipping Instructions
Serum to Archive	NIDDK Repository	V10,15,18,21 (V24,27,30)[1] (Subs Tx Day 75[3], 365)[2]	4 mL Blood	(1) 4-mL Gold SST	Ship in batches at least quarterly.
TAT Complex, C3a, c-peptide	Rudbeck Laboratory	V03[5] (Subs Tx Day 0)[2]	10 mL Total Blood @ V03 2 mL at Pre-immunosuppression, immediately pre-tx, 15, 60, 180 min post-tx	(5) 2-mL Lavender top EDTA Vacutainer	Ship on dry ice (minimum 5 kg) in batches at least quarterly.

[1]Visits as needed for subsequent islet transplant(s). See Appendix 2 – Year Two Schedule of Events
[2]Endpoint assessments for subsequent transplant(s). See Appendix 3 – Subsequent Transplant Schedule of Events
[3]If third transplant occurs less than 75 days after the second transplant, the 75 day endpoint data for the second transplant will not be collected
[4]Do not collect these samples at Day 75 for subjects with confirmed graft failure
[5]Collect these samples beginning on Day -2 (pre-IS) for Visit 03
[6]1-Year Additional Follow-up

3. THROMBIN-ANTITHROMBIN COMPLEX (TAT), C3a, AND C-PEPTIDE

- Refer to the General Lab manual for instructions regarding blood draws, processing and shipping
- Subjects randomized to CIT-04 do not receive immunosupression on Day -2. For those subjects, the Pre Immunosuppression sample, labeled Pre IS, is drawn on day 0 prior to the administration of Belatacept, Daclizumab or MMF; the Pre-Transplant sample, labeled Pre-Tx, is drawn immediately prior to the start of the islet infusion

4. BELATACEPT TROUGH LEVEL

- Complete test **prior to** Belatacept infusion.
- Collect 2 ml Blood into 3.5 ml Gold Serum Separator (SST) tube
- Allow to clot at room temperature for 15 – 30 minutes
- Centrifuge for 15 minutes at 1000 xg (approximately 3000 RPM) in a refrigerated centrifuge
- Transfer at least 0.5 ml serum into a purple cap cryovial
- Immediately freeze at -20° C or colder
- Follow packing instructions outlined in section 4 of General Lab Manual
- Ship samples monthly on dry ice. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (Appendix 5 in General Lab Manual)
- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. NOTE: you will need to expand the columns in the specimen list to show all the data before printing. An automatic email will be sent to:

  Susan.Steever@bms.com

  MG-PK-Manifest@bms.com

  maria.edwards@richmond.ppdi.com

  Kathy.zelinsky@bms.com
  Mehmooda.shaikh@bms.com

- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form and fax a copy of the form including the airbill tracking number to the laboratory at +1-804-253-1104.
- Shipments must be made Monday through Thursday.

PPD Development
Attn: Maria Edwards
2244 Dabney Rd
Richmond, VA 23230
Tel: 804-359-1900
Fax: 804-253-1104

5. IMMUNOGENICITY SAMPLE

- Collect immunogenicity samples **prior to** belatacept infusion. For subjects who discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.
- Collect 4 ml Blood into 4ml Gold Serum Separator (SST) tube
- Allow to clot at room temperature for 15 – 30 minutes
- Centrifuge for 15 minutes at 1000 xg (approximately 3000 RPM) in a refrigerated centrifuge
- Transfer at least 2 ml serum into a white cap cryovial
- Immediately freeze at -20° C or colder
- Follow packing instructions outlined in section 4 of General Lab Manual
- Ship samples quarterly on dry ice. If shipping from Canada to U.S., please complete an Expanded Service International Air Waybill (With Dry Ice) (Appendix 5 in General Lab Manual)
- On the day of the shipment, print the Specimen List by clicking the View Shipping Report button in the Specimen Tracking System and place a copy in the shipping box. NOTE: you will need to expand the columns in the specimen list to show all the data before printing. An automatic email will be sent to:

  Susan.Steever@bms.com
  zoe.tzogas@bms.com
  Leec4@labcorp.com
  albanej@labcorp.com
  mcgrata@labcorp.com
  Mehmooda.shaikh@bms.com
  Yaskand@Labcorp.com

- If you are unable to print the Specimen List from the Specimen Tracking System on the day of shipment, complete a Specimen Submission Form and fax a copy of the form including the airbill tracking number to the laboratory at +1-804-253-1104.
- Shipments must be made Monday through Wednesday.

Tandem Labs
Attn**:** Dania Yaskanin
115 Silvia Street
West Trenton, NJ 08628
Phone: 609.228.0243
Fax: 609.434.1028

Note: On visits 14 and 21 you will need to draw both the Belatacept Trough Level and the Immunogenicity sample. On these 2 visits you can draw 5 mL of blood in 1 – 5mL SST tube. Then follow the processing directions in sections 3&4, aliquot 0.5 mL serum in the purple cap cryovial and 2 mL into the white cap cryovial. Shipper above instructions.

6. CIT-04 KIT COMPONENTS

VISIT 03 **Day 0**	**KIT #3**	**TAT, C3a, C-Peptide** (5) 2-mL EDTA Vacutainer Tube (5) 1.8-mL Cyrogenic Vial **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial	**Immunogenicity sample** (1) 4-mL Gold SST (1) 4.0-mL white cap Cryogenic Vial
VISIT 04 – 07 **Day 4, 7, 14, 21** **[Subs Tx Day 4, 7] (if needed)**	**KIT #4**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	
VISIT 08 **Day 28**	**KIT #5**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial	**GFR** (5) 2-mL Na Heparin Tubes (5) 1.8-ml Cryogenic Vials **Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial
VISIT 09 **Days 56**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial
VISIT 10 **Day 75** ***Secondary Endpoint*** **[Subs Tx Day 75] (if needed)**	**KIT #7**	**[1]Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[1,2]MMTT : Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **[1]FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials	**GFR** (5) 2-mL Na Heparin Tubes (5) 1.8 ml Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10-mL Na Heparin Vacutainer tubes **Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial
VISIT 11 **Day 84**	**KIT #4**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	

VISIT 12,13 **Day 112, 140**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial
VISIT 14 **Day 168**	**Kit #8**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level /Immunogenicity sample** (1) 5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial (1) 4.0-mL white cap Cryogenic Vial
VISIT 15 **Day 196**	**KIT #9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10 mL Na Heparin Vacutainer tubes **Belatacept Trough Level** (1) 3.5 mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial
VISIT 16, 17 **Days 224, 252**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial
VISIT 18 **Day 280**	**KIT #9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10 mL Na Heparin Vacutainer tubes **Belatacept Trough Level** (1) 3.5 mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial
VISIT 19, 20 **Days 308, 336**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial

VISIT 21 **Day 365 / Month 12** ***Secondary Endpoint*** **Subs Tx Day 365 (if needed)**	**KIT #10**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **FSIGT** (24) 3.5-mL Gold SST (58) 1.8-mL Cryogenic Vials **Atherogenic Profile** (1) 8.5 mL Gold SST (4) 1.8 mL Cryogenic Vials	**GFR** (5) 2-mL Green-top Vacutainer Tubes (5) 1.8-ml Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10-mL Na Heparin Vacutainer tubes **Belatacept Trough Level /Immunogenicity sample** (1) 5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial (1) 4.0-mL white cap Cryogenic Vial
VISIT 22, 23 **Day 392, 420**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial
VISIT 24 **Day 448**	**KIT #9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10 mL Na Heparin Vacutainer tubes **Belatacept Trough Level** (1) 3.5 mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial
VISIT 25, 26 **Day 476, 504**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial

VISIT 27 **Day 532**	**Kit #11**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials [2]**MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10-mL Na Heparin Vacutainer tubes **Belatacept Trough Level /Immunogenicity sample** (1) 5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial (1) 4.0-mL white cap Cryogenic Vial
VISIT 28, 29 **Day 560, 588**	**KIT #6**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**Belatacept Trough Level** (1) 3.5 mL Gold SST (1)1.8-mL purple cap Cryogenic Vial
VISIT 30 **Day 616**	**KIT #9**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials [2]**MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **HBA1C** (1) 2-mL EDTA Vacutainer Tube	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Serum to Archive** (1) 4-mL Gold SST Vacutainer Tube (3) 1.8-mL Cryogenic Vials **RNA to Archive (NIDDK)** (3) 3-mLTempus RNA Tube **PBMC and Plasma to Archive** (3) 10 mL Na Heparin Vacutainer tubes **Belatacept Trough Level** (1) 3.5 mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial
Visit 51, 52 **M13 (post final tx),** **M14 (post final tx)**	**KIT #4**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	
Visit 53 **M15 (post final tx)**	**KIT #13**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	**HBA1C** (1) 2-mL EDTA Vacutainer Tube
Visit 54, 55 **M16 (post final tx),** **M17 (post final tx)**	**KIT #4**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	

Visit 56 **M18 (post final tx)**	**KIT #14**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HBA1C** (1) 2-mL EDTA Vacutainer Tube **Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial
Visit 57, 58, 59 **M19 (post final tx),** **M20 (post final tx)** **TBD (extra visit)**	**KIT #4**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	
Visit 60 **M21 (post final tx)**	**KIT #13**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	**HBA1C** (1) 2-mL EDTA Vacutainer Tube
Visit 61, 62 **M22 (post final tx),** **M23 (post final tx)**	**KIT #4**	**Belatacept Trough Level** (1) 3.5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial	
Visit 63 **M24 (post final tx)**	**KIT #15**	**Albumin/Creatinine Ratio** (1) Urine Specimen Container (1) 4.0-mL Cryogenic Vial **HBA1C** (1) 2-mL EDTA Vacutainer Tube **GFR** (5) 2-mL Green-top Vacutainer Tubes (5) 1.8-ml Cryogenic Vials **Atherogenic Profile** (1) 8.5 mL Gold SST (4) 1.8 mL Cryogenic Vials **Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Belatacept Trough Level /Immunogenicity sample** (1) 5-mL Gold SST (1) 1.8-mL purple cap Cryogenic Vial (1) 4.0-mL white cap Cryogenic Vial
Visit 64 **Y2 (post initial tx)**	**KIT#16**	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **Immunogenicity sample** (1) 4-mL Gold SST (1) 4.0-mL white cap Cryogenic Vial	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **[2]MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials

4 or 8 weeks post last dose of Belatacept	**KIT #12**	**Immunogenicity sample ____ week post last dose** (1) 4-mL Gold SST (1) 4.0-mL white cap Cryogenic Vial **(note which visit on the requisition)**	
Reduced Follow-Up **(Year 1 and 2 post Initial transplant)**	**KIT #50**	**90 min C-peptide post MMTT, Serum Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials	**HBA1C** (1) 2-mL EDTA Vacutainer Tube **Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial
Reduced Follow-Up **(Monthly and Quarterly)**	**KIT #50X**	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8-mL Cryogenic Vial	
Subs Tx Day 0 **(if needed)**	**KIT #51**	**TAT, C3a, C-Peptide** (5) 2-mL EDTA Vacutainer Tube (5) 1.8-mL Cyrogenic Vial	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial
Suspected Graft Failure	**Kit 50Z**	**Fasting Serum Glucose, C-Peptide, Creatinine** (1) 3.5-mL Gold SST (2) 1.8-mL Cryogenic Vials **MMTT: Stimulated Glucose and C-peptide** (2) 3.5-mL Gold SST (4) 1.8-mL Cryogenic Vials	**Alloantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial **Autoantibody** (1) 3-mL Red-top Vacutainer Tube (1) 1.8 mL Cryogenic Vial

[1]Do not collect these samples at Day 75 for subjects with confirmed graft failure
[2]The kit contains two 3.5mL Gold SST tubes for the MMTT. Only one of these two tubes will be filled (at 90 minutes), unless it is suspected that the subject has suffered graft failure (in which case, the second tube should be filled at 60 minutes). If there is no suspicion of graft failure, the 60 minute tube and aliquots associated with the 60 minute draw can be discarded

See Appendix 1 for Kit Supply Order Form

6. CIT-04 MAXIMUM RESEARCH BLOOD VOLUME TABLE

CIT04 - MAXIMUM RESEARCH BLOOD VOLUME TABLE																						
TIME POINTS/VISITS																						
	Days					Weeks																
TIMING OF STUDY PARTICIPATION	SCRN	BL	TX 0	3	4	1	2	3	4	8	(Day 75)	12	16	20	24	28	32	36	40	44	48	52
VISIT	1	2	3	3a	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BLOOD VOLUMES																						
LOCAL LABORATORY ASSESSMENTS																						
CBC (WBC + Diff & Plat)	5	5	5			5	5	5	5	5	5		5	5		5			5			5
Chemistry (P18 + Mg or P20)	4	4	4			4	4	4	4	4	4		4	4		4			4			4
Lipids	4	4									4					4			4			4
Thyroid Function	4	4																				
Serum b-HCG (females)	4	4			4		4		4	4		4	4	4	4	4	4	4	4	4	4	4
PSA	4	4																				4
Serology	7	7																				
EBV IgG	2																					
CMV IgG, CMV IgM		4																				
Coagulation (PT, PTT, INR)	5	5	5																			
Blood Type & HLA		11																				
Crossmatch		10																				
PRA by flow cytometry (Sub Tx)		10																				
Fasting and 2 post-prandial (1-3 hrs) c-pep				9		9																
CMV by PCR		4									4				4							
EBV py PCR		4																				
CENTRAL LABORATORY AND METABOLIC ASSESSMENTS																						
GFR (5 timed specimens/timept; 2 ml each)	10	10							10		10											10
HbA1c (central + local)	4	4									4					4			4			4
Fasting glucose & c-pep / serum creatinine	2	2							2	2	2		2	2	2	2	2	2	2	2	2	2
Insulin modified FSIGT (c-pep, insulin, gluc)		48									48											48
60, 90 min c-pep, gluc (MMTT) / serum creat	4																					
90 min c-pep, gluc (MMTT) / serum creat											2					2			2			2
Belatacept Trough Level					2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Atherogenic Profile		8.5																				8.5
CENTRAL MECHANISTIC ASSAYS																						
Alloantibody	2	2									2					2			2			2
Autoantibody		2									2					2			2			2
TAT, c-peptide & C3a			10																			
Immunogenicity samples			4												4							4
CENTRAL ARCHIVED SAMPLES																						
Serum		4									4					4			4			4
PBMC & Plasma		30									30					30			30			30
RNA		9									9					9			9			9
TOTALS (mls)	61.0	199.5	28.0	9.0	6.0	20.0	15.0	11.0	27.0	17.0	132.0	6.0	17.0	17.0	16.0	74.0	8.0	8.0	74.0	8.0	8.0	148.5
BL - WK 6 TOTAL (mls)	315.5																					
YEAR TOTAL (mls)	910.0																					

Appendix 1: Kit Supply Order Form

Protocol CIT- 04: Please complete form and fax to University of Iowa @+1-319-353-3960

Site Name & #:	_____	CIT Protocol:	_____
Shipping Address:			
Order Date:		Due Date @ Site:	_____
Requested By:	_____	Requestor's phone:	_____
Requestor's FAX:	_____	Requestor's email:	_____

Kit(s) #	**QUANTITY**
Kit(s) # _____	_____
Kit(s) # _____	_____
Kit(s) # _____	_____
Kit(s) # _____	_____
Kit(s) # _____	_____

You will receive an initial supply of kits for 10 participants upon notice of your site activation. The initial supply of kits will include (1) Kit #1 through Kit # 5, per subject.

Please check your kits' expiration dates and DO NOT order more than a 6 month supply of kits.

Appendix 2: Year One Schedule of Events

Appendix 1: Year One Schedule of Events

Time points (in days relative to transplant)	SCR	WL / BL[1]	0[2]	3	4	7	14	21	28	56	75	84	112	140	168	196	224	252	280	308	336	365
Visit Number	01	02	03	03a	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
Visit Windows (in days)	N/A	N/A	N/A	N/A	N/A	+/-2	+/-2	+/-2	+/-3	+/-3	+/-5	+/-3	+/-3	+/-3	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5
Equivalent Week	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	W8	N/A	W12	W16	W20	W24	W28	W32	W36	W40	W44	W48	W52
GENERAL ASSESSMENTS																						
Informed Consent	X[3]	X[4]																				
Med/Diabetes Hx & Demographics	X																					
Eval of Inclusion / Exclusion	X	X																				
Mammogram (females >35)	X	X-yrly																				
Retinopathy Evaluation[5]	X	X-yrly[6]																				X
Physical Exam	X	X-yrly	X			X	X	X	X	X	X		X	X		X			X			X
QOL		X-q3mo									X					X			X			X
Chest X-Ray	X	X-yrly																				X
Abdominal US (Pelvis/Liver	X	X-yrly				X																X
ECG	X	X-yrly																				X
Cardiac Stress Test or Angiogram	X																					
PPD	X	X-yrly																				X
AE/Hypo Event/Toxicity Assess		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LOCAL LABORATORY ASSESSMENTS																						
CBC (WBC + Diff & Plat)	X	X-q6mo	X			X	X	X	X	X	X		X	X		X			X			X
Chemistry[7]	X	X-yq6mo	X			X	X	X	X	X	X		X	X		X			X			X
Lipids	X	X-q6mo									X					X			X			X
Thyroid Function (TSH)	X	X-yrly																				
Pregnancy test (WOCBP)	X	X[8]			X[9]		X[9]		X[9]	X[9]		X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]	X[9]

Time points (in days relative to transplant)	SCR	WL / BL[1]	0[2]	3	4	7	14	21	28	56	75	84	112	140	168	196	224	252	280	308	336	365
Visit Number	01	02	03	03a	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
Visit Windows (in days)	N/A	N/A	N/A	N/A	N/A	+/-2	+/-2	+/-2	+/-3	+/-3	+/-5	+/-3	+/-3	+/-3	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5
Equivalent Week	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	W8	N/A	W12	W16	W20	W24	W28	W32	W36	W40	W44	W48	W52
LOCAL LABORATORY ASSESSMENTS (Con't)																						
Serology[10] (Hep B/C, HIV)	X	X-yrly																				X
EBV IgG	X																					
CMV IgG, CMV IgM		X-yrly[11]																				X[11]
Coagulation (PT, PTT, INR)	X	X-yrly	X																			
Blood Type		X[12]																				
HLA		X																				
Crossmatch		X[13]																				
Fasting & post-prandial c-pep[14]				X		X																
Glucose (immediately post-tx)			X[15]																			
PRA by flow cytometry		X[16]																				
CMV by PCR		X									X				X							
EBV by PCR[17]		X																				
CENTRAL LABORATORY ASSESSMENTS																						
First morning spot urine[18]	X	X							X		X											X
GFR	X	X-yrly							X		X											X
HbA1c	X	X-q3mo									X					X			X			X
Fasting serum gluc/c-pep & creat[19]	X	X							X	X	X		X	X	X	X	X	X	X	X	X	X
Insulin modified FSIGT[19]		X-yrly[6]									X											X
90 min[20] c-pep/gluc (MMTT)[19]	X										X					X			X			X
Atherogenic Profile[21]		X																				X
LOCAL METABOLIC ASSESSMENTS																						
Glycemic Stability (CGMS)[19]		X[6]									X											X

Time points (in days relative to transplant)	SCR	WL / BL[1]	0[2]	3	4	7	14	21	28	56	75	84	112	140	168	196	224	252	280	308	336	365
Visit Number	01	02	03	03a	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
Visit Windows (in days)	N/A	N/A	N/A	N/A	N/A	+/-2	+/-2	+/-2	+/-3	+/-3	+/-5	+/-3	+/-3	+/-3	+/-3	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5	+/-5
Equivalent Week	N/A	N/A	N/A	N/A	N/A	W1	W2	W3	W4	W8	N/A	W12	W16	W20	W24	W28	W32	W36	W40	W44	W48	W52
BSR eCRFs[19,22]	X	X-q6mo									X					X			X			X
CALCULATED METABOLIC ASSESSMENTS																						
MAGE		X-q6mo									X					X			X			X
LI	X	X-q6mo									X					X			X			X
Clarke Score	X	X-q6mo														X						X
HYPO	X	X-q6mo									X					X			X			X
Beta Score		X									X					X			X			X
C-peptide (gluc X creat) ratio	X	X							X	X	X		X	X	X	X	X	X	X	X	X	X
IMMUNOSUPPRESSION LEVELS																						
Belatacept trough levels[23]					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MECHANISTIC ASSAYS																						
Alloantibody	X	X-q6mo[24]									X					X			X			X
Autoantibody[25]		X									X					X			X			X
TAT, C3a, & c-peptide		X	X[26]																			
Immunogenicity samples[27]			X[28]												X							X
ARCHIVED SAMPLES																						
Serum		X									X					X			X			X
PBMC & Plasma		X									X					X			X			X
RNA		X									X					X			X			X

[1] WL = Waiting List. BL = Baseline. Repeat assessments as indicated (i.e. yrly, q3mo), while subject is on the waiting list. All one-time WL/BL assessments should be completed on Day -2 whenever possible, but always prior to start of immunosuppression. For WL/BL repeat assessments, record results from test done closest to the start of immunosuppression will be used as the baseline value.
[2] Day 0 = the day of transplant.
[3] *Informed consent #1* includes information on CIT04 and the multi-center Phase 3 protocol (CIT07)
[4] *Informed Consent #2* includes information specific to CIT04. IC # 2 must be signed immediately after randomization.
[5] Retinopathy eval includes fundoscopic pictures for WL/BL assessments and Y1. Screening retinopathy evaluation should be done per site-specific standards. If pupils cannot be dilated, then a manual ophthalmologic evaluation can be substituted.

[6] These can be collected after subject is considered protocol eligible and has been moved to the transplant wait list, as time allows.
[7] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus
[8] Complete serum pregnancy tests within 72 hours prior to initiation of study medication.
[9] Complete test prior to Belatacept infusion. Confirm negative result prior to administering Belatacept
[10] Serology includes: HBc Ab, HBs Ab, HBs Ag, HCV Ab, and HIV. Do not repeat Hepatitis B tests if HBs Ab was previously positive.
[11] Repeat only if previous test was negative.
[12] Repeat for subsequent transplant(s)
[13] Sample used for crossmatch may be obtained up to 60 days prior to the start of immunosuppression, as long as there is no evidence of infections or transfusions since the time the sample was drawn. Repeat crossmatch for subsequent transplants.
[14] C-peptide should be done locally and drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.
[15] Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.
[16] Subsequent transplants only. Local result used to determine eligibility for subsequent transplants only.
[17] EBV by PCR should only be done post-randomization if reactivation is suspected.
[18] First morning spot urine includes: albumin, protein, and creatinine
[19] Do not collect for participants with graft failure. Results of tests performed at the time of graft failure will be used for day 75 endpoint calculations.
[20] MMTT should include 60 and 90 minute c-peptide and glucose measurements for the screening visit and as necessary when determining graft failure.
[21] Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
[22] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements
[23] Complete test prior to Belatacept infusion.
[24] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw. Central PRA result, current within 6 months, is used to determine subject eligibility for first transplant
[25] Autoantibody testing includes GAD, IA-2, and IAA.
[26] TAT & c-peptide: pre-tx, 15, 60, 180 min post-tx
[27] Collect immunogenicity samples prior to belatacept infusion. For subjects that discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.
[28] Collect immunogenicity sample prior to dosing with immunosuppression medications.

Appendix 3: Continuation of Appendix 1 Schedule of Events (Subjects with Subsequent CIT Transplants)

Appendix 2: Continuation of Appendix 1 Schedule of Events (Subjects with Subsequent CIT Transplants)

Subjects who receive a subsequent islet transplant should continue their study visits according to this Schedule of Events until one year (365 +/- 5 days) after the final islet transplant. Additional visits - outlined in Appendix 3 - will also need to be completed after each subsequent transplant. After day 365 post final transplant, subjects should stop following this schedule, and complete the follow-up visits outlined in Appendix 4: *Schedule of Events for 1-Year Additional Follow-Up*.

Time points (in days relative to 1st transplant)	392	420	448	476	504	532	560	588	616
Visit Number	22	23	24	25	26	27	28	29	30
Visit Windows (in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5
Equivalent Week	W56	W60	W64	W68	W72	W76	W80	W84	W88
GENERAL ASSESSMENTS									
Physical Exam			X			X			X
QOL			X			X			X
AE/Hypo Event/Toxicity Assessment	X	X	X	X	X	X	X	X	X
LOCAL LABORATORY ASSESSMENTS[1]									
CBC (WBC + Diff & Plat)			X			X			X
Chemistry[2]			X			X			X
Lipids			X			X			X
Pregnancy test (WOCBP)[3]	X	X	X	X	X	X	X	X	X
CENTRAL LABORATORY ASSESSMENT									
HbA1c			X			X			X
Fasting serum glucose & c-peptide & serum creat[4]	X	X	X	X	X	X	X	X	X
90 min[5] c-peptide/glucose (MMTT)[4]			X			X			X
Atherogenic Profile[6]	*1 year post-final transplant*								
LOCAL METABOLIC ASSESSMENTS									
BSR eCRFs[4,7]			X			X			X
CALCULATED METABOLIC ASSESSMENTS									
MAGE			X			X			X
LI			X			X			X
Clarke Score						X			
HYPO			X			X			X

Appendix 2: Continuation of Appendix 1 Schedule of Events (Subjects with Subsequent CIT Transplants)

Subjects who receive a subsequent islet transplant should continue their study visits according to this Schedule of Events until one year (365 +/- 5 days) after the final islet transplant. Additional visits - outlined in Appendix 3 - will also need to be completed after each subsequent transplant. After day 365 post final transplant, subjects should stop following this schedule, and complete the follow-up visits outlined in Appendix 4: *Schedule of Events for 1-Year Additional Follow-Up*.

Time points (in days relative to 1st transplant)	392	420	448	476	504	532	560	588	616
Visit Number	22	23	24	25	26	27	28	29	30
Visit Windows (in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5
Equivalent Week	W56	W60	W64	W68	W72	W76	W80	W84	W88
GENERAL ASSESSMENTS									
Physical Exam			X			X			X
QOL			X			X			X
AE/Hypo Event/Toxicity Assessment	X	X	X	X	X	X	X	X	X
LOCAL LABORATORY ASSESSMENTS[1]									
CBC (WBC + Diff & Plat)			X			X			X
Chemistry[2]			X			X			X
Lipids			X			X			X
Pregnancy test (WOCBP)[3]	X	X	X	X	X	X	X	X	X
CENTRAL LABORATORY ASSESSMENT									
HbA1c			X			X			X
Fasting serum glucose & c-peptide & serum creat[4]	X	X	X	X	X	X	X	X	X
90 min[5] c-peptide/glucose (MMTT)[4]			X			X			X
Atherogenic Profile[6]	*1 year post-final transplant*								
LOCAL METABOLIC ASSESSMENTS									
BSR eCRFs[4,7]			X			X			X
CALCULATED METABOLIC ASSESSMENTS									
MAGE			X			X			X
LI			X			X			X
Clarke Score						X			
HYPO			X			X			X

Time points (in days relative to 1st transplant)	392	420	448	476	504	532	560	588	616
Visit Number	22	23	24	25	26	27	28	29	30
Visit Windows (in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5
Equivalent Week	W56	W60	W64	W68	W72	W76	W80	W84	W88
CALCULATED METABOLIC ASSESSMENTS (continued)									
Beta Score			X			X			X
C-peptide (glucose X creatinine) ratio	X	X	X	X	X	X	X	X	X
IMMUNOSUPPRESSION LEVELS									
Belatacept trough levels[8]	X	X	X	X	X	X	X	X	X
MECHANISTIC ASSAYS									
Alloantibodies[9]			X			X			X
Autoantibodies[10]			X			X			X
Immunogenicity samples[11]						X			
ARCHIVED SAMPLES									
Serum			X			X			X
PBMC & Plasma			X			X			X
RNA			X			X			X

[1] EBV by PCR should only be done post-randomization if reactivation is suspected.
[2] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus
[3] Confirm negative result prior to administering Belatacept.
[4] Also collect as necessary to perform graft failure. Do not collect after graft failure has been confirmed.
[5] MMTT should include 90 minute c-peptide and glucose measurements, add 60 minute as necessary when determining graft failure.
[6] Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
[7] Blood sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[8] Complete test prior to Belatacept infusion. For pregnancy test, confirm negative result prior to administering Belatacept.
[9] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw.
[10] Autoantibody testing includes GAD, IA-2, and IAA.
[11] Collect immunogenicity samples prior to belatacept infusion. For subjects that discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.

Appendix 4: Subsequent Transplant Schedule of Events

Appendix 3: Subsequent Transplant Schedule of Events

The following immediate post-transplant and endpoint assessments should be completed for subjects who receive a subsequent islet transplant. If any of the visits below fall within an acceptable window of a follow-up visit on the Year One Schedule of Events (Appendix 1) or Year Two Schedule of Events (Appendix 2), the assessments may be added to that follow-up visit so that a separate visit does not need to occur.

Time point (in days relative to most recent infusion)	0[1]	3	4	7	75	365
Visit Number	TBD	TBD	TBD	TBD	TBD	TBD
Visit Window (in days)	N/A	N/A	N/A	+/- 3	+/- 5	+/- 14
Equivalent Week (post most recent infusion)	N/A	N/A	N/A	W1	N/A	W52
GENERAL ASSESSMENTS						
Physical Exam	X			X	X	X
QOL					X	X
Chest X-Ray						X
Abdominal US (Pelvis/Liver)				X		X
ECG						X
PPD						X
AE/Hypo Event/Toxicity Assess	X		X	X	X	X
LOCAL LABORATORY ASSESSMENTS						
CBC (WBC + Diff & Plat)	X			X	X	X
Chemistry[2]	X			X	X	X
Lipids					X	X
Coagulation (PT, PTT, INR)	X					
Blood Type & HLA	X[3]					
Crossmatch	X[4]					
PRA by flow cytometry	X[5]					
Fasting & post-prandial c-peptide[6]		X		X		
Glucose (immediately post-transplant)[7]	X					
Pregnancy test (WOCBP)[8]	X					X
CMV by PCR[9]					X	
CENTRAL LABORATORY ASSESSMENTS						
First morning spot urine[10]					X	X
GFR					X	X
HbA1c					X	X
Fasting serum glucose/c-peptide & creat[11]					X	X
Insulin modified FSIGT[11]					X	X
90 min c-pep/glucose (MMTT)[11]					X	X
Artherogenic Profile[12]						X
LOCAL METABOLIC ASSESSMENTS						
Glycemic Stability (CGMS)[11]					X	X
BSR eCRFs[11,13]					X	X
CALCULATED METABOLIC ASSESSMENTS						
MAGE					X	X
LI					X	X
Clarke Score						X
HYPO					X	X
Beta Score					X	X

LEA29Y Emory Edmonton Protocol (LEEP)

Time point (in days relative to most recent infusion)	0[1]	3	4	7	75	365
Visit Number	TBD	TBD	TBD	TBD	TBD	TBD
Visit Window (in days)	N/A	N/A	N/A	+/- 3	+/- 5	+/- 14
Equivalent Week (post most recent infusion)	N/A	N/A	N/A	W1	N/A	W52
CALCULATED METABOLIC ASSESSMENTS (continued)						
C-peptide glucose creatinine ratio					X	X
IMMUNOSUPPRESSION LEVELS						
Belatacept trough levels[14]			X	X	X	X
MECHANISTIC ASSAYS						
Alloantibody[15]	X				X	X
Autoantibody[16]					X	X
TAT, C3a, & c-peptide	X[17]					
Immunogenicity samples[18]						X
ARCHIVED SAMPLES						
Serum					X	X
PBMC & Plasma					X	X
RNA					X	X

[1] Day 0 = the day of transplant.
[2] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO_2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus
[3] Repeat for subsequent transplant(s).
[4] Sample used for crossmatch may be obtained up to 60 days prior to islet infusion, as long as there is no evidence of infections or transfusions since the time the sample was drawn.
[5] PRA by flow cytometry should be performed locally prior to any subsequent transplant. Local result used to determine eligibility for subsequent transplants only.
[6] C-peptide should be done locally and drawn fasting, and twice between 1-3 hrs post-prandial on Day 3 and Day 7 post-transplant.
[7] Finger stick glucose should be done locally and drawn every hour for the first 6 hours immediately post-transplant.
[8] Perform pregnancy test and confirm negative result prior to each belatacept infusion.
[9] EBV by PCR should only be done post-randomization if reactivation is suspected.
[10] First morning spot urine includes: albumin, protein, and creatinine.
[11] Also collect as necessary to confirm graft failure. Do not collect after graft failure has been confirmed.
[12] Atherogenic profile consisting of fasting lipid panel (TG, TC, HDL, LDL, non-HDL), C reactive protein, serum amyloid A, apolipoprotein A1 and apolipoprotein B. If blood is drawn locally, sample should be sent from local lab to study site and then shipped to the central laboratory (Univ of Washington).
[13] Blood Sugar Record (BSR) eCRF is completed using information gathered from subject diary logs, glucometer download data, and insulin requirements.
[14] Complete test prior to belatacept infusion. For pregnancy test, confirm negative result prior to administering belatacept.
[15] For each transplant, complete alloantibody assessment every 6 months and again on Day -2, regardless of the most recent draw.
[16] Autoantibody testing includes GAD, IA-2, and IAA.
[17] TAT & c-peptide: pre-infusion, 15, 60, and 180 min post-infusion.
[18] Collect immunogenicity samples prior to belatacept infusion. For subjects that discontinue belatacept, collect additional immunogenicity samples at 4 weeks and 8 weeks post last dose.

Appendix 5: Schedule of Events for 1-Year Additional F/U

Appendix 4: Schedule of Events for 1-Year Additional Follow-Up

Time point (Equivalent weeks after 'Day 365 post final transplant' visit)	W4 (AY)	W8 (AY)	W12 (AY)	W16 (AY)	W20 (AY)	W24 (AY)	W28 (AY)	W32 (AY)	W36 (AY)	W40 (AY)	W44 (AY)	W48 (AY)	W52 (AY)	Y2[1] (AY)
Visit Number (relative to final islet transplant)	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Visit Window (specified in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±30
GENERAL ASSESSMENTS														
Physical Exam						X							X	X
Telephone Consult			X							X				
QOL													X	X
AE /Hypoglycemic Events/Toxicity Assessment			X			X				X			X	X
LOCAL LABORATORY ASSESSMENTS														
CBC (WBC + Diff & Plat)			X			X				X			X	
Chemistry[2]			X			X				X			X	
Lipids						X							X	
Pregnancy test (WOCBP)[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	
CENTRAL LABORATORY ASSESSMENTS														
First morning spot urine[4]						X							X	
GFR													X	
HbA1c			X[5]			X				X[5]			X	X
90-min c-pep/glucose (MMTT)[2]						X							X	X
Atherogenic Profile													X	
LOCAL METABOLIC ASSESSMENTS														
Glycemic Stability (CGMS)[2]													X	X

Clinical Islet Transplantation (CIT) Protocol CIT-04 CONFIDENTIAL Page 113 of 116

[1] Two years post initial transplant.

[2] Also collect as necessary to confirm graft failure. Do not collect after graft failure is confirmed.

LEA29Y Emory Edmonton Protocol (LEEP) Version 7.0 (October 24, 2011)

Time point (Equivalent weeks after 'Day 365 post final transplant' visit)	W4 (AY)	W8 (AY)	W12 (AY)	W16 (AY)	W20 (AY)	W24 (AY)	W28 (AY)	W32 (AY)	W36 (AY)	W40 (AY)	W44 (AY)	W48 (AY)	W52 (AY)	Y2[1] (AY)
Visit Number (relative to final islet transplant)	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Visit Window (specified in days)	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±5	±30
CALCULATED METABOLIC ASSESSMENTS														
Clarke Score													X	
IMMUNOSUPPRESSION LEVELS														
Belatacept Trough Levels	X	X	X	X	X	X	X	X	X	X	X	X	X	
MECHANISTIC ASSAYS														
Autoantibody						X							X	
MECHANISTIC ASSAYS (Cont'd)														
Alloantibody						X							X	
Immunogenicity Samples													X	X

[1] Since belatacept is infused every 28 days, an additional infusion visit will be required during the second year. Since the timing is dependent on final transplant date, this visit can be shifted as needed.
[2] Chemistry includes: Sodium, albumin, magnesium, chloride, potassium, alk phosphatase, total bilirubin, CO2, creatinine, ALT (SGPT), BUN, gamma GT, glucose, AST (SGOT), calcium, phosphorus.
[3] Perform pregnancy test and confirm negative result prior to each belatacept infusion.
[4] First morning spot urine includes: albumin, protein, and creatinine.
[5] Can be drawn locally.

Appendix 6: Supply Order Form: Bulk Supply (shipping supplies)

Protocol CIT-99: Please complete form and fax to University of Iowa @+1-319-353-3960

Site Name & #:		CIT Protocol:	
Shipping Address:			
Order Date:		Due Date @ Site:	
Requested By:		Requestor's phone:	
Requestor's FAX:		Requestor's email:	

SHIPPING SUPPLIES	
Shipping Container – ambient kit	
Shipping Container – refrigerated kit	
Shipping Container – frozen kit	
STP-317 ambient gel packs	
FedEx Airbills to University of Washington—DRY ICE	
FedEx Airbills to University of Washington—COLD PACK	
FedEx Airbills to University of Pennsylvania	
FedEx Airbills to Barbara Davis Center	
FedEx Airbills to Rudbeck Laboratory	
FedEx Airbills to NIDDK Repository	
FedEx Airbills to ITN Central Cell Processing Core Facility	
FedEx Airbills to ITN RNA Isolation Core Facility	
FedEx Airbills to University of Minnesota	
FedEx Airbills to Tandem Lab	
FedEx Airbills to PPD	