

Supplementary Exhibit 2 (v1.0)

Note: CITR data/results quoted herein reflect a previous data lock (Jan 2017). A more recent data lock (Feb 2022) containing a greater number of participants was utilized for final manuscript analyses. This manuscript presents main cohort results from described analyses performed as possible, based on data availability.

Islet transplantation vs. standard care for type 1 diabetes from the Collaborative Islet Transplant Registry and the Type 1 Diabetes Exchange Clinic Registry

Statistical Analysis Plan (V0.12 25JAN2018)

NB: All CITR data/results quoted herein are from the Jan 2017 CITR data lock.

1. Background and Study Objectives

Allogeneic islet transplantation is an evolving treatment for type 1 diabetes, in particular for those individuals with long standing disease experiencing problematic hypoglycemia manifest by hypoglycemia unawareness and severe hypoglycemia events. The majority of clinical trials for allogeneic islet-alone transplantation (ITA) have involved single cohorts with small numbers of subjects, and when comparator groups have been involved, randomization has not been possible due to ethical concerns for on-going severe hypoglycemia events in a non-transplanted population. These ITA cohort studies have unequivocally demonstrated protection from hypoglycemia afforded by islet transplantation, with 3 multi-center national trials in patients experiencing severe hypoglycemia reporting a composite endpoint of HbA1c < 7.0% and freedom from severe hypoglycemia at one-year met in > 80% of islet transplant recipients. Nonetheless, islet transplantation comes with the cost of chronic immunosuppression to prevent alloimmune rejection and autoimmune recurrence, and so understanding the long-term benefits and risks associated with islet transplantation compared to on-going standard care is critically important. In particular, both diabetes and the immunosuppression required to support a functioning islet graft may impact kidney function, and whether the anticipated improvement in glycemic control expected with islet transplantation may offset any decline in kidney function associated with the immunosuppression regimen is unknown.

The Collaborative Islet Transplant Registry (CITR) represents the majority of islet transplantation activity in North American, Australia, and JDRF-sponsored programs in Europe, and presently includes 877 (CITR 10th Annual Report, in progress) islet transplant alone recipients. **We propose a case-control comparison of islet-alone transplantation (ITA) from the Collaborative Islet Transplant Registry (CITR) to standard care for type 1 diabetes of similar severity from the T1D Exchange using group-matched five-year cohorts in the US (with the possibility of presenting also the non-US ITA 5-year cohort) on primary clinical outcome measures of glycemic control and severe hypoglycemia events, and safety measures including mortality, kidney function, and diabetes complications progression.**

This is the Statistical Analysis Plan for the proposed investigation, to be undertaken jointly between the CITR investigators and the T1D Exchange investigators.

2. Inclusion/Exclusion and Group-Match Criteria

a. CITR ITA Inclusion Criteria

- Diagnosis of type 1 diabetes
- Islet transplant alone
- ≥1 year of follow up available post initial infusion
- No previous or concurrent pancreas or kidney transplant

From those subjects who meet the above criteria, the following main cohort will be analyzed:

- “CITR-ITA-with SHE” U.S. ITA recipients with at least one episode of severe hypoglycemia involving seizure or loss of consciousness in the year prior to transplant (N=58)
- We will retain those with *at least one episode of severe hypoglycemia requiring assistance* for possible future analysis

The following cohorts will also be drawn from the CITR data and held for possible analysis:

- *CITR-ITA-1 U.S. ITA recipients with at least one episode of severe hypoglycemia which required assistance (CITR definition) in the year prior to transplant (N=203)*
- *CITR-ITA-2 U.S. ITA recipients with no episodes of severe hypoglycemia in the year prior to transplant (N=37)*
- *CITR-ITA-3 Non-U.S. ITA recipients with at least one episode of severe hypoglycemia in the year prior to transplant (N=198)*
- *CITR-ITA-4 Non-U.S. ITA recipients with no episodes of severe hypoglycemia in the year prior to transplant (N=44)*

b. T1DX Controls Inclusion Criteria

- Diagnosis of type 1 diabetes
- No known prior islet, pancreas, or kidney transplant
- ≥1 year of follow up available

From those subjects who meet the above criteria, the following main cohort will be analyzed:

- “T1DX-with SHE involving seizure/LOC and no prior pancreas, islet or kidney transplant” --patients with at least one episode of severe hypoglycemia involving seizure/LOC in the year prior to registration (N=500)

These main cohorts (“CITR-ITA-with SHE” and “T1DX-with SHE”) will be group matched (mean±SD) on the baseline measures shown in the following table.

Table 1. Baseline characteristics of ITA cohorts to use as group-match criteria for the corresponding T1DX cohorts (Mean (SD))

	Main Cohort	These cohorts will not be used initially for comparison with T1DX; this information is just retained			
	US-ITA-SHE	ITA-1	ITA-2	ITA-3	ITA-4
Mean (SD)	U.S. Islet Transplant Recipients with ≥1 severe hypoglycemic events involving seizure or loss of consciousness at baseline (N=58)	U.S. Islet Transplant Recipients with ≥1 severe hypoglycemic events requiring assistance at baseline (N=203) – Includes the main cohort	U.S. Islet Transplant Recipients without severe hypoglycemic events at baseline (N=37)	Non-U.S. Islet Transplant Recipients with severe hypoglycemic events at baseline (N=198)	Non-U.S. Islet Transplant Recipients without severe hypoglycemic events at baseline (N=44)
Recipient Age (yrs)	42 (9)	44 (10)	45 (9)	46 (10)	40 (11)
Diabetes duration (yrs)	29 (10)	29 (11)	26 (11)	30 (12)	22 (10)
BMI	23.2 (2.1)	23.7 (2.5)	23.2 (3.1)	23.7 (3.0)	24.4 (3.4)
Baseline Daily Insulin Use (Units)	33.5 (11.2)	32.8 (10.9)	34.6 (11.6)	38.7 (14.0)	42.0 (15.7)
CKD-EPI-eGFR	91.6 (17.0)	91.2 (18.3)			
% Female	69%	67%	73%	57%	59%

CITR data as of 01/06/2017

Table 2. Timing of last infusion and total number of infusions for CITR ITA Cohorts			
Cohort	US-ITA-SHE (N=58)	ITA-1 (N=203)	ITA-2 (N=37)
	n		
Time from 1 st to last Infusions			
0-1 year	44	172	28
1-2 years	9	18	4
>2 years	5	13	5
Number of Infusions			
1-3	58	203	35
>3	0	0	2

Table 3A. Availability of Outcome measures at follow-up for US-ITA-SHE Cohort (N=58)					
	N with data at Year Post Last Infusion				
Outcomes	1	2	3	4	5
Absence of severe hypoglycemic events with seizure or loss of consciousness	49	41	33	34	28
Hypoglycemia Awareness	53	45	36	36	28
C-peptide ≥ 0.3 ng/mL	57	52	49	46	45
Fasting Blood Glucose of 60-140 mg/dL	52	43	35	30	29
HbA1c %	53	46	38	33	32
Daily Insulin Dose	45	38	31	32	29

Table 3B. Availability of Outcome measures at follow-up for <u>ITA-1</u> Cohort (N=203)					
	N with data at Year Post Last Infusion				
Outcomes	1	2	3	4	5
Absence of severe hypoglycemic events with seizure or loss of consciousness	156	127	104	101	73
Hypoglycemia Awareness	127	104	78	68	53
C-peptide ≥ 0.3 ng/mL	194	180	160	152	128
Fasting Blood Glucose of 60-140 mg/dL	174	141	111	95	76
HbA1c %	164	137	112	91	65
Daily Insulin Dose	136	92	69	62	53

Table 3C. Availability of Outcome measures at follow-up for <u>ITA-2</u> Cohort (N=37)					
	N with data at Year Post Last Infusion				
Outcomes	1	2	3	4	5
Absence of severe hypoglycemic events with seizure or loss of consciousness	32	26	24	15	14
Hypoglycemia Awareness	34	28	22	16	15
C-peptide ≥ 0.3 ng/mL	34	32	29	25	23
Fasting Blood Glucose of 60-140 mg/dL	34	27	23	16	15
HbA1c %	33	30	27	20	15
Daily Insulin Dose	30	26	22	16	14

3. General Statistical Considerations

The primary efficacy and safety outcomes are discussed in Section 4. below. The bolded ones comprise the primary efficacy outcomes for ITA in CITR, and will be analyzed at baseline and 1, 2, 3, 4, and 5 years post last islet transplant for the ITAs or post registry for T1DX, with all available data at each annual time point from the defined cohorts.

Based on outcome estimates available from the current CITR ITA cohort, the following table shows the minimum detectable difference in prevalence or in group means between ITA and T1DX at 5-years with at least 0.80 power, at $\alpha=0.05$, for each of the five specified outcomes.

Table 4. Minimum detectable difference in prevalence (percentage) or mean in primary outcomes at 5-years between T1DX-SHE (N=1000) and US-ITA-SHE/S-LOC, with at least 0.80 power and alpha=0.05

Primary Outcomes				
Absence of severe hypoglycemic events with seizure or loss of consciousness	C-peptide ≥ 0.3 ng/mL	Fasting Blood Glucose of 60-140 mg/dL	HbA1c %	Daily Insulin Dose
7%	21%	7%	0.6	6.4

Missing data. In accordance with standard methods of CITR utilized in annual reports and previous publications, when directly observed data are missing, but graft function is known to have been previously lost and not restored, insulin independence will be set as dependent and basal C-peptide will be set at 0.0. For T1DX data, c-peptide and fasting blood glucose values at baseline will be carried forward. Otherwise, missing data will be treated as missing at random in computations and modeling. No other imputation will be done unless indicated and agreed by both CITR and T1DX statisticians.

Analyses will be performed using SAS 9.4.

Baseline characteristics. The following characteristics at baseline will be summarized descriptively for each cohort and compared between corresponding ITA and T1DX cohorts:

- Continuous baseline variables
 - Age (years)
 - Duration of diabetes (years)
 - BMI
 - Number of severe hypoglycemia events (SHE) with seizure or loss of consciousness in prior year
 - Number of episodes of diabetic ketoacidosis (DKA) in prior year
 - Basal C-peptide, as available
 - HbA1c
 - Insulin requirements (units per day)
 - Self-monitoring of blood glucose (times per day)
 - Continuous glucose monitoring (days per week)
 - CKD-EPI-eGFR
 - Urine albumin to creatinine ratio
- Categorical baseline variables
 - Sex
 - Insulin delivery method
 - Prior intervention for retinopathy progression

Continuous variables will be summarized by mean and standard deviation and categorical variables will be summarized by percent (see Tables 3 Shell). In addition, tests of means (two-sample *t* test) or proportions (Pearson chi-square), respectively, will be performed comparing islet transplant cohort and pancreas transplant cohort to the T1DX control cohort) to identify any significant differences at baseline. Islet preparation characteristics and islet donor characteristics will be summarized with descriptive statistics for the CITR cohort (see Table 4 Shell).

Additional exploratory analyses, such as interactions, will be performed as indicated by the results of the planned analyses.

4. Analysis of Efficacy and Safety Outcomes

Efficacy Outcomes

- 1. HbA1c % (continuous measure), and prevalence of HbA1c, < 6.5%, and < 7.0% targets**
- 2. Basal C-peptide (≥ 0.3 ng/mL)**
- 3. Absence of severe hypoglycemic events involving seizure or loss of consciousness (SHE/S-LOC)**
- 4. HbA1c <7.0% with absence of SHE**
5. Hypoglycemia awareness (defined as aware, partially aware, or unaware)
6. Insulin requirements in units per day
- 7. Insulin independence (prevalence), as available**
8. Insulin delivery (pump, MDI, or none)
9. Glucose monitoring (SMBG, CGM)

Safety Outcomes

- Mortality
- Estimated glomerular filtration rate (eGFR; CKB-Epi equation)
- Urine albumin to creatinine ratio
- Progression of nephropathy (defined by need for dialysis or kidney transplantation with month/year as available)
- Progression of retinopathy (defined by retinal photocoagulation [laser], intravitreal injection, or vitrectomy treatment for diabetic retinopathy with month/year as available)
- Progression of neuropathy
- Progression of cardiovascular disease (defined by CV event with month/year as available)

For the ITA cohort, the categorical outcomes will be analyzed as percentage “successful” outcome at each yearly time point post last infusion, shown as bar charts of prevalence rates (see Figure 1 Shell and Example Figure 1); for the T1DX cohort comparable outcomes will be computed at annual time points post enrollment. Comparisons will be made by general

estimating equations with repeated subjects (multiple annual visits per patient), forcing cohort into the model and adjusting for covariates.

Unadjusted odds ratios and odds ratios adjusted for any significant differences in baseline characteristics comparing corresponding ITA and T1DX cohorts will be presented with 95% confidence intervals.

Continuous outcomes including HbA1c, fasting glucose, insulin requirements, eGFR, and urine albumin to creatinine ratio will be analyzed as continuous variables, and exhibited as box-plots (see Figure 2 Shell and Example Figure 2). Comparisons between ITA and T1DX cohorts will be done using mixed models with repeated measures with adjustment for covariates.

Time-to-event outcomes including mortality and progression of retinopathy, neuropathy, and cardiovascular disease will be analyzed using Kaplan-Meier plots (see Figure Shell and Example Figure 3). The Wilcoxon test will be used to determine significant differences in time to event between islet transplant cohort and the T1DX control group.

5. Table Shells and Figures

Table 5: Baseline characteristics of the main cohorts

	CITR ITA Recipients (N=)	T1DX Controls (No Islet Transplant) (N=)
Age (years)		
Sex (% female)		
Duration of Diabetes (years)		
BMI		
Number of SHE in prior year		
C-peptide (fasting)		
HbA1c		
Average glucose, random/as available		
Insulin requirements (units per day)		
Insulin delivery method (% pump or MDI)		
Self-monitoring of blood glucose (times per day)		
Continuous glucose monitoring (days per week)		
Estimated glomerular filtration rate		
Urine albumin to creatinine ratio		
Prior intervention for retinopathy progression (%)		

Table 5: Islet and islet donor characteristics

	US-ITA-SHE/S-LOC	ITA-1	ITA-2	ITA-3	ITA-4
	U.S. Islet Transplant Recipients with ≥ 1 severe hypoglycemic events with seizure or loss of consciousness at baseline (n=58)	U.S. Islet Transplant Recipients with ≥ 1 severe hypoglycemic events requiring assistance at baseline (n=)	U.S. Islet Transplant Recipients without severe hypoglycemic events at baseline (n=)	Non-U.S. Islet Transplant Recipients with severe hypoglycemic events at baseline (n=)	Non-U.S. Islet Transplant Recipients without severe hypoglycemic events at baseline (n=)
	Donor characteristics				
Mean Donor Age (yrs)					
Donor BMI					
Donor Blood Type O					
Donor Cause of Death: Trauma					
Donor Cause of Death: CVA					
Donor given insulin					
Donor given steroids					
Donor given vasopressors					
Donor given transfusions intra-operatively					
Donor given transfusions prior to surgery					
	Islet characteristics				
Hours Cold Ischemia					
Hours Culture Time (0-max)					
Islet Viability					

Stimulation Index					
Purity					
IEQs Infused (1,000s, over all infusions)					
IEQs Infused/kg Recipient Weight (over all infusions)					
Total Volume All Infusions (ml)					
Total Donor Beta Cells (1000s)					
Total Donor Beta Cells(1000s)/kg Recipient					
Total Insulin Content of Islets					
Embedded Islets (%)					
Total Islet Particles (final preparation) (1000)					
IEQ/Islet Particle Ratio					
Total DNA (µg)					
Total Endotoxin Infused (EU)					
Total Endotoxin Infused (EU)/kg Recipient					

Figure 1 Shell: Barcharts

Outcome												
	CITR-ITA						T1DX-Control					
O.R. (95% CI)	U.S. Islet to control											
Year	0	1	2	3	4	5	0	1	2	3	4	5
% with Outcome												
N												
Unadjusted O.R. (95% CI)	Compared to control						Reference					
Adjusted O.R. (95% CI)	Compared to control						Reference					

Example Figure 1: Barcharts

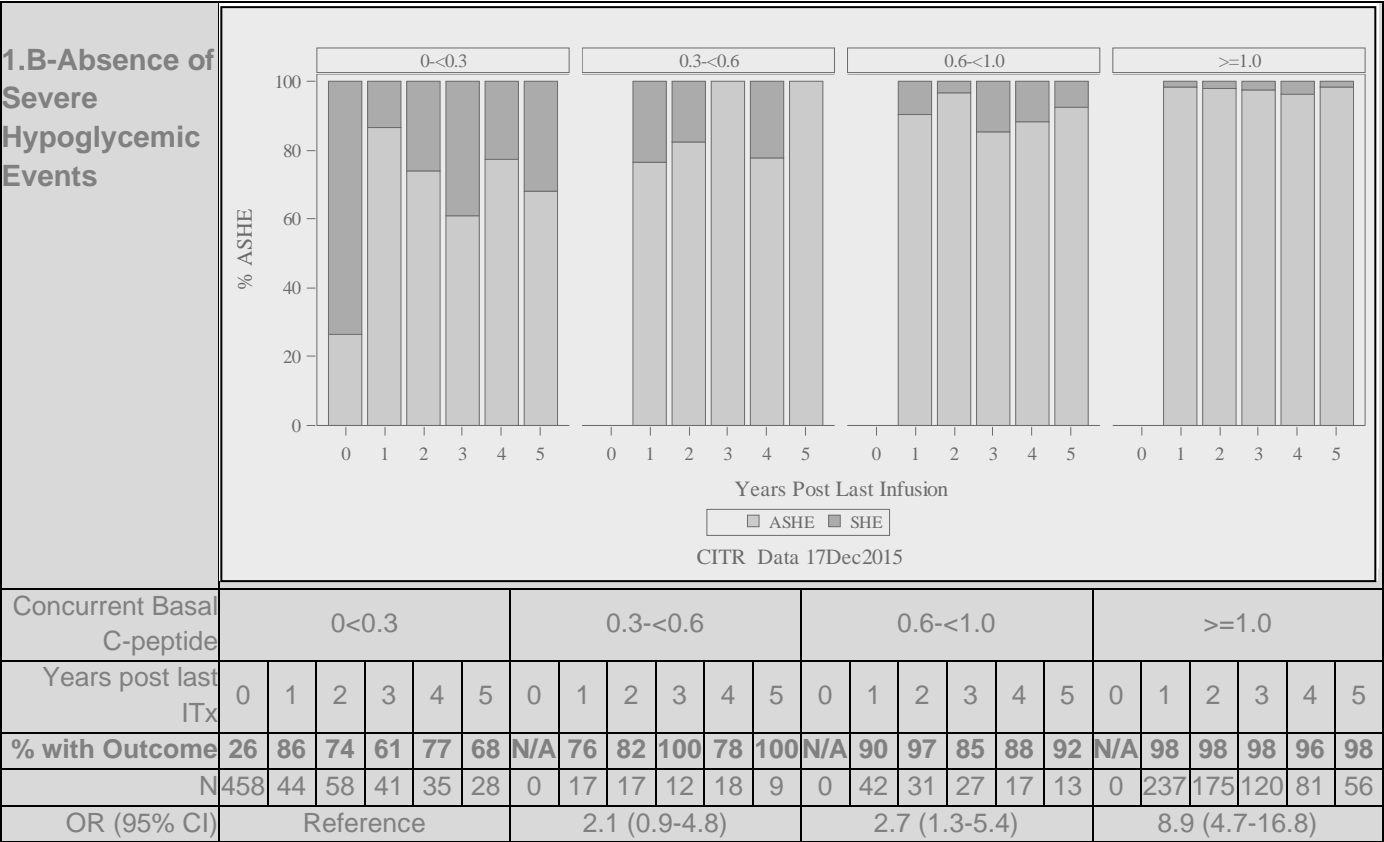


Figure Shell 2: Box-plots

Outcome												
	U.S. Islet Transplant						Control					
O.R. (95% CI)	U.S. Islet to control											
Year	0	1	2	3	4	5	0	1	2	3	4	5
Median												
N												
Unadjusted O.R. (95% CI)	Compared to control						Reference					
Adjusted O.R. (95% CI)	Compared to control						Reference					

Example Figure 2: Box-plots

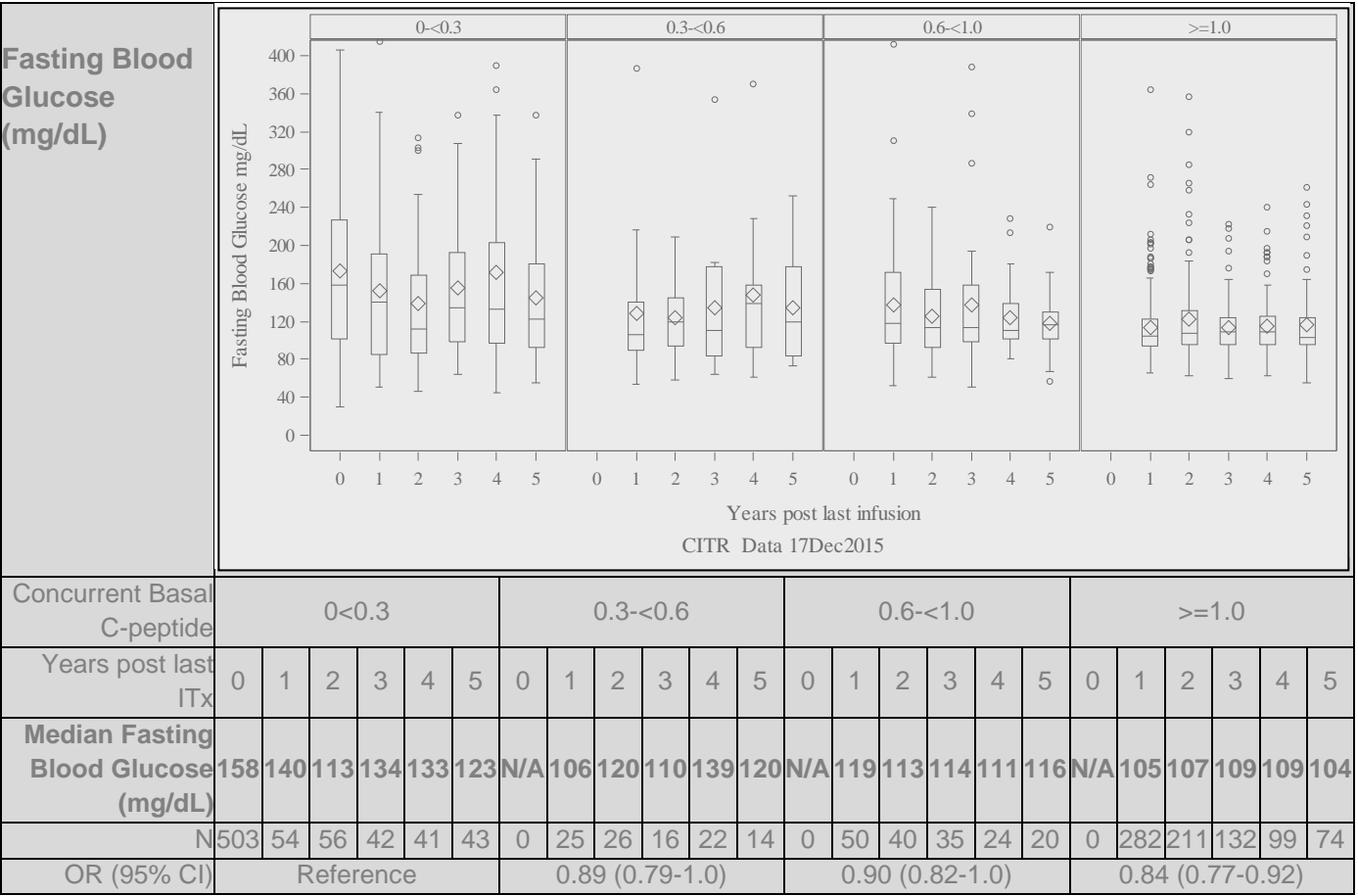


Figure Shell 3: Kaplan-Meier plots

	CITR-ITA vs. T1DX-Control
Wilcoxon p-value	

Example Figure 3: Kaplan-Meier plots

