May 01, 2024

A Prospective Randomized Placebo-Controlled Double Blind Clinical Trial to Evaluate the Safety and Efficacy of CLBS03 (Autologous Ex Vivo Expanded Polyclonal CD4+CD25+CD127lo/-FOXP3+ Regulatory T-cells [Tregs]) in Adolescents with Recent Onset Type 1 Diabe

DOI
dx.doi.org/10.17504/protocols.io.n92ld8zb7v5b/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.n92ld8zb7v5b/v1

Protocol Citation: Stephen Gitelman, Kurt Griffin 2024. A Prospective Randomized Placebo-Controlled Double Blind Clinical Trial to Evaluate the Safety and Efficacy of CLBS03 (Autologous Ex Vivo Expanded Polyclonal CD4+CD25+CD127lo/-FOXP3+ Regulatory T-cells [Tregs]) in Adolescents with Recent Onset Type 1 Diabe. **protocols.io https://dx.doi.org/10.17504/protocols.io.n92ld8zb7v5b/v1**

Protocol status: Working
We use this protocol and it's working

Created: April 29, 2024

Last Modified: May 01, 2024

Protocol Integer ID: 99083

Funders Acknowledgement:
Caladrius CRA
Sanford Health CRA

Abstract

This protocol details a prospective randomized placebo-controlled double blind clinical trial to evaluate the safety and efficacy of CLBS03 (autologous ex vivo expanded polyclonal $CD4^{+}CD25^{+}CD127^{lo/-}FOXP3^{+}$ regulatory T-cells [Tregs]) in adolescents with recent onset Type 1 Diabetes Mellitus.

Guidelines

4. SCHEDULE OF STUDY ASSESSMENTS

	Screening Visit	Pre-Tmt	Treatment			Follow-up									
			Pre-Inf	Inf	Post-Inf										
Visit Number	**1**	**2**	**3**	**3**	**3**	**4[a]**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13[b]**
Week of Trial (approx.)	**-4**	**-2**	**0**	**0**	**0**		**1**	**2**	**4**	**13**	**26**	**39**	**52**	**78**	**104**
Day of Trial	**-79 to -23**	**-16 to -15**	**0**	**0**	**0**		**7**	**14**	**28**	**91**	**182**	**273**	**364**	**546**	**728**
Visit Window							**±1**	**±3**	**±7**	**±7**	**±14**	**±14**	**±14**	**±14**	**±14**
Informed Consent/Assent	X														
Medical History	X														
Concomitant Medications	X	X	X				X	X	X	X	X	X	X	X	X
Physical Examination[c,d]	X		X							X	X		X	X	X
Vital Signs[e]	X	X	X		X		X	X	X	X	X	X	X	X	X
Confirmation of Eligibility		X													
Randomization[f]		X													
Adverse Events		X	X	X	X		X	X	X	X	X	X	X	X	X
Contact Subject for Diary Completion[g]			X						X	X	X	X	X	X	X
Dispense (D)/Collect (C) Subject Diary[h]		D	C					D	D/C	D/C	D/C	D/C	D/C	D/C	C
Blood Draw for Treg Collection/IP manufacture[i]		X													
Investigational Product (IP) infusion[j]				X											
Hematology[k]	X		X[l]				X	X	X	X	X	X	X	X	X

[a] Visit 4 was removed with protocol version 6.0
[b] In the event that a subject prematurely discontinues the study, the site should make all efforts to bring the subject back to the site to complete Visit 13 procedures.
[c] Complete physical exam to be performed at screening and Visit 13, and limited physical examination at Visits 3, 8, 9, 11, and 12.
[d] Tanner stage will be evaluated at screening (Visit 1). Tanner stage will be re-evaluated at 12 and 24 months if not already documented as ≥stage III at a previous exam.
[e] At Visit 3, height and weight will not be included in the post-infusion vitals assessment.
[f] Subjects will be randomized once all screening procedure results are available and it's confirmed that the study eligibility requirements are met based on screening. Eligibility requirements will be confirmed at Visit 2 prior to the blood draw for Treg collection.
[g] To be performed approximately 4 days prior to the visit specified.
[h] Subjects will capture glucose levels and insulin (both basal and bolus doses) use in a provided diary during the 3-day period prior to the visit. Subjects on an insulin pump will bring a printout of their insulin pump activity on the day of the visit. Subjects using continuous glucose monitoring (CGM) will download data at each clinic visit or as otherwise arranged.
[i] Blood volume being drawn based on the subject's weight at the time of blood draw (at pretreatment visit, Visit 2).
[j] IP infusion must be performed within 100 days of T1DM diagnosis. IP dose will be based on the subject's weight at the time of screening (Visit 1). Subjects will be administered acetaminophen and diphenhydramine hydrochloride prior to the infusion of IP.
[k] Coagulation assays are only done at screening
[l] At treatment visit, blood sample will be collected and sent to site's local laboratory; results must be reviewed by the investigator prior to IP infusion.

	Screening Visit	Pre-Tmt	Treatment			Follow-up									
			Pre-Inf	Inf	Post-Inf										
Visit Number	**1**	**2**	**3**	**3**	**3**	**4[a]**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13[b]**
Week of Trial (approx.)	**-4**	**-2**	**0**	**0**	**0**		**1**	**2**	**4**	**13**	**26**	**39**	**52**	**78**	**104**
Day of Trial	**-79 to -23**	**-16 to -15**	**0**	**0**	**0**		**7**	**14**	**28**	**91**	**182**	**273**	**364**	**546**	**728**
Visit Window							**±1**	**±3**	**±7**	**±7**	**±14**	**±14**	**±14**	**±14**	**±14**
Clinical Chemistry	X						X			X	X		X	X	X
Human Immunodeficiency Virus (HIV) 1/2, Hepatitis B, Hepatitis C, and Human T-lymphotropic Virus (HTLV) 1/2	X														
Epstein-Barr Virus/Cytomegalovirus (EBV/CMV) Serology	X														
Whole-blood Quantitative Polymerase Chain Reaction (PCR) for EBV/CMV Viral Load	X														
Serum-autoantibodies for Eligibility	X														
Hemoglobin A1c (HbA1c)			X							X	X	X	X	X	X
Blood Collection for Exploratory Assays[m]	X		X				X	X	X	X	X	X	X		X
Blood Collection for Regulatory T-cells Count	X														
QuantiFERON® Tuberculosis (TB) Test or Purified Protein Derivative (PPD) Skin Test	X														
Mixed Meal Tolerance Test (MMTT) – Insulin Connecting Peptide (C-peptide)/Glucose (2-hour)										X				X	
MMTT - C-peptide/Glucose (4-hour)	X										X		X		X
Urinalysis	X						X			X	X		X	X	X
Urine Pregnancy Test[n]	X	X	X												X

APPENDICES

1. ADA Criteria for Diabetes Diagnosis

The following criteria are taken from the 2017 ADA publication on classification and diagnosis of diabetes:41

Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*

OR

2-h plasma glucose (PG) ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test(OGTT). The test should be performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

OR

A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardized to the DCCT assay.*

OR

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

Per ADA Standards of Medical Care-2017, blood glucose rather than A1c should be used to diagnose acute onset of type 1 diabetes in individuals with symptoms of hyperglycemia.

*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

2. Mixed Meal Tolerance Test

- The mixed meal tolerance test (MMTT) measures the level of C-peptide (an amino acid which is released by the pancreas in amounts equal to insulin) and glucose.
- The level of C-peptide in the blood indicates how much insulin the pancreas is producing. A beverage called Boost® High Protein (HP) Nutritional Drink (a milkshake-like drink containing carbohydrate, fat and protein) will be administered.
- Boost HP will raise blood glucose levels and cause the body to release insulin, as a meal would.
- In general, if a participant has a known food allergy to one or more components of Boost HP, the participant should not be enrolled and the site should notify the Sponsor/designee immediately.
- The MMTT should be performed by study staff adequately trained and authorized by the PI to perform the procedure including the PI, sub-investigators, or study nurses/coordinators trained in the performance of IV procedures.
- The MMTT should be performed in the morning, with the first blood draw (time point) between 7:00 am and 10:00 am (when blood glucose is more likely to be within the target range).
- The 4-hour MMTT should take 250 minutes to perform, and the 2-hour MMTT should take 130 minutes.

- The table below outlines the time points at which the blood sample will be taken from the subject for MMTT-stimulated C-peptide/glucose measurement.

Time	2-hour MMTT	4-hour MMTT
–10	X	X
0	X	X
Participant drinks Boost®		
15	X	X
30	X	X
60	X	X
90	X	X
120	X	X
150		X
180		X
210		X
240		X

In preparation for the visit, each participant should:

- Fast for at least 10 hours (but not more than 16 hours) before the test. Participants should not eat or drink anything except water. This means no coffee, tea, soda, cigarettes, alcohol, or chewing gum during the fasting period.
- Refrain from vigorous exercise during the fasting period.
- Refrain from working the night before the morning of the test.
- Discontinue taking any prescription medications that must be taken daily (guidelines on insulin use are provided below).

- Carbohydrates should not be restricted from the diet before the test. As a general guideline, for each of the 3 days prior to the visit where a MMTT will be done, it is recommended that adolescent subjects should consume at least 150 grams of carbohydrates.
- Most diets include greater amounts of carbohydrates and there is no need to alter the participant's diet unless he/she has been on a carbohydrate-restricted diet. Participants should also be instructed to drink plenty of liquids during the 3 days prior to the test.

Target Glucose Level at the Start of the MMTT

- The target glucose level at the start of the test is between 70 and 200 mg/dL. Regular insulin or short acting insulin analogues may be used up to 6 and 2 hours before the test, respectively (see below), to achieve the desired glucose level.
- The principal investigator and the study participant should discuss the individual situation for insulin administration to attain the goal of meter capillary glucose values within the range of 70-200 mg/dL at the start of the test.
- Subjects should be instructed to check their blood glucose by meter at home 2 hours before the start of the test so that marked hyperglycemia can be treated with a short-acting insulin analogue.
- Alternatively, participants who arrive at the research unit with elevated blood glucose can receive additional short-acting insulin analogues at the time of their arrival, if the test itself does not start until at least 2 hours after insulin

administration and occurs before 10 a.m.
- If a subject's blood glucose is below the limit (70 mg/dL) prior to performing the MMTT, the participant should be treated according to local practice, and the MMTT should be rescheduled (within the allowed visit window).

Insulin Before the Test

- Short-acting insulin analogues (such as lispro or aspart) may be administered up to 2 hours before the test.
- Regular insulin may be administered up to 6 hours before the test.
- Intermediate-acting insulin (such as neutral protamine Hagedorn [NPH]) may be administered on the evening before the MMTT, but not on the morning of the test. Participants managed with intermediate-acting insulin (NPH or Lente) should administer their usual dose on the evening before the MMTT, but not on the morning of the test.
- Long-acting basal (such as glargine) insulin or continuous subcutaneous insulin infusion may be administered before, during and after the test as usual. Participants on glargine may take their usual injection at the appropriate time, and those on continuous subcutaneous insulin infusion may continue with their usual basal settings.

IV Placement During the Test

- The IV should be in place for the duration of the test and must be flushed after each draw with saline solution or heparin flush.
- The participant should remain sitting or resting in bed quietly throughout the test until the test is completed. However, he or she may engage in quiet, nonstrenuous activities, such as reading, playing cards, or watching TV. The participant may walk to the bathroom between blood draws if necessary.

Boost HP to be Administered

Calculate the amount of Boost HP to be administered using the formula below. To convert from pounds to kilograms: pounds ÷ 2.2 = kg. There is no need to calculate after 60 kg, as this meets the minimum weight requirement for the full volume of 360 mL.

Boost HP dose= 6 kcal/kg body weight, at 1 kcal/mL to a max of 360 kcal (360 mL)

Amount of Boost HP to be administered = 6 mL x ___kg = ___ mL

3. BMI z-score

Age (months)	BMI z-score +2.25 SD Males	BMI z-score +2.25 SD Females
95.5	23.69	24.62
96.5	23.85	24.76
97.5	24.00	24.89
98.5	24.15	25.03
99.5	24.31	25.17
100.5	24.46	25.31
101.5	24.61	25.45
102.5	24.77	25.59
103.5	24.92	25.73
104.5	25.07	25.86
105.5	25.22	26.00
106.5	25.37	26.14
107.5	25.52	26.28
108.5	25.67	26.42
109.5	25.82	26.56
110.5	25.97	26.70
111.5	26.11	26.84
112.5	26.26	26.98
113.5	26.40	27.12
114.5	26.55	27.26
115.5	26.69	27.39
116.5	26.83	27.53
117.5	26.97	27.67
118.5	27.11	27.81
119.5	27.24	27.94
120.5	27.38	28.08
121.5	27.51	28.22
122.5	27.64	28.35
123.5	27.77	28.49
124.5	27.90	28.63
125.5	28.03	28.76
126.5	28.16	28.89
127.5	28.28	29.03
128.5	28.40	29.16
129.5	28.52	29.30
130.5	28.64	29.43
131.5	28.76	29.56
132.5	28.88	29.69
133.5	28.99	29.82
134.5	29.10	29.95
135.5	29.21	30.08
136.5	29.32	30.21
137.5	29.43	30.34

Age (months)	BMI z-score +2.25 SD Males	BMI z-score +2.25 SD Females
138.5	29.53	30.47
139.5	29.64	30.60
140.5	29.74	30.72
141.5	29.84	30.85
142.5	29.93	30.98
143.5	30.03	31.10
144.5	30.12	31.23
145.5	30.22	31.35
146.5	30.31	31.47
147.5	30.39	31.60
148.5	30.48	31.72
149.5	30.57	31.84
150.5	30.65	31.96
151.5	30.73	32.08
152.5	30.81	32.20
153.5	30.89	32.32
154.5	30.96	32.44
155.5	31.04	32.56
156.5	31.11	32.68
157.5	31.18	32.79
158.5	31.25	32.91
159.5	31.32	33.03
160.5	31.38	33.14
161.5	31.45	33.26
162.5	31.51	33.37
163.5	31.57	33.49
164.5	31.63	33.60
165.5	31.69	33.72
166.5	31.75	33.83
167.5	31.80	33.94
168.5	31.86	34.05
169.5	31.91	34.17
170.5	31.96	34.28
171.5	32.01	34.39
172.5	32.06	34.50
173.5	32.11	34.61
174.5	32.15	34.72
175.5	32.20	34.84
176.5	32.24	34.95
177.5	32.29	35.06
178.5	32.33	35.17
179.5	32.37	35.28
180.5	32.41	35.39

	BMI z-score +2.25 SD	BMI z-score +2.25 SD
Age (months)	Males	Females
181.5	32.45	35.50
182.5	32.49	35.61
183.5	32.53	35.72
184.5	32.57	35.83
185.5	32.61	35.94
186.5	32.64	36.06
187.5	32.68	36.17
188.5	32.72	36.28
189.5	32.75	36.39
190.5	32.79	36.51
191.5	32.82	36.62
192.5	32.86	36.73
193.5	32.89	36.85
194.5	32.93	36.96
195.5	32.96	37.08
196.5	33.00	37.20
197.5	33.03	37.31
198.5	33.07	37.43
199.5	33.11	37.55
200.5	33.14	37.67
201.5	33.18	37.79
202.5	33.22	37.92
203.5	33.26	38.04
204.5	33.29	38.16
205.5	33.33	38.29
206.5	33.37	38.42
207.5	33.42	38.54
208.5	33.46	38.67
209.5	33.50	38.81
210.5	33.55	38.94
211.5	33.59	39.07
212.5	33.64	39.21
213.5	33.69	39.35
214.5	33.74	39.49
215.5	33.79	39.63
216.5	33.85	39.77

4. List of Changes from CLBS03-P01 v6.0 to CLBS03-P01 v7.0

Location	Change	Rationale
Throughout protocol	Editorial changes made to improve readability and reduce ambiguity. These changes are not otherwise highlighted in this change table.	Administrative and clerical
1. Study Synopsis; 6.1 Study Design Overview; 6.1.2 Justification for Study Population; 6.4.1 Inclusion Criteria; 17.3 BMI z-score	Expansion of allowable age range from 12 to under 18 years to 8 to under 18 years.	To allow exploration of Treg therapy in younger children and to allow them access to this potentially effective therapy. Lowering of the allowable age was recommended by the DSMB.
1. Study Synopsis; 6.4.1 Inclusion Criteria	Lowering of weight requirement for study participants from 40 kg to 30 kg.	To parallel the expansion of age range.
4. Schedule of Study Assessments; 6.3.4 Continuous Glucose Monitoring; 6.8 Outcome Measures; 7. Study Visit Assessments; 9.6 Diabetes Management; 9.7 Subject Diaries; 12.2.3 Continuous Glucose Monitoring	Addition of continuous glucose monitoring to the protocol.	Subjects are encouraged to use continuous glucose monitoring devices to collect around the clock data on glucose levels. CGM data will be examined for frequency of low and high values and for overall variability.

BACKGROUND AND SIGNIFICANCE

1 Summary

1.1 It is hypothesized that CLBS03 or autologous ex vivo expanded, polyclonal $CD4^+CD25^+CD127^{lo/-}FOXP3^+$ regulatory T-cells (Tregs) administered to participants with recent onset Type 1 Diabetes Mellitus (T1DM) will be safe and effective in preserving β-cell function. This protocol describes an exploratory Phase 2 trial to assess this therapy.

1.2 Autologous, polyclonal $CD4^+CD25^+CD127^{lo/-}FOXP3^+$ Tregs will be expanded using a protocol that was established utilizing anti-CD3/anti-CD28 beads plus interleukin-2 (IL-2).

1.3 Subjects will receive target doses of 2.5 and 20 x 10^6 cells/kg BW, based on the results of one Phase 1 study in adults and one Phase 1 study in children, where it was demonstrated that it was feasible to reliably expand these cell numbers, and the infusions were well tolerated with potential for efficacy.

1.4 The primary objective of this exploratory study is to assess the safety and potential efficacy of CLBS03 to modify the T1DM disease course including preservation of β-cell function and improvements in measures of disease severity as compared with placebo in adolescents with recent onset T1DM.

2 Clinical and Scientific Rationale

2.1 Type 1 Diabetes Mellitus

- T1DM is one of the most common and costly pediatric diseases.[2] It is estimated that up to 1 million Americans are living with T1DM[3] and more than 18,000 children are diagnosed with T1DM annually in the US.
- The incidence of T1DM has been increasing by approximately 3% annually in the US and abroad.[4] Currently, the number of youth (age <20 years) with T1DM is projected to increase by at least 23% by 2050.[5]
- T1DM results from autoimmune destruction of pancreatic β-cells by islet-specific T-cells.[6-8] This destructive process takes place over a period of months, before and after the onset of clinical diabetes.[9]
- While interruption of this destructive process before disease onset can prevent clinical disease,[10] cessation of autoimmune cell destruction even after disease onset provides for the possibility of clinical benefit.[11]

- The importance of maintaining glycemic control in T1DM is well established in epidemiologic observations and prospective studies.
- Results from these studies demonstrated that intensive treatment slowed the loss of β-cell function (as measured by C-peptide in subjects with residual β-cell function) and maintained substantially lower mean HbA1c for 5 to 6 years while lowering the risk for development of microvascular complications.[12]
- Treatments for T1DM specifically aimed at preserving residual β-cell function may allow subjects to more effectively manage their disease and prevent long-term diabetic complications.
- As reported by the Diabetes Control and Complications Trial (DCCT), current standards of care aim to reduce the incidence and progression of T1DM- related complications by initiating early, aggressive treatments to maintain a level of HbA1c as close to normal as possible.[13]

2.2 The Role of Tregs in T1DM

- Efforts to prevent or reverse T1DM have been limited by the absence of tolerogenic drugs that can be used both safely and effectively. Relatively non-specific therapies such as anti-cluster of differentiation 3 (anti-CD3) monoclonal antibodies (mAbs) can be efficacious, but have associated side effects.
- Two substantial findings have emerged: first, that short-term immune regulation of T-cells can have a long-term effect on disease progression; and second, that immunomodulatory anti-T-cell agents induce a T-regulatory cell subset that is likely to be responsible for the long lived efficacy of T-cells, in both animal and human studies.[14, 15]
- Recently, Couri and colleagues reported that transplantation of autologous nonmyeloablative hematopoeitic stem cells in participants newly diagnosed with T1DM led to a preservation of β-cell mass and insulin-independence for up to four years.[16]
- This study demonstrated that immune tolerance can be regained; however, the adverse events associated with the stem cell transplant procedure were considerable.
- There is mounting evidence that polyclonal Tregs have the potential to alter the course of T1DM relatively safely, as will be detailed below.
- Tregs play a central role in the protection from diabetes. Diabetes is accelerated in non-obese diabetic (NOD) mice depleted of CD4+CD25+ Treg.[17, 18]
- Similarly, removal of proliferative signals necessary for Treg development or survival, such as IL-2, exacerbates diabetes in NOD mice.[18] Recently, Bettini et al., and Tang et al. showed that the number and function of Treg cells were perturbed in NOD mice,[19, 20] and transforming growth factor- β (TGF-β) was reduced.[19]
- D'Alise et al. identified defects in Treg transcripts in locations associated with diabetic pathogenesis in NOD mice (lamina propria and pancreatic lymph node).[21] Experimental work suggests that therapies that augment the number or function of Tregs have beneficial effects on the progression of T1DM.

- Grinberg-Bleyer et al. found that IL-2 reverses established diabetes in NOD mice by a local effect on pancreatic Tregs.[22] Tang et al. showed that infusion of Tregs into NOD mice will prevent disease onset and even reverse diabetes in mice with hyperglycemia.[23]
- Finally, the safety of the polyclonal Tregs—a primary consideration in designing the clinical development plan—has been demonstrated in T1DM,[24, 25] and in graft-versus-host disease (GvHD).[26]
- Clinical adoptive transfer of polyclonal expanded Tregs—selected and expanded in a method comparable or similar to the manufacturing process planned for CLBS03—was evaluated in two Phase 1 clinical trials, with one trial in adults with T1DM and one trial in children with T1DM.
- Doses up to 40 x 10^6 cell/kg body weight (BW) were administered to the adult population, and up to 30 x 10^6 cells/kg BW were administered to children younger than 18 years old.
- These two clinical trials provided evidence of tolerability and safety of adoptive expanded polyclonal Tregs transfer in both adults and children with T1DM.
- Given the potential impact of benefit, observed through efficacy biomarkers, the two clinical trials support further evaluation of CLBS03 in this disease population.
- Details of the two Phase 1 clinical trials will be discussed in Step 4 below, and are also detailed in the CLBS03 Investigator's Brochure (IB).

2.3 Description of Investigational Product

- Tregs, consitituting the active ingredient of CLBS03, will be isolated from the whole blood of individuals with T1DM, followed by ex vivo selection and expansion by a process based on preclinical and Phase 1 clinical experience as described in Step 4.1.
- These cells will be prepared using a Good Manufacturing Practice (GMP) process as described in the IB, Manufacturing.

3 Non clinical Experience

3.1

Note

Extensive nonclinical studies characterizing human Tregs have provided the framework for developing a robust Treg cell therapy product:

In vitro studies characterizing Tregs in normal and T1DM subjects have demonstrated that sufficient numbers of $CD4^+CD25^+CD127^{lo/-}$Tregs can be isolated from T1DM subjects, and that these cells retain the hallmarks of bona fide Tregs following the process of ex vivo expansion for 14 days.

3.2 Analyses of human Tregs have demonstrated that selection of cells based on a combination of CD4, CD25, and CD127 ($CD4^+CD25^+CD127^{lo/-}$) results in a highly purified population of cells that are greater than 90% $FOXP3^+$(a transcription factor that is critical throughout life to maintain the Treg population).

3.3 Tregs can be readily expanded using anti-CD3 and anti-CD28-coated beads supplemented with IL-2. This expansion process has been shown to increase and restore the suppressive properties of Tregs.

3.4 Studies of human Tregs transplanted in humanized mouse models demonstrate that these cells retain their functional activity in vivo.

3.5 $CD4^+CD25^+CD127^{lo/-}$ Treg cells represent a stable, committed lineage that does not exhibit effector functions or the ability to transdifferentiate into effector cell types.

Note

A detailed discussion of the nonclinical data outlined above is provided in the CLBS03 IB.

4 Clinical Experience

To date, several clinical studies have been conducted with Treg based cell therapies in T1DM, GvHD, and Crohn's disease. The two Phase 1 studies, one study in adults with T1DM and one study in adolescents with T1DM, are discussed in detail below.

4.1 US Phase 1 Trial in Adults with T1DM

- This was a Phase 1 prospective, open-label, uncontrolled, dose-escalating trial of autologous expanded polyclonal Tregs (investigational product manufacturing process comparable to that planned for CLBS03) in adults with T1DM (Study title: A Phase 1 Safety Trial of $CD4^+CD127^{lo/-}CD25^+$ Polyclonal Treg Adoptive Immunotherapy for the Treatment of Type 1 Diabetes, www.clinicaltrials.gov identifier: NCT01210664) conducted by the University of California, San Francisco (UCSF) and Yale University (Yale).
- This study evaluated the safety and feasibility of autologous, expanded polyclonal Tregs in treating adults with established T1DM (onset ranging from 4.5 to 23.5 months), and is being evaluated out to 60 months.[27]
- Twenty-six patients were screened, 16 met eligibility criteria and were enrolled, and 14 subjects received a single infusion of polyclonal Tregs. After signing the informed consent form (ICF), 400 mL of blood were drawn from each subject followed by separation and expansion of Tregs according the methods prospectively described in the protocol at the UCSF GMP facility.

- The study involved four dosing cohorts: 1) three subjects were treated in the first dosing cohort with 5 x 10^6 cells, 2) three subjects were treated in the second dosing cohort with 40 x 10^6 cells, 3) four subjects were treated in the third dosing cohort (target dose of 320 x 10^6cells, average received dose of 360 x 10^6 cells), and 4) four subjects were treated in the fourth dosing cohort (target dose of 2600 x 10^6 cells, average received dose of 2700 x 10^6 cells).
- The highest dose represents approximately 20% of the total number of Tregs (13 x 10^9 cells) that are predicted to exist in normal individuals.[28]
- The mean follow-up at the time of data cutoff for the publication was 124 weeks (cohort 1: 182 weeks; cohort 2: 156 weeks; cohort 3: 104 weeks; and cohort 4: 78 weeks).
- The two subjects who did not receive expanded Tregs (due to failure of Treg product to meet release criteria) were not included in the data analysis.

1. Safety

- To date, (124 weeks follow-up), a total of 144 AEs have been reported of which 4 were reported as serious. Ninety-one events were judged as mild in severity, 42 were judged as moderate, 9 were judged as severe, and 2 were judged as life-threatening.
- The 11 events judged as severe or life-threatening largely reflected metabolic abnormalities of underlying diabetes. Four serious adverse events in two patients were reported: one patient had three episodes of serious hypoglycemia 14, 248, and 463 days after Treg infusion; and another patient had one episode of diabetic ketoacidosis 67 days after Treg infusion.
- No opportunistic infections or malignancies were observed. One subject developed grade 2 pharyngitis and had low-copy number CMV detected on day 7, but not detected at day 28, due to a presumed new infection with CMV occurring before receiving Treg cells. There was no apparent relationship between adverse events and Treg dose (refer to table S2 in the Bluestone et al. publication[27]).
- In general, administration of autologous, expanded polyclonal Tregs, comparable to CLBS03, was found to be safe and well tolerated in this Phase 1 dose-escalating trial through the mean follow-up period of 124 weeks.

2. Efficacy

- Impact of Treg administration on MMTT-stimulated C-peptide and other indices of metabolic control (HbA1c and daily dose insulin [DDI]) was evaluated.
- MMTT-stimulated C-peptide levels (measured by 4-hour area under the curve [AUC] for MMTT-stimulated C-peptide) generally remained stable over time and remained unchanged at 1 year and even after 2 years in dose cohorts 1 and 2.
- Three of four subjects in cohort 3 and three of the fours subjects in cohort 4 showed a decline in MMTT-stimulated C-peptide of more than 50% over 78 weeks of follow-up. DDI

also appeared to be stable for almost all subjects over time and there was no apparent decrease in DDI in comparison to baseline values.
- HbA1c levels appeared to be stable over time (except for one subject whose levels went from 6.2% at screening to 12.6% at week 13), with no clear deterioration in glucose control.
- Given the small number of subjects in each cohort, the overall changes in MMTT-stimulated C-peptide in this study fall within the expected decline observed in the natural history of the disease.
- However, the sample size was too small to make a clear statement about stabilization or decay in MMTT-stimulated C-peptide in treated subjects.

Note

A detailed discussion of the clinical data outlined above and a list of all AEs is provided in the CLBS03 IB.

4.2 Gdańsk Medical University (GMU) Phase 1 Trial in Children with New Onset T1DM

- This was a Phase 1, prospective, open-label, non-randomized, parallel matched group trial of autologous, expanded polyclonal Tregs (IP manufacturing process similar to that planned for CLBS03) in children aged 5-18 years with new onset T1DM, diagnosed within the preceding 2 months.[25]
- The trial enrolled 12 subjects in the treatment arm, and 10 untreated control subjects matched for age, sex, and disease duration. Twenty-four months follow-up is planned, and 12 months follow-up has been completed and the results published.
- The trial is being conducted at a single center at Gdańsk Medical University in Poland, and is designed to be a pilot trial to investigate safety and efficacy of Tregs.
- Three doses were administered based on body weight: single dose of 10 x 10^6cells/kg for 3 subjects, single dose of 20 x 10^6 cells/kg for 3 subjects, and two doses totaling 30 x 10^6cells/kg separated by 6-9 months for 6 subjects.
- In subjects administered 2 doses, the second dose was administered to subjects with good clinical and metabolic response to the first dose of Tregs [fasting C-peptide >0.4 ng/mL and/or DDI <0.5 IU/kg BW], who presented symptoms of disease progression after ≥6 months of follow-up.
- Protocol predefined release criteria were specified and met for all dosed subjects.[24, 25]

1. Safety and Feasibility

- There were no severe or serious AEs reported. There were no AEs suggestive of a transfusion reaction related to the administration of Tregs.

- In general, administration of autologous, expanded polyclonal Tregs, similar to CLBS03, in this dose escalation trial appeared to be safe and well tolerated.
- For feasibility, all planned doses for the treatment cohort met the pre-specified release criteria.

2. Efficacy

- In addition to safety, the primary efficacy endpoints of the trial were remission as defined as DDI ≤0.5 IU/kg, and fasting C-peptide > 0.5 ng/mL 1 year after enrollment. One year after inclusion in the study, 8 out of 12 subjects (66%) still met the protocol predefined criteria of clinical remission (DDI ≤0.5 IU/kg BW) and fasting C-peptide levels > 0.5 ng/mL.
- In addition, compared to the non-treated control individuals, insulin doses (DDI/kg BW) were significantly lower in Treg-treated individuals at 4 months (intermediate efficacy point) and one year after commencing the trial (primary endpoint) (p=0.04 and p=0.02, respectively).
- Fasting C-peptide levels were significantly higher in Treg-treated individuals (4 months: p=0.01 and 1-year: p=0.01). HbA1c levels were the lowest in subjects treated with two doses of Tregs, intermediate in those treated with one dose and the highest in untreated individuals (p=0.01).
- In addition, when data for the treated and untreated subjects were analyzed together after 4-month and one-year follow-up, it was observed that C-peptide levels correlated with percentage of Tregs in peripheral blood (4 months, p=0.04; 1 year, p=0.01).
- This Phase 1 trial provides preliminary evidence supporting the safety, efficacy and biologic activity of single and multiple doses of Tregs in children with new onset T1DM, in comparison with concurrent matched controls.

Note

A detailed discussion of the clinical data outlined above is provided in the CLBS03 IB.

STUDY DESIGN

5 **Overview**

- This will be a double-blinded, multi-center, exploratory trial assessing a single infusion of one of 3 treatments: CLBS03 2.5 x 10^6 cells/kg BW, CLBS03 20 x 10^6 cells/kg BW, and placebo. The study will include adolescent subjects (aged 8 to less than 18 years old) with recent onset of T1DM.
- Due to potential manufacturing and processing variabilities, subjects may not receive exactly 2.5 or 20 x 10^6 cells/kg BW.

- Subjects in the dose group referred to as 2.5 x 10^6 cells/kg BW will receive a dose ranging from 2 x 10^6 to 3 x 10^6 cells/kg BW.
- Subjects in the dose group referred to as 20 x 10^6 cells/kg BW will receive a dose ranging from 2 x 10^6 cells/kg BW to 24 x 10^6 cells/kg BW depending on the manufacturing yield of Investigational Product from the subject's blood.
- A screening visit will be conducted to determine eligibility. Subjects who continue to be eligible will be randomized prior to the blood draw at Visit 2 to manufacture the investigational product. Approximately 6 mL/kg up to 540 mL of blood will be collected.
- The blood will be shipped to PCT, a Caladrius Company, for manufacturing the autologous investigational product. The investigational product manufacturing takes approximately 14 days.
- Once the manufacturing is completed, the investigational product will be shipped to the site for infusion, which must occur prior to product expiration. Subjects will be infused on Day 0.
- Post-treatment visits will occur on days 7, 14, 28, 91, 182, 273, 364, 546, 728. There will be a total of 12 to 13 visits (including the screening and pretreatment visits).
- Approximately 111 subjects will be randomized to one of 3 treatment groups in a 1:1:1 ratio:

1. 2.5 x 10^6 cells/kg BW
2. 20 x 10^6 cells/kg BW
3. Placebo

- Randomization will be done centrally with stratification by baseline MMTT-stimulated C-peptide value. Enrollment will be completed in 2 parts. For Part 1 approximately 18 subjects will be initially randomized, at a 1:1:1 ratio, to all treatment arms.
- After subjects enrolled in Part 1 have reached the 28-day post-treatment visit, a safety review will be conducted. If the safety profile is deemed acceptable, enrollment in Part 2 will be allowed.
- An additional safety review will take place once subjects enrolled in Part 1 have reached the 3-month post-treatment visit.
- The additional subjects will be enrolled in Part 2 and randomized to all treatment arms at a ratio of 1:1:1 to reach a total enrollment of approximately 111 subjects.
- A planned interim analysis will be conducted after approximately 50% of the total planned sample size have completed the Week 26 visit.
- Safety, diabetes control, β-cell function, and immune function will be assessed over the course of the study.
- All subjects are expected to be on intensive diabetes management (refer to Step 21) consistent with the American Diabetes Association (ADA) standard of care.
- Study participants' planned visit schedule is outlined in Section 4, Schedule of Assessments.

5.1 **Justification for Dose**

- CLBS03 will be administered in a single dose administration to study subjects, at dose levels of 2.5 or 20 x 10^6 cells/kg BW.
- The lowest tested doses in the Phase 1 studies, in adults and children with T1DM, were approximately 0.1 x 10^6 cells/kg BW and 10 x 10^6 cells/kg BW, respectively.
- These were based on semi-quantitative allometric scaling of animal data, accumulated safety information from preceding clinical studies in other populations, and quantitative understanding of the Treg population in humans (Step 4 Clinical Experience).
- Using an IP comparable or similar to CLBS03, up to 38.9 x 10^6 cell/kg BW, were evaluated in adults, and 20 x 10^6 cells/kg BW in children in a single administration.
- Additionally, 30 x 10^6 cells/kg BW were evaluated in children administered 2 separate doses, 6-9 months apart. In both studies all doses tested were well-tolerated, and appear to be safe, based on AE and safety laboratory analyses.
- As discussed above, preliminary mechanistic analyses from the cohorts in the above mentioned adult Phase 1 study showed no significant changes in T helper (Th) subsets, $CD4^+$ or $CD8^+$ effector cell differentiation, T-cell activation, B-cell subsets or monocytes.
- Deuterium labeling utilized for a subset of subjects in the same study also confirmed the lineage stability of infused Tregs. Taken together, it appears that all doses tested were not associated with manifestations of Treg effector transdifferentiation that can pose a clinical safety concern.
- In the planned study, inclusion of the low dose of 2.5 x 10^6 cells/kg BW is to investigate a wider range of dose for this exploratory study, while supporting the available manufacturing stability data.
- The high dose of 20 x 10^6 cells/kg BW was conservatively selected based on the highest single dose administered to children which appears to be safe (with the 30 x 10^6 cells/kg BW multiple dose profile providing a safety margin), as well as accounting for manufacturing feasibility considerations.
- Hence, from a safety perspective, the 2 proposed doses appear to be safe and well tolerated.
- From an efficacy perspective, the high dose is supported by the Gdańsk Medical University Phase 1 study that was conducted in children less than 18 years old, the relevant population for this study.
- In comparison to concurrent controls, children administered with Tregs were able to maintain higher fasting C-peptide levels, but despite some preliminary evidence pointing to efficacious dose response, the relatively small sample size rules out any robust conclusions.
- In this study, two children achieved clinical remission (as defined by DDI ≤0.5 IU/kg, and fasting C-peptide > 0.5 ng/mL 1 year after enrollment) in the higher dose group, in comparison to one child in the lower dose group, but the small sample number prohibits a meaningful dose dependency assessment.
- Additionally, 5 of those receiving two doses of Tregs (for a total of 30 x 10^6 cells/kg BW) achieved remission. In summary, 8 of those in the active treatment arms achieved

remission while 2 subjects in the concurrent control groups achieved remission.

- The low dose of 2.5 x 10^6 cells/kg BW was chosen to provide an approximately 10-fold multiplication factor between the low dose and the high dose group, and to account for the possibility that lower doses may be active, as suggested by the adult study.
- In conclusion, the above safety and preliminary efficacy analyses justify the evaluation of the 2 proposed doses in this study.

5.2 Justification for Study Population

- Adolescent subjects aged 8 to less than 18 years old with recent onset T1DM will be eligible for enrollment in this study. Details of the inclusion and exclusion criteria are included in Step 8.1 and 8.2.
- T1DM most commonly presents in childhood with incidence rates increasing from birth, and peaking between 5-7 years of age and at or near puberty.[4, 29, 30]
- The increasing incidence of T1DM throughout the world is especially marked in young children.[31]
- Registries in Europe suggest that recent incident rates of T1DM were highest in the youngest age-group (0-4 years).[32]
- Incidence rates decline after puberty and appear to stabilize in young adulthood (15-29 years). Those under the age of 18 are most often afflicted,[33] but an approximately equal number of adults over 18 are thought to develop the disease, though incidence in older people receives less media or research attention.[34]
- The T1DM disease course varies from adolescents to adults. Beta-cell destruction in adults appears to occur at a much slower rate than in young T1DM cases, often delaying the need for insulin therapy after diagnosis.[35]
- Younger adolescents in particular appear to have a more robust and aggressive autoimmune process with more rapid progression to disease and a shorter remission (or honeymoon) phase.[36]
- Not surprisingly, there have been suggestions of efficacy differences in younger vs. older subjects in clinical studies of anti-CD3 and anti-CD20 mAbs, with higher efficacy trends observed in younger cohorts.[37, 38]
- According to a statement from the ADA, adolescents with T1DM have characteristics and needs that dictate different standards of care from adults with T1DM.
- The management of diabetes in adolescents must take into account the major differences between adolescents of various ages and adults.[39]
- For example, insulin doses based only on body size are likely to be incorrect; the consequences of hypoglycemic events are distinctly different between adults and adolescents; and risks for diabetic complications are likely influenced by puberty.[40]
- The rationale for studying childern and adolescents is detailed in the Caladrius Biosciences authored document titled “Prospect of Direct Benefit” (reference on file) that was submitted to the FDA in support of the Investigational New Drug (IND) #016232.

- As discussed above, there is sufficient evidence from the two Phase 1 studies that autologous ex vivo expanded polyclonal Treg therapy (manufactured comparably or similar to CLBS03) is safe and well tolerated in adults with established T1DM, and in adolescents with new onset T1DM.
- Additionally, Treg therapy appears to be potentially efficacious especially in adolescents.
- Taken together, the critical yet unmet medical need in adolescents with new onset T1DM, underpinned by a more aggressive autoimmune process, and with evidence for safety, tolerability, and potential for efficacy in adolescents, support studying the safety and efficacy of CLBS03 in a prospective, randomized, placebo-controlled, exploratory Phase 2 clinical study.

6 Objectives

6.1 Primary Objective:

The primary objective of this study is to assess the safety and potential efficacy of CLBS03 (either of the two doses 2.5 or 20 x 10^6 cells/kg BW) to modify the T1DM disease course,including preservation of β-cell function and improvements in measures of disease severity, as compared with placebo in adolescents with recent onset T1DM.

6.2 Secondary Objective:

The secondary objective is to assess the efficacy of CLBS03, including additional measures of T1DM severity, and to evaluate the effect of CLBS03 on the pathologic autoimmune response underlying T1DM and on the general immune responsiveness through 104 weeks.

7 Study Endpoints

7.1 Primary Efficacy Endpoint

The primary study endpoint is the 4-hour MMTT-stimulated C-peptide AUC mean at 26 and 52 weeks.

7.2 Secondary Efficacy Endpoints

- Secondary efficacy endpoints include the 2-hour MMTT-stimulated C-peptide AUC mean at 13, 26, 52 and 78 weeks and the 4-hour MMTT-stimulated C-peptide AUC mean at 104 weeks.
- Additional metabolic evaluations will include a comparison between the study treatment groups in DDI, severe hypoglycemia, HbA1c, fasting and post-prandial blood glucose levels, and proportion of subjects who achieve partial or complete remission.
- Additional information on the secondary efficacy endpoints can be found in Step 37.2.

7.3 Safety Endpoints

- Adverse events, including SAEs and events of special interest (see Section Adverse event reporting and safety monitoring), will be assessed in both treatment arms through week 104.
- Proportion of subjects in which the infusion was prematurely stopped or paused because of adverse events will also be assessed for each treatment arm.

7.4 Continuous Glucose Monitoring

- Serum glucose data from continuous glucose monitoring (CGM) devices will be examined for a subset of patients who are willing to use a continuous glucose monitoring device and have access to a continuous glucose monitoring device.
- As the science of CGM is still evolving and there is no consensus on the best way to examine these data, these analyses are considered exploratory.
- Particular attention will be paid to observations of low and high glucose levels and the pattern of fluctuations in serum glucose over the course of the study.

7.5 Exploratory Biomarker Assays

- Retained biological samples including genetic samples may be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment.
- Samples may also be utilized to further evaluate those subjects responsive to therapy and to elucidate treatment mechanisms. The results of these future analyses, and any mechanistic studies, will not be made known to the participant.
- Retained biological samples will be collected for future analyses according to the schedule of assessments table (Section 4 and step 7.5). The following analyses may be conducted on the retained samples collected:

1. Assessment of immune cell subset and intracellular cytokine profile: Cryopreserved peripheral blood mononuclear cells (PBMCs) will be analyzed using multi-parameter flow cytometry to assess changes in various immune cell subsets following expanded Treg administration.

- Overall changes in peripheral blood leukocyte composition (B-cell, monocytes, natural killer [NK] cells, T-cells, etc.) will be determined.
- Of particular interest will be the enumeration of activated, naïve, memory, and regulatory T-cell populations as defined by combinations of cell surface markers. Additionally, the intracellular cytokine profile will be assessed via flow cytometry to determine if cytokine expression is altered by the investigational therapy;
- for example, a shift in Th subset (Th1, Th2, and Th17) cytokine production. This analysis will include intracellular staining for regulatory cytokines in the regulatory and effector T-cell

subsets.

2. Cytokine analysis: Pro-inflammatory cytokines (platelet derived growth factor (PDGF), IL-1ß, Il-4, IL-5, IL-6, IL-12, IL-13, IL-33, tumor necrosis factor (TNF)-α) and anti-inflammatory cytokines (TGF-ß, IL-10) will be analyzed.

3. Autoantibody analysis: Key markers of autoimmune processes directed against pancreatic islets include anti-glutamic acid decarboxylase (GAD) 65, anti-insulin (IAA), anti-islet cell (ICA)-512/insulinoma antigen (IA)-2, and anti-zinc transporter protein (ZnT)-8 autoantibodies.

- Detection of these autoantibody combinations has proven to be an accurate predictor of T1DM in several natural history studies. Shifts in the immunoglobulin (Ig) isotype titers may indicate a change in the type of T-helper cell responses to the autoantigen.
- For example, increases in titers of anti-GAD IgE, IgG2, or IgG4 antibodies could indicate a shift to a more regulatory-type profile following Treg infusion.
- This study will test the hypothesis that successful treatment will be associated with a reduction in the titer or change in the isotype of diabetes-related autoantibodies.

4. Whole blood deoxyribonucleic acid (DNA) epigenetic studies: DNA purified from whole blood will be used to measure the frequency of Tregs in peripheral blood by means of epigenetic analysis.

- Analysis will be performed using the methylation-sensitive quantitative polymerase chain reaction (qPCR) method developed by Epiontis (Berlin, Germany).
- This assay targets the demethylated state of the Treg-specific demethylation region (TSDR) of the FOXP3 gene – a highly specific marker of Tregs.
- The proportion of $CD4^{+}CD25^{+}CD127^{lo/-}$cells determined by flow cytometry strongly correlates with the proportion of demethylation of FOXP3 TSDR measured by qPCR.
- The proportion of demethylated FOXP3 will be normalized to the proportion of demethylated CD3 DNA.
- The FOXP3 to CD3 ratio as determined by epigenetic analysis of methylation state strongly correlates with the ratio of Treg to $CD3^{+}$ cells as measured by flow cytometry.

5. Whole blood human leukocyte antigen (HLA) genotyping: DNA collected from participants will be used to perform sequence-based HLA typing.

- A complete class I and class II haplotype may be performed, including fine typing of the DQB and DRB regions.
- Genotyping for single nucleotide polymorphisms (SNPs) in selected immune-response genes may also be performed.
- The results of genotype analyses may be used to correlate with disease progression and therapeutic responses (tolerance induction).

6. Whole blood genomics studies: Ribonucleic acid (RNA) purified from whole blood will be used to examine expression of genes of interest.

7. CLBS03 tracking by stable isotope labeling: The persistence of infused Tregs will be assessed by stable isotope enrichment in Tregs purified from peripheral blood using stable isotope labeling with mass spectrometric analysis.

- During ex vivo expansion, the ^{2}H label from deuterated glucose (^{2}H-glucose) contained in the cell culture medium is incorporated into the deoxyribose moiety in replicating DNA through the de novo purine nucleotide synthesis pathway.
- Following infusion of stable isotope-labeled Tregs, peripheral blood will be sampled according to the schedule of events. The total number of Tregs in peripheral blood will be measured at each time point by flow cytometry.
- Cell pellets of Tregs purified from PBMCs by fluorescence activated cell sorting will be frozen and stored until batched for stable-isotope enrichment analysis.
- Following isolation and hydrolysis of genomic DNA, the isotopic enrichment of the purine deoxyribonucleosides in Tregs sorted from whole blood will be assessed by gas chromatography/mass spectrometry.
- The change in the ^{2}H enrichment of labeled cells in the total peripheral Treg pool will be assessed at times specified in the Schedule of Study Assessments (Section 4).

8. Functional cell based assays on cryopreserved samples: Cryopreserved PBMCs will be studied using in vitro cell culture techniques to study T-cell functionality in treated participants.

- Frozen PBMCs will be thawed and Treg will be isolated by flow-based sorting using CD4, CD25 and CD127 (and in some cases CD45RA and CD45RO) antibodies.
- Treg will be assayed for their ability to suppress polyclonal T-cell responses using either carboxyfluorescein diacetate succinimidyl ester (CFSE) or ^{3}[H]-thymidine assays.
- Cytokines in the culture supernatants will be assessed.

9. Additional exploratory assays: Additional mechanistic assays may be implemented as guided by the efficacy and safety findings of the study utilizing retained biological samples collected (including DNA).

- A schedule of the planned exploratory assays is shown below, in Step 7.6. For lower weight subjects, a modified blood collection schedule will be followed from screening to day 28 to comply with current guidelines for blood collection (see the Central Laboratory Manual).

7.6 Schedule of Exploratory Biomarker Assays

	Screening Visit	Pre-Tmt	Treatment			Follow-up									
			Pre-Inf	Inf	Post-Inf										
Visit Number	**1**	**2**	**3**	**3**	**3**	**4***	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Week of Trial (approx.)	**-4**	**-2**	**0**	**0**	**0**		**1**	**2**	**4**	**13**	**26**	**39**	**52**	**78**	**104**
Day of Trial	**-79 to -23**	**-16 or -15**	**0**	**0**	**0**		**7**	**14**	**28**	**91**	**182**	**273**	**364**	**546**	**728**
Treg Isotope Tracking & Total Treg Number†							X	X	X	X	X	X	X		X
HLA and SNP Genotyping											X				
Immune Cell Subset and Intracellular Cytokine Profile Analysis	X		X				X	X	X	X	X	X	X		X
Cytokine Analysis	X		X				X	X	X	X	X	X	X		X
Serum-based Autoantibody Analysis (post-screening)													X		X
Whole Blood DNA Epigenetic Studies	X		X				X	X	X	X	X	X	X		X
Whole Blood Genomic Studies (from RNA)	X		X				X	X	X	X	X	X	X		X
Functional Cell Based Assays	X		X				X	X	X	X	X	X	X		X

8 Study Population

8.1 Inclusion Criteria:

Subjects who meet ALL of the following criteria are eligible for this study:

1. Male and female subjects between the ages of 8 to less than 18 at the time of screening.
2. Diagnosis of T1DM within 100 days of IP administration (treatment visit) according to ADA criteria41 (see Appendix 17.1). For this study, diagnosis based on A1c alone is not acceptable.
3. Positive for at least one islet cell autoantibody (GAD65; IAA [if obtained prior to 10 days after the onset of insulin therapy]; ICA-512/IA-2, ZnT8).
4. Peak MMTT-stimulated C-peptide level > 0.2 pmol/mL (at the screening visit).
5. Weight of ≥30 kg at the time of screening.
6. Body mass index (BMI) z-score less than 2.25 at the time of screening (see Appendix 17.3).
7. For females of childbearing potential, a negative urine pregnancy test is required at Screening and prior to the blood draw for Treg collection at Visit 2. A negative pregnancy test is also required prior to infusion of the treatment at Visit 3.
8. Females of child bearing potential (i.e., females who have reached puberty or have had their first menstrual bleeding) must: a) be surgically sterile, or b) be willing to practice an acceptable method of birth control for the duration of their participation in the study. Acceptable methods of birth control are: oral contraceptive tablets, hormonal implant device, hormonal patch, intrauterine device, diaphragm and contraceptive cream or foam, condom with spermicide, or abstinence.

9. Males must agree to use a reliable and acceptable method of contraception for the duration of their participation in the study. Acceptable methods of contraception are: condom with spermicide, or abstinence.
10. Willing and medically acceptable to postpone live and killed vaccine immunizations for one year and 3 months after Visit 3, respectively.
11. Subject signing the study ICF. As the subject will be a minor at the time of consent, the subject must provide written assent with the parent or legally authorized representative signing the ICF.
12. Able to comply with and undergo procedures as required for the study.

8.2 **Exclusion Criteria‡:**

1. Hemoglobin less than 12.0 g/dL at the time of screening.
2. Leukocytes <3,000/µL; neutrophils <1,500/µL; lymphocytes <800/µL; platelets <100,000/µL at the time of screening.
3. Regulatory T-cells present in peripheral blood at <20 cells per µL as determined by flow cytometry at screening.
4. Use of non-insulin pharmaceuticals (that may affect glycemic control) 15 days or 5 half-lives prior to screening, whichever is longer.
5. Use of systemic corticosteroids or other immunomodulatory drugs 4 weeks or 5 half-lives before study treatment, whichever is longer.
6. Serious (requiring hospitalization or an antibiotics course of duration greater than 10 days) bacterial, viral, or fungal infections, or clinically significant opportunistic infections within 90 days prior to Visit 3.
7. History of malignancy or serious uncontrolled (in the judgement of the investigator) cardiovascular, nervous system, pulmonary, renal, or gastrointestinal disease.
8. Have serologic evidence of current or past viral infection: HIV, hepatitis B, hepatitis C, and HTLV 1/2.
9. Positive QuantiFERON® TB test or PPD skin test, history of tuberculosis, or active TB infection.
10. Active infection with EBV as defined by EBV viral load ≥10,000 copies per mL of whole blood.
11. Active infection with CMV as defined by CMV viral load ≥10,000 copies per mL of whole blood.
12. Diagnosis of liver disease as defined by alanine aminotransferase (ALT) >3x the upper limit of age-determined normal (ULN) or total bilirubin >1.5 x ULN.
13. Pregnant or breast-feeding females.
14. Vaccination with a live virus 8 weeks prior to Visit 3.
15. Vaccination with a killed virus 3 weeks prior to Visit 3.
16. Subjects who have participated in an investigational drug study within 90 days prior to the screening visit.
17. Subjects previously treated with a Treg based cell therapy.
18. Known history of allergy to gentamicin.

19. Any other condition which, in the opinion of the investigator, may preclude the subject from safe participation in the study or compromise data integrity.

8.3 Protocol Deviations/Violations:

Protocol violations and deviations will be summarized with descriptive statistics by category. A listing of all events will also be included.

Protocol deviations include, but are not limited to the following:

- Enrolling subjects in violation of eligibility criteria designed to ensure a specific subject population
- Failing to collect data necessary to interpret primary endpoints
- Subject's refusal to complete scheduled research activities
- Out-of-window study visits or study procedures

9 Description of Treatment Groups

This protocol will enroll a total of approximately 111 participants who will be randomly assigned using a 1:1:1 allocation ratio to the two treatment arms of CLBS03 (2.5 x 10^6 cells/kg BW, 20 x 10^6 cells/kg BW) or placebo.

10 Treatment Assignment and Double Blinding

- The study will be conducted as a double blind study. The participants and blinded study personnel will not be informed regarding the treatment assignment until the end of the study (after the end of study database lock), unless required by the DSMB or in case of emergency unblinding for safety reasons (see below).
- The investigator and investigator site personnel will also be blinded as to subject treatment assignment. Laboratories and other vendors performing assays for this protocol will also be blinded except for the laboratory performing the exploratory assays.
- The sponsor personnel, including the study medical monitor or designee, involved in the study conduct will also be blinded to subject treatment assignment.
- The final randomization schedule will be generated prior to study enrollment by an unblinded statistician.
- In an emergency situation where knowledge of the study treatment is critical to subject safety and will influence choice of treatment, the study treatment for that subject can be unblinded.
- The Investigator should make all reasonable efforts to notify the medical monitor and/or study manager prior to unblinding.
- If unable to contact the medical monitor and/or study manager, the investigator must inform the medical monitor and/or study manager as soon as possible after unblinding.

11 Investigational Product

Additional information on the handling of investigational product, including instructions for infusion, can be found in the Investigational Product: Collection and Infusion Manual.

11.1 Description of IP

- The investigational product refers to either dose of CLBS03 or placebo and will be manufactured at PCT, a Caladrius company.
- The CLBS03 cell product (autologous, ex vivo expanded Treg cells) will be prepared using a Good Manufacturing Practice (GMP) process as described in the CLBS03 IB.
- Briefly, buffy coat leukocytes are isolated using a SynGenX-LAB device and labeled with anti-CD4 microbeads for positive selection of $CD4^+$ cells using a CliniMACS instrument.
- Cells are then stained with fluorescent-conjugated monoclonal antibodies to CD4, CD25 and CD127. The target $CD4^+CD25^+CD127^{lo/-}$ Treg cells are then purified by flow cytometric sorting on a Becton Dickinson Influx™ cell sorter and expanded in culture for 13 to 14 days using anti-CD3/CD28 microbeads (Dynabeads® CD3/CD28 CTS) and IL-2, resulting in an approximately 1,000-fold expansion of Treg cells.
- At the end of the 14-day culture period, washing and volume reduction are performed on a LOVO® cell processing device, and anti-CD3/CD28 Dynabeads are removed using a CliniMACS® instrument.
- Cells are then re-suspended in sterile USP infusion solution (0.5% human albumin USP in an equal mixture of USP Plasma-Lyte A and USP 5% dextrose / 0.45% sodium chloride [NaCl]) for intravenous infusion into the same (autologous) patient.
- The placebo includes the infusion solution only, composed of 0.5% human albumin USP in an equal mixture of USP Plasma-Lyte A and USP 5% dextrose / 0.45% sodium chloride [NaCl].

11.2 Packaging and Storage

- CLBS03 (approximately 100 mL) will be contained in a sterile 150-mL infusion bag. The investigational product will be masked with an opaque cover to blind the treating medical staff and the study subject as to the possible identity of the product (Treg cells or placebo).
- CLBS03 is express-shipped to the clinical site in a shipping container validated to maintain temperature between 2 and 10°C.
- CLBS03 will be stored in the shipping container in a secure, limited access area maintained at room temperature until immediately prior to administration.

11.3 Labeling

Labeling will comply with country-specific requirements for investigational product. The investigational product will be identified as a new drug, limited by federal law to investigational use.

Labels for the investigational product will minimally contain the following:

- Protocol number
- Subject number
- Product name
- Storage temperature
- Expiration date/time
- Caution statement(s) (i.e., “Caution: New Drug—Limited by Federal (or United States) law to investigational use”)
- Sponsor information
- Other additional information may be displayed as required by federal laws and regulations.

11.4 IP Infusion

- The IP infusion procedure is described in detail in the IP Collection and Infusion Manual for the study. The site must not infuse the IP until it has been released for infusion by PCT Quality Assurance.
- Investigational product must be infused on the same day of receipt and prior to the expiration date and time.
- All study subjects will be observed at the investigator site on the day of IP infusion (Day 0).
- Prior to infusion, the investigator will evaluate the subject and ensure that all study assessments are completed (including review of any medical history, laboratory results, and previous/ongoing symptoms that would preclude IP infusion) per the Schedule of Study Assessments (Section 4) and reach a positive decision to proceed with the infusion.
- Approximately 30-90 min prior to initiation of transfusion, all study participants will be administered up to 650 mg oral acetaminophen (10-15 mg/kg/dose) and 25 mg to 50 mg oral diphenhydramine, depending on body weight (25 mg for subjects <50 kg and 50 mg for subjects ≥50 kg), and provided there are no known previous allergies.
- If the subject is allergic to either medication, the investigator can substitute an appropriate alternative regimen (e.g., ibuprofen, nonsteroidal anti-inflammatory drug).
- Study personnel should wait until email receipt from PCT Quality Assurance (QA) regarding release for infusion before administration of the above listed medications.
- The IP dose will be manufactured based on the subject’s weight at the time of screening (Visit 1). The study drug will be administered via a peripheral intravenous (IV) line primed with saline per the site’s established standard operating procedures.
- The study drug may be administered by peripheral IV line with a 23 gauge (or larger bore as tolerated) needle over approximately 30 minutes (± 10 minutes; actual infusion time may vary by subject).
- Following IP infusion, the infusion bag, tubing and peripheral IV line will be flushed with normal saline to ensure complete dose is infused.
- Subjects will be closely monitored for safety during and after the completion of the infusion of the study drug.

- Clinical monitoring will include monitoring vital signs (see Step 15.3 Vital Signs) prior to IP infusion, and again 2 hours post IP infusion.
- The investigator may implement additional procedures or increase the time or close monitoring if required for subject's safety.
- Emergency medical equipment and appropriately trained personnel, including advanced cardiac and life support (ACLS and/or PALS) crash cart, must be immediately available onsite.

11.5 Slowing or Pausing the Infusion

- No infusion reactions from autologous expanded Treg infusion have been noted to date, in either Phase 1 study (i.e., UCSF or GMU studies).
- Nonetheless, subjects will be monitored carefully until the resolution of infusion reaction in the case any infusion reaction, such as cytokine release syndrome (CRS) (or individual components including nausea, headache, tachycardia, hypotension, rash, or shortness of breath) occurs.
- If such an event occurs, it will be handled in the following manner:

1. Mild Reactions (Grade 1)

For mild (grade 1) reactions per the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE)[42], the cell infusion will be continued. The investigator shall take one or more of the following actions, depending on the type of the reaction:

- Administer additional doses of antihistamine, and acetaminophen or ibuprofen.
- Reduce the rate of infusion by 50% or more.
- For chills and rigors, meperidine may be considered.

2. Moderate or More Severe Reactions (Grade 2 or Higher)

- For moderate or more severe reactions (grade 2 or higher), the study infusion will be interrupted. The investigator may administer additional medications as needed to provide symptomatic relief, such as anti-pyretics, antihistamine, bronchodilators, or normal saline.
- If there is not prompt resolution of these events within 30 minutes, glucocorticoids may be considered at a dose of 0.5 mg/kg methylprednisolone intravenously, and may be repeated every 6 hours as needed if moderate or more severe reactions persist.
- If the reaction disappears or becomes NCI-CTCAE grade 1, then the infusion may be restarted (prior to IP expiration). If the reaction reoccurs after the restart of the infusion at NCI-CTCAE grade 2 or higher, then the infusion will be permanently discontinued.
- If severity persists at NCI-CTCAE grade 2 or higher despite the above medications for more than 3 hours, then the infusion will be permanently discontinued.
- The above guidelines are meant to help the investigator with the clinical management of the subject.

- The investigator should attempt to contact the study medical monitor in case of additional questions or concerns.
- Finally, the investigator has the right to modify the above guidelines or follow a different course of action if deemed necessary to safeguard the subject's safety.

11.6 Investigational Product Accountability

- The investigator will ensure that the IP is stored as specified in the protocol (Step 11.2) and that the storage area is secured, with access limited to authorized study personnel.
- The investigator will maintain records that the IP was received, including the date received, IP identity code, date of manufacture or expiration date, amount received, and disposition.
- Records will be maintained that include the subject's initials and subject identification code (SIC), dispensation date, and amount dispensed.
- All remaining partially used and/or unused IP will be returned to the sponsor or sponsor's representative or destroyed with the permission of the sponsor in accordance with applicable laws and study site procedures.
- If the IP is to be destroyed, the investigator will provide documentation in accordance with sponsor's specifications.

12 Outcome Measures

- During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, and overall health and well-being (see Schedule of Assessments, Section 4).
- Investigator or his/her delegate will be required to review, critically evaluate, and record the amount of insulin subjects have used, as well as glucose levels, during the 3-day period immediately preceding pre-specified study visits (for additional details, see Step 21 Intensive Diabetes Management and Section 4. Schedule of Assessments).
- Diaries will be provided to participants at specified study visits and reviewed and collected at the next visit. Subjects on an insulin pump will provide outputs of their insulin pump activity for the diary days.
- Depending on the participants' age, a parent or guardian may oversee the collection of data on the diary or assist with making outputs of insulin pump activity available to the investigator or his/her delegate.
- Subjects who volunteer to undergo CGM and with access to a CGM device will provide downloads on a regular basis.
- Generally, these downloads will occur at the time of diary data collection but downloads may occur at other visits or may occur without a clinic visit either by mailing a used glucose sensor to the clinic for download or by providing a download via the internet.
- Methods for data transfer will be established with patients and/or a parent or guardian on a case-by-case basis.

13 Study Timeline

13.1 **Study Duration**

Total study duration for a subject is approximately 115 weeks, including screening, pretreatment, treatment, and study follow-up visits. All randomized subjects will be followed for safety and efficacy for approximately 104 weeks after treatment.

13.2 **Visit Windows**

All scheduled study visits must occur within the time limits specified in Table 6-1. Visits that occur outside of the specified windows will be considered protocol deviations.

Table 6-1: Visit Windows

	Visit Day(s)	Visit Windows
Visit 1: (screening)	-79 to -23	day -79 to day -23
Visit 2: (pretreatment)	-16 to -15	no window
Visit 3: (treatment)	0	no window
Visit 4:	Removed beginning with Protocol Version 6.0	
Visit 5:	7	±1 day
Visit 6:	14	±3 days
Visits 7 and 8:	28 and 91	±7 days
Visits 9 through 13:	182, 273, 364, 546, and 728	±14 days

STUDY VISIT ASSESSMENTS

14 Additional details on study procedures can be found in Section Study procedures.

14.1 **Screening Visit (Visit 1, Day -79 to -23):**

- Administer informed consent and assent
- Collect demography: gender, date of birth (age), race, and ethnicity
- Collect medical history (including date of initial type 1 diabetes diagnosis according to ADA criteria [see Appendix 17.1])
- Collect concomitant medications and concurrent procedures
- Perform complete physical examination, including Tanner stage evaluation
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature

- Advise on iron supplementation (recommended to take one week prior to the pretreatment visit and up to visit 7, if eligibility is continued)
- Administer Tdap and inactivated flu vaccine, if required
- Perform urine pregnancy test (on female subjects of childbearing potential)
- Review inclusion and exclusion criteria
- Assess adverse events
- Blood draw for:

1. Safety: Hematology, clinical chemistry
2. Virus: HIV, hepatitis B, hepatitis C, HTLV 1/2, EBV, CMV, quantitative PCR for EBV and CMV viral load
3. Diabetes confirmation with serum autoantibodies: GAD, IAA, ICA/IA-2, ZnT8
4. Treg cell concentration in blood for manufacturing suitability (at PCT)
5. Select exploratory assays (see Step 7.6)

- TB test: Blood draw for QuantiFERON® or PPD skin test

1. PPD skin test should be evaluated 48 and/or 72 hours after test performed

- Perform 4-hour MMTT-stimulated C-peptide/glucose (see Section Mixed Meal Tolerance Test)
- Collect urine sample for urinalysis
- After the screening visit, randomize the subject once all screening procedure results are available and it's confirmed that the study eligibility requirements are met based on screening. Eligibility requirements will be confirmed at Visit 2 prior to the blood draw for Treg collection/IP manufacture.

14.2 Pretreatment Visit (Visit 2, Day -16 or -15)

- Collect concomitant medications and concurrent procedures
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Perform urine pregnancy test (on female subjects of childbearing potential)
- Review inclusion and exclusion criteria
- Assess adverse events
- Review diabetes assessments expectations, dispense subject diary, and instruct on how to complete the subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- For subjects using CGM, discuss options for data download
- Confirm subject eligibility and perform blood draw for Treg collection (On day of collection, blood must be shipped to PCT for IP manufacturing; blood volume being drawn based on the subject's weight at the time of blood draw)

14.3 Treatment Visit (Visit 3, Day 0)

Prior to IP infusion

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary
- Collect concomitant medications and concurrent procedures
- Perform limited physical examination
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Perform urine pregnancy test (on female subjects of childbearing potential)
- Review inclusion and exclusion criteria
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Blood draw for:

1. Hematology (results must be reviewed prior to IP infusion)
2. HbA1c
3. Select exploratory assays (see Step 7.6)

- Administer acetaminophen and diphenhydramine within 30-90 minutes of infusion (dose based on body weight [refer to Step 11.4 IP Infusion]); study personnel should wait until email receipt from PCT Quality Assurance (QA) regarding release for infusion before administration of the above listed medications

1. IP Infusion

- Infuse investigational product (IP dose will be based on the subject's weight at the time of screening (Visit 1).
- Assess adverse events

2. After IP infusion

- Perform vital signs 2-hours post-infusion: blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events

14.4 Day 1 (Visit 4) - NO LONGER USED BEGINNING WITH PROTOCOL VERSION 6.0

- Collect concomitant medications and concurrent procedures
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Blood draw for:

1. Hematology
2. Select exploratory assays (see Step 7.6)

14.5 Day 7 (Visit 5)

- Collect concomitant medications and concurrent procedures
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature

- Assess adverse events
- Blood draw for:

1. Hematology
2. Clinical chemistry
3. Select exploratory assays (see Step 7.6)

- Collect urine sample for urinalysis
- Download CGM data if applicable

14.6 **Day 14 (Visit 6)**

- Collect concomitant medications and concurrent procedures
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Dispense subject diary
- Download CGM data if applicable
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- Blood draw for:

1. Hematology
2. Select exploratory assays (see Step 7.6)

14.7 **Day 28 (Visit 7)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Dispense subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- Blood draw for:

1. Hematology
2. Select exploratory assays (see Step 7.6)

14.8 **Day 91 (Visit 8)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures
- Perform limited physical examination

- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Dispense subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- Blood draw for:

1. Hematology
2. Clinical chemistry
3. HbA1c
4. Select exploratory assays (see Step 7.6)

- Perform 2-hour MMTT-stimulated C-peptide/glucose (see Section Mixed Meal Tolerance Test)
- Collect urine sample for urinalysis

14.9 **Day 182 (Visit 9)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures
- Perform limited physical examination
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Dispense subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- Blood draw for:

1. Hematology
2. Clinical chemistry
3. HbA1c
4. Select exploratory assays (see Step 7.6)

- Perform 4-hour MMTT-stimulated C-peptide/glucose (see Section Mixed Meal Tolerance Test)
- Collect urine sample for urinalysis

14.10 **Day 273 (Visit 10)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures

- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Dispense subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for
- the diary days to the next visit
- Blood draw for:

1. Hematology
2. HbA1c
3. Select exploratory assays (see Step 7.6)

14.11 **Day 364 (Visit 11)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures
- Perform limited physical examination
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Dispense subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- Blood draw for:

1. Hematology
2. Clinical chemistry
3. HbA1c
4. Select exploratory assays (see Step 7.6)

- Perform 4-hour MMTT-stimulated C-peptide/glucose (see Section Mixed Meal Tolerance Test)
- Collect urine sample for urinalysis

14.12 **Day 546 (Visit 12)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures
- Perform limited physical examination
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable

- Dispense subject diary
- For subjects on an insulin pump, instruct the subject to bring outputs of pump activity for the diary days to the next visit
- Blood draw for:

1. Hematology
2. Clinical chemistry
3. HbA1c

- Perform 2-hour MMTT-stimulated C-peptide/glucose (see Section Mixed Meal Tolerance Test)
- Collect urine sample for urinalysis

14.13 **Day 728/Early Exit (Visit 13)**

- Contact subject approximately 4 days prior to scheduled visit to remind subject to complete subject diary and if using a pump, bring outputs of pump activity for the diary days
- Collect concomitant medications and concurrent procedures
- Perform complete physical examination
- Perform vital signs: height, weight, blood pressure, pulse rate, respiration rate and temperature
- Assess adverse events
- Collect and assess subject diary; download CGM data if applicable
- Blood draw for:

1. Hematology
2. Clinical chemistry
3. HbA1c
4. Select exploratory assays (see Step 7.6)

- Perform 4-hour MMTT-stimulated C-peptide/glucose (see Section Mixed Meal Tolerance Test)
- Collect urine sample for urinalysis
- Perform urine pregnancy test (on female subjects of childbearing potential)

Note

In the event that a subject prematurely discontinues the study, site personnel should make all efforts to bring the subject back to the site to compete Visit 13 procedures.

STUDY PROCEDURES

15 See the schedule of assessments (Section 4) for additional information on study procedures performed over the course of the study.

15.1 Medical, Medication, and Non-Drug Therapy-History

- At screening, the subject's medical history will be described for the following body systems including past surgeries, and start and end dates, if known: eyes, ears, nose, and throat; respiratory; cardiovascular; gastrointestinal; musculoskeletal; neurological; endocrine; hematopoietic/lymphatic; dermatological; and genitourinary.
- All medical history 30 days prior to screening will be recorded on the eCRFs (refer to the eCRF Completion Guidelines document for additional information).
- Beyond the 30-day window, the following should be documented, but, not limited to:

1. T1DM onset
2. Past hospitalizations
3. Past surgeries or medically significant procedures
4. History of unusual/atypical infections
5. History of serious bacterial, viral, fungal or other opportunistic infections
6. Allergies (including eczema, asthma, and any food and drug allergies)
7. History of other clinically significant diseases/ conditions as deemed by the investigator

- All medications taken and non-drug therapies received from 30 days before screening until completion/termination will be recorded on the concomitant medications and non-drug therapies electronic case report forms (eCRFs). Past vaccinations should also be documented.

15.2 Physical Examinations

- At screening and subsequent study visits, a physical examination will be performed. A complete physical exam assessment includes general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological.
- Limited physical examinations will be symptom-directed. The physical examinations should be performed by the same investigator each time, whenever possible.
- Tanner stage will be evaluated at screening (Visit 1). Tanner stage will be re-evaluated at 12 and 24 months if not already documented as ≥ stage III at a previous exam.
- At screening, physical examination abnormalities deemed clinically significant by the investigator, should be recorded as medical history.
- At subsequent study visits, if a new abnormal or worsened abnormal pre-existing condition is detected, the condition will be recorded as an adverse event.

15.3 Vital Signs

- Vital signs will include body temperature (ºC), respiratory rate (breaths/min), pulse rate (beats/min), systolic and diastolic blood pressure (mmHg), height, and weight.

- Blood pressure and pulse rate measurements will be measured after the subject has rested for at least 5 minutes. Blood pressure should be performed in the same position and measured using the same arm for the duration of the study when possible.
- On the day of IP infusion, vitals will be taken before infusion and again 2-hours post-infusion (vital signs assessment 2 hours post-infusion will not include height and weight).
- BMI z-score will be determined for eligibility using the charts provided in Appendix 17.3. To determine BMI z-score, the subject's BMI, weight and gender must be available. BMI can bederived using the formula below:

1. BMI = (weight in kg)/(height in m)^2, OR
2. BMI = (weight in lb)/(height in in)^2 x 703

15.4 Hematology

- The hematology panel will consist of complete blood count (hemoglobin, hematocrit, erythrocytes [i.e., red blood cell count], and leukocytes [i.e., white blood cell count]) with differential (i.e., basophils, eosinophils, lymphocytes, monocytes, and neutrophils), platelet counts, prothrombin time (PT; sec) or international normalized ratio (INR), and activated partial thromboplastin time (APTT; sec).
- Coagulation assays will only be performed at baseline. A limited hematology assessment will be performed at the treatment visit (Day 0), which will only include the complete blood count (CBC) with differential, and platelet counts. Hematology can be evaluated in either the fed or fasted state.

15.5 Clinical Chemistry

- The clinical chemistry panel will consist of C-reactive protein (CRP), creatinine, estimated glomerular filtration rate (e-GRF), creatine kinase (CK), electrolytes (sodium, potassium, chloride, calcium, magnesium, and phosphate), bicarbonate, total protein (T-pro), albumin, ALT, total bilirubin (T-bil), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), total cholesterol (t-cho), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), and glucose.
- Clinical chemistry will be evaluated in the fasted state.

15.6 Hemoglobin A1c (HbA1c)

HbA1c can be evaluated in either the fed or fasted state.

15.7 Other tests

- At screening, blood may also be collected for the tests described below.
- Blood will be collected to determine the regulatory T-cell count to satisfy the requirement for manufacturing of investigational product.
- Virology testing (including human immunodeficiency virus [HIV] 1/2, hepatitis B, hepatitis C, human T-lymphotropic virus [HTLV] 1/2, Epstein-Barr virus [EBV], cytomegalovirus [CMV])

will be performed for eligibility determination.
- Whole-blood quantitative polymerase chain reaction (PCR) for EBV and CMV viral load will also be assessed as part of the screening procedures and then as necessary after treatment only when a subject is symptomatic.
- Additionally, QuantiFERON test or PPD skin test may be performed to rule out tuberculosis.
- For eligibility determination, an autoantibody analysis will be performed to ensure that the subject is positive for at least one islet cell autoantibody as specified in the inclusion criteria.

15.8 Mixed Meal Tolerance Test (MMTT)

- The MMTT will be performed in the morning, where a mixed meal will be administered and blood collected up to 250 minutes.
- The 4-hour MMTT should take 250 minutes to perform, and the 2-hour MMTT should take 130 minutes.
- Refer to the Appendix (Section Mixed Meal Tolerance Test) for details on the MMTT.

15.9 Urinalysis

The urinalysis panel will consist of protein, occult blood, glucose, urobilinogen, bilirubin, ketones, and sediments.

15.10 Pregnancy Testing

Urine pregnancy testing for human chorionic gonadotrophin (hCG) will be performed on female subjects of childbearing potential.

SUBJECT MANAGEMENT

16 Informed Assent/Consent and Enrollment

Subjects will be enrolled when they have provided informed assent/consent to participate in the study, met all inclusion and none of the exclusion criteria, completed the screening visit, and have been randomized.

17 Subject Identification Code (SIC)

- The subject identification code will be a 3-digit site number-3-digit subject number (e.g., 111-002) reflecting the order in which ICFs were signed.
- For example, the third subject who signed an informed consent form at study site 002 will be identified as Subject 002-003.
- All study documents and related material (e.g., electronic case report forms [CRFs], clinical documentation, sample containers, drug accountability logs, etc.) will be identified with the SIC.

18 **Screening**

- Enrolled subjects must meet all inclusion criteria and none of the exclusion criteria, and have completed all screening procedures prior to the blood draw for Treg collection at Visit 2. Subjects who have failed the screening process and have not been randomized will be recorded as a screen failure.
- Re-screening of subjects is allowed, at the discretion of the investigator with prior approval from the study sponsor, unless there are clinical implications that impact the ability of subject to meet selection criteria change.

19 **Randomization**

- Subjects will be randomized once all screening procedure results are available and it's confirmed that the study eligibility requirements are met based on screening.
- Eligibility requirements will be confirmed at Visit 2 prior to blood draw for Treg collection/IP manufacture.
- Randomization of eligible subjects will be performed using the Interactive Response Technology (IRT) system. Sites will log into the IRT system (Clincapture) to obtain the randomization code for the subject.
- The randomization allocation ratio will be 1:1:1. Randomization will be done centrally with stratification by screening MMTT-stimulated C-peptide AUC mean value.
- Details regarding the randomization assignment will be discussed in the Statistical Analysis Plan (SAP)

20 **Blood Collection for CLBS03 Manufacturing**

- Blood will be collected (approximately 6 mL/kg up to 540 mL based on weight at Visit 2 [pretreatment visit]) from the designated study subject via phlebotomy for Treg isolation and expansion to generate the CLBS03 cell product.
- Blood is collected into standard CPD (citrate-phosphate-dextrose) blood collection bag(s) following standard clinical procedures.
- The bag(s) is labeled with the subject's unique subject randomization code / identifier number.
- The blood is packaged in a shipping box validated to maintain temperature between 15 to 30°C and express-shipped with a temperature monitoring device to the PCT (Mountain View, CA) facility for processing of Treg cells.
- Blood collected that is not used for CLBS03 manufacturing (i.e., blood from control subjects) may be used for optimizing Treg processing techniques, such as Treg expansion and stability extension of the CLBS03 products.
- Additional details on the blood collection process can be found in the Investigational Product: Collection and Infusion Manual.

21 **Diabetes Management**

- During the study, all participants are expected to be receiving intensive management of their diabetes, and HbA1c will be assessed every 3 months to evaluate metabolic control. The goal of treatment will be to maintain the HbA1c level as close to normal as possible, without frequent occurrence of hypoglycemia.
- All individuals should strive for targets in accordance with current ADA recommendations, with HbA1c levels of <7.5% in children under the age of 19 years, and with preprandial glucose levels of 90-130 mg/dL, postprandial levels of less than 180 mg/dL, and bedtime levels of 90-150 mg/dL.[39]
- All participants will be expected to take a sufficient number of daily insulin injections to meet the glycemic targets. In general, the expectation is that all participants will receive at least four injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion.
- All participants will be expected to manage their diabetes with an insulin pump if possible. Glucose levels should be checked frequently, at a minimum of 4 times per day (before each meal and at bedtime).
- Subjects who are willing and have access to a continuous glucose monitoring device are strongly encouraged to monitor their blood glucose in this way. The outputs from these devices will occur at regular intervals and will be used in exploratory data analyses.
- Depending on the participants' age, parent or guardian involvement to obtain these device outputs may be necessary. Subjects will not be permitted to use non-insulin pharmaceuticals for glycemic control.
- The primary responsibility for diabetes management will reside with the treating or referring diabetes care provider.
- The study team will provide close additional support through regular interaction, and records of the glucose levels and insulin dosing will be evaluated by the study team at regularly scheduled study visits.

22 **Subject Diaries**

- Participants will be required to record the amount and the type of insulin they have used, as well as blood glucose levels during the 3-day period immediately preceding each pre-specified study visit.
- Subject diaries will be provided to participants at select study visits (see Section 4) and reviewed and collected at the next visit. Subjects will be instructed to document all insulin administrations and glucose assessments during the 3-day period.
- Depending on the participants' age, a parent or guardian may oversee the collection of data on the diary. Subjects on an insulin pump will bring outputs of their insulin pump activity for the diary days.
- Subjects using CGM will provide data downloads. Sites will contact the subject for diary completion approximately 4 days prior to the visit.

- Depending on the participants' age, parent or guardian involvement to obtain these device outputs may be necessary.

23 **Procedures for Monitoring Subject Compliance**

All study procedures are to be performed by the investigator or under his/her designation to a sub-investigator or study team member.

24 **Withdrawal from Study**

- Discontinuation of, or partial treatment does not require subject withdrawal from participation in the study. Any subject may voluntarily withdraw consent for continued participation and data collection.
- Reasons for discontinuation will be reported on the appropriate eCRF, including: screen failure, adverse event (e.g., death), discontinuation by subject (e.g., lost to follow-up [defined as 3 documented unsuccessful attempts to contact the subject], dropout), physician decision (e.g., pregnancy, progressive disease, non-compliance with IP/protocol violation(s), recovery), study terminated by sponsor, or other (reason to be specified by the investigator, e.g., technical problems).
- Regardless of the reason, all data available for the subject up to the time of discontinuation should be recorded on the appropriate eCRF. The data collected on withdrawn subjects may be used in the analysis and included in the clinical study report.
- Discontinuation (i.e., complete withdrawal from study participation) may be due to dropout (i.e., active discontinuation by subject) or loss to follow-up (i.e., discontinuation by subject without notice or action).
- Additionally, the investigator and sponsor have the discretion to discontinue any subject from the study if judged that continued participation would pose an unacceptable risk for the subject.
- In the event that the IP cannot be manufactured to meet release criteria, the subject will be terminated from the study prior to receiving treatment.
- In the event of subject discontinuation from treatment due to an AE, additional clinical and/or laboratory investigations (that are beyond the scope of the required study observations/assessments) may be performed as part of the event evaluation.
- These investigations will take place under the direction of the investigator in consultation with the sponsor, and the details of the outcome may be reported to the appropriate regulatory authorities by the sponsor or its designee.
- In the event that a subject prematurely discontinues the study, site personnel should make all efforts to bring the subject back to the site to compete Visit 13 procedures.

PARTICIPANT SAFETY

25 **Risks and Potential Risks from Tregs**

- The autologous nature of Tregs that constitute CLBS03, and the equivalency in biologic function robustness between the expanded cells and freshly isolated Tregs, diminishes the possibility of a significant toxicity potential. Primary pharmacology animal studies also support this conclusion.
- Because the potential toxicity of $CD4^{+}CD25^{+}CD127^{lo/-}$ Treg cell therapy cannot be evaluated in currently available mouse models, the data from early phase clinical trials provide the most relevant and clinically meaningful assessment of potential toxicity.
- Based on this early clinical data, and other biologic and experimental considerations, the following risks outlined in the subsections below are the most relevant to CLBS03 administration.

25.1 T-Cell Suppression

- There is accumulating data related to the clinical use of Tregs to treat T1DM and other autoimmune diseases.
- In mice, Tregs are known to suppress naïve T-cell responses to antigens. Less is known about ongoing immune responses, especially to viruses and bacteria; it is not known whether Tregs will alter protective immunity.
- In the GMU Phase 1 trial, previously immunized adolescents treated with Tregs (similar to CLBS03) maintained protective titers against hepatitis B virus (HBV) and rubella viruses; only one child was inadvertently immunized against HBV and mounted an appropriate serological response.
- To date, no significant safety issues were identified in clinical trials with Tregs. In addition, appropriate subject selection criteria and clinical monitoring will be implemented in the CLBS03 clinical development program.
- It is advised that age appropriate vaccinations are current for enrolled subjects before receiving study drug (CLBS03 or placebo) (see Step 8.2, Exclusion Criteria). Subjects with active infections or CMV/EBV viremia will have to clear them before receiving study drug.
- At this time, subjects with certain chronic infections will be excluded from participation in the development program.

25.2 Transfusion Reaction

- Side effects reported from previous human trials involving T-cell infusions include transient fever, chills, and/or nausea.
- Subjects will be administered acetaminophen and diphenhydramine hydrochloride prior to the infusion of Tregs (See Step 11.4, IP Infusion). These medications may be repeated every six hours as needed.
- Participants will not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol®) or dexamethasone (Decadron®) unless deemed necessary by the medical team for subject safety.
- If corticosteroids are required for an acute infusion reaction, an initial dose of 0.5 mg/kg methylprednisolone should be used.

25.3 Loss of Immune Surveillance and Risk for Lymphoproliferative Disease

- T lymphocytes are one major component of tumor surveillance and it is possible that cells that inhibit T lymphocytes could impair this tumor surveillance function. There is lack of conclusive evidence linking adoptive transfer of Tregs to cancer promotion.
- The relationship between Tregs and cancer have been shown in preclinical and clinical observations; however, these observations were in established cancers, remain contradictory, and do not support a causal relationship of Tregs in de novo cancer promotion.[43-45]
- A relatively small degree of immune suppression is expected from a single administration of Tregs, but the experience from the two Phase 1 clinical trials minimizes this concern. Complications such as lymphoproliferative disease are a possible but unlikely concern with a single Treg infusion.
- The Phase 1 T1DM study follow up data show no evidence of EBV reactivation (except for one subject who developed a primary CMV infection at the time of the Treg infusion and cleared the infection quickly through a normal clinical course).
- As indicated in the exclusion criteria above (Step 8.2), subjects with evidence of EBV/CMV infections will have to clear viremia before receiving study drugs. In addition, close monitoring will be implemented during the clinical development program.
- Additionally, the long term follow-up of all treated participants will determine whether there is evidence of an increase in the frequency of tumors. To date, most subjects have completed more than 1 year follow up, and no issues have been noted in either Phase 1 clinical trial.
- Additional follow-up for subjects in both trials is currently ongoing and any significant findings related to this risk will be monitored in the clinical development program.

25.4 Worsening Diabetes

- It is possible that Tregs could have an anti-islet effect and thus worsen diabetes. This may occur independently of whether the functional phenotype of the Tregs changes from a regulatory to an effector phenotype after in vivo adoptive transfer.
- However, this is likely to be mitigated by the relatively higher ratio of T-regulatory cells, the polyclonal nature of the infused cells, low islet antigen-specific precursor frequency in the preparation, and endogenous regulatory mechanisms.
- To date, there is no significant clinical evidence to support this potential, but any sign of worsening diabetes will be closely monitored by utilizing MMTT-stimulated C-peptide as an efficacy endpoint in the clinical development program for T1DM.
- During the course of the trial, MMTT-stimulated C-peptide levels will be monitored by the DSMB.

25.5 Reproductive Risks

- There may be an unexpected risk to an unborn or nursing child. Pregnant and breastfeeding women will be excluded from participation in the study. Pregnancy testing will be performed on female subjects of childbearing potential and test results must be negative prior to treatment.
- Females of child bearing potential (i.e., females who have reached puberty or have had their first menstrual bleeding) must: a) be surgically sterile, or b) be practicing an acceptable method of birth control for the duration of their participation in the study.
- Acceptable methods of birth control are: oral contraceptive tablets, hormonal implant device, hormonal patch, intrauterine device, diaphragm and contraceptive cream or foam, condom with spermicide, or abstinence.
- Males must also agree to use a reliable and accepted method of contraception for the duration of their participation in the study. Acceptable methods of birth control are: condom with spermicide, or abstinence.
- At every study visit the sexual activity and contraceptive use of participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, s/he will be counseled about the need to use a reliable and acceptable form of birth control.
- Female subjects will also be required to undergo a urine pregnancy test at the screening visit (Visit 1, Day -79 to -23), pretreatment visit (Visit 2, Day -16), treatment visit (Visit 3, Day 0), and Visit 13 (Day 728).
- A positive pregnancy test will result in the subject being discontinued from the study. Refer to Step 34 for details in the event of subject pregnancy, and refer to Step 24, Withdrawal from Study for details regarding subject discontinuation.

26 **Procedure-Related Risks**

- The amount of blood required to be drawn for Treg collection/IP manufacture, and safety and efficacy monitoring could represent potential safety risks. There were no reports of related serious or severe AEs associated with blood volumes collected in the GMU Phase 1 trial in children.
- Hemoglobin levels will be monitored throughout the trial. Additionally, the investigator(s) will recommend iron supplements to subjects who successfully complete the screening visit.

27 **Concomitant and Prohibited Medications**

The following medications are not permitted throughout the duration of the study:

- Use of non-insulin pharmaceuticals that affect glycemic control.
- Use of systemic corticosteroids or other immuno-modulatory drugs. Topical corticosteroid and nasal steroid use should be consulted with the study sponsor prior to use.
- Vaccination with a live virus one year after Visit 3.
- Vaccination with a killed virus 3 months after Visit 3.

28 **Expected Side Effects and Adverse Events**

- Mild upper respiratory tract infections could be expected since they were commonly observed in the adult Phase 1 study.
- Mild drop in hemoglobin could be expected following blood collection for Treg collection/IP manufacture. This procedure was not associated with severe or serious AEs in the GMU Phase 1 study in adolescents. Hemoglobin will be monitored and supplementary iron will be administered.
- AEs related to metabolic control of T1DM are to be expected in this subject population, but at comparable rates to that for an age-matched population with new onset T1DM.

ADVERSE EVENT REPORTING AND SAFETY MONITORING

29 **Adverse Event**

- All AEs will be recorded from the time of consent until study completion or discontinuation. An AE is defined as any untoward medical occurrence in a subject treated with the IP, regardless of a causal relationship with the treatment.
- An AE can therefore be any unfavorable and unintended sign (including clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an IP, whether or not related to the IP.
- Any clinically significant abnormal laboratory finding can be repeated before being reported as an AE. Any medical occurrence that leads to dosing interruption, subject discontinuation, or administration of concomitant medication for treatment will be considered an AE.
- Each untoward medical occurrence experienced before the first treatment procedure (i.e., from the time of signed informed consent up to but not including the IP infusion) will be described on the AE eCRF.
- Any untoward medical occurrence that qualifies as an SAE, regardless of study drug administration, will be recorded as an SAE on the eCRF, and reported according to the sponsor as outlined in Step 30 below.
- As hypoglycemic events are part of the natural course of T1DM, severe hypoglycemia (defined as an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions) will be reported as an AE.
- Non-severe hypoglycemia deemed clinically significant by the investigator may also be reported as an AE.

30 **Serious Adverse Event**

A serious adverse event is defined as any adverse event resulting in any of the following outcomes:

1. Outcome is fatal/results in death
2. Is life-threatening (at the time of the event)

3. Requires hospitalization or results in prolonged hospitalization
4. Results in persistent or significant disability/incapacity
5. Results in congenital anomaly/birth defect
6. Is a medically important event that may not be immediately life-threatening or require hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the definitions above (e.g., infection, allergic reaction, etc.)

- SAEs will be followed until resolution, medically stabilized, or 30 days after the end-of-study visit, whichever comes first.
- All SAEs are to be reported to INC Research within 24 hours of becoming aware of the event.
- To report an SAE or event of special interest, fax the report form to 1-877-464-7787 or email it to INCDrugSafety@increresearch.com.

31 **Events of Special Interest**

Events of special interest will be captured for this study and include the following:

1. Infusions prematurely stopped or paused due to an adverse event
2. Severe hypoglycemia defined as an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions
3. Infusion-related reactions resulting in discontinuation of infusion or requiring medical treatment
4. Infections with severity of Grade 3 or worse; or any opportunistic infections (Grade 3 defined by NCI-CTCAE as severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living)
5. Ketoacidosis
6. Cancer-related events
7. C-peptide failure (defined as the first occurrence of 2-hour or 4-hour MMTT-stimulated C-peptide concentration <0.2 pmol/mL). This will not be considered or reported as an AE as the information will be derived from C-peptide measures at the time of data analysis.

- Events of special interest (except for C-peptide failure) will be followed similarly to SAEs and reported to INC Research employing the same process as for SAEs (see Step 30).

32 **Grading Event Severity and Causality**

The investigator will assess the severity of each AE using his/her clinical expertise and judgment based on the most appropriate description below, as recommended by NCI-CTCAE Version 4.03.[42]

1. **Grade 1** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2. **Grade 2** Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
3. **Grade 3** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
4. **Grade 4** Life-threatening consequences; urgent intervention indicated.
5. **Grade 5** Death related to AE.

- Causality is a determination of whether there is a reasonable possibility that the IP is etiologically related to/associated with the AE.
- Causality assessment includes for example, assessment of temporal relationships, dechallenge/rechallenge information, association (or lack of association) with underlying disease, presence (or absence) of a more likely cause, and physiological plausibility.
- For each AE, the investigator will assess three causal relationships to assess whether the AE was related to the blood collection procedure, the infusion procedure, or the investigational product.
- The investigator will use his/her clinical expertise and judgment according to the following most appropriate algorithm for the circumstances of the AE:

32.1 Not related (both circumstances must be met)

- Is due to underlying or concurrent illness, complications, concurrent treatments, or effects of concurrent drugs
- Is not related (i.e., does not follow a reasonable temporal relationship or has a much more likely alternative etiology).

32.2 Unlikely related (either one or both circumstances are met)

- Has little or no temporal relationship
- A more likely alternative etiology exists

32.3 Possibly related (both circumstances must be met)

- Follows a reasonable temporal relationship
- An alternative etiology is equally or less likely compared to the potential relationship

32.4 Probably related (both circumstances must be met)

- Follows a strong temporal relationship
- Another etiology is unlikely or significantly less likely

33 **Pre-existing Diseases**

- Pre-existing diseases that are present before entry in to the study (as described in the medical history) will not be recorded as AEs, but will be documented as part of the subject's initialmedical history.
- Pre-existing diseases that manifest with the same severity, frequency, or duration after IP exposure also will not be recorded as AEs, but will be documented in the subject initial history.
- However, when there is an increase in the severity or duration of a preexisting disease, the event must be described as an AE, and recorded on the eCRF.

34 **Pregnancy**

- Pregnancy is not considered to be an AE or an SAE, unless a complication occurs that meets the requirements for an AE or SAE, but must be reported on a pregnancy report form. Female subjects who are pregnant or likely to become pregnant are excluded from this study.
- In the event a subject becomes pregnant during the study, a pregnancy report form must be completed to capture potential drug exposure during pregnancy, and the pregnancy must be reported within 24 hours of notice.
- For subjects who exit the study prematurely, pregnancies occurring up to 90 days after study treatment should be reported.
- Any pregnant subject must be followed until the outcome of her pregnancy is known (i.e., normal delivery, abnormal delivery, spontaneous/voluntary/therapeutic abortion).
- The pregnancy (i.e., the mother and the fetus) must be followed through delivery with regard to outcome.

35 **Adverse Event Reporting and Monitoring**

- All AEs will be recorded from the time of consent until study completion or discontinuation. For subjects who exit the study prematurely, SAEs occurring up to 90 days after study treatment should be reported.
- All AEs will be described using the sign, symptom, or medical diagnosis (where available, medical diagnosis is preferred over symptom) on the AE eCRF in standard medical terminology in order to avoid the use of vague, ambiguous, or colloquial expressions.
- Each AE will be defined as a SAE or non-serious AE according to the definitions in Section Adverse event reporting and safety monitoring.
- Both serious and non-serious AEs will be followed until resolution, medically stabilized or 30 days after the end-of-study visit, whichever occurs first.
- All SAEs are to be reported to the INC Research within 24 hours of becoming aware of the event.

36 **Assessment of Adverse Events**

Each AE will be evaluated by the investigator for:

1. Seriousness as defined in Step 30
2. Severity as defined in Step 31
3. Causal relationship to IP exposure as defined in Step 31

- For each AE, the outcome (e.g., recovering/resolving, recovered/resolved, recovered/resolved with sequelae, not recovered/not resolved, fatal) and action taken will also be recorded on the AE eCRF.
- Recovering/resolving AEs will be followed until resolution, medically stabilized, or 30 days after the end-of-study visit, whichever comes first.

STATISTICAL CONSIDERATIONS

37 Enrollment will be completed in 2 parts.

- For Part 1 approximately 18 subjects will be initially randomized, at a 1:1:1 ratio, to all treatment arms. After subjects enrolled in Part 1 have reached the 28-day post-treatment visit, a safety review will be conducted.
- If the safety profile is deemed acceptable, enrollment in Part 2 will be allowed. An additional safety review will take place once subjects enrolled in Part 1 have reached the 3-month post-treatment visit.
- The additional subjects will be enrolled in Part 2 and randomized to all treatment arms at a ratio of 1:1:1 to reach a total enrollment of approximately 111 subjects.
- The randomization allocation ratio will be 1:1:1 and will be done centrally with stratification by screening MMTT-stimulated C-peptide AUC mean value.

37.1 **Analysis Populations**

- Several analysis populations will be defined for this trial for the summaries and analyses of the study data.
- The Intent-to-Treat (ITT) population will consist of all randomized subjects who receive a study treatment.
- Subjects will be analyzed according to the treatment to which they were randomized, even if they received a different treatment or less than a full dose of the assigned treatment.
- The ITT population will be used for subject demographics and efficacy analyses.
- The modified Intent-to-Treat (mITT) population will consist of all randomized subjects who receive a study treatment and have at least one post-treatment assessment, with their treatment group designation defined by the actual numbers of Treg cells dosed, which will be further defined in the SAP. This population will be used for efficacy analyses.

- The Per-Protocol (PP) population will consist of all randomized subjects who receive the correct dose of the randomized study treatment and have at least one post-treatment assessment, have MMTT-stimulated C-peptide levels assessed at day 182 (week 26), and do not have any major protocol violations.
- This population will exclude subjects who receive less than the intended treatment dose. The PP population will be used for subject demographics and as a supportive analysis for the primary and secondary efficacy analyses.
- The Safety population will consist of all subjects who receive study treatment, analyzed according to the treatment they received.
- Further details on the definitions of the analysis populations will be provided in a prospectively developed statistical analysis plan (SAP).

37.2 Study Endpoints

- **Primary Endpoints**

The primary study endpoint is the 4-hour MMTT-stimulated C-peptide AUC mean at 26 and 52 weeks.

- **Secondary Endpoints**

1. The 2-hour MMTT-stimulated C-peptide AUC mean at 13, 26, 52, 78 and 104 weeks and the 4-hour MMTT-stimulated C-peptide AUC mean at 104 weeks.
2. Additional metabolic evaluations will include a comparison between the study treatment groups in:

- DDI as measured by U/kg BW at weeks 4, 13, 26, 39, 52, 78 and 104.
- Severe hypoglycemia, defined as an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions, occurring from the time of treatment through weeks 13, 26, 52, 78 and 104.
- HbA1c levels measured at 13, 26, 39, 52, 78 and 104 weeks.
- Fasting blood glucose levels at weeks 13, 26, 52, 78 and 104 (based on MMTT).
- Post-prandial (2-hour post meal) blood glucose levels at weeks 13, 26, 52, 78 and 104 (based on MMTT).
- Proportion of subjects who achieve partial remission, as defined by persistent reduction in exogenous insulin administration preceding weeks 13, 26, 52, 78 and 104. This will be further defined in the SAP.
- Proportion of subjects who achieve complete remission, as defined by freedom from exogenous insulin adminsitration preceding weeks 13, 26, 52, 78 and 104. This will be further defined in the SAP.

- **Continuous Glucose Monitoring**

1. Serum glucose data from CGM devices will be examined for a subset of patients who are willing to use a CGM device and have access to a continuous glucose monitoring device.
2. As the science of CGM is still evolving and there is no consensus on the best way to examine these data, these analyses are considered exploratory.
3. Particular attention will be paid to observations of low and high glucose levels and the pattern of fluctuations in serum glucose over the course of the study. Additional details will be provided in the SAP.

- **Exploratory Biomarker Assays**

1. Exploratory analyses will be conducted to assess the effect of CLBS03 on the pathologic autoimmune response underlying T1DM and on general immune responsiveness.
2. The following parameters may be evaluated (see Step 7.5 for further details on the assays): 1) assessment of immune cell subset and intracellular cytokine profile, 2) cytokine analysis, 3) autoantibody analysis, 4) whole blood DNA epigenetic studies, 5) whole blood genomic studies, 6) CLBS03 tracking by stable isotope labeling, 7) functional cell based assays on cryopreserved samples, and 8) potential additional exploratory assays.
3. Descriptive and analytical statistical methods will be used to assess the significance of any observations. Additional details will be provided in the SAP.

- **Safety Endpoints**

1. The primary safety endpoints are treatment emergent adverse events (TEAE), SAEs, and events of special interest.
2. Additional safety and tolerability endpoints include clinical laboratory tests, physical examination findings, concomitant medications, and vital signs.

37.3 Demographic and Baseline Characteristics

Demographic and relevant baseline characteristics will be presented and summarized descriptively by treatment for the various analysis populations.

37.4 Statistical Analysis

1. General Considerations

- **Baseline:** Baseline refers to the last measurement collected on or prior to study treatment at Visit 3. Subjects with missing baseline data will have their baseline values imputed by the mean baseline value of subjects with baseline values.

- **Type 1 Error for Multiple Comparisons:** This is a Phase 2 exploratory study and thus there will be no adjustment of type 1 errors arising from multiple comparisons. All statistical

analyses will be conducted at the 2-sided 0.05 significance levels.

All collected data will be listed. A detailed SAP will be written and approved prior to database lock.

2. Analyses of the Primary Endpoints

- The AUC mean of 4-hour MMTT-stimulated C-peptide is defined as the AUC for the 4-hour MMTT-stimulated C-peptide divided by the actual time span the samples are collected during the target 4-hour period.
- The AUC will be calculated using the trapezoidal rule. Similarly, the AUC mean of 2-hour MMTT-stimulated C-peptide is defined as the AUC for the 2-hour MMTT-stimulated C-peptide divided by the actual time span the samples are collected during the target 2-hour period.
- The unit for AUC mean is pmol/mL.
- Data will be examined for normality and may or may not be log-transformed according to best statistical practice. In the event of log-transformation, the primary endpoint of 4-hour AUC mean of MMTT-stimulated C-peptide will be log-transformed by the formula $\log_e(x+1)$ before analyses[1].
- The baseline value of AUC mean of MMTT-stimulated C-peptide is collected at screening (Visit 1).
- The change in AUC mean of MMTT-stimulated C-peptide from baseline to Week 26 and Week 52 will be summarized descriptively by treatment.
- The mean values will be transformed back to the original scale by the formula exp(y)-1 to yield the inversed mean and inversed median.
- The 2-hour AUC mean of MMTT-stimulated C-peptide will be summarized similarly.
- The change in 4-hour AUC mean of MMTT-stimulated C-peptide from baseline Week 26 and Week 52 will be analyzed by a mixed-effect model with repeated measures (MMRM).
- The MMRM model will include the change in AUC mean of MMTT-stimulated C-peptide as the dependent variable; treatment, C-peptide stratum, visit, and treatment-by-visit interaction as fixed effects; and subject as a random effect.
- Baseline 4-hour AUC mean of MMTT-stimulated C-peptide will be included as a covariate. All observed AUC mean of MMTT-stimulated C-peptide data from post-baseline scheduled visits (including early termination visits) will be included in the MMRM analysis.
- In addition, if a subject's last available measurement during the 52-week assessment period is from an unscheduled visit or early termination visit, the value will be programmatically mapped to the next closest scheduled visit and included in the MMRM analysis.
- The MMRM model will employ an unstructured covariance matrix unless it does not converge; alternative covariance structures will then be examined and the Akaike information criterion (AIC) will be used to determine the best fit.
- The least squares (LS) mean of the change from baseline in log-transformed 4-hour AUC mean of MMTT-stimulated C-peptide will be calculated for each treatment at each visit together with the 95% confidence intervals.

- The LS mean difference between each treatment and placebo of the change from baseline in log-transformed 4-hour AUC mean of MMTT-stimulated C-peptide will also be calculated at each visit together with the 95% confidence intervals.
- The above LS means and the 95% confidence intervals will be transformed back to the original scale using the formula exp(y)-1 to yield the inversed LS means and the corresponding 95% confidence intervals.
- A regression analysis will also be conducted to study the dose-response relationship between the actual dose (actual number of cells per kg) and the primary endpoints of AUC means.
- If log-normally distributed, the AUC means will be log-transformed as in the primary analysis before being analyzed. The regression model will also include other exploratory variables that may have an impact on the primary endpoint.
- The list of variables will include age, time since diagnosis of T1DM, gender, body weight at baseline, baseline MMTT-stimulated C-peptide, baseline HbA1c, etc.
- A step-wise model selection method may be used to eliminate variables that are deemed not important or be confounded with other key variables. More detail will be provided in the SAP.

3. Analysis of the Secondary Endpoints

- The changes in the secondary endpoints of 2-hour AUC mean of MMTT-stimulated C-peptide from baseline will be analyzed in a similar manner as described above for the analysis of the primary endpoint whereby the response variable and the independent variables will include values at 13, 26, 52, 78, and 104 weeks.
- The change in the secondary endpoint of 4-hour AUC mean of MMTT-stimulated C-peptide from baseline will be analyzed in a similar manner as described above for the analysis of the primary endpoint whereby the response variable and the independent variables will include values at 104 weeks.
- The daily dose of insulin and HbA1c will be summarized and analyzed similarly as for the primary efficacy endpoints without the log-transformation.
- Severe hypoglycemia will be summarized by incidence and event rate adjusted by patient year of observation.
- The change from baseline in the secondary endpoints of fasting plasma glucose and the 2-hour post-prandial glucose measured by MMTT at Weeks 13, 26, 52, 78, and 104 will be analyzed in a similar manner as for the analysis of the secondary endpoint of the 2-hour AUC mean C-peptide.
- Proportion of subjects who achieve partial remission will be summarized by visit and analyzed for treatment comparisons using Fisher’s exact tests by visit.
- In addition, logistic regressions will be used to analyze the proportion by visit whereby the baseline 4-hour AUC mean MMTT-stimulated C-peptide will be included as a covariate.
- Proportion of subjects who achieve complete remission will be summarized and analyzed in the same manner as for partial remission.

4. Analysis of the Safety Endpoints

- The analysis of safety data will be performed for the Safety Population unless otherwise stated. The primary safety and tolerability endpoints are the incidence of TEAEs.

37.5 Adverse Events

- Treatment-emergent adverse events will be defined as those occurring during or after administration of randomized study treatment on Visit 3 or existing prior to the time of and worsening after the time of the dose of randomized study treatment through study termination or early termination.
- Adverse events prior to the dose of randomized study treatment on Visit 3 will be classified as pretreatment (non-treatment-emergent).
- Treatment-emergent AEs will be summarized by treatment, system organ class, and preferred term defined by the Medical Dictionary for Regulatory Activities (MedDRA®).
- The number of events, the number of subjects, and the percent of subjects who experienced at least one TEAE will be presented for each system organ class and for each preferred term by treatment group.
- TEAEs that lead to early withdrawals and serious TEAEs will be summarized in the same manner. Additional details will be provided in the SAP.

37.6 Clinical Laboratory Evaluations

- All hematology, clinical chemistry and urinalysis data will be listed by treatment, subject, and visit, including scheduled and unscheduled/repeat measurements. Laboratory assessments that are outside of normal ranges will be flagged.
- Baseline values, the values at each visit, and changes from baseline values will be summarized for each of the quantitative laboratory assessments by treatment.
- Shift tables of hematology, clinical chemistry, and urinalysis results will be used to summarize changes from baseline to study termination (or early termination).

37.7 Vital Signs and Physical Examinations

Vital signs at baseline, each scheduled visit, and changes from baseline values will be summarized by treatment. Clinical significant physical examination findings will be listed.

37.8 Sample Size and Power Estimation

- The goal of this study, assuming that safety is acceptable, is to determine if there is sufficient evidence of clinically meaningful bioactivity to proceed with further development.
- Since there are no prior data in the current study population, sample size estimation was performed based on projected difference in the decreases in the 4-hour AUC mean MMTT-stimulated C-peptide in the control and active treatment groups at the Week 52 visit.
- The following assumptions are used in sample size estimation for this study:

1. The baseline 4-hour AUC mean MMTT-stimulated C-peptide value is approximately 0.768 pmol/mL for all three groups.
2. The root mean square error (RMSE) or the within treatment group standard deviation for the end value of 4-hour AUC mean for all treatments at Week 52 (day 364) is σ=0.204 at the $\log_e(x+1)$ scale. The information comes from Table 5 in Lachin et al.[1]
3. The sample size calculation is conducted at the $\log_e(x+1)$ scale.
4. All statistical testing is done at a two-sided significance level of 0.05.

- A sample size of 33 subjects per treatment group will provide approximately 80% power to detect a 0.2 pmol/mL difference in the 4-hour AUC mean MMTT-stimulated C-peptide between an active treatment and the placebo with a two-sample t-test at a two-sided 0.05 significance level.
- A sample size of 33 subjects per treatment group will also provide at least 85% power to show a statistically significant correlation between the log-transformed 4-hour AUC mean MMTT-stimulated C-peptide at the Week 52 visit and the actual dose (actual number of cells per kg) assuming that the true correlation is 0.3. Statistical significance of the correlation is equivalent to statistical significance of the dose-response (regression) analysis of the log-transformed 4-hour AUC mean MMTT-stimulated C-peptide at the Week 52 visit with respect to the actual dose.
- Additionally, the same sample size will provide approximately 66% power for detecting a 50% attenuation in the decrease of the 4-hour AUC mean MMTT-stimulated C-peptide between an active treatment and the placebo with a two-sample t-test for the log-transformed 4-hour AUC mean MMTT-stimulated C-peptide at a 2-sided significance level of 0.05.
- Assuming a 10% dropout rate by week 26, 37 subjects per group need to be randomized.

37.9 **Interim Analysis Plan**

- A formal interim analysis allowing for early termination for futility will be conducted. The study will not be stopped for demonstrating statistically significant efficacy at the interim analysis. Therefore, there will be no adjustment of type 1 error associated with the interim analysis.
- As the target sample size is 111 subjects, the planned interim analyses will be conducted after approximately 50% of the total planned sample size have observed Week 26 assessments.
- The detailed definition of the futility criterion will be defined in the prospective SAP that will be finalized prior to the formal interim analysis.

DATA MANAGEMENT

38 **Study Documentation and Case Report Forms (CRFs)**

- The data collection tool for this study will be sponsor defined eCRFs to be completed by study-site personnel. The investigator will maintain complete and accurate study

documentation in a separate file.

- Study documentation may include medical records, records detailing the progress of the study for each subject, signed informed consent forms, drug disposition records, correspondence with the Institutional Review Board (IRB) and the study monitor/sponsor, screening and consent information, CRFs, SAE reports, laboratory reports, subject diaries, data clarifications requested by the sponsor or designee, and any other documentation deemed relevant and pertinent to the study and the study subjects.
- Subject data necessary for analysis and reporting will be entered into a validated database or data system in accordance with Title 21 of the Code of Federal Regulations (21 CFR) Part 11.
- Clinical data management will be performed by a sponsor assigned data management vendor in accordance with applicable data management vendor standards and data cleaning procedures.
- The investigator is responsible for the procurement of data and for the quality of data recorded on the eCRFs. The eCRF will be electronically signed by the investigator listed on Form FDA 1572.
- The handling of data by the sponsor and the data management vendor, including data quality assurance, will comply with regulatory guidelines (e.g., International Council on Harmonisation Guideline for Good Clinical Practice E6 [ICH GCP]) and the standard operating procedures of the sponsor or designee.
- Data management and control processes specific to the study will be described in the Data Management Plan.

39 **Document and Data Retention**

The investigator will retain study documentation and data (electronic case report forms) in accordance with applicable regulatory requirements and the document and data retention policy, as described in the Data Management Plan or other supporting documents as applicable.

40 **Direct Access to Source Data/Documents**

- The investigator/study site will cooperate and provide direct access to study documents and data, including source documentation for monitoring by the study monitor, audits by the sponsor or sponsor's representatives, review by the Ethics Committee, and inspections by applicable regulatory authorities, as described in the Clinical Study Agreement.
- If contacted by an applicable regulatory authority, the investigator will notify the sponsor of contact, cooperate with the authority, provide the sponsor with copies of all documents received from the authority, and allow the sponsor to comment on any responses, as described in the Clinical Study Agreement.

ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICEI

41 **Statement of Compliance**

- This study will be conducted in accordance with this protocol, the ICH GCP, 21 CFR, and applicable national and local regulatory requirements.
- Before enrollment of subjects into this study, the IRB will review and approve/give favorable opinion on the protocol, ICF, any promotional material/advertisements, and any other written information to be provided to the subjects.
- The IB will be provided to the IRB for review. The IRB's composition or a statement that the IRB's composition meets applicable regulatory criteria will be documented.
- If the protocol or any other information given to the subject is amended, the revised documents will be reviewed and approved/given favorable opinion by the IRB, where applicable.
- The protocol amendment will only be implemented upon the sponsor's receipt of approval and, if required, upon the sponsor's notification of applicable regulatory authority(ies) approval.

42 **Participating Centers**

- Participating clinical sites must have an appropriate IRB governance since they are actively engaged in research and provide informed consent.
- The protocol and consent/assent forms will be approved for the sponsor by a central IRB review prior to release to participating sites.
- Health Insurance Portability and Accountability Act (HIPAA) and applicable local regulations will be followed by each participating institution in accordance with each institution's requirements.
- The participating sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.
- The investigator is required to keep accurate records to ensure the conduct of the study is fully documented.
- The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants participating in this study.
- Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information).
- Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals. The investigational site will normally be notified in advance of auditing visits.

43 **Informed Consent/Assent**

- Written informed consent will embody the elements of informed consent as described in the Declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all

applicable laws and regulations.
- Investigators will enroll subjects according to the study eligibility criteria and in compliance with 21CFR50. The investigator will exercise no selectivity so that no bias is introduced from this source.

44 **Study Subject Confidentiality**

- The investigator will comply with applicable subject privacy regulations/guidance as described as per HIPAA and any additional local regulations.
- The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from subjects. When a subject participates in this study at more than one site, sharing of this information is required.
- Sharing of information obtained during this study between clinical centers and affiliates will be done to assure subject understanding and consent, safety, and adherence to protocol. Medical and research records will be maintained at each site in the strictest confidence.
- However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.
- Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information).
- Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals.
- Study records with the study subject's information for internal use at the clinical sites will be secured at the study site during the study.
- At the end of the study, all records will continue to be kept in a secure location for at least 2 years post market approval in the applicable region. There are no plans to destroy the records.
- Retained samples including genetic samples could be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment (e.g. via biomarker screening).
- Samples can also be utilized to further evaluate those subjects responsive to therapy to elucidate treatment mechanisms.
- The results of these future analyses, and any mechanistic studies, will not be made known to the participant.

45 **Risks and Benefits**

The risks of this study are presented in this protocol, the Investigator's Brochure and the informed consent form. There is no guaranteed benefit to subjects for their participation in the

study.

46 **Ethics**

- The study protocol, along with the required informed consent forms, will be approved by each participating institution's IRB prior to the initiation of any research procedures (at the site).
- In addition to details described in the sections above (informed consent, confidentiality, and risks and benefits), the investigators should review and consider ethical ramifications in the design and development of this protocol, in accordance with 21 CFR 50.
- The investigators will make every effort to minimize and monitor risks and discomforts to participants throughout the course of the study.

STUDY ADMINISTRATION

47 **Monitoring**

- Throughout the course of the study, the study monitor will make frequent contacts with the investigator. This will include telephone calls and on-site visits.
- The study will be routinely monitored to ensure compliance with the study protocol and the overall quality of data collected.
- During the on-site visits, the eCRFs will be reviewed for completeness and adherence to the protocol. As part of the data audit, source documents will be made available for review by the study monitor.
- The study monitor may periodically request review of the investigator study file to assure the completeness of documentation in all respects of clinical study conduct.
- The study monitor will verify that each patient has proper consent documentation from the patient and/or patient's authorized representative for study procedures and for the release of medical records to the sponsor, FDA, other regulatory authorities, and the IRB.
- The study monitor will also verify that assent was obtained for patients not capable of providing informed consent or that documentation is provided by the investigator explaining why the patient was unable to provide assent.
- The investigator or appointed delegate will receive the study monitor during these on-site visits and will cooperate in providing the documents for inspection and respond to inquiries.
- In addition, the investigator will permit inspection of the study files by authorized representatives of the regulatory agencies.
- On completion of the study, the study monitor will arrange for a final review of the study files after which the files should be secured for the appropriate time period.

48 **Medical Monitor and Data Safety Monitoring Board (DSMB)**

- All adverse events will be recorded on the adverse event forms, and the treatment related SAEs will be sent to the IRB, per their reporting requirements, and to sponsor.

- The study medical monitor or designee will review all adverse event reports, masked to treatment assignment. Further details are captured in the study medical monitoring plan.
- The DSMB is responsible for monitoring the safety of the trial subjects, and data integrity during periodic and ad hoc reviews.
- Following these reviews, it can recommend to the sponsor and the study executive committee the continuation of study as planned, modification of the protocol, or the stopping of the study.
- The sponsor will ensure the timely communication of the safety and efficacy data to the DSMB according to the agreed upon schedules or within reasonable time if requested for an ad hoc analysis.
- The DSMB will meet periodically during scheduled and ad hoc meetings as specified in its charter.
- The DSMB will be provided blinded data initially. Unblinded data may be provided if requested for specific considerations. Following enrollment of approximately 18 subjects in Part 1, the 28-day follow-up data will be assessed for safety by the DSMB as pre-specified in its charter.
- Enrollment in the study will be paused and will not resume until the DSMB has completed this review and communicated its recommendation to the study executive committee to continue the study as planned, continue with protocol modifications, or stop the study.
- Details of the DSMB membership, frequency of the meeting, data sharing/review procedures, and voting process will be outlined in the DSMB Charter.

Testing Procedure

49 Confirm that all prerequisite requirements of the test as detailed above are met prior to starting the test (i.e. participant has fasted, that fasting glucose is between 70 mg/dL and 200 mg/dL (will also be verified by in clinic by finger stick prior to testing), and that any insulin use prior to the test meets the specified guidelines).

50 Please refer to the Laboratory Manual for instructions on sample processing.

51 Check blood glucose level by finger stick and proceed with test if within range.

52 Establish IV line (after each blood draw, saline lock IV or run saline drip to keep open).
The first sample should be taken at least 10 minutes after establishing the IV line (this is the '–10 minute' sample).

53 After at least 10 minutes, the second sample should be taken just before the participant drinks the Boost HP (this is the '0 minute' sample). Immediately administer the Boost HP and instruct the participant to consume within 5 minutes (keep the Boost HP out of the reach of the participant so that you can control the start time). Start timer at beginning of the Boost HP

drink. Patient can drink water after finishing the Boost HP and freely throughout the remainder of the test.

54 The remaining samples should be drawn per the collection schedule based on the start time of the Boost HP drink.

55 It is critical to adhere to the collection schedule as closely as possible and to accurately record the collection times. However, it will not be considered a protocol deviation if a specimen is collected at an incorrect time.

56 At the conclusion of the test, check blood glucose and administer insulin as per participant's standard insulin plan.

57 Discontinue IV.

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